			APPLE HORTICULTURE/POSTHARVEST RESEARCH REVIEW Wednesday, January 23, 2019	
Time	**Page**	**PI**		**Yrs**
8:00		Hanrahan	Welcome - Introductions and housekeeping	
			Final Reports	
8:15	22	Amiri	Improved risk assessment and management of apple postharvest diseases	16-18
	29	Zhu	Ozone in apple storage: microbial safety and decay management: ***Written report only***	16-17
	38	Critzer	Understanding/managing the food safety risk of packline brushbeds: ***Written report only***	17
			Continuing Projects: 1:30 - 4:00	
1	49	Ganjyal	Improving food safety by hot air impingement drying: ***No-cost extension***	15-17
1	53	Ganjyal	Complying with the FSMA preventative controls for human food rule: ***No-cost extension***	17-18
1	60	Zhu	Control of *Listeria* on processing surfaces in apple packing facilities	17-19
1	67	Critzer	Systems-based approach for improved packinghouse sanitation	18-20
1	73	Critzer	Utility of rapid tools to assess cleanliness in apple packinghouses	18-19
1	78	Zhu	Fate of Listeria on apples at ozone and controlled atmosphere storage	18-20
1	86	Musacchi	WA38 fruit and dry matter for fruit quality/consumer preference: ***Out-of-cycle funding***	17-19
1	93	Hanrahan	Improving apple fruit quality and postharvest performance	*Internal*
	100	Rudell	Reducing scald after long-term CA storage	
	107	Rudell	Risk assessment for delayed sunburn and sunscald	
1	114	Evans	Apple scion breeding program: **Final report with 1-yr. extension**	18-20
	123	Evans	Apple scion breeding program	
2	130	Kalcsits	Control of fruit size and bitterpit on Honeycrisp using irrigation	17-19
2	138	DuPont	How do we measure and manage soil health for productive orchards?	17-19
2	145	Amiri	Evaluation of fungicide application methods: ***Final report***	*16-17*
2	154	Kalcsits	How does fruit acclimation to sunburn affect sunburn management?	*18-19*
2	161	Kalcsits	Optimizing light and water for orchards covered with netting	18-19
2	169	Lewis	WA38 demonstration trial block	18-19
2	175	Schmidt	Crop load and canopy management of WA tree fruit	*Internal*
2	182	Schmidt	WTFRC apple pesticide residue study	*Internal*
	186	Harper	Understanding decline on select apple scion-rootstock combinations: ***Written report only***	18-19
	192	Willett	Apple rootstock and scion evaluation; ***Written report only***	*Internal*

FINAL PROJECT REPORT
WTFRC Project Number: AP-16-106

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

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Total Project Funding: $150,000

Other funding sources: None

WTFRC Collaborative expenses: None

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

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

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

OBJECTIVES

This report summarizes results in the final year of a three-year project assessing the impact of rootstock cultivar on the soil microbiome, specifically examining root exudates and rhizosphere (root-zone) soil microbiome changes. The project objectives per the project proposal are below:

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

The significant **year one** findings reported in 2016, included a) defining specific root exudate metabolites differing among rootstocks G.41, G.935, M.9Nic29, and M.26, b) establishing that root exudate quantity correlates to rootstock vigor / tree size, c) delineating preliminary data regarding rhizosphere pH, and d) demonstrating that new apple seedling growth in replant soil is altered according to the genotype of the previously planted rootstock.

Significant findings for **year two** (2017) included a) determining that rootstock genotype-specific fungal rhizosphere communities differing from the no-tree soil control developed within 6 weeks after planting, b) phenolic compounds exuded from roots can inhibit pathogen growth, c) root exudates can lower soil pH, presumably due to contributions from organic acids and hydrogen ions.

SIGNIFICANT FINDINGS YEAR 3

Year three expanded upon Objectives 1, 2, and 3, to incorporate the assessment of the impact of the scion on the rhizosphere microbiome. Objective 4 was completed year two.

The significant findings for year three were:

1. In controlled environment/greenhouse studies, apple scion cultivar did not have a detectable cultivar-based effect on the root-zone microbial community during the first two seasons of growth after bud-grafting. Scions assessed were ‘Honeycrisp’, ‘Granny Smith’, and ‘G.41’, grafted onto rootstock cultivar ‘G.41’.
2. Evidence indicates that rootstocks can maintain fungal endophytes inside root tissue without symptoms of disease, even under sterile tissue culture conditions. Rootstock core fungal endophytes included genera *Ilyonectrica*, *Serendipita*, *Lasiosphaeria*, *Leptodontidum*, and *Paraglomus*. Potential beneficial biological functions are detailed in results and discussion. Rootstocks tested were ‘G.935’ and ‘M.26’.
3. In a greenhouse experiment, metabolites released by tree roots within the first growing season were shown to differ with the scion cultivar bud-grafted on the apple rootstock G.41 (even though no impact of these compounds was detected for the associated root-zone microbial community). Cultivar-based differences were more profound in metabolites with potential to inhibit pathogen growth than in metabolites that would promote overall microbial growth in

the root-zone. Scions assessed were ‘Honeycrisp’, ‘Granny Smith’, and ‘G.41’, grafted onto rootstock cultivar ‘G.41’.

4. When microbes were specifically excluded from the experiment (that is, micropropagated trees grown in sterile conditions with no natural root-zone microbial populations), more compounds that could be utilized as substrate by the rhizosphere microbiome than substrates with potential to inhibit pathogens differed between rootstock cultivars M.26 and G.935.
5. Assessment of metabolites produced by rootstocks in axenic conditions indicated that phloridzin and sorbitol are among the more abundant metabolites produced by apple roots; testing the impacts of these metabolites on the soil microbial community indicates that sorbitol has a significant effect on both the bacterial and fungal community structure, while phloridzin influenced only the fungal community structure and to a lesser extent than did sorbitol.
6. Extension roots and fibrous roots of apple rootstocks possess different phenolic compound profiles which also differ according to rootstock cultivar. Cultivars tested were ‘M.26’ and ‘G.41’.

The discussion will consider these results in the context of previous studies on the apple soil and microbiome, address how results impact future studies on the soil/rhizosphere/endosphere microbiome, rootstock breeding, and future directions.

METHODS

Finding 1: Apple scions ‘Granny Smith’ and ‘Honeycrisp’ were grafted on rootstock cultivar ‘G.41’, with ‘G.41’ grafted onto ‘G.41’ serving as a control. Root-zone (rhizosphere) soil was collected and analyzed for differences in the fungal microbial communities utilizing molecular methods. Briefly, soil DNA was extracted and the internal transcribed region of ribosomal DNA (a region commonly used to identify fungi) was specifically amplified from the soil fungal community via polymerase chain reaction. The resulting amplicons were digested with restriction enzymes and DNA fragments were subjected to terminal fragment length polymorphism (T-RFLP) analysis to establish a fungal community profile.

Finding 2: DNA extraction and molecular analysis to assess endophyte presence in axenically micro-propagated trees was performed using previously published methods [1]. Extracted DNA was subsequently submitted to a metagenomics sequencing service (Molecular Research, Shallowater, TX).

Finding 3: Nursery grown 3/8” diameter G.41 rootstock liners were planted in sterilized sand in pots and maintained under greenhouse conditions. Scions (Honeycrisp, Granny Smith, and G41) were bud-grafted onto the rootstock liners shortly after planting. Several months after grafting, root exudates were collected via a root dip method and processed similar to methods in outlined in Leisso et al. [2]. Samples were analyzed for biochemical compounds that could function either as growth substrate for microbes or could potentially inhibit microbes in the root zone.

Finding 4: M.26 and G.935 rootstock stem tissue was multiplied axenically via sterile micropropagation and treated to induce rooting (**Figure 1**). Three months after root initiation, rooted plantlets were subjected to a sterile root dip process to collect exudates. Exudates were filtered, flash frozen, concentrated, and analyzed via liquid chromatography – mass spectrometry. Full methods are detailed in a published article [1].

Figure 1. A sterilely propagated rootstock and collection of root exudate metabolites via a 'root dip' system.

Finding 5: Soil from two locations was treated periodically with phloridzin and sorbitol solutions. DNA was extracted from the soil and ribosomal DNA fragments from fungal and bacterial microorganisms were specifically amplified via polymerase chain reaction, digested with restriction enzymes, and fragments analyzed to establish a community profile (T-RFLP analysis).

Finding 6: rootstock liners ('M.26' and 'G.41') were planted in pasteurized sand and maintained in a growth chamber. Six weeks after planting trees were removed from the pots, and roots were divided into separate samples classed as either "extension" or "fibrous" roots, taking 3 samples of each root type per tree resulting in a total of 36 samples. "Extension" roots for metabolite assessment were attached to the main stem and generally larger than 1 mm in diameter; "fibrous" roots were attached to extension roots and had a greater number of branches per length and overall were smaller than 0.5 mm in diameter with a fibrous morphology. Roots were flash frozen in liquid nitrogen and metabolites expected to have inhibitory activity toward plant pathogenic organisms were analyzed. The experiment was repeated with a longer tree growth period prior to root tissue collection.

RESULTS & DISCUSSION

Significant finding 1. The apple scion did not have profound detectable effect on the fungal rhizosphere microbiome in terms of overall diversity based upon species count (**Figure 2**) or in composition (**Figure 3**) over the first two seasons of growth after bud-grafting. For brevity, data on bacteria are not shown but overall results have a similar interpretation. Scions assessed were 'Honeycrisp', 'Granny Smith', and 'G.41', grafted onto rootstock cultivar 'G.41'.

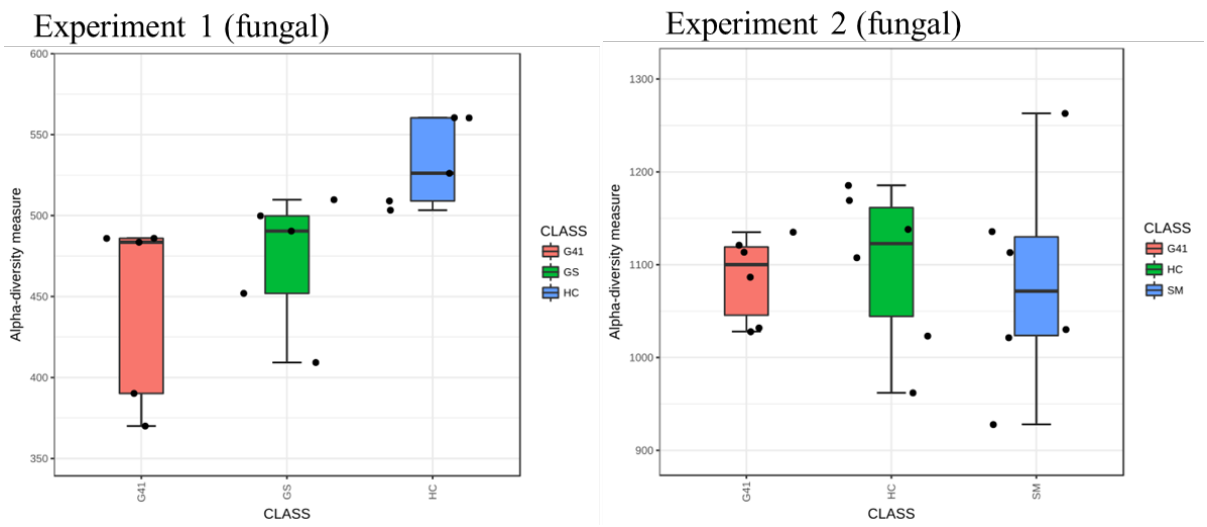


Figure 2. Fungal microbiome diversity in terms of species count.

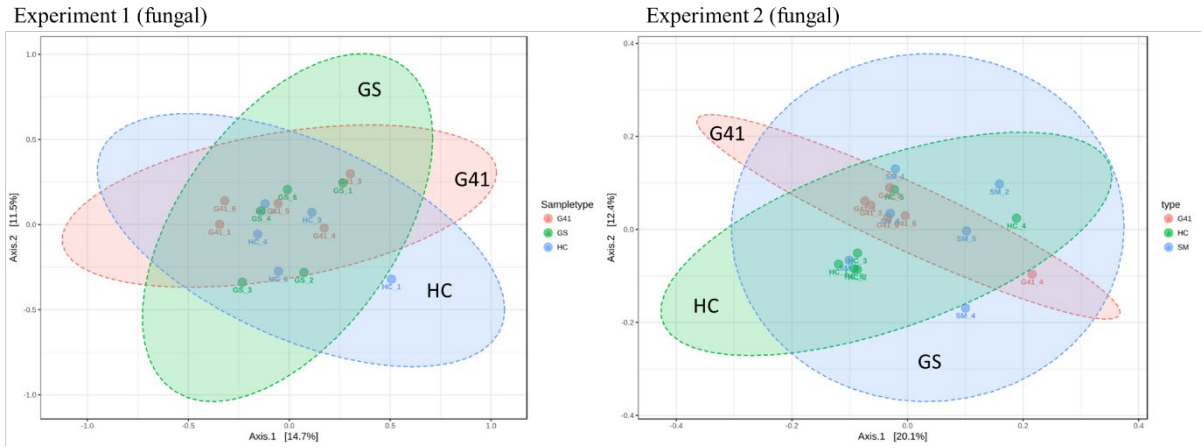
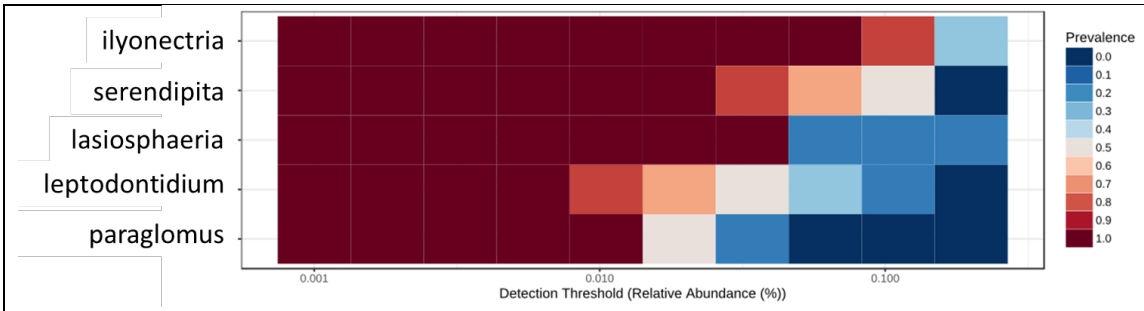


Figure 3. Fungal community composition detected in the rhizosphere of G.41 rootstock was not influenced by scion cultivar. Data represent principal component analysis of operational taxonomic unit (OTU) data derived through amplicon sequence analysis of fungal ribosomal DNA. GS = Granny Smith; HC = Honey Crisp; G.41 = G.41 as scion grafted on G.41.

Significant finding 2. Rootstock core fungal endophytes included members of the genera *Ilyonectrica*, *Serendipita*, *Lasiosphaeria*, *Leptodontidum*, and *Paraglomus*. Biological functions are detailed **Figure 4.** Rootstocks tested were G.935 and M.26.



The most common fungal genera present as endophytes in both G.935 and M.26 are indicated above. Counts of each genera and relative presence in all samples are shown: relative number of sequence reads above the detection threshold are indicated on the x-axis combined with color to indicate the prevalence of the genera in the samples assessed. Genera present in a greater number of samples are indicated in red; genera present in fewer samples are indicated in blue.

	Ilyonectria sp.	*Seredipita sp.*	*Lasiosphaeria sp.*	*Leptodontidium sp.*	*Paraglomus sp.*
Biological function	Genera include non-symptomatic root endophtyes [3] Can be plant pathogens Can be associated with diverse hosts [4]	Genera has been demonstrated to enhance biomass yield and drought tolerance [5]	Endophtyic fungus; biological function unknown [6]	Endophytic fungal genera; biological impacts of some genera include increasing root length; genera tends to be host specific [7]	Related genera can increase nitrogen and phosphorus uptake [8] More common in organically managed systems [9]

Figure 4. The core community of fungal endophytes detected by molecular methods in apple roots and respective biological function according to published literature.

Significant finding 3. Differential effects of the scion on apple root exudate compounds derived from G.41 rootstock were detectable in the first season of growth according to scion for bud-grafted apple trees. In general, total exudates were more profound in metabolites that would inhibit pathogen growth than those that would promote overall growth in the microbiome (**Figure 5**). Scions assessed were 'Honeycrisp', 'Granny Smith', and 'G.41', grafted onto rootstock cultivar 'G.41'.

Sugars, organic acids, sugar alcohols (untargeted PLSDA)

Phenolics and a few triterpenoids (untargeted PLSDA)

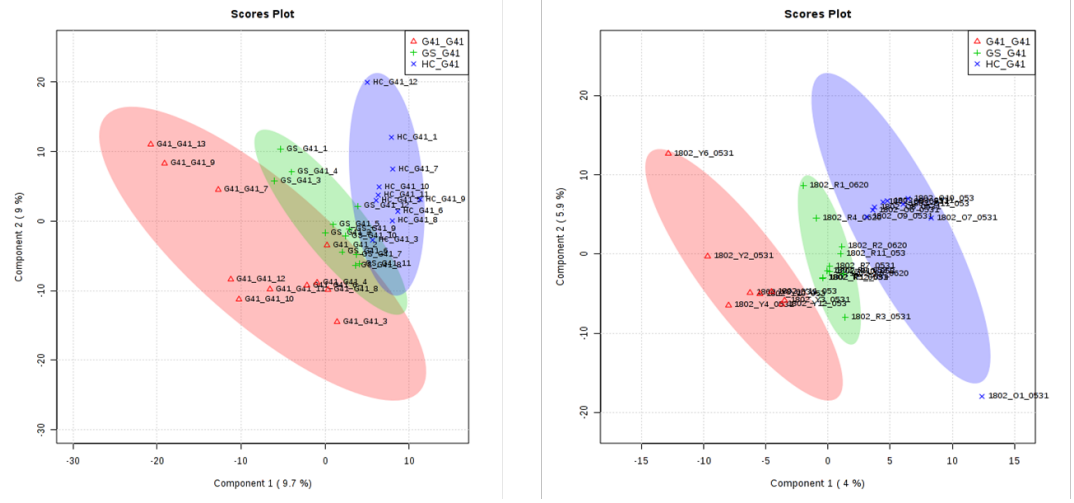


Figure 5. Apple scions had an effect on rootstock G.41 root exudate composition, however these differences did not bring about corresponding differences in composition of the rhizosphere microbiome. GS = Granny Smith; HC = Honeycrisp

Significant finding 4. In a separate experiment, where microbes were specifically excluded from the experimental system, more compounds that could feed the rhizosphere microbiome than inhibit pathogens differed between rootstock cultivars. Rootstock cultivars assessed were 'M.26' and 'G.935' (data not shown; see publication [1]).

Significant finding 5. Assessment of metabolites produced by rootstocks in axenic conditions indicated phloridzin and sorbitol are among the more abundant metabolites produced by roots; testing the impacts of these metabolites on the soil microbial community indicated that sorbitol has a significant effect on both the bacterial (**Figure 6**) and fungal community structure, while phloridzin influenced only the fungal community structure and to a lesser extent than did sorbitol.

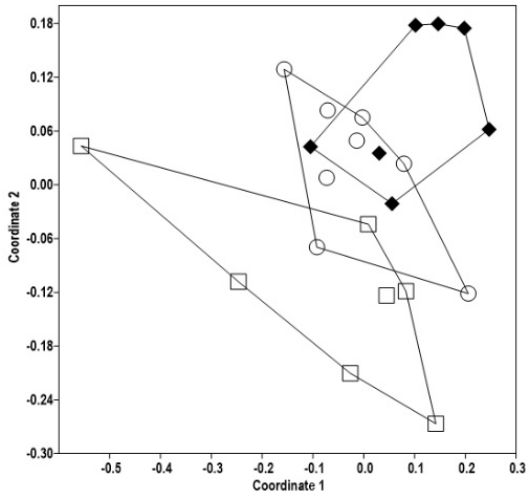


Figure 6. Effect of apple rootstock metabolites phloridzin and sorbitol on relative composition of the soil bacterial community. Phloridzin and sorbitol were added (0.5 ml) to independent orchard soil samples and bacterial community composition was determined by T-RFLP analysis. The bacterial community in sorbitol treated soil (♦) differed significantly in structure from both the control ($P = 0.0021$) and phloridzin treated ($P = 0.0162$) soil. □ = control; ○ = phloridzin; ♦ = sorbitol

Significant finding 6. Extension roots and fibrous roots have different phenolic compound profiles which also differ according to rootstock cultivar (**Figure 7**). Cultivars tested were M.26 and G.41.

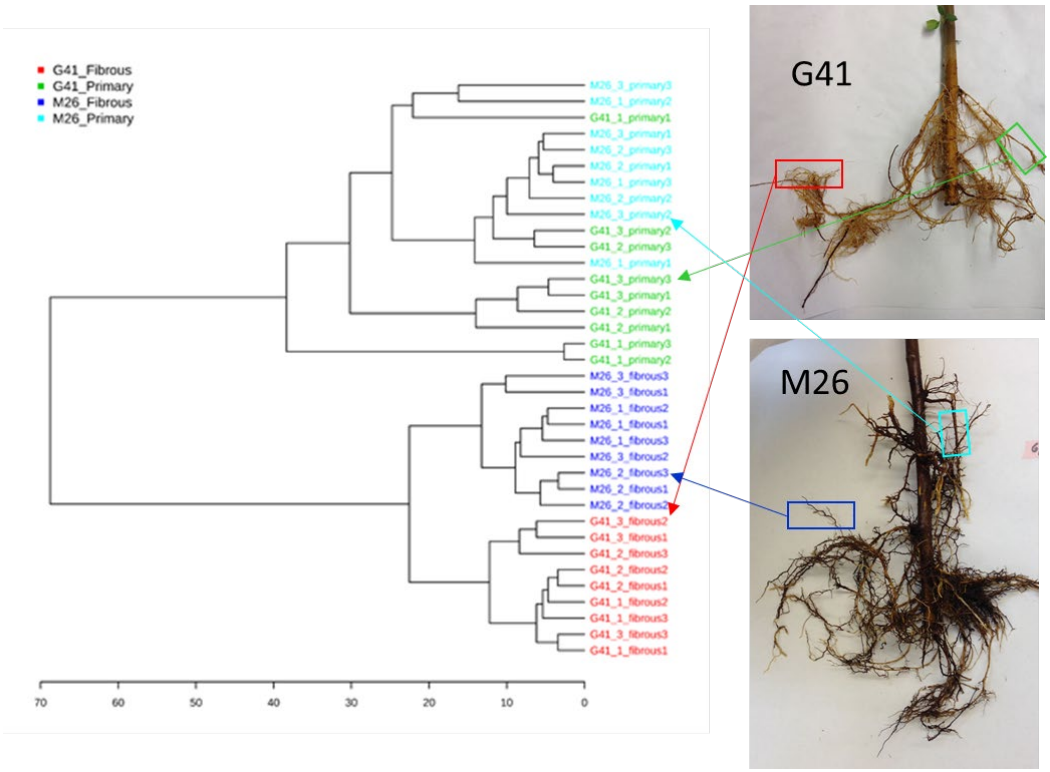


Figure 7. A dendrogram summarizes differences among phenolic root metabolite samples according to both root type (extension and fibrous) and rootstock cultivar (M.26 and G.41).

Discussion and summary

In the final year of the project, a number of experiments were completed to address project goals. *Scion* cultivar-specific impacts on the rhizosphere microbiome were not readily detected within the first two years of growth after bud-grafting. We previously established that *rootstock* cultivars do impact root-zone microbial communities [11], and that effects of rootstock cultivars on soil microbial communities carry over in the soil to affect newly planted trees [12]. The potential practical implications of the latter finding may include more intensive pre-plant treatments and post-treatment testing for soils where the previous orchard was grafted onto disease-intolerant rootstocks, e.g., many of the Malling series.

The present study indicated that the apple scion can affect root exudates qualitative and quantitatively, likely through translocation of photosynthetic products from the leaves to the roots. However, the differential metabolic composition of root exudates did not result in a detectable differentiation in composition of the rhizosphere microbiome of young trees. This remains an area where further research could lend insight into scion x rootstock cultivar compatibility, and into

tailoring scion and rootstock cultivar choices based upon knowledge of site-specific soil conditions and biology.

Endophytes (microorganism which grow within the tissues of a plant) were detected in roots of apple rootstock cultivars even when reared under axenic conditions. Cataloging and determining possible function for these microorganisms remains an area of active research. Ongoing research has demonstrated that composition of the endophytic microbiome differs in a rootstock genotype-dependent manner (Mazzola and Van Horn, unpublished). The assertion that there are additional (microorganism's) genomes, with potential impacts on tree growth, to consider when growing and obtaining trees for an orchard planting is a fascinating outcome.

Analysis of metabolic composition of extension roots and fibrous roots revealed differing levels of phenolic compounds according to both type of root and rootstock cultivar. Phenolic compounds can be involved in root disease resistance, and as it has been demonstrated that the fibrous roots are generally more involved in pathogen attack [10], this result offers a target for breeding and disease tolerance assessment.

In addition to the significant findings detailed above, results indicated that environmental factors also impact apple root exudates, both quantitatively and qualitatively. While unintentional in the present study, this result calls for continued assessment of environmental variables, especially temperature and water relations, in relation to tree and soil interactions.

REFERENCES

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12. Mazzola, M. and Hewavitharana, S. S. 2018. *Advances in understanding tree fruit-rhizosphere microbiome relationships for enhanced plant health*. Chapter 1, *In*, Achieving sustainable cultivation of temperate zone tree fruits and berries. Burleigh Dodds Science Publishing. Cambridge, UK.

Executive summary: Managing rhizosphere/soil microbiology via apple rootstock biochemistry

This research program addressed three priorities outlined in the 2016 Apple Horticulture and Postharvest research needs assessment: i.) Understanding and management of soil health and productivity in conventional and organic systems (critical priority) and ii.) Soil health and productivity – Interaction of rootstocks with rhizosphere microbiology (high priority) and contributes to additional priorities including iii.) apple replant (high priority) and iv.) improved scion and rootstock genetics (medium priority).

Results contributed to addressing all of the targeted priorities. The project determined that environmental factors, as well as scion genotype and vigor impact apple root exudates, both quantitatively and qualitatively. Additionally, the project demonstrated that rootstock genotype is a critical factor in determining composition of the rhizosphere microbiome, which has implication for functional activities of these microbes and their effects on tree growth and health, including the inhibition of pathogens by certain phenolic compounds. Effects of the scion on composition of the rhizosphere microbiome are not consistently detectable, at least when examined using rootstock liners over the first two seasons of growth under controlled environment conditions. Rootstock cultivar choices also influence soil pH, an attribute reported to be a primary determinant of bacterial community composition. Rootstock vigor impacts the quantity of exudates added to the soil, with more vigorous rootstocks releasing more exudates. Root exudate metabolic profiles can differ among both scion and rootstock genotypes in several biochemical classes, including phenolic compounds, sugars, sugar alcohols, organic acids, amino acids, and triterpenoids.

Practical implications include:

1. When considering orchard planting decisions, rootstock has greater immediate impact on soil / tree interface, but the scion can impact compounds released through the roots into the soil. The full implications of the latter remain to be discerned.
2. In an orchard replant situation, the previous rootstock cultivar can impact soil biology, with apple replant disease susceptible cultivars (including many of the Malling series) leading to a more “pathogen-rich” microbiome.
3. Future research could assess the efficacy of tailoring rootstock decisions to site-specific conditions including soil type, soil biology, general climactic trends regarding temperature and precipitation; a subtext to this vision is further defining rootstock cultivar characteristics according to their optimal growth and health conditions.
4. Rootstock metabolite contrasts among genotypes can inform apple rootstock breeding programs either for disease tolerance or for supporting a beneficial microbiome.
5. The knowledge that endophytes persist in apple trees opens further questions regarding their influence on tree nutrient sequestration, growth, and transmission mechanisms, especially from a nursery production perspective.

FINAL PROJECT REPORT

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

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Collaborators: Lee Kalcsits, Corina Serban, WSU TFREC, Wenatchee

Other funding sources

Agency Name: USDA, ARS
Amount awarded: $76,386
Notes: in-kind, GS-9 technician

Total Project Funding: $161,873

Budget History:

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

RECAP ORIGINAL OBJECTIVES

This project was developed based on previous work to identify factors influencing 'Honeycrisp' response in the postharvest environment. These included interactions among delayed cooling, rapid CA establishment, CA composition, and use of 1-MCP. The relative success in reducing risk of chilling disorder development (soft scald, soggy breakdown) using delayed cooling, reduced bitter pit from rapid CA and/or 1-MCP, and moderate CA O_2 concentration with low CO_2 showed the value for a postharvest management approach very different from that typically used for other varieties. Those results also suggested additional work to optimize and determine limits for the use of rapid CA for 'Honeycrisp' bitter pit management as well as to determine if similar practices influence disorders and fruit quality of other varieties. The increase in organic production and associated need for appropriate postharvest management strategies was another consideration for these studies. Disorders previously managed using DPA and/or 1-MCP require other control techniques, for chilling disorders the use of delayed cooling could be an option to reduce injury development. Delayed cooling increases the risk of fruit quality loss for varieties with softening as a primary concern during storage, but higher initial storage temperature can also reduce the risk of CO_2 injury. Establishing CA during conditioning was hypothesized to be a means to reduce the risk of quality loss but this strategy also posed risk for inducing other disorders due to the potential for low O2 injury at relatively warm temperatures. The studies were therefore designed to determine impacts of conditioning with or without CA on chilling and other disorders as well as fruit quality.

SIGNIFICANT FINDINGS

Objective 1 (Identify optimum controlled atmosphere conditions during 'Honeycrisp' conditioning) partially met, conditions under which CO_2 injury can occur not identified.
Objective 2 (Determine impacts of CA established during temperature conditioning on fruit quality and disorder development of 'Gala', 'Fuji', and 'Granny Smith' apples) fully met as positive and negative consequences of treatments were identified, potential for commercial use exists.
Objective 3 (Compare how 1-MCP and rapid CA establishment during temperature conditioning impact disorders and fruit quality) fully met, results consistent over 7 orchard years.

- 'Honeycrisp' bitter pit incidence is consistently reduced by one week in CA established during conditioning followed by storage in air.
- Bitter pit was reduced in some lots by treatment with 1-MCP alone or in combination with CA during conditioning.
- CA established during 'Honeycrisp' conditioning did not provoke development of internal or external disorders.
- No CO_2 disorders developed through 8 months storage of 'Honeycrisp' apples stored in CA with up to 4% CO_2 during conditioning.
- CA during conditioning of 'Fuji', 'Gala', and 'Granny Smith' has potential to reduce disorders without fruit quality loss.
- CA storage of 'Gala' at 31°F increased internal browning and shrivel compared to fruit stored at 33°F.
- CA oxygen concentration (0.5 or 1%) did not influence quality compared to storage temperature during 'Gala' CA storage.
- Conditioning 'Fuji' and 'Granny Smith' reduced development of core browning compared to fruit held continuously at 33°F.

RESULTS & DISCUSSION

Optimum CA conditions during 'Honeycrisp' conditioning

Oxygen concentration during conditioning: The objective was to determine if bitter pit control is possible with relatively high oxygen concentration (5-15%) during conditioning. CA (all with 0.5% CO_2) was established 1 day after receipt and after 1-MCP treatment. Fruit were stored in air at 37°F after conditioning. There was some bitter pit reduction due to the CA treatments, but a clear trend was not established due to results for fruit held at 5% O_2 (Figure 1). Additional experiments would provide a better indication of the potential for this protocol.

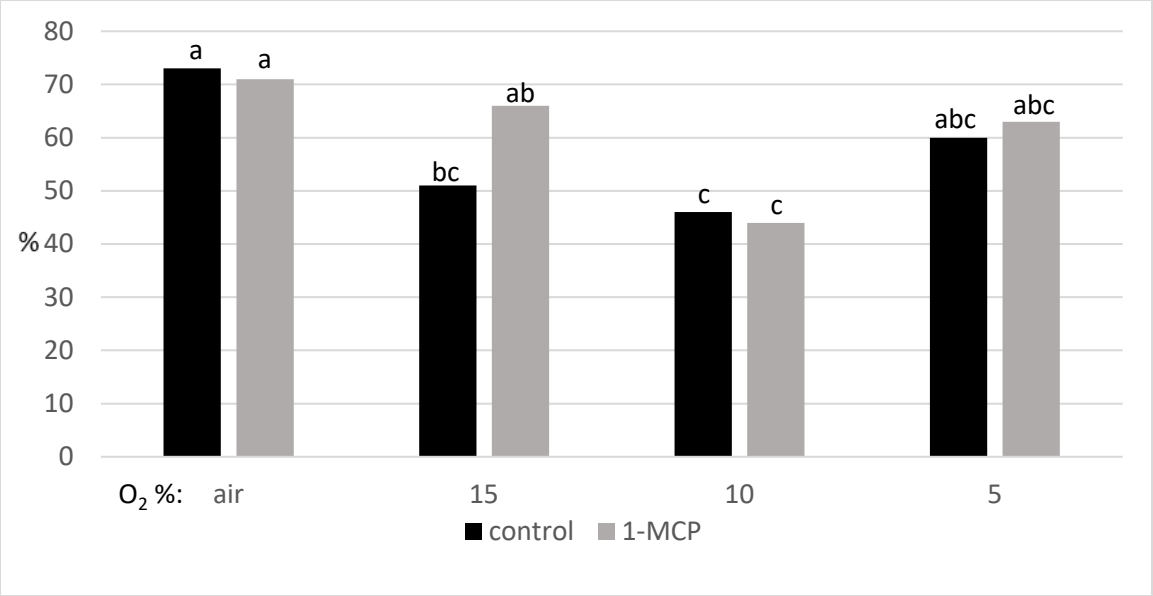

Figure 1. 'Honeycrisp' bitter pit incidence after 4 months cold storage plus 7 days at 68°F. Fruit were conditioned at 50°F for 7 days after receipt, 1-MCP applied day of receipt, CA established 1 day after receipt and held through a week, then fruit stored in air at 37°F for 4 months.

Carbon dioxide conditions during 'Honeycrisp' conditioning

'Honeycrisp' can be sensitive to CO_2 during storage. In the previous project CO_2 accumulation in air during conditioning did not provoke CO_2 injury (6 lots, 2 seasons). In the current project, fruit during conditioning were held in CA (2% O_2 throughout or 2 days at 3% then 2% O_2) with up to 4% CO_2. Exposing fruit to up to 2% CO_2 during conditioning, then storage in air (Table 1) or CA (2.5% O_2, 0.5% CO_2) (Table 2) after conditioning did not cause injury or reduce efficacy for bitter pit management. Each table contains means for 2 orchards. Use of 1-MCP did increase development of peel blotch and cavities for some treatments. This result for 1-MCP is different from our previous research where CO_2 exposure during conditioning was in air. Where DPA was applied prior to 1-MCP, no cavities developed. With the sample population to date, risk of injury from high CO_2 CA during conditioning is low where 1-MCP has not been used. While the results do not rule out CO_2 risk during conditioning, the cumulative lack of injury (experiments over 4 years, two years this project, two previous project) in work with 10 orchard lots not treated with 1-MCP suggests short CO_2 exposures at 50°F may not present a high risk for CO_2 injury.

CA	BP %	Peel blotch %	Lenticel breakdown %	Core browning %	Internal browning %	Cavities %
2% O_2, 0.5% CO_2	33b	0b	0b	3ns	3ns	1b
1-MCP	37ab	23a	7ab	0	3	12a
3/2% O_2, 0.5% CO_2	47ab	0b	0b	1	1	9ab
1-MCP	45ab	20a	6ab	4	4	15a
DPA, 1-MCP	25b	13ab	0b	0	3	0b
3/2% O_2, 1% CO_2	55a	0b	10a	1	3	4b
1-MCP	27b	24a	6ab	3	4	14a
3/2% O_2, 2% CO_2	52a	0b	4ab	3	8	8ab
1-MCP	32b	14a	10a	3	3	10ab

Means followed by different letters are significantly different, $p \leq 0.05$. ns: not significant

Table 1. 'Honeycrisp' fruit quality after 4 months in air plus 7 days at 68°F. Fruit were held at 50°F for 7 days then at 37°F in air. 1-MCP applied the day fruit received, CA established the following day.

CA	BP %	Peel blotch %	Lenticel breakdown %	Core browning %	Internal browning %	Cavities %
air	53a	0ns	0ns	0ns	1ns	2ns
3/2% O_2, 0.5% CO_2	31b	3	2	0	2	6
3/2% O_2, 1% CO_2	26b	0	1	0	0	2
3/2% O_2, 2% CO_2	27b	1	1	0	2	4
3/2% O_2, 4% CO_2	29b	3	1	1	1	2

Means followed by different letters are significantly different, $p \leq 0.05$. ns: not significant

Table 2. 'Honeycrisp' fruit quality after 4 months in CA plus 7 days at 68°F. Fruit were held at 50°F for 7 days then at 37°F. CA was established the following day. O_2 was held for 2 days at 3%, then reduced to 2%. After 7 days all CA treatments were 2% O_2 with 0.5% CO_2.

CA during conditioning of other varieties

'Gala' CA during conditioning

Over the two years with two orchards each year, CA during conditioning of 'Gala' provided similar results for quality management compared with CA begun 7 days after receipt for fruit held continuously at 33°F (Tables 3,4). Delaying CA for conditioned fruit resulted in unacceptable firmness loss and more cavities in the first year and more cortex senescent browning in both years. Less senescent browning developed in CA and 1-MCP fruit supporting this is an aging-related disorder. Peel blotch occurred only in the second year and only in fruit held continuously at 33°F supporting initial temperature as a factor in development of this disorder. The general conclusion is conditioning 'Gala' can reduce disorder development but CA is needed to avoid excessive firmness loss.

Initial Temp °F	Atm	MCP	Lbs	TA %	Senescent browning %
50	CA9d	No	12.7c	0.286d	8c
	CA1d	No	15.7b	0.302bc	0d
	Air	No	9.9e	0.193f	34a
	CA9d	Yes	17.1a	0.309ab	0d
	CA1d	Yes	17.4a	0.319a	1d
	Air	Yes	16.0b	0.295bcd	9c
33	CA9d	No	16.0b	0.292cd	3cd
	air	No	11.5d	0.203b	21b
	CA9d	Yes	17.3a	0.296bcd	1d
	Air	Yes	15.6b	0.262e	0d

Means followed by different letters are significantly different, $p \leq 0.05$.

Table 3. 'Gala' disorders and quality after 6 months plus 7days at 68°F, year 1, means of two orchard lots. Fruit were held at 50 (7 days) or 33°F at receipt, 1-MCP applied day of receipt. CA was 1% for both O_2 and CO_2, was established 1 or 9 days after receipt.

Initial Temp °F	Atm	MCP	Lbs	TA %	Stem end Browning %	Cavity %	Peel blotch %	Senescent browning %
50	CA9d	No	16.2c	0.303a	16ab	9a	0b	0b
	CA1d	No	16.9ab	0.310a	4cd	2bc	0b	0b
	Air	No	10.4d	0.203c	10bc	0c	0b	15a
	CA9d	Yes	16.6bc	0.310a	12ab	4b	0b	3b
	Air	Yes	16.9ab	0.303a	11ab	2bc	0b	0b
33	Air	Yes	17.0ab	0.284b	17a	3bc	6a	0b
	Cad1	Yes	17.2a	0.313a	1d	1c	4a	0b

Means followed by different letters are significantly different, $p \leq 0.05$.

Table 4. 'Gala' disorders and quality after 6 months plus 7days at 68°F, year 2, means of two orchard lots. Fruit were held at 50 (7 days) or 33°F at receipt, 1-MCP applied day of receipt. CA was 1% for both O_2 and CO_2, was established 1 or 9 days after receipt.

Temperature and O_2 optimization for 'Gala' CA storage. CA storage of organic 'Gala' has potential challenges for firmness management. We evaluated storage temperature (33 or 31°F) and CA O_2 concentration (1 or 0.5%, both with 0.5% CO_2), and CA establishment date (1 or 7 days after receipt, year 2 only) as factors that influence quality management. For two orchards in two production seasons, results showed temperature but not the O_2 concentrations used influenced fruit quality and disorders of fruit stored 6 months plus 7 days at 68°F (Tables 5,6). In both years fruit stored in 31°F had higher firmness but titratable acidity and soluble solids content (not shown) were not influenced consistently by temperature or CA O_2. Some disorders increased at the lower storage temperature including shrivel, stem-end browning, and senescent browning although the trends were not consistent across years for all disorders. Splitting incidence decreased with delayed CA establishment. The results indicate 'Gala' storage performance at 31°F and/or with 0.5% O_2 is not consistently enhanced compared to 33°F and 1% O_2, and that rapid CA may enhance splitting potential.

Temp °F	O_2 %	Lbs	Titr. Acid %	Shrivel %	Stem-end browning %
33	0.5	16.7b	0.307ns	0b	2b
	1.0	16.6b	0.300	0b	1b
31	0.5	17.3a	0.315	6a	10a
	1.0	17.5a	0.315	6a	6a

Means followed by different letters are significantly different, $p \leq 0.05$. ns: not significant

Table 5. 'Gala' quality (means for two lots) after CA storage at two temperatures and two O_2 concentrations, 2016 crop. CA CO_2 concentration was 0.5% for all treatments. CA was established one day after fruit were received. Fruit stored 6 months plus 7 days at 68°F.

Temp °F	O_2 %	Lbs	Titr. Acid %	Splitting %	Peel blotch %	Shrivel %	Senescent browning %
33	0.5 day1	16.7b	0.308a	7ab	1b	1b	0b
	1.0 day1	16.9ab	0.296a	9a	3ab	2b	0b
	0.5 day7	17.0ab	0.299a	1c	5ab	1b	1b
	1.0 day7	16.7ab	0.301a	1c	6ab	1b	0b
31	0.5	17.1ab	0..301a	6ab	9a	19a	0b
	1.0	17.3a	0.311a	5ab	6ab	19a	1b
33	air	11.6c	0.199b	3bc	1b	0b	13a

Means followed by different letters are significantly different, $p \leq 0.05$.

Table 6. 'Gala' quality (means for two lots) after CA storage at two temperatures and two O_2 concentrations, 2017 crop. CA CO_2 concentration was 0.5% for all treatments. CA was established one or seven days after fruit were received. Fruit stored 6 months plus 7 days at 68°F.

'Fuji' conditioning

Reducing the risk of CO_2 injury during CA from watercore at harvest can be accomplished by delaying establishment of CA for 'Fuji'. Delayed CA can result in some quality loss, particularly titratable acidity, that would be notable after long term storage. As DPA for internal browning control cannot be used with organic fruit, we evaluated conditioning at 50°F to reduce the risk of CO_2 injury when CA is imposed close to harvest. Fruit were held at 50°F for 7 days then 34°F, or at 34°F continuously. CA was established after 1 or 9 days. Fruit were held in CA after conditioning was completed through 8 months plus 7 days at 68°F. Watercore was present in year two (90% incidence, mean rating of 3.3 with a scale of 1-4, 4 being severe). Fruit with CA started 1 day after receipt during conditioning in both years had less core browning after 8 months compared to fruit held continuously at 34°F and CA established 1 day after receipt (Tables 7,8). However, delaying CA for fruit held continuously at 34°F resulted in core browning incidence like that of conditioned fruit with CA at day 1. Consistent impacts on SSC, TA, and firmness were not observed although values were higher after 8 months in year two for conditioned fruit (Table 8). While these results show conditioning and rapid CA provide similar disorder management to normal cooling and delayed CA, the question remains regarding how conditioning might impact fruit with a higher risk of CO_2 injury sensitivity at harvest compared with lots used in this study.

Month	Initial Temp °F	Atm.	Core browning %	SSC %	TA %	Lbs
4	50/34	CA d9	6c	12.9bc	0.199a	13.2b
		CA d1	3c	12.7c	0.186ab	13.5b
		Air	30b	13.2ab	0.132c	10.9c
	34	CA d9	4c	12.5c	0.183b	13.4b
		CA d1	8c	13.5a	0.187ab	14.3a
		Air	87a	11.7d	0.109d	10.4c
8	50/34	CA d9	5b	13.2a	0.127b	13.2a
		CA d1	1d	12.8ab	0.135ab	12.8ab
		Air	82a	12.2c	0.062d	12.2c
	34	CA d9	3cd	12.5bc	0.133ab	12.5bc
		CA d1	12c	12.2c	0.144a	12.5bc
		Air	84a	10.9d	0.080c	10.9d

Means followed by different letters are significantly different, $p \leq 0.05$.

Table 7. 'Fuji' disorders and quality after storage plus 7days at 68°F, 2016 crop. Fruit were held at 50 (7 days) or 34°F at receipt. CA O_2 was 3% for 2 days then 1.5%, CO_2 was 0.5% throughout. CA was established 1 or 9 days after receipt.

Month	Initial Temp °F	Atm.	Core browning %	Cavities %	SSC %	TA %	Lbs
4	50/34	CA d9	0	6ns	15.2ab	0.323c	16.7b
		CA d1	0	6	16.2a	0.346b	17.3ab
		Air	0	1	15.2ab	0.250d	14.4c
	34	CA d9	0	8	15.8ab	0.379a	17.1ab
		CA d1	0	3	16.0ab	0.340bc	17.8a
		Air	0	1	15.1b	0.238d	15.1c
8	50/34	CA d9	0b	7ab	15.5b	0.317a	16.4c
		CA d1	0b	4ab	16.7a	0.310ab	17.4ab
		Air	39a	1b	15.6b	0.164d	13.6d
	34	CA d9	1b	10a	16.4a	0.298bc	18.1a
		CA d1	6b	4ab	15.2b	0.289c	16.7bc
		Air	39a	0b	15.1b	0.143e	14.2d

Means followed by different letters are significantly different, $p \leq 0.05$.

Table 8. 'Fuji' disorders and quality after storage plus 7days at 68°F, 2017 crop. Fruit were held at 50 (7 days) or 34°F at receipt. CA O_2 was 3% for 2 days then 1.5%, CO_2 was 0.5% throughout. CA was established 1 or 9 days after receipt.

'Granny Smith' CA during conditioning

Several factors influence superficial scald development on 'Granny Smith'. Most important in PNW fruit are storage duration, CA O_2 concentration, how long after harvest CA is established, and how long and under what temperature fruit are held after removal from CA. Scald is a chilling injury in that symptoms are unlikely to occur in fruit held at relatively warm temperature. Organic 'Granny Smith' storage relies on ultralow O_2 for superficial scald control. Core browning is another chilling injury that can be reduced by CA but may also respond to conditioning at harvest. In these experiments fruit were stored at 50°F for 7 days then 33°F or at 33°F continuously. CA (1% O_2, 1% CO_2) was established 1 or 9 days after receipt (conditioned fruit where CA was established 9 days after receipt were lost due to cold room failure).
At 4 months conditioned fruit was like that held continuously at 33°F except with statistically less but commercially unacceptable superficial scald on fruit stored in air (Table 9). After 8 months, quality of conditioned and control fruit was similar except that conditioned CA fruit had the highest acidity and much less core browning compared to fruit held continuously at 34°F. However, superficial scald was not controlled by 1% O_2. A current study is repeating this experiment with the addition of a 0.5% O_2 CA treatment. Summary results will be submitted in summer, 2019.

Months	Initial °F	Atm	SSC %	TA %	Lbs	Core Browning %	Superficial Scald %
4	50	CA day 1	12.2a	0.546a	17.7a	0b	0c
		Air	11.0d	0.431c	14.7c	61a	69b
	33	CA day 9	11.8b	0.536a	16.9b	0b	0c
		CA day 1	11.6c	0.533a	17.7a	0b	0c
		Air	11.4c	0.461b	14.8c	54a	92a
8	50	CA day 1	11.5a	0.513a	16.7a	3d	76c
		Air	11.0b	0.329d	10.4b	8d	82bc
	33	CA day 9	11.6a	0.447c	17.1a	60a	96a
		CA day 1	11.7a	0.485b	17.1a	31c	49d
		Air	9.5c	0.263e	10.5b	44b	90ab

Means followed by different letters are significantly different, $p \leq 0.05$.

Table 9. 'Granny Smith' fruit quality and disorders after 4 or 8 months plus 7 days at 68°F. Fruit were held at 50°F for 7 days after receipt then at 33°F, or at 33°F continuously. CA (1% O_2, 1% CO_2) was established 1 or 9 days after receipt (conditioned fruit where CA was established 9 days after receipt were lost due to cold room failure).

Bitter pit reduction from short-term CA established during 'Honeycrisp' conditioning

Work in the previous project showed CA established during 'Honeycrisp' conditioning and held through 4 months results in less bitter pit without increased development of internal disorders. The focus on the current project was to evaluate efficacy of short CA duration, CA during conditioning only or with an additional week after conditioning as fruit cooled. Results from 7 orchard years over

3 years consistently showed less bitter pit for fruit in CA for 1 week, with 2, 3, or 4 weeks (3 and 4 week results not shown) not significantly better than 1 week (Figure 2). Unlike previous results, 1-MCP did not enhance bitter pit reduction for CA fruit (Table 1). CA established during conditioning then storage in air did not enhance internal disorder development. Variation among lots existed in bitter pit incidence as well as the amount of reduction due to CA, but in most cases at least some bitter pit reduction was observed.

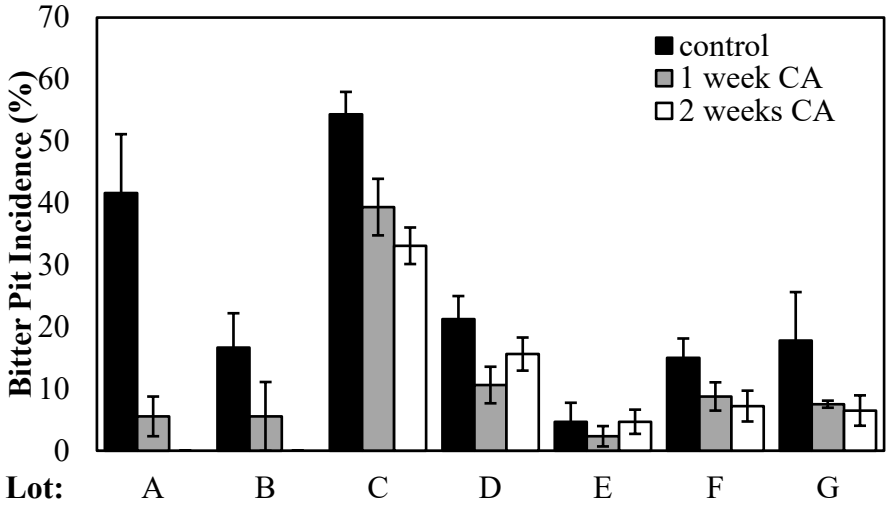

Figure 1. 'Honeycrisp' bitter pit incidence in 5 orchard lots over 3 seasons. Fruit were held at 50°F for 7 days then at 37°F. CA was established after one day in 50°F. O2 was initially All CA fruit held in air after removal from CA and stored for 4 months plus 7 days at 68°F.

	1-MCP			Control		
Weeks CA	0	1	2	0	1	2
Bitter pit	20.4ab	14.5b	12.6b	27.9a	12.5b	11.9b
Soft scald	0.3ns	0.3	0.5	0.2	0.6	0.6
Soggy breakdown	0.3ns	0.1	0.3	0.6	0.4	0.7
Cavities	0.5ns	0.8	1.1	0.2	0.4	2.4

Means followed by different letters are significantly different, $p \leq 0.05$. ns: not significant

Table 1. Physiological disorders in 'Honeycrisp' apples after 4 months storage, means for 7 lots. 1-MCP (1 ppm) applied the day fruit were received.

The results suggest a brief period after harvest in CA can be sufficient to reduce bitter pit development without enhancing internal disorder risk. While this pattern was consistent throughout this study, situations where orchard factors including maturity, and postharvest factors including cooling and CA dynamics may increase disorder development risk cannot be ruled out. What the results from this and previous work may indicate is that bitter pit management from CA may be most likely the sooner CA is established after harvest. What remains to be determined are factors and situations, if any, that enhance risk of internal disorder development from CA established during conditioning.

EXECUTIVE SUMMARY

Meeting the postharvest challenges of 'Honeycrisp' has expanded the technical expertise of Washington apple industry and research personnel. 'Honeycrisp's' sensitivity to chilling and susceptibility to bitter pit have been addressed through management practices that had not been considered for other varieties. Delayed cooling is commercially usable due in part to the lack of the need to manage 'Honeycrisp' texture, a primary postharvest objective for most legacy apple varieties. The efficacy of delayed cooling to reduce 'Honeycrisp' chilling susceptibility exacerbates bitter pit development, while establishing CA during conditioning reduces bitter pit. While CA is known to impact the disorder in other bitter pit prone varieties, the short duration (1 week) of CA needed to reduce 'Honeycrisp' identified in this project has not been widely recognized. This finding is the most significant outcome of this project. Adopting non-standard postharvest conditions for a high value, new variety pushed the comfort of some postharvest personnel, applying those concepts to other varieties could be equally as challenging.

Lessons from 'Honeycrisp' postharvest management, temperature conditioning, CA during conditioning, were the basis for this project. High volume, existing varieties with established postharvest systems are being challenged to adapt to organic production. The low O_2 CA fruit sensing technologies, efficient CA generation equipment, tight rooms and high capacity refrigeration systems provide the pieces to accomplish chemical-free apple storage management. What is needed is how to integrate their use to meet the specific needs of varieties with different objectives for postharvest management. The general conclusion of this project is that CA during temperature conditioning reduces the negative impacts of delayed cooling on fruit quality and can benefit disorder management for all the varieties we evaluated in some way. 'Honeycrisp' bitter pit, 'Gala' peel blotch, 'Fuji' and 'Granny Smith' core browning all were reduced by conditioning. Also notable is the lack of disorders induced by CA during conditioning. The lack of CA induced disorders is likely due to lowered CO_2 sensitivity at higher temperatures, and no fermentation at the O_2 settings evaluated.

A question considering all the results is whether the benefits of this protocol are sufficient in relation to established practices for changes in standard methods to be considered. We also recognize the commercial challenges of filling rooms rapidly. Note that none of the experiments with 'Gala', 'Fuji', or 'Granny Smith' have been pilot tested. The specific temperature, CA settings, and timing combinations evaluated may be useful to consider incremental changes in established practices as well as potential areas for further research. For example, 'Gala' peel blotch developed more on fruit held initially at a lower temperature. Humidity, rate of temperature decrease, and use of other temperatures between 33 and 50^{o}F were not evaluated leaving a question as to other ways to reduce this disorder based on how refrigeration is managed. Another positive is all this work was conducted with existing equipment in our facility. For industry that could mean changes in postharvest protocols rather than facility upgrades are what is needed to implement new procedures. Additional research that builds on results for each variety may be appropriate based on the degree to which specific disorders continue to be a management challenge for organic as well as conventional production. That work now has at least some path suggested that does not rely on identification of new technology or new chemistries.

FINAL PROJECT REPORT
WTFRC Project Number: AP-16-105

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

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

Other funding sources: None

WTFRC Collaborative Expenses: None

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

Budget 1
Organization Name: WSU **Contract Administrator:** Katy Roberts/Kim Rains
Telephone: 509-335-2885/509-293-8803 **Email address:** arcgrant@wsu.edu / kim.rains@wsu.edu

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

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

OBJECTIVES

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

SIGNIFICANT FINDINGS

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

- 325 grower lots were surveyed across all apple growing regions in central Washington between January and June, 2016 and 2017. Blue and gray molds were predominant and accounted for 48 and 25% of total decay, respectively.
- The "export" quarantine pathogens were found at about 10% of total decay with Bull's eye rot being the most predominant one in this group. Speck rot (Phacidiopycnis) was at about 1% whereas Sphaeropsis was found very sporadically.
- The newly reported Lambertella rot, now known as Yellow rot was found in 34% of lots surveyed with an incidence ranging from 2 and 40% per grower lots. The newly reported pathogen, Phacidium rot, was not found sporadically.

Objective 2: *Evaluate risks related to fungicide resistance*

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

- Nearly 6,000 isolates of *Penicillium expansum* (blue mold) and 4000 isolates of *Botrytis cinerea* (gray mold) were collected from different packinghouses in 2016 an d2017. These isolates were tested for sensitivity to 6 fungicides: thiabendazole (Mertect), pyrimethanil (Penbotec) and fludioxonil (Scholar) for both *P. expansum* and *B. cinerea* and to pyraclostrobin + boscalid (Pristine) and fluxapyroxad (Merivon) for *B. cinerea* only.
- Resistance of *P. expansum* to thiabendazole (Mertect) and pyrimethanil (Penbotec) was found in about 60% and 52% of the 325 lots surveyed, respectively.
- Resistance of *B. cinerea* to pyrimethanil, TBZ, Pristine, and fluxapyroxad (Merivon) was found in 55%, 46%, 38% and 15% of the 325 lots surveyed, respectively.
- Populations of *B. cinerea* and *P. expansum* with reduced sensitivity to fludioxonil were found in several packinghouses. These populations are controlled by the label rate of the fungicide. However, continuous use of Scholar and related products can cause these populations to become actually resistant.

- A total of 325 decay and resistance profiles were shared with the participating packers and growers before the beginning of the new season to allow them change strategies and spray regimes based on decays and resistance found at their locations.

Objectives 3:

- Genes known to increase the risk for fungicide resistance development in *B. cinerea* seem to be up-regulated (increased expression) by cold temperatures usually used to store apples which may increase the risk of selecting for resistance especially for Penbotec and Scholar.
- For *P. expansum*, the expression of some genes is lowered at cold temperatures while other genes could be up-regulated.
- Further Research is needed to better assess the risk and find solutions that may help with reducing the expression of the genes involved.

Objectives 4:

- The efficacy of Academy (fludioxonil + difenoconazole) was evaluated against 10 major apple pathogens in vitro (lab) and on fruit
- Preliminary results indicate that Academy would be effective against blue mold, gray mold, yellow rot, Speck rot ad moderately effective against Mucor.
- A second-year study is going on to further confirm the efficacy levels seen in 2017-18 season.
- Currently, Academy is only available for drench application and the waste management process for difenoconazole has limited the use of this new fungicide in commercial packinghouses. If a formulation for a dry application is developed (expectation for the 2020 season), Academy can be a valuable tool to add to the few existing fungicides.

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

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

RESULTS AND DISCUSSION

Objective 1. *Postharvest diseases prevalence*

Blue and gray molds accounted for almost 72% of total decay observed with blue mold being predominant with 48% of total decay (Figure 1-left). Blue mold was detected in 157 of the 160 lots surveyed versus 132 lots for gray mold. A majority of lots had less than 20% incidence of gray mold, whereas a higher number of lots had between 40 and 80% blue mold (Figure 1-right). Besides these two mains decays, bull's eye rot was found in 52 lots at frequencies ranging from 1 to 75%, whereas the statewide frequency was 4.3%. The frequency of the "crabapple diseases" Speck rot and Sphaeropsis rot was 2.5 and 1.4%, respectively. It is possible that better management practices, including pruning and appropriate fungicide sprays, resulted in such low frequencies compared to those reported when these two pathogens were first described in the state. Additional minor pathogens included Alternaria rot (2.9%) and the newly reported yellow rot (2%). Other minor or non-identified decays accounted for 14.3% of total decay.

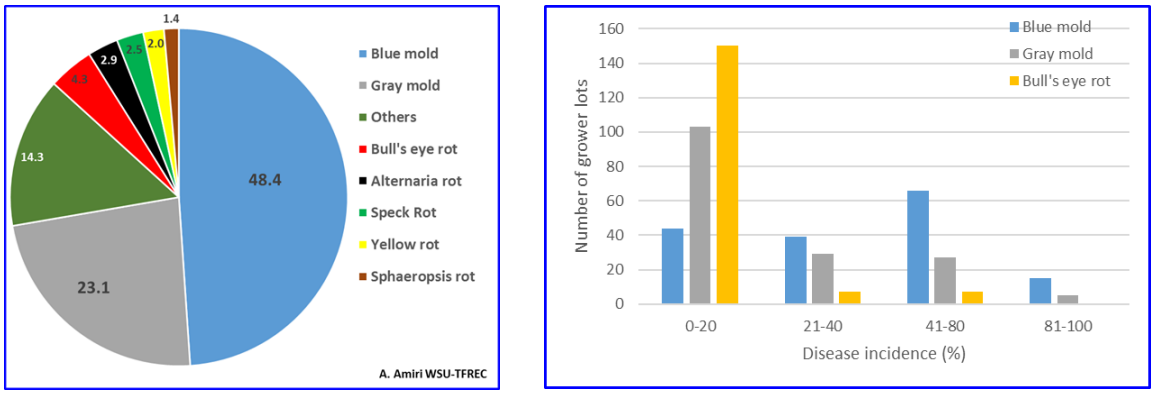

Figure 1. Overall incidence of major postharvest diseases found in Washington in 2016 (left) and incidence distribution of blue mold, gray mold and Bull's eye rot among grower lots (Right).

Objective 2. *Fungicide resistance occurrence and frequencies*

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

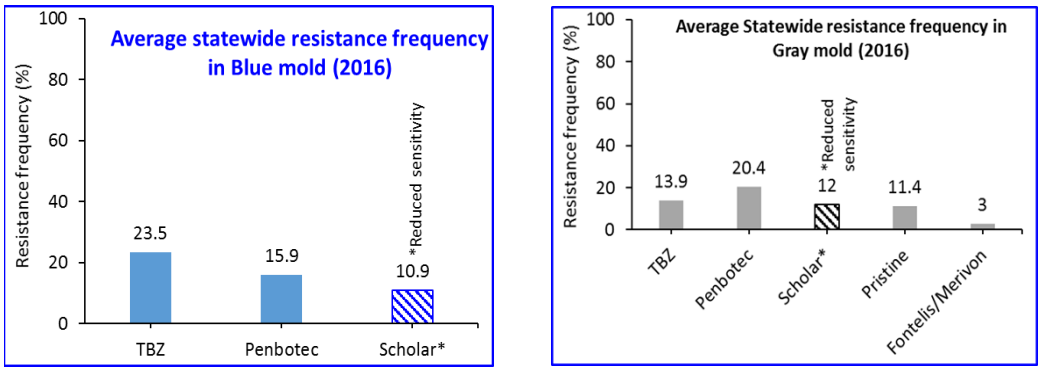

Figure 2. Overall resistance frequencies to major pre- and postharvest fungicides in blue mold (left) and gray mold (right) observed statewide in 2016.

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

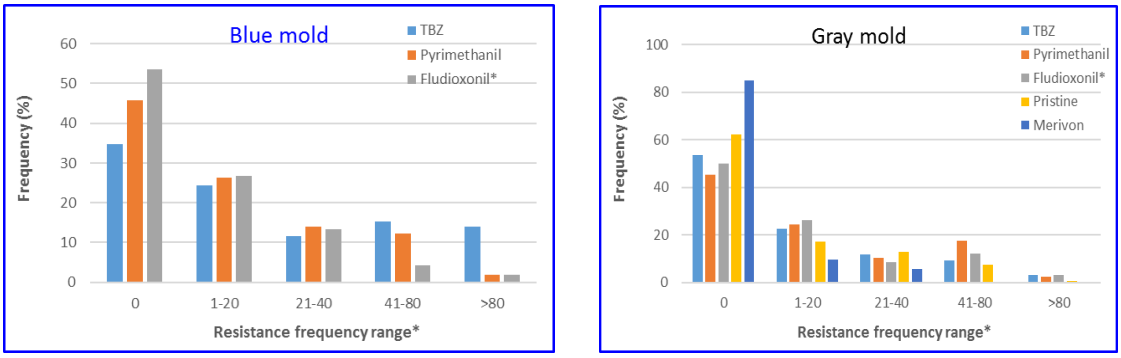

Figure 3. Resistance frequency distribution in blue mold (left) and gray mold (right) observed statewide in 2016. * for fludioxonil, it is only reduced sensitivity.

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

In pome fruit, fungicides are applied at harvest and fungicide residue are constant (do not degrade) over a long storage period at 33°F to 36°F. Therefore, if the fungus is present on the fruit/bin or in the room, the contact between the fungicide and the fungus is continuous which may increase the selection pressure and cause more resistance to occur. We evaluated the expression of genes known to be involved in fungicide resistance in both *B. cinerea* and *P. expansum* at 34°F (1°C) and 36°F (20°C) over a 6-month period. Our preliminary results show that the expression of the studied genes seems to be increased at low temperature when using pyrimethanil (PYR) or fludioxonil (FDL) especially in resistance isolates. It seems also that the low temperature alone, without the fungicide, may cause an increase in the expression of the genes especially in *Botrytis*. More experiments are ongoing to better understand the risks and look at other potential genes and results will be presented at the Apple Review Day in January 2019.

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

Trials were conducted in the 2016-17 season to evaluate the efficacy of Difenoconazole and Academy against major postharvest diseases. The same trial has been reconducted in the 2017-18 season. Difenoconazole (alone) was applied at harvest on fruit artificially inoculated before harvest and after harvest (for Lambertella, Penicillium and Mucor only). Results on Figure 4 indicate that after 4 months of storage, most of preharvest fungicides are controlled, whereas Lambertella and Penicillium were significantly reduced compared to the control. Incidence of Mucor rot was reduced from 80% in the control to 50% in the difenoconazole-treated fruit.

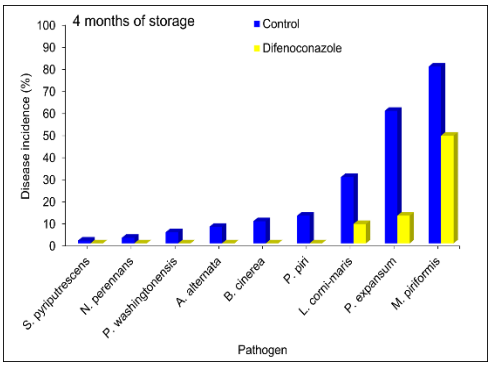

Figure 4. Efficacy of Difenoconazole against 9 pathogens

Objective 5: *Prevalence of yellow and Phacidium rots and their sensitivity to pre- and postharvest fungicides*

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

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

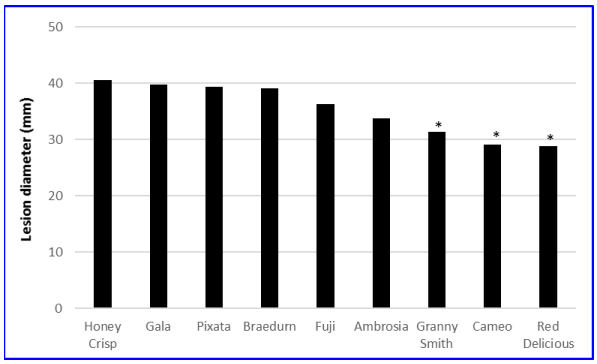

Figure 5. Susceptibility (expressed as lesion diameter) of most common apple cultivars to yellow rot (Amiri et al. Plant Disease, 2017). * indicate cultivars significantly less susceptible.

Yellow rot is totally controlled by fludioxonil at label rate while pyrimethanil provided a high efficacy (>94% control) on fruit wounded and inoculated with the fungus (Figure 6). To the contrary, TBZ failed to provide any efficacy against yellow rot. This is not due to fungicide resistance but rather to inherent inefficacy of this group of fungicide against yellow rot. It is not clear yet if *L. corni-maris* infect fruit pre- or postharvest, however the preharvest fungicides Topsin-M (same group as TBZ) and Pristine (pyraclostrobin + boscalid) may not provide adequate control if fruit are infected in the orchard.

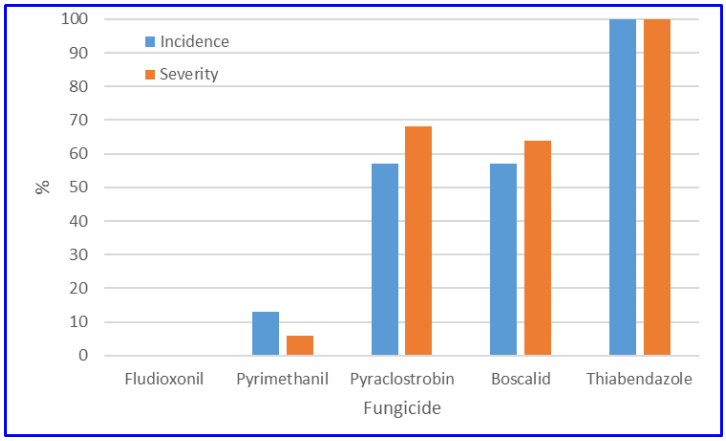

Figure 6. Efficacy of pre- and postharvest fungicides against yellow rot on apple fruit (Amiri et al. Plant Disease, 2017).

Executive summary

The long-term known apple diseases in addition to the emerging new pathogens, mostly reported from Washington, have put a tremendous pressure on growers and packers in recent years in term of improving management and reducing losses. We planned for a first step consisting of knowing the exact and real risks and their extent to develop sustainable solutions. We conducted two years of statewide decay surveys combined with a statewide fungicide resistance monitoring to assess any possible impact in recent shifts in cultural and management practices and climate change on emergence or exacerbation of pathogen populations or increased resistance frequencies. Such information was highly needed to improve fruit production sustainability. Fungicide resistance monitoring programs will be crucial to pinpoint location-specific problems, evaluate the potential impact of environmental conditions between regions and different spray regimes on resistance development. Moreover, the impact of target and no-target sprays in orchards as well as storage conditions on decay control efficacy and potential resistance development problems need to be assessed to improve disease management.

Summary of findings: We have a better understanding of the risks caused by apple pathogens in term of occurrence, distribution and importance. More specifically, we acquired new knowledge about the emerging quarantine pathogens (*Lambertella, Phacidiopycnis, Lambertella, Phacidium*) in term of importance, control and susceptibility of major cultivars. One the major outcomes from the project is a better assessment of existing risks of fungicide resistance in pome fruit systems in Washington. Although, resistance has emerged to most fungicides, levels of resistance can be considered lower compared to other crop systems. However, caution is needed to avoid catastrophic scenarios due to control failure is resistance continue to increase because rationale solution and practices are not implemented. We have also evaluated the efficacy of the newest postharvest fungicide, difenoconazole, against major diseases which will make it a useful tool in the future due of its efficacy and different mode of action suitable for fungicide resistance management.

Project Outcomes:

- Peer reviewed publications: 5 (3 more pending)
- Extension publications: 5
- Professional presentations: 8
- Extension presentations: 25

Future Directions:

- Develop specific management programs for major disease: Blue mold, gray mold and Bull's eye rot.
- Better understand the epidemiology of preharvest pathogens to enhance management in storage.
- Continue research efforts in assessing the effect of storage conditions on decay and fungicide resistance development and develop solutions to them.
- Acquire more knowledge about the emerging (quarantine) pathogens in term of epidemiology and best management practices.

FINAL PROJECT REPORT

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

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Cooperators: Allan Brothers. Inc., Stemilt Growers LLC.,

Total Project Request: **Year 1:** $104,707 **Year 2:** 108,515 **Year 3: no request**

Other funding sources: None

WTFRC Collaborative expenses

Item	2016	2017	2018
Wages	5,000	5,000	
Benefits	2,000	2,000	
Total	7,000	7,000	**0**

Budget 1: Meijun Zhu

Organization Name: WSU-Pullman **Contract Administrator:** Katy Roberts
Telephone: (509) 335-2885 **Email address:** katy.roberts@wsu.edu

Item	2016	2017	2018[1]
Salaries[1]	$22,000	$22,880	
Benefits	$10,193	$10,601	
Wages[2]	$27,565	$28,667	
Benefits	$5,623	$5,848	
Supplies[3]	$25,326	$26,219	
Travel[4]	$2,000	$2,300	
Miscellaneous[5]	$5,000	$5,000	
Total	$97,707	$101,515	**0**

[1]**Footnotes:** No cost extension for 2018

RECAP OF ORIGINAL OBJECTIVES

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

SIGNIFICANT FINDINGS

1. *Listeria monocytogenes* is a tough foodborne pathogen. A limited reduction of *L. monocytogenes* on apple surfaces occurred during 12 weeks of refrigerated storage either at 1°C/33°F, 4°C/39.2°F or 10°C/50°F. There was about ~1 Log reduction when stored at room temperature (22 °C / 71-72 °F) at ambient relative humidity (RH, 40-50%).
2. We determined a ~3-Log reduction of *Listeria* on Fuji apples after 30 weeks of cold storage under a commercial RA and CA storage environment. Continuously low dose ozone gas application (87.0 ± 38.8 ppb) in CA storage generated an additional 2-Log reduction.

   Additional 2-week storage under RA beyond their respective initial storage treatments had little influence on *L. innocua* survival.
3. Natural bacterial counts of Fuji apples stored at CA/RA remained stable throughout storage. Continuous low dose ozone gradually resulted in about 1-Log reduction after 30-week storage.
4. Indigenous yeasts/molds (Y/M) count of un-inoculated Fuji apples stored in RA remained relatively stable during first 12 weeks of storage. By the end of the 30-week storage, the Y/M count of RA stored apples increased about one log. The Y/M count of Fuji apples in CA room remained relatively stable over 30 weeks of storage. There is about 0.6-Log reduction of Y/M count in Fuji apples of CA with ozone storage during first 12 weeks. Nevertheless, the inhibitory effect of ozone was compromised with prolonged storage time.
5. Continuous low doze ozone gas used at this study had no negative influence on apple visual quality, including both external and internal disorders during 6-month CA cold storage.
6. Ozone at 60-80 ppm did not reduce the incidence of blue mold, gray mold and bull's eye rot and reduced severity (lesion diameters) very slightly (10 to 20%) on wounded and artificially inoculated Fuji and Granny Smith after 4 months of storage.
7. A negative impact (increased decay) on Mucor infection was seen in the 2017-18 season in fruit inoculated with Mucor at harvest.
8. The efficacy of ozone at 60-80 ppb was slightly higher (4 to 90% reduction) on the density of spores of *Penicillium expansum* and *Botrytis cinerea* on non-wounded fruit but was not effective against *Neofabraea perennans.*
9. Ozone at 60-80 ppb reduced nesting of gray mold (Botrytis) by 60 and 40%, respectively, compared to a non-ozone treatment.
10. Ozone at 60-80 ppb did not reduce residue levels of TBZ, pyrimethanil or fludioxonil on the surface of the fruit after 5 months of storage and did not reduce the efficacy of the fungicides against *P. expansum* (Blue mold) after 8 months of storage.
11. Data show that cold storage with continuously low dose ozone can be an additional hurdle for controlling *Listeria* on apple fruits; however, it can not completely eliminate *Listeria*.
12. A systematic/hurdle approach is needed to ensure apple microbial safety.

RESULTS AND DISCUSSION

Objective 1

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

Currently, there is barely any information available on how easily *L. monocytogenes* survives on fresh apples under different storage conditions. Thus, we first did a short- term storage study with *L. monocytogenes* established on fresh apples of selected varieties (Fuji, Granny Smith) under different storage temperatures. In the study, we include both high and low inoculation level of *Listeria* on fresh apples and chose the following storage temperatures per stated reasons.

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

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

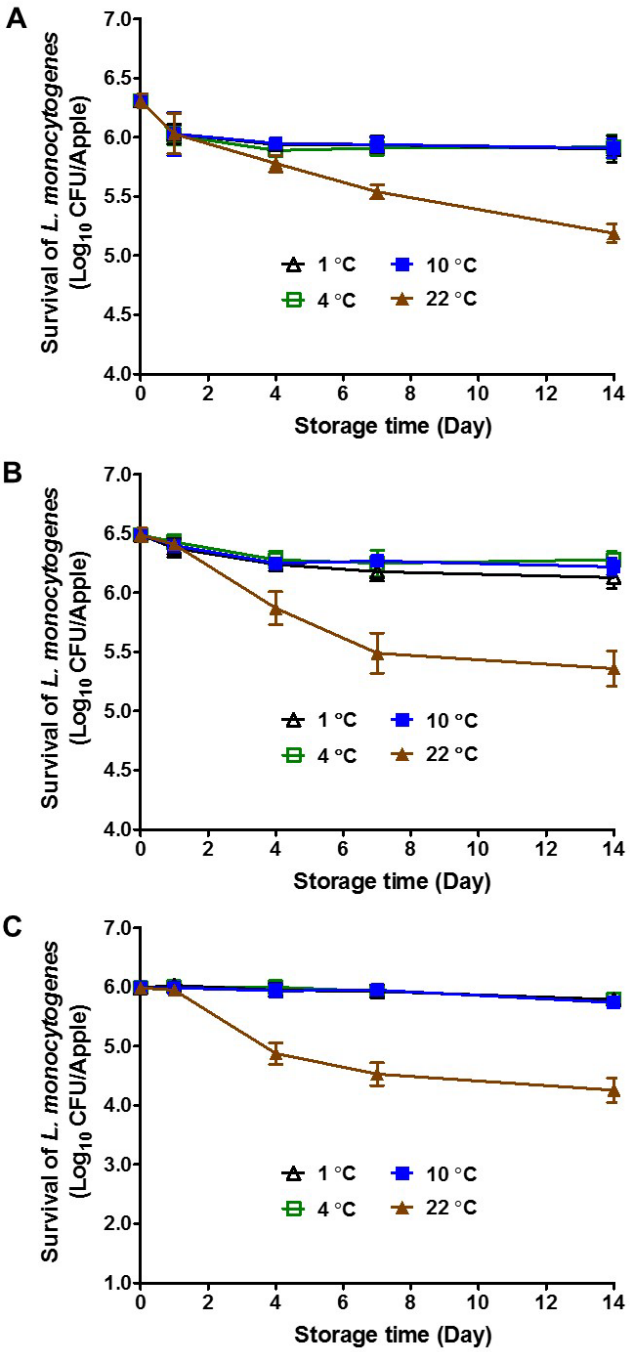

Fig. 1. Fate of *Listeria monocytogenes* on fresh apples during short-term storage under different temperatures when inoculated at 1×10^6 CFU/apple. A. Organic Granny Smith; B. Conventional Granny Smith; C. Conventional Fuji. Mean ± SEM, n=12.

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

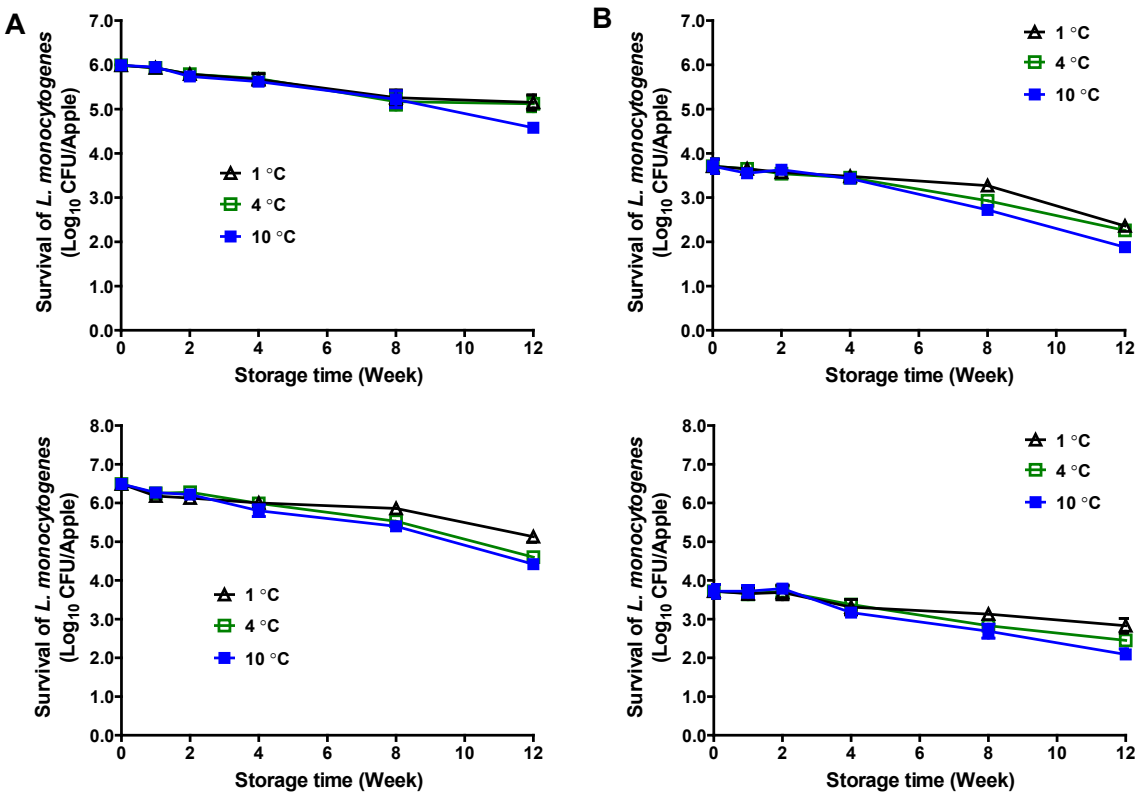

Fig. 2. Survival of *Listeria monocytogenes* on Fuji (AB) and Granny Smith apples (CD) during 3-month cold storage. AC. Apples were inoculated at ~1×10^6 CFU/apple; BD. Apples were inoculated at 1×10^4 CFU/apple. Mean ± SEM, n=12

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

We further conducted an 18-week cold storage experiment in a typical commercial apple facility using *L. innocua* inoculated apples in which Fuji apples were inoculated and established to 7.05 ± 0.02 Log_{10} CFU/apple before being subjected to RA, CA, and CA with 50 ozone gas. During the 6-week storage, *L. innocua* populations on apples under RA and CA storage decreased by ~2.0 Log_{10} CFU/apple (Fig. 1B). Supplementation of CA with continuous gaseous ozone at a concentration of 50.0 ± 28.5 ppb resulted in an additional ~1.7 Log_{10} CFU/apple reduction after 6 weeks (Fig. 3). Upon further 12-week storage, a gradual reduction of *L. innocua* on apples under all three storage conditions was observed. RA, CA, and CA with 50 ppb ozone storage for 18

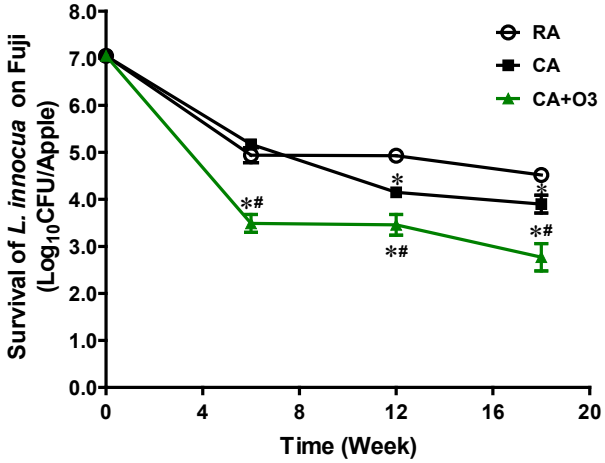

Fig. 3. Survival of *Listeria* on Fuji apple under 18-week commercial cold storages. RA: refrigerated atmosphere (33°F); CA: controlled atmosphere (33°F, 2% O2, 1% CO2); CA + O3: CA with ~50.0 ppb ozone. Mean ± SEM, n=40. In survival curve: *significant difference from RA ($P < 0.05$), #significant difference from CA ($P < 0.05$).

weeks reduced *L. innocua* on Fuji apples by 2.5, 3.1, and 4.2 Log_{10} CFU/apple, respectively (Fig. 3).

To confirm the promising antimicrobial effect of low-dose continuous ozone application in CA storage, we next conducted a 30 -week cold storage experiment in a typical commercial apple facility using *L. innocua* inoculated apples in which Fuji apples were inoculated and established to 6.20 ± 0.06 Log_{10} CFU/apple before being subjected to RA, CA, and CA with 87 ± 39 ppb ozone gas. A rapid reduction of 1.4-1.8 Log_{10} CFU/apple was observed within the first 3 weeks in all three storage conditions (Fig. 4). In the following 15-week storage, *L. innocua* on Fuji apples under RA and CA storage behaved similarly with about a 2.6 Log reduction. Interestingly, by the end of the 30-week storage, bacterial count on apples in RA decreased by ~0.9 Log_{10} CFU/apple compared with 18-week, while that in CA storage remained the same (Fig. 3). For CA storage with 87 ppb ozone gas, *L. innocua* population was reduced by ~5.0 Log after 24-week storage and remained at the similar level till 30-week. To mimic packing facility practice, apples in CA and CA with ozone storage were moved to RA by the end of the 30-week storage, at which time *L. innocua* was enumerated. Additional 2-week storage under RA beyond their respective initial storage treatments had little influence on *L. innocua* survival (Fig. 3).

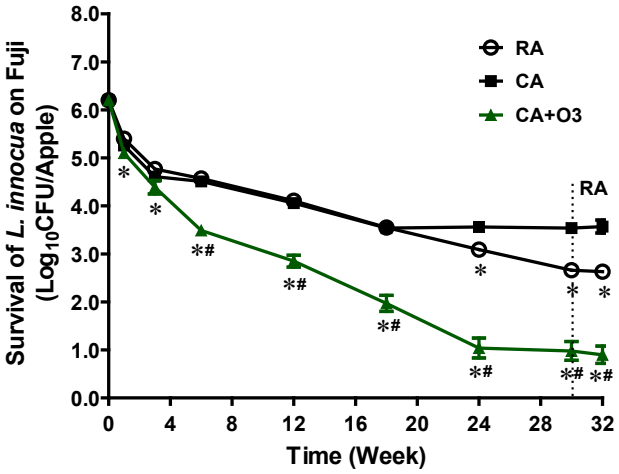

Fig. 4. Survival of *Listeria* on Fuji apple under commercial cold storages. RA: refrigerated atmosphere (33°F); CA: controlled atmosphere (33°F, 2% O2, 1% CO2); CA + O3: CA with ~87 ppb ozone. Mean ± SEM, n=40.

3. Natural microbial reduction on apple fruit surfaces under different cold storage conditions

Another set of Fuji apple fruits (non-waxed and non-inoculation) was subjected to different storage conditions (RA, CA and CA with 87 ± 39 ppb ozone), total plate count (TPC) and yeasts/molds (Y/M) count were evaluated as described above. The initial TPC and Y/M count were 3.44 ± 0.07 and 4.77 ± 0.07 Log_{10} CFU/apple, respectively (Fig. 5). TPC remained stable on Fuji apples in RA and CA storages throughout storage (Fig. 5A). Continuous application of ozone at low doses gradually achieved ~1.0 Log_{10} CFU/apple reduction of TPC after 30-week storage (Fig. 4A). At 12 weeks, Y/M count in RA

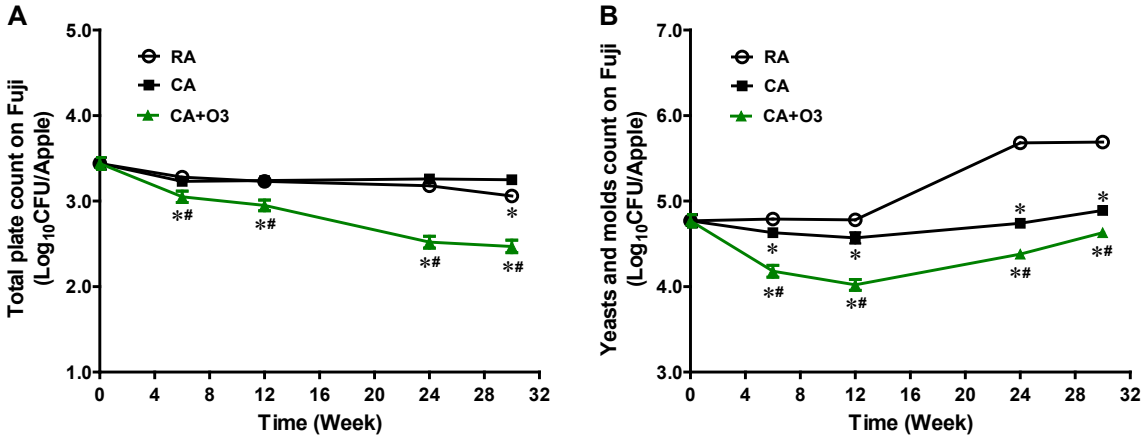

Fig. 5. Fuji apple decay during cold storages. A. TPC. B. Y/M count. RA: refrigerated atmosphere (33°F); CA: controlled atmosphere (33°F, 2% O2, 1% CO2); CA + O3: CA with ~87 ppb ozone. Mean ± SEM, n=40.

and CA storage remained relatively stable, while the Y/M count in CA + O_3 was reduced by ~ 0.6 Log_{10} CFU/apple (Fig. 5B). Nevertheless, the inhibitory effect of CA ± O_3 was compromised with prolonged storage time. By the end of the 30-week storage, the Y/M count of apples in CA and CA ± O_3 reached 4.89 ± 0.05 and 4.63 ± 0.04 Log_{10} CFU/apple, respectively (Fig. 5B). Y/M count on apples under RA storage stayed at the same level within the first 12-week storage, increased by ~1.0 Log_{10} CFU/apple from the 12th to the 24th week of storage, then remained constant in the last 6 weeks of storage (Fig. 5B).

Objective 2

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

Quality parameters of apple fruits under different storage treatments were assessed both at harvest and after storage. Firmness decreased after storage for all apples, and no difference was found between CA and CA + O_3 storage. Apples subjected to RA storage had a significantly lower firmness than those in CA with or without gaseous ozone storage. Compared to harvest levels, TSS levels did not change in apples post-6-month storage in RA, CA, or CA + O_3. TA after storage decreased to between one third to two thirds of the TA at harvest. TA reduction in apples was significantly mitigated by CA storage, while addition of ozone had no impact on TA (Table 1).

Table 1. Fuji apples quality parameters at harvest and after 6 months of cold storage and ripen at RT for 7 days.

Treatment	Weight (g)	Diameter (in)	Firmness (lbs)	TSS (% Brix)	TA (% malic acid)
At harvest					
	204 ± 10.1	3.0 ± 0.1	15 ± 0.2	12.7 ± 0.3	0.307 ± 0.01
After 6 months storage					
RA	188 ± 4.6^{a}	2.97 ± 0.03^{a}	10.0 ± 0.4^{b}	12.4 ± 0.8^{a}	0.173 ± 0.01^{b}
CA	183 ± 3.3^{a}	2.92 ± 0.03^{b}	13.8 ± 0.3^{a}	12.5 ± 0.5^{a}	0.232 ± 0.02^{a}
CA + O_3	186 ± 4.3^{a}	2.95 ± 0.02^{ab}	13.7 ± 0.4^{a}	12.4 ± 0.8^{a}	0.220 ± 0.02^{a}

Means within a column with no common letter differ significantly ($P < 0.05$). TSS: total soluble solids; TA: titratable acidity; Mean ± SEM, n=40.

The incidence of external and internal disorders was visually evaluated at the end of each storage treatment. Overall, the parameters evaluated for either external disorders or internal disorders were not significantly different among apples stored under RA, CA, or CA + O_3 (Table 2 & 3). No ozone burn,

Table 2. External disorders analysis for Fuji apples after 6 months of cold storage and ripen at RT for 1 and 7 days.

Treatment	External disorders (%)						
	Ozone burn	Superficial scald	Lenticel decay	Visible decay	Sunburn	Russet	CO_2 damage
1 day at RT							
RA	0^{a}	15 ± 2.2^{a}	0^{a}	1 ± 0.4^{a}	23 ± 4.4^{a}	5 ± 2.8^{a}	0^{a}
CA	0^{a}	5 ± 5.3^{a}	0^{a}	1 ± 0.9^{a}	20 ± 2.3^{a}	5 ± 1.3^{a}	0^{a}
CA + O_3	0^{a}	1 ± 0.4^{a}	0^{a}	1 ± 0.4^{a}	22 ± 2.9^{a}	7 ± 2.3^{a}	0^{a}
7 days at RT							
RA	0^{a}	16 ± 6.2^{a}	0^{a}	1 ± 4.3^{a}	22 ± 2.1^{a}	5 ± 2.1^{a}	0^{a}
CA	0^{a}	4 ± 2.5^{a}	0^{a}	0^{a}	16 ± 3.8^{a}	7 ± 2.4^{a}	0^{a}
CA + O_3	0^{a}	1 ± 0.4^{a}	0^{a}	0^{a}	23 ± 1.9^{a}	8 ± 1.9^{a}	0^{a}

Means within a column with no common letter differ significantly ($P < 0.05$). Mean ± SEM, n=200.

lenticel breakdown, decay, or CO_2 damage was found in any apples subjected to 6-month of low-dose ozone gas storage (Table 2). No watercore, internal browning, or cavity was observed in apples stored under CA or CA + O_3. A small number of apples under RA stored were found to have watercore and internal browning, but the incidence rate was not significantly different from those fruit kept under CA storage (Table 3).

Table 3. Internal disorders analysis for Fuji apples at harvest and after 6 months of cold storage and ripen at RT for 7 days.

Treatment	Internal disorders (%)		
	Watercore	Internal browning	Cavity
At harvest			
	0^a	0^a	0^a
After 6 months of cold storage			
RA	3 ± 0.4^a	10 ± 1.0^a	0^a
CA	0^a	0^a	0^a
CA + O_3	0^a	0^a	0^a

Means within a column with no common letter differ significantly ($P < 0.05$). Mean ± SEM, n=200.

Objective 3

1. Efficacy of ozone on artificially wounded and inoculated fruit and non-wounded fruit

After 60 days of storage, incidence of blue mold, gray mold and bull's eye rot was reduced by 52, 45 and 60%. After 90 days, respective reduction decreased to 18, 12 and 20%, respectively (Fig. 6A). On non-wounded fruit, ozone reduced spore loads of blue mold, gray mold and bull's eye by 78, 62 and 4%, respectively after 90 days of storage. After 180 days,

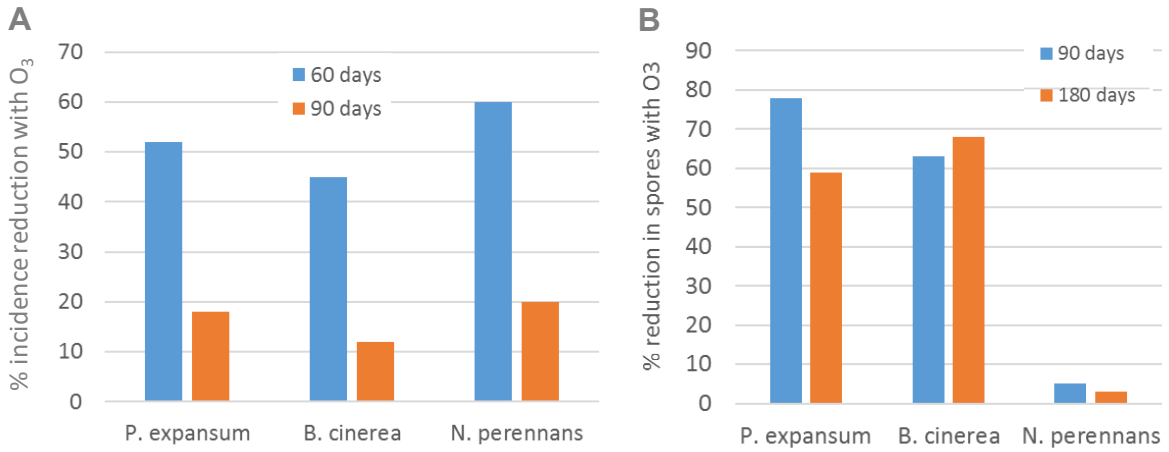

Fig. 6. Reduction of incidence of blue mold, gray mold and bulls eye rot on wounded fruit (A, wounded fruit) and of spore load on non-wounded fruit (B, surface inoculation) treated with ozone relative to non-ozonated fruit.

reduction of blue mold spores decreased to 58% but remained steady for the two other pathogens (Fig. 6B).

2. Efficacy of ozone in reducing nesting of Botrytis cinerea (gray mold)

Ozone at 60 ppb reduced nesting of gray mold (*Botrytis*) from 50% in the CA room without ozone to 20% on Fuji apples after 6 months of storage, versus 33% when ozone was applied at 80 ppb (Fig. 7).

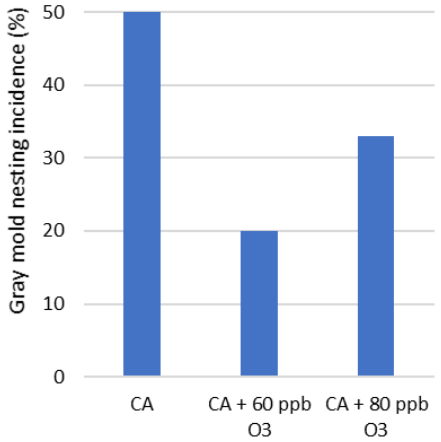

Fig. 7. Efficacy of continuous ozone application in reducing nesting of Botrytis cinerea (gray mold) after 6 months of storage

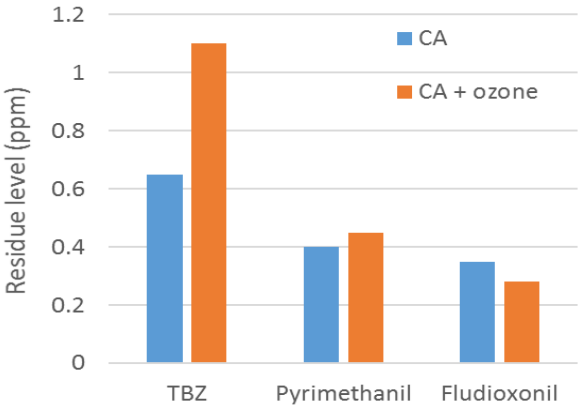

Fig. 8. Residue levels of TBZ, pyrimethanil, and fludioxonil on Fuji fruit stored inn CA and CA + ozone (80 ppb) for 6 months.

3. Interaction of postharvest fungicides with a low dose of ozone on non-wounded fruit

Gaseous ozone at 60-80 ppb did not decrease residue levels of TBZ, pyrimethanil or fludioxonil after 6 months of storage (Fig. 8).

EXECUTIVE SUMMARY

The overall goal of the proposed studies was to evaluate the antimicrobial efficacy of low dose continuous gaeous ozone against *L. monocytogenes* contaminated apples, as well as apple fungal pathogens during commercial cold storage, and to further evaluate its impacts on apple fruit quality and its interaction with fungicides over an extended period of storage.

Listeria is a tough foodborne pathogen to eliminate once it has contaminated apple surface. At ambient/low relative humidity (RH), limited reduction of *L. monocytogenes* on apple surfaces occurred during 12 weeks of refrigerated storage either at 1°C/33°F, 4°C/39.2°F or 10°C/50°F. However, a 6-month commercial cold storage (RA and CA) with high RH decreased *L. innocua,* a non-pathogenic surrogate of *L. monocytogenes* count on Fuji apples by 2.5-3.0 Log_{10} CFU/apple. Continuous application of gaseous ozone at ~87 ppb in CA storage facilitated *L. innocua* reduction and resulted in ~5.0 Log_{10} CFU/apple reduction after 30-week storage, and inhibited apple residence microflora. Furthermore, continuous low dose ozone gas used in this study had no negative influence on Fuji apple visual quality, including both external and internal disorders during 6-month CA cold storage. Data collectively show that cold storage with continuous low dose ozone can be an additional hurdle for controlling *Listeria* on apple fruits; however, it can not completely eliminate *Listeria*. A systematic/hurdle approach is needed to ensure apple microbial safety.

Furthermore, ozone at the tested concentration is beneficial in reducing the inoculum load on fruit surfaces and nesting of some pathogens such as *Botrytis* and *Phacidiopycnis*, which resulted in an overall reduction of 15 to 20% in disease incidence. Low dose ozone may also be effective in controlling infections that starts on fresh wounds and therefore should not be viewed as a replacement for fungicides in conventional packinghouses. At the concentrations evaluated in this study ozone is safe to use on fruit treated with fungicides at harvest, as no reduction in residue levels was observed after 6 months of storage.

In summary, continuous low-dose ozone gas has the potential to be applied during long term apple cold storage, as well as other fresh produce industries with similar practices as a supplemental intervention method to ensure fresh produce safety and control decay.

FINAL REPORT

Project Title: Understanding and managing the food safety risk of packline brushbeds

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*Faith Critzer will assume PI in January 2018.

Cooperators: Eight packing facilities in Washington

Total Project Request: Year 1: $51,966

WTFRC Collaborative expenses:

Item	2017
Salaries & Benefits	$2,736
Wages	$3,472
Benefits	$729
Travel	$4,180
Total	**$11,117**

Footnotes: Salary and benefits for Ines Hanrahan; Wages and benefits for intern.

Budget 1
Organization Name: WSU
Telephone: 509-335-2885
Contract Administrator: Katy Roberts
Email address: arcgrants@wsu.edu

Item	2017
Wages	$6,256
Benefits	$1,314
Equipment	$11,000
Supplies	$16,707
Travel	$5,574
Total	**$40,850**

Footnotes: Wages and benefits for technical assistant; Equipment is incubator ovens, plate counter and pipettes; Supplies for microbial test plates and sponges.

1. Objectives

1. Compare current brush cleaning and sanitation procedures in eight apple packing houses in Washington to determine the effectiveness of these procedures.
2. Determine if fruit sanitation practices are adequate to reduce the risk of cross-contamination from wax brushes during a production shift.
3. Determine if wax brushes are a commercially significant source of spoilage organisms (yeasts and molds)
4. Determine if there is a difference between the packing organic and conventional fruit in the above objectives.
5. Conduct appropriate food safety extension outreach with the apple packing industry

Significant Findings

- Aerobic plate counts were not correlated with populations of coliforms and *E. coli.* r^2=0.17 and 0.018, respectively. This indicates that there is very little utility in testing for APCs on food contact surfaces as they are readily in the environment and of no connection to indicators which are more indicative of sanitary concerns (*E. coli* or coliforms).
- Clean out of place (COP) steam cleaning was very effective in reducing microbial counts on packing line brushes – aerobic colony counts were 1700 times lower than the average of the other five packing facilities.
- Extremely low populations of *E. coli* were found in all facilities across both years, indicating that current sanitation practices are significantly reducing the risk of harborage. Setting sanitary performance metrics for food contact surfaces (zone 1) based upon *E. coli* or coliform populations would be recommended for tree fruit packers.
- False positives for *Listeria* spp. are common when relying solely on selective and differential media. Subsequent steps should involve conformation utilizing standardized methods, such as PCR. No samples were confirmed positive for *Listeria* spp. in this study.

Methods

Six representative apple packing facilities in Washington were selected for this project in 2017, partly based on responses from a project pre-survey. Packing facilities are numbered to maintain confidentiality (Table 1). Brushes and other packing line surfaces (oven rollers, drying oven walls, belts, curtains and transfer rubbers) were swabbed (3M™ Quick Swab) both before and after a production shift. The focus of the study was drying and wax brushes, but also included other brushes and surfaces in the wet area of the packing line.

Fruit samples were taken off the line before and after the brushbed at the start and end of the production shift; 10 fruit were taken at each location. Swabs and fruit were stored in a cooler box with ice packs during transportation from Yakima or Wenatchee, stored in a refrigerator overnight, and plated the following morning at WSU IAREC in Prosser. Fruit were placed in buffered peptone water incubation pouches for 1 h before plating.

The following microbial tests were conducted on swabs using 3M Petrifilm™ plates: aerobic colony count, coliform/*E.coli*, environmental *Listeria*, and yeasts and molds following the 3M Petrifilm methods for each test. The same tests were conducted on fruit samples, except that environmental *Listeria* and coliforms/*E.coli* testing were omitted. Enumeration was done using a 3M Petrifilm Plate Reader. Samples were diluted 1:10 using Butterfields solution for APC and yeast and molds if high microbial loads were anticipated.

In 2018, the project was continued with four facilities (two from 2017 and two which were new to the study) to collect more data on microbial populations of food contact surfaces (Table 2). The methodology was modified slightly for inclusion of sponge swabs with Dey Engley (D/E)

neutralizing buffer, increasing the surface area to 1ft^2 for *Listeria* spp., and identification of *Listeria* species through selective enrichment with PCR confirmation targeting the *iap* gene.

Table 1: Packing facility numbers and description sampled in 2017

	Packing Facility Number					
	1	**2**	**3**	**4**	**5**	**6**
Relative Age of Line	Newer	Older	Newer	Newer	Older	Newer
Wet/Dry Separation	Yes	No	Yes	No	No	Yes
Hygiene Monitoring	Yes	No	Yes	Yes	Yes	Yes
Brush CIP/COP	CIP & COP	CIP	CIP & COP	CIP	CIP	COP
Brush Cleaning Method	Chlorine foam	Chlorine foam	Chlorine foam	Chlorine foam	Chlorine foam	Steam
Sanitizer during Production	Ozone, PAA, ClO_2	PAA	Ozone, PAA, ClO_2	PAA	Ozone	PAA

CIP, Clean in Place; COP, Clean out of Place; PAA, peracetic acid; ClO_2 chlorine dioxide.
Newer lines < 5 years old; Older lines >15 years old

Table 2: Packing facility numbers and description sampled in 2018

	1	**2**	**3**	**4**
Relative Age of Line	Newer	Older	Newer	Older
Wet/Dry Separation	Yes	No	No	No
Hygiene Monitoring	Yes	Yes	Yes	Yes
Brush CIP/COP	CIP & COP	CIP	CIP & COP	CIP
Brush Cleaning Method	Chlorine foam	Chlorine foam	Chlorine foam	Chlorine foam
Sanitizer during Production	Ozone, PAA, ClO_2	Chlorine, Ozone, PAA	Chlorine, PAA	Chlorine, PAA

CIP, Clean in Place; COP, Clean out of Place; PAA,peracetic acid; ClO_2 chlorine dioxide.
Newer lines < 5 years old; Older lines >15 years old

Results & Discussion
Environmental Listeria

It should be noted when relying upon differential and selective media, as was used in this study, there are frequently false positive isolates identified that when confirming with secondary tests like PCR, are actually not *Listeria* spp. but rather Enterococci, such as *Enterococcus faecium* or *Enterococcus*

faecalis. Therefore, we cannot definitively state if the isolates from 2017 were in fact *Listeria* spp. Only facility 5 (older line) had environmental *Listeria* detections in 2017. These detections were on:

- a transfer rubber at the end of shift (10/2),
- soap brushes and a felt fabric transfer curtain at the start of shift (10/30), and
- a wax brush at end of shift on 10/30.

This facility had high aerobic colony counts at the start and end of shifts (Figure 2).

Of the four facilities which were sampled in 2018, none were positive for environmental *Listeria* spp. after sanitation and with 4 hrs of production startup (n=156). Brushes and transfer points were identified as common targets for sampling, but were not observed to be harborage points.

Coliforms & *E.coli*

Coliforms were detected at least once at all packing facilities at the start of the production shift (Figure 1, 2017 data). Areas that regularly tested positive for coliforms at the start of the production shift were:

- Wax brushes
- Transfer brushes after the drying oven
- Bin filler brushes
- Transfer brushes in general

Coliforms were also detected in all of the facilities during 2018 sampling, but no significant associations were made by material or unit operation with populations ranging from 14-700 CFU per 25 cm^2.

There were four *E.coli* detections in 2017:

- Facility 1 on a wax brush under the wax applicator at the start of the shift.
- Facility 2 on repair tape on a spacer bar.
- Facility 5 on a transfer rubber – the same date (10/2) and location where environmental *Listeria* was detected (see above) – and one fruit sample at the start of shift after going over the brushbed.

These three facilities had the highest average aerobic colony counts (Figure 2).

Three of the four facilities in 2018 had sites which were positive for *E. coli* in 2018.

- Twenty-two of 156 sites tested positive for *E. coli* in 2018 with populations remaining very low (1-5 CFU/25cm^2)
- Sites were evenly divided between dry (sorter, oven rollers, packing tables) and wet (spray bars, dump tank) areas of the packing line.

Aerobic Bacteria

Aerobic plate counts (APCs) are considered of very little utility for monitoring cleanliness within the food industry given that they have no linkage to food safety and many times encompass bacterial populations which may be more resistant to sanitizers and heat than target organisms of quality or safety concerns. There were no significant correlations found between APCs and *E. coli* or coliforms. If using APC as a sanitation performance metric, it is important to set baseline populations for each surface. When evaluating 2017 data, Facility 6 had aerobic colony counts 3 orders of magnitude lower at the start of shift and 2 orders of magnitude lower at the end of shift than the other five facilities – the APCs at the end of the production shift at facility 6 were often lower than the APCs at the start of the shift at other facilities. Their success demonstrates that it is possible to clean a

packing line to very low counts, and reduce these by 2-3 $\log_{10}$ values with COP steam cleaning and a multi-hurdle approach during a production shift.

Facilities 3 and 6 (2017) had the lowest average aerobic colony counts on the packing line (Figure 2), and also had the lowest aerobic colony counts on fruit (Figure 4). Facility 5 had high counts throughout the line and consequently the fruit from that facility had the highest counts. General comments regarding specific areas on packing lines are given below in Table 2.

When evaluating data for 2018, recovery of total aerobic bacteria was significantly different at sites along the processing line (p=0.0179). A post-hoc analysis revealed that recovery was higher at spray bar sites (Mean=3,255) than at dryer (Mean=999), dump tank (Mean=718), or sorter (Mean=484) sites, but indistinguishable from packaging (Mean=1,735) or wax bar (Mean=1,227) sites (LSD test). No significant differences were found between the four facilities.

Table 2: Comments on cleaning and sanitation procedures for zones 1 and 2 areas on apple packing facilities.

Area	General Comments
Soap and sanitizer brushes	Need attention during cleaning
Drying brushes	ATP swab first brushes; highest APC there, decreasing down bed
Wax brushes	All CIP lines have high APCs, especially under the wax applicator. ATP swab brushes under the wax applicator.
Oven rollers	Lower concern, but high residues indicate higher APCs
Post-oven transfer brushes	Can have high loads, need more attention during cleaning and sanitation
Alignment brushes	Lower concern
Bin filler brushes	Some concern, need more attention during cleaning and sanitation
Transfer brushes	Some concern, need more attention during cleaning and sanitation
Other surfaces	Other surfaces, like fruit pushers, oven walls, etc. require attention during cleaning and sanitation in the worse performing packing facilities. Surfaces like tape, foam, cloth and rubber should ideally be removed from the line because they are potential harborage sites for food pathogens.

APC, aerobic plate count; CIP, Clean in Place

Molds

The mold counts on the packing lines generally increased during the production shift and correlated with the aerobic colony counts. Facilities 3 and 6 having lower mold counts and facilities 1, 2, and 5 having higher loads (Figure 5 and Figure 6). This will vary by lot and storage duration, however, and requires longer term monitoring. Facility 1 did not have good mold control over the brushbed and consequently through the shift, with both mold and aerobic counts increasing over time on fruit (Figure 6). Yeast counts followed a similar trend to molds so data were not presented for brevity. Good cleaning and sanitation practices not only reduce food safety risks, but may improve returns by reducing rejections of packed fruit with an extended storage period – such as exports or in a high production season.

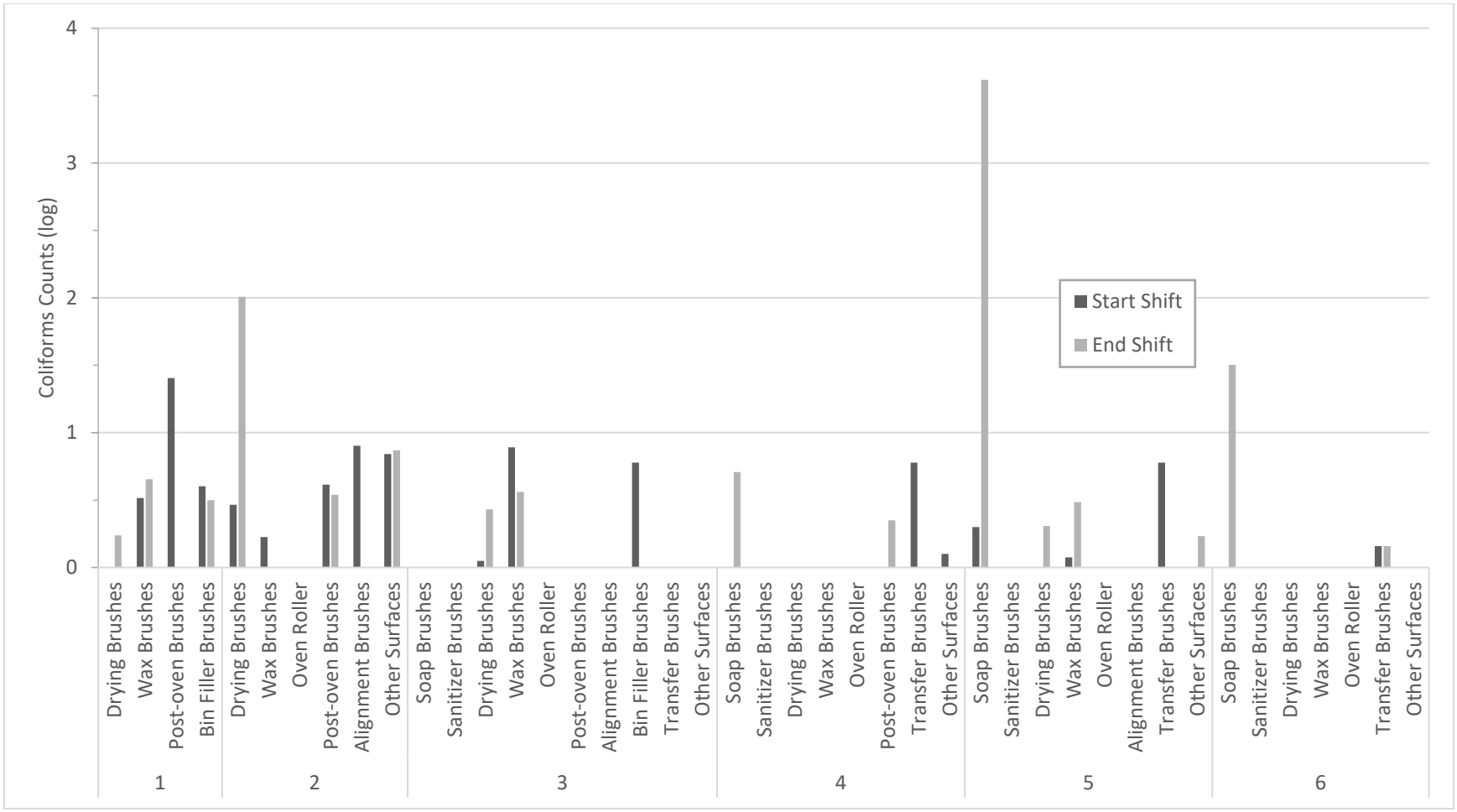

Figure 2: Coliform counts on the packing lines of six particpating packing facilities (2017).

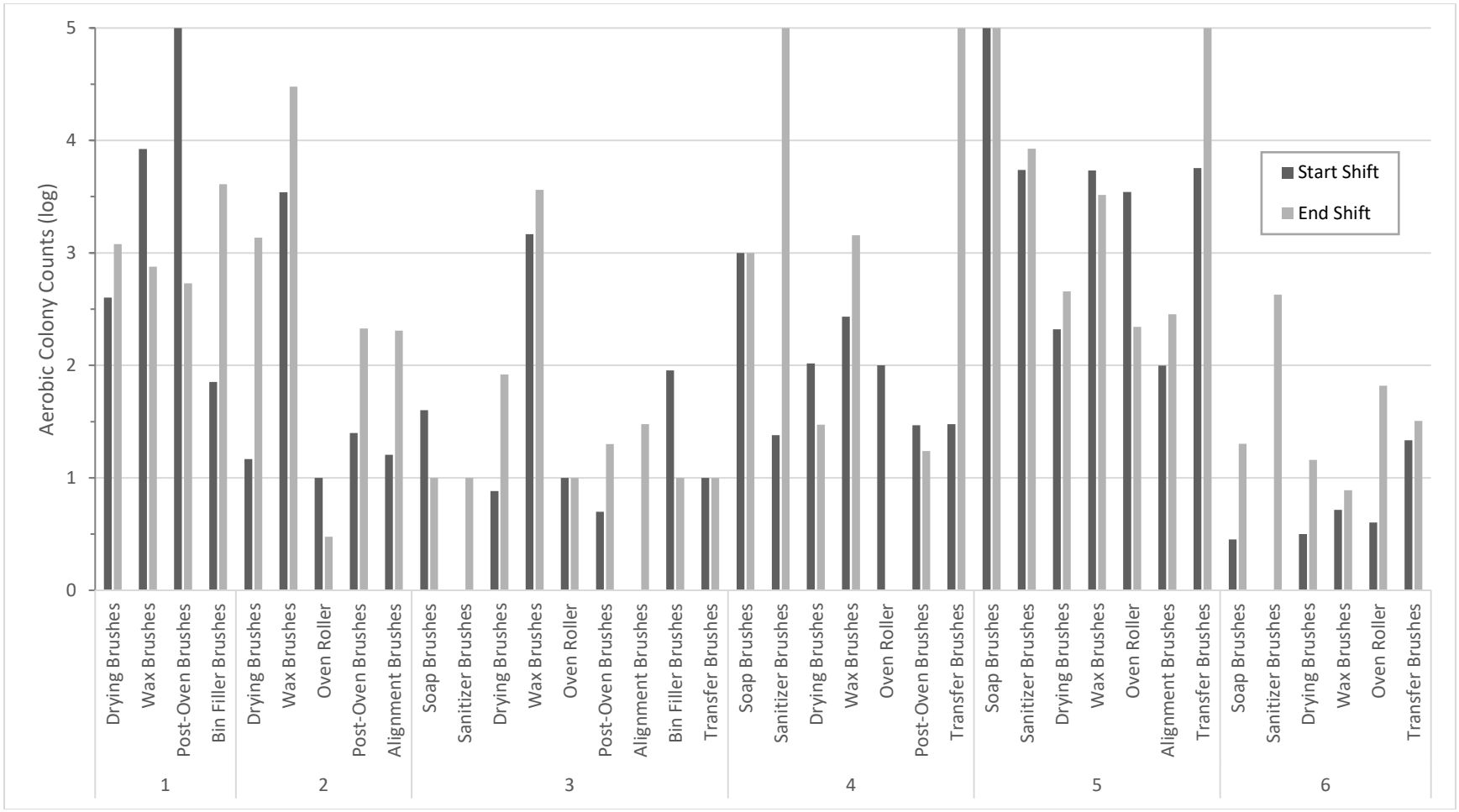

Figure 3: Aerobic colony counts on the packing lines of six particpating packing facilities (2017).

Figure 4: Aerobic colony counts on selected packing line surfaces at four of the participating packing facilities (2017).

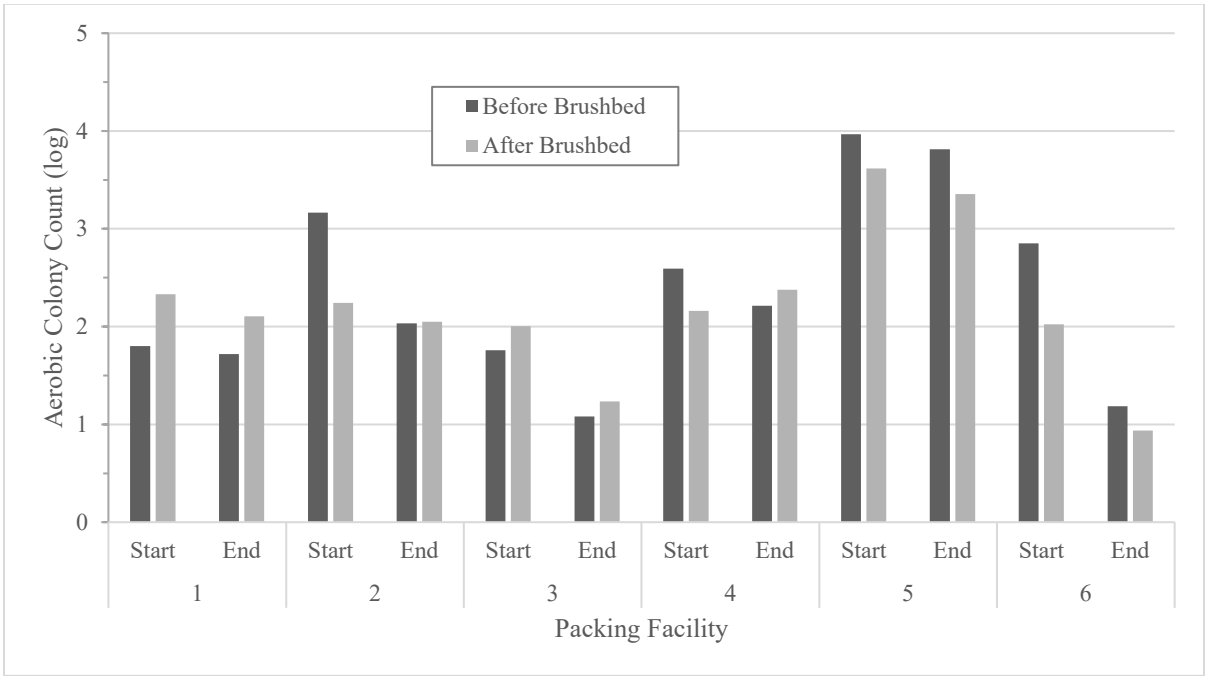

Figure 5: Aerobic colony counts on apple fruit at the start and end of shift, sampled before and after the brushbeds of the six participating packing facilities (2017).

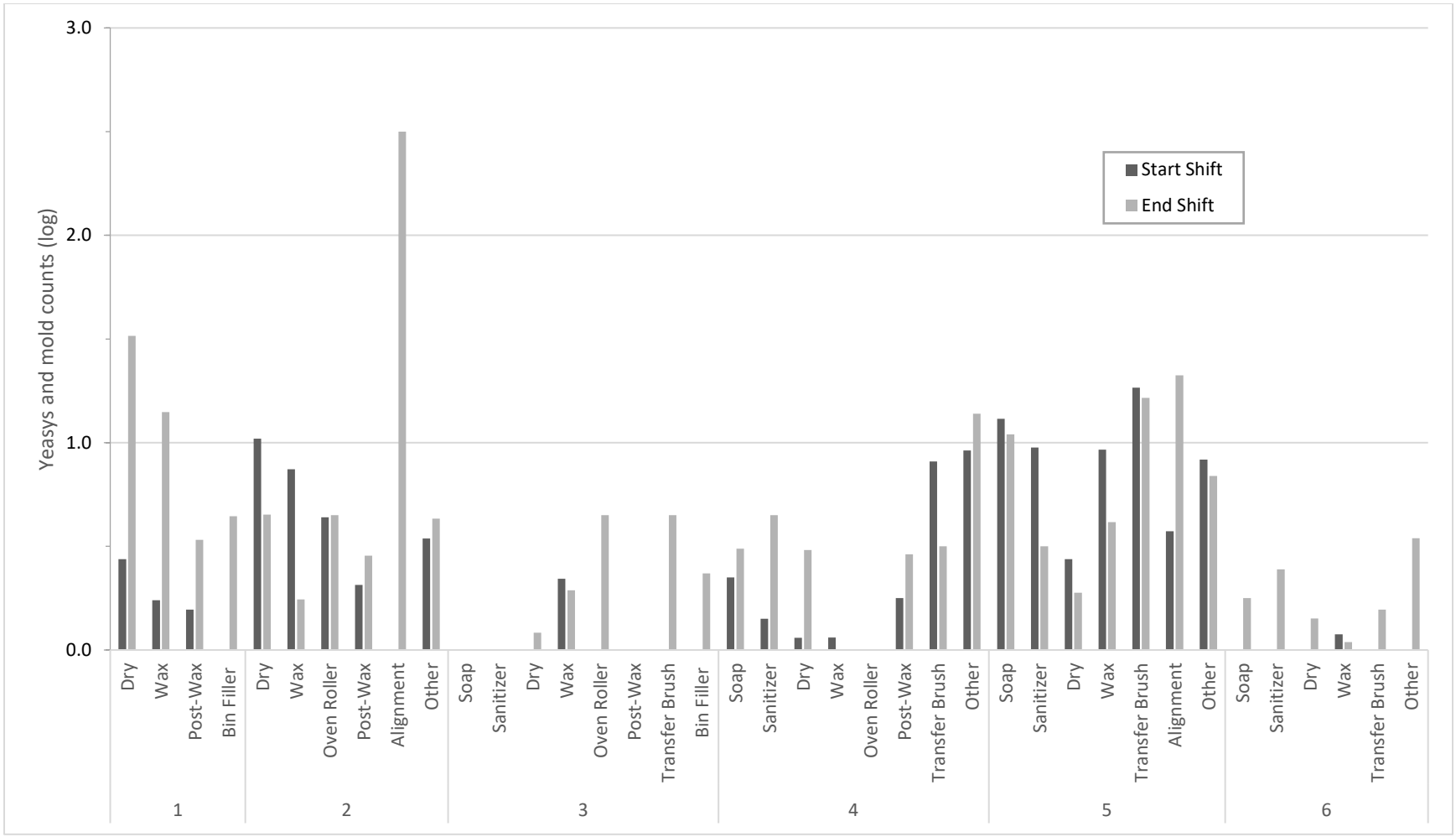

Figure 6: Mold counts on the brushes and other surfaces of packing lines of six particpating packing facilities (2017).

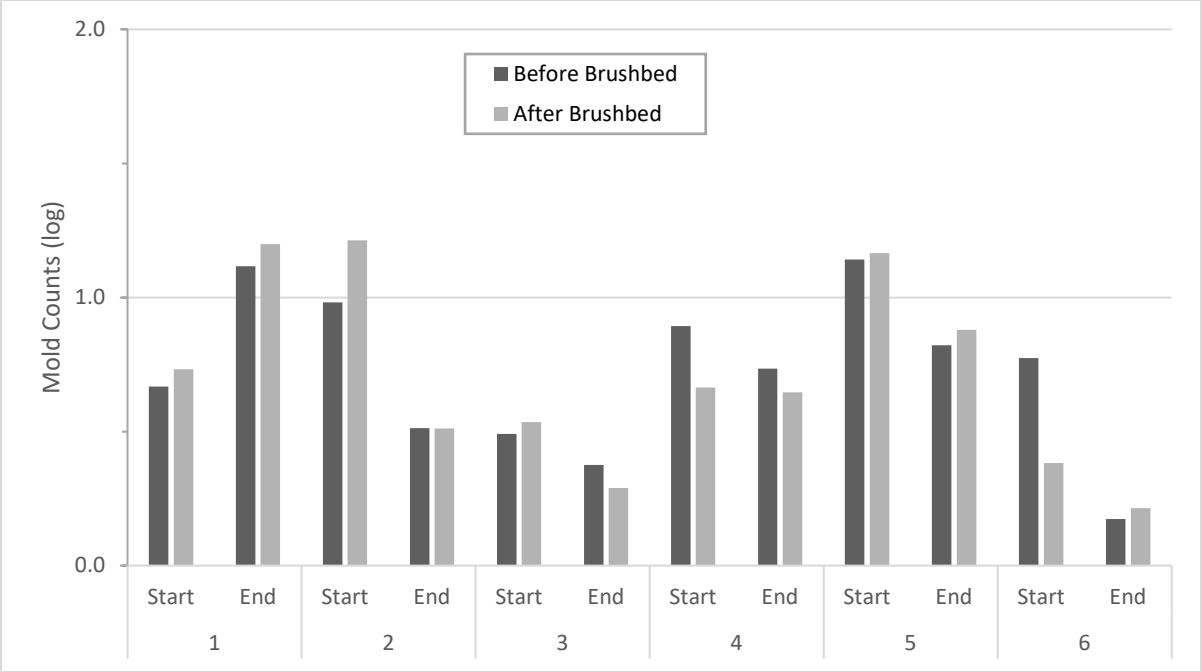

Figure 7: Mold counts on fruit at the start and end of shift, taken before and after the brushbeds of the six participating packing facilities (2017).

Conclusion

Generally speaking, there is little value in testing surfaces for aerobic mesophilic bacteria (APC) as high populations are regularly recovered and are not correlated with populations of indicators more commonly employed for food safety (generic *E. coli* or coliforms). Cleaning practices dictated recovery of microbial populations to a greater extent than the age of the facility. This is encouraging as capital outlay for new facilities and packing lines are significant and not an option for many operations. COP steam cleaning of brushes resulted in a significant reduction in microbial counts, but has been noted to reduce the life of brushes. Some key points to improving hygiene levels in packing facility, and reducing food safety risk are: a motivated, properly equipped sanitation crew with attention to detail and sufficient time to clean and sanitize the packing facility, a validated hygiene monitoring system, an appropriate sanitizer monitoring system and protocol, and leadership from management to continually improve hygiene levels in a facility.

These results only provide a snap shot at each packing facility. To be effective, a food safety program requires daily attention, and long term planning for continual improvement. These assays, excluding the environmental Listeria test, can be done easily at a packing facility and the results used to improve cleaning and sanitation procedures at the facility.

Executive Summary

Aerobic plate counts (APCs), coliforms and *E.coli*, environmental *Listeria*, and yeasts and molds samples were taken at six apple packing facilities in Yakima and Wenatchee between August and October 2017. These facilities were representative of the types of packing lines currently in Washington. The brushbed was swabbed before and after a production shift. Fruit samples were also taken at the same times, before and after going over the brushbed. Microbial tests were performed using 3M Petrifilm™ plates. In 2018, four facilities were sampled (two from 2017 and two new facilities) with sampling of food contact surfaces (zone 1) to determine populations of APC, *E.coli*, coliforms and *Listeria* spp..

APCs were not correlated with populations of more common food safety indicator organisms (*E. coli*, coliforms and *Listeria* spp.). However, APCs may be a cost effective means of measuring cleaning and sanitation efficacy, with appropriate baseline establishment. When evaluating data from 2017 data, **APCs** at the start of production were lower in the three newest lines, but results show that it is possible to clean older facilities to levels comparable to those of newer lines. **Yeasts and molds** correlated with APCs, suggesting that evaluation of these populations may also help decrease post-packing decay – particularly on fruit with an extended post-packing storage duration. **Coliforms** were detected on all the packing lines in 2017 and 2018. ***E.coli*** was sporadically detected at the three facilities in 2017 and all facilities in 2018. There may be better utility in testing zone one surfaces for these organisms compared to APC as an indication of sanitation efficacy. This is due to the fact that target foodborne pathogens share similar inactivation behavior to these organisms, whereas APCs will detect many bacteria which are not a concern for quality or safety and may be more resistant to our cleaning and sanitizing practices. **Environmental *Listeria*** was only detected on one older line in 2017, however these positives were not confirmed. No *Listeria* spp. were detected in 2018 demonstrating efficacy of current practices used in facilities of all ages employing many different sanitation practices.

It is clear from this study that detailed attention and evaluation of cleaning and sanitizing efficacy should be conducted by all facilities. Regardless of sanitizers used or age of a facility, very low populations of common food safety indicators (coliform, generic *E. coli,* and *Listeria* spp.) were observed amongst eight facilties. It is key that facilities continually evaluate their sanitation programs and inclusion of testing for indicators associated with foodborne pathogens will help to mitigate risks and identify areas which may need more frequent or intensive sanitation. Coliform or *E. coli* populations could be determined through in-house testing and may be the best organisms to indicate the efficacy of a sanitation program from a food safety perspective. APCs will enumerate any organism which can grow at 98°F and is tolerant to air. There are many organisms which will be enumerated on APC which are not a food safety or quality risk. However, higher yeast and mold counts did align with higher APCs and may assist when trying to control for cross-contamination onto fruit.

CONTINUING PROJECT REPORT **YEAR**: 3 of 3
WTFRC Project: AP-15-103 No Cost Extension

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

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

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

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

Organization Name: WSU **Contract Administrator:** Katy Roberts

Telephone: (509) 335-2885 **Email address:** arcgrants@wsu.edu

Item	2016	2017	2018	2019
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	**0**

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

RECAP OF ORIGINAL OBJECTIVES

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

1) Determine the impingement drying characteristics for broad range of wax coated apples and the impact on quality of the apples with in-plant testing.
2) Study the effectiveness of impingent drying in reducing the microbial levels in apples.
3) Develop scale-up strategies for commercial packing lines and complete the energy efficiency analysis.

In 2019 we would like to continue to explore the potential of impingent drying in reducing the microbial levels in apples. Tests conducted during 2018 gave us promising results and improved bacterial reduction. We want to optimize the treatment to get the maximum bacterial reduction, considering the important aspects such as limited time of cleaning process, and the delicate nature of the apple peel.

SIGNIFICANT FINDINGS

- As proposed in the original proposal, the first objective has been completed during the first year of the project and part of the 2nd objective has also been completed, during the year 2016. With the results from the year 2016, we did not see more than 1 log reduction of *L. innocua* (a surrogate strain of *L. monocytogenes*) with high temperature conditions in the dyer.
- We took a close look at the apple surface using microscopy. We identified many micro sized cracks with higher concentrations in the calyx and the stem bowl areas. These micro cracks are actually big size for the Listeria spp. and other pathogens, but too small for the water or other chemicals to enter. We did more research in this matter and have published an article titled "*Apple Peel Morphology and Attachment of Listeria Innocua through Aqueous Environment as shown by Scanning Electron Microscopy"* in the Food Control journal.
- In the year 2018 we performed more tests using surfactants which helped to increase bacteria kill as well as it helped to dry the apples faster.

METHODS

We are providing here only the methods used for the data shown in this report. The drying protocols, experimental procedure for microscopy and contact angle testing were provided in the previous reports, and the original proposal.

Experimental procedure for microbial testing

Whole apples were pulled out from the refrigerated storage and placed on a bench top overnight to equilibrate to room temperature. Apples were inoculated with L. innocua by submerging 15 apples in 5 L of inoculum solution (approximately concentration of 10^7 CFU/mL). Apples were carefully turned around in the inoculum for 10 min to uniformly spread the inoculum, then air-dried at room temperature for minimum of one hour before analysis.

Apple samples (approximately 3 x 3 x 1 cm cubes) were cut using sterilized knife from either core (calyx and stem bowl cavities) or side sections. Apple samples were placed in sterile stomacher bags with filter (Fisher Scientific, Pittsburgh, PA), weighed, mixed with 25 ml of sterile PBS with 0.2% Tween 20 solution and homogenized, using a Stomacher 400 Lab-blender (Steward Limited, London, UK). Each sample was homogenized for 2 min at 300 rpm, allowed to rest for 1 min, and homogenized again for 2 min at 300 rpm. Homogenized suspensions were serially diluted in sterile PBS and spread-plated with a turntable and glass rod on Modified

Oxford agar (MOX; Difco, BD, Sparks, Md., U.S.A.).

Preparation of cleaning solutions

Three types of surfactants were used: cationic lauric arginate (LAE) (CYTOGUARD ™ LA20, 20 v/v%, A&B Ingredients, Fairfield, NJ, U.S.A), anionic sodium dodecyl sulfate (SDS) (Sigma-Aldrich, St. Louis, MO, U.S.A) and nonionic Tween 20 (T20) (Sigma-Aldrich, St. Louis, MO, U.S.A), alone and combined with peracetic acid (PAA) (Pace International, Wapato, WA, U.S.A). The antimicrobial treatments were prepared by adding LAE, SDS, and T20, respectively to the water to obtain solutions with a final surfactant concentration of 0.1% w/w. For the solutions with PAA, the concentration of PAA was set at 80 ppm. PAA concentration was measured using titration kit (LaMotte, Chestertown, MD, U.S.A).

Cleaning procedure

Applied treatments are summarized in Figure 1. Each time three inoculated, untreated apples were subjected to microbial enumeration as a control.

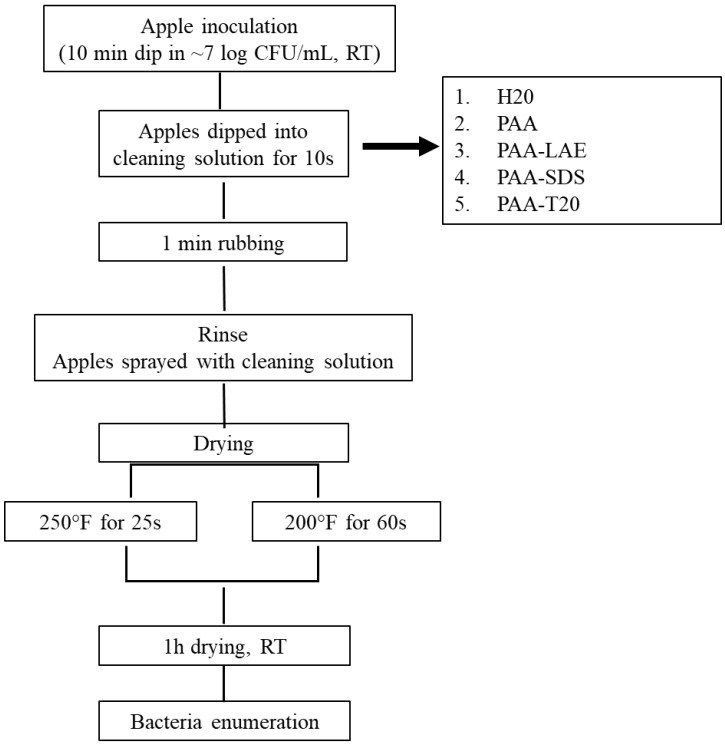
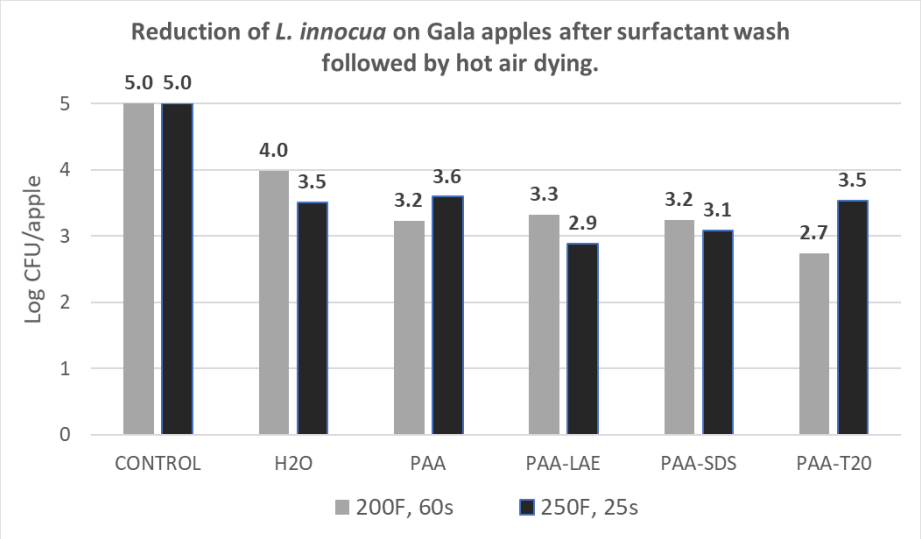


Figure 1. Cleaning treatments procedures.

RESULTS & DISCUSSION

- Use of surfactant helped to reduce bacterial count (Figure 2). Maximum log reduction was observed when apples were treated with peracetic acid combined with Tween 20 surfactant and dried for 60s at 200°F.



- Additionally, application of surfactants resulted in enhanced drying of the solutions from surface of the apples, when compared with water and peracetic acid. Apples were almost dry even when the time of drying was only 25s.
- Applied treatments did not cause damage of the apples, such as heat burn. We measured the temperature of the apple surface immediately after drying and it did not exceed 104°F. This temperature can be normally observed during sunny summer days.

CONCLUSIONS FROM THE STUDIES SO FAR

- The morphology of the apple skin/peel, specifically including the microcracks play a bigger role in harboring the microbes.
- Application of surfactants combined with sanitizer followed by drying with hot temperature air helped to decrease bacterial count on apple surface. However, bacteria are attached to the microcracks in stem bowl cavity which may protect them from contact with cleaning solution for time long .
- 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.

CONTINUING PROJECT REPORT **YEAR**: 2 of 2
WTFRC Project: AP-17-104 No Cost Extension

PROJECT TITLE: Complying with the FMSA preventive controls for human food rule

PI: Girish M. Ganjyal
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City/State/Zip: Pullman, WA, 99164

Ph.D. Student: Ms. Ewa Pietrysiak

COOPERATORS: Claudia Coles (WSDA), Ines Hanrahan (WTFRC) and Various Packing Houses.

Budget: **Year 1:** $48,711 **Year 2:** $50,260

Other funding sources: The PI has some funds from the WSDA-SCBG program to support the one training on the PSFA-PCHF one day training. The event coordinator's (Cathy Blood) time will be covered through the WSDA grant.

Budget 1
Organization Name: WSU **Contract Administrator:** Katy Roberts
Telephone: 509-335-2885 **Email address:** arcgrants@wsu.edu

Item	2017	2018	2019
Salaries	28,418	29,555	
Benefits	2,580	2,683	
Wages	7,261	7,552	
Benefits	452	470	
Training Materials	5,000	5,000	
Supplies	2,000	2,000	
Travel	3,000	3,000	
Miscellaneous	$0	$0	
Plot Fees	$0	$0	
Total	48,711	50,260	**0**

Footnotes:
The majority of the funding requested is to cover graduate student wages. Funds are also requested for wages to support an undergraduate student to help with the project. Funds are requested to cover travel costs related to the project work, such as trips to the packing facilities in Wenatchee and Yakima for the work related to first objective and for the trainings. Funds are also requested to cover supplies and training material costs that will be provided to the training attendees.

RECAP OF ORIGINAL OBJECTIVES

In this project, we proposed to conduct a thorough assessment of a range of apple packing lines and identify the common practices that can be improved. These assessments were further to be utilized to develop model food safety plan for the apple packing process.

The specific objectives of the proposal are as detailed below:

4) Develop a thorough assessment of the current apple and pear packing process and packing house environments.
5) Develop model food safety plans, for both apple and pear packing processes, to help comply with the FSMA-PCHF rule.
6) Summarize the peer reviewed literature available on the different technology interventions that are currently used in different produce industries.
7) Offer one, 2.5-day training, on the FSMA-PCHF rule with an emphasis on apple and pear packing process, and two additional, 1-day trainings, focused on the implementation of the FSMA-PCHF rule.

We have completed about 80% of the work on this project. The model food safety plan was developed and shared with industry and the review on food safety interventions was submitted to peer review journal. One of the last objectives was the offering of two specially developed PCHF training for apple packing houses. We have completed one of these trainings, with tremendous success and feedback. We will be offering one more (potentially two) during 2019 (spring). We will provide full report on this project by fall 2019.

SIGNIFICANT FINDINGS

<u>The following were the major findings from the 2nd year of work:</u>

<u>Assessment of current packing facility practices:</u>

- Most of apple packing houses fall under the FSMA Product Safety Rule; however, the customers often expect them to comply with Preventive Control for Human Food Rule which requires development and implementation of the food safety plan.
- The food safety practices vary significantly across the industry.
- The cleaning and sanitation of the packing line is of crucial importance, however, is often difficult because of insufficient amount of time and problems with rotation of employees.
- Apple packing houses across the WA State are very motivated to improve food safety in their facilities. The biggest challenges identified by food safety managers were;
    - Design of facility and equipment.
    - Limited time for cleaning and sanitizing due to high production rate.
    - The availability of water and the amount of water that would be used to conduct a proper sanitation of the flume piping and pump systems.
    - Restricted capacities of waste water allowed for municipal sewage system.
    - Budget limitation.
    - Personnel unawareness and high turnover.

<u>Food safety model plan development</u>:

- We have completed the model food safety plan for a typical apple packing house.
- The observations of the current practices from the first objective were incorporated into this plan.
- We presented this plan at the Hort Show in December in Pasco, WA.
- We have also shared this plan with the attendees of our first 1-day special FSMA-PCHF class on apple packing houses.

Summary of literature on different interventions

- This objective aimed to facilitate implementation of the new FSMA regulation in apple packing facilities. Current FSMA-PCHF regulations require the interventions in food safety to be based on scientific data. It is essential for the apple packing industry to find appropriate peer reviewed literature to support the use of these technologies.
- This review will provide the fresh apple packing industry with peer reviewed literature on the effectiveness of these technologies. This will serve as useful information for the apple packers as they make decisions on the use of the different interventions and will also be useful for developing the food safety plans.
- Additionally, supplementary information such as the possible routes of produce contamination, bacteria attachment, bacteria resistance mechanisms, and the mode of action of the common decontamination agents has been described in this review manuscript.
- Manuscript titled, "*Food Safety Interventions to Control Listeria Monocytogenes in Fresh Apple Packing Industry: A Review"*, has been submitted for publication to a peer reviewed journal.

Trainings:

- As a part of the WSDA grant, we did offer one 2.5-day FSMA-PCHF class at a highly subsidized rate in the Tricities area during the year 2017.
- This class was at full capacity of 30 people and was well received. We utilized the examples of the fresh produce industries to tailor this course more towards the apple packing house.
- In the year 2018, we developed the 1-day special edition apple packing house FSMA-PCHF training. This training was directed towards the attendees, who would have already completed the standard 2.5 days training from the FSPCA.
- The first, 1-day class, was organized on November 2nd, in Yakima, WA. We had 33 attendees in this class. The class was very well received, based on the feedback we got from the attendees.
- We will be offering one more of these 1-day classes in the year 2019.

Overall, we are on track to complete all the proposed project objectives. We have requested a 1-year no-cost extension for this project, to finish up the remaining parts of the proposed objectives. This mainly includes the one, 1-day training, that will be offered in the year 2019 and finishing up the review article on the interventions used in the fresh produce industry to control *Listeria*. As we are working on this project, we are discovering new things that may help the industry in addressing the food safety concerns. We hope to report all our findings in early 2019 in the final report.

METHODS

Objective #1: Develop a thorough assessment of the current apple and pear packing process and packing house environments.

The thorough assessment of the current apple and pear packing process was done by visiting many packing lines and having detailed discussions and completing the detailed survey on food safety practices by the experts in the different packing houses.

Importance was given to each and every step of the packing process during this thorough assessment process. For each of the steps, best practices were documented. The information gathered in this assessment were the base for developing model food safety plan. They allowed to

create a detailed flow chart with description for each step in the process, perform hazard analysis, and indicate best practices for each of the steps.

Objective #2: Develop a model food safety plan for apple and pear packing processes to help comply with the FSMA Preventive Controls for Human Food Rule.

- The model food safety plan for apple packing house has been completed.
- This was circulated within the network of packing houses, through the WTFRC for comments.
- This was also submitted to the FSPCA for approval. The FSPCA gave us the technical feedback on it, but they are holding off on the idea of publishing it as a model plan, as they said there are still in discussions on whether the FDA will officially put apple packing houses under PCHF or not.
- We have finalized the model food safety plan and shared with the WTFRC and also with the attendees in our first 1-day apple packing house special editions FSMA-PCHF training.
- This model food safety plan model can help all the packing houses to create or modify food safety plan and meet new regulation requirements and their customer requirements.

Objective #3: Summarize the peer reviewed literature available on the different technology interventions that are currently used in different produce industries.

- Literature review on mode of action and effectiveness of technologies used in fresh produce industry was conducted.
- Reviewed literature was discussed and presented in form of table (Figure 1).
- More than 50 scientific publications were reviewed.
- Furthermore, supplementary information such as the possible routes of produce contamination, bacteria attachment, bacteria resistance mechanisms, and the mode of action of the common decontamination agents were described (Figure 2).

Table 1. Methods for reduction *L. monocytogenes* in fresh produce.

Method	Conditions	Target	Log count reduction	Impact on produce quality	Reference
Chemical					
Chlorine	200 ppm, 5min	Apples	0.6	NA	Beuchat et al. (1998)
		Tomatoes	1.0	Not analyzed	
	100 or 200 ppm, 5 min	Apples	> 5	Triangle test (n=50) showed significant difference ($P < 0.05$) in sensory perception after treatment with 200 ppm	Rodgers et al. (2004)
		Cantaloupes	> 5	Not analyzed	
		Strawberries	> 5	Triangle test (n=50) showed no difference in sensory perception after both treatments	
	200 ppm, 2 min	Green coconuts	2.7	NA	Walter, Nascimento and Kuaye (2009)
	150 ppm, 2 min	Apples	1.8*	NA	Kim et al. (2018)
	100 ppm, 5 min	Cantaloupes	1.9	NA	Singh, Hung and Qi (2018)
	100 ppm, 5 min	Romain lettuce	1.7	NA	
ClO_2	3 ppm, 30 min	Green peppers	> 6	NA	Han et al. (2001)

Figure 1. Table (in part) with summary of literature review findings. (Pietrysiak et al., in review).

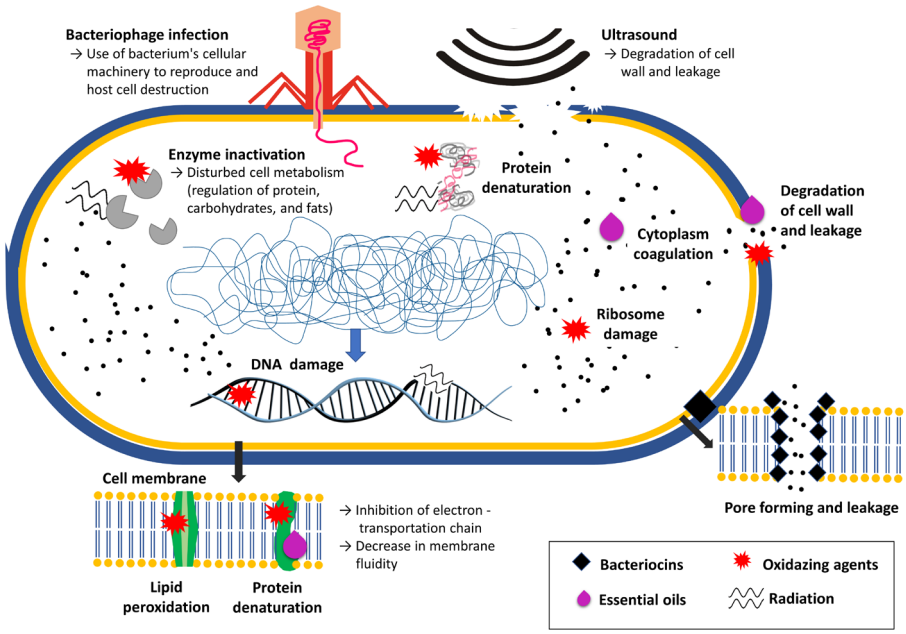

Figure 3. Mechanism of bacteria inactivation by various antimicrobial agents (Pietrysiak et al., in review).

Objective#4: Offer one, 2.5-day training, on the FSMA-PCHF rule with an emphasis on apple and pear packing process, and two additional, 1-day trainings, focused on the implementation of the FSMA-PCHF rule.

We offered 1-day training on implementation of the FSMA-PCHF rule with an emphasis on apple packing process on November 2nd in Yakima. This 1-day training has been designed specifically for the attendees who have gone through the FSPCA standard Preventive Controls for Human Food (PCHF) Course. During the training we shared with participants food safety plan model, draft of literature review and presentation slides.

RESULTS & DISCUSSION

Objective #1: Develop a thorough assessment of the current apple and pear packing process and packing house environments.

- The cleaning and sanitation of the packing line is of crucial importance, however, is often difficult because of insufficient amount of time and problems with rotation of employees.
- Visited apple packing houses across the WA State are very motivated to improve food safety in their facilities. The biggest challenges identified by food safety managers were:
    - Design of facility and equipment.
    - Limited time for cleaning and sanitizing due to high production rate.
    - The availability of water and the amount of water that would be used to conduct a proper sanitation of the flume piping and pump systems.
    - Restricted capacities of waste water allowed for municipal sewage system.
    - Budget limitation.
    - Personnel unawareness and high turnover.

Objective #2: Develop a model food safety plan for apple and pear packing processes to help comply with the FSMA Preventive Controls for Human Food Rule.

- The model food safety plan consists on hazard analysis for each step of apple packing process.
- We identified the appropriate preventive controls for managing the hazards.
- Majority of the hazards can be addressed by GMPs and sanitation preventive controls.
- We do not recommend including process preventive control, because of inability to perform validation study. Food safety system in apple packing facility should be focused on controlling and reducing risk of fruit contamination.

Objective #3: Summarize the peer reviewed literature available on the different technology interventions that are currently used in different produce industries.

- *L. monocytogenes* is a persistent, highly pathogenic microorganism which can pose a high risk in fresh produce operations. Abundant amounts of water used during the apple packing process, and the presence of wet surfaces and difficult to clean equipment, provide ideal conditions for Listeria growth and numerous paths for produce contamination.
- Removal of bacteria from surface of the apple is difficult due to its morphology. The irregular shape of apples, and the presence of microstructures on the apple peel surface, provide ideal conditions for bacterial attachment. Bacteria harbored in the microstructures may be protected from cleaning interventions.
- Attacking bacteria by several different mechanisms through hurdle technology may help to improve the apple decontamination efficiency.
- Significant research is still needed for the development of effective strategies of reducing microbial loads on fresh apples. Critical aspects that should be considered include morphological characteristic of apples, conditions and scale of the packing process, and influence of the interventions on apple quality.

Objective #4: Offer one, 2.5-day training, on the FSMA-PCHF rule with an emphasis on apple and pear packing process, and two additional, 1-day trainings, focused on the implementation of the FSMA-PCHF rule.

- The one 2.5 day training as a part of the WSDA-SCBG was completed in 2017.
- We developed the, special, 1-day training, for the FSMA-PCHF, directed towards the apple packing houses.
- We offered the first class on November 2nd, 2018 in Yakima, WA, with 33 attendees.
- The training was well received with great feedback from them.
- During the training, we also assisted some of the packers with their food safety plans.
- Many questions were answered during this training.
- We will be offering one more of this training with openings for 50 attendees in the year 2019, before wrapping up the project.

CONCLUSIONS FROM THE STUDIES SO FAR

- The biggest challenges identified by food safety specialists are difficult (or impossible) to clean equipment, very limited time for cleaning and sanitizing, use and treatment of water, budget limitation, and personnel unawareness and high turnover due to high production rate.
- Based on (i) visits to various facilities; (ii) survey outcomes; (iii) scientific literature review; (iv) comments from industry and FSPCA the food safety plan model was finalized.
- The apple peel surface morphology play a significant role in decreasing decontamination treatments efficiency.
- More research is needed to develop optimum method for apple decontamination.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: AP-17-102

Project Title: Control of *Listeria* on processing surfaces in apple packing facilities

PI: Meijun Zhu
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City/State/Zip: Pullman/WA/99164

Co-PI: Ines Hanrahan
Organization: WTFRC
Telephone: 509-669-0267
Email: hanrahan@treefruitresearch.com
Address: 2403 S. 18th St., Suite 100
City/State/Zip: Yakima, WA 98903

Cooperators: Stemilt Growers LLC.; McDougall & Sons; Hansen Fruit; Washington Fruit; Allan Bros Fruit; Josh Tucker, Pace International; Guardian Manufacturing, Inc.

Budget: **Year 1**: 98,447 **Year 2**: 101,752 **Year 3**: **105,882**

Other funding sources None

WTFRC collaborative expenses:

Item	2017	2018	**2019**
Salaries	$1,573	$2,172	$2,172
Benefits	$1,049	$1,305	$1,305
Wages	$2,750	$2,750	$2,750
Benefits	$825	$825	$825
Total	$6,197	$7,052	**$7,052**

Footnotes: Dr. Hanrahan is committing to spend 2%/year of her time on this project. Mendoza will supervise Wenatchee based team members (0.5% of her time in year 1, 2% in years 2&3). Timeslip wages are calculated at $11/hr. plus benefits for 250 hours/year.

Budget 1: Meijun Zhu
Organization Name: WSU-Pullman **Contract Administrator:** Katy Roberts
Telephone: (509) 335-2885 **Email address:** arcgrants@wsu.edu

Item	2017	2018	**2019**
Salaries[1]	$13,562	$19,889	$20,685
Benefits[1]	$4,386	$6,094	$6,338
Wages[2]	$38,054	$30,773	$32,003
Benefits[2]	$3,248	$3,300	$3,432
Supplies[3]	$26,000	$26,644	$27,872
Travel[4]	$2,000	$3,000	$3,500
Miscellaneous[5]	$5,000	$5,000	$5,000
Total	$92,250	$94,700	**$98,830**

Footnotes:
[1]Postdoc research associate and professor's salaries plus benefits.
[2]PhD graduate student stipends and undergraduate assistant wages plus benefits.
[3]Bacteria culture media, reagents and consumable supply cost
[4]Travel funds for industrial sampling and experiments.
[5]Funds are requested to partially cover the Biosafety Level 2 facility and equipment maintaining fees.

OBJECTIVES

1. Assess the antimicrobial efficacy of ozonated water against *L. monocytogenes* biofilm on different surfaces, which will be further comprehensively compared with commonly used sanitizers, quaternary ammonium compounds, chlorine dioxide and peroxyacetic acid.
2. Examine the antimicrobial efficacy of steam alone and in combination with selected sanitizers against *L. monocytogenes* biofilm on different surface materials.
3. Validate antimicrobial efficacy of superheated steam and/or selected sanitizers in apple packing lines using parameters selected based on laboratory testing.

PRELIMINARY DATA AND SIGNIFICANT FINDINGS

1. Different *L. monocytogenes* strains have a different biofilm formation ability.
2. In general, the 2-day-old single strain *L. monocytogenes* biofilm formed on polystyrene surface was susceptible to all tested sanitizers. For each tested sanitizer, increasing concentration significantly enhanced their efficacy.
   - Ozonated water for 1-min at 2.0 and 4.0 ppm resulted in 3.4 and 4.1 log reduction.
   - QAC 1-min intervention at 200 and 400 ppm resulted in 3.2 and 3.6 log reduction.
   - Chlorine 1-min intervention at 100/200 ppm and chlorine dioxide at 2.5/5.0 ppm resulted in ~2.0/3.1 and ~2.4/3.8 log reduction.
   - PAA 1-min treatment at 80/160 ppm achieved ~3.6/4.8 log reduction.
3. With the exception of PAA, efficacy of all tested sanitizers against *L. monocytogenes* mixed strain biofilm on polystyrene surface was reduced when compared to single strain *L. monocytogenes* biofilm.
4. Efficacies of all tested sanitizers against 7-day-old biofilm were reduced when compared 2-day-old biofilm. PAA was relatively less influenced by age of biofilm.
5. Biofilm removing ability of all tested sanitizers decreased dramatically against aged biofilm on surface with organic matter. Antimicrobial efficacy of PAA was less impacted compared with other sanitizers. PAA at 160ppm and 1min contact still resulted in ~3 log reduction against 7-day-old biofilm on polystyrene surface conditioned with organic matter.
6. PAA had a similar efficacy against biofilm formed on different food contact surfaces, including stainless steel, polyvinyl chloride (PVC), polyester (PET), low density polyethylene (LDPE) and rubber surface.
7. Organic matter impacted efficacy of PAA against *L. monocytogenes* biofilm on all tested food contact surfaces at similar degree.

METHODS

We have established methods for proposed Objective 1 studies as detailed in the following.

Objective 1: Assess the antimicrobial efficacy of ozonated water against *L. monocytogenes* biofilm on different surfaces, which will be further comprehensively compared with commonly used sanitizers, quaternary ammonium compounds, chlorine dioxide or peracetic acid.

1. Strain selection

To elucidate the impact of strain variability on biofilm formation and sanitizer's antimicrobial efficacy, six strains of *L. monocytogenes* were evaluated. These *L. monocytogenes* strains were either outbreak strains or processing plant/food isolates. They have been kept at -80°C until used.

2. Selection and preparation of food contacting surfaces

Stainless steel, PVC, PET and PE along with polystyrene were selected representing most commonly used surface materials. The selected surface sheet was cut into coupons for *Listeria* biofilm growth. Polystyrene biofilm was conducted in sterile 96-well plates.

Organic matter conditioning: The above surfaces were cleaned and exposed with 1:10 diluted apple juice or whole milk prior to be subjected to *Listeria* biofilm growth and sanitizer treatments.

3. *Listeria* biofilm formation on different surface materials

Inoculum preparation: Before inoculation, respective strains were twice activated in TSBYE broth, washed and re-suspended in Modified Welshimer's Broth (MWB) broth to achieve the target population density.

Biofilm formation on polystyrene surface in 96 well plate: A100 µl of the above prepared cultures were transferred into each well of 96 well microtiter plate. The plates were covered and incubated at room temperature (22°C/72°F) for 2 or 7 days statically to form biofilm using our established method, 6 wells per sanitizer treatment of each experimental day.

Biofilm formation on different surfaces: All four surface coupons (conditioned with/without organic matter) will be transferred to *Listeria* suspension in MWB broth prepared as described above and incubated at room temperature (22°C/72°F) for 2 or 7 days statically to form biofilm.

4. Sanitizer intervention against *Listeria* biofilm on different surfaces.

Sanitizer solution concentration: Ozonated water was used at 2.0 and 4.0 ppm, representing commonly used levels practiced in apple packing lines. Quaternary ammonium compound (QAC), chlorine dioxide and peroxyacetic acid (PAA) are commonly used for surface antimicrobial intervention. QAC (Stop It, Pace International) was prepared with water at a concentration of 200 and 400 ppm. PAA (Shield-Brite PPA 12.0, Pace International) was evaluated at 80, 160 and 200 ppm. Chlorine (Accu-Tab, Pace International) was used at 100 and 200 ppm, pH6.8. Chlorine dioxide was generated on site and used at 2.5 and 5.0 ppm.

Antimicrobial intervention: Wells of polystyrene plates or coupons of the selected surface bearing *Listeria* biofilm cells were rinsed with sterile distilled water, then subjected to respective sanitizer treatments at appropriate concentrations for 1 or 5 min. Untreated control wells with biofilm were subjected to distilled water instead of sanitizer solution treatments.

5. Microbiological analysis.

Detachment of biofilm from surface: Wells or coupons subjected sanitizer treatments were rinsed with Dey/Engley (D/E) neutralizer broth once, subsequently, sterile water. Then biofilm of respective surfaces was detached from surface into sterile PBS by sonication or vigorous vortexing. Control wells/coupons were treated with sterile water instead of sanitizer solutions.

Bacterial enumeration: The detached cell suspensions were serially diluted in sterile PBS and plated in duplicate Tryptic Soy Agar (TSA) with yeast extract (TSAYE) agar plates. Colonies that had formed on the plates were counted after 48 h of incubation at 37°C (98°F).

Objective 2: Examine the antimicrobial efficacy of steam alone and in combination with selected sanitizers against *L. monocytogenes* biofilm on different surface materials

Biofilm grown on different surface, intervention, detachment and enumeration will be conducted as described in Objective 1 studies. The Objective 1 outcomes will guide standardization of sanitizer

concentration in relation to the variable residence time. To mimic the harshest condition, 7-day-old biofilm grown on different surfaces will be used in Objective 2 study.

For steam treatment, different surface coupon bearing 7-day-old biofilm will be subjected 100-160 °C (212 – 320 °F) for 5-50 seconds at a distance of 20-60 mm.

Objective 3: Validate antimicrobial efficacy of steam treatment and/or selected sanitizers in apple packing lines using parameters selected based on laboratory testing

Methods developed in Objective 1 and 2 studies will be used for Objective 3 studies. The outcomes of Objective 1 & 2 studies will guide standardization of sanitizer concentrations in relation to the variable residence time. Additional methods will be updated in year 3.

RESULTS AND DISCUSSION

1. Antimicrobial efficacy of selected sanitizers against single strain *L. monocytogenes* biofilm

We further compared the biofilm formation ability among the six *L. monocytogenes* strains. There was no clear link between biofilm formation and the serotype of the selected strains (Fig. 1C). NRRL B-33385, a 4b human clinic isolate had the lowest population density in the biofilm, while the *L. monocytogenes* environmental isolate (NRRL B-33466) showed the highest biofilm forming ability among all strains tested (Figure 1).

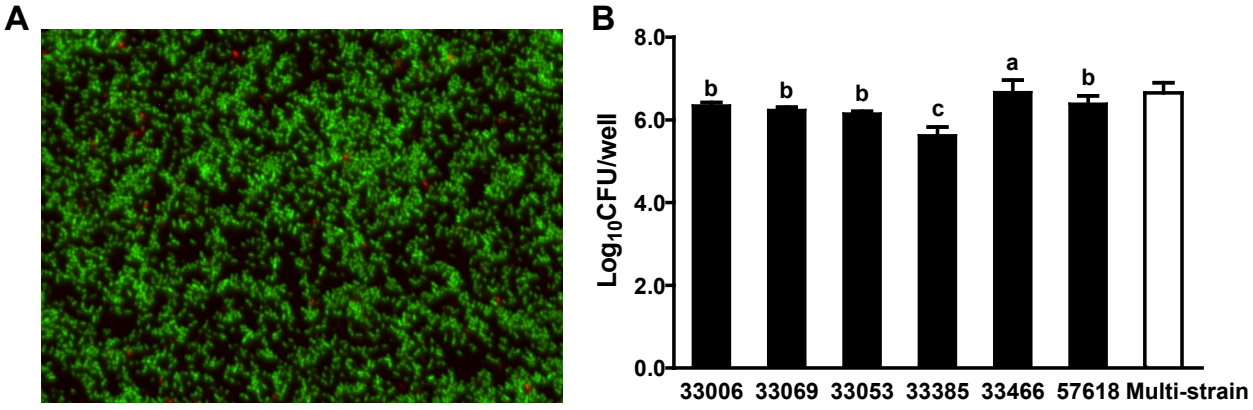

Figure 1. Biofilm forming ability of different *L. monocytogenes* strains on Polystyrene surface. A: BacLight Live/Dead staining of *Lm* biofilm grown in MWB; B: Enumeration of biofilm of different *Lm* strains. Mean ± SEM. Bars topped with same letter are not different at $P < 0.05$.

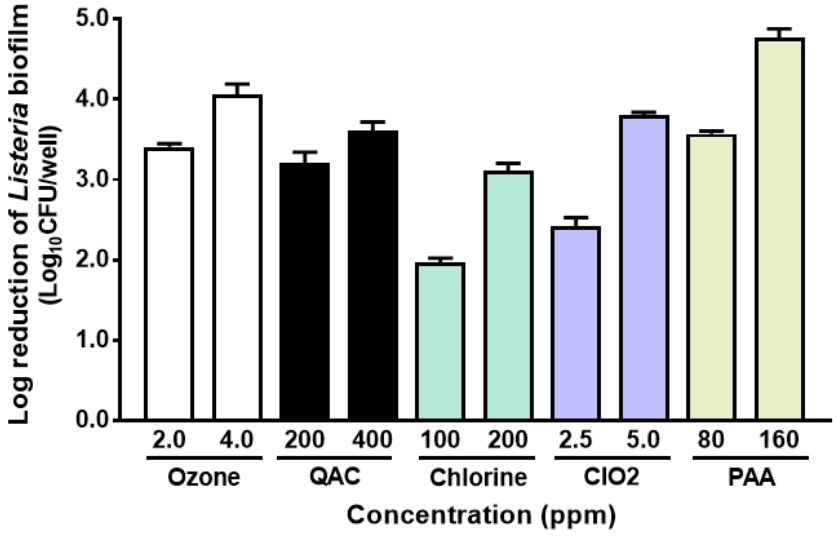

Figure 2. Antimicrobial efficacy of selected sanitizer intervention against 2-day-old *L. monocytogenes* single strain biofilm. Ozone: Ozonated water; QAC: Quaternary ammonium compounds; ClO2: Chlorine dioxide; PAA: Peroxyacetic acid. Mean ± SEM, n=3.

In general, the 2-day-old single strain *L. monocytogenes* biofilm was susceptible to all tested sanitizers (Figure 2). Treatment with ozonated water for 1-min at 2.0 and 4.0 ppm resulted in a 3.4 and 4.1 log reduction of single strain biofilm grown on polystyrene surfaces, respectively (Figure 2). QAC 1-min intervention at 200 and 400 ppm resulted in a 3.2 and 3.6 log reduction of biofilm, respectively. Chlorine 1-min intervention at 100/200 ppm and chlorine dioxide at 2.5/5.0 ppm resulted in ~2.0/3.1 and ~2.4/3.8 log reduction of *L. monocytogenes* (Figure 2). PAA 1-min treatment at 80 and 160 ppm achieved ~3.6 and 4.8 log reduction of *L. monocytogenes* in single strain biofilm, respectively. For each tested sanitizer, increasing concentration significantly enhanced their efficacy (Figure 2).

2. Antimicrobial efficacy of selected sanitizers against mixed strain *L. monocytogenes* biofilm

Antimicrobial efficacies of all sanitizers except PAA against mixed strain *L. monocytogenes* biofilm were reduced when compared to single strain *L. monocytogenes* biofilm (Figure 3). Furthermore, antimicrobial efficacies of all sanitizers against 7-day-old biofilm were reduced when compared to 2-day-old biofilm; antimicrobial efficacy of PAA was relatively less affected by age of bioflim (Figure 3). Organic matter of apple origin conditioning dramatically influenced efficacy of all sanitizers against biofilm on polystyrene plates (Figure 4). Compared with other sanitizers tested, efficacy of PAA was least impacted. PAA 1-min treatment at 160ppm resulted in ~ 3 log reduction against 7-day-old biofilm conditioned with organic matter; further increase of PAA concentration seemed not quite effective (Figure 4).

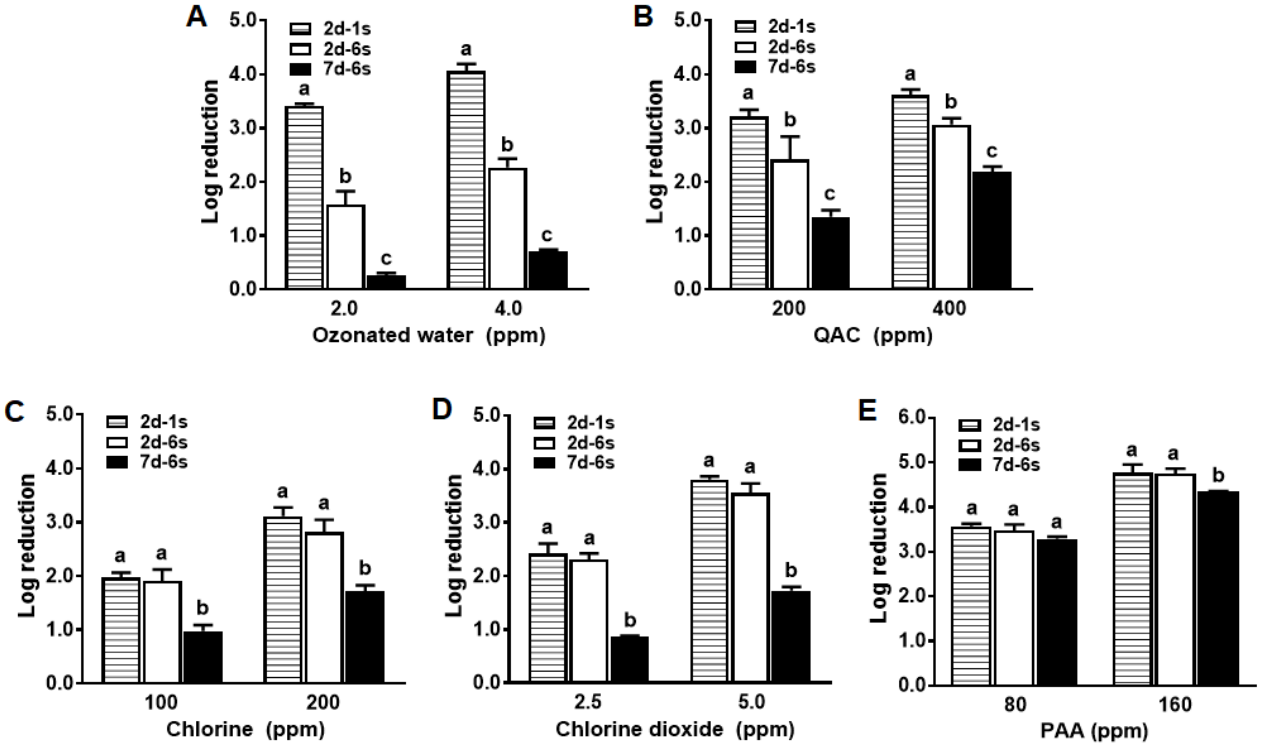

Figure 3. Antimicrobial efficacy of selected sanitizer intervention against biofilm of *Listeria monocytogenes* mixed strains. 2d, 2-day-old biofilm; 7d, 7-day-old biofilm. A: Ozonated water; B: QAC; C: Chlorine; D: Chlorine dioxide; E: PAA. Mean ± SEM, n=3.

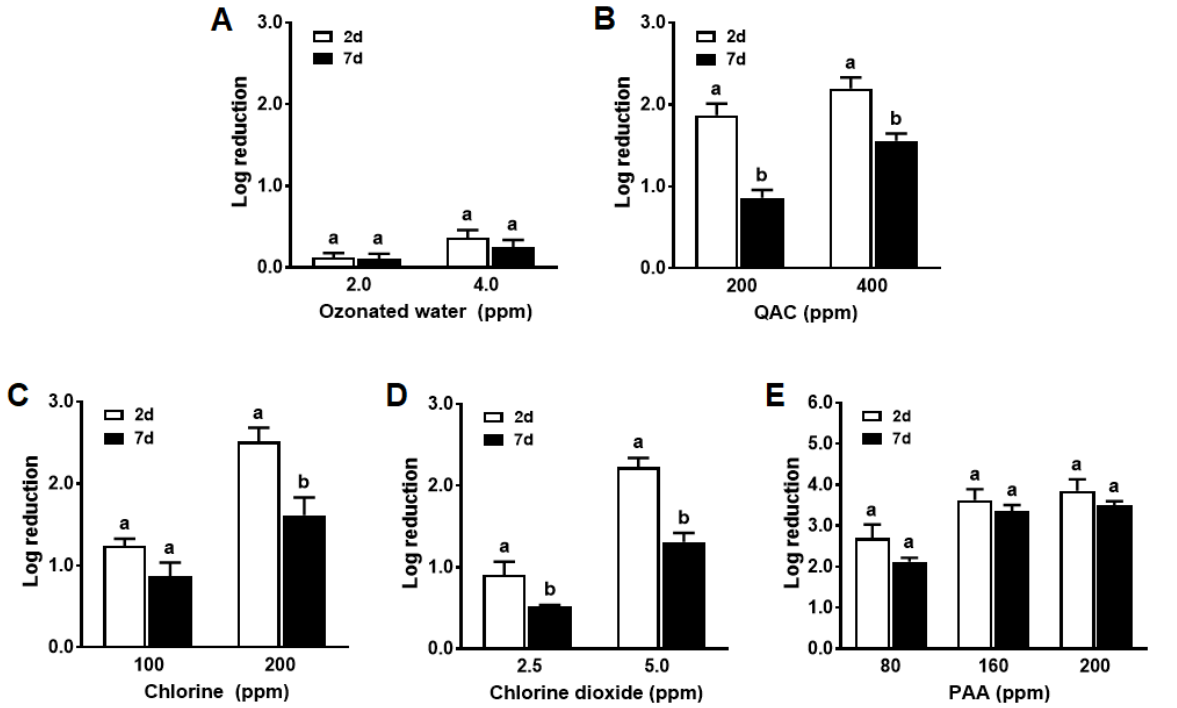

Figure 4. Efficacy of selected sanitizer intervention against mixed strain *L. monocytogenes* biofilm in the presence of organic matter. 2d: 2-day-old biofilm; 7d: 7-day-old biofilm. A: Ozonated water; B: Chlorine; C: Chlorine dioxide; D: QAC; E: PAA. Mean ± SEM, n=3.

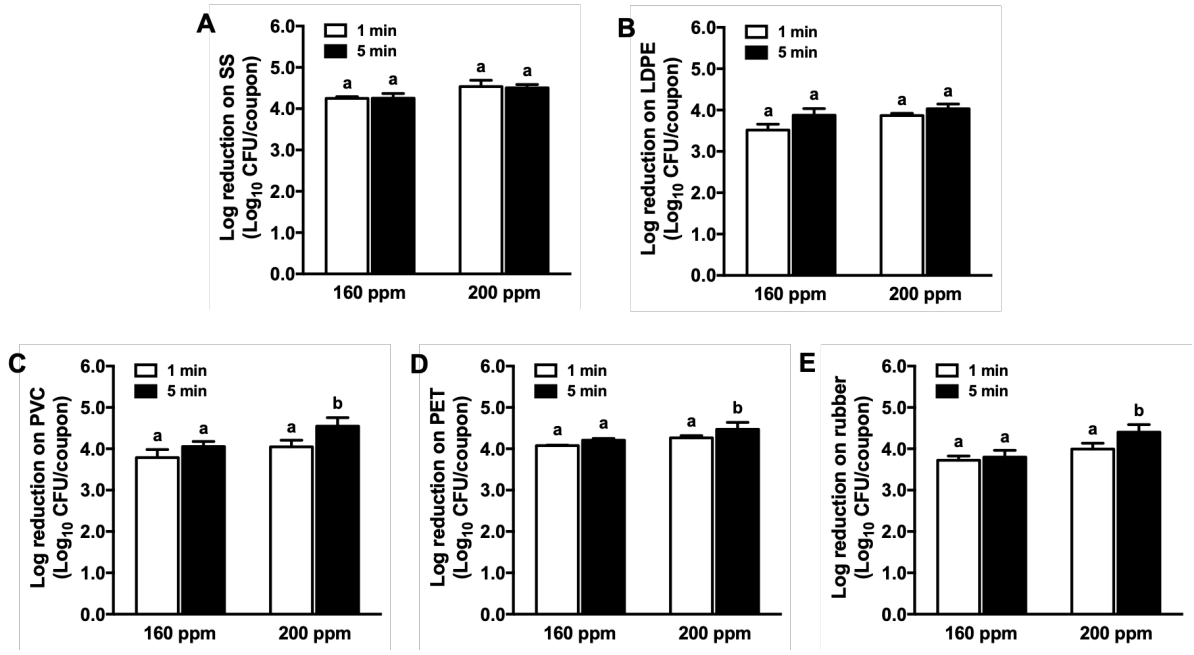

Figure 5. Efficacy of peroxyacetic acid (PAA) against 7-day-old *L. monocytogenes* mixed strain biofilm on clean food-contact surfaces. (A) SS: stainless steel; (B) LDPE: low-density polyethylene; (C) PVC: polyvinyl chloride; (D) PET: polyethylene terephthalate; (E) Rubber. Each surface was treated with sanitizers for 1 or 5 min at 22°C. Means ± SEMs, n=3. [a,b] Bars topped with the same letter are not significantly different at $P \leq 0.05$.

3. Efficacy of PAA against 7-day-old *L. monocytogenes* mixed strain biofilm on different food contact surface.

PAA at 160 – 200 ppm and 1 or 5 min contact time had a similar efficacy against 7-day-old *L. monocytogenes* mixed strain biofilm formed on different food contact surfaces, including stainless steel, PVC, PET, LDPE and rubber surfaces (Figure 5). Organic matter impacts efficacy of PAA against *L. monocytogenes* biofilm on different food contact surface at a similar degree (Figure 6).

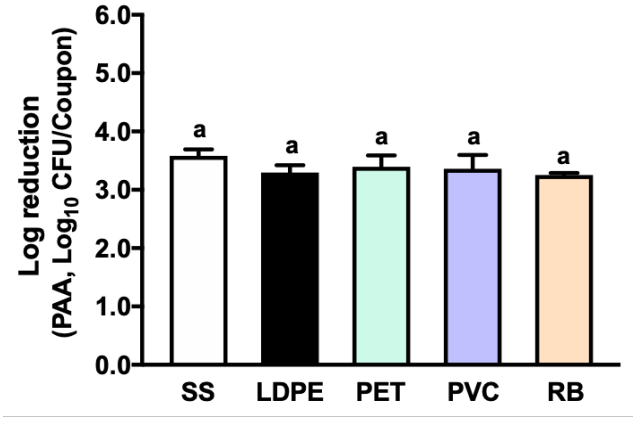

Figure 6. Efficacy of 160ppm PAA against 7-day-old *L. monocytogenes* mixed strain biofilm on food-contact surfaces conditioned with apple juice. (A) SS: stainless steel; (B) LDPE: low-density polyethylene; (C) PVC: polyvinyl chloride; (D) PET: polyethylene terephthalate; (E) Rubber. Each surface was treated with sanitizers for 1 or 5 min at 22°C. Means ± SEMs, n=3. [a,b] Bars topped with the same letter are not significantly different at $P \leq 0.05$.

CONCLUSIONS

Eradication of *L. monocytogenes* biofilm in the processing surface is challenging. Antimicrobial efficacies of sanitizers against *L. monocytogenes* biofilm were dramatically impacted by biofilm stage, strains present and cleaniess of surfaces. Data up to now indicate that PAA is less impacted by biofilm age and the presence of organic matter on surface, has a similar efficacy against *L. monocytogenes* biofilm on different food contact surfaces. More studies are ongoing to evaluate efficacies of the selected sanitizers in combination with heat treatment against biofilms formed on different food contact surfaces.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project: AP-18-101

Project Title: Systems-based approach for improved packinghouse sanitation

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Cooperators: Washington apple packinghouses and Jacqui Gordon (WSTFA)

Total Project Request: **Year 1:** 67,369 **Year 2: 71,399** **Year 3:** 58,209

Other funding sources

In kind support: donations for sanitizers, detergents, and food contact surfaces will be sought to defray the cost of this research.

WTFRC Budget:

Item	2018	**2019**
Salaries	4,050	4,131
Benefits	1,337	1,363
Wages	4,500	4,703
Benefits	1,485	1,552
RCA Room Rental		
Shipping		
Supplies		
Travel	500	500
Plot Fees		
Miscellaneous		
Total	11,872	**12,249**

Footnotes:

Salaries/Benefits: estimate of percent of time spent for Mendoza (5%) and Hanrahan (1%), a 33% benefit rate and 2% annual increases.

Wages/Benefits: calculated based on expected staff wage adjustments proportional to the WA state minimum wage increases (2018=$11.50, 2019=$12.00), approx. 350 hours

Travel: in state travel for Hanrahan (lodging in Wenatchee)

Budget 1
Organization Name: Washington State University
Contract Administrator: Katy Roberts/Karen Kniep Blanton
Telephone: (509) 335-2885/(509)786-9285
Email address: arcgrants@wsu.edu/prosser.grants@wsu.edu

Item	2018	**2019**	2020
Salaries	26,274	27,509	28,807
Benefits	2,373	2,468	2,566
Wages	6,000	8,112	5,192
Benefits	600	811	519
Equipment			
Supplies	19,250	19,250	21,125
Travel	1,000	1,000	
Miscellaneous			
Plot Fees			
Total	55,497	**59,150**	58,209

Footnotes: Salaries: $26,274, $27,509, and $28,807 is requested in years 1, 2 and 3 , respectively, for a Graduate Research Assistantship for a PhD student to work on all objectives.

Benefits: $2,373, $2,468, and $2,566 is requested in years 1, 2 and 3, respectively, for benefits tied to the Graduate Research Assistantship for a PhD student to work on all objectives.

Wages: $6,000 in year 1, $8,112 in year 2 and $5,192 in year three are requested for hourly wages for student employee to conduct experiments as relating to the surface characteristics of the different types of materials used on packing lines from an engineering point of view.

Benefits: $600 in year 1, $811in year 2 and $519 in year three are requested for benefits of the student employee.

Supplies: Supply costs of $19,250 in year 1, 19,250 in year 2 and 21,125 in year 3 are requested to purchase disposable supplies such as glassware, microbiological media, Petri dishes, pipettes, and PCR reagents tied to objectives 1 and 3.

Travel: $1,000 is requested in years 1 and 2 for mileage and associated travel costs at a rate of $0.535/mi and adhering to all university policies for per diem associated with overnight travel.

Objectives

1. Identify harborage points and niches for *Listeria monocytogenes* indicator organism (*Listeria* spp.) on food contact surfaces in produce packinghouses.
2. Rank surfaces based upon prevalence of indicator organisms to identify material types and design features with the greatest likelihood of harborage.
3. Evaluate standard design features from a microbiological and engineering perspective to determine if alternative sanitation practices can compensate for less than ideal hygienic design.

Significant Findings

To this point, four of six facilities have been sampled once. One hundred and fifty-six sites have been sampled in total. *Listeria* spp. have not been isolated from any sites. Forty-four more sampling event will occur throughout 2019 and 2020 before completion of the project.

Methods

Objective 1. Identify harborage points and niches for *Listeria monocytogenes* indicator organism (*Listeria* spp.) on food contact surfaces in produce packinghouses (years 1-3).

Packinghouse selection. Commercial apple packinghouses have been recruited into the study (Figure 1). Six packinghouses have been enlisted into the study and will be sampled once quarterly during packing season for a total of eight data collection points per facility. Random identifiers have been established for all participants in order to ensure complete anonymity. An interview has been conducted with the appropriate personnel to determine cleaning and sanitizing practices utilized within the operation and general management strategies for the packinghouse. Additionally, the interviewer has described the purpose of the study, what data will be collected, and how outcomes will be conveyed to the packinghouse operator. The facility, including the packing line, will be diagramed and sampling points will be pre-determined, with a minimum of 30 and maximum of 50 per each facility. A picture will capture the exact location of each sampling site and will be utilized when conveying results.

Figure 1. *Listeria* species sampling overview of apple packinghouses during 2018 and 2019 harvesting season.

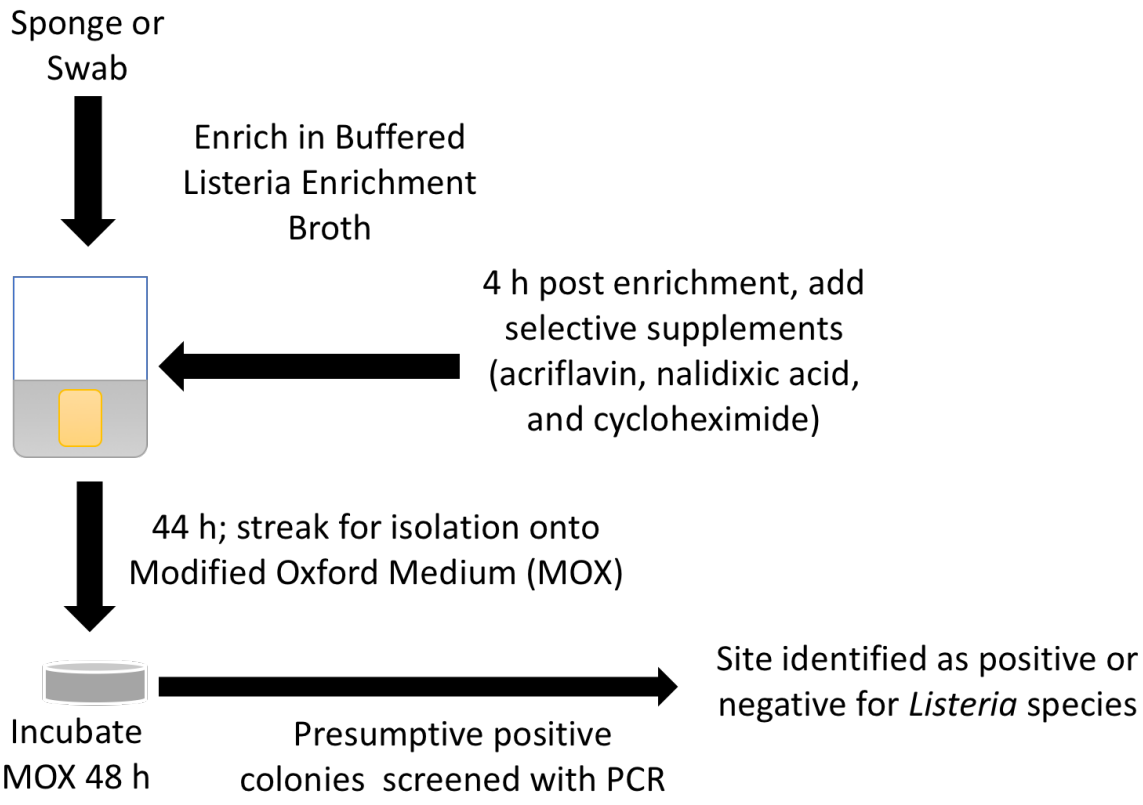
Six apple packinghouses	• Sampled during 2018 and 2019 season

In each packinghouse	• Interview with sanitation crew and observation of sanitation event • Packinghouse will be diagramed, sampling sites determined and pictures taken to record sites • Bimonthly sampling after sanitation • 30 to 50 sites comprised of zone 1 (direct food contact) and zone 2 (adjacent to zone 1) surfaces will be sampled • Each site swabbed to determine presence of *Listeria* species (indicator for *L. monocytogenes*)

Surface sampling methods. Sampling was coordinated to occur after a sanitation event and within 4 hrs of startup to align with current FDA guidance. A pre-moistened sterile sponge is being utilized to sample a 100 cm^2-area or as large a space as is permissible for smaller surfaces.

Isolation of *Listeria* species. Bacteria are eluted in D/E neutralizing buffer, enriched in Buffered Listeria Enrichment Broth (BLEB) with antibiotic supplements, and confirmed through polymerase chain reaction (PCR) targeting the *iap* gene (Figure 2). This approach will identify only *Listeria* sensu strictu as a group (*Listeria* species) and will not identify *Listeria monocytogenes* specifically. Included in this grouping are *L. monocytogenes*, *L. seeligeri*, *L. marthii*, *L. ivanovii*, *L. welshimeri*, and *L. innocua*. In draft guidance for industry for control of *Listeria monocytogenes*, the FDA has recommended testing both food contact surfaces (e.g. zone 1) and non-food contact surfaces (e.g. zones 2-4) for *Listeria* species [1]. Additionally, the FDA acknowledges that a positive test result for *Listeria* spp. on a surface does not establish the presence of *Listeria monocytogenes*, but rather that the conditions are conducive for presence of *Listeria monocytogenes*. By testing for *Listeria* species, we will be able to determine what surfaces are conducive for potential harborage of *Listeria monocytogenes* without jeopardizing the production schedules of partnering packinghouses.

Figure 2**.** Sample processing to determine presence or absence of *Listeria* species (environmental indicator for *L. monocytogenes*).



Statistical analysis. Non-parametric methods will be utilized to analyze the categorical data of presence or absence of *Listeria* spp. recovered based upon the surface type (e.g. stainless steel, painted steel, hard plastic, PVC belting, vinyl-like belting, cloth belting, PVC rollers, brush rollers, and foam rollers), unit operation, and any significant differences between facilities where similar surfaces are found at specific unit operations.

Objective 2. Rank surfaces based upon prevalence of indicator organisms to identify material types and design features with the greatest likelihood of harborage (year 3).

Review for hygienic design features. Outcomes from the statistical analysis in objective 1, combined with pictures of sampling locations and measurements taken from surfaces within packinghouses will be analyzed to evaluate hygienic design features of equipment with significantly more prevalence of *Listeria* spp. on food contact surfaces. Surfaces will be ranked by type and unit operation based upon likelihood of *Listeria* spp. presence.

Objective 3. Evaluate standard design features from a microbiological and engineering perspective to determine if alternative sanitation practices can compensate for less than ideal sanitary design (Year 2-3).

Outcomes from objective one and two will also be used to identify less than ideal standard design features commonly found in packinghouses. These design features will be replicated in the lab, where they can be soiled with microorganisms, and various sanitation practices evaluated to determine if they can mitigate less than ideal hygienic design. This will be extremely beneficial given that the cost of design improvements may be prohibitive in the short-term, but alternate sanitation strategies could prove to be effective.

Selection of surfaces for further evaluation. The research team will collectively identify surfaces from the outcome of objective two which should be further evaluated for alternative sanitation practices. No fewer than six, but no greater than ten surfaces will be evaluated in this objective. The surfaces will be characterized for roughness, contact angle for water drops on the surface and surface morphology using microcopy techniques. Each surface will be purchased new from suppliers and sterilized to remove background microflora prior to inoculation. When possible, used surfaces of the same material will be included also.

Inoculation of surfaces with *Listeria* species. *L. seeligeri*, *L. marthii*, *L. ivanovii*, *L. welshimeri*, and *L. innocua* will be individually grown in Tryptic Soy Broth with Yeast Extract (TSBYE) at 32°C (89.6°F) for 24 h with three successive transfers prior to inoculation of Tryptic Soy Agar (TSA) plates with each individual strain. TSA will be incubated at 32°C (89.6°F) for 24 h to achieve a lawn of each species of *Listeria*. Each plate will be flooded with 10 ml of Buffered Peptone Water (BPW) to harvest cells. Each *Listeria* species will be combined to create a five-species cocktail for inoculation. Surfaces will be spot inoculated with 100 10µL spots.

Incubation of inoculated surfaces. To allow for bacterial attachment, each surface will be incubated in an environmental chamber for 24 h at two temperatures and humidity levels determined from packinghouse conditions observed in objective 1.

Treatment of surfaces. Surfaces will be exposed to three treatments in addition to a no treatment control and one commonly used sanitation protocol that will be determined by the research team based upon outcomes of objective 1 and 2, knowledge of current industry practices, and other research projects also focusing on this topic (e.g. Blakey brush bed sanitation). Example treatments include the use of alternative sanitizers (e.g. steam or quaternary ammonia compounds), use of alternating sanitizers, or extended exposure to sanitizers. All experiments will be replicated three times with three samples evaluated per replicate (n=9).

Enumeration/isolation of *Listeria* species. After treatment, surfaces will be hand massaged for 30 s in 50 mL BPW with 1% Tween 20 to remove attached *Listeria* species. The rinsate will be

serially diluted and direct plated in duplicate on MOX. Plates will be incubated for 24 h at 32°C (89.6°F) prior to enumeration.

For instances where the population of attached *Listeria* species are below the level of detection, the remaining BPW Tween 20 rinsate will be enriched as described in objective one to determine the presence or absence of *Listeria* species.

Statistical analysis. A mixed model with mean separation will be used to determine where significant differences in populations exist to infer if alternate sanitation practices result in significantly fewer *Listeria* species. Additionally, a non-parametric procedure will be utilized to determine which treatments are significantly less likely to result in *Listeria* species positive surfaces.

Results and Discussion

A total of 242 samples (n=156 after sanitation and 156 after startup within 4 hrs of running have been analyzed in four packinghouses. At this time, no samples have been positive for *Listeria* spp. which is a very encouraging preliminary result. Each facility will be sampled a total of eight times at a frequency of once every three months to determine if temporal differences are observed.

Citations

1. FDA, *Control of Listeria monocytogenes in Ready-To-Eat Foods: Guidance for Industry*, HHS, Editor. 2017: https://www.fda.gov/downloads/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/UCM535981.pdf#page=39.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project: AP-18-103

Project Title: Utility of rapid tools to assess cleanliness in apple packinghouses

PI: Faith Critzer
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Co-PI: Ines Hanrahan
Organization: WTFRC
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City/State/Zip: Union Gap, WA 98903-1637

Total Project Request: Year 1: 55,956 **Year 2: 56,525**

WTFRC Budget:

Item	2018	**2019**
Salaries	3,900	3,978
Benefits	1,287	1,313
Wages	3,350	3,503
Benefits	1,106	1,156
RCA Room Rental		
Shipping		
Supplies		
Travel	500	500
Plot Fees		
Miscellaneous		
Total	10,143	**10,450**

Footnotes:
Salaries/Benefits: estimate of percent of time spent for Mendoza (3%) and Hanrahan (2%), a 33% benefit rate and 2% annual increases.
Wages/Benefits: calculated based on expected staff wage adjustments proportional to the WA state minimum wage increases (2018=$11.50, 2019=$12.00), approx. 250 hours
Travel: in state travel for Hanrahan (lodging in Wenatchee)

Budget 1
Organization Name: Washington State University
Contract Administrator: Katy Roberts/Karen Kniep Blanton
Telephone: (509) 335-2885/(509)786-9285
Email address: arcgrants@wsu.edu/prosser.grants@wsu.edu

Item	2018	**2019**
Salaries	32,440	34,107
Benefits	2,373	2,468
Wages		
Benefits		
Equipment		
Supplies	10,000	8,500
Travel	1,000	1,000
Miscellaneous		
Plot Fees		
Total	45,813	**46,075**

Footnotes:
Salaries: In year 1, $32,440, and year 2, $34,107, is requested for a Graduate Research Assistantship for a PhD student to work on all objectives.

Benefits: $2,373 and $2, 468 are requested for benefits tied to the Graduate Research Assistantship for a PhD student to work on all objectives for years 1 and 2, respectively.

Supplies: Supply costs of $10,000 in year 1 and $8,500 in year 2 are requested to purchase disposable supplies such as swabs, sponges, glassware, microbiological media, Petrifilm, pipettes, and PCR reagents tied to objective 1.

Travel: $1,000 is requested in years 1 and 2 for mileage and associated travel costs at a rate of $0.535/mi and adhering to all university policies for per diem associated with overnight travel.

Objectives:

1. Determine the correlation of ATP or carbohydrate swabs to populations of indicator microorganisms (aerobic plate counts, total enterobacteriaceae, coliforms, and *E.* coli) in typical packinghouse settings on zone 1 (food contact) surfaces.
2. Model thresholds for accepting and rejecting a surface cleanliness for ATP and carbohydrate residues and resulting populations of indicator microorganisms based upon material type.

Significant Findings

To this point, no significant correlations have been observed with any of the indicator organisms (aerobic plate counts, total Enterobacteriaceae, coliforms, or *E.* coli) and rapid screening tests (ATP and carbohydrate residue). However, there have only been 4 of 24 sampling events thus far, so the data set is limited at this point in time.

Methods

Objective 1. Determine the correlation of ATP or carbohydrate swabs to populations of indicator microorganisms (aerobic plate counts, coliforms, and *E.* coli) in typical packinghouse settings on zones 1 and 2.

Packinghouse selection. Commercial apple packinghouses in Washington were recruited into the study. Six packinghouses were enlisted into the study and were sampled once a quarter during packing season in years 1 and 2. Identifiers have been utilized for all participants in order to ensure anonymity. An interview was conducted with the appropriate personnel to describe the purpose of the study, what data will be collected, and how outcomes will be conveyed to the packinghouse operator. The facility was diagramed, and sampling points have been predetermined in all six facilities. A picture has captured the exact location of each sampling site and is being utilized when conveying results.

Surface sampling methods. Sampling has been coordinated to occur after a sanitation event. For microbiological analysis, a pre-moistened sterile sponge has been utilized to sample a 25 cm^2-area. For ATP and carbohydrate swabs, surfaces adjacent to those for microbiological sampling will be used to swab a 25 cm^2-area.

ATP determination. An ATP luminometer and accompanying swabs have been utilized to determine the ATP present in the given surface area expressed as reflective light units (RLU).

Glucose and lactose presence. The SpotCheck Plus Glucose and Lactose Residue swab (Hygiena) have been used to determine if there is presence of either of these sugars on the surface. The results will be categorized as pass (no color change=0), moderate fail (light green=1), and severe fail (dark green=2).

Microbiological isolation. Bacteria are eluted in D/E neutralizing buffer and surface plated onto Petrifilm E. coli/Coliform Count Plates (to enumerate *E. coli* and coliforms), Petrifilm Enterobacteriaceae Count Plates (to enumerate total Enterobacteriaceae), Petrifilm Aerobic Count Plates (to enumerate aerobic, mesophilic bacterial counts).

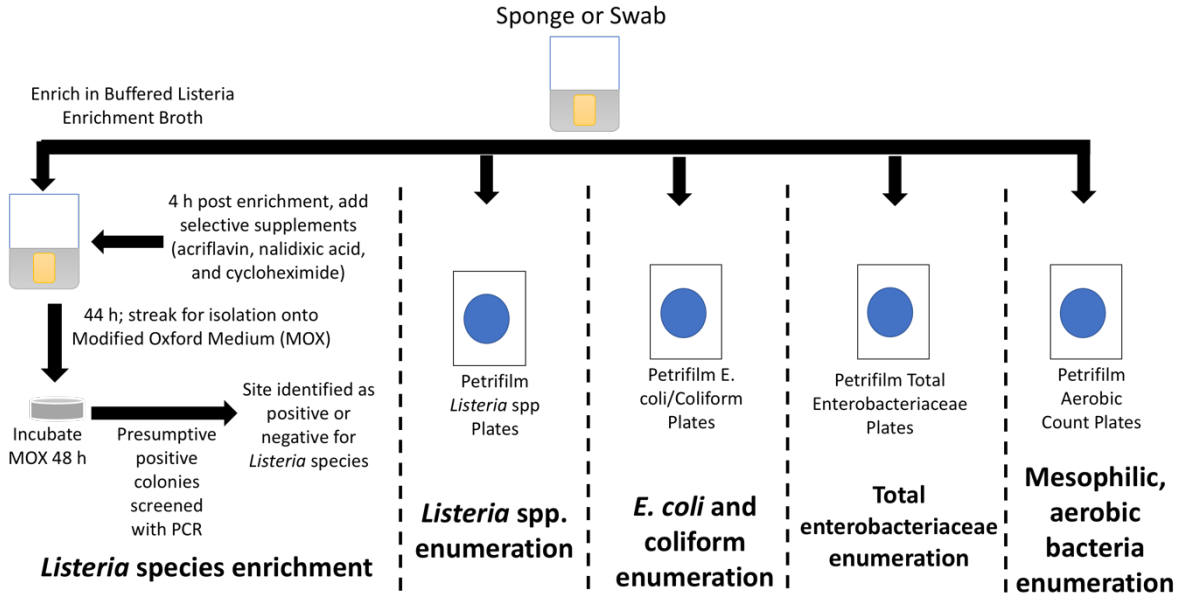

Figure 1. Sample processing to determine presence or absence and populations of *Listeria* species (environmental indicator for *L. monocytogenes*) as well as populations of *E. coli*, coliforms, total Enterobacteriaceae and aerobic plate counts which have also been commonly used as indicators of surface cleanliness.

Statistical analysis. The correlation coefficient will be determined for each indicator test (ATP, glucose and lactose swab, *E. coli*, coliforms, and aerobic plate count) and *Listeria* species.

Alterations to original design of experiments. Due to a high prevalence of Enterococci present on food contact surfaces, it was determined that the methodology for enumerating *Listeria* spp. would always overestimate the population as Enterococci (*Enterococcus faecalis* or *Enterococcus faecium*) cannot be differentiated on selective and differential media. Therefore, enumeration of listeria was abandoned as it is was not going to accurately reflect populations of *Listeria* spp.

Objective 2. Model thresholds for accepting and rejecting a surface cleanliness for ATP and carbohydrate residues and resulting populations of indicator microorganisms based upon material type.

Statistical analysis. Whenever indicators are utilized for making risk-based decisions, many firms wrestle with what thresholds should be established for action (e.g. re-clean surface). Based upon outcomes of objective 1, equations will be evaluated in year two for any moderate to highly correlated indicator to determine the threshold at which the likelihood of having *Listeria* spp. present significantly increases.

Expected outcomes. Results from this objective will allow operators to determine evidence-based thresholds when adopting a rapid strategy such as ATP or glucose/lactose presence in addition to other microbiological enumeration methods (APC, Enterobacteriaceae, *E. coli,* or coliform populations).

Results and Discussion

Six facilities have been enrolled in the study, with 28-50 zone 1 sampling sites identified per facility. Each facility is set to be scheduled once per quarter with sampling commencing in fall of 2018. At this point, four facilities have been sampled with a total of 156 sampling sites represented. The mean values of each indicator separated by unit operation are shown below in Table 1.

Table 1. Mean populations for various indicators

Unit Operation	APC	Coliform	E. coli	Enterobacteriaceae	ATP (NTU)	Carbohydrate residue frequency[1]
	Colony Forming Units (CFU)/100cm^2					
Dump tank	718	128	3	743	333	0 = 7 1 = 3 2 = 3
Brushes beneath spray bars	3255	23	2	24	290	0 = 18 1 = 4 2 = 8
Wax brushes	1227	14	1	16	49	0 = 4 1 = 3 2 = 2
Oven/dryer	999	86	4	54	305	0 = 14 1 = 7 2 = 11
Sorter	484	12	3	17	900	0 = 7 1 = 8 2 = 13
Packing	1735	12	5	31	854	0 = 2 1 = 4 2 = 6

[1] 0=pass; 1=moderate fail; 2=severe fail

As was expected, populations of Enterobacteriaceae, coliforms and generic *E. coli* are very low throughout the system compared to total aerobic plate counts. Additionally, APC populations remain relatively stable throughout the entire packing line regardless of if the unit operation is wet or dry. Of note, populations of coliforms or Enterobacteriaceae on roller surfaces passing through the oven were higher than surfaces just adjacent upstream (wax brushes) or downstream (sorter cups). As more data is collected, we will expect that these trends may change, so results should be understood to be entirely preliminary.

Citations

1. FDA, *Control of Listeria monocytogenes in Ready-To-Eat Foods: Guidance for Industry*, HHS, Editor. 2017: https://www.fda.gov/downloads/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/UCM535981.pdf#page=39.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: AP-18-104

Project Title: Fate of *Listeria* on apples at ozone and controlled atmosphere storage

PI: Meijun Zhu
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Address: 2403 S. 18th St., Suite 100
City/State/Zip: Yakima, WA 98903

Cooperators: Allan Brothers. Inc., Stemilt Growers LLC., Guardian Ozone Inc., Stemilt Growers LLC and Allan Bros Fruit, Guardian Manufacturing, Inc. AgroFresh Inc.

Total Project Request: **Year 1:** 118,779 **Year 2: 121,797** **Year 3:** 125,404

Other funding sources: None
WTFRC Budget:

Item	2018	2019	2020
Salaries[1]	4,141	4,224	4,308
Benefits[1]	1,367	1,394	1,422
Wages[2]	4,500	4,703	5,267
Benefits[2]	1,485	1,552	1,738
RCA Room Rental	8,316	8,316	8,316
Travel	500	500	500
Total	20,309	**20,689**	21,551

Footnotes:
[1]Salaries/Benefit for WTFRC staff support.
[2]Wages/Benefits for research intern support
[3]RCA room sharing with Stemilt
[4]Travel cost for transferring of fruit from Wenatchee to Pullman

Budget: Meijun Zhu
Organization Name: WSU-Pullman **Contract Administrator:** Katy Roberts
Telephone: (509) 335-2885 **Email address:** arcgrants@wsu.edu

Item	2018	2019	2020
Salaries[1]	37,124	38,609	40,154
Benefits[1]	12,412	12,909	13,424
Wages[2]	15,340	15,953	16,592
Benefits[2]	1,094	1,137	1,183
Equipment			
Supplies[3]	25,500	25,500	25,500
Travel[4]	2,000	2,000	2,000
Miscellaneous [5]	5,000	5,000	5,000
Plot Fees			
Total	98,470	**101,108**	103,853

Footnotes:
[1]Postdoc research associate and professor's salaries plus benefits.
[2]PhD graduate student partial stipends and undergraduate assistant wages plus benefits.
[3]Bacteria culture media, reagents and consumable supply cost
[4]Travel funds for industrial sampling and experiments.
[5]Funds are requested to partially cover the Biosafety Level 2 facility and equipment maintaining fees.

OBJECTIVES

1. Assess fate of *Listeria* on apple surfaces stored under RA and CA with continuous low doses of ozone.
2. Examine survival of natural microorganisms on apple surfaces stored under RA and CA with continuous low doses of ozone.
3. Evaluate impacts of ozone in the storage environment on final fruit quality

SIGNIFICANT FINDINGS

1. We determined 2.2-2.8 Log reduction of *Listeria innocua* on Granny Smith apples (GSA) after 36 weeks of cold storage under a commercial RA and CA storage environment. Surprisingly, apple subjected to RA had significantly less *L. innocua* compared to those stored at CA room.
2. Continuously low dose ozone gas application (87.0 ± 38.8 ppb) in CA storage generated an additional 2-Log reduction of *L. innocua* on GSA.

   Additional 2-week storage under RA beyond their respective initial storage treatments had little influence on *L. innocua* survival.
3. The natural bacteria level of GSA apples stored at CA/RA remained stable during 12-week storage. It slightly increased in both RA/CA during subsequent storage, while remaining similar or slightly decreased in GSA under CA with continuous low dose ozone.
4. Indigenous yeasts/molds (Y/M) count of un-inoculated GSA apples stored in RA remained relatively stable during first 12 weeks of storage. By the end of the 30-week storage, the Y/M count of RA stored apples increased about 0.6 log. The Y/M count of GSA apples in CA room remained relatively stable over 30 weeks of storage. There is about 0.8-Log reduction of Y/M count in GSA of CA with ozone storage during first 12 weeks. Nevertheless, the inhibitory effect of ozone was compromised with prolonged storage time.
5. During 30-week CA storage, continuous low dose ozone gas had no negative influence on the visual quality of GSA without MCP-1 pre-treatment. However, prolonged continuous low dose ozone gas in CA storage in combination with ozone negatively impacted the visual quality of GSA pretreated with MCP-1, which warrants further research.

METHODS

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

Objective 1: Assess fate of Listeria on apple surfaces stored under RA and CA with continuous low doses of ozone.

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

A 3-strain *L. innocua* cocktail was prepared via mixing equal numbers of each respective strain suspension.

Unwaxed and unbruised GSA apples at commercial maturity were individually and separately inoculated to establish 1×10^6 CFU/apple of 3-strain *Listeria* cocktail strains through dipping inoculation and held at room temperature for 24 h prior to different storages.

2. Cold storage treatments in a commercial packing facility

Granny Smith apples inoculated with ~1×10^6 CFU/apple of *L. innocua* were randomly separated into three groups and subjected to three different storages: refrigerated air (RA, 33 °F), controlled atmosphere (CA, 33 °F, 2 % O_2, 1 % CO_2), and CA with a low dose (~90 ppb) ozone (CA+O_3) for up to 30 weeks. Apples under different storage conditions were sampled at 0, 1-, 3-, 6-, 12-, 18-, 24-, and 30-week of storage to analyze the survival of *L. innocua* on fresh apples.

3. Microbial analysis

At each sampling day, apples under the respective storage condition were sampled and transferred to sterile bags with 10 ml of 0.1% buffered peptone water in Whirl-PAK bag to release attached microorganism, then serial diluted. Appropriate dilutions were plated on agar plates. Plates were incubated at 35˚C (95˚F) for 24 -48h and enumerated manually.

Enrichment was done when *L. innocua* level was under the detection limit of 10 CFU/apple following our previous publication (Sheng et al., 2018).

Objective 2. Examine fate of natural microorganisms on apple fruit surfaces when stored in refrigerated air, controlled atmosphere in the presence or absence of ozone.

1. Cold storage treatments in a commercial packing facility

Non-waxed, non-inoculated GSA apples were subjected to different storage conditions (RA, CA and CA+O_3) as described previously. Apples were sampled at 0-, 6-, 12-, 24, and 30-week of storage for total plate count and yeast and mold enumeration.

2. Survival microorganism analysis

At each sampling day, apple was sampled and transferred to a sterile bag with 10 ml of 0.1% buffered peptone water in Whirl-PAK bag, rub to release attached microorganism, then serial diluted. The appropriate dilution was plated onto TSAYE plates for total plate count and potato dextrose agar (PDA) plates for yeasts and molds, respectively. TPC colonies were counted manually after incubation at 35°C (95˚F) for 48h, and PDA plates were counted after incubation at room temperature for 5 days.

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

1. Fruit quality analysis

Fruit maturity and quality measurements such as firmness, total soluble solids and titratable acidity were performed at harvest, after storage and following an additional week of storage at room temperature. Briefly, fruit firmness was assessed with a fruit texture analyzer using a 1 cm diameter probe on a peeled area of ~3 cm^2 on both sun and shade side of the apples. Total soluble solids (TSS) were evaluated using Atago PR-32 digital brix refractometer. Titratable acidity (TA) of fruit juice was measured with a potentiometric titrator. Measurements of each parameter were repeated four times independently with a sample size of 10 apples per replication per storage regimen.

2. Disorder analysis

The incidence of disorders was assessed after cold storage followed by one day at room temperature (RT) for external disorders and 7 days at RT for both internal and external disorders. The absence or

presence of the following external disorders was visually inspected and recorded: ozone burn, superficial scald, lenticel decay, visible decay, sunburn, russet, and CO_2 damage. Apples were sliced 3 times to determine the presence of any internal disorders including watercore, internal browning, or cavities. Sample size for both external and internal disorder analysis were 50 apples per replication per storage regimen, with 4 replicates for each analysis.

RESULTS AND DISCUSSION

1. Survival of *L. innocua* on GSA under commercial cold storage.

We conducted a cold storage experiment in a typical commercial apple facility using *L. innocua* inoculated apples in which GSA apples were inoculated and established to 6.09 ± 0.07 Log_{10} CFU/apple before being subjected to RA, CA, and CA with 87 ppb ozone gas.

During 3 weeks of cold storage, *L. innocua* was reduced by 1.0-1.4 Log_{10} CFU/apple on GSA stored in RA, CA, and CA plus O_3 with a die-off rate of 0.35-0.45 Log_{10} CFU/apple/week (Figure 1). A single log reduction is equal to 10-fold or 90% reduction in *Listeria* population on fresh apple. Die-off rate significantly decreased during the subsequent 15-week storage, which was 0.02 Log_{10} CFU/apple/week in RA and CA storages while 0.7 Log_{10} CFU/apple/week in CA + O_3. During the prolonged storage of 18-36 weeks, die-off rate in CA + O_3 maintained while that in RA and CA decreased to 0.07 and 0.04 Log_{10} CFU/apple/week, respectively. By the end of the storage, *L. innocua* on GSA was reduced by 2.9, 2.2 and 4.6 Log_{10} CFU/apple in RA, CA, and CA + O_3, respectively (Figure 1). Upon 36-week storage, most of the apples were confirmed to contain *L. innocua* by enrich-ment and plating method.

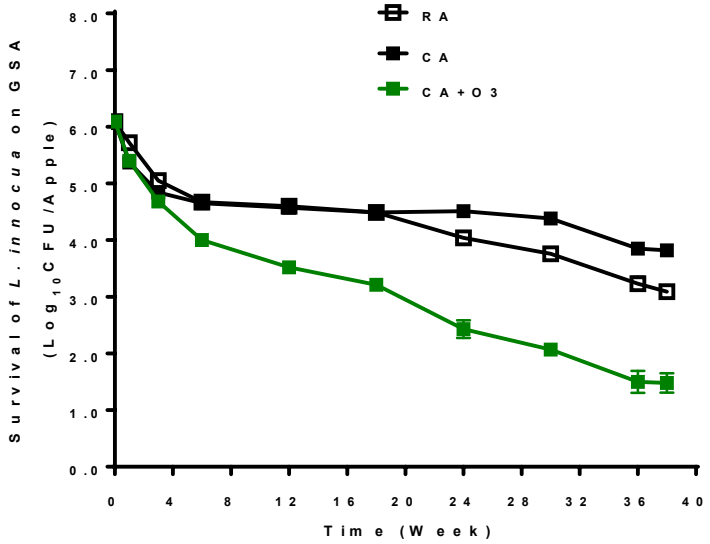

Figure 1. Survival of *Listeria* on Granny Smith apple under commercial cold storages. RA: refrigerated atmosphere (33°F); CA: controlled atmosphere (33°F, 2% O2, 1% CO2); CA + O3: CA with ~87±39 ppb ozone. Mean ± SEM, n=40.

2. Fate natural microorganisms on apple fruit surfaces when stored in refrigerated air, controlled atmosphere in the presence or absence of ozone

Resident bacteria, mold, and yeast cause postharvest decay of apples (Janisiewicz and Korsten, 2002), which were assessed during the storage. Another set of Granny apple fruits (non-waxed and non-inoculation) was subjected to different storage conditions (RA, CA and CA with ozone), and total plate count (TPC) and yeasts/molds (Y/M) count were evaluated as described above. The initial TPC on GSA in 2016 was 3.75 ± 0.04 Log_{10} CFU/apple (Figure 2A). TPC on GSA maintained similar levels during 30-week storage under CA and RA, while that on CA + high O_3 was significantly reduced by ~ 0.4 Log_{10} CFU/apple (Figure 2A). The initial Y/M on GSA was 4.97 ± 0.03 Log_{10}

CFU/apple, which gradually increased by ~0.6 Log_{10} CFU/apple in RA, maintained the population in CA, and decreased by ~0.8 Log_{10} CFU/apple in CA + high O_3 during the first 12 weeks and increased to ~4.6 Log_{10} CFU/apple by the end of 30 weeks (Figure 2B).

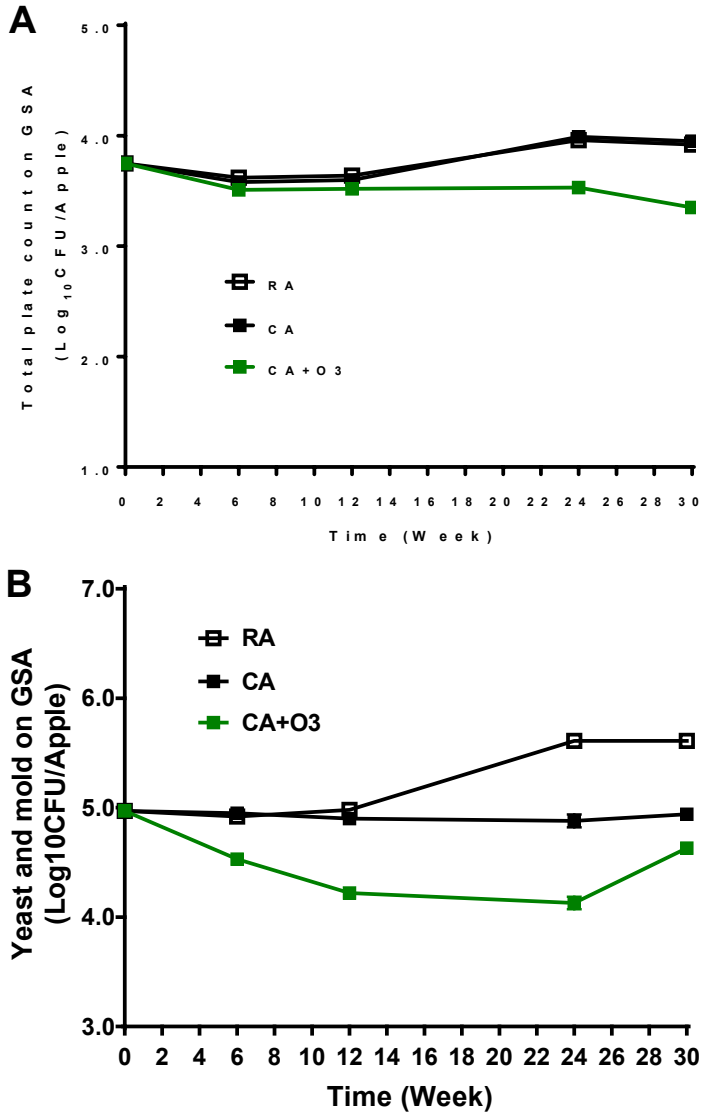

Figure 2. GSA apple decay during 30-week cold storages. A. TPC. B. Y/M count. RA: refrigerated atmosphere (33°F); CA: controlled atmosphere (33°F, 2% O2, 1% CO2); CA + O3: CA with ~87±39 ppb ozone. Mean ± SEM, n=40.

3. Effects of continuous low dose ozone in cold storage environment on final fruit quality

Quality parameters of apple fruits under different treatments were assessed both at harvest and after storage. Firmness decreased after storage for all apples, and no difference was found between CA and CA + O_3 storage. Apples subjected to RA storage had a significantly lower firmness than those in CA with or without gaseous ozone storage (Table 1). Compared to harvest levels, total soluble solids (TSS) levels did not change in apples post-6-month storage in RA, CA, or CA + O_3 (Table 1). Titratable acidity (TA) after storage decreased compared to fruit at harvest. TA reduction in apples was significantly mitigated by CA storage, while addition of ozone had no impact on TA (Table 1).

Table 1. Granny Smith apples* quality parameters at harvest and after cold storage

Treatment	Weight (g)	Diameter (cm)	Firmness (lbs)	TSS	TA
At harvest					
RA/CA/CA+O_3	228.0 ± 14.0	8.08 ± 0.10	16.0 ± 0.3	11.5 ± 0.3	0.710 ± 0.05
6-month post-storage					
RA	229.0 ± 9.7[a]	8.26 ± 0.03[a]	9.0 ± 0.7[b]	11.7 ± 0.5[a]	0.464 ± 0.02[a]
CA	233.0 ± 14.6[a]	8.28 ± 0.03[b]	15.3 ± 0.0[a]	12.5 ± 0.2[b]	0.656 ± 0.02[b]
CA + O_3	222.0 ± 12.3[a]	8.10 ± 0.02[ab]	15.1 ± 0.3[a]	11.6 ± 0.2[a]	0.613 ± 0.02[b]

Granny Smith apples were not treated with 1-methycyclopropene before subjecting to respective cold storage; TSS: total soluble solids, expressed in % Brix. TA: titratable acidity, express in % malic acid. NA: not available, data not collected. Ozone was adjusted to the target concentration from the 3rd and 6th week of cold storage. [ab]Means within a column with no common letter differ significantly ($P < 0.05$). Mean ± SD; n=40.

Table 2. External disorders analysis for Granny Smith apples after 6 months of cold storage and ripened at RT for 1 and 7 days.

Treatment	Ozone burn	Superficial scald	Lenticel decay	Visible decay	Sunburn	Russet	CO_2 damage
1 day at RT							
RA	0[a]	34 ± 2.7[b]	15.0 ± 1.9[a]	1.0 ± 1.0[a]	1.0 ± 0.5[b]	NA*	1.0 ± 0.5[a]
CA	0[a]	1.0 ± 0.4[a]	2.0 ± 1.1[b]	1.0 ± 0.4[a]	10 ± 2.8[a]	NA	1.0 ± 0.5[a]
CA + O_3	1.0 ± 0.8[a]	1.0 ± 0.5[a]	2.0 ± 2.2[b]	0 ± 0.4[a]	1.0 ± 0.4[b]	NA	2.0 ± 1.2[a]
7 days at RT							
RA	0[a]	52.0 ± 2.9[a]	26.0 ± 2.5[a]	19.0 ± 2.1[a]	3.0 ± 0.9[a]	9.0 ± 2.2[a]	2.0 ± 1.3[a]
CA	0[a]	1.0 ± 0.5[b]	3.0 ± 2.7[b]	1.0 ± 0.4[b]	9.0 ± 2.7[b]	4.0 ± 1.1[a]	2.0 ± 1.2[a]
CA + O_3	3.0 ± 1.5[a]	0 ± 2.6[b]	0 ± 0.4[b]	0 ± 0.4[b]	6.0 ± 1.9[ab]	11.0 ± 3.3[a]	3.0 ± 1.5[a]

[ab]Means within a column with no common letter differ significantly ($P < 0.05$). Mean ± SD; n=200.

Table 3. Internal disorders analysis for Granny Smith apples at harvest and after 6 months of cold storage and ripened at RT for 7 days.

Treatment	Watercore	Internal browning	Cavity
At harvest			
RA/CA/CA + O_3	0[a]	0[a]	0[a]
6-month post-storage			
RA	0[a]	70.2 ± 1.2[a]	0[a]
CA	5.0 ± 0.5[a]	0[b]	0[a]
CA + O_3	10.0 ± 1.2[a]	0[b]	0[a]

[ab]Means within a column with no common letter differ significantly ($P < 0.05$). Mean ± SD; n=200.

The incidence of external and internal disorders was visually evaluated at the end of each storage treatment. Overall, the parameters evaluated for either external disorders or internal disorders were not significantly different among apples stored under RA, CA, or CA + O_3. No ozone burn, lenticel breakdown, decay, or CO_2 damage was found in any GSA without MCP-1 subjected to 6-month of low-dose ozone gas storage (Table 2). Continuous low-dose ozone gas application in 6-month cold storage did not cause additional watercore, internal browning or cavity on Granny Smith apples, though. A small number of apples under CA+ O_3 storage were found to have watercore, but the incidence rate was not significantly different from those fruit kept under CA storage (Table 3). However, our preliminary study indicated that low dose ozone gas in prolonged (9-month) CA storage negatively impacted visual quality of GSA pretreated with MCP-1. Currently, we are conducting additional studies to discern interaction among apple varieties, storage time, low ozone gas and MCP-1 application.

4. Conclusion

Continuous low dose ozone gas used at this study caused additional ~2 log reduction of Listeria on fresh GSA surface over 6-month of CA storage and had no negative influence on the visual quality of GSA. Impacted of low dose ozone application at prolonged CA storage such as 9-month CA storage for GSA or other apple varieties treated with MCP-1 will be further assessed in the following years.

REFERENCES

Janisiewicz, W.J., Korsten, L., 2002. Biological control of postharvest diseases of fruits. Annual Review of Phytopathology 40, 411-441.

Sheng, L., Hanrahan, I., Sun, X., Taylor, M.H., Mendoza, M., Zhu, M.-J., 2018. Survival of *Listeria innocua* on Fuji apples under commercial cold storage with or without low dose continuous ozone gaseous. Food Microbiology 76, 21-28.

CONTINUING PROJECT REPORT **YEAR**: 16 months of 18
WTFRC Project Number: AP-17-108

Project Title: 'WA38' fruit size and dry matter for fruit quality/consumer preference

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Other funding sources: None

Total Project Funding: $170,198

Budget History:
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Item	2017	2018	**2019**
Salaries	24,000	24,960	
Benefits	8,597	8,941	
Equipment¥	6,000		
Supplies			
Travel	6,500	6,500	
Miscellaneous[1]	36,350	38,350	
Plot Fees	5,000	5,000	
Total	86,447	83,751	**0**

Footnotes:
[1] WSU sensory evaluation facility fees
¥ Originally, we requested funds for an Amilon starch meter. Instead in December 2017 our Minolta color meter broke and Konica Minolta would no longer service it. We asked permission from Mike Willet to use the equipment money to buy a new Minolta Colorimeter CR-400 (no request of increase in current year budget).

OBJECTIVES

1. ***Identify the distribution of fruit size and dry matter in both a young (1st crop in 2018) and a mature orchard (4th crop in 2017 and 5th crop in 2018).***
2. ***Correlate fruit quality parameters of selected fruit categories (by size and dry matter) to consumer preference.***

SIGNIFICANT FINDINGS

1. ***Identify the distribution of fruit size and dry matter in both a young (1st crop in 2018) and a mature orchard (4th crop in 2017 and 5th crop in 2018).***
    - Using a non-destructive predictive model, dry matter was estimated at harvest in 2017 and 2018 among young (1st crop 2018) and mature (4th crop 2017 and 5th crop 2018) orchards.
    - Distribution of predicted dry matter and fruit size were highly significantly dependent within each orchard ($P < 0.001$, Chi-square test of independence) with the exception of the 1st cropping of a commercial 'WA38'/G41 young orchard in 2018.
    - Predicted dry matter % increases with fruit size. This effect was most pronounced in established mature orchards relative to young orchards (0.016, 0.314, and 0.114 R^2 for 1st, 4th, and 5th cropping years, respectively).
2. ***Correlate fruit quality parameters of selected fruit categories (by size and dry matter) to consumer preference.***
    - Sorting fruit by size and predicted dry matter % produced distinct groups of fruit in terms of both instrumental quality and consumer liking. These groups were largely consistent in these attributes both at 1- and 6-months post-harvest.
    - In aggregate between rootstocks and evaluation periods, high predicted dry matter fruits were characterized by significantly more red overcolor coverage and red intensity of overcolor relative to lower dry matter fruits, as well as higher actual dry matter and soluble solids content.
    - While liking of each fruit classification was generally high and differences small due to exceptional overall eating quality of 'WA38', high (15.00-15.99%) predicted dry matter fruits were more favored by consumers over mid (14.00-14.99%) and low (13.00-13.99%) dry matter fruits in respect to apple flavor and overall liking in aggregate between rootstocks and evaluation periods.
    - Liking of dry matter interacted with liking of large fruits – larger, higher dry matter fruits were also significantly more favored in terms of apple flavor and overall liking.

METHODS

1. ***Identify the distribution of fruit size and dry matter in both a young (1st crop in 2018) and a mature orchard (4th crop in 2017 and 5th crop in 2018).***

Non-Destructive Dry Matter Prediction Model

Procedure described in 2017 continuing report.

At-Harvest Size and Dry Matter Distribution of Young and Mature Orchards

To examine dry matter distribution as a response to orchard age, rootstock, and fruit size, four orchards were evaluated non-destructively for predicted dry matter at-harvest using the model previously described in year 1 report. In 2017, the 4th crop (mature) of Sunrise Orchard (SRO, 'WA38' on G41 and M9-NIC29 rootstocks) was harvested. In 2018, Sunrise WA38 block (5th crop) was again harvested with the addition of a Granny Smith on M9-T337 top-worked to 'WA38' in 2016 (2018 being its 1st crop). Additionally, two new commercial 'WA38'orchards trained to spindle– one budded on G41 and the other on M9-NIC29 – were harvested as their 1st cropping. Representative Fancy and Extra Fancy subsamples of fruit from each orchard were selected for dry matter prediction

and sorted in to size classes under the following classifications: Small = 70-75 mm or ~113-88 apples/box (U.S. apple box equivalent to 19 kg apples), Medium = 80 mm or ~80-72/box, Large = 85-95 mm or ~64-<48/box, and Extra Large = 100+mm or < 48/box. Fruit 65mm or smaller (163/case) were not considered marketable fruit for the purpose of this study, however in 2018 an "Extra Small" size category was used for fruit 65mm.

Fruit Sorting for Quality and Consumer Testing

Following predicted dry matter and fruit size classification, apples from the 2017 Sunrise Orchard harvest were divided in to low (13.00 – 13.99%), moderate (14.00 – 14.99%), and high (15.00-15.99 %) predicted dry matter categories and small (70-75mm), medium (80mm), and large (85-95mm) fruit size categories. From these categories, fruit were randomly assigned in equal proportion to either instrument fruit quality evaluation or consumer testing groups, and within these groups, either split in a 1 or 5-months post-harvest evaluation period. Fruit were stored at 32°F under regular atmosphere conditions until quality evaluation and consumer testing.

Fruit sorting by dry matter predicted at 2018 harvest produced higher dry matter categories than at harvest 2017 so we modified the classification with "very high" dry matter classes (i.e. 16.00-16.99%, 17.00-17.99%, 18.00-18.99% predicted dry matter). Combinations of orchard age-size-predicted dry matter apples were sorted in to evaluation periods as done in 2017. Data collection for these evaluations of 2018 harvest is ongoing, the results of which will be reported in the final report in 2020.

2. ***Correlate fruit quality parameters of selected fruit categories (by size and dry matter) to consumer preference.***

Instrumental Fruit Quality

Fruit quality was assessed 1- and 5-months post-harvest on the basis of red blushed overcolor (%), maximum red and background color (CR-300 Colorimeter, Konica Minolta, Toyko, Japan), firmness (Digi-Test2, Mohr, Richland, WA, USA), soluble solids concentration (°Brix, PAL-1, Atago, Bellevue, WA, USA), starch index (1 to 6 WTFRC scale), actual dry matter (%), titratable acidity (% Malic Acid), and pH, among others.

Consumer Panels

'WA38' apples were received on November 15th, 2017 and March 26th, 2018 for consumer evaluation at 1- and 5-months post-harvest, respectively, and placed in 38°F storage at the WSU School of Food Science in Pullman, WA. Fruit from regular cold storage were brought up to room temperature 24 hours before analysis, washed in cool water and dried with paper towels. Apples were cut into equal 1/8 parts with the seed core removed and placed on a white paper plate. From this samples, consumers were asked about their acceptance of the apple slice appearance, aroma, firmness, crunchiness, juiciness, sweetness, sourness, apple flavor and overall liking using a 9-point hedonic scale (1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like very much and 9=like extremely). For each period of evaluation, consumers anonymously tested up to 5 sliced fruit samples a day for each day of testing until all combinations of rootstock, fruit size, and predicted dry matter were exhausted. 94 consumers participated in the 1-month, and 97 for the 5-month post-harvest evaluation – a total of 1,965 responses.

RESULTS & DISCUSSION

1. ***Identify the distribution of fruit size and dry matter in both a young (1st crop in 2018) and a mature orchard (4th crop in 2017 and 5th crop in 2018).***

Non-Destructive Dry Matter Prediction Model
Results described in 2017 continuing report.

At-Harvest Size and Dry Matter Distribution of Young and Mature Orchards

Figure 1 depicts predicted 2018 dry matter distribution among 1% dry matter classifications. As shown, younger orchards generally produced larger proportions of higher dry matter fruits relative to the more mature orchard. This is due to their low cropping densities in 2018 as well as from being of first cropping maturity, increasing the allocation of dry matter on a per-fruit basis. The large proportion of high dry matter fruits in the first cropping year is likely transitory and will even out as the orchard matures. Distributions of predicted dry matter between rootstocks were not shown to be starkly different, however factors such as rootstock age, orchard management strategies, and site conditions between orchards may be contributing to overall distributions and thus limiting our ability to make direct comparisons based on rootstock and cropping year alone.

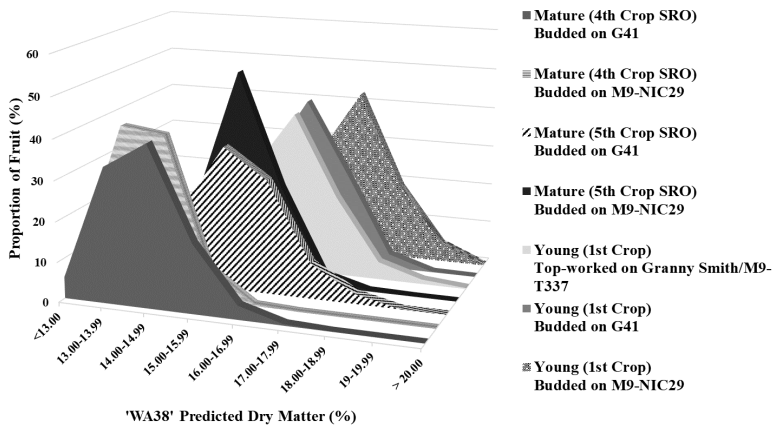


Figure 1: Predicted dry matter (%) distribution among 'WA38' orchards both mature (SRO) and young as determined at-harvest using Felix F-750 Produce Quality Meter.

Table 1 depicts the distributions of predicted dry matter among size classes among orchards and cropping years. Chi-square test of independence indicated highly significant dependence of dry matter on fruit size at $P < 0.001$ with the exception of the 1st crop of young commercial 'WA38'/G41 orchard which was non-significant. Without consideration of fruit size, dry matter was also shown to be dependent on orchard/cropping year combination at a highly significant level ($P < 0.001$, not shown). This supports the previous observation that dry matter production varies by orchard age, but the effects of the 1st cropping may be somewhat random as indicated by the independence of dry matter and fruit size of young commercial 'WA38'/G41 (**Table 1**). Dry matter shifts among fruit sizes and rootstocks and orchards will follow the crop load and the age of the orchards.

Fruit weight (as proxy for fruit size) and dry matter are linearly related, but this relationship varies among cropping years and rootstocks (**Figure 2**). Predicted dry matter was more highly associated with fruit weight in mature orchards than young orchards (0.016, 0.314, and 0.114 R^2 for 1st, 4th, and 5th cropping years, respectively).While these relationships may be considered poor in terms of R^2, it is notable that both the spread and minimum observed value of predicted dry matter appears to relate strongly to fruit weight – as fruit weight increases, the spread of possible predicted dry matter values decreases in range and the minimum value of predicted dry matter strongly increases. In other words, as fruit weight increases, the possibility of obtaining a higher dry matter fruit is greater due to lower variability and higher minimum dry matter. As for rootstocks, M9-NIC29 produced more robust associations between fruit weight and predicted dry matter than G41 and top-worked Granny Smith on M9-T337, though overall these relationships were quite poor (0.012, 0.01, 0.12 R2 for G41, GS/M9-T337, and M9-NIC29, respectively).

Table 1: Proportion of fruit (%) in predicted dry matter (%) classes among orchards, cropping years and fruit sizes (S, Small = 70-75, M, Medium = 80, L, Large = 85-95, XL, Extra Large = 100+mm). Trend column shows proportion of fruit belonging to each predicted dry matter class as a bar chart. Asterisks indicate significance of Chi-square Test of Independence within rootstock:cropping year distributions. Rows and columns with all zero values were excluded from the test. * = P < 0.05, ** = P < 0.01, *** = P < 0.001.

		Predicted Dry Matter (%)									
Rootstock:Cropping Year	**Size**	**< 13.00**	**13.00-13.99**	**14.00-14.99**	**15.00-15.99**	**16.00-16.99**	**17.00-17.99**	**18.00-18.99**	**19-19.99**	**> 20.00**	**Trend**
Mature (4th Crop) * Budded on G41**	**S**	17	47	28	6	2					
	M	4	55	33	7	1					
	L	1	13	50	29	6	1				
	XL										
Mature (4th Crop) * Budded on M9-NIC29**	**S**	17	50	29	5						
	M	5	37	49	8	1					
	L		18	59	21	2					
	XL										
Mature (5th Crop) * Budded on G41**	**S**	19	30	26	6	11	9				
	M		18	41	24	9	6	2			
	L		2	18	39	31	8	3			
	XL			7	37	32	20	5			
Mature (5th Crop) * Budded on M9-NIC29**	**S**		10	42	19	16	13				
	M		2	33	43	19	3				
	L			18	54	25	3				
	XL			10	64	23	3				
Young (1st Crop) * Top-worked on Granny Smith/M9-T337**	**S**	1	1	12	26	34	14	8	3	1	
	M			15	27	37	17	3	1		
	L			4	25	44	23	4			
	XL					33	67	0			
Young (1st Crop) Budded on G41	**S**		1	8	26	40	22	4			
	M		1	2	23	44	27	3			
	L			2	17	55	26				
	XL										
Young (1st Crop) * Budded on M9-NIC29**	**S**		1	2	7	24	27	28	10		
	M			1	7	16	45	23	9		
	L			1	6	31	47	14	1		
	XL				25	38	25	13			

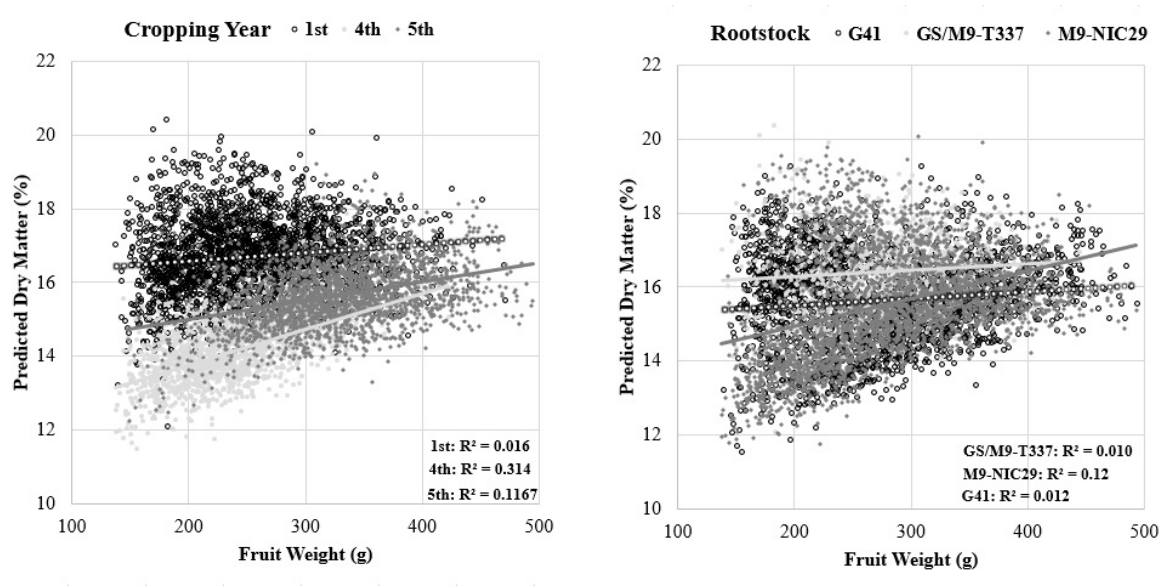


Figure 2: Linear regression of predicted dry matter (%) on fruit weight (g) by cropping year (left) and rootstock (right). All regressions were statistically significant at P < 0.01 or greater.

Fruit Sorting for Quality and Consumer Testing

Shown in **Figure 1**, not all class combinations were available in sufficient proportions for both quality and consumer evaluation, and in these scenarios (e.g. low dry matter in large fruit), instrumental quality was prioritized over consumer evaluation. Additionally, classes used for 2017 harvest of Sunrise Orchard (low, mid, high dry matter) needed to be modified to accommodate 2018 harvests as substantial portions of fruit belonged to groups outside these classes. New classes will be described in more detail in the final report following quality and consumer evaluation of 2018 harvests of both young and mature orchards.

2. ***Correlate fruit quality parameters of selected fruit categories (by size and dry matter) to consumer preference.***

Instrumental Fruit Quality

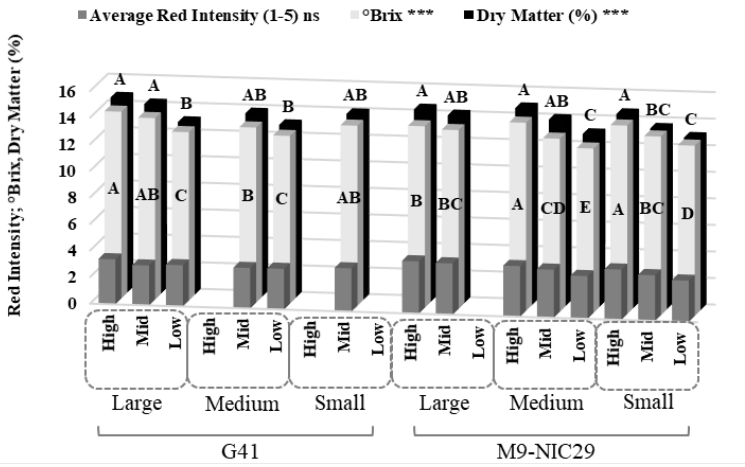


Figure 3: Average values for instrumental quality 5 months post-harvest including average red intensity (1-5), dry matter (%), and soluble solids content (°Brix) among fruit size:dry matter categories. Significant difference in means indicated by different letters via SNK. * = P < 0.05, ** = P < 0.01, *** = P < 0.001.

Figure 3 depicts results of some instrumental quality parameters among 2017 harvest fruit size:dry matter categories evaluated 5-months post-harvest. Though not statistically significant, average red intensity tends to increase with predicted dry matter content independent of fruit size. This may be due to increased sun exposure leading to higher amounts of photosynthates accumulation resulting in higher dry matter content of the fruit. Just as for quality evaluation of 1-month post-storage fruits, destructive dry matter and soluble solids content were both significantly uniquely higher in high dry matter fruits relative to lower dry matter fruits within each size class. This is to be expected as categories were defined by dry matter, to which soluble solids is closely correlated. However, the values of actual dry matter and soluble solids among the various classifications is not as strictly segregated as observed 1-month post-harvest shown in 2017 continuing report. This is likely due to the physio-chemical evolution of the fruit throughout storage where we expect dry matter and soluble solids content to increase as water is lost from the fruit during storage, leading to inflation of the dry matter and soluble solids metric over time during storage. From 2018 harvest, we will be able to see how these relationships hold true across varying levels of orchard maturity.

Consumer Panels

Table 3 shows average consumer responses to 'WA38' 1- and 5-months post-harvest to liking categories of crunchiness, juiciness, apple flavor, and overall liking among fruit size:dry matter categories. Consumer liking among these attributes generally improved with increasing fruit size and dry matter, but vary among evaluation time and among attributes. For example, consumers indicated significant differences in liking among fruit size: dry matter categories in terms of crunchiness 1-month post-harvest, but not 5-months post-harvest. Perhaps the exceptional at-harvest firmness of 'WA38' and its ability to retain this firmness through storage masks the subtle at-harvest differences among fruit classifications over time, making these differences undetectable by consumers.

Conversely, apple flavor and overall liking were not significantly different at-harvest, but emerged as significantly different at 5-month post-storage. For these attributes, larger and high predicted dry matter fruits were favored over smaller and lower predicted dry matter fruits. This would seem to indicate that dry matter, when implemented at-harvest, can be a predictor of future overall sensory eating quality. Additionally, juiciness, firmness, and sweetness were generally more favored as fruit size and predicted dry matter increased, but less reliably and often at a non-significant magnitude (not shown).

Table 3: Average consumer liking scores obtained 1- and 5-months post-harvest and pooled data of two consumer tests for perceiving liking of firmness, crunchiness, juiciness, apple flavor, and overall liking among fruit size:dry matter categories regardless the rootstock. Significant difference in means indicated by different letters via SNK. * = P < 0.05, ** = P < 0.01, *** = P < 0.001.

		Predicted Dry	Average Consumer Liking (1-9)							
Evaluation	**Fruit Size**	**Matter**	**Crunchiness**		**Juiciness**	**Apple Flavor**		**Overall Liking**		
1 mo. Post-Harvest	**Large**	**High**	7.44	AB	7.60	7.10		7.00		
		Mid	7.65	A	7.58	7.12		7.17		
		Low	-		-	-		-		
	Medium	**High**	-		-	-		-		
		Mid	7.45	AB	7.65	7.23		7.16		
		Low	7.45	AB	7.62	6.95		6.95		
	Small	**High**	-		-	-		-		
		Mid	7.19	B	7.49	7.00		7.00		
		Low	7.37	AB	7.46	6.86		6.86		
			*		NS	NS		NS		
6 mo. Post-Harvest	**Large**	**High**	7.59		7.48	7.31	A	7.30	A	
		Mid	7.42		7.49	7.16	AB	7.17	AB	
		Low	-		-	-		-		
	Medium	**High**	-		-	-		-		
		Mid	7.30		7.35	6.93	AB	6.96	AB	
		Low	7.28		7.30	7.06	AB	6.98	AB	
	Small	**High**	-		-	-		-		
		Mid	7.16		7.22	7.05	AB	7.03	AB	
		Low	7.29		7.20	6.76	B	6.80	B	
			NS		NS	**		*		
Pooled	**Large**	**High**	7.51	A	7.54	7.20	A	7.15	A	
		Mid	7.53	A	7.53	7.14	A	7.17	A	
		Low	-		-	-		-		
	Medium	**High**	-		-	-		-		
		Mid	7.37	AB	7.50	7.08	AB	7.06	AB	
		Low	7.37	AB	7.46	7.01	AB	6.96	AB	
	Small	**High**	-		-	-		-		
		Mid	7.18	B	7.35	7.03	AB	7.02	AB	
		Low	7.33	AB	7.33	6.81	B	6.83	B	
			**		NS	**		**		

However, due to the apparent complexity of interactions between rootstocks, fruit sizes, and predicted dry matter, not all combinations were available for consumer testing in the 2017 harvest, producing the partial results shown in **Table 3**. From **Figure 1** we see much broader distributions of predicted dry matter from harvest 2018 both from Sunrise Orchard and with the addition of three new young orchards. As such, consumer panels conducted from 2018 harvest will show consumer responses to a larger variety of predicted dry matter classes than what was produced in 2017 which will allow us to further evaluate consumer preferences. Additionally, future testing will give an indication of how much consumers are willing to pay for unit increases in predicted dry matter as determined from a "willingness-to-pay" questionnaire included in the 2018 ballot.

CONTINUING PROJECT REPORT **YEAR:** 2018

Project Title: Improving apple fruit quality and postharvest performance

PI: Ines Hanrahan
Organization: Washington Tree Fruit Research Commission
Telephone: 509-669-0267
Email: hanrahan@treefruitresearch.com
Address: 1719 Springwater Ave
City/State/Zip: Wenatchee, WA, 98801

Cooperators:

- WTFRC internal program: Manoella Mendoza, Mackenzie Perrault, Marcella Galeni
- Others: Rob Blakey, Corina Serban, Hannah Walters (Stemilt), misc. grower collaborators, WTFRC seasonal crew and interns, Garrett Bishop (GS Long), Dirk Köpcke (Germany)
- Defect guide: TJ Mullinex (Good Fruit Grower), Darrel Kilgore, Matt Ziegler, Wendy Jones, Karen Lewis (WSU), Rob Blakey (Stemilt)
- WA 38 starch scale review, panel: Lauren Gonzalez (GS Long), Suzanne Bishop (Allan Bros.), Jim Mattheis (USDA-ARS), Kate Evans + team (WSU-Wenatchee), Bill Wolk (BC)

Other funding sources
Majority of supplies and fruit donated by industry cooperators (approx. value: $2,500); GS Long covered the cost for fruit mineral analysis of WA 38 ($6,000), WSU Postharvest Fruit School supplied $6,000 to complete initial draft of defect guide.
Interreg Project: Storage defect mobile app (under review, TBD)
Partners: Forschungsanstalt Agroscope, Hochschule Weihenstephan, Landwirtschaftskammer Niedersachsen, Esteburg Obstbauzentrum Jork, Internetagentur Bodensee, Marktgemeinschaft Bodenseeobst, Württembergische Obstgenossenschaft, Versuchszentrum Laimburg, Universität Bozen, Hortgro Science, South Africa

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

Item	**2018**		
Salaries	4,165		
Salary benefits	1,708		
Wages	15,000		
Wage benefits	7,950		
RCA rental	0		
Equipment + supplies	500		
Travel	500		
Total net costs	**29,823**		

Footnotes:
Salaries: incl. proportional time spent on outlined projects for Mendoza (7% with 41% benefits rate); NOT included 6% of Hanrahan and 1% of Schmidt time

Note: This is a final report. Additional summaries of the WA 38 starch scale, mineral analysis for green spot, and Honeycrisp induced bitter pit will be prepared separately and posted to the WTFRC database. Information transfer related to each project will include Fruit Matter article, Good Fruit Grower, and talks at grower meetings.

OBJECTIVES

1. Serve on WSU Tree Fruit Extension team as postharvest specialist
    a. Development of an apple defect guide
    b. Development of a WA 38 starch scale (1-6)
    c. Organization of the 2018 Postharvest Fruit School
2. Field test methods to induce bitter pit in Honeycrisp.
3. Expand collaborative efforts with other research programs working on fruit quality management.

SIGNIFICANT FINDINGS

Objective 1: *WA38* develops two predominant *starch* patterns, which appear in a 6:4 ratio. WA38 converts starch very slow back to sugar. The reaction time for the iodine solution to color the starch crystals is significantly longer compared to other industry specific varieties.
A *Postharvest fruit school* was carried out by the Extension team. It was held in two locations (Prosser, Wenatchee) in March of 2018.
A new apple defect guide was developed. It will contain the following modules: web-based content featuring rotating defects with call-outs, a set of five posters, a laminated booklet.

Objective 2: None of the methods tested to *induce bitter pit in Honeycrisp* consistently predicted bitter pit in storage.

Objective 3: We continue to build productive and dynamic research and outreach partnerships with a number of cooperators on projects relevant to enhance pre and postharvest apple quality management.

METHODS

Induced bitter pit

Four orchards were selected (4 orchards = all methods). 20 representative trees were selected in each orchard. Before the apples were harvested, the amount of Bitter Pit (BP) was assessed per tree. All apples were harvested between the 2nd and 5th wire on the west or south side of the tree. The apples were picked a few days prior to first commercial harvest. For the PennState method ([https://extension.psu.edu/fruit-disorders-new-tool-to-assess-the-potential-for-bitter-pit-in-honeycrisp]) five typical terminal shoot measurements were taken per tree. In all orchards, a total of 230 apples (passive method: 40, Ethephon method: 40, PennState method: 20, untreated control (UTC): 120 and maturity sample: 10) were harvested.
Within the next day of harvest, fruit was prepared as follows:

1. All fruit were washed in 77°F (25°C) tap water.
2. The fruit were left to air dry.
3. Ethephon Method:
    a. A plastic box was filled with 2 gal (7.6 l) of water (temperature: 77°F) and 0.5 oz (15 ml) of Ethephon (1 gal= 0.25 oz) was mixed in.
    b. Every apple was dipped (2 sec) in the prepared solution.
    c. The samples were laid to dry on trays layered with paper towels.
4. PennState Method:
    a. A commercial fruit dryer was preheated to 160°F (71°C).
    b. A fruit peeler was used to remove a 3/8” wide (1 cm) strip of peel from around the circumference at the calyx end of the fruit.
    c. Samples were put on a drying tray and dried for 9 hours at 160°F.
    d. After 9 hours the samples were stored in Ziploc® bags and sent to a laboratory for a nutrition analysis.

5. Passive Method:
   a. Apples were kept in trays at room temperature and no further steps were added to this method.
6. All apples from these three methods were placed in apple boxes and stored at room temperature for 3 weeks.
7. The fruit was evaluated after one, three, five, eight, eleven, fourteen and twenty-one days.
8. The UTC fruit was stored following the Honeycrisp storage recommendations [http://treefruit.wsu.edu/article/honeycrisp-storage-recommendations-revisited/] and apples were evaluated after 2, 4, 6, 8 and 12 weeks of cold storage.

WSU Tree Fruit Extension team postharvest activities:

WA 38 starch scale:

Fruit was picked at various locations from Sept. 20-Oct. 10, 2018 . After harvest, the fruit was stored at 33°F. In mid-November, a sample of 40 fruit was stored at room temperature, to advance maturity. Apples were prepared, and pictures taken between 10/1/2018 and 12/06/2018.

To prepare the apples:

1. The apples were cut in half through the equator.
2. Scrape the surface to create an even canvas for photographs.
3. The freshly cut apple surface was dipped in iodine solution to fully cover cut flesh.
4. After 30 mins, when the pattern was fully developed, excess moisture was removed from the fruit surface with a paper towel.
5. A picture was taken under consistent light environment (i.e. a photo box).
6. The pictures were evaluated and grouped.

We have used an industry focus group (field men, QC personnel, R&D, scientists) to gather feedback on various stages of the actual starch scale and on potential best layout options.

RESULTS & DISCUSSION

In 2018, the fruit quality program has continued to focus part of its effort on Honeycrisp fruit quality. Based on the membership of WTFRC staff in the WSU Tree Fruit Extension team, Hanrahan directed the development of a WA 38 starch scale and served as co-organizer of the 2018 Postharvest Fruit School. On August 17, 2018 Dr. Hanrahan accepted a new position as Executive Director of the WTFRC. As a direct result of her new appointment she will phase out her direct involvement in applied horticultural experiments and has resigned from the WSU Tree Fruit Extension Team.

Induced bitter pit

After 4-8 weeks of exponential rise in bitter pit incidence, the expression of additional bitter pit in remaining fruit slowed down and eventually approached zero (see Figure 1). After a 12-week storage period, Honeycrisp apples from orchard 1 showed one apple with symptoms (1%), from orchard 2, 25 apples (25%), from orchard 3, 27 apples (22%) and from orchard 4, 34 apples (28%) had developed bitter pit.

In orchard 1 and 3, none of the methods predicted bitter pit. In orchard 2, only the Ethephon method predicted presence of bitter pit, but it underestimated the observed severity since there was an average of bitter pit three times bigger in storage. In orchard 4, all methods predicted a certain amount of bitter pit after 12 weeks in storage. PennState method was the most accurate in orchard 4.

One aspect of bitter pit prediction that is not considered in any of the current methods involves the amount of fruit left in the orchard due to bitter pit incidence at the time of harvest. The average in-field loss from bitter pit in 2018 was relatively low, between 0% and 4%. In orchard 1, we determined

that no fruit was left in the field due to bitter pit. Orchard 2 had more in-field bitter pit with an average of 2%. Orchard 3, 1% was left due to bitter pit in field, and orchard 4 had 4% of the crop with discernable bitter pit symptoms before harvest. After 12 weeks in storage, between 1% and 28% of the apples harvested symptom free had developed bitter pit. Orchard 1 developed 1%, orchard 2 25%, orchard 3 22%, and orchard 4 28% bitter pit in storage. This means that one cannot predict storage bitter pit potential based on field symptom expression alone. For example: All orchards had low bitter pit incidence in the field (0-4%). However, the second orchard developed 25% bitter pit in storage, while 28% of fruit from the fourth orchard developed bitter pit. Orchard one had the lowest preharvest loss due to bitter pit orchard 2 was one of the worst orchards for bitter pit development in storage.

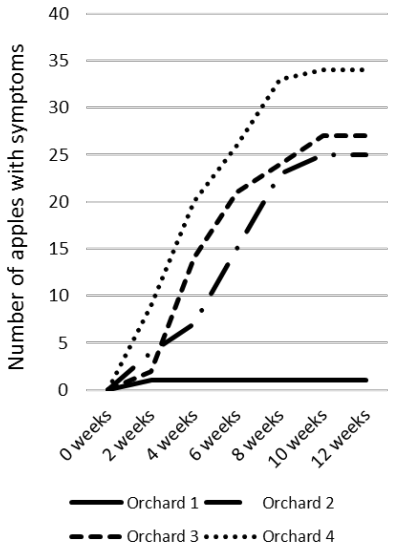


Figure 1: Amount of untreated control fruit developing bitter pit during 12 weeks of storage (1 week at 50°F followed by 11 weeks at 36°F). n=120.

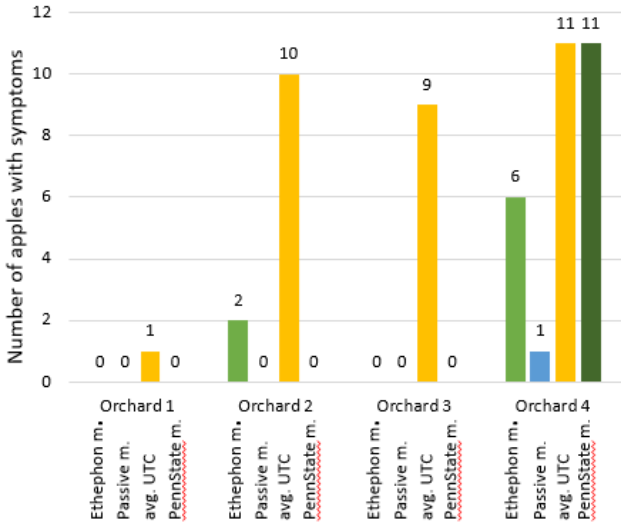


Figure2: Comparison of four methods to predict bitter pit in Honeycrisp apples, n=40, error bars=SD. (UTC= untreated control)

WSU Tree Fruit Extension team postharvest activities:

WA 38 starch scale:

For the new starch scale, 707 apple samples were cut in 2018; 486 in October, 200 in November and 21 in December. To achieve a surface reflecting the middle of the core, WA 38 apple fruit were cut, unlike other industry specific varieties, right through the equator and the surface was scraped to have a clean pattern. During the experiment, two predominant patterns were observed. The commonly known "flower" pattern (see Figure 3), which is characterized by 5 "flower petals" growing with advancing maturity. The other pattern was named "radial" (see Figure 4), and it can be compared with a "sunrise". The radial pattern clears larger parts of the cut surface in a radial fashion with advanced maturity. The patterns appeared in a 6:4 ratio in 2017 and in a 3:7 ratio in 2018.

Another observation was the slow disappearance (aka slow fruit ripening) of dark color and consequently starch. This was similar to observations from previous years with cv. WA38 apples at the WTFRC. In the new starch scale this fact was considered and a scale with half point increments was developed (see Figure 5).

The experiment also showed that the starch patterns took longer to develop, compared to other industry specific varieties. Depending on fruit temperature and maturity, the development of a complete starch staining took up to one hour. In most cases the pattern developed fully after 28-30 minutes if the fruit is warm (74°F), 50-60 minutes if the fruit came from cold storage. Starch readings

are possible in less than 5 minutes. In comparison, Granny Smith took up to 10 minutes, when the fruit came from cold storage and 2.5 minutes, when the fruit was warm (70°F).

Figure 3: Wilson chart (4x6 inches) for cv. WA38 "flower" pattern.

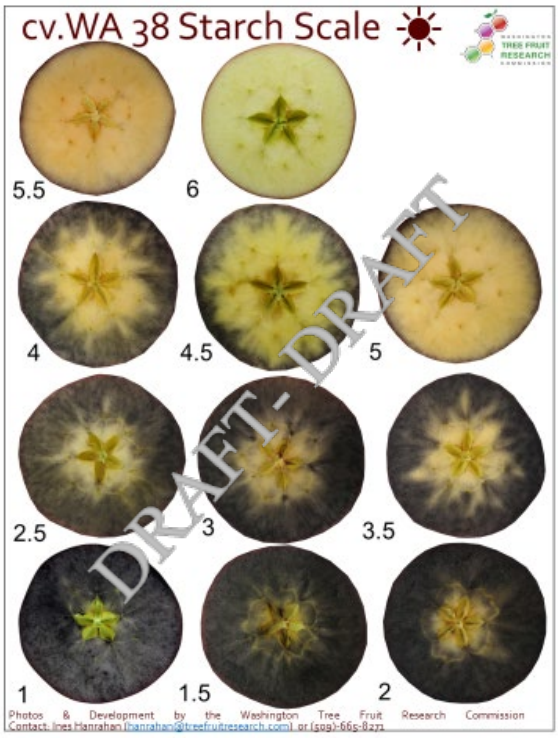


Figure 4: Wilson chart (4x6 inches) for cv. WA38 "radial" pattern.

This year, we incorporated feedback received from our industry focus group. The scale was refined by incorporating half steps for “radial” pattern.
We also discovered that the presence of water core, sunburn and green spot interferes with the starch pattern. All of them showed starch clearing patterns similar to Honeycrisp starch pattern (figure 6).

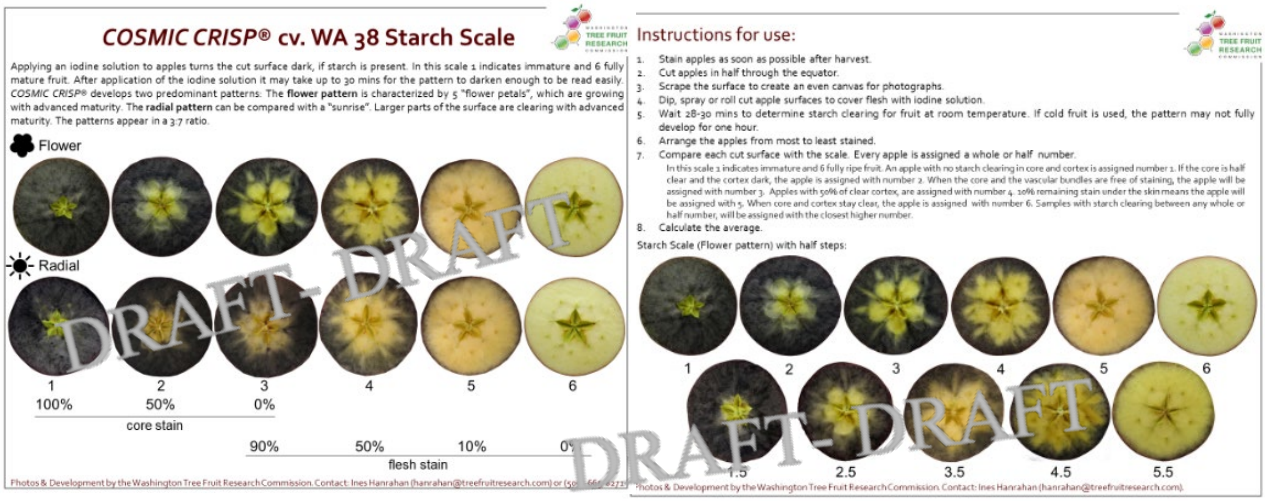
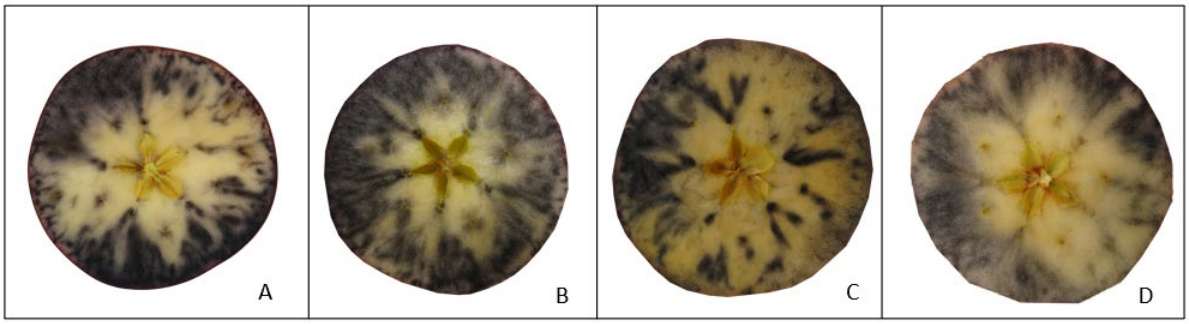


Figure 5: cv. WA38 starch scale and "flower" pattern scale with half steps.

Presence of water core and green spot revealed an uneven maturity on the starch pattern. It was noticeable that sometimes the pattern was not even, showing more maturity on one side of the cut. We thought it might be related to sun exposure. In general, apples show advanced maturity (starch clearing) on the sun exposed side of the fruit. For WA 38 we could not attribute uneven starch clearing patterns to differences in sun exposure.



A= water core; B= sunburn; C= green spot; D= uneven maturity

Figure 6: Examples of unusual starch patterns.

We have been observing the starch movement through the vertical cut. The starch in the stem bowl cleared ahead of the remainder of the fruit. The calyx end has delayed starch clearance. We hypothesize that even before reaching full maturity, WA38 is likely to have splits, and the vertical starch pattern may predict the likelihood of splits in the stem bowl. But we will have to finish data analysis to confirm. Along with all of our observations, we have been observing the starch degradation of WA38 apples in storage, during 8 weeks in a cold room at 36°F.

Apple Defect guide:

During the 2016-2017 and the 2017-2018 storage seasons samples were collected from local packers to complete an initial set of 106 defects (~10,000 photographs). Fruit is grouped by variety, defect, and severity of symptoms. TJ Mullinax of the Good Fruit Grower photographed samples of fruit, including views of external and internal symptoms, and additional photographs were taken on a rotating platform. Darrell Kilgore and Matt Ziegler from the WSU CAHNRS Video & Photography Department produced 76 rotations. These will be used in an online guide with rotatable photographs with annotations and descriptions. Wendy Jones is leading the development of a five-poster series (insect, preharvest, postharvest, Honeycrisp, decay), a website interface and a waterproof hard copy with photographs and defect descriptions. Depending on resources, a Spanish version and an APP could be produced at a later stage. Starting during the 2017-18 storage season Blakey and Hanrahan are creating (i) descriptive text for each disorder, (ii) annotated call outs to appear on points of interest on the rotating photographs, (iii) a sorting system including cross linking, common misidentification, and links to additional sources, (iiii) poster content.
The project was funded partially by the WSU Tree Fruit Extension team, with costs to be recovered from selling posters and the hard copy version. It was agreed that any profits would accrue to the Tree Fruit Postharvest Extension Specialist. Karen Lewis has taken over as PI since Blakey left WSU employment.

We have been asked to partner with a group of European scientists to integrate the disorder guide with tools that are in existence in Europe. An Interreg project proposal has been prepared and project funding is currently pending.

Postharvest fruit school:

A Postharvest fruit school was developed by the WSU Extension Tree Fruit Team and held in two locations (Prosser, Wenatchee) March 20-22 of 2018. Fruit Schools are designed to delve deep into topics important to the industry using a combination of presentation, discussion, hands-on activities, and demonstration. Regional, national, and international speakers (12 invited speakers) were equally located at both Wenatchee and Prosser to facilitate interaction with attendees. To make the meeting affordable, we secured 13 sponsors (>$40,000). The first day focused on the principles of postharvest science and management. The second day addressed crop specific problems with sessions for apples and berries (morning), and cherries and pears (afternoon). The third day consisted of warehouse tours in both the Yakima valley and Wenatchee locations.

Collaborative research

We continue to build productive and dynamic research and outreach partnerships with a number of cooperators on projects relevant to pre and postharvest fruit quality management (Table 1).

Table 1. 2017 Hanrahan/WTFRC collaborations on pre-and post- harvest fruit quality projects.

COLLABORATOR(S)	PROJECT	HANRAHAN ROLE
2018 (continuing and new)		
Evans	WSU breeding: P3	Collaborator storage evaluation
Univ. of Talca*	Superficial scald control	Contract project
Lewis/Blakey	NEW WSU Apple Defect Guide	CO-PI

*project costs completely covered by companies/external projects

In addition, Hanrahan and her team participated in several events geared towards organic production to develop specific knowledge and expertise in this area. Further, since roughly 40% of all apple trees to be planted in 2018 will belong to one of ± 36 clubs, the team visited established plantings, obtained samples to observe and taste and gathered information on horticultural challenges, maturity, storability etc.

Lastly, Dr. Hanrahan went on a trip oversees to Europe (Italy, Germany: January) to participate in grower meetings, meet with collaborators, and to visit WA 38 plantings. In August she participated in the NE postharvest meetings ahead of the American Society of Horticultural Science Annual Meeting in Washington DC. The information gathered on those trips has been shared with the WTFRC board and management, local collaborators, the extension team, and industry stakeholder groups (Pomclub, NCWFA) through a variety of means (report, ppts, personal visits). Further, we utilized the information to optimize our information transfer for the postharvest fruit school.

CONTINUING PROJECT REPORT **YEAR:** 3 of 3
WTFRC Project Number: AP-16-101 No-cost extension

Project Title: Reducing scald after long-term CA storage

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

Budget: **Year 1**: $30,690 **Year 2**: $63,095 **Year 3**: $72,508

Collaborators: Brenton Poirier, Ed Valdez, Loren Honaas, Girish Ganjyal, Ines Hanrahan, Heidi Hargarten

WTFRC Collaborative expenses:

Item	2016	2017	2018	2019
Salaries				
Benefits				
Wages				
Benefits				
RCA Room Rental	$6,300	$6,300	$6,300	
Shipping				
Supplies				
Travel				
Plot Fees				
Miscellaneous				
Total	$6,300	$6,300	$6,300	**0**

Footnotes: Costs for 1 RCA room

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

Item	2016	2017	2018	2019
Salaries	$18,338	$39,004	$41,344	
Benefits	$6,052	$12,871	$13,644	
Wages				
Benefits				
Equipment				
Supplies				
Travel				
Miscellaneous *		$11,220	$11,220	
Plot Fees				
Total	$24,390	$75,695	$66,208	**0**

Footnotes: One-third of instrument service contract

OBJECTIVES:
1. Identify rapid, stress provoking, at-harvest treatments that reduce scald levels during a prolonged supply chain.
2. Validate changes in peel chemistry as indicators of efficacy for stress-based scald treatments.
3. Determine how at-harvest treatments that provoke stress impact other fruit quality factors.
4. Determine if post-storage reduction of ethylene action is a feasible post-storage scald control technique.

Goals and activities for continuing work:
Determine how post-storage applications of 1-MCP and DPA impact peel chemistry and quality traits throughout a simulated supply chain as compared with immediate postharvest applications.
Determine the feasibility of integrating post-storage hot water treatments as a scald management strategy for organic apples.

SIGNIFICANT FINDINGS:
1. Scald induction is cumulative and rapidly imposed, effective CA reduces the rate of induction.
2. Depending upon how much scald induction has occurred, post-CA 1-MCP and hot water treatments can reduce scald in the cold chain to varying degrees.
3. At-harvest delayed cold storage (2 d) or intermittent warming, or hot water treatment reduces scald better on more mature fruit but only during air storage.
4. Hot water treatment following effective CA storage (3 months 0.5% O_2 CA storage) but not at-harvest, reduces scald during the post-CA storage cold chain.
5. At-harvest hot water or acclimation treatments do not reduce scald following effective CA because scald induction continues once fruit are removed from CA and enter the cold chain.
6. 1-MCP treatment following effective CA storage (3 or 6 months 0.5-0.8% O_2 CA storage) reduces scald in the subsequent cold chain.
7. Cold chain temperature following CA storage for organic Granny Smith should be below 37 °F and as close to 33 °F as possible for prolonged periods and optimally not above 45 °F on retail display.
8. New scald risk assessment biomarkers based on gene expression indicate if storage environment was effective after up to 6 months (Honaas Lab).

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

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

Year one focused on the development and characterization of temperature conditioning and stress amendment treatments for scald reduction including delayed cooling, intermittent warming, initial heat shock with an impingement oven, impact injury, and the use of chemical stressors. We also focused on optimizing post-CA supply chain conditions for scald reduction by testing the effectiveness of post-storage 1-MCP treatments and optimizing supply-chain storage temperatures. These experiments included metabolic profiling efforts, monitoring of scald risk assessment biomarkers, and assessment of quality traits and scald development.

Year 2 (includes objectives 1, 2, and 3)

Heat treatments. Granny Smith apples were harvested from Sunrise Research Orchard on October 6th (average Brix of 10.1, starch index of 2.5 out of 6) and October 20th (Brix:11.4, starch index: 3.5). Apples from each harvest were subjected to three different heat treatments prior to storage: (1) hot water submergence (118°F for 3 min); (2) warm air (100°F for 72 h); and (3) hot air (108°F for 24 h). Untreated controls were included for both storage conditions.

Delayed DPA in different CA environments.

Granny Smith apples harvested from Sunrise Orchard on October 17th were stored at 33°F in air and three different CA storage conditions: (1) 2% O_2 (0.5% CO_2); (2) 1% O_2 (0.5% CO_2), and (3) 0.5% O_2 (0.5% CO_2). Apples were treated with DPA immediately following harvest, and additional treatments were performed on separate apples at 1 week, 2 weeks, 4 weeks, 6 weeks, 8 weeks, 3 months, 4 months, and 6 months postharvest. Untreated controls were included for each storage condition. All apples were moved to 37°F air storage after 6 months for continued evaluation.

Post-storage 1-MCP treatment with CA storage delays.

Granny Smith apples were placed into CA storage (33°F, 0.6% O_2:0.5% CO_2) immediately after harvest or following a 2 week or 4 week delay. After 6 months, all apples were removed from CA and placed in 37°F air storage. Apples from each of the three storage conditions were treated with 1-MCP (1 ppm for 12h) immediately upon removal from CA. Separate sets of apples from immediate CA storage were treated with 1-MCP following a 1 day or 2 day delay after removal from CA. Untreated controls were included for each CA storage condition.

Post-storage heat treatment and wounding experiments.

Granny Smith apples were placed into CA storage (33°F, 0.6% O_2:0.5% CO_2) immediately after harvest. After removal from CA at 3 months, apples were subjected to either hot water (118°F for 3 min) or warm air (68°F for 2 days) temperature treatments. Additional apples underwent an injury treatment of bruising with a 5.6 g ball-bearing dropped from a height of 38 cm or were punctured with a syringe.

Post-CA supply chain optimization.

Granny Smith were sampled from 4 lots stored commercially (multiple rooms) at 0.6-0.8% O_2:1% CO_2 for 6 months. Within 2 days following removal from CA, apples were placed in 33 °F, 35 °F, or 37 °F air for two months, then moved to a simulated retail temperature of 68°F for one week. Two additional temperatures (55°F and 45°F) were used for apples that were stored 33°F. Scald evaluations were performed monthly and following the 1 week of simulated retail storage.

Year 3 (includes objectives 2, 3, and 4)

The post-storage hot water treatment and post-storage 1-MCP treatment with CA storage delays are being repeated using fruit from three orchards (protocols listed above). An additional experiment was included for post-storage DPA treatment with CA storage delays, using an identical experimental design. Changes in peel chemistry, quality traits, and scald development will be evaluated throughout a simulated supply-chain period. These data will be compared with apples that received hot water and 1-MCP treatments immediately after harvest to determine the relative effectiveness post-CA treatments.

RESULTS AND DISCUSSION

A number of stress-inducing or acclimation treatments were employed (ozone, nitrogen dioxide, hydrogen peroxide, superoxide, non-bruising physical stressor, bruising, intermittent warming, hot water, irradiant heat) in year 1 with or without a 2 day waiting period at 68°F. Bruising, intermittent warming, hot water, and the 2 day waiting period were the only at-harvest treatments with consistent

impact and only during air storage but not following CA. Treatments that effectively reduced scald after CA were reducing post-storage cold chain temperature and post-storage 1-MCP. Much of the following year focused on scald reduction during the post-CA period, repeating experiments from year 1 and determining why at-harvest treatments that reduce scald during air storage immediately after harvest do not reduce scald following ultra-low O_2 CA.

Post-storage 1-MCP treatment reduces scald during a prolonged cold chain following CA storage.
Year 1 results revealed that a 12 h 1 ppm 1-MCP treatment reduced scald during a 4 month cold chain following 3 and 6 months ultra-low (ULO) O_2 (0.5% O_2) CA storage (not shown) in our storage chambers. In Year 2, again scald was reduced following 3 months ultra-low O_2 CA storage (0.5% O_2; Fig. 1, left and center) as well as in multiple lots of commercially CA stored Granny Smith (0.6-0.8% O_2; Fig. 1, right)

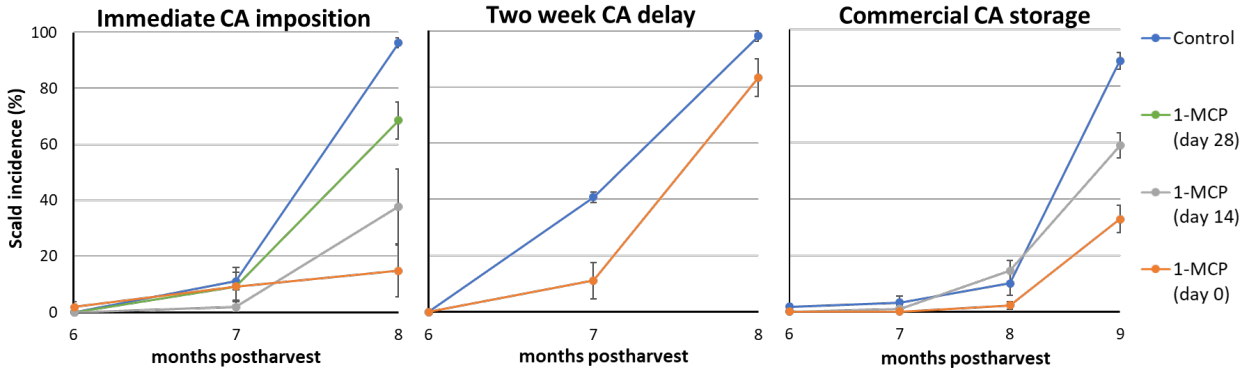


Fig. 1. Post-CA (0.5% O_2: 0.5% CO_2)-storage 1-MCP treatment reduces scald during a 2-month post-storage supply chain in multiple seasons. Post-storage 1-MCP treatment also reduced scald following commercial storage at (0.6% O_2). Control diminishes as 1-MCP treatment is delayed up to 28 days following removal from storage (left). A similar loss of scald control occurs when CA imposition (at harvest) is delayed for 2 weeks (right) indicating rapid establishment of ultra-low O_2 CA delivers the best outcomes. Error bars = standard error.

Post-storage (0.5% O_2–3 months) heat treatment reduces scald development. The consensus of our at-harvest stress and wounding treatments intended to reduce scald during a prolonged cold chain revealed that scald control using this general strategy only reduced scald development during air storage. However, as in most of our past work, once fruit were removed from CA it would eventually develop scald after months in air at 33 °F and stress or wounding treatments at harvest had little impact on latter cold chain scald where we proposed to reduce scald. It appears that scald reduction conferred by these at-harvest treatments was lost over a long period of ultra-low oxygen CA.

Consequently, year 2 experiments focused on determining why this was not working and if and when these sorts of scald mitigation strategies would provide any benefit. We found that scald reduction mediated by warm air, hot air, or hot water treatments was more effective for controlling scald on relatively more mature (at harvest) apples (Fig. 2). As in Year 1, it impacted scald incidence during air storage and not following CA storage during a prolonged cold chain. However, hot water after removal from 3 months CA reduced scald beyond 5 months in air at 33 °F (Fig. 2). This may be an easily integrated cost-effective scald control measure.

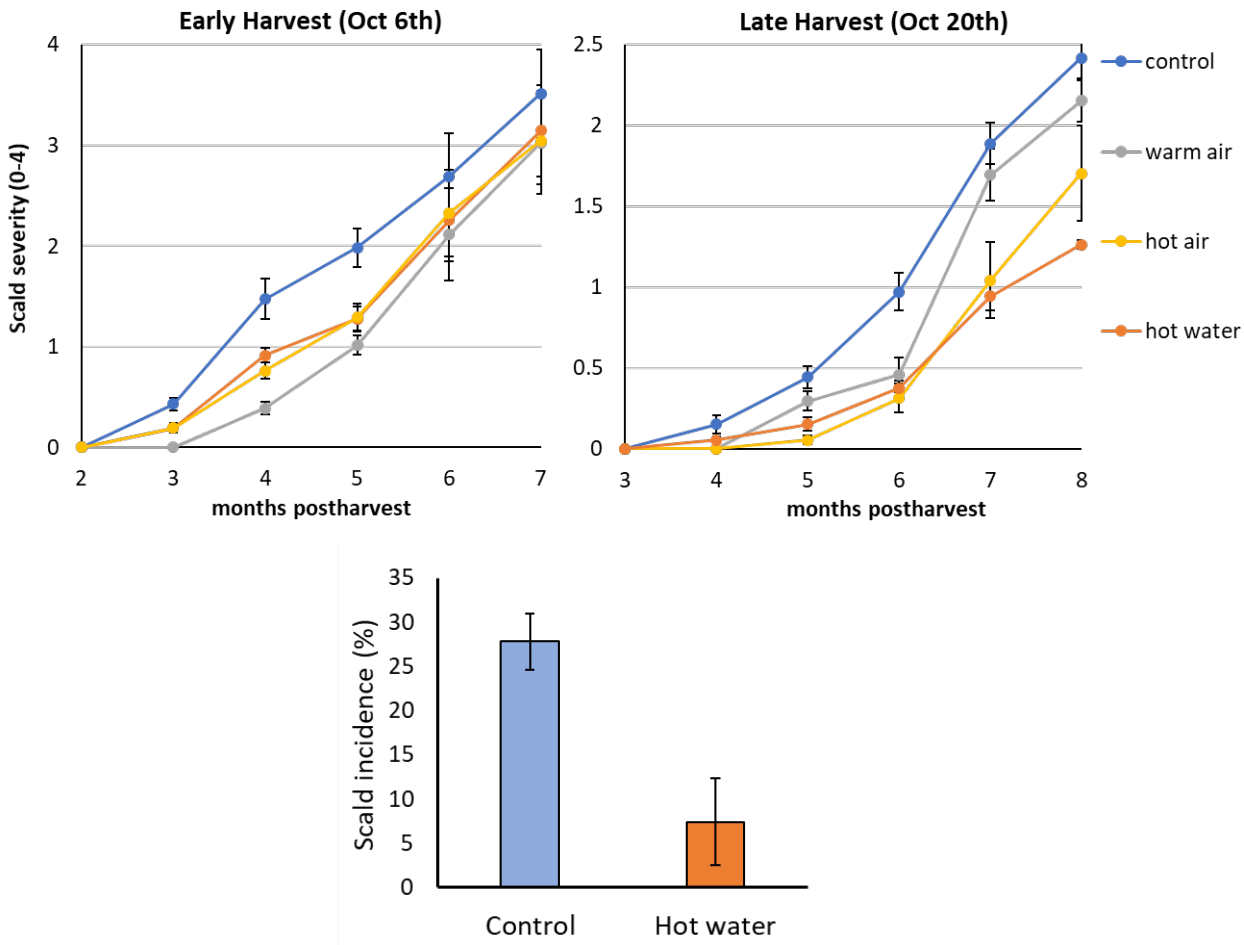


Fig. 2. At-harvest hot water, hot air and warm air treatments reduce scald during air storage but not following CA storage (0.5% O_2: 0.5% CO_2). Scald reduction using these strategies was most effective on mature fruit (Top left and top right). Post-CA-storage hot water treatment reduced scald up to 5 months following 3 months CA storage (bottom). Error bars = standard error.

Scald induction period is prolonged by ultra-low controlled atmosphere storage

Our results demonstrating the effectiveness 1-MCP and hot water treatment following immediately applied ULO contradict conventional understanding of scald control. It was previously considered difficult, if not impossible, to control superficial scald if the treatment was not applied within the first few weeks of cold storage. This typically meant assuring susceptible cultivars were drenched or otherwise treated with DPA and/or gassed with 1-MCP soon after harvest or upon placing fruit into the cold. It is well known in the scientific literature and from our previous experience that scald control using DPA or 1-MCP treatment wanes after apples have been placed in air storage. Control weakens after 1-2 weeks of storage, and 1-2 months is the longest delay with any measurable control. However, in this way, we can define the period during which cumulative cold stress causes scald, and this is fairly regular year-to-year. This event can be defined as the "scald induction period" or when the invisible injury that later leads to superficial scald occurs.

When apples are produced without these crop protectants, scald control relies on effective control by establishing and maintaining the appropriate atmosphere, typically the lowest affordable O_2 setting that does not cause other disorders. More conventional CA settings (1% O_2 and above) do not provide the same scald control as often evinced by scalded apples as fruit approach 6 months storage.

Quick room loading and establishment of proper CA atmosphere before too much of the scald induction period has transpired is critical when depending on CA for scald control. However, once effective CA is established, what happens in terms of this scald induction period?

Unlike DPA and 1-MCP treatment, ultra-low O_2 CA storage typically does not confer scald control as effectively following long term CA. Typically scald begins to appear on fruit from 2-4 months following removal from storage if apples are maintained at proper temperatures (see below) or about the same amount of time it takes untreated apples to develop scald if stored in air—the scald induction period. If this were the case—if scald induction were delayed, at least in part, until after CA storage—we could expect at-harvest treatments that impact scald to also be effective if employed following storage, as we report here where scald outcome is improved following post-storage 1-MCP and hot water treatment (Figs. 1 and 2).

Other findings indicate that effective CA prolongs scald induction. First, DPA treatment remains partially effective up to 6 months in CA at 1.0% O_2 as opposed to 2-4 weeks in air (Fig. 3–left) indicating the scald induction period is indeed delayed even at these suboptimal scald control conditions. Delaying CA establishment for up to 2 weeks reduces scald control conferred by 1-MCP treatment immediately upon removal from effective CA (Fig. 1–center). Also, delaying post-CA 1-MCP treatment up to 1 month successively reduces scald reduction (Fig. 1–left). This demonstrates the cumulative nature of scald induction which begins at harvest, then transpires for 2-4 weeks more upon removal from CA storage. Finally, physical wounding following ULO CA causes the same scald clearing as that observed after air storage (but not CA) if wounding occurs at-harvest. Taken altogether, as proper CA conditions better control scald, the opportunities for scald control after storage approach those at harvest.

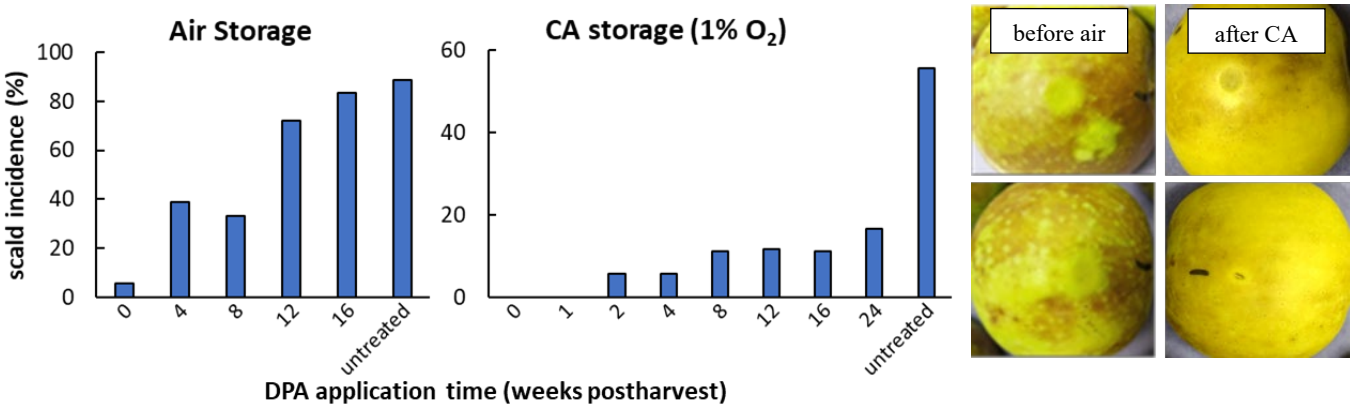


Fig. 3. Delayed DPA treatment during CA (1% O_2: 0.5% CO_2) and air storage demonstrates how CA prolongs the scald induction period (left). Physical wounding prior to air storage or following CA storage causes scald clearing (right). Physical wounding at harvest does not cause the same clearing on fruit stored in CA unless it occurs after CA.

Post-CA storage temperatures are critical for scald reduction in a crop protectant restricted cold-chain. Results from the previous year indicated that after commercial CA storage the optimal supply-chain temperature for minimizing scald was somewhere between 33-37°F (Fig. 4, left). Our most recent experiments determined to optimize supply-chain temperature and study the impact of subsequent retail temperature on scald development using 4 organic Granny Smith lots after six months of commercial CA storage (0.6-0.8% O_2). Consistent with the previous year's experiment, lowering the storage temperature from 37°F to 33°F decrease scald development, but the largest benefit came from decreasing the retail storage temperature (Fig. 4, right). A moderate decrease in scald was observed when temperatures were decreased from 68°F to 55°F after only one week, and

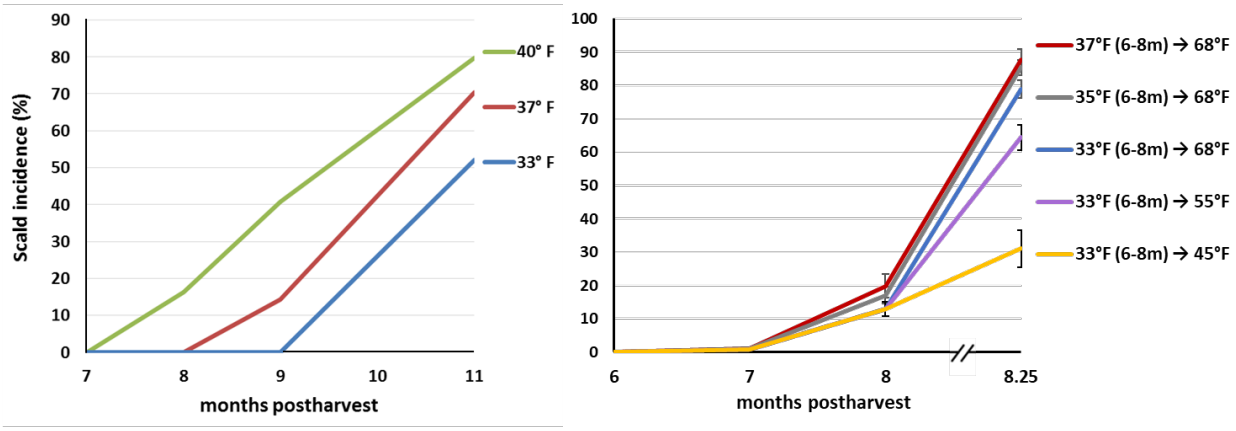


Fig. 4. Cooler temperatures reduce scald on commercial Granny Smith lots following storage at 0.6-0.8% O_2:1% CO_2 for 6 months. During year 1, 33, 37, and 40 °F was tested (left). In Year 2, we focused on determining the optimum temperature between 33 and 37 °F for 2 months post-storage and, then, best simulated retail temperature between 45 and 68 °F for 1 week starting at 8 months after harvest.

further decreasing the temperature to 45°F resulted in a dramatic (~2.5-fold) reduction in scald occurrence. A great deal of effort is expended to maintain apples at the lowest possible temperature during storage, but dramatic increases in scald development arising from increased retail temperature may ultimately nullify the benefits of CA and low storage temperatures.

Conclusions

Where control options are limited by regulation or customer requirement, scald control strategies rely on advanced CA storage technologies and, where cold chains are months beyond removal from storage, soft controls such as acclimation and hot water. When scald is controlled by ULO, the induction period is prolonged or even delayed until after storage. This may afford new options with respect to post-storage 1-MCP treatment if a shift in marketing strategy is necessary but also makes it necessary to apply acclimation or temperature treatments that can reduce scald until after removal from CA. A critical activity when employing post-storage scald control strategies is estimating how much of the induction period has occurred during CA storage as any delay in CA atmosphere establishment, period of sub-optimal O2 settings, or extended storage period may negatively influence any post-storage scald treatment. Using the protocol for monitoring scald risk (Blakey and Rudell, 2017) may be one way of assessing how effectively a particular strategy is controlling scald induction up to 3 months in storage. Finally, cold chain temperature, including retail storage is especially critical for maintaining scald free fruit where crop protectants are unavailable.

Publications

Blakey, R. and D.R. Rudell. 2017. Superficial scald risk assessment assay for apples. WSU Extension Bulletin FS287E. (www.extension.wsu.edu/publications/)

CONTINUING PROJECT REPORT **YEAR:** 3 of 3
WTFRC Project Number: AP-16-102 **No-cost extension**

Project Title: Risk assessment for delayed sunburn and sunscald

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Budget: **Year 1**: $67,427 **Year 2**: $70,865 **Year 3**: $72,595

Collaborators: Christine McTavish, Omar Hernández, Brenton Poirier, Loren Honaas

Other funding sources

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

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

Item	2016	2017	2018	2019
Salaries	$40,757	$43,342	$44,620	
Benefits	$13,450	$14,303	$14,755	
Wages				
Benefits				
Equipment				
Supplies				
Travel	$2,000	$2,000	$2,000	
Miscellaneous*	$11,220	$11,220	$11,220	
Plot Fees				
Total	$67,427	$70,865	$72,595	**0**

Footnotes: One-third instrument service contract

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

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

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

Remaining goals and activities:
Determine if sunscald-like injury induced by heating in the orchard has similar natural chemical levels in the cutin and peel when compared to sunscalded material. Determine the timing of sun stress events before harvest that provoke sunscald. Alter cutin chemistry artificially using different natural chemicals impacted by the sun and determine the impact on cutin structure. Continue to evaluate how heat alone can impact sunscald incidence, cuticle structure, and cuticle chemistry.

SIGNIFICANT FINDINGS:
1. Peel appearance (symptoms) changes in different ways during storage depending upon cultivar.
2. Peel chemistry changes differentially depending upon pre-harvest sun exposure, indicating continued stress on the exposed side of the fruit during cold storage.
3. Sun exposure alters cutin consistency and may alter cutin structure, thereby altering epidermal cell protection.
4. Heating in the orchard can lead to symptoms or similar appearance and etiology to sunscald.
5. Heating in the orchard can inhibit sunscald development.
6. Simple UV reflectance imaging targeting metabolites associated with light exposure can non-destructively detect relative sun exposure.
7. Identifying changes in multiple metabolic targets detected in the UV-vis-NIR range may be a reliable basis for non-destructive detection of sun stress.

Methods:
2016-2017 (primarily Objective 1)
The influence of the pre-harvest light environment alone and during the transition to cold storage on peel metabolism is different among apple cultivars. Granny Smith, Gala, September Fuji, and Honeycrisp were picked at commercial harvest (see 2017 continuing report). At 0, 2, 4, and 8 weeks (and then monthly until 6 months) sun damage and/or sunscald incidence was monitored and peel chemistry data were analyzed to determine differential changes between the exposed and unexposed sides of the fruit upon chilling stress, possibly linking specific changes in peel chemistry with specific delayed conditions. Additional samples consisting exclusively of sunscald tissue were analyzed to determine where peel chemistry was most linked with the disorder.

2017-2018 (Objectives 1 and 2)
At 4, 2, and 1 week(s) before commercial harvest, 180 hand-thinned Granny Smith apples in the Sunrise experimental orchard were heated on both the sun facing and shaded sides to 130 ºF for 3 minutes (60 apples per timepoint). Fifteen fruit were bagged with green sleeved paper apple bags at each time point and remained so until harvest. Another 15 fruit per timepoint were heated to 130 ºF on the sun facing and shaded sides and then bagged until harvest at each timepoint for a grand total of 270 apples (90 apples treated per timepoint, excluding controls). Four days before harvest, 45 additional fruit were treated at 130, 115, and 100 ºF (15 apples at each temperature). Unheated controls were included at every treatment date. Injury on each fruit was tracked using image analysis

as well a rating system (see 2017 continuing report). Peel from the front and back was sampled at 0, 3, and 6 months and specifically from injured zones to determine the levels of compounds most associated with injury, as well as if compounds associated with sunscald in year 1 were more associated with sun stress, heat stress, or a combination. Peel from both heat damage and sunscald from unheated fruit were compared.

2018-2019 (Objectives 1-3)
Twenty-one hand thinned Granny Smith apples per timepoint were heat treated as detailed above to 125 ºF for 3 min at 2 months, 1 month and 2 weeks prior to harvest to clarify last season's results using an optimized temperature. Additionally, 100 apples were bagged at each timepoint to determine when sun damage leading sunscald may have occurred for a total of 363 apples for the experiment. Temperature optimization improved our assessment of orchard heat and the bagging trial was improved by a better plot design.

A technique to profile cutin composition using Granny Smith peel powder samples from year 1 was developed and these samples analyzed. Briefly, free metabolites are washed from the peel using multiple solvents. The resulting powder is freeze dried followed by incubation in an enzyme buffer to rid the sample of pectin, cellulose, and hemicellulose leaving primarily cutin. The enzyme buffer is changed 5 times over 2 weeks. The cutin is then hydrolyzed, partitioned, the fractions resuspended, and analyzed using 2 different LC-MS analyses. This analysis detected expected aliphatic and hydroxycinnamoyl components as well as some entirely novel monomers.

Samples were taken from sunscalded, shade side, and sun side undamaged peel from the 2017-2018 heat treated apples using a biopsy punch and frozen in cryo-matrix for cryo-sectioning, staining, and confocal microscopic imaging of the cuticle layer. The samples were sectioned by Dr. Loren Honaas using the Leica Cryo Jane. The cuticle was stained and imaged using a Zeiss LSM. Images provide a detailed physical 3-D physical assessment of the cuticle.

Sunburned Granny Smith apples were selected from a commercial lot. Apples were warmed overnight to room temperature and sealed in 1gallon pickle jars and the CO_2 concentration was raised using $^{13}CO_2$ to 2% for 3 min. Apples were removed from jars and peeled immediately and at 4, 8, 24, 48, and 36 h. Peel metabolites were analyzed from frozen peel powder using our polar analysis for sugars, organic acids, amino acids (Leisso et al., 2016) to detect presence of the label through pathways responsible for life-sustaining energy production in the peel. This evaluation will be repeated at 3, 6, and 9 months.

Sunscald prediction model, Vis-NIR reflectance characterization, and targeted near UV imaging
Using 90 Granny Smith fruit from 5 different lots, a model to predict delayed sunscald after 6 months 33 ºF air storage based on the degree of sun exposure at harvest has been developed for Chilean apple producers by the Torres laboratory. Ten other Granny Smith lots were picked from bins for analysis using a Vis-NIR reflectance spectrometer at harvest. Sunscald ratings were taken at 4 months of air storage. Vis-NIR spectra from 108 fruit from each lot were incorporated into existing models from Chile and were tested against actual sunscald incidence. An additional tool was developed using a camera adapted to detect specific wavelengths of light. Target metabolites associated with sunscald risk absorb light in specific wavelengths. Peel will be taken from regions of different absorbance in this bandpass (region of the spectrum) to verify if target peel chemicals are represented by absorption (darkness).

RESULTS AND DISCUSSION:

Storage disorder symptoms resulting from solar stress are cultivar specific

Evaluation of the sun damage incidence indicated differential changes of appearance depending on sun exposure of Honeycrisp and Granny Smith (Fig. 1). For example, Honeycrisp developed severe lenticel blotch on the sun side of many fruit and Granny Smith developed sunscald during the 6 months air storage. These changes and the timing of the changes in every one of these cultivars reference those from multiple previous studies. It is important to note that solar radiation also includes heat which is thought to be a principal cause of sunburn rather than only ultraviolet/visible light. Delayed sunscald, defined by the progressive darkening or browning of the exposed side, is thought to be the continuation of the effects of pre-harvest irradiation well into storage and perhaps cold stress.

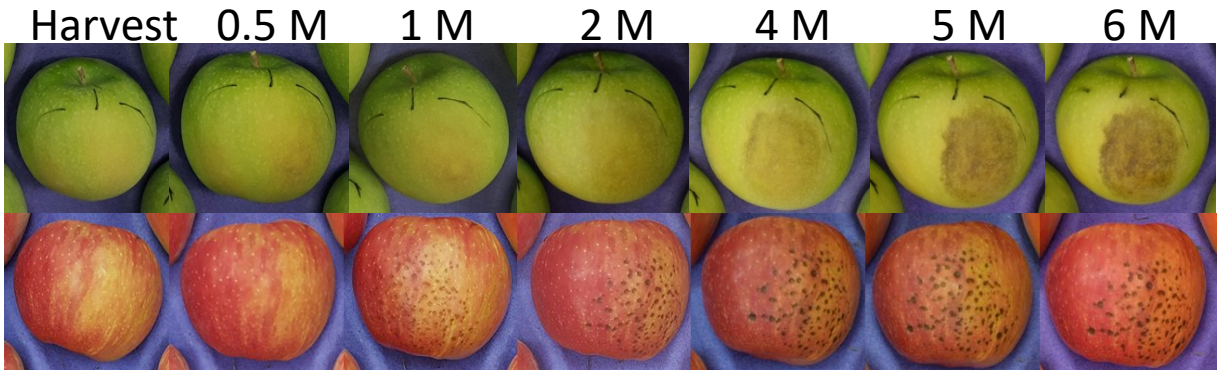

Fig. 1. Examples of different peel disorders occurring on the sun side of different cultivars. Highlighted disorders include (top to bottom) sunscald on Granny Smith and lenticel blotch on Honeycrisp. In each case, the injury was not present at harvest and began to develop after 1 month in 33 ºF air storage.

Sunscald development both caused and inhibited by heat

During the first season of our trial we noticed that a disorder similar in appearance to sunscald occurred following 3 months of air storage on peel that contacted a heated surface prior to storage. Our subsequent treatments in year 2 using a heat gun (130ºF for 3 min) produced severe injury immediately following treatment at all treatment dates (1 month prior to harvest, 2 weeks, 1 week) in most, but not all, cases. Also, in most cases, the injury worsened during the 6 months of air storage, and had a pronounced "halo" of less colored (both red and green) peel around it (Fig. 2). In a few cases, where injury was not as severe, it had an appearance typical of sunscald. The most interesting result of all was the sunscald-free tissue within the halo where heat injury overlapped with natural sunscald on the sun side of a few apples (Fig. 2B). It appears that peel that was damaged by the sun and subsequently treated with the heat gun was rendered resistant to developing sunscald.

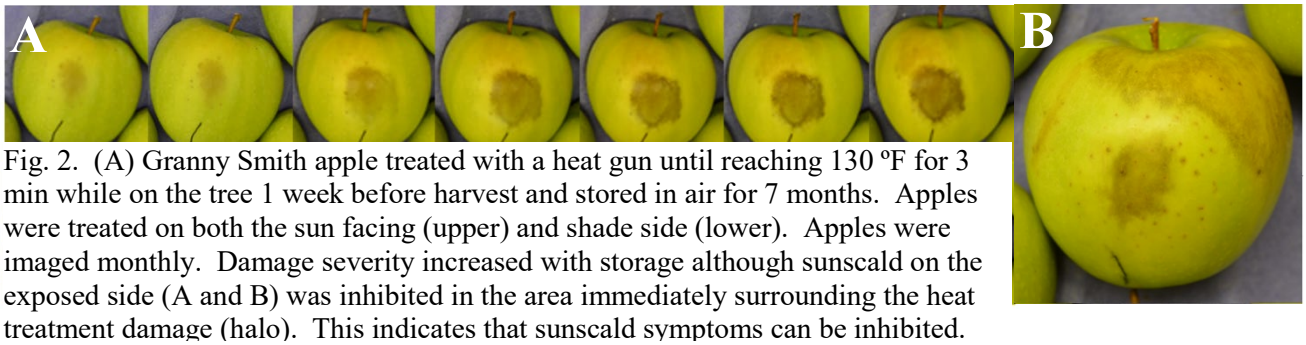

Fig. 2. (A) Granny Smith apple treated with a heat gun until reaching 130 ºF for 3 min while on the tree 1 week before harvest and stored in air for 7 months. Apples were treated on both the sun facing (upper) and shade side (lower). Apples were imaged monthly. Damage severity increased with storage although sunscald on the exposed side (A and B) was inhibited in the area immediately surrounding the heat treatment damage (halo). This indicates that sunscald symptoms can be inhibited.

The experiment was refined in year 3 using an improved orchard block design as well as an optimized heat treatment temperature (125ºF). Results-to-date support those from year 2 although, the symptoms provoked by the lower temperature heat treatment appeared approximately a month into storage and, in many cases, were similar in appearance to sunscald on both sides of the fruit. Prior to symptom development, the treated region with greater chlorophyll degreening surrounded by a halo of slightly more discolored peel was apparent, appearing much like slightly sun damaged peel in the orchard. Sunscald also began to appear on the sun side of some un(heat)treated apples at the same time.

These results indicate heat likely contributes more to sunscald incidence than ultraviolet/visible light and, as is the case with sunburn, warmer air temperatures enhance the risk. However, unlike sunburn, this injury can occur in areas where there is little evidence of sun-stress at harvest. In year 3, heat treatment largely simulated sunscald symptom appearance and disease progression. Another unexpected result is that the heating can also provoke an either physical and/or chemical response that renders a portion of the peel resistant to developing sunscald. This indicates that heat can actually correct an earlier sunscald-provoking event, although there is still no evidence of what conditions make one event damaging and the other curative. Ongoing activities are attempting to answer this and what physical and chemical changes occur upon heating, as well as how closely related the heat gun and sun provoked symptoms are to each other.

Differences of natural peel chemistry during storage are linked with sun exposure
To investigate any difference in chemical composition during the first 6 months of storage caused by the combination of cold stress and sun exposure, peel from either side of each cultivar was sampled during storage as outlined above. Sun exposure impacted levels of a number of chemicals produced by multiple metabolic pathways and residing in different layers of apple peel. Aside from many of the differences in levels of both water soluble and oily pigments, levels of compounds residing in the wax layer, cellular membranes, and aroma differed depending upon sun exposure. Using a statistical analysis that finds the main influence of experimental factors (treatments or differences in appearance we expect or employ in our tests), we determined that peel differed depending upon sun exposure. We distinguished peel according to sun exposure for all cultivars on this basis. Peel chemistry continued to diverge between sides of the fruit during storage. Honeycrisp and Fuji were more mature when picked and the contrast between sides was not as dramatic. Some of the major differences were the elevated levels of pigments and related compounds including quercetin glycosides known to be associated with light exposure and sun damage. Other compounds included volatile metabolites that are linked with oxidative stress caused by high light in leaves, suggesting oxidative events responsible for the genesis of these compounds continue after the fruit have been removed from the orchard. Possibly some of the most striking differences were levels of compounds that potentially modify the structure of the wax and cutin layer on the outside of the fruit (Fig. 3A–inset).

Cutin chemistry is altered by sun exposure and changes continue during storage
In order to answer questions about actual changes to the cutin, we developed a method to extract and hydrolyze this otherwise insoluble natural fruit coating from the frozen peel powder samples to evaluate the freely soluble chemicals. Like the freely extractable peel chemicals, the chemicals comprising ‘Granny Smith’ cutin were very different depending upon sun exposure. The main components of apple cutin are lipophilic fatty acids linked together producing a polyester polymer similar to some plastics. Also, comprising a small part of this polymer are hydroxycinnamates of which ferulic acid was higher in the cutin of the sun side when compared to the shade side. Ferulic acid is purportedly higher with sun exposure of waxy leaves but this is the first report of that in apple. Ferulic acid may provide some sun protection in the UV-B range. Some new cutin components were also discovered that are enhanced in the sun side peel. Like the acylated hydroxycinnamates, these

appear to be built from acylated monomers that are knitted into the cutin polymer. Even though it has been hypothesized that the hydroxycinnamates confer some sun protection in other species and organs, the impact that these chemical constituency changes have on cutin structure and function is not necessarily clear. Other evidence indicates changes in relative concentration of these could alter the "breathability" or consistency of these layers at different temperatures.

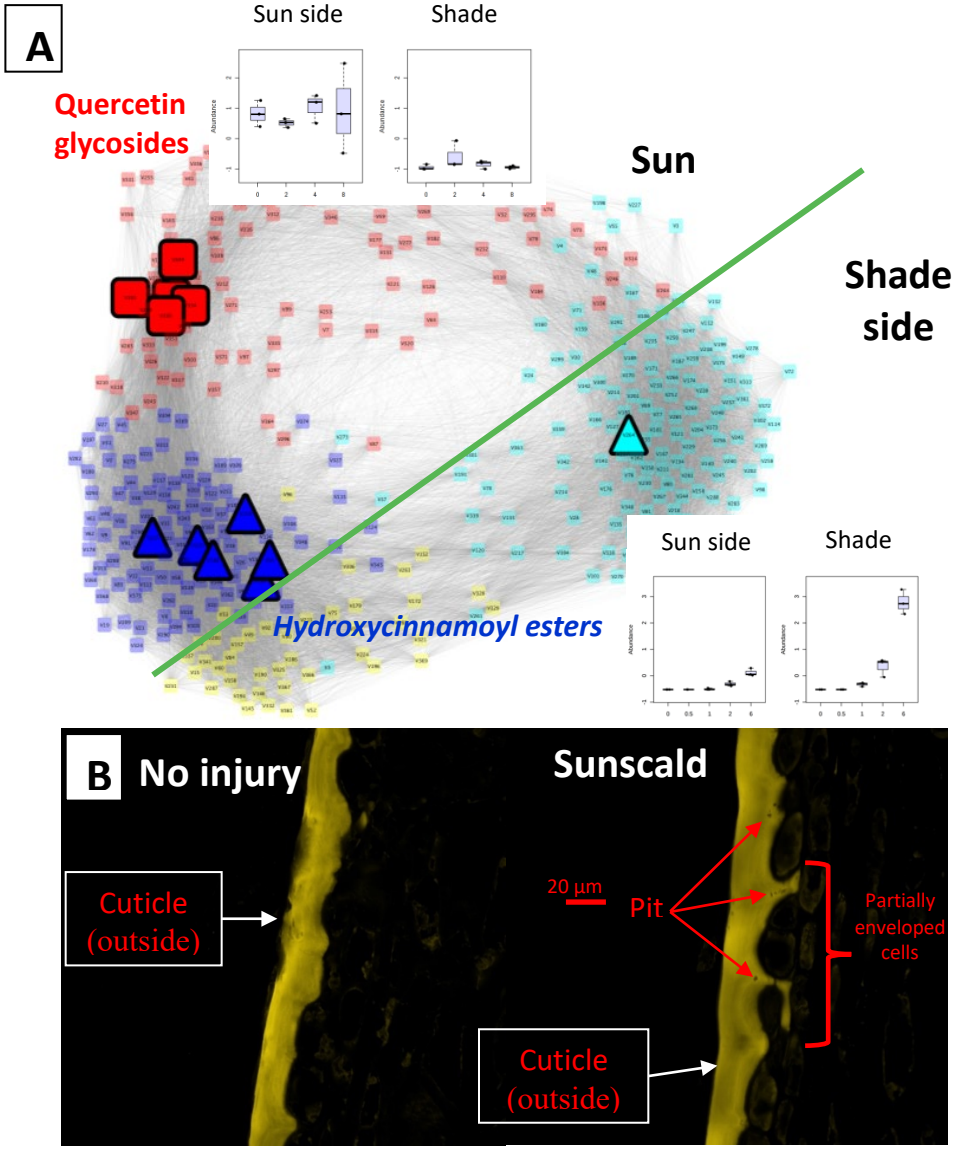

Fig. 3. (A and insets) Examples of natural peel chemicals with higher levels in the sun facing side of Granny Smith peel. These include quercetin glycosides which are a potential target for non-destructive detection as well as hydroxycinnamoyl esters that are likely building blocks of cutin, the external "plastic" coating around plant organs. These and other chemicals with a similar modification appear to be related to sun stress. We have also found that these compounds are actually integrated into the cutin itself. Their impact on the structure and function is unknown. (B) Preliminary micrographic evidence (Auromine O stain, 40X, 2 µm optical section) indicates cutin is deposited deeper in the peel and there are more defects (pits) in the layer when tissue is sunscalded.

Micrographic analysis of sunscalded cuticle reveals extra cuticle deposition and pitted cutin
Preliminary results from our micrographic analysis of Granny Smith cuticle with sunscald reveals at least one physical difference compared with healthy peel on either side of the apple. While results were inconclusive regarding cuticle thickness, areas where the outer layer of epidermal cells are transected or even enveloped by cutin are more prevalent in sunscalded peel after 6 months of storage in air (Fig. 3B). Also, cutin deposited in many of sub-epidermal regions contained pits and channels appearing weakened or degraded. Results point to a change in cutin deposition as an outcome of surface damage and cutin quality, possibly as an outcome of cutin composition. We will employ multiple methods to verify this, including additional microscopy, cutin analysis, and artificially alter cutin composition during storage.

Non-destructive detection of natural chemicals at harvest linked with sun stress
Sunscald prediction models using Vis/NIR reflection of both non-sunburned and sunburned apples at harvest (Grandón et al, in press) were developed by Dr. Torres's group. These models focus on relatively depleted levels of chlorophyll (green color) and anthocyanin (red color) as well as enhanced levels of carotenoid (yellow and orange color) associated with peel at risk for developing sunscald. These models were tested on lots from two local packing sheds. Both models tested revealed significant differences in accumulated reflectance between fruit that did and did not develop sunscald after storage (not shown) indicating they may be useful, with further development, alone or as part of a non-destructive prediction test for Granny Smith in Washington state.

Better non-destructive sun-related postharvest disorder assessment tools will likely focus on additional target metabolites that are even more tightly linked with sun stress compromised peel. Quercetin glycosides have a unique absorption spectrum (less interference), therefore, we chose to target this area of the near ultraviolet spectrum to image peel according to light exposure. We modified a digital camera to acquire images within this absorption range. Our preliminary results indicate that peel on the exposed side is darker or absorbs more light within this spectral band ostensibly due to the elevated presence of quercetin glycosides, and fruit from internal portions of the tree, while still maintaining the contrast between sides, are overall lighter than external fruit (Fig. 4). Vis-NIR along with UV-Vis spectroscopy or imaging is expected to provide a solid non-destructive basis for assessing sun-related disorder risk at harvest.

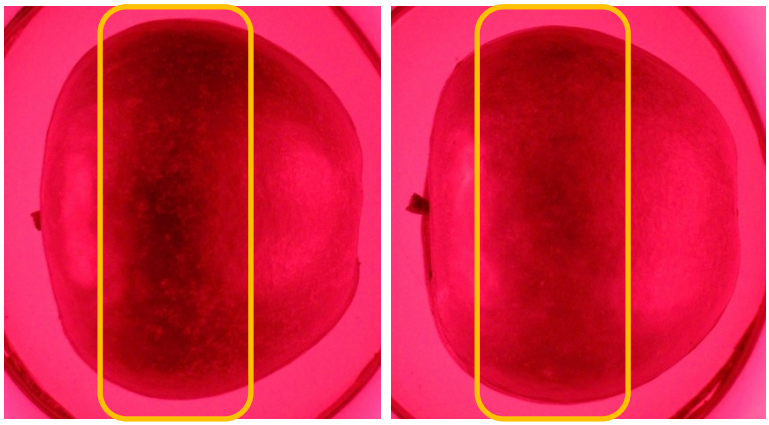

Fig. 4. Granny Smith apple sun side (left) and shade side (right) after one month of storage using a modified camera that displays the absorption spectrum representing quercetin glycosides, one group of target compounds highlighted in our metabolic analysis. Notice the sun side is darker (between the glare caused by the light source on the tops and bottoms of the fruit) indicating the target compound related to higher light exposure may be elevated which could not be visually determined without the aid of the filter. We are investigating this as a basis for sorting apples non-destructively according to their relative sun exposure.

FINAL PROJECT REPORT
WTFRC Project Number: AP15-102A

(This is a final report on the 3-year 2015-2017 funding cycle)

Project Title: Apple scion breeding

PI: Kate Evans
Organization: WSU TFREC
Telephone: 509-663-8181 x245
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Co-PI (2): Cameron Peace
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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

Other funding sources

Agency Name: WTFRC Apple Review
Amount awarded: $107,000 (2015-2018)
Notes: "Combining fire blight resistance and horticultural quality in Washington apples" PI: Norelli. Co-PI: Evans. Synergistic project to identify sources of fire blight resistance.

Agency Name: USDA-CSREES Specialty Crops Research Initiative
Amount awarded: $5.72M (2015-2018 with 1 more year 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.

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

Budget History:

WTFRC Collaborative expenses:

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

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

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

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

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

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

ORIGINAL 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.

SIGNIFICANT FINDINGS

1. Fifty-eight new families were made in 2015-2017 with approximately 79,800 seeds produced in the WSU Apple Breeding Program (WABP).
2. Seedlings from approximately 43,500 seeds from 2014-2016 crosses were grown in the greenhouse.
3. Approximately 26,000 seedlings were screened with DNA markers for fruit quality; just over 15,000 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 10,500 seedling/M.9 trees were produced in the nursery for planting in Phase 1 seedling orchards in 2016-2018.
5. The final count of new Phase 1 trees planted in 2015-2017 was approximately 11,080.
6. Promising selections already in Phase 2 trials (planted in 2007-2016) at three evaluation sites in Central Washington were evaluated for productivity and fruit quality.
7. Twenty-nine new promising selections (on Geneva 41 rootstock) were planted at three evaluation sites in Phase 2 trials in 2015-2017.
8. Three new promising Phase 3 selections were planted (on Geneva 41 rootstock) at the Quincy site.
9. One new promising Phase 2 selection was top-worked onto trees at the Phase 3 Quincy site but was subsequently rejected in the 2017 BPAC meeting.
10. Fruit was harvested and evaluated through storage for new Phase 3 selections. Fruit from the two most advanced selections was available for sampling at the WA Hort Show and at the January 2018 WTFRC Apple Review.
11. A total of seven WA 38 field days were held in 2015-2017.
12. 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.
13. The Apple Cultivar Licensing Committee continued to provide input regarding the release strategy for WA 38 and future releases from the WABP.
14. WA 38 was the focus of a WA Hort Show session (12.5.16)
15. *S*-incompatibility alleles of most parents and selections were deduced using whole genome DNA profiling data.

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 2015 and revisited at the 2016 Review dinner in the form of a clicker survey. Current target priorities were reported in the 2017 continuing report.

DNA test information was used was used to help design crosses for the 2015, 2016 and 2017 seasons and approximately 26,000 seedlings from this project period were screened with DNA tests for fruit quality. DNA tests used were as follows: Ma-indel (acidity, crispness, bitter pit resistance), Md-PG1$_{SSR}$10kdb (firmness), Md-LG1Fru-SSR (fructose content), and Md-ACS1-indel (firmness/storage). Almost 60% of these seedlings were culled in the greenhouse as they were predicted to have less than favorable fruit quality.

Approximately 9,000 seedlings were inoculated with fire blight in the greenhouse to select for resistance.

S-incompatibility alleles for the majority of WABP parents and advanced/elite selections were determined using whole-genome DNA profiles. This information is particularly useful when designing crosses to eliminate the risk of trying to make a fully incompatible cross combination.

Seedlings of 'WA 2' × 'White Angel' from the 2014 crossing season were germinated and sent to Pullman to be raised. 'White Angel' has a different source of resistance to mildew than *M. zumi.* Fei Xiong Luo (a WSU graduate student) developed a DNA test for this 'White Angel' genetic source (Luo et al., submitted) based on genetic map information previously published by Evans and validated by the new seedling population.

Crosses were made between WA 38 and a wild apple relative to introgress fire blight resistance into the breeding program germplasm, using the three *Malus sieversii* accessions selected from the Norelli project ('Fire blight resistance and fruit quality in new Washington cultivars', CP-15-100) as having the best fruit quality. Several hundred seeds were produced from each cross combination which were screened for fire blight resistance in 2017.

All seedling tree blocks (Phase 1 plantings) are now at Columbia View orchard and the breeding program team has been working closely with Cameron Burt (TFREC farm manager) to improve management practices with a focus on improving efficiency and increasing the number of individuals that fruit by year 3. Several hundred seedling selections have been evaluated through the timespan of this project. Evaluation of stored fruit from the 2017 season was completed in the no-cost extension of early 2018.

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

Fruit from 1,041 seedling trees were harvested and evaluated during the 2014-2017 seasons. In addition, fruit from 324 'keeper' selections were evaluated (1,166 samples in total), plus fruit from 81 Phase 2 selections and controls (3,189 samples).

All samples were routinely bar-coded at harvest and then tracked through post-harvest evaluation. Data at the end of the season were analyzed with 'Elite Advance' software, trait by trait, and top-ranking individuals were selected using a combination of these data and breeding team discussion.

The DA meter (T.R. Turoni, Italy) was tested in the 2014 season (data analysed within this project) for determining harvest maturity of Phase 2 selections. This testing confirmed that the results varied

depending on skin and flesh color (white/cream/green hues). While the DA meter is a possibly useful tool for determining harvest maturity on single varieties, the diversity of the breeding program germplasm reduces its applicability. Jamie Coggins (a WSU Masters student) demonstrated that an alternative instrument, the Felix F750 Quality meter, can be used to accurately predict dry matter content and soluble solids content of Phase 2 advanced selections. Prediction models obtained from the Felix were considerably less robust for estimating acidity content or firmness. Consequently, we will have to continue to use destructive measurements to evaluate these important traits. Correlation analysis of the dry matter data with other sensory and instrumental measures is on-going, with the aim of replacing laborious destructive methods.

As a result of collaboration with Dorrie Main's NRSP10 Big Data project, all sensory data collection has been successfully transitioned into the Tablet-based FieldBook App, streamlining the last remaining part of the program that had required manual data entry.

New selections were made for both Phase 2 and Phase 3. Twenty-nine promising seedling selections were planted in Phase 2 during this project. With seven older selections (pre-2015) remaining in Phase 2, there are currently 36 advanced selections in Phase 2 or Phase 3.

Three selections were added to Phase 3 on G.41 rootstock (2015 and 2017 planting) using the staggered start system of planting initially at only one site prior to adding a second site. Fruit was harvested on four elite selections in Phase 3 from the Quincy site and three from the Prosser site. Performance results for three elite selections were discussed at the November 2017 BPAC meeting and fruit samples were provided for tasting. The observation of a relatively large proportion of culled fruit at harvest (23% total yield) of one of these, a 'Honeycrisp' × 'Cripps Pink' selection, validated performance problems that were experienced in the 2016 season. Consequently, the decision was taken to discontinue this selection. Sufficient volumes of fruit from two other 'Golden Delicious'-season elite selections (another 'Honeycrisp' × 'Cripps Pink' selection and a 'Cripps Pink' × 'Honeycrisp' selection) enabled samples to be provided at multiple stakeholder meetings in the 2017/18 season.

The breeding program benefits from input from the Breeding Program Advisory Committee (BPAC) particularly in terms of the horticultural aspects of Phase 3 elite selections. An annual meeting in November each year has provided the opportunity to obtain BPAC feedback on the commercial potential of Phase 3 selections and provide input on the future relationship between the WABP and its Washington stakeholders.

Thanks to Dave Allan and Sarah Franco in Prosser, Scott Driscol, and Dale Goldy for the Quincy trial and Ray Fuller for the Phase 2 planting in Chelan. Also thanks to AgroFresh for providing 1-MCP, Stemilt for accommodating our complex needs through the storage season, and Legacy fruit packers.

WABP Publicity

Numerous fruit samples of WA 38 and Phase 3 selections were distributed to the grower industry, allied technologies industry, and other target audiences. Field days were organized to showcase WA 38 at several stages of the growing season each year. Video: http://treefruit.wsu.edu/videos/wa-38-cosmic-crisp-field-day/

'Market to Market' Iowa public TV attended and filmed a WA 38 field day in 2016.

WA 38 horticulture and commercialization was featured in a full afternoon session of the 2016 WA Hort Show (Washington Horticultural Association Show) in December. 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).

Talks and Posters

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 annual conference, New Orleans.

September 2015 – A SeedWorld interview with Dr. Evans "The challenges of apple breeding" was featured online. (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 Show in Yakima, WA. Dr. Evans also presented a talk 'Developing and implementing new technologies for and from the WSU pome fruit breeding program'.

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

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

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

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

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

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

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

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

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

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

Nov 2016 – The breeding program hosted a Fruit Evaluation class of students from Wenatchee Valley College.

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

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

Aug 2017 – National Association of Plant Breeders conference, Davis, CA. (*Coggins*, *Evans grad student*) poster: 'Utilizing dry matter and Near-Infrared spectroscopy for selection in the WSU apple breeding program.'

Aug 2017 – WSU CSS 512 Field Crop Breeding students tour of the apple breeding program (*Evans*).

Sep 2017 – Pacific Science Center WellBeing Curiosity Day, Seattle, WA (*Schonberg, Kostick, Evans team*) Apple breeding program fruit evaluation.

Sep 2017 – American Society for Horticultural Science annual conference 2017, Waikoloa, HI. (*Peace*): 'What you see is what you can improve: Breeding utility of genome-wide haplotype mosaics'.

Sep 2017 – NRSP10: Bioinformatic and Database Resources for Specialty Crops workshop, American Society for Horticultural Science Annual Conference 2017, Waikoloa, HI. (*Peace*): 'NRSP10 resources for translational tree fruit research'.

Sep 2017 – American Society for Horticultural Science annual conference 2017, Waikoloa, HI. (*Coggins, Evans graduate student*): 'Utilizing visible/Near-infrared spectroscopy as a non-destructive phenotyping method in the WSU apple breeding program'.

Oct 2017 – Our Valley Our Future seminar series, Wenatchee Valley College, WA. (*Evans*): 'Apple breeding 101'.

Nov 2017 – The breeding program hosted the Fruit Evaluation class of students from Wenatchee Valley College. (*Kostick & Coggins, Evans graduate students*)

Nov 2017 – NC-140 multi-state project annual meeting, Wenatchee, WA. (*Kostick*): 'Identifying sources of fire blight resistance and associated heritable loci in apple'.

Dec 2017 – Fruit of advanced selections was available for tasting at the Washington State Horticultural Association Show in Wenatchee, WA.

Dec 2017 - Washington State Horticultural Association Show, News flash presentations, Kennewick, WA. (*Barritt, Coggins, Kostick*): 'Cosmic Crisp® cv WA 38 and Sunrise Magic® cv WA 2: different opportunities to satisfy consumers', 'Utilizing visible/near-infrared spectroscopy as a non-destructive phenotyping method in the WSU apple breeding program', 'Identifying sources of fire blight resistance and associated heritable loci in apple'

<u>Scientific Papers</u>

Chagné D, Vanderzande S, Kirk C, Profitt N, Weskett R, Gardiner SE, Peace CP, Volz RK, Bassil NV. Validation of SNP markers for fruit quality and disease resistance loci in apple (*Malus* × *domestica* Borkh.) using the OpenArray® platform. *Horticulture Research* (accepted)

Evans K, Peace C. (2017). Advances in marker-assisted breeding of apples. In: *Achieving Sustainable Cultivation of Apples*. Ed. K. Evans. Burleigh Dodds Science Publishing, Cambridge, UK. (ISBN: 978 1 78676 032 6) pp 165-194

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

Harshman J, Evans K, Allen H, Potts R, Flamenco J, Aldwinckle HS, Wisniewski M, Norelli JL. (2017) Fire blight resistance in wild accessions of *Malus sieversii*. *Plant Disease* 101:1738-1745.

Harshman J, Evans K, Hardner C. (2016) Cost and accuracy of advanced breeding trial designs in apple. *Horticulture Research* 3:16008. DOI 10.1038/hortres.2016.8

Howard NP, van de Weg E, Bedford DS, Peace CP, Vanderzande S, Clark MD, Teh SL, Cai L, Luby JJ. (2017) Elucidation of the 'Honeycrisp' pedigree through haplotype analysis with a multi-

family integrated SNP linkage map and a large apple (*Malus* × *domestica*) pedigree-connected SNP data set. *Horticulture Research* 4:17003.

Luo F, Luo F, Sandefur P, Evans K, Peace C. A DNA test for routinely predicting mildew resistance in descendants of crabapple 'White Angel'. *Molecular Breeding* (submitted)

Peace C. (2017) DNA-informed breeding of rosaceous crops: Promises, progress, and prospects. *Horticulture Research* 4:17006.

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

Vanderzande S, Piaskowski JL, Luo F, Edge-Garza DA, Klipfel J, Schaller A, Martin S, Peace C. (2018) Crossing the finish line: How to develop diagnostic DNA tests as breeding tools after QTL discovery. *Journal of Horticulture* 5:228.

EXECUTIVE SUMMARY

The Washington State University apple breeding program (WABP) continues to evolve to meet the demands of the Washington industry. Involvement of the industry is vital at several key parts of the program: priority trait discussion, independent evaluation for grower-friendly selections, and commercialization decisions (both selections and mechanisms).

The initial uptake of the latest release from the WABP 'WA38' has been unprecedented. Estimates of current plantings in Washington exceed 6 million trees. New elite germplasm continues to move through the cultivar development pipeline, being evaluated through the same mechanisms as 'WA 38'; this detailed evaluation is essential to provide grower confidence in new variety uptake. Almost 80,000 apple seeds were produced as a result of controlled crosses within this project cycle, with approximately 26,000 of them screened with an array of DNA tests shortly following germination. In addition, approximately 9,000 seedlings were inoculated with fire blight to determine their resistance as part of a continued effort to introgress fire blight resistance into elite germplasm.

The WABP continues to implement new technology whenever available and appropriate, and has tested two new non-destructive phenotyping methods within this project cycle. Although initial data was not promising, we hope to further evaluate these techniques possibly in collaboration with other North American apple breeding programs.

The WABP team has taken every opportunity to present data from the program at a wide range of events as well as participate in several broadcasted interviews. We will endeavor to build on current success as we move forward into the 2018 season.

CONTINUING PROJECT REPORT **YEAR**: 1 of 1
WTFRC Project: AP-18-105 **No-cost extension**

Project Title: Apple scion breeding program

PI:	Kate Evans	**Co-PI (2):**	Cameron Peace
Organization:	WSU TFREC	**Organization**:	WSU
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Address:	1100 N. Western Ave	**Address**:	PO Box 616414
City/State/Zip:	Wenatchee WA 98801	**City/State/Zip**:	Pullman WA 99164

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

Total Project Request: **Year 1:** $268,142 no-cost extension granted

Other funding sources

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.

Agency Name: WTFRC Apple Review
Amount requested: $107,000 (2015-2018 with no cost extension)
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: $10.0M (2014-2019)
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.

WTFRC Budget:

Item	2018	2019
Salaries[1]	10,935	
Benefits	3,609	
Wages[2]	15,000	
Benefits	5,000	
RCA Room Rental[3]	12,600	
Supplies[4]	500	
Travel	500	
Total	48,144	**0**

Footnotes:
[1]Estimate of percent of time spent for Mendoza (10%) and Hanrahan (6%), a 33% benefit rate and 2% annual increases.
[2]Based on expected staff wage adjustments proportional to the WA state minimum wage increases (2018=$11.50, 2019=$12.00, 2010=$13.50)
[3]2 rooms @ $6,300 p.a.
[4]Consumables for fruit quality lab (KOH, distilled water, iodine solution etc.)
[5]In-state travel for Hanrahan (mainly lodging in Wenatchee)

Budget 1
Organization Name: Washington State University **Contract Administrator:** Katy Roberts/Kim Rains
Telephone: 509-335-2885/509-293-8803 **Email address:** katy.roberts@wsu.edu/kim.rains@wsu.edu

Item	2018
Salaries[1]	64,469
Benefits	25,629
Wages[2]	24,381
Benefits	2,309
Orchard establishment supplies	20,000
Genotyping supplies	20,000
Travel[3]	13,910
Miscellaneous (virus testing)	3,000
Plot Fees	8,800
Total	**182,498**

Footnotes:
[1]Salaries for Agricultural Research Technologist (Bonnie Schonberg@ 1.0 FTE) and for 3 months for genetic screening technician (to be appointed @ 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:** Brett Adams
Telephone: 509 787 1555 **Email address:** brett@willowdrive.com

Item	2018
Seedling propagation	32,500
Phase 2 & 3 trees	5,000
Total	**37,500**

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.

SIGNIFICANT FINDINGS

1. Twenty-two new families were made in 2018 with approximately 70,000 seeds produced in the WSU Apple Breeding Program (WABP).
2. Seedlings from approximately 14,500 seeds from 2017 crosses were grown in the greenhouse.
3. Approximately 8,000 seedlings were screened with DNA markers for fruit quality; almost 4800 were culled leaving the remaining seedlings to be transplanted to Willow Drive nursery.
4. Approximately 3,400 seedlings were screened for resistance to fire blight in the greenhouse; almost 70% survived and were transplanted to the orchard for a second inoculation.
5. Seedlings at Willow Drive were propagated on M.9 rootstocks for future orchard evaluation. Approximately 3,000 seedling/M.9 trees were produced in the nursery for planting in Phase 1 seedling orchards in 2019.
6. The final count of new Phase 1 trees planted in 2018 was approximately 2,250.
7. Promising selections already in Phase 2 trials (planted in 2007-2017) at three evaluation sites in Central Washington were evaluated for productivity and fruit quality.
8. Twenty-two new promising selections (on Geneva 41 rootstock) were planted at three evaluation sites in Phase 2 trials in 2018.
9. Fifteen promising selections made in 2017 were propagated in 2018 for planting in 2020 Phase 2 trials at three diverse sites in Central Washington.
10. Fruit was harvested on four elite selections in Phase 3.

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. Introgression of resistance to mildew or resistance to fire blight remains a target in the crossing program.

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 on 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 or an external service provider 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 on G.41 rootstock and planted 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 new 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 Ines Hanrahan. 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. 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. All data will be recorded making use of the Field Book App and bar-code labels. 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 RCA 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 input from the Breeding Program Advisory Committee (BPAC) particularly in terms of the elite selections in Phase 3. 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, providing multiple opportunities to sample fruit and publishing reports usually in the Good Fruit Grower.

Weather-related events can cause potential problems both in terms of the failure of the crossing program (frost damage to bloom) or the lack of quality fruit data if the crop is affected by, e.g., frost or hail damage. The risks from these potential problems are mitigated by spreading the crossing program and the test plantings across the state. Rodent pressure in the greenhouse and orchard has increased. Appropriate rodent control measures will be applied. TFREC refrigerated storage for the WABP is now fully alarmed in case of potential high or low temperature threats.

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.

Crosses for this season were designed taking into account all the available DNA test information as well as phenotypic trait knowledge. DNA testing focused on the Ma-indel test (acidity, crispness, bitter pit resistance) and worked very efficiently to reduce population sizes. In addition, five progenies were screened with the LG8a acidity test, one with the Md-LG1Fru-SSR test for fructose content and two for the Md-ACS1-indel storage/firmness test.

Approximately 3,400 seedlings were screened in the greenhouse for resistance to fire blight. These seedlings were the result of crosses combining fire blight resistance from the cultivars Fiesta and Splendour (both with known resistances) with three fire blight resistant WABP selections. The total survival rate was almost 70% indicating an overall increase in resistance from earlier crossing generations. Resistant individuals were planted in the Columbia View orchard and re-inoculated later in the season.

Stored fruit from the 2018 harvest is still being evaluated for fruit quality and storage potential.

Twenty-two new Phase 2 selections were planted at the WSU Columbia View orchard in spring 2018. This has re-established Phase 2 at CV, following several years of planting at WSU Sunrise orchard, to consolidate operations following the hiring of a new farm worker at CV using WA 38 royalty income.

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

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

Fruit was harvested on four elite selections in Phase 3 from the Quincy site (three from the Prosser site). Fruit is currently still in storage and will be assessed through to the summer of 2019.

Thanks to Dave Allan and Sarah Franco in Prosser, Scott Driscol and Dale Goldy for the Quincy trial and Ray Fuller for the Phase 2 planting in Chelan. Also thanks to AgroFresh for providing 1-MCP, Stemilt for accommodating our complex needs through the storage season and to Legacy fruit packers.

WABP Publicity

WA 38 and breeding program coverage included in the following:

NWPB (Nov 2018) https://www.nwpb.org/2018/11/26/like-that-year-round-crisp-apple-thank-a-scientist-or-warehouse-possibly-in-wenatchee

KSPS (Nov 2018) https://youtu.be/Uzg9MjlVTtY

Produce Business Journal (August 2018) A star is born

Good Fruit Grower (June 2018) Post-Cosmic question: What's next?

The Produce News (June 2018) Exciting research from Pace International's record-breaking Postharvest Academy

Wenatchee World (June 2018) Big impacts

KCTS9 (June 2018) Washington's new apple could be an industry game-changer

Fresh plaza (May 2018) Working as a state for Cosmic Crisp®

Ag Info podcast (May 2018) Cosmic Crisp® exceeds expectations

Popular Science (May 2018) I developed a sturdier, crisper, and yummier apple

Capital Press (April 2018) Cosmic Crisp® plantings beat estimate

Presentations:

Mar 2018 – Washington Farm Bureau visit. (*Evans*): 'WSU apple breeding program'.

Apr 2018 – UC Davis Plant Breeding Seminar Series, UC Davis, CA. (*Evans*): 'Development and application of DNA-informed breeding in the WSU apple breeding program.'

June 2018 – Korean nursery group visit. (*Evans*): 'WSU apple breeding program.'

June 2018 – IRTA/Portuguese grower visit, (*Evans*): 'Introduction to TFREC and the WSU apple breeding program.'

Sept 2018 – International New Varieties Network, Sunrise orchard field visit. (*Evans*): 'WSU pome fruit breeding program.'

Oct 2018 – International Pome Fruit Alliance visit, (*Evans*): 'WSU pome fruit breeding program.'

Dec 2018 - Washington State Tree Fruit Association (WSTFA) 114th annual meeting (*Kostick* [Evans grad student]): 'Identifying elite sources of fire blight resistance in apple.'

Dec 2018 - Washington State Tree Fruit Association (WSTFA) 114th annual meeting (*Kostick*): 'Fire blight susceptibility; apple cultivar survey.'

Peer-reviewed publications

Jung S, Lee T, Cheng C-H, Buble K, Zheng P, Yu J, Ficklin S, Gasic K, Scott K, Frank M, Ru S, Hough H, Evans K, Peace C, McFerson J, Coe M, Staton M, Wegrzyn J, Main D. (2018) 15 years of GDR: new data and functionality on the Genome Database for Rosaceae. *Nucleic Acids Research* gky1000.

Kostick S, Evans K. (2018) Apple. In: (K. Gasic, J.E. Preece, and D. Karp, eds.) Register of New Fruit and Nut Cultivars List 49. HortScience 53(6): 748-750. doi.org/HORTSCI1049fn-18.

Desnoues E, Norelli JL, Aldwinckle HS, Wisniewski ME, Evans KM, Malnoy M, Khan A (2018) Identification of novel strain-specific and environment-dependent minor QTLs linked to fire blight resistance in apples. *Plant Molecular Biology Reporter* doi.org/10.1007/s11105-018-1076-0

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project: AP-17-101

Project Title: Control of fruit size and bitter pit in Honeycrisp using irrigation

PI: Lee Kalcsits
Organization: Washington State University
Telephone: 509-293-8764
Email: lee.kalcsits@wsu.edu
Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

Cooperators: Michelle Reid, WSU, Jeff Cleveringa, Oneonta Starr Ranch; Dave Allan and Suzanne Niemann, Allan Brothers

Total Project Request: **Year 1:** $53,442 **Year 2:** $74,199 **Year 3: $84,972**

Other funding sources

Agency Name: Lawrence Berkeley National Lab, Berkeley, CA
Amt. awarded: ~$32,000
Notes: This is work in collaboration with scientists at the University of California, University of Kentucky, and Lawrence Berkeley National Lab to look at how irrigation regimes change fruit structure, porosity and how it relates to quality. The funding supports beamline access to make measurements in the spring and fall.

Agency Name: Canadian Light Source Synchrotron, Saskatoon, SK
Amt. awarded: ~$30,000
Notes: This is work in collaboration with scientists at the Canadian Light Source to look at how irrigation regimes change fruit vasculature in developing fruit. The funding supports beamline access to make measurements in the summer.

Agency Name: Pacific Northwest National Lab, Richland, WA
Amt. awarded: ~$20,000
Notes: This is work in collaboration with scientists at the PNNL to look at how irrigation regimes change fruit vasculature in developing fruit. The funding supported instrumentation access to make measurements in the fall of 2017 from these experiments.

Budget 1

Organization Name: TFREC-WSU **Contract Administrator:** Katy Roberts/Kim Rains
Telephone: 509-335-2885/509 663 8181 (221) **Email address:** arcgrants@wsu.edu/kim.rains@wsu.ed

Item	2017	2018	**2019**
Salaries[1]	18,000	46,026	47,867
Benefits	7,942	10,809	11,306
Wages[2]	0	5,223	12,192
Benefits	0	141	1,607
Equipment[3]	8,000	0	0
Supplies[4]	17,000	9,500	9,500
Travel	2,000	2,000	2,000
Miscellaneous	0	0	0
Plot Fees	500	500	500
Total	53,442	74,199	**84,972**

Footnotes:
[1] Salaries are budgeted to support a research technician at 50% for three years and the salary for a M.S. student for two years.
[2] Wages provide summer salary for a M.S. student and a summer student for year 3
[3] Equipment in year 1 will be for the purchase of a pressure bomb to measure stem water potential
[4] Supplies are for irrigation set-up supplies in year 1 combined with lab consumables, leaf and fruit nutrient testing and fruit quality analysis for years 1-3.

OBJECTIVES

1. Test how early, middle and late-season deficit irrigation affects fruit size, quality and return bloom in Honeycrisp.

2. Identify whether bitter pit occurrence can be reduced by reducing fruit size in a bitter pit susceptible orchard.

3. Develop horticultural indicators (e.g., visual indicators, stem water potential and/or soil moisture) for monitoring plant water status to guide the deployment of deficit irrigation for the control of fruit size.

SIGNIFICANT FINDINGS

- Irrigation cannot correct for nutrient imbalances. For commercial sites, bitter pit was low when nutrient balance was achieved. However, bitter pit was still high for sites with nutrient imbalances.
- Stomatal conductance was strongly affected by irrigation regime.
- Photosynthesis decreased during periods of water limitation indicating a change in stomatal conductance and plant water-status.
- Midday leaf water potential increased during drought treatments and then recovered once irrigation was brought back to normal. When using stem water potential as an indicator for watering Honeycrisp, -1.2 to -1.5 MPa can be used as a watering trigger depending on crop load and forecasted fruit size.
- When water limitations were applied, fruit size was reduced in all treatments but environment seemed to impact the degree of size reductions and periods with low evaporative stress will be harder to achieve a response (cooler, cloudy, or smoky periods).
- Bitter pit was lower when water was limited during the middle and later part of the season (45-105 DAFB).
- Red color increased when water was limited later in the season (75-105 DAFB)

METHODS

Experimental site and tree management

An experiment was set up at the WSU Sunrise research orchard using 240 Honeycrisp trees on M9-T337 that were planted in 2015 at a spacing of 3’ x 12’ (1,210 trees/acre). The soil is an alluvial shallow sandy loam soil. The trees filled their canopy space in 2015 and 2016. The first year crop was in 2017. Using a randomized complete block design, irrigation regimes were used that will withhold irrigation either early, middle or late in the season and compare it to a fully watered control. In 2018, trees were sprayed with calcium starting in June at standard commercial rates.

Experimental design and irrigation treatments

The irrigation system at Sunrise was controlled with a variable speed pump drive and electrovalves. Using exclusion valves and by-pass lines, the entire block was appropriately randomized. Irrigation was applied using emitters at 1 foot spacing at 0.42 gal/hour and supplemented with microsprinkler irrigation to maintain the grass between rows. The well irrigated control was irrigated four times per day for 30 minutes. This was significantly above evapotranspiration demand.

The early irrigation deficit where irrigation was reduced by approximately 80-90% from 15-45 days after full bloom (DAFB), middle irrigation deficit with irrigation was reduced by approximately 80-90% from 45-75 DAFB and late irrigation deficit where irrigation was reduced by approximately 80-90% from 75-105 DAFB. Full bloom occurred on May 3rd, 2017 and on April 27th, 2018. All treatments were returned to the well-watered irrigation schedule after the predetermined deficit irrigation period.

Tree Selection

Sample trees were selected for uniformity. Bloom clusters were counted to continue checking uniformity. Three trees were selected from each replicate with uniformity in fruit load, trunk cross-sectional area (TCSA), and height.

Physiological Measurements

At the beginning, middle, and end of each deficit irrigation period, physiological measurements were made including mid-day leaf water potential and photosynthesis. Plant water status, measured as Ψ_{md} was assessed using a 3005 Series Plant Water Status Console (Soilmoisture Equipment Corp, Goleta, CA, USA). Leaves used for measurement of Ψ_{md} were bagged for at least one hour in silver reflective bags to equalize the leaf and xylem water potential before readings were taken. Ψ_{md} was measured around solar noon. Leaf gas exchange was measured using a LI-6400XT infrared gas analyzer (Li-COR, Lincoln, NE, USA). Reference carbon dioxide concentration was set at 400 ppm, leaf temperature at 25 °C (77 °F), and photosynthetic photon flux density (PPFD) to 1500 μmol CO_2 m^{-2} s^{-1}.

Harvest and Fruit Quality

All of the fruit was harvested from sample trees on September 6th in 2017 and August 30th in 2018. The total amount of fruit from each tree was weighed in the field and counted. Then, 20 fruits were randomly subsampled from each tree, 10 for at-harvest fruit quality and then 10 fruit for quality evaluation after storage. The storage samples were placed in regular atmosphere (RA) for 3 months. Quality analysis was performed two days after harvest testing for standard quality metrics including color, firmness, soluble solids content, titratable acidity, starch and mineral analysis.

Commercial Orchard Sampling

In 2019, five commercial orchard blocks were used for testing out deficit irrigation on bitter pit in Honeycrisp. Growers implemented irrigation practices using sensor-based information in a way that worked best for their operations. Then, just prior to commercial harvest, two boxes of fruit were taken from each orchard using fruit not exhibiting bitter pit symptoms. One box was analyzed for fruit quality at harvest using quality metrics described above. Fruit samples were also analyzed for mineral nutrient composition. The other box was stored for three months in regular atmosphere at 33°F and then bitter pit incidence and fruit quality were analyzed again.

RESULTS

Despite early thinning in the 3rd leaf in 2017, there was still a biennial bearing pattern in the experimental orchard block we are using for this experiment. There were no significant differences in return between deficit treatments and the normally watered control. However, the middle deficit period did have lower cluster counts compared to the early deficit periods and will be something we will track into 2019 for the ‘on’ year (Figure 1).

At WSU Sunrise which has a shallow sandy loam soil, the maximum volumetric soil water content was approximately 35% vol/vol. Water was turned off at the beginning of these three periods in the associated treatments. Volumetric soil water content was allowed to decrease until it reached approximately 12% vol/vol. At this point, water was turned on for 1-2 hours every 3-4 days to deliver small amounts of water but still keeping soil volumetric water content well below the well-watered control (Figure 2). We used soil moisture to determine when to water, not when visual symptoms were present. These periodic water limitations translated into real responses in the tree. At the end of the early, middle, and late water-limitation periods, leaf photosynthetic rates were lower than the well-watered controls. After the water limitation periods, the trees quickly recovered and photosynthetic rates increased back to levels that were not signficantly different than the controls. Midday leaf water potential followed a similar pattern where the highest leaf water potential was observed during periods when there were water limitations and water potential quickly increased again once irrigation patterns returned to normal (Figure 3).

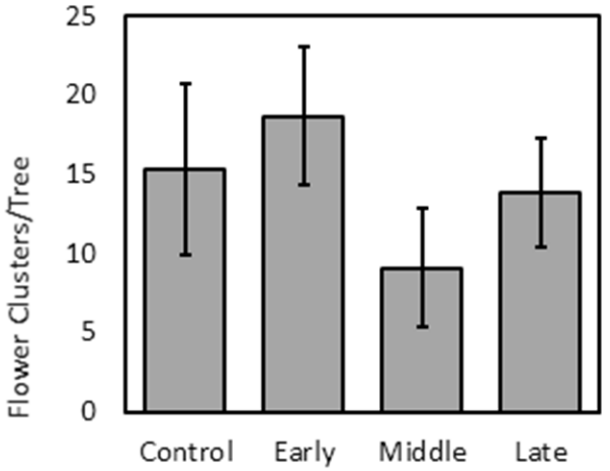
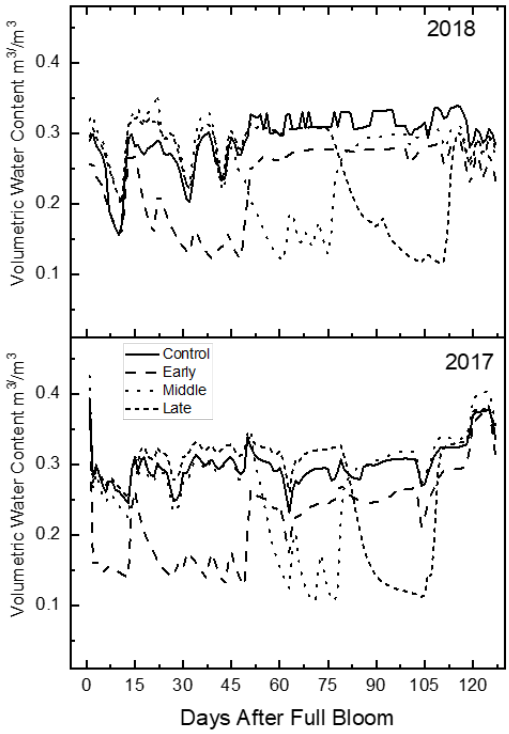


Figure 1. *Flower clusters per tree for trees that were exposed to early, middle, and late season irrigation deficits compared to a normally watered control. Error bars denote standard error (N= 3 replicates).*

Environmental conditions appear to directly influence the response to stress. During the middle and late deficit periods in 2018, drought responses were lower than 2017. We think this might be because cooler temperatures were present during the middle deficit period that limited stem water potential decreases. Heavy smoke then influenced the responses seen during the end of the late period. Environmental conditions need to be taken into account when deciding on watering patterns, whether your operation is deficit irrigating or not.

In 2018, fruit size had a much different response than 2017, particularly under the early deficit irrigation treatment. Because the crop load was lower in 2018 than in 2017, overall fruit weight was greater in 2018. Despite showing similar patterns in stem water potential during the early period, fruit size was the smallest for trees exposed to early water deficits. June 2017 was much warmer than June 2018 which could have altered fruit growth during this stage. Since cooler temperatures were present when the trees under early deficit irrigation were returned to full irrigation, fruit expansion may have been slower at this time as well.

In 2017, red color was greater in fruit from trees that were water limited compared to the well-watered control. This was also true in 2018 but the differences were not significantly different. Fruit from trees that were exposed to water deficits late in the season had an average overall red color classification of 2.94 in 2017 and 3.04 in 2018. A color classification of 3 is where 50-75% of the fruit is red. Firmness, and soluble solids content were also highest in fruit harvested from trees that were exposed to water deficits late in the season (Table 1). This was also observed in 2018 but were not significantly different than the fully irrigated control. In contrast, fruit from trees within the well-watered control had the lowest firmness, and soluble solids content. Fruit from trees exposed to water deficits either early or mid-season were in-between fruit from the well-watered control and fruit from trees that were water-limited later in the season.



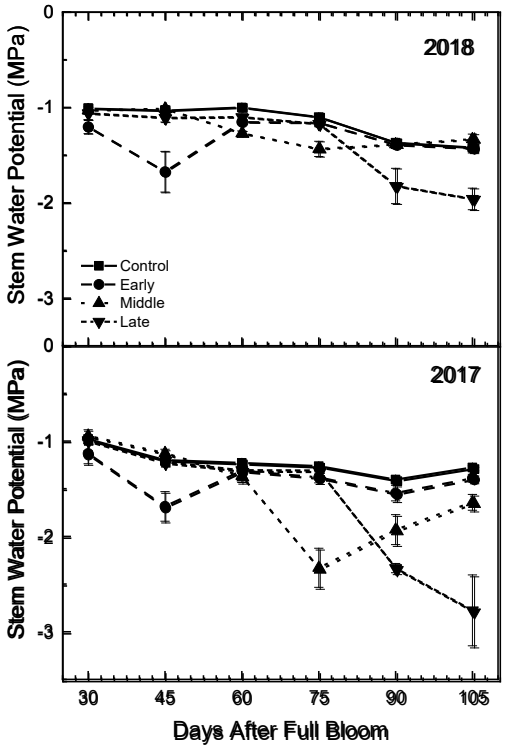


Figure 2 (left). *Volumetric water content (m^3/m^3) during the 2017 growing season (N=3) for trees within a well-watered control (solid grey line), early water limitation (dashed line; 15-45 DAFB), middle water limitation (solid black line; 45-75 DAFB), and later water limitation (dotted black line; 75-105 DAFB). Light grey, medium grey, and dark grey squares represents the early, middle and late periods of water limitation, respectively.*

Figure 3 (right). *Mean midday leaf water potential (ψ_{md}) during the 2017(below) and 2018 (above) growing season (N=3) for trees within a well-watered control (solid grey line), early water limitation (dashed line; 15-45 DAFB), middle water limitation (solid black line; 45-75 DAFB), and later water limitation (dotted black line; 75-105 DAFB). Higher values indicate greater water stress. Light grey, medium grey, and dark grey squares represents the early, middle and late periods of water limitation, respectively.*

Table 1: *Fruit quality after storage averages for each treatment from 90 fruits per treatment.*

	2017				2018			
Treatment	**Weight (g)**	**Color Class (1-4)**	**Firm (lb)**	**Brix (%)**	**Weight (g)**	**Color Class (1-4)**	**Firm (lb)**	**Brix (%)**
Control	255 a	2.6 b	17.1 a	13.8 a	326 a	2.7 a	16.0 a	14.9 a
Early	247 ab	2.7 ab	17.4 a	15.3 c	269 a	2.9 a	16.4 ab	15.7 a
Middle	226 b	2.3 b	18.1 a	14.5 b	299 a	2.8 a	17.4 b	15.4 a
Late	209 c	2.9 a	19.6 b	15.6 c	305 a	3.0 a	16.3 ab	15.2 a

Bitter pit incidence was high immediately after harvest in fruit from trees that were water-limited early in the season where 24% of the fruit had bitter pit. This was also true for 2018 as well where bitter pit was the highest in the early treatment. Bitter pit incidence at harvest was low in the well-watered control and for fruit from trees that were exposed to water deficits in either the middle or later part of the growing season with 6%, 1%, and 7%, respectively. After 3 months of RA storage at 2 °C (36 °F), bitter pit incidence increased in all treatments. Despite calcium sprays in 2018, the low crop load strongly stimulated bitter pit development. Periodic water limitations had a significant impact on bitter pit incidence. Overall, middle and late-season deficits reduced bitter pit incidence compared to the control. Bitter pit averaged approximately 58% in both years for the well-watered control. Late season water limitations limited bitter pit to approximately 40%. Environmental differences between 2017 and 2018, particularly during the early and middle season treatments may have contributed to the differences in post storage bitter pit incidence between 2017 and 2018. These patterns will be monitored closely in 2019 to see if risk assessments based on temperatures during fruit development follow the same patterns as 2017 and 2018.

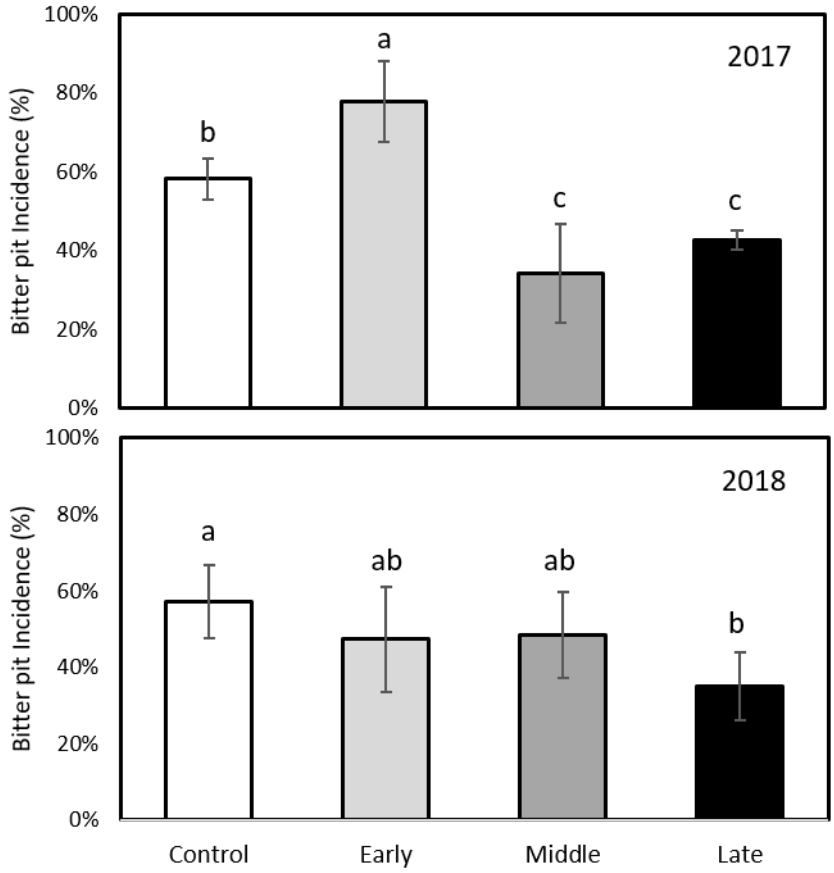


Figure 3. *Bitter pit incidence (%) for fruit sampled from trees exposed to water limitations early (dotted; 15-45 DAFB), middle (striped; 45-75 DAFB), or late (diagonal lines; 75-105 DAFB) season compared to a well-watered control after 3 months of regular atmosphere storage at 2 °C (36 °F). Vertical bars represent the SE of the total (N=3). Letters denote significant differences determined using Tukey's HSD test.*

COMMERCIAL ORCHARDS

Three of the commercial orchard sites had less than 10% bitter pit incidence (Figure 4). Fruit size averaged 88-100, 64-72, 64-72, 88, and 72 box sizes for orchards A, B, C, D, and E, respectively. For the two orchards with high bitter pit incidence, the K:Ca ratio was above 35, whereas the K:Ca ratio for the other three sites was below or equal to 20 (Table 2). These differences in ratio were driven by both low levels of Ca and high levels of potassium. This demonstrates that irrigation cannot be used as a mediation tool when nutrients are not correctly balanced.

Table 2. *Fruit calcium, potassium, and magnesium concentrations and associated ratios for five commercial orchard sites deploying deficit irrigation*

Orchard Site	Ca (%)	K (%)	Mg (%)	K/Ca	K+Mg/Ca
A	0.045	0.611	0.033	13.6	14.3
B	0.018	0.817	0.042	45.4	47.7
C	0.042	0.625	0.03	14.9	15.6
D	0.036	0.708	0.039	19.7	20.8
E	0.027	0.674	0.033	25.0	26.2

Industry Outreach

Since the start of this project, Lee Kalcsits has given 10 presentations to the Washington State apple producers. These have included state, regional, and grower-specific discussions. Additionally, Tianna DuPont organized an irrigation field day in June 2017 attended by approximately 50 industry members that Lee Kalcsits presented at. Michelle Reid, the M.S. student working on this project has presented this research at national meetings and also provided three talks in 2018 to industry members.

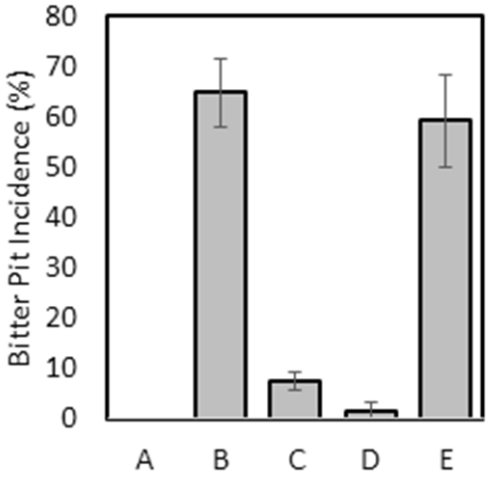


Figure 4*. Post storage bitter pit incidence for five commercial orchard employing deficit irrigation strategies in Washington State. Fruit placed into storage was bitter pit free.*

2019 PLANS

- Repeat the experiment at Sunrise research orchard when a higher crop load is expected. Bloom thinning will be used to manage crop load early return bloom will be tracked for an 'on' year.
- Fruitlets and fruit from the 2017 and 2018 experiments will be analyzed for structure including cell density, size, and number at the Canadian Light Source Synchrotron.
- Continue to work with interested grower orchards with soil moisture monitoring already in place to regulate irrigation during the growing season in one of their orchard blocks. They can choose their management strategy as long as their soil moisture values can be reported to my group. My lab group will then come and sample fruit. This will be compared against a random sample from orchards with no deficit irrigation practices.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project: AP-17-103

Project Title: How do we measure and manage soil health for productive orchards?

PI: S. Tianna DuPont
Organization: Washington State University
Telephone: (509) 293-8758
Email: tianna.dupont@wsu.edu
Address: 1100 N. Western Ave
City/State/Zip: Wenatchee/WA/98801

Co-PI: Lee Kalcsits
Organization: Washington State University
Telephone: (509) 663-8181
Email: lee.kalcsits@wsu.edu
Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

Cooperators: orchardist site hosts

Total Project Request: Year 1: $48,884 **Year 2**: $51,258 **Year 3**: **$51,686**

Other funding sources: None

WTFRC Collaborative Expenses: None

Budget 1
Organization Name: Washington State University **Contract Administrator:** Katy Roberts/Kim Rains
Telephone: 509-335-2885/509-293-8803 **Email address:** katy.roberts@wsu.edu/kim.rains@wsu.edu

Item	2017	2018	**2019**
Salaries[1]	$24,600	$25,584	$26,607
Benefits[2]	$9,740	$10,130	$10,535
Wages	0	0	0
Benefits	0	0	0
Equipment	0	0	0
Supplies[3]	$10,272	$11,272	$10,272
Travel[4]	$4,272	$4,272	$4,272
Miscellaneous	0	0	0
Plot Fees	0	0	0
Total	$48,884	$51,258	**$51,686**

Footnotes:
[1]Salaries for a 25% scientific assistant (Kalcsits) and a 33% scientific assistant (DuPont).
[2]Benefits at 44.1% for scientific assistant (Kalcsits) and 37% for scientific assistant (DuPont).
[3]Goods and services include soil nutrient analysis, soil quality analysis, plant tissues tests, fruit quality analysis, sampling and lab materials.
[4]Travel to collect soil, yield, and fruit quality samples from farm sites.

OBJECTIVES

1. Test the relationship between soil quality and fruit productivity.
2. Determine which of a suite of 21 soil quality indicators are appropriate for tree fruit production systems in the irrigated west.
3. Increase grower understanding of soil quality indicators, what they mean, and how to use the information they provide to improve management.

SIGNIFICANT FINDINGS

- Estimated 40 lb packs/acre was greater in grower identified sites with high productivity than paired sites with self-identified low productivity.
- Soil quality indicators measured fell over a wide range.
- Either root health indicators or available water indicators are often low in sites with constricted yield/fruit quality.
- Soil nutrient availability metrics scored high for most sites tested with the exception of pH which had pH above optimum for multiple sites.

METHODS

Site description: To date 72 orchard plots were soil sampled. Of these plots 34 plots (17 matched pairs) were well matched with available/measured yield data. A subset of plots (11 pairs, 22 plots) was sampled for fruit yield and fruit quality.

Matched plots were on the same general soil type with matching variety, tree age and training system. One plot in each pair was high performing based on grower description and the other site in the matched pair was underperforming.

Soil sampling: Fifty to one hundred soil probe subsamples to an 8-inch depth just inside the drip line of the canopy were taken for nutrient, soil health and nematode analysis. Four four-inch deep intact soil cores were taken for bulk density analysis. Five intact cores two inches deep by two-inch diameter were taken for micro-arthropod analysis. Water infiltration was measured by timing the length of time for water to fully infiltrate when one inch of water was added to a 10 inch diameter ring pounded 2 inches into the ground.

Soil health analysis: Compaction, and water infiltration were measured in the field (for methods see DuPontNew). Micro-arthropods were measured using a modified Berlese-Tullegren funnel extraction. Nematodes were extracted using a combination of decanting, sieving and Baermann funnel methods, counted and identified. An apple seedling bioassay (adapted from Laurent 2008) and a bean bioassay (Cornell 2010) were performed. Nutrient analysis, aggregate stability, soil protein, respiration, active carbon, water holding capacity and potentially available N were analyzed by Cornell University or Oregon State University Soil Health lab (per Clune et al 2016).

Soil health indicator scores were calculated as follows:

Soil organic matter, active carbon, aggregate stability, ace protein, potentially mineralizable nitrogen, bean bioassay and compaction are calculated as a scoring function of the normal distribution where p is the probability (between 0 and 1) that a measured value x will fall at a given position in the interval (+∞, -∞), and μ is

$$p = f(x,\mu,\sigma) = \frac{1}{\sigma\sqrt{2\pi}} \int_{-\infty}^{+\infty} e \frac{-(x-\mu)^2}{2\sigma^2} dx$$

the indicator mean and σ the standard deviation following Fine et. al. 2017.[1] Means and standard deviations derived from Fine 2017 based on 3,762 samples.

Water infiltration and the apple bioassays are calculated using the above scoring function of the normal distribution with means and standard errors derived from the current dataset.[2] Bulk density (BD) ratings are calculated according to Fayez 2017 Y= max x/(1+(x/mean x)^slope), where less than 1.5 g/cm^3 is desirable. [3]

Lesion nematode scores were calculated based on thresholds where 20-70 lesion nematodes per 500 g may cause crop damage; and 80+ will likely cause damage in young trees.[4,5] Nematode Structure Index and Basal Index were calculated according to Yeates et al (1993) Bongers (1990), and Bongers and Bongers (1998).

pH ratings were calculated based on a normal curve where the optimal range is 6 to 6.5.[6] Phosphorus ratings were calculated based on a curve where the upper threshold is 200, the upper baseline is 100, the lower optimum is 10, the upper optimum is 60 and the slope is 0.0428 (Glover, Reganold, and Andrews 2000).[5,7] Potassium scores were calculated based on an optimum range of 100 to 250 ppm where the slope for values less than 100 is 3.1416 and for values greater than 250 is 0.428.[5,8]

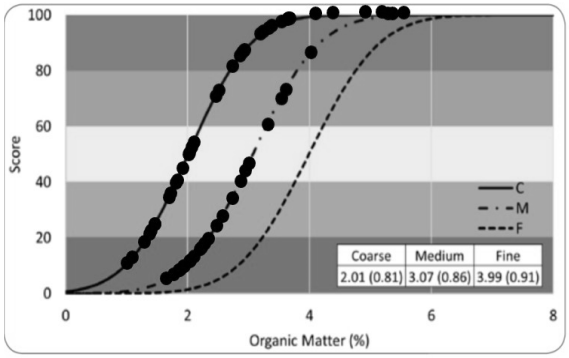
[1] Score OM =IF(A="coarse", (100*(NORM.DIST(X,2.01,0.81,1))),(100*NORM.DIST(A,3.07,0.86,1)))
ACE score =IF(A="coarse",(100*NORM.DIST(X,7.4,3.5,1)),(100*NORM.DIST(X,6.5,3.3,1)))
PMN score = 100*(NORM.DIST(BU6,15,7,1))
WSA=IF(A="coarse",(100*NORM.DIST(CC6,40,23,1)),(100*NORM.DIST(X,31,18,1)))
Surface compaction =100*(1-(NORM.DIST(X,164,94,1)))
Subsurface compaction =100*(1-NORM.DIST(X,300,110,1))
Bean bioassay score =100*(1-NORM.DIST(X,4.5,1,1))
[2] Infiltration =100*(1-(NORM.DIST(X,2.3,1.65,1)))
Apple bioassay score =100*(NORM.DIST(X,0.7,0.27,1))
[3] BD=(1/(1+(X/1.5)^2.5))*100
[4] Lesion nematode score=(1-(1/(1+((90-0.01)/(X-0.01))^((2*0.01)*(90+X-2*0.01)))))*100
[5] $y = \frac{1}{[1+(\frac{LR-LT}{x-LT})^{\frac{2S(LR+x-2\times LT)}{x-LT}}]}$
[6] Ph rating =(+(1/((2*(3.1416))^0.5))*2.7183^(-((X-6.25)^2)/(2*((6-2.1)/3)^2)))*250
[7] Phosphorus score=IF(X>100, (1/(1+((200-100)/(X-0))^((2*-0.0428)*(200+X-2*0))))*100, IF(X<=29, (1/(1+((7-1)/(X-0))^((2*0.0428)*(7+X-2*0))))*100, 100))
[8] Potassium score =IF(X>250, (1/((2*(3.1416))^0.5))*2.7183^(-((X-250)^2)/(2*(-180)^2))*250, IF(X<=150, (1/(1+((60-1)/(X-0))^((2*0.0428)*(60+X-2*0))))*100, 100))

RESULTS

Of 72 plots soil sampled to date, 34 were well matched sites (17 pairs) with grower available/measured yield data and 11 pairs, 22 plot were sampled for fruit yield and fruit quality. Figures 1 to 8 show the results of soil quality measurement for some of the more interesting measurements compared to scoring function curves. Individual dots indicate the value for that index at a single site. For most indices we have measured a range of values from high to low. For some indices such as water stable aggregates (WSA), potentially mineralizable nitrogen (PMN) and ACE protein index (Figures 2&3), Washington Orchard soils have tended to score in the lower end of the scale compared to the scoring curve functions which were developed in the East. Conversely, subsurface hardness (Figure 4) tends to score high (low compaction levels). Root health indicators based on both apple seedling growth in pasteurized and unpasteurized soil as well as bean root bioassays display a normal distribution (Figure 5). Soil nutrient availability metrics scored high for most sites tested with the exception of pH which had pH above optimum for multiple sites (Figure 8). Either root health indicators or available water indicators are often low in sites with low packs/A.

Bins per acre and packs per acre were larger in grower identified high productivity sites compared to low productivity sites consistent with grower identification in most sites (61 bins per acre high, 43 bins per acre low; 1232 packs per acre high; 809 packs per acre low). Fruit crop load based on trunk cross sectional area was not significantly different between sites with high or low productivity (Table 1). In general, changes in yields were primarily associated with a lack of canopy fill where low canopy in-fill for low sites translated to about a 50% reduction in gross yields and packed boxes per acre (Figure 9). Although packs per bin were 1.5 less for low sites than high sites, this was not statistically significant. Fruit quality was less affected by the productivity levels for an orchard. In general, fruit quality in low versus high sites were consistent with lighter crop loads for low sites and advanced ripening and in most cases, these changes were not significant.

Figure 1. Organic Matter Indicators



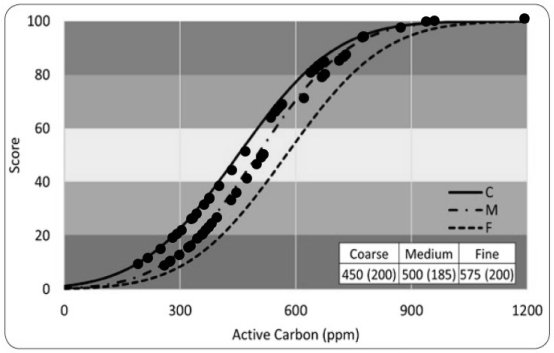
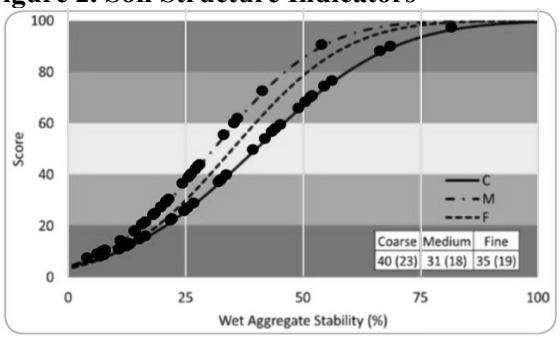


Figure 2. Soil Structure Indicators



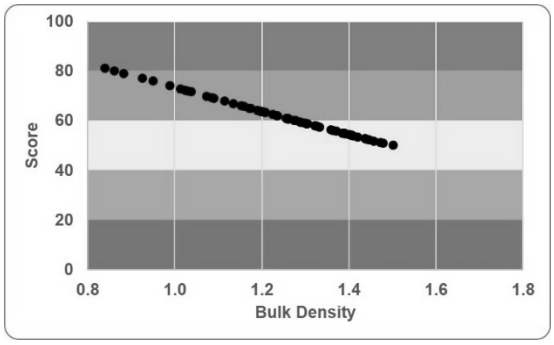
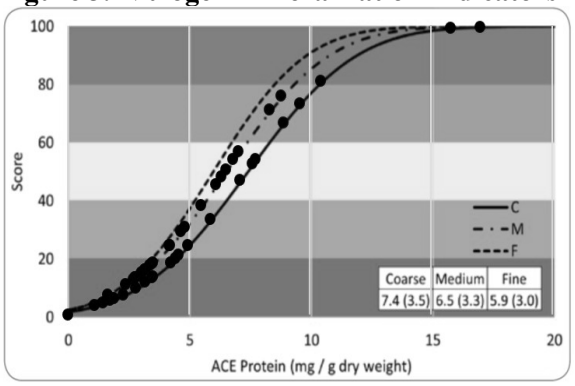


Figure 3. Nitrogen Mineralization Indicators



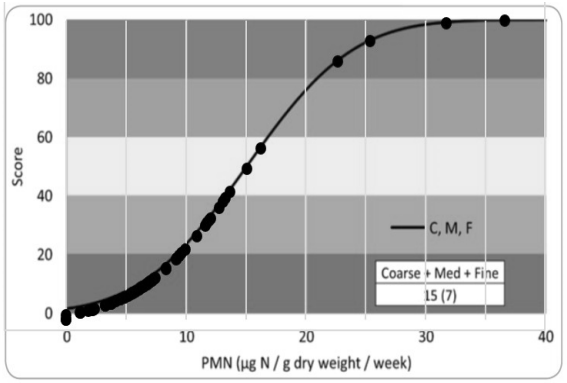
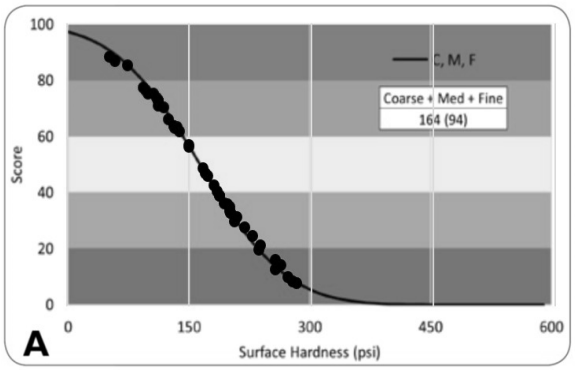


Figure 4. Soil Compaction Indicators



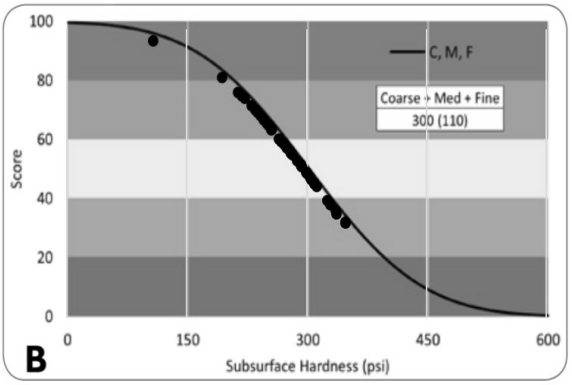
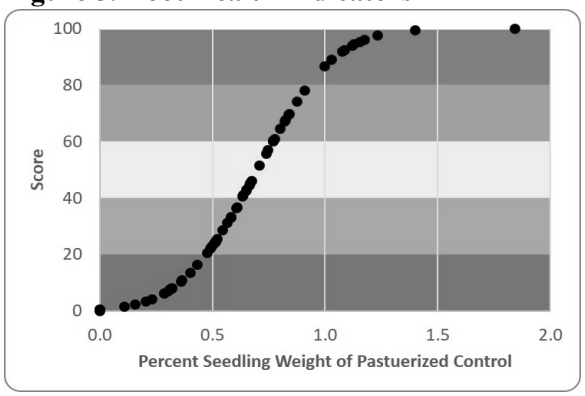


Figure 5. Root Health Indicators



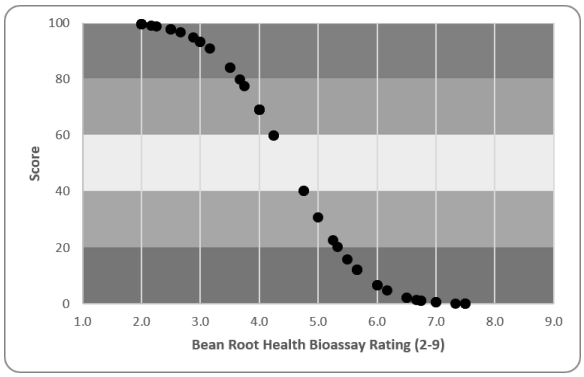
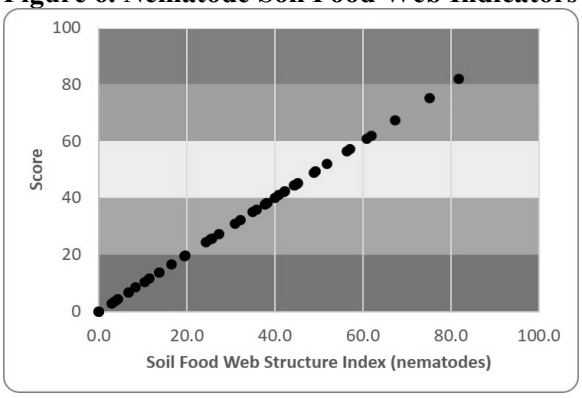


Figure 6. Nematode Soil Food Web Indicators



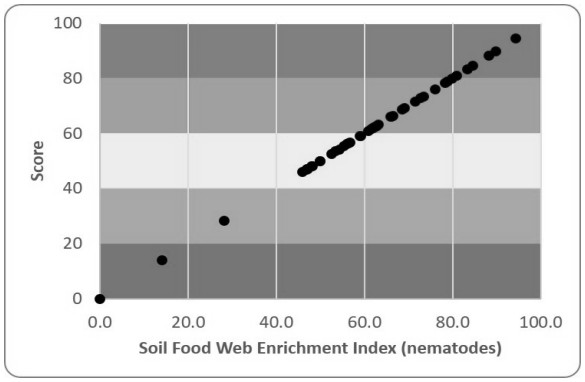
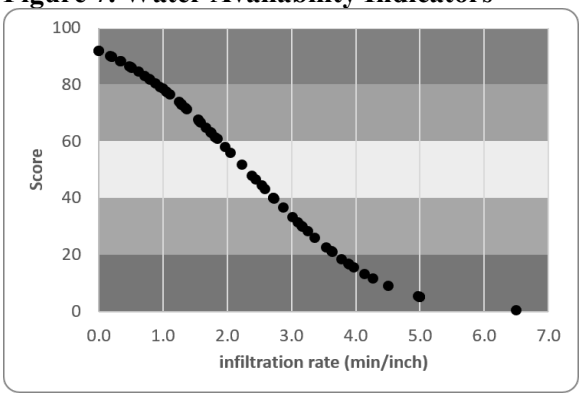


Figure 7. Water Availability Indicators



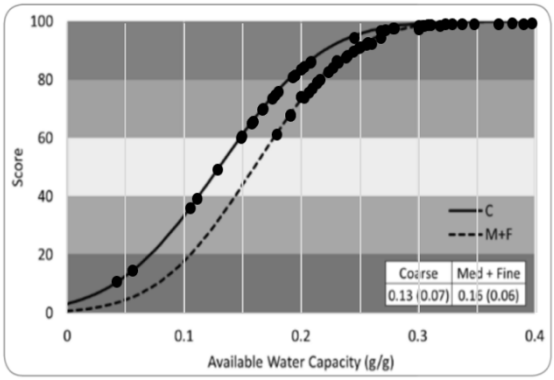
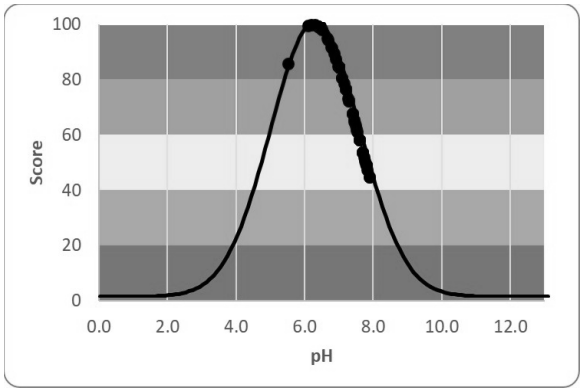
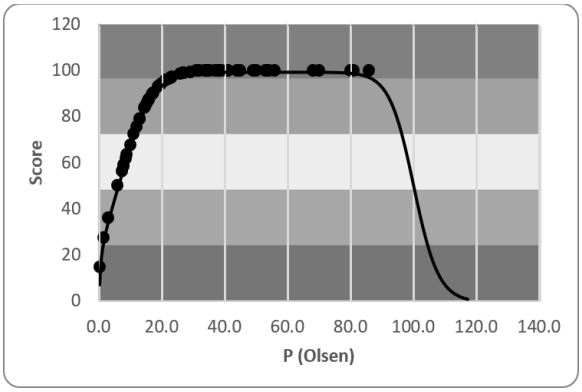
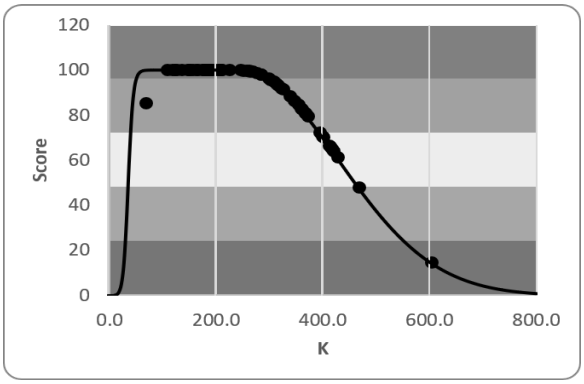


Figure 8. Nutrient Indicators







Fruit Quality

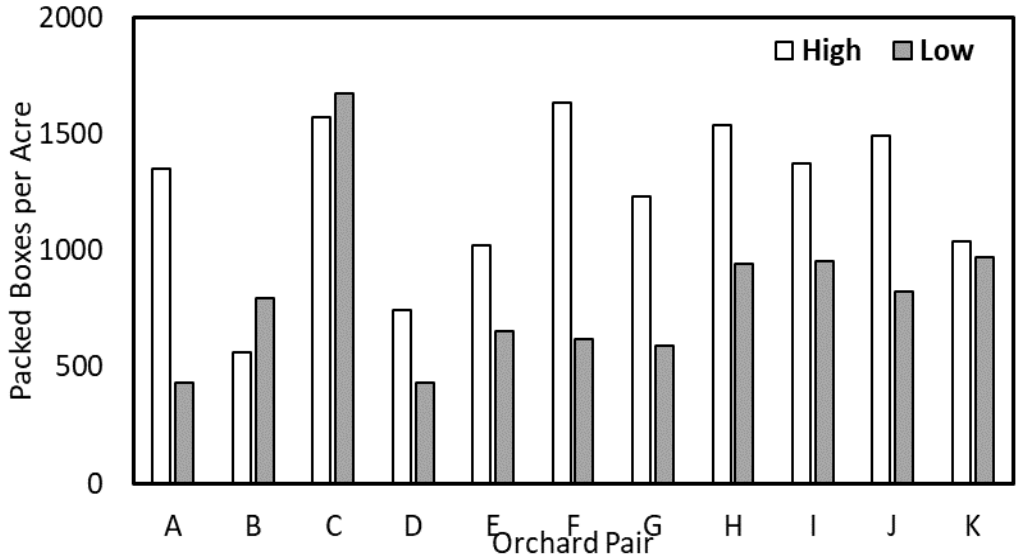


Figure 9. Estimated packed boxes per acre for 11 orchard pairs that were self-identified with either high or low productivity.

Table 1. Yield and pack out metrics for sites with either low or high productivity (N=11). Letters indicate a significant difference between the pairs determined using Tukey's HSD test ($\alpha = 0.05$).

	Fruit Weight (g)	Crop Load[1]	Bins acre^{-1}	Packs acre^{-1}	Packs bin^{-1}
High	173 a	4.40 a	61 a	1232 a	20.5 a
Low	186 a	4.05 a	43 b	809 b	19.1 a
P-Value[2]	0.39	0.71	0.026	0.010	0.18

[1] Expressed as fruit per cm^2 trunk cross sectional area
[2] P-values of less than 0.05 are considered significant

Table 2. Mean fruit quality metrics for sites with either low or high productivity (N=11). Letters indicate a significant difference between the pairs determined using Tukey's HSD test ($\alpha = 0.05$).

	Color[1]	Background Color[2]	Starch[3]	SSC (°Brix)	Firmness (lb)
High	3.10 a	2.25 a	3.69 a	12.89 a	19.0 a
Low	3.37 a	2.42 a	3.88 a	11.69 b	17.8 a
P-Value	0.25	0.40	0.81	0.046	0.24

[1] Color scale developed for each bi color cultivar where increasing numbers indicate improved red color coverage
[2] Scale developed where increasing numbers indicate loss of green background color during ripening
[3] Based on a scale of 1-6 where 6 indicates that starch has completely cleared out

FINAL REPORT
WTFRC Project Number: AP-16-104A

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

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City/State/Zip:	Wenatchee/WA/98801	**City/State/Zip:**	Puyallup/WA/98371

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

Total Project Request: **Year 1**: $70,950 **Year 2**: $73,767

Other funding sources: *None*

Budget 1.(Achour Amiri)
Organization name: WSU-TFREC **Contact Administrator:** Carrie Johnston; Joni Cartwright
Telephone:509-335-4564; 509-663-8181 x221 **Email:**carriej@wsu.edu;joni.cartwright@wsu.edu

Item	2016	2017
Salaries[1]	39,000	40,560
Benefits	15,639	16,265
Wages[2]	2,592	2,696
Benefits	260	270
Supplies[3]	4,000	4,000
Travel	1,500	1,500
Miscellaneous[4]	1,000	1,000
Plot Fees	0	0
Total	63,991	66,291

[1] Salaries are for a PostDoc (Vikas Koundal, 1.0 FTE) at 40.1% benefit rate.
[2] Wages are for an hourly person for 12 weeks.
[3] Supplies include Petri dishes and microbiological media for lab use
[4] Miscellaneous include residue level analysis.

Budget 2 (R. Karina Gallardo)
Organization name: WSU SES PREC **Contact Administrator:** Karen Proudfoot
Telephone: 253-445-4507 **Email:** karen.proudfoot@wsu.edu

Item	2016	2017
Salaries[1]	$3,820	$3,973
Benefits[1]	$1,247	$1,297
Travel[2]	$1,900	$1,090
Miscellaneous[3]	$0	$1,116
Total	$6,967	$7,476

[1]Salaries are for Research Associate (6.25% FTE). Benefits calculated at 32.65%.
[2]Travel in Year 1 is for meeting with individual packers in Yakima and Wenatchee. Travel in Year 2 is for meetings with scientists and industry stakeholders in Wenatchee to report the data gathered and economic analysis.
[3]Miscellaneous includes poster and publication of study results.

OBJECTIVES

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

2. Investigate (a) residue levels related to each method and (b) impact on fungicide resistance development.

3. Evaluate the effect of different rates of fludioxonil and pyrimethanil on efficacy, residue levels, and resistance risks.

4. Evaluate the economic impact of each application method by estimating costs and benefits.

SIGNIFICANT FINDINGS:

- Overall, clean drench (<500 bins/tank) provided a better efficacy in controlling decays after 6 to 8 months of storage.
- Decay on artificially inoculated wounds developed earlier on fruit treated with a fungicide through a dry application (fog or aerosol) compared to a wet (drench/dip) application.
- In commercial rooms without wounding or artificial inoculation, decay incidence was slightly higher in rooms treated dry compared to those with drenched fruit especially in susceptible cultivars such as Fuji. Differences vary between lots depending on preharvest sprays.
- Efficacy of aerosol or fog in controlling decay was similar for TBZ, fludioxonil and pyrimethanil.
- Fungicide residue levels were uniform between bins sampled on the truck and within the range between the MRL (maximum residue level) and LMR (lower limit for efficacy).
- Inconsistency in fungicide residue levels was common in rooms treated via aerosol or fog. Bins in the center and the back of the room have lower residues then those at the front of the room. Also, bins at the top of the pile have higher residue levels than those in the middle or the lower level of the pile.
- An economic study based on the price of a bin (between $4 and $6) and corelated with decay incidence estimated lost revenues could be double in rooms (2,000 bins) with dry application in susceptible cultivars whereas a difference of a $1,000 to $3,000 was estimated for less susceptible cultivars such as Red Delicious.

METHODS

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

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

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

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

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

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

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

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

Because no commercial room or RCA room (Stemilt) were not available, this objective will be conducted in 2017 at Pace and Decco facilities in Yakima. We will test the effect of reducing the rate of Flud and Pyr applied through fog or aerosol on efficacy and residue levels. Trials will be conducted in small cold rooms at Pace facility (Wapato) and at Decco facility (Yakima). An experiment will be conducted on wounded and inoculated fruit exactly as described in objective 1. For the semi-commercial trial using natural infections, five fruit bins will be used per room. Wounded and unwounded fruit will be placed in same room and fogged with pyrimethanil (ecoFog^{TM}-160, Pace Int) or fludioxonil (ecoFOG80, Pace Int.), or aerosoled with fludioxonil

(Scholar EZ, Syngenta) at full, 75, and 50% label rate. Fruit will then be stored in a CA room at 1°C (33°F) for 3 months (wounded fruit) and 6 months (for unwounded fruit) before checking incidence and severity as explained in objective 1. Residue levels will be assessed on wounds and whole fruit as explained in objective 2.

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

RESULTS AND DISCUSSION

Decay development on artificially wounded and inoculated fruit.

In fall 2016, Gala, Fuji and Red Delicious apples were inoculated and then either drenched, fogged or aerosolled. Fruit used for fog or aerosol were placed at the front of the rooms where fungicide residue levels are expected to be adequately high. On all cultivars, decay developed after 70 days for fog or aerosol, whereas drenched fruit showed significant decays after 140 days. For blue and gray molds, decay reduction with drenched ranged between 35 to 90% compared to dry application.
Non-inoculated wounds treated with different fungicides and methods were cut and subjected for fungicide residue analyses. Residue levels found on wound were similar to levels on fruit surface (cuticle). This indicates that something other than the fungicide concentration affect decay control by fungicides applied through fog or aerosol.

In 2018, trials were conducted to evaluate the efficacy of fludioxonil + TBZ and pyrimethanil applied via the three methods above. *Penicillium expansum* (blue mold) was better controlled by dry application compared to drench but *Botrytis* (gray mold) and *Neofabraea* (bull's eye rot) were better controlled by drench. Results were overall similar to those obtained in the 2016-17 season.

Decay development on naturally infected fruit at commercial packinghouses.

In the 2016-17 season, Fuji fruit and Red Delicious apples grown conventionally were harvested, stored and treated with fungicides following standard procedures used in commercial packing houses. Rooms were opened after 5 to 7 months and 15 bins located at the front (6 bins), center (3 bins) and back (6 bins) of each room were used. Decayed fruit were separated healthy fruit to determine decay incidence.
For Red Delicious, Fludioxonil + TBZ was applied through drench, fog or aerosol. Decay incidence was 0.52, 0.70 and 0.84%, respectively.
For Fuji apples, 3 different lots were used. Total decay incidence ranged between 0.32% and 2.2% in drenched fruit and between 0.58% and 9.3% for fog and aerosol with FDL + TBZ. For Penbotec (PYR), decay rate ranged between 0.7 to 1.8 for drench and from 0.27 and 5.2% for fog.

In the 2017-18 season, trials at two commercial packinghouses included Fludioxonil + TBZ applied via fog or aerosol in comparison with drench including Fuji, Golden Delicious and Gala. Results showed slightly higher decay rates in rooms with dry (for or aerosol) compared to drench but differences were not always statically different.

While the overall trend seems to indicate increased decay rate following dry application, there are nuances that should be taken into consideration:

- In susceptible cultivars such as Fuji and Gala, there were significant differences between lots. When orchard disease pressure is high, drench seem to provide a better efficacy than dry applications.

- ❖ Therefore, management should start from orchard especially for diseases such as gray mold and particularly bull's eye rot, especially in lots with a known history of disease problems.
- ❖ Drench used in our trials on Red Delicious was similar to standard packinghouse practices (700 to 1000 bins). But for Fuji, our drench can be considered as a "fresh" drench because the fungicide solution was only recycled for about 100 bins. When a fungicide solution gets "dirtier", the risk for cross-contaminations with Mucor or blue mold to occur may be higher but efficacy should not decrease as long as there is enough active ingredient (fungicide) in the solution.

Fungicide residue levels on fruit drenched, fogged or aerosolled

Drench:
All fruit sampled from drenched lots showed residue levels at or slightly above the lowest level required for the fungicide to be effective (LMR). We sampled from three bins on the truck (top, middle and bottom) and there were no significant differences in term of residue levels.

Fog:
Residue levels for Pyrimethanil (a.i.) were above the LMR and below the MRL at the front and center of the room but lower than LMR in floor bin regardless of the position of the room.
Significant variability in term of residue of TBZ and fludioxonil (FDL) was observed between bins next to the ceiling compared to the floor bins and between the front and the back of the room. Residue higher than MRL were seen at ceiling and lower then LMR at back and center of the rooms.

Aerosol:
We tested only TBZ and FDL. Overall, fungicide distribution throughout the room was more consistent compared to fog. For FDL residue levels were all above LMR and below MRL (max levels allowed) but TBZ residues were lower than minimum required (LMR) level in all rooms tested in 2016 but higher than LMR in most cases in 2017-18.

2017 vs. 2018: Fungicide residue level patterns were similar between the two seasons except that the LMR for TBZ through Aerosol improved throughout the room (higher than LMR) from last season.

Evaluate the economic impact of each application method by estimating costs and benefits.

Methods: Drenching is the status quo method to prevent and control postharvest diseases for apples in controlled atmosphere storage. Usually, packing houses perform in-house drenching; however, they hire outside companies for thermofogging and aerosolization. To compare the costs of the different methods, we collected two years' data on fungicide costs (material and application costs) and decay rates for each method using Penbotec or Fludioxonil+TBZ as fungicide.

Assumptions: The following are used in the analysis:
- We considered two models for a drencher – basic model at $50,000, and advanced model at $100,000/unit. The annual costs for maintenance and repair, water and power costs per 2,000 bins, and hourly labor cost are shown in Table 1.
- A drencher runs 500 bins per hour, and drenching is done for 12 weeks between mid-August to early November. The maximum capacity of a drencher is estimated at 240,000 bins per season (i.e., 500 bins/hour *times* 8 working hours/day *times* 5 working days/week *times* 12 weeks/season).
- The alternative to drenching, aerosol or thermofogging, is also applied between mid-August to early November.

- Data were collected for two years but no differences were observed across years. Therefore, representative costs and revenues for 2 years are reported.

Table 1. Costs of different models of a drencher.

	Basic model	Advanced model
Initial investment	$50,000	$100,000
Maintenance and repair ($/year)	$2,000	$2,000
Water and power (2,000 bins)	$500	$500
Labor ($/12 weeks)	$4,500	$8,000

Results: Fungicide costs are estimated for different treatment methods (Table 2). If drenching only, using Penbotec is cheaper compared to using Fludioxonil+TBZ. Note that drenching includes the cost of the fungicides, water and power, labor, and capital. A more detailed calculation of the annual drenching cost is provided in the appendix.

For thermofogging, the cost ranges from $8,000 to $10,000 per 2,000 bins if using Penbotec, and $8,000 to $12,000 per 2,000 bins if using Fludioxonil+TBZ. For aerosolization, the fungicide costs are the same regardless of the fungicide product used. Thermofogging and aerosolization are both custom work which means their respective costs include material and application costs.

Table 2. Cost of fungicide application by different treatment methods ($ per 2,000 bins).

Chemicals	Drench		Fog		Aerosol
	Basic model	Adv. model	Lower rate	Higher rate	
Penbotec	$4,449	$4,501	$8,000	$10,000	$8,000
Fluodioxonil+TBZ	$4,649	$4,701	$8,000	$12,000	$8,000

Table 3 shows the estimated revenues of 'Fuji' and 'Red Delicious' that are lost due to decay in fruits while in storage. This considers an average price of $530/bin for 'Fuji' and $420/bin for 'Red Delicious'. Compared to the other treatment methods, *drench* using Fludioxonil+TBZ has the smallest amount of revenues lost for 'Fuji' (i.e., $11,554 – $11,660 /2,000 bins) and 'Red Delicious' (i.e., $4,368 /2,000 bins).

Table 3. Comparison of lost revenues due to decay given different treatment methods to 'Fuji' and 'Red Delicious'.

Method	Chemical	Decay rate (per 2,000 bins)		Lost revenues ($/bin)	
		Fuji	Red Delicious	Fuji	Red Delicious
Drench	Penbotec	1.49%	0.52%	$7.90	$2.18
Drench	Fludioxonil+TBZ	1.09%–1.10%	0.52%	$5.78-$5.83	$2.18
Thermofogging	Penbotec	3.80%	0.70%	$20.14	$2.94
Thermofogging	Fludioxonil+TBZ	2.95%–3.92%	0.84%	$15.64-$20.78	$3.53
Aerosolization	Fludioxonil+TBZ	3.92%	0.84%	$20.78	$3.53

Table 4 shows the estimated foregone revenues as percentage of the average gross return or price per bin of 'Fuji' and 'Red Delicious'. Considering an average price of $530/bin and $420/bin for 'Fuji' and 'Red Delicious', respectively, the foregone revenues in drenching is 0.5% of the average price of 'Red Delicious' and between 1.1%-1.5% of the average price of 'Fuji'. These proportions are highest in aerosolization for 'Fuji' (3.92%), and in both thermofogging and aerosolization for 'Red Delicious' (0.84%).

Table 4. Comparison of lost revenues given different treatment methods, as percentage of the average prices of 'Fuji' and 'Red Delicious'.

Method	Chemical	Lost revenues (%)	
		Fuji	Red Delicious
Drench	Penbotec	1.49%	0.52%
Drench	Fludioxonil+TBZ	1.09%-1.10%	0.52%
Thermofogging	Penbotec	3.80%	0.70%
Thermofogging	Fludioxonil+TBZ	2.95%-3.92%	0.84%
Aerosolization	Fludioxonil+TBZ	3.92%	0.84%

Given the lower overall costs of fungicide application, lower decay rates, and lower foregone revenues in drenching relative to the alternatives, it is more profitable to invest on a drencher (considering both variable and fixed costs) than pay custom work for thermofogging or aerosolization. Comparing the two chemicals applied in drenching 'Fuji', better results or lower decay rates were observed with Fludioxonil+TBZ compared to Penbotec. Also 'Red Delicious' responded with less percentage of decay than 'Fuji' across all treatments.

Appendix
Calculation of annual drenching cost, excluding chemical solution

	Drencher - Basic Model			Drencher - Advanced Model		
	Annual Cost	Cost per bin	Cost per 2,000 bins	Annual Cost	Cost per bin	Cost per 2,000 bins
Interest cost[A]	$1,250	$0.01	$10.42	$2,500	$0.01	$20.83
Depreciation cost[B]	$5,000	$0.02	$41.67	$10,000	$0.04	$83.33
Maintenance and repair	$2,000	$0.01	$16.67	$2,000	$0.01	$16.67
Labor[C]	$9,600	$0.04	$80.00	$9,600	$0.04	$80.00
Water and power			$500			$500
Total cost			**$648.75**			**$700.83**

Notes:

Initial investment (Basic)	$50,000
Initial investment (Advanced)	$100,000
Interest	5%
Salvage value	$0
Useful life	10
Labor rate ($/hour)	$20
Total number of bins drenched[D]	240,000

A. Interest Cost is calculated as: (Total Purchase Price + Salvage Value)/2 x Interest Rate.
B. The depreciation cost is calculated as straight-line depreciation: (Total Purchase Price – Salvage Value)/Years of Use.
C. Labor hours for 12 weeks: 8 hours per day *times* 5 days per week *times* 12 weeks = 480 hours
A drencher runs 500 bins per hour. The capacity constraint is estimated as follows: 500 bins per hour *times* 8 working hours per day *times* 5 working days per week *times* 12 weeks per season = 240,000 bins per season.

ACKNOWLEDGMENTS

We thank the WTRC for funding this crucial project. We also thank the participating packers for allowing us to use access their rooms to conduct the work planned. This a challenging work to do and their patience and collaboration has been much appreciated. Moreover, we are very thankful to our collaborators from Pace and Decco who provided a tremendous support with application and residue analyses.

OTHER OUTCOMES

Talks

- **Amiri.** Comparison of wet and dry fungicide applications to control apple postharvest disease. 69th International symposium of crop Protection, Ghent, Belgium, May 2017.
- **Koundal & Amiri.** Comparison of fungicide application methods to control apple post-harvest diseases. WTFA-HortShow, December 2017.
- **Koundal & Amiri.** Comparison of drench and thermonebulization of fungicides to control post-harvest diseases in apple. American Phytopathological Society, Pacific division meeting, Portland, June, 2018.
- **Amiri.** Benefit and Risks of Drench and Thermonebulization of Fungicides to Control Storage Decays. WTFA-HortShow, December 2018.
-

Abstracts and Manuscripts:

Amiri & Koundal. 2018. Is thermonebulization the solution to control postharvest decays of pome fruit. International Congress of Plant Pathology, Boston, July-August, 2018.

Koundal & Amiri. 2019. Thermonebulization of fungicides to control major postharvest disease of apple fruit. (In preparation).

EXECUTIVE SUMMARY

This is the first study in its genre comparing two approaches (dry vs. wet) and three methods (Drench vs. Aerosol vs. Fog) side by side for postharvest fungicide applications in semi-controlled and controlled environments. Although more research is needed to understand all the aspects of the different methods, findings from this study allow to make some general conclusions:

- Decay on artificially inoculated wounds developed earlier on fruit treated with a fungicide through a dry application (fog or aerosol) compared to a wet (drench/dip) application except for blue mold.
- An average of 5 to 10 days delay in applying the fungicide via dry application after harvest has been noticed throughout our survey in hundreds of lots through most packinghouses.
- Under commercial conditions, decay incidence was slightly higher in rooms treated dry compared to those with drenched fruit especially in susceptible cultivars such as Fuji. Differences vary between lots depending on preharvest management
- Efficacy of aerosol or fog in controlling decays was similar for all three current postharvest fungicides.
- Fungicide residue levels were uniform between bins sampled on the truck and within the range between the MRL (maximum residue level) and LMR (lower limit for efficacy).
- Inconsistency in fungicide residue levels was common in rooms treated via aerosol or fog. Bins in the center and the back of the room have lower residues then those at the front of the room. Also, bins at the top of the pile have higher residue levels than those in the middle or the lower level of the pile.

Implications to the industry and future trend: It is estimated that about 60% of apple packers currently apply fungicides dry (aerosol or fog) and the number may vary from a season to another and from early (August) versus late (October-November). It is difficult to predict in which direction the industry will move in the next years or decade, especially for food safety matters, however, the choice to go dry or wet to control decay can be challenging to make and should take into consideration efficacy, safety and security (fungicide residue above MRL), cultivars, length of storage and costs. Our preliminary data indicate that dry application may be more suitable to reduce blue mold and Mucor rot because inoculum of these pathogens present on bins are less recirculated compared to drench. However, there might be a higher risk for the remaining diseases, such as gray mold, bull's eye rot, speck rot, etc.., which initiate infections in the orchards and which frequencies were higher in lots treated dry, especially that a delay of 7 to 10 days after harvest is common in packinghouses currently. Packers who prefer to use drenchers are advised to implement enhanced sanitation practices to reduce risks of contamination but future research will be needed to help them deal will food safety issues related to the drenchers. Thermo-nebulization (aerosol or fog) of fungicides depends on the chemistry of the formulated fungicide and the physical barriers (mainly bins) inside the room. Companies that develop these technologies should put more efforts in improving the technologies in term of power energy to provide and end product that diffuses uniformly and quickly in the room. The formulations of the fungicides can be improved to enhance fungicide distribution and residue levels: heavy chemistries are more difficult to fog/aerosol than lighter ones. An effort may also be required from the packers in the way they stack the bins. Although this requires further research, leaving some space between bin piles, especially in the middle and the back of the room may improve fungicide distribution and residue levels. Future research will be needed to evaluate new technologies, such as Actimist from AgroFresh, coming into the market as the application device may differ and provide completely different results. Conducting research on this issue is challenging for scientists because of some limitations but collaborations of all parties are highly recommended and needed.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project: AP-18-100

Project Title: How does fruit acclimation to sunburn affect sunburn management?

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Cooperators: Brenda Castaneda

Total Project Request: **Year 1:** 86,621 **Year 2:** **87,846**

Other funding sources

None

Budget: Kalcsits, Waliullah, Waite
Organization Name: TFREC-WSU **Contract Administrator:** Katy Roberts/Kim Rains
Telephone: 509-335-2885/509 663 8181 (221) **Email address:**
arcgrants@wsu.edu/kim.rains@wsu.ed

Item	2018	**2019**
Salaries[1]	49,920	51,917
Benefits[2]	18,201	18,929
Travel[3]	1,500	1,500
Goods and Services[4]	17,000	15,500
Total	86,621	**87,846**

Footnotes:
[1,2] Salaries and 36.5% benefits for Post-Doctoral Research Associate (Dr. Sumyya Waliullah, now Dr. Jessica Waite)
[3]For frequent travel to orchard site (Quincy) where trials are being conducted
[4]Goods and services include irrigation supplies, fruit respiration chamber, basic physiological and molecular lab supplies including molecular biological enzymes and chemicals for gene expression analysis and pigment analysis, liquid nitrogen tank rental and lab consumables.

OBJECTIVES

1. Identify how acclimation to high light and near sunburn threshold temperatures influences fruit susceptibility to sunburn

In 2018, we conducted experiments looking at the effect of temperatures on fruit susceptibility to sunburn. We also added some measurements to identify fruit surface temperature patterns during fruit development and under different environmental conditions. In 2019, these experiments will be run again and we will also assess the impact of removing netting mid-season on photooxidative sunburn development. Dr. Sumyya Waliullah left for a new position at the University of Georgia after completing the summer experiments. Since then, Dr. Jessica Waite has joined the Kalcsits lab and has taken over responsibilities for this project under the guidance of Lee Kalcsits.

2. Link physiological and biochemical changes in the fruit to sunburn development.

In 2018, we completed the major field experiments that will also be done again in 2019. Currently, samples that were collected during the summer are being processed for gene expression analysis and pigment quantification. This will be completed prior to the start of the 2019 field season. We expect to repeat some of this sampling in 2019.

3. Use information provided on temperature and light conditions that stimulate natural resistance to guide evaporative cooling and sunburn protectant applications and reduce losses to sunburn

Here, we will be testing the use of automated evaporative cooling triggers when air temperature is either 85 or 90 °F. We will track water use, soil moisture, and fruit surface temperature through continuous loggers. We will also test supplemental protectant applications under netting.

SIGNIFICANT FINDINGS

1. **Fruit are less susceptible to sunburn early in the season due to differences in stomatal conductance (transpiration) and pigmentation.**

2. **Honeycrisp has elevated fruit surface temperatures compared to Granny Smith or WA38 under the same light and heat conditions. This leads to elevated sunburn risk compared to the other two cultivars tested.**

3. **Exposure to near threshold temperatures in June induced important processes that led to increased sunburn resistance in July and August. Sudden temperature changes or practices that keep fruit surface temperatures cooler may lead to increased susceptibility to sunburn if the light or heat conditions suddenly change.**

METHODS

Objective 1: The experiment took place at Sunrise research orchard on trees covered by 20% pearl netting to reduce the exposure to high light intensities before the start of the experiments. Thirty sample trees for each cultivar were selected early in 2018 for uniform size and crop load and thinned to appropriate commercial crop loads. To manipulate fruit surface temperature (FST) on fruit in the field, methods that were adapted from those developed by Larry Schrader were used to increase the FST to 110-113 °F, 114-119 °F, or 120-124 °F for 45 to 60 minutes under full sunlight (30 fruit for each treatment plus an additional 90 fruit for the 110-113 °F treatment). These temperatures were

chosen to bracket the threshold temperature for sunburn browning (114-119 °F). The fruit surface temperature was measured using thermal imaging. Afterward, representative fruit samples (15 fruit) were taken from each temperature treatment for physiological, molecular, biochemical, and non-destructive and destructive pigment analysis described in Objective 2.

Two weeks later, the 90 fruit from the coolest treatment group (110-113 °F), which were not damaged by sunburn were exposed again to the three subsequent radiant heat treatments to assess whether the fruit had become acclimated to higher temperatures (Fig. 1). Afterwards, representative fruit samples were taken for physiological, molecular, biochemical and non-destructive and destructive pigment analysis. A separate subset of fruit was exposed to below, at, or above threshold temperatures three weeks prior to harvest for Honeycrisp to capture different physiological changes that may occur during fruit ripening and cause changes in sunburn susceptibility. However, smoke during this period prevented us from retrieving the fruit at harvest. As a result, we have samples near maturity that have been exposed to below, at and above threshold fruit surface temperatures that will provide information on physiological changes within the fruit. This information will guide sampling for 2019 where we will analyze gene expression of stress pathway components, such as phenylpropanoid and anthocyanin pathway genes, as well as known components of stress memory and acclimation processes.

Objective 2: This objective was focused on teasing apart physiological and developmental changes to fruit during sunburn development. We focused on three areas of interest: (1) how differences in stomatal function during fruit development relate to regulation of fruit surface temperature, (2) physiological changes that occur during sunburn development, and (3) physiological changes that occur that may be linked to acclimation to heat and light.

During fruit development, stomatal function decreases, and subsequently the cooling ability of the fruit decreases, making it more susceptible to sunburn. We measured transpiration in developing fruitlets of Honeycrisp, WA38, and Granny Smith at six different time points during the season using a LI-6400XT (LI-COR, Lincoln, NE) with an adapted chamber for sampling fruit. Corresponding fruit surface temperature measurements augmented with local orchard climate data will provide information on the importance of fruit stomata and lenticels in limiting fruit surface temperature. Additionally, to capture changes occurring during sunburn development, we have supplemental information on biochemical and gene expression changes that occurred from the controlled heating experiments. These will be processed from December 2018 to April 2019.

Objective 3: This will be started in 2019. Temperature activated solenoids will be installed at Sunrise research orchard in a Honeycrisp block. Three replicates of each treatment (85 °F cooling activation, 90 °F cooling activation, and an uncooled control) will be used for these experiments**.** Fruit surface temperatures will be continuously monitored during these periods using custom-built infrared thermometers connected to a cellular data-logger. Sunburn incidence will then be tracked throughout the season along with soil moisture conditions.

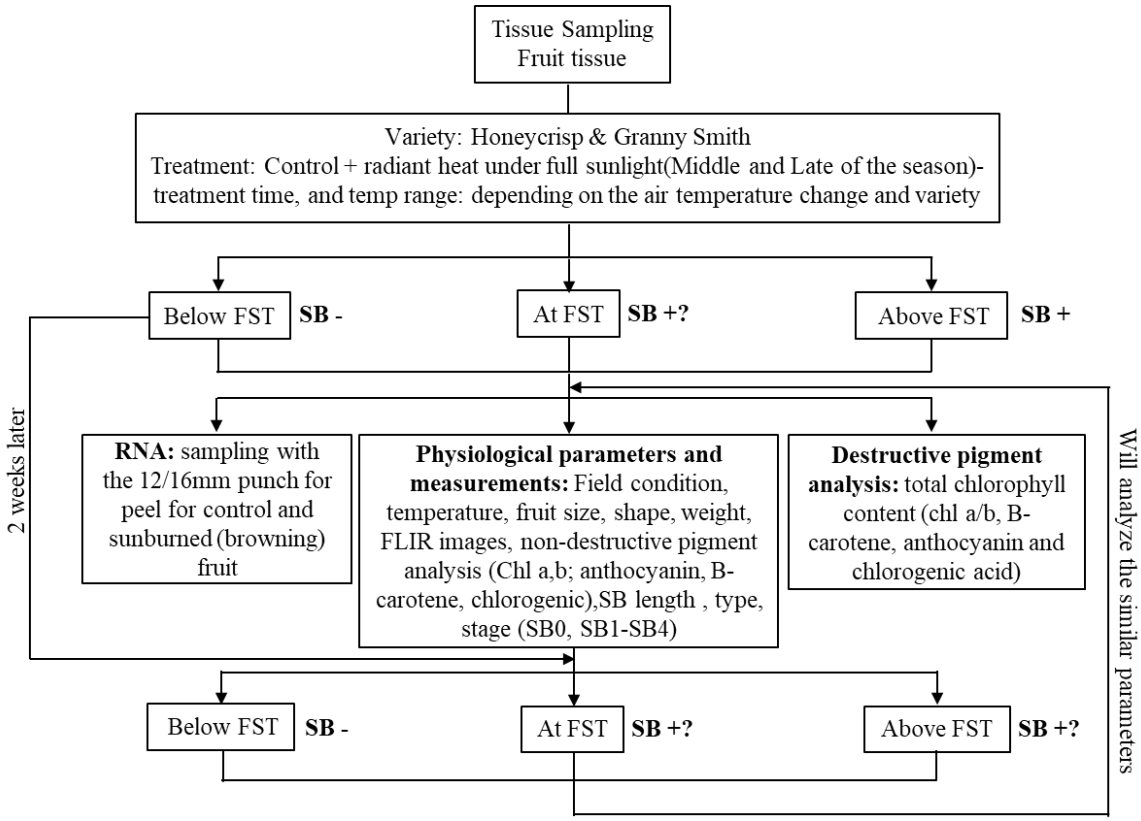

Fig. 1. Experimental layout for experiments conducted in 2018 (1) fruit acclimation to high temperature and (2) mechanisms underlying sunburn resistance and development in developing fruits. FST = Fruit surface temperature threshold.

RESULTS & DISCUSSION

Fruit surface temperatures monitored throughout the 2018 growing season indicate that there are cultivar level differences in response to light and air temperature (Figs. 2-4). Honeycrisp apples maintain greater fruit surface temperatures under similar conditions, and under the most extreme temperature and light conditions can vary by as much as 10 °F more than Granny Smith or WA38. Approximately three-quarters of the variation in fruit surface temperature can be explained by two variables: air temperature and light intensity. For experiments conducted to determine whether fruit can physiologically acclimate to elevated fruit surface temperatures, fruit that was exposed to near threshold fruit surface temperatures in June when temperatures were relatively cool showed reduced sunburn compared to fruit that was exposed to normal conditions (Fig. 5). When the temperature rapidly increased from highs in the mid-70s to about 100 °F in early July, sunburn incidence and severity was greater for unexposed fruit (Fig. 5). This was true for both Honeycrisp and Granny Smith; although overall incidence was lower for Granny Smith than Honeycrisp, which further supports the observations that Honeycrisp, with elevated fruit surface temperatures, is more susceptible to sunburn. Fig. 6 shows two images of sunburn development in untreated fruit but no sunburn development in adjacent fruit that was exposed to near threshold temperatures. This is evidence of physiological changes occurring within the fruit that increase resistance to sunburn, and will be explored more in depth in the winter of 2018 and in 2019.

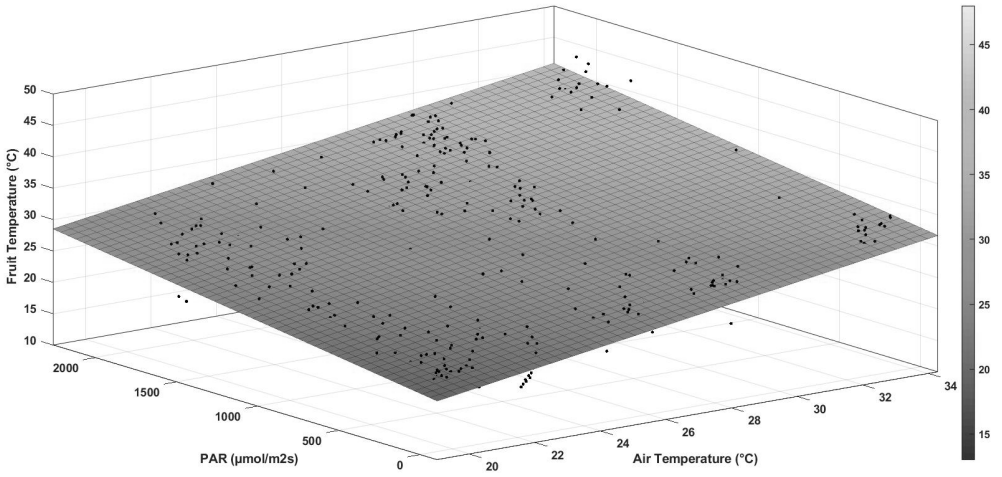

Fig. 2. Fruit surface temperature of WA38 apples as a function of light intensity at the fruit surface and air temperature. Each point represents one fruit measured from June 1 through to August 24, 2018. The surface represents the best fit model using air temperature and light intensity to explain fruit surface temperature

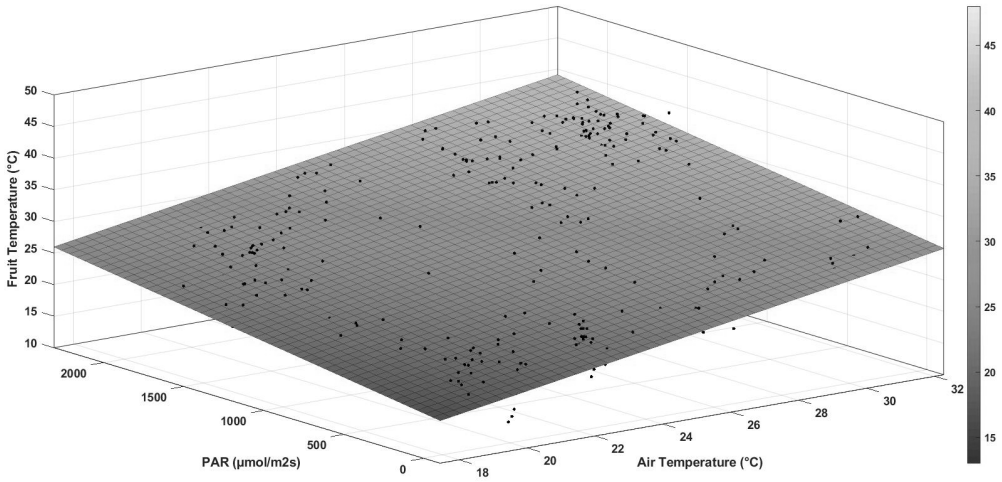

Fig. 3. Fruit surface temperature of Granny Smith apples as a function of light intensity at the fruit surface and air temperature. Each point represents one fruit measured from June 1 through to August 24, 2018. The surface represents the best fit model using air temperature and light intensity to explain fruit surface temperature

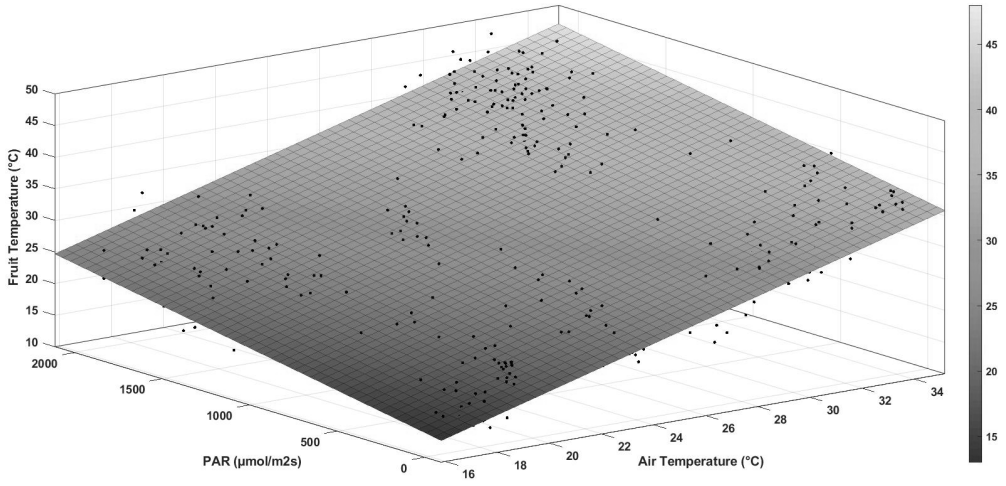

Fig. 4. Fruit surface temperature of Honeycrisp apples as a function of light intensity at the fruit surface and air temperature. Each point represents one fruit measured from June 1 through to August 24, 2018. The surface represents the best fit model using air temperature and light intensity to explain fruit surface temperature

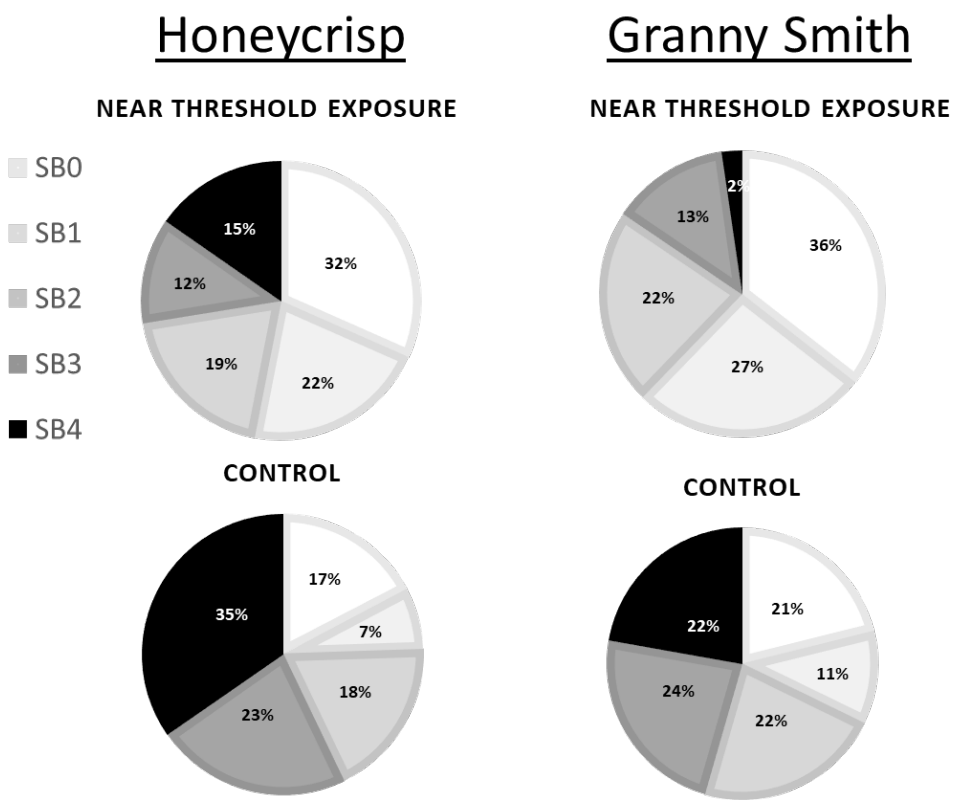

Fig. 5. Fruit sunburn severity of Honeycrisp or Granny Smith apples (N=90) after being exposed to sunburn inducing temperatures in July that were either exposed to near sunburn threshold temperatures in June or only exposed to cooler ambient conditions that were present in June, 2018. The sunburn scale used was a 5-point scale where SB0 is where there is no sunburn present and SB5 is where there is browning formed on the fruit surface.

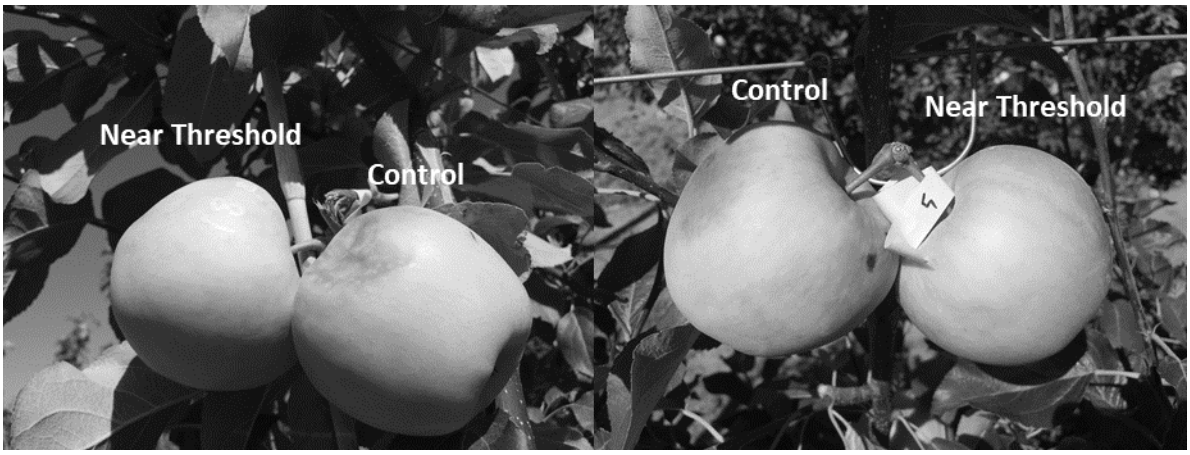

Fig. 6. Fruit sunburn development after sudden increased in temperature during early July 2018. Adjacent fruit was either left untreated or exposed to elevated, near sunburn threshold fruit surface temperatures for one hour in June 2018. Fruit that was part of the untreated control suffered more severe sunburn than fruit that was exposed to near-threshold temperatures.

From measurements of stomatal function during fruit development, we observed that transpiration rapidly decreased in fruit during the month of June and slowly began to decrease as fruit continued to grow and mature. While changes in pigmentation during sunburn have been well documented, the changes in pigmentation that provide further protection for fruit have been less documented. Fig. 7 shows changes in reflectance during fruit development where reflectance increases as fruit matures meaning that it absorbs less energy. Specifically, it reflects more green and red light. There were also differences between interior or exterior fruit where exposed fruit reflects more red light and non-exposed fruit reflects more green light. However, this will be confirmed based on a larger dataset that is being processed in December.

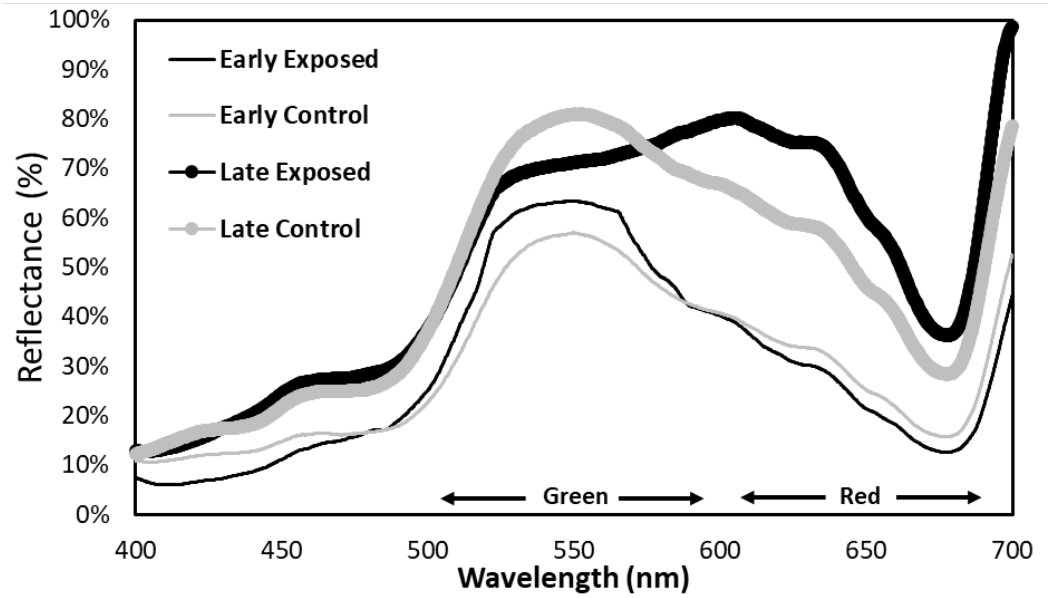

Fig. 7. Spectral reflectance (% of incoming energy) for Honeycrisp before and after a rapid heating event for fruit that was either exposed to high radiation pressure (exposed) versus low radiation pressure (control).

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project: AP-18-102

Project Title: Optimizing light and water for orchards covered with netting

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Cooperators: Manoella Mendoza (WTFRC): Extenday USA Inc

Total Project Request: **Year 1:** $99,921 **Year 2: $89,176**

Other funding sources

Agency Name: Extenday USA Inc
Amt. requested/awarded:
Notes: In-kind contribution of protective netting materials, reflective ground cover, consumables for installation, field visits to identify trial sites and consultancy services at the trial establishment.

WTFRC Budget:

Item	2018	2019
Salaries	7,000	7,000
Benefits	3,000	3,000
Wages	1,000	1,000
Benefits	600	650
Shipping	150	180
Travel	500	500
Total	12,250	**12,330**

Footnotes:

Budget 1
Organization Name: TFREC-WSU **Contract Administrator:** Katy Roberts/Kim Rains
Telephone: 509-335-2885/509 663 8181 (221) **Email address:** arcgrants@wsu.edu/kim.rains@wsu.ed

Item	2018	**2019**
Salaries[1]	49,920	51,917
Benefits[2]	18,201	18,929
Equipment[3]	13,550	-
Supplies[4]	3,000	3,000
Travel[5]	3,000	3,000
Total	87,671	**76,846**

Footnotes:
[1]Salary for 100% Postdoc Research Fellow (Kalcsits)
[2]Benefits rate @ 36.5%
[3]Purchase Flow32-1K Sap Flow System
[4]Lab consumables
[5]Travel to Sunrise and field cooperator sites

OBJECTIVES

1. Determine the optimal shade percentage for the most common cultivars under protective netting in WA (Honeycrisp and Granny Smith)
2. Test whether reflective ground fabrics improve light penetration under protective netting to improve fruit quality, flower bud formation, return bloom, and fruit set in 'Honeycrisp' and 'Granny Smith' apple.
3. Quantify changes in water needs for orchards under protective netting in 'Honeycrisp' apple.

SIGNIFICANT FINDINGS

- In 'Granny Smith', the higher shade factors (17% and 24%) had higher sunburn incidence reduction compared to 10%, all the shade factors significantly reduced sunburn compared to the uncovered control.
- In 'Honeycrisp', 10%, 17%, and 24% shading reduced sunburn incidence significantly compared to the uncovered control
- For 'Honeycrisp', 24% shade increased the incidence of bitter pit compared to an uncovered control
- In 'Honeycrisp', 10%, 17%, and 24% shade factor reduced red color coverage compared to the uncovered control, but no differences were observed between the different shade factors.
- In 'Granny Smith', red blush was reduced under 17% and 24% shading compared to 10% shading.
- Extenday® and Mylar® improved red coloration significantly compared to protective netting without reflective ground cover as a control.
- Under protective net, Mylar® had significantly higher sunburn incidence compared to Extenday ®.
- Red blush incidence at harvest under netting was reduced in 'Granny Smith' when Extenday® was deployed early in the season at full bloom for ≈7 weeks
- 17% protective net reduced overall water use in 'Honeycrisp' by reducing tree transpiration and soil evaporation by somewhere between 15 and 30%

METHODS.

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

5th 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. The netting was first deployed in the spring of 2015. In 2018, the existing netting was removed and replaced with 10%, 17% and 24% white protective netting between (4-6) June 2018. Full bloom was on 19 April 2018. Weather equipment for monitoring environmental conditions was installed on 29th June 2018.

Site 2: McDougall & Sons, Inc., Mattawa, WA.

12th leaf 'Granny Smith' on M26; trees were trained on a 4-wire tall spindle trellis and spaced at 3' X 12'. The trees were planted in winter 2006. Full bloom was on 18 April 2018. Protective netting (10%, 17% and 24%) was deployed 05th of May for the shade factor trial and 2nd of July for the reflective ground cover trial. The block used for the trial was not under protective netting before. Weather equipment for monitoring environmental conditions was installed on 8th of May 2018 only in the reflective ground cover trial.

Objective 1: Determine the optimal shade percentage for the most common cultivars under protective netting in WA (Honeycrisp and Granny Smith)

At both Quincy and Mattawa, 10%, 17% and 24% protective netting was deployed in 'Honeycrisp' and 'Granny Smith'. A randomized complete block design was used with five replications per treatment in 'Honeycrisp' and four replications per treatment in 'Granny Smith'. Leaf gas exchange, leaf spectral reflectance, leaf chlorophyll fluorescence and plant water status were measured at 30 and 60 days after deployment. Meteorological conditions were measured at the Quincy site, namely; solar radiation, ambient temperature and relative humidity (above and in-canopy). Fruit quality was assessed at harvest and fruit was run through a test commercial grade packing line. Fruit quality after 3 months of regular cold storage was conducted on 12-12-18 (Honeycrisp) and 12-19-18 (Granny Smith) and will be included in the final report.

Objective 2: Test whether reflective ground fabrics improve light penetration under protective netting to improve fruit quality, flower bud formation, return bloom, and fruit set in 'Honeycrisp' and 'Granny Smith' apple.

The trial was conducted under 17% white neutral protective net for both 'Honeycrisp' and 'Granny Smith' respectively. The treatments in 'Honeycrisp' were protective netting without reflective ground cover as a control and protective netting with late reflective ground cover deployed ≈6 weeks (Extenday®) and ≈2 weeks (Mylar®) before harvest. The treatments in 'Granny Smith' were protective netting without reflective ground cover as a control and protective netting with early reflective ground cover (Extenday®) at cell division from full bloom until 22nd June 2018 (≈ 7 weeks). A randomized complete block design was used with five replications per treatment in 'Honeycrisp' and four replications per treatment in 'Granny Smith'. Ecophysiological measurements comprising of leaf gas exchange, leaf spectral reflectance, leaf chlorophyll fluorescence and plant water status were done at 4 weeks after installation in 'Granny Smith' and 1 day before harvest in 'Honeycrisp'. Meteorological conditions were measured at the Mattawa site, namely; ambient temperature and relative humidity (above and in-canopy) and soil moisture. Fruit quality was assessed at harvest and fruit was run through a test commercial grade packing line. Fruit quality after 3 months of regular cold storage is going to be conducted on 12-12-18 (Honeycrisp) and 12-19-18 (Granny Smith).

Objective 3: Quantify changes in water needs for orchards under protective netting in 'Honeycrisp' apple.

The experiments were conducted in a 'Honeycrisp' orchard under 17% neutral white protective netting at Quincy, WA. The Dynagage Flow32-1K Sap Flow system with SGEX Exo Stem gages was used to measure tree water use. The two treatments were 17% neutral protective white net and an uncovered control. Four sap flow sensors were installed in each treatment to monitor water use. Trunk diameter was measured at the height where each sap flow sensor was installed. Sap flow was then be normalized per trunk cross-sectional surface area. Measurement of evapotranspiration from the orchard floor was done using microlysimeters. The microlysimeters were pushed into the soil either by hand. After removing the microlysimeter from the field, cleaning soil from the outside, and trimming the soil even with the bottom, a cap was used to seal the bottom of the cylinder. The microlysimeter was then weighed, put in an outer envelope and placed in a preformed hole in the soil. Following exposure to environmental conditions for 24 hours, the microlysimeter was removed from the hole and outer envelope and its mass is determined again. The difference between the two masses divided by the circular cross-sectional area of the cylinder was the cumulative soil evaporative flux density during the time period.

RESULTS & DISCUSSION

There were no differences in sunburn incidence between the different shade factors in 'Honeycrisp' (Figure 1). In 'Granny Smith', the two highest shade factors (17% and 24%) reduced sunburn

incidence probability compared to 10% shade netting. From these preliminary first-year results, shade factor does not seem to matter in a blushed cultivar like 'Honeycrisp', whereas in a green cultivar like 'Granny Smith', higher shade factors could help increase the amount of sunburn protection offered.

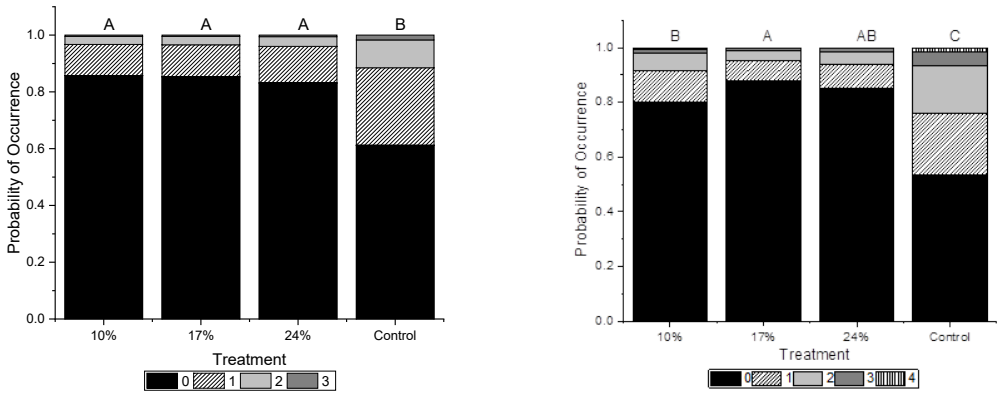

Figure 1. Probability analysis of sunburn occurrence in 'Honeycrisp' (left) and 'Granny Smith' (right) apple grown under 10%, 17% and 24% protective netting compared to an uncovered control. (0-5 score, with 0 having no sunburn and 5 the most severe).

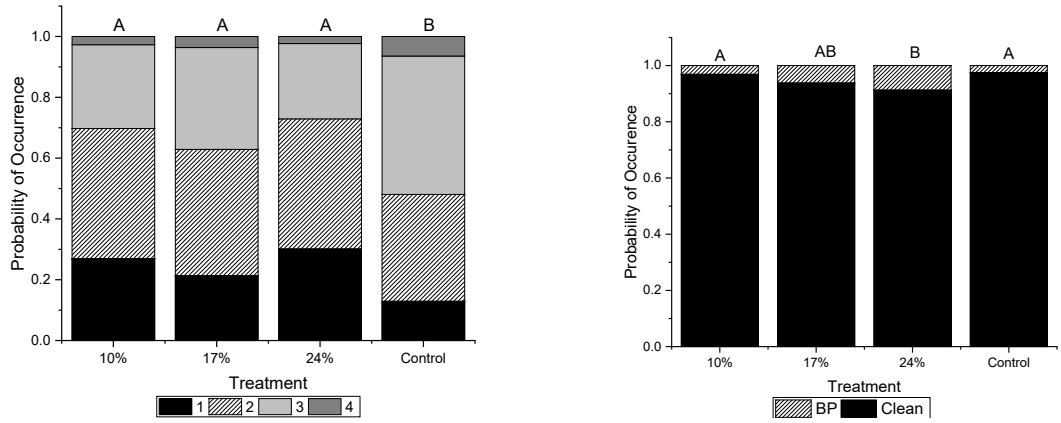

Figure 2. Probability analysis of red color coverage (1=0-25%, 2=26-50%, 5=51-75%, 4=>76% red coloration) (left) in 'Honeycrisp' and bitter pit incidence (right) in apple grown under 10%, 17% and 24% protective netting compared to an uncovered control

All shade factors reduced the number of fruit with >50% red color coverage compared to the control (Figure 2). There were no significant differences between the shade factors in of red fruit coloration. The highest shade factor (24%) increased the incidence of bitter pit in 'Honeycrisp' compared to the control and 10% shade factor (Figure 2). In 'Granny Smith', preliminary results from our study seem to indicate that shade factor played an important part in the occurrence of red blush (Figure 3). The incidence of red blush occurrence was significantly reduced under 17% and 24% shade factor compared to 10% shade factor and the control (Figure 3). Light is needed for the synthesis of anthocyanin which cause red blush in 'Granny Smith', the higher shade factors reduced the light sufficiently to reduce the synthesis of anthocyanins.

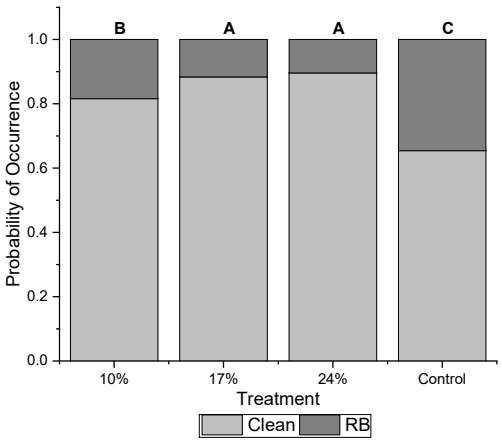

Figure 3. Probability analysis of red blush occurrence in 'Granny Smith' apple grown under 10%, 17% and 24% protective netting compared to an uncovered control (RB=red blush)

Table 1. The effect of 10%, 17% and 24% protective net on fruit quality of 'Honeycrisp' apple at harvest at Quincy, WA

Treatment	Fruit firmness	Total Soluble solids	Titratable acidity	Fruit weight (g)
Control	15.83	13.59 a	0.67	260.72
10% Shade	15.67	13.03 b	0.66	246.17
17% Shade	15.42	13.00 b	0.67	271.05
24% Shade	15.49	12.89 b	0.68	282.58
P value	0.4239	0.0029	0.8489	0.3027

Table 2. The effect of 10%, 17% and 24% protective net on fruit quality of 'Granny Smith' apple at harvest at Mattawa, WA

Treatment	Fruit firmness	Total soluble solids	Titratable acidity	Fruit weight (g)
Control	17.4 a	13.14 ab	1.03	228.82
10% Shade	17.6 a	13.40 a	1.06	235.29
17% Shade	16.9 b	13.05 b	1.08	228.46
24% Shade	16.3 c	12.91 b	1.01	226.49
P value	0.0001	0.0064	0.5407	0.7407

In 'Honeycrisp', shade factor had no effect on fruit firmness, titratable acidity and fruit weight whilst total soluble solids were reduced under all the shade factors compared to the uncovered (Table 1). In 'Granny Smith' 17% and 24% shade factor reduced fruit firmness compared to control (Table 2). 10% shade factor had significantly higher total soluble solids compared to 17 and 24% in 'Granny Smith'. Titratable acidity and fruit weight were not affected by shade factor in 'Granny Smith'. Total soluble solids were significantly reduced under 17% and 24% shade factor compared to 10% shade factor in 'Granny Smith'.

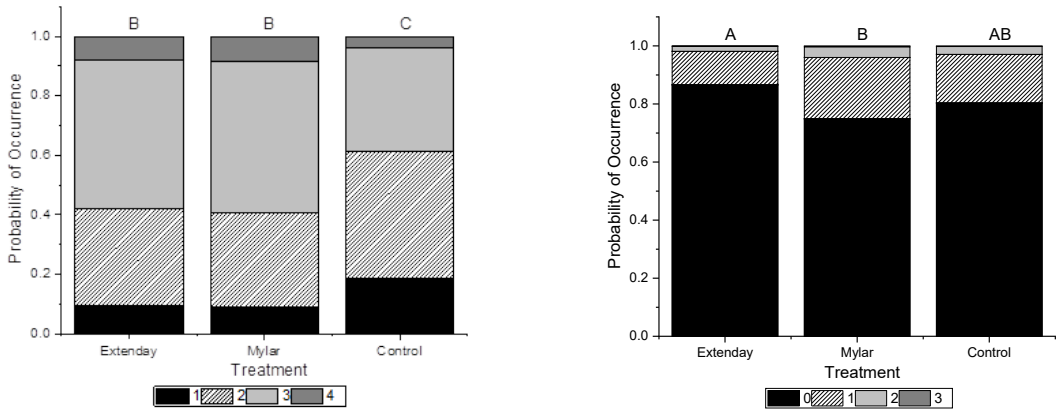

Figure 4. Probability analysis of red color coverage (1=0-25%, 2=26-50%, 5=51-75%, 4=>76% red coloration) (left) and sunburn incidence (0-5 score, with 0 having no sunburn and 5 the most severe) (right) in 'Honeycrisp' apple grown under 17% protective netting with Extenday® and Mylar® reflective ground covers compared to a control with grass cover.

Both Extenday® and Mylar® significantly increased the proportion of fruit with >50% red coloration in 'Honeycrisp' compared to the control with grass cover under 17% protective netting (Figure 4). The use of reflective ground covers under protective netting could be a potential solution to solve the problem of poor red color coverage that has been reported in previous studies. Under 17% protective netting, Mylar® significantly increased the sunburn incidence compared to the Extenday®

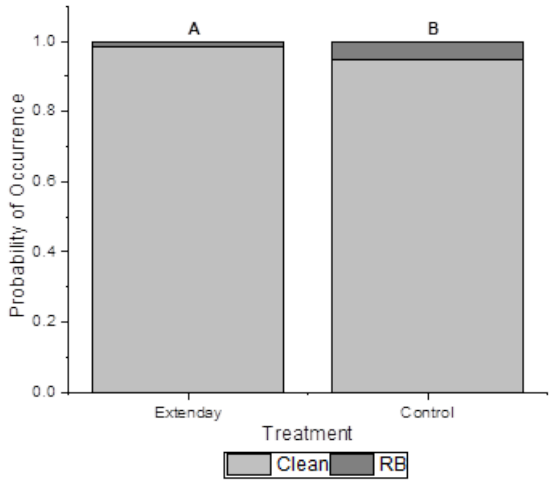

Figure 5. Probability analysis of red blush incidence in 'Granny Smith' apple grown under 17% protective netting with Extenday® reflective ground cover from full bloom compared to a control with grass cover.

The incidence of red blush was reduced in 'Granny Smith' apple that had been exposed to high light conditions earlier in the growing (Figure 5). These results seemed to indicate that exposure of fruit to increased light amounts increased the capacity of the fruit peel to deal high light conditions later on in the season.

Table 3. The effect of reflective ground covers on incoming, reflected and diffuse photosynthetically active radiation measured 1.5m above the ground in a 'Honeycrisp' apple orchard under 17% pearl protective net at Quincy, WA

Treatment	Center of drive row			Tree Canopy	
	Incoming PAR	Reflected PAR	Diffuse PAR	Reflected PAR	Diffuse PAR
Control	1296	50.02 c	60.84 c	15.72 c	22.34 b
Extenday®	1289	418. 82 b	414.96 b	148.36 b	79.84 a
Mylar®	1307	449.34 a	530.12 a	206.20 a	99.28 a
P value	0.7628	0.001	0.0001	0.001	0.0001

Reflective ground covers increased the amount of reflected and diffuse photosynthetically active radiation (PAR) in the center of the drive row and the tree canopy (Table 3). Increasing the amount of PAR penetrating the tree canopy is very important as light penetration into the canopy is linked to improved fruit quality through improvement in fruit red color in blushed cultivars. Light is needed for the formation of anthocyanins which give fruit their red color.

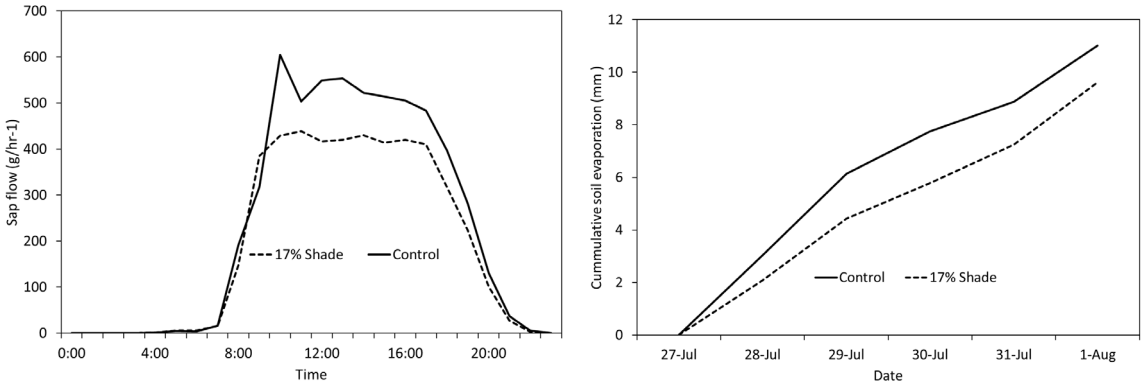

Figure 6. Influence of protective netting on whole tree water use (left) and cumulative soil evaporation (right) measured in 'Honeycrisp' orchard under 17% protective netting at Quincy

Protective netting reduced whole tree transpiration compared to an uncovered control. In addition, soil evaporation was reduced under protective netting compared to an uncovered control. This ties in with previous research which showed improved soil moisture status under protective netting. This has implications where orchards under netting will require different irrigation management than exposed orchards. Additionally, in regions with junior water rights, growers using protective netting may be better able to protect their trees and improve survival and productivity under water limitations compared to exposed orchards.

2019 Plans

In 2019, we will continue with the three stated objectives in order to validate our results from the first season. We also still need to measure flower bud formation, return bloom, and fruit set in both cultivars after the first year of our project. We plan to hold a field day at the trial site for growers and other interested industry representatives. We plan to work with AWETA in order to get better pack out data from our trials. They have indicated that they would be willing to let us use their modern test packing line.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project: AP-18-106

Project Title: WA 38 demonstration trial block

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City/State/Zip: Wenatchee, WA 98801

Cooperators: Burrows Tractor – Sunnyside, Jeff Sample

Total Project Request: 43,614 **Year 1:** 30,514 **Year 2: 13,100**

WTFRC Budget:

Item	2018	2019
Salaries	~~6,136~~ 8247	
Benefits	~~4,264~~ 2797	
Wages	3,125	
Benefits	1,656	
RCA Room Rental	583	
Supplies	200	
Travel	250	
Total	~~16,214~~ 16,858	**0**

Footnotes:
Salaries/Benefits: 104 hours each for Mendoza and Hanrahan, 41% benefit rate
Wages/Benefits: 200 hours @ $11.50, 50 hours @ $16.50, 53% benefit rate
RCA room rental: 1/9th of one CA room for 10 months @ $6,300/year
Travel: in state travel between Yakima or Wenatchee to Prosser

Budget 1
Organization Name: WA State Univ. Contract Administrator: Katy Roberts
Telephone: 509.335.2885 Email address: arcgrants@wsu.edu

Item	2018	**2019**
Wages	1,000	**1,000**
Equipment	2,200	**1,000**
Supplies	1,200	**1,200**
Travel	2,500	**2,500**
Miscellaneous	2,400	**2,400**
Plot Fees	5,000	**5,000**
Total	14,300	**13,100**

Footnotes: Equipment: Temperature and moisture sensor + data logger.
Supplies: Materials to build Rhizotrons and stablish new irrigation system.
Miscellaneous: Soil and tissue analyses
Travel: Lewis to / from Prosser: Moses Lake

OBJECTIVES

1. Provide opportunity for industry horticulturists to demonstrate/debate and teach/learn canopy management strategies for WA 38 in vertical, angled, single stem and bi-axis trees.
2. Demonstrate and field evaluate impact of sprayable and netting products for sunburn mitigation and fruit finish
3. Demonstrate the use of mechanical hedgers and platforms
4. Determine best commercial picking scenarios for optimum fruit quality and long-term storage potential.
5. Evaluate the effect of drape net, spray-able sunburn protectant compound and no mitigation on nutrient status and root development
6. Demonstrate soil and root growth across rootstocks
7. Conduct field days and document best management practices. Contribute to body of knowledge using all methods and informational platforms.

METHODS

Objective # 1 Canopy Management and Crop load
We will recruit industry cooperators to prune and train the block on an annual basis. We will include mechanical hedging in the rows that have been hedged since year 2. All trees currently trained using "bending" treatments will be converted to "narrow" robot ready architectures and will be available for robot harvest. We will find balance between the necessary conversions and the goal of producing sufficient yield of high quality fruit. We will develop pruning rules that are transferable and executable by growers.

We will document bloom dates and pollinizer bloom dates in relation to other varieties/pollinizers in nearby orchards. With industry, we will prune to bud counts and evaluate crop load.

Objective # 2 Sunburn / Fruit Finish
We will cover 1/3 of trees (angled and vertical) with drape net to evaluate and demonstrate impact of netting on incidence of sunburn, fruit finish and tree vigor. We will use a sunburn protectant on 1/3 of the block to evaluate and demonstrate impact of product on incidence of sunburn and fruit finish. One third of the block will be used as control.

Objective # 3 Mechanization
In cooperation with vendors, we will use platforms in the block for demonstration and efficiency purposes. We will also use and demonstrate hedgers and harvest robots. Field days will be held for demonstration purposes.

Objective # 4 Fruit Quality and Storage Potential
The starting date for picking is reached when an average of starch index of 2 (scale 1.0 to 6.0) is reached. After that, harvest will be done weekly for 3 more weeks. For each pick, WA 38 apples will be sampled for 4 different purposes, to understand the variation of internal fruit quality on the basis of the harvest dates one month after harvest (exactly 30 days after each of the picks) as well as on Nov. 28nd when, fruit are supposed to be sold (hypothetical). On Nov. 28 industry representatives are invited to come to the WTFRC lab and have an informal panel test on the different fruit by picking day. After harvest fruit is stored in refrigerates air storage at 36 F until analysis.

Within the dataset of P3 block in Quincy, we are selecting 20 trees that have medium-high number of fruit per tree (count beginning of September) with a range of fruit from 87 to 166 per tree and a crop load between 1.9 to 3.7 fruit/cm^2 TCSA. For each of the 4 harvests (picks) we will have 5 trees available as reps.

Objective # 5 Evaluate the effect of drape net and no mitigation on nutrient status and root development.
a. We will adjust and correct nutritional status of the block before the establishment of treatments according to the soil supply (soil chemical analyses) and tree demand.
b. To evaluate root growth, we will build two root evaluation structures in the soil (Rhizotrons), one on each rootstock of the control treatment to follow root growth pattern and differences.
c. Two sets of moisture and temperature sensor will be placed in the soil under the drape net and the control treatment at root depth to determine temperature or moisture differences between treatments.
d. Evaluations:

- Nutritional status of the tree will be evaluated with standard foliar tissue analyses
- Root growth will be measured by collecting soils + roots in 4 points around each experimental unit and determining root biomass and nutritional status
- Temperature of first roots tips for each rootstock – monitoring dynamic of root growth
- Fruit quality and nutritional levels

Objective #6 Soil and Root growth analyses demonstration
Field day to show the Rhizotron structure and evaluation system. A guide to build a Rhizotron and how to evaluate root growth.

Objective #7 Conduct field days and document best management practices. Contribute to body of knowledge using all methods and informational platforms.

RESULTS & DISCUSSION

Objective # 1 Canopy Management and Crop load
Industry cooperators were recruited to prune the block on April 4th. Due to the historic high vigor of the block, the pruning strategy was to leave as much fruiting wood as possible. Mechanical hedging was delayed until temperatures reached 100°F to reduce fire blight potential infection. On July 13th all rows were hedged. Trees trained with "bending" are in transition to "narrow" robot ready canopies. Full bloom time was between April 25th to 28th. The crop load was low probably due to several factors that reduced pollination, such as; low pollinizer density due to fire blight infection and whole tree removals, 0,21 inches of rain between April 27th and 28th and high winds (up to 32.5 mph).
Despite the pruning strategy to leave as many flowers as possible, crop load was approximately 12 bins, equivalent to 25 bins/acre at a spacing of 2420 trees/acre.
Pruning strategy during winter 2018/19 will aim to promote growth and breaks in weaker trees and/or blind wood. Vigorous shoot will be minimally pruned with clean cuts to promote light.

Objective # 2 Sunburn / Fruit Finish
Due to fire blight incidence and subsequent need to conduct intense scouting during 2018, the drape net demonstration was not established. Fire blight conditions in upcoming season will dictate if we demonstrate drape net in 2019. Sunburn protectants were utilized during the season with five sprays between July 27 and August 29. The commercial products utilized were Parka (wax based product) and Eclipse ™ (Calcium carbonate derived Ca (25%) + B (0.1%)). The latter stays in the fruit for a long period and requires additional cleaning at harvest.

Objective # 3 Mechanization
In cooperation with vendors, we used platforms in the block for pruning and harvest. Mechanical hedging process (July 13th) was video recorded and shared in social media, followed by a field tour on July 20th with growers.

Objective # 4 Fruit Quality and Storage Potential
On August 17, 2018 Ines Hanrahan, the lead investigator of this portion of the project, assumed a new position. Subsequently, Dr. Sara Serra and Stefano Musacchi have offered to take on leadership for this project piece. Protocols were developed to reflect industry needs expressed through the PVM Quality Standards Committee and previous experience in both WTFRC and WSU labs. WA 38 apples internal quality will vary depending on the date of harvest. Little is known about the optimum picking date and how to monitor the fruit once received at the storage facility to arrive to that moment. The easiest way to implement a maturity assessment industry-wide to do is through starch degradation assessment. The iodine test is widely utilized from grower to packer too and it could be a feasible tool to adopt. At the time of the report, the experiment was not completed. A report will be made available to WTFRC upon completion of this portion of the project in Spring 2019. In order to substantiate the data set developed in this project, a new proposal has been crafted and will be presented at the 2019 Apple Horticulture and Postharvest Research Review.

The Quality Standard Advisory group met on November 28, 2018 to visually and organoleptically evaluate fruit. Fruit samples have been made available to interested marketing desk for further evaluation. Members of this group will report their experience to the Marketing Committee lead by Robert Kershaw.

Objective # 5 Evaluate nutrient status and root development.
Soils samples were obtained during spring for chemical analyses following the standard methods recommended for western soils (Miller et al 2013). Mineral deficiencies were obtained for phosphorous (10 mg/kg), sulphur (8 mg/kg), zinc (0.50 mg/kg) and boron (0.12 mg/kg). All other nutrients were adequate according to the standards for WA tree fruit industry (http://treefruit.wsu.edu/orchard-management/soils-nutrition/fruit-tree-nutrition/).
Nutrients were adjusted in the soil with 100 lbs of Mono Ammonium Phosphate (MAP)/acre, 25 lbs of $ZnSO_4$/acre and 2 lbs of B/acre. Nutrient management was followed by leaf tissue analyses during the summer (standard recommendation for apples). Sample obtained from the most representative trees on each training system showed adequate levels of all nutrients except for Ca levels only in V trellis trained trees, despite the rootstock. Trees on V trellis have half of the spacing of trees trained to multi stem or spindle, thus Ca deficiency in the V trellis could be associated to higher tree density, root spacing and/or water balances. A separate sample was obtained from a tree with chlorosis and weak growth, that showed deficiency levels of all macro nutrients (N, P, K, Ca, Mg), probably due more to absorption problems of the particular tree. This exercise allows us to demonstrate the utility of soil and tissue sampling for nutrient diagnostic in an apple block.
b. To evaluate root growth, two root windows were stablished in the block, one on each rootstock to follow root growth pattern and differences. Root growth timing did not show differences between G41 and M9 nic 29. Both root systems showed initial growth approximately 30 DAFB. Root growth was sustained during Jun and July 11th followed by a high rate of root death (root browning) between July 11th and August 31th, coinciding with an increase soil temperature period (above 75 °F). During the month of September new growth was observed with shorter period of growth. Root growth timing and growth rate evaluation will continue during 2019.
c. Two sets of moisture and temperature sensor were placed in the soil at 10 inches' depth and 10 feet between each other. The soil texture corresponds to a silt loam with a bedrock at approximately 80 inches from the surface.

We kept the soil water moisture content between 33 and 19% of the volumetric water content VWC (33% VWC is equivalent to 100% water holding capacity in a silt loam soil), with exception of the period between bloom and July 12, were moisture was kept below 30% to reduce favorable conditions for fire blight infection. The first irrigation was on May 16th, 21 days after full bloom.

Objective #6 Soil and Root growth analyses demonstration
Field day to show the Rhizotron structure and evaluation system. A guide to build a Rhizotron, and how to evaluate root growth.

During 2018 we evaluated the development of the root windows (Rhizotron) as a tool to evaluate initial root growth and period of growth in relation to development. Rhizotrons were built with two different acrylic type of window; Lexan UV Polycarbonate sheet 1/8 inch, and Lexan UV Polycarbonate sheet ¼ inch, and a wooden frame. The 1/8-inch window was too weak to resist the soil and ended up bending. The window material needs to be resistant to the force of the soil and water and stay strait to facilitate root growth measurement. A plywood cover should be place on top of the Rhizotron to avoid light inside the box, as it will inhibit root growth. The collection of data was done by taking pictures of the roots and then evaluating the root timing in relation with developmental growth. We were able to obtain preliminary data during 2018 (as described above).

During 2019 we will develop additional windows in each rootstock in a replicated design to evaluate differences between G41 and M9 nic 29 under two different root management strategies (to be determine according to soil nutrient analyses and tree vigor during spring).

Objective #7 Conduct field days and document best management practices. Contribute to body of knowledge using all methods and informational platforms.

Field days;
June 28th – Roza Tour: Open to public (20 p)
July 24th WA 38 tour – South Central WA growers (20 p)
September 11 – Roza Tour (112 p)
September 13th - National Crop Insurance Services (10 p)
October 1 National Association of State Departments of Agriculture (NASDA) (36 p)

Reference
Miller, R.O, R. Gavlak, D. Horneck. 2013. Soil, Plant and Water Reference Methods for the Western Region. WREP 125

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

Project Title: Crop load and canopy management of WA tree fruit

PI: Tory Schmidt
Organization: WTFRC
Telephone: (509) 665-8271 x4
Email: tory@treefruitresearch.com
Address: 1719 Springwater Ave.
City/State/Zip: Wenatchee, WA 98801

Cooperators: Ines Hanrahan, Manoella Mendoza, Mackenzie Perrault, Gerardo Garcia, Harold Ostenson, Adama, Fine Americas, Marrone Bioscience

Requested WTFRC Funds for Project:

Item	2018	2019	2020
Salaries	5950	6130	6310
Benefits	2440	2510	2590
Wages	25,000	27,500	30,250
Benefits	13,250	14,580	16,040
RCA Room Rental			
Shipping			
Supplies	1500	1500	1500
Travel	1000	1000	1000
Plot Fees	5040	4400	4600
Miscellaneous	500	500	500
Total gross costs	54,680	58,120	62,790
Anticipated Income (contracts and gift grants)	67,560	55,000?	60,000?
Total net costs	(12,880)	**3120**	2790

Footnotes:
Salaries: 10% of Mendoza (with 41% benefits); not included in salaries: 20% of Schmidt time
Increase in wages & benefits include increase in WA minimum wage through 2020
Supplies include tractor/sprayer fuel & maintenance, spray suits, occasional chemical purchase, etc.
Plot fees assume use of 2 blocks at WSU Sunrise Research Orchard

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

OBJECTIVES:

1. Determine best use practices for metamitron including appropriate rates, timings, use of adjuvants, and weather considerations.
2. Explore other novel bloom and postbloom chemical thinning programs utilizing new chemistries and/or new use patterns for existing products, especially those approved for organic use.
3. Explore new uses of plant growth regulators to help manage apple crop load and orchard canopy systems.

2018 SIGNIFICANT FINDINGS:

No treatments reduced fruit set in a chemical bloom thinning trial on Gala, including our first tests with Regalia, a biofungicide approved for organic use (Table 1)

The most efficacious options for chemical bloom thinning of apple continue to be spray oil + lime sulfur programs (Table 2)

Metamitron can effectively reduce fruit set and boost fruit size in WA conditions (Tables 3, 4)

Metamitron efficacy can be promoted by tank mixing with non-ionic surfactants (Table 3)

Metamitron treatments demonstrate greater reductions in fruit set when 1-2 warm, cloudy days are experienced within a week after application (Table 3)

Warm temperatures combined with low light conditions following applications of postbloom thinners can amplify treatment effects, potentially resulting in over-thinning (Table 3)

Thinning efficacy of BA can be improved with use of surfactants (Table 3)

Either metamitron or BA + NAA programs are as effective as any postbloom thinning program featuring carbaryl (Table 4)

2017 applications of GA_3 or GA_7 failed to reduce 2018 return bloom in a single trial on Scilate (Table 5), but both programs have been effective in prior studies; a new GA_7 product should be available for this use pattern in time for the 2019 season and is expected to be approved for organic use

Collaborative research efforts continue to help develop new models, information, and technologies to improve crop load management of WA apples

BACKGROUND:

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

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

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

BLOOM THINNING:

For years, chemical bloom thinning programs in Washington have predominantly featured lime sulfur or combinations of lime sulfur and horticultural spray oils. While these programs have been largely efficacious for most growers, there have been few alternative chemistries that have demonstrated potential as cost-effective chemical thinners, especially for organic growers. After hearing anecdotal reports of reduced fruit set in some commercial organic apple blocks and in pathology research trials by Regalia, a biofungicide derived from extracts of knotweed, we decided to test the material as a chemical thinner in 2018.

We applied 4 different programs of Regalia, evaluating different formulations, concentrations, and a tank mix with a standard summer spray oil. Since we had no precedent regarding effective thinning rates, our trial rates were derived from best guesses based on those anecdotal reports. Unfortunately, none of the treatments in our Gala trial demonstrated any reductions in fruit set, including our industry standard program of summer oil + lime sulfur (Table 1). We observed no clear phytotoxicity in treated trees, aside from some light speckling on flower petals and there were no clear effects on fruit finish at harvest. Interestingly, we documented increases in fruit size from one Regalia treatment and the oil + lime sulfur treatment despite no reductions in fruit set; we made no observations of powdery mildew strikes in this mildew-prone block, but fruit size may have been boosted in these plots by improved overall tree health and increased carbon fixation by trees which were sprayed with these programs with fungicidal benefits.

Table 1. Crop load and fruit quality effects of bloom chemical thinning programs. WTFRC 2018.

Treatment	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size	Russet free fruit	Return bloom
		%	*%*	*g*		*%*	*%*
Gala / M.9 Nic 29 - Rock Island							
Regalia - 5% 128 fl oz	95 ns	38 ns	39 b	152 a	119	89 a	
Regalia - 5% 256 fl oz	99	36	39 b	149 ab	122	61 b	
Regalia - 5% 128 fl oz + W-E 440 oil 128 fl oz	84	42	39 b	151 ab	120	61 b	
Regalia - 12% 106 fl oz	87	44	35 b	150 ab	121	63 ab	
Lime sulfur 320 fl oz + W-E 440 oil 128 fl oz	86	33	49 a	166 a	109	bad data	
Control	109	32	39 ab	138 b	132	89 ab	

Table 2 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 2. Incidence and percentage of results significantly superior to untreated control. Apple chemical bloom thinning trials. WTFRC 1999-2018.

Treatment	Fruitlets/100 blossom clusters	Harvested fruit size	Return bloom[1,2]
ATS	15 / 60 (25%)	10 / 63 (16%)	4 / 55 (7%)
NC99	15 / 32 (47%)	7 / 34 (21%)	2 / 28 (7%)
Lime sulfur	26 / 58 (45%)	12 / 52 (23%)	9 / 52 (17%)
CFO + LS	62 / 115 (54%)	27 / 106 (25%)	22 / 105 (21%)
JMS + LS	14 / 24 (58%)	8 / 23 (35%)	4 / 22 (18%)
WES + LS	15 / 31 (48%)	5 / 30 (17%)	4 / 29 (14%)
ThinRite	7 / 22 (32%)	0 / 23 (0%)	0 / 12 (0%)

[1]Does not include data from 2018 trials.
[2] (no. blossom clusters year 2/sample area) / (no. blossom clusters year 1/sample area)

POSTBLOOM THINNING:

Our primary focus for postbloom chemical thinning research continues to be to identify and develop alternatives to carbaryl, which faces regulatory scrutiny as well as mounting pressure from elements of the consumer market seeking to reduce overall use of broad-spectrum pesticides. Most of our recent work has featured 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.

Results from 2018 trials (Table 3) continue to demonstrate the efficacy of various metamitron formulations (ADA 46701 and ADA 46343), rates, and timings across multiple apple varieties and locations throughout Washington. Metamitron thinning effects tend to be increased with the addition of a non-ionic surfactant (i.e. Regulaid) in the spray tank. While we typically have found relatively consistent dose responses with metamitron, that trend was not as clear in 2018 trials, where lower product rates sometimes produced as much thinning as higher concentrations.

One pattern that continues to be clear is that the response of trees sprayed with metamitron is considerably influenced by weather conditions for several days after application. In all trial sites, trees experienced 1-2 days of warm (70-75F), cloudy weather within a week of their 8-10mm application timings; plots sprayed at these early timings experienced greater reductions in fruit set than plots which received identical sprays at later timings. Interestingly, most trial sites experienced a day of cool (60F), rainy weather a few days after later applications (14-18mm) but did not demonstrate as much thinning as treatments which had been sprayed earlier and experienced similar cloudy conditions, but at warmer temperatures. As we have seen several times in recent years, a standard industry thinning program featuring NAA overthinned Granny Smith in a trial near Rock Island, but most metamitron treatments showed more modest thinning (Table 3); this result further indicates that metamitron products may not be as prone to overthinning in challenging weather conditions than postbloom thinners currently enjoying broad use in industry.

Historically, we have rarely seen clear thinning from solo applications of benzyladenine (BA) products, but our 2018 results corroborate other recent findings that the addition of certain proprietary surfactants to Exilis can significantly improve efficacy (Table 3). These findings are quite encouraging and warrant further investigation. An initial attempt at postbloom thinning with dilute oil + lime sulfur applications (Orondo Gala) was not effective and should be repeated in 2019.

Table 3. Crop load and fruit quality effects of postbloom thinning programs. WTFRC 2018.

Treatment	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size	Russet free fruit
		%	%	g		%
Fuji / M.9 - Wapato						
ADA 46701 2.4 lbs @ 8-10mm	21 cd	82 b	15 cd	291 abc	62	83 ns
ADA 46701 2.2 lbs @ 14-16mm	63 ab	61 cd	22 bc	255 cd	71	93
ADA 46701 2.4 lbs @ 8-10mm & 2.2 lbs @ 14-16mm	10 d	91 a	9 d	294 ab	62	79
Exilis 9.5SC 25.6 oz	54 b	56 d	35 a	266 abcd	68	93
Exilis 9.5SC 25.6 oz + Surfactant A 32 oz	22 cd	81 ab	16 cd	297 a	61	83
Exilis 9.5SC 25.6 oz + Surfactant T 64 oz	29 cd	75 bc	20 bcd	272 abcd	67	81
Sevin 4F 36 oz + Fruitone L 3 oz	32 c	73 bc	23 bc	256 bcd	71	91
Control	76 a	51 d	28 ab	249 d	73	91
Gala / M.9 – Frenchman Hills, Quincy						
ADA 46701 2.7 lbs @ 8-10 mm	43 d	64 a	30 c	213 ab	85	85 bc
ADA 46701 2.2 lbs @ 14-16 mm	85 ab	37 de	45 a	185 ab	98	90 ab
ADA 46701 2.7 lbs @ 8-10mm & 2.7 lbs @ 14-16mm	43 c	63 a	32 c	212 ab	86	98 a
Exilis 9.5SC 25.6 oz	71 bc	40 cde	50 a	180 b	101	85 bc
Exilis 9.5SC 25.6 oz + Surfactant A 32 oz	62 cd	47 bcd	45 ab	184 ab	99	86 bc
Exilis 9.5SC 25.6 oz + Surfactant T 64 oz	55 cd	53 abc	40 bc	192 ab	95	70 c
Sevin 4F 36 oz + Fruitone L 3 oz	54 cd	57 ab	34 c	220 a	83	91 ab
Control	95 a	31 e	50 ab	182 b	100	86 bc
Gala / M.26 - Orondo						
ADA 46701 2.3 lbs @ 8-10mm	73 c	51 a	32 abc	198 ab	92	59 ns
ADA 46701 2.4 lbs @ 14-16mm	128 a	33 c	30 abc	200 ab	91	53
ADA 46701 2.3 lbs @ 8-10mm & 2.4 lbs @ 14-16mm	88 bc	49 ab	24 c	199 ab	91	51
ADA 46701 2.3 lbs + Fruitone L 2 oz	84 bc	49 a	29 bc	202 ab	90	71
CFO 1 gal + LS 1 gal @ 8-10mm	104 ab	37 abc	36 ab	179 bc	101	54
CFO 0.5 gal + LS 0.5 gal @ 8-10 & 14-16mm	112 ab	32 c	36 ab	182 abc	100	43
Sevin 4F 32 oz + Fruitone L 2 oz	101 abc	34 bc	41 a	207 a	88	61
Control	91 bc	42 abc	34 abc	170 c	107	61
Granny Smith / M.9 – Rock Island						
ADA 46701 2 pt @ 8-12mm	30 bcde	71 bcd	27 bcd	219 ns	83	89 ns

ADA 46701 2 pt @ 14-18mm	40 abcde	64 bcde	33 abc	220	83	88
ADA 46701 2 pt @ 8-12 & 14-18mm	44 abcd	62 bcde	34 abc	220	83	93
ADA 46701 2.7 pt @ 8-12mm	70 a	48 de	40 abc	219	83	90
ADA 46701 2.7 pt @ 14-18mm	65 ab	57 bcde	27 bcd	225	81	84
ADA 46701 2.7 pt @ 8-12 & 14-18mm	47 abcd	58 cde	37 abc	220	83	95
ADA 46701 3.33 pt @ 8-12mm	51 abcd	55 de	40 abc	205	89	94
ADA 46701 3.33 pt @ 14-18mm	43 abcd	60 cde	37 abc	192	95	98
ADA 46701 3.33 pt @ 8-12 & 14-18mm	56 abc	51 de	43 ab	198	92	94
ADA 46701 3.33 pt + Reg 32 oz @ 8-12mm	48 abcd	58 bcde	37 abc	222	82	96
ADA 46701 3.33 pt + Reg 32 oz @ 14-18mm	27 cde	76 bc	22 bcd	216	84	90
ADA 46701 3.33 oz + Reg 32 oz @ 8-12 & 14-18mm	17 de	84 b	16 cd	221	82	90
ADA 46343 3.33 lb + Reg 32 oz @ 8-12mm	41 abcd	63 bcde	34 abc	236	77	93
ADA 46343 3.33 lb + Reg 32 oz @14-18mm	43 abcd	62 bcde	34 abc	218	83	95
Sevin 4F 36 oz + Fruitone L 3 oz	4 e	97 a	3 d	236	77	83
Control	67 a	40 e	54 a	213	85	81

Table 4 demonstrates the strong performance of BA + NAA programs and metamitron products as compared to other postbloom thinning options over the course of all our studies across varieties and locations.

Table 4. Incidence and percentage of results significantly superior to untreated control. Apple chemical postbloom thinning trials. WTFRC 2002-2018.

Treatment	Fruitlets/100 blossom clusters	Harvested fruit size	Return bloom[1,2]
BA	6 / 27 (22%)	0 / 28 (0%)	0 / 24 (0%)
Carb + BA	33 / 91 (36%)	10 / 89 (11%)	13 / 86 (15%)
Carb + NAA	25 / 73 (34%)	18 / 71 (25%)	11 / 67 (16%)
BA + NAA	18 / 40 (45%)	9 / 39 (23%)	7 / 36 (19%)
Metamitron	13 / 24 (54%)	8 / 23 (35%)	5 / 18 (28%)

[1]Does not include data from 2018 trials.

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

GIBBERELLIC ACID FOR BLOOM INHIBITION:

Over many years of trials, we have established that multiple applications of modest concentrations of GA_3 can be effective at reducing return bloom across multiple apple varieties as a tool for mitigation of biennial bearing. In the absence of GA products registered for this use pattern, we focused most of our work on GA_3 products because of their relatively low price point. Despite ample data demonstrating their efficacy, the registrants of these products have been reluctant to add this use pattern to their labels, primarily due to the abundance of competitive generic products in the market and relatively poor prospects for making a return on investment for such a label amendment.

In recent years, however, we have been testing a new formulation of GA_7 from Fine Americas alongside our standard GA_3 programs. GA_7 is known to be a more potent isomer than GA_3 in terms of inhibiting floral initiation and can produce analogous results at lower concentrations. Our preliminary trials with this GA_7 product have suggested potential to be an effective tool for reducing return bloom, but we need more experience with it to determine the most efficacious rates and timings. Table 5 below details the results of a 2017 trial on Scilate where no GA program reduced return bloom, including our standard GA_3 (Falgro) programs.

According to Fine Americas, their GA_7 product should clear EPA registration shortly and be available for use in the spring of 2019; the product will be called "Arrange" and is expected to be approved for organic use. This product could become a very important tool in managing biennial bearing blocks, especially for organic apple growers who have very limited chemical tools for managing crop load.

Table 5. Effects on tree vigor, fruit size, and return bloom of GA applications. WTFRC 2017.

Treatment	2017 harvest fruit weight	2017 relative box size	2018 return bloom	2018 return bloom per CSA
	g		*%*	*clusters/cm²*
Scilate / M.9 Nic.29 - Yakima				
Arrange 25ppm	173 b	105	3877 ns	1.7 ns
Arrange 100ppm	174 b	104	3488	1.5
Falgro 2XLV 100ppm	183 ab	99	3314	1.7
Falgro 2XLV 200ppm	176 ab	103	3914	1.6
Novagib twice; Arrange 3785ml	176 ab	103	2867	1.7
Novagib 4 times; Arrange 1893ml	178 ab	102	4560	1.5
Novagib 4 times; Arrange 3785ml	188 a	97	3438	1.4
Control	178 ab	102	3892	1.6

COLLABORATIVE CROP LOAD MANAGEMENT RESEARCH:

"Optimizing light and water for orchards covered with netting" (AP-18-102; PI: Kalcsits) – support for labor intensive data collection, harvest sampling, and postharvest fruit quality analysis; also support for project leadership team including sharing of relevant WTFRC projects and protocols, as well as editing of project manuscripts

"Development and validation of a precision pollination model" (TR-16-102; PI: DeGrandi-Hoffman) – coordination of local data collection for bee foraging, bloom phenology, and fruit sampling activity at sites near Yakima and Chelan; active member of project leadership team (project funded through WTFRC technology committee)

"Developing and validating models for tree fruit" (TR-17-102; PI: Jones) – coordination of data collection for fruit growth at 39 blocks throughout Central Washington (primarily Golden Delicious, Fuji, and Honeycrisp); help with outreach activities for new horticultural models (project funded through WTFRC technology committee)

CONTINUING REPORT **Year: 2018**

Project Title: 2018 WTFRC apple pesticide residue study

PI: Tory Schmidt
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Since 2011, the Washington Tree Fruit Research Commission (WTFRC) has conducted annual trials to evaluate pesticide residues on 'Gala' apples. This year, we applied seven insecticide/acaricides, four fungicides, and one plant growth regulator with a Rears Pak-Blast sprayer according to either an "aggressive" protocol intended to simulate a worst-case scenario with the highest possible residues while observing label guidelines (maximum label rates at minimum retreatment and pre-harvest intervals) or a "standard" protocol following more typical industry use patterns for rates and timings. Each treatment protocol was sprayed at both 100 (concentrate) and 200 (dilute) gallons of water per acre while holding the rate of pesticide per acre constant. Fruit samples were collected at commercial maturity on August 29 and delivered the next day to Pacific Agricultural Labs (Sherwood, OR) for chemical residue analysis.

TRIAL DETAILS

- 11th 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 or 200 gal / acre
- All pesticides applied with 8 oz Regulaid / 100 gal water / acre
- No measurable precipitation recorded during trial except 0.01" of rain on Aug 25 (4 days before harvest)

Measured residues vs. maximum residue levels (MRLs) for uniformly applied STANDARD industry apple pesticide programs in 100 or 200 gal water/acre utilizing typical rates, timings, and retreatment intervals. 'Gala'/M.9 Nic.29, Rock Island, WA. WTFRC 2018.

Chemical name	Trade name	Application rate	Application timing(s)	100 gal/acre	200 gal/acre	US MRL[1]	Lowest export MRL[1]
		oz per acre	*dbh*	*ppm*	*ppm*	*ppm*	*ppm*
Ethephon	Ethephon 2SL	36	58	**0.22**	**0.35**	5	0.01 (UAE)
Spinetoram	Delegate WG	7	35 & 21	0.022	.031	0.2	0.05 (many)
Cyantraniliprole	Exirel	13.5	35 & 21	0.16	0.22	1.5	0.8 (many)
Spinosad	Entrust	3	35 & 21	0.029	0.091	0.2	0.1 (many)
Tolfenpyrad	Bexar	27	35 & 21	**0.46**	**0.65**	1	0.01 (many)
Myclobutanil	Rally 40WSP	10	35 & 21	**0.27**	**0.41**	0.5	0.01 (UAE)
Novaluron	Rimon	32	35 & 21	0.38	0.52	3	2 (CAN, TAI)
Fluxapyroxad	Merivon	5.5	28	0.054	0.11	0.8	0.8 (Canada)
Pyraclostrobin	Merivon	5.5	28	0.033	0.071	1.5	0.5 (many)
Etoxazole	Zeal	2	28	0.054	**0.089**	0.2	0.07 (many)
Difenoconazole	Inspire Super	12	28	**0.025**	**0.024**	5	0.01 (India)
Cyprodinil	Inspire Super	12	28	**0.061**	**0.054**	1.7	0.05 (INDO)
Ziram*	Ziram 76DF	96	21	1.26	0.36	7	2.5 (Taiwan)
Fenpropathrin	Danitol	18	14	**0.33**	**0.41**	5	0.01 (many)

[1] Top markets for WA apples with established MRLs; 16 October 2018. 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

Measured residues vs. maximum residue levels (MRLs) for uniformly applied AGGRESSIVE industry apple pesticide programs in 100 or 200 gal water/acre utilizing maximum labeled rates, and minimum preharvest and retreatment intervals. 'Gala'/M.9 Nic.29, Rock Island, WA. WTFRC 2018.

Chemical name	Trade name	Application rate	Application timing(s)	100 gal/acre	200 gal/acre	US MRL[1]	Lowest export MRL[1]
		oz per acre	*dbh*	*ppm*	*ppm*	*ppm*	*ppm*
Ethephon	Ethephon 2SL	36	35	**0.42**	**0.57**	5	0.01 (UAE)
Spinosad	Entrust	3	21 & 7	0.085	**0.11**	0.2	0.1 (many)
Etoxazole	Zeal	3	14	**0.081**	**0.13**	0.2	0.07 (many)
Spinetoram	Delegate WG	7	14 & 7	**0.062**	**0.084**	0.2	0.05 (many)
Cyantraniliprole	Exirel	20.5	14 & 5	0.20	0.40	1.5	0.8 (many)
Fluxapyroxad	Merivon	5.5	7 & 1	0.32	0.51	0.8	0.8 (Canada)
Pyraclostrobin	Merivon	5.5	7 & 1	0.30	0.47	1.5	0.5 (many)

[1] Top markets for WA apples with established MRLs; 16 October 2018. http://www.nwhort.org/AppleMRLs.html, https://www.globalmrl.com/

NOTE: Residue results for several materials are not reported in this table due either erroneous application or lack of product.

DISCUSSION

As in the previous 7 years of studies, no residue from a pesticide applied following label-recommended rates and timings exceeded the US Environmental Protection Agency's tolerance. Pesticides which produced residues above MRLs for important export markets included **Ethephon 2SL, Bexar, Rally 40WSP, Zeal, Inspire Super, Danitol, Entrust, and Delegate WG**. In most cases, these potentially problematic findings have less to do with the actual amount of residue generated by these products than with the fact that some nations have very stringent MRLs; these tolerances are frequently set at the limit of quantitation (LOQ), or smallest amount that can be reliably measured by modern analytic methods, essentially creating a *de facto* ban on importation of apples treated with these products. Growers hoping to market their fruit to such nations should consider avoiding use of those materials altogether.

In general, we found higher residues in 2018 from dilute (200 gal water/acre) than concentrate (100 gal water/acre) applications, suggesting that the higher carrier volume improved coverage. This trend is consistent with our results from comparing 200 gal/acre (concentrate) vs. 400 gal/acre (dilute) applications in a 2016 cherry study, but counter to results from our 2017 apple and 2018 cherry studies, where concentrate applications generally produced higher residues than dilute. These contradictions make interpretation of our cumulative data set quite difficult; simply put, our results to date have shown no consistent effect of water carrier volume on 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. We encourage

growers and consultants to stay abreast of current information on international MRLs, which often change in response to trade negotiations and/or political developments. For more information, visit the Northwest Horticultural Council website at www.nwhort.org.

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, or a guarantee of similar results regarding residues for any user. Apple growers should consult their extension team members, crop advisors, and warehouses to develop responsible pest control programs.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project: AP-18-110

Project Title: Understanding decline on select apple scion-rootstock combinations

PI:	Dr. Scott Harper	**Co-PI (2):**	Dr. Alice Wright
Organization:	Washington State University	**Organization**:	Washington State University
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City/State/Zip:	Prosser WA 99350	**City/State/Zip**:	Prosser WA 99350

Cooperators: Washington apple growers.

Total Project Request: $116,180 **Year 1:** $60,200 **Year 2: $55,979**

Other funding sources
None

Budget
Organization Name: Washington State University **Contract Administrator:** Katy Roberts
Telephone: 509-335-2885 **Email address:** arcgrants@wsu.edu

Item	2018	**2019**
Salaries	24,585	25,568
Benefits	17,415	18,511
Wages	-	-
Benefits	-	-
Equipment	-	-
Supplies	16,700	11,900
Travel	1500	**-**
Miscellaneous	-	**-**
Plot Fees	-	**-**
Total	60,200	**55,979**

Footnotes: Salaries and Benefits include a postdoc at 0.10 FTE, and an MS student. Tuition for the student is not included in this proposal.

OBJECTIVES

The objective of this project is to *determine whether a virus or viral-like pathogens are associated with decline and dieback on G.935 rootstock*. At present there is no clear association of a virus or virus-like pathogen with the expression of decline and/or dieback, only inconsistent findings of endemic viruses. Therefore we propose to take a systematic approach to clearly identifying what pathogens are present in declining plants. This project looks to the future of the apple industry in the U.S., for understanding the cause of today's problems is key to ensuring that they do not reoccur.

SIGNIFICANT FINDINGS

- Plants exhibiting decline symptoms have reduced roots systems with the cortex and phloem of the root tissue showing necrosis. Necrotic streaking is, in severe cases visible up to the graft union, but not above. The scions die back due to the root loss.

- Newly reported viruses have been found in association with disease symptoms, as have endemic viruses. This suggests a potential synergistic reaction.

- Similar disease symptoms have been observed on other rootstocks; while not as severe, the same viruses were found to be present, suggesting that this disease is not isolated to G.935.

- Five new viral species have been identified in some of the symptomatic trees.

METHODS

Determine whether a virus or viral-like pathogens are associated with decline and dieback on G.935

The goal of this project is to identify candidate viruses or viral-like organisms present in apple cultivars on G.935 rootstock that are exhibiting decline and dieback symptoms. Diseased plants will undergo a brief physiological examination to determine whether disease symptoms are consistent, and/or whether they can be attributed to other, non-viral causes. Following this, disease and asymptomatic plants will be first screened by RT-PCR for common endemic and recently discovered viruses, then representative samples submitted for high-throughput sequencing. The resulting reads will be passed through a data analysis pipeline, and candidate viruses identified. Non-diseased trees, and trees on other apple rootstocks, will be examined using the same methodology to identify which viruses are likely pathogens, versus those which are present but not a direct cause of the disease.

RESULTS AND DISCUSSION

Objective: Determine whether a virus or viral-like pathogens are associated with decline and dieback on G.935 rootstock

Throughout the summer and fall of 2018, trees exhibiting decline symptoms were collected from growers and nurseries in north-central Washington State. Honeycrisp cultivars on G.935 rootstock were the focus of collection efforts, as these have been found to most commonly exhibit the decline and dieback symptoms in the second, and sometimes first, leaf stages (Figure 1a). A total of 31 samples representing five Honeycrisp lines, or lines with significant Honeycrisp parentage were collected. Also included were 6 asymptomatic samples from two lines plus an un-grafted, certified G.935.

From observation of the diseased samples we found that the root systems were much smaller than healthy plants of the same age, sometimes severely so. Feeder roots were sparse, with soft, flexible tissue rather than expected 'carrot-like' texture of an asymptomatic G.935. External necrosis was visible on the taproot and secondary roots, which was evident up to the graft union when the bark was removed (Figure 1b). Sections of these tissues revealed necrosis in the cortex, phloem, and phloem fibers (Figure 1c). One plant showed stem pitting/grooving symptoms characteristic of *Apple stem grooving virus* or *Apple stem pitting virus* infection, and three showed foliar chlorosis typical of *Apple mosaic virus* infection. None of the above symptoms were evident on asymptomatic Honeycrisp on G. 935, although interestingly milder necrosis and poor root development was observed on one Honeycrisp tree on Nic-29 rootstock.

Figure 1. A) A tree with decline symptoms next to a healthy tree. B) Necrosis of the phloem tissue at the graft union. C) Cross section of a root showing necrosis (brown areas).

RT-PCR based screening of the symptomatic plants revealed the presence of all nine endemic and newly-reported apple-infecting viruses tested in one or more of the trees (Table 1). No single virus species was present in all plants, which is to be expected given differences in titer, distribution within the plant, and sampling time. Therefore, virus frequencies were examined to give an indication of which viral species could be associated with the diseases observed. *Apple chlorotic leaf spot virus* (ACLSV), *Apple mosaic virus* (ApMV), *Apple rubbery wood associated virus 2* (ARWaV2), *Apple stem grooving virus* (ASGV) and, *Apple stem pitting virus* (ASPV) were all found to occur in over 50% of the samples collected. Of these, only ARWaV-2 is newly reported, whilst the other four are endemic to Washington State. Citrus concave gum-associated virus (CCGaV), a newly reported close relative off ARWaV-2 was in ~45% of the trees, as was Apple hammerhead viroid (AHVd).

However, when we compared these frequencies to viruses present in asymptomatic Honeycrisp samples of G.935, as well as a certified G.935 stock, a different pattern emerged (Table 2). ApMV and ASGV were present in nearly all samples, suggesting that by they alone are not responsible for the disease observed. The other viruses, ACLSV, ASPV, ARWaV-2, CCGaV and AHVd were all in approximately 15-30% of asymptomatic samples tested. One caveat to be observed with a study of this nature is that asymptomatic samples may not truly be healthy and free of disease, but may be expressing mild or initial stages of the disease.

Table 1. Pooled results of the RT-PCR and HTS screening of disease-expressing samples of Honeycrisp cultivars of G.935 rootstock from Washington State. Viruses are as follows: *Apple chlorotic leaf spot virus* (ACLSV), *Apple green crinkle associated virus* (AGCaV), *Apple mosaic virus* (ApMV), *Apple rubbery wood associated virus 1 and 2* (ARWaV1 and ARWaV2), *Apple stem grooving virus* (ASGV), *Apple stem pitting virus* (ASPV), *Citrus concave gum associated virus* (CCGaV), and *Apple hammerhead viroid* (AhVd).

Sample	ACLSV	AGCaV	ApMV	ARWaV1	ARWaV2	ASGV	ASPV	CCGaV	AhVd
1	+	+	+	-	-	+	+	-	-
2	+	+	+	-	-	-	+	-	+
3	+	-	+	-	+	-	+	-	+
4	+	+	+	-	+	+	+	+	+
5	+	-	-	+	+	+	+	+	+
6	+	+	-	-	+	+	+	+	+
7	+	+	-	-	+	+	+	+	-
8	+	-	-	-	-	-	-	+	-
9	-	-	-	-	-	-	-	+	-
10	+	-	-	-	-	-	-	-	-
11	+	-	-	-	+	+	+	+	-
12	+	-	-	-	+	+	+	+	-
13	-	-	-	-	-	+	-	-	-
14	-	-	+	-	+	+	+	-	-
15	-	-	+	-	+	+	+	+	-
16	-	-	-	-	+	+	+	+	-
17	-	+	+	-	+	+	+	+	+
18	-	-	+	-	-	+	-	-	-
19	+	-	+	-	+	+	+	-	+
20	+	-	+	-	+	+	+	-	+
21	+	-	+	-	+	-	+	-	+
22	-	-	+	-	-	-	-	+	+
23	+	-	+	-	-	-	+	-	+
24	-	-	+	-	-	-	-	-	+
25	-	-	+	-	-	+	-	-	-
26	+	-	+	-	-	+	+	-	+
27	-	-	+	-	-	+	-	-	+
28	+	-	+	-	+	+	+	+	-
29	+	-	+	-	+	+	+	-	-
30	+	-	+	-	+	+	+	+	-
31	+	-	+	-	+	+	+	-	+

Table 2. Pooled results of the RT-PCR and HTS screening of asymptomatic samples of Honeycrisp cultivars of G.935 rootstock from Washington State.

Sample	ACLSV	AGCaV	ApMV	ARWaV1	ARWaV2	ASGV	ASPV	CCGaV	AhVd
G.935	-	-	+	-	-	-	-	-	+
1	+	+	+	-	+	+	+	-	-
2	+	-	+	-	+	+	+	+	-
3	-	-	+	-	-	+	-	+	+
4	-	-	+	-	-	+	-	-	+
5	-	-	+	-	-	+	-	-	-
6	-	-	+	-	-	+	-	-	-

These data indicate that ACLSV, ASPV, and ARWaV-2 are likely associated with the disease observed. CCGaV may also be involved, as it was present in a few samples were ARWaV-2 was absent, and often in coinfection. It is possible that either or both of these viruses can induce disease on an infected plant. Interestingly, this effect, while rootstock specific, may not be unique to G.935, as mild-yet-similar necrosis and dieback symptoms were observed on one tree on Nic-29 rootstock. This tree contained the same combination of viruses as the diseased G.935 stock (Table 3). For the purposes of comparison we also looked at three Honeycrisp on Pajam 2 rootstock, and while they were heavily infected, they showed no sign of disease (Table 2).

Table 3. Results of RT-PCR screening of Honeycrisp samples on other common rootstock lines.

Rootstock	Disease	ACLSV	AGCaV	ApMV	ARWaV1	ARWaV2	ASGV	ASPV	CCGaV	AhVd
Nic-29	Yes	+	-	+	-	+	+	+	-	-
Pajam 2	No	-	-	+	-	-	+	+	+	-
Pajam 2	No	+	-	-	-	-	+	+	+	-
Pajam.2	No	+	-	+	-	+	+	+	+	-

Finally, this year we examined four disease expressing samples using either root or shoot tissue, as well as two asymptomatic samples by HTS. Analysis of the libraries identified named apple-infecting viruses which are listed in Tables 1 and 2, as well as 13 new or uncharacterized viruses that could only identified down to the genus level (Table 3). Of these, five are likely environmental viruses, that is, viruses infecting or present in non-apple hosts such as bacterial, invertebrates, or fungi. Two, members of the *Potexvirus* and *Partitivirus* genera infect both plants and fungi, therefore no clear conclusion can be made as to their role. The six remaining, putative members of the *Goravirus, Ilarvirus, Carlavirus, Sobemovirus, Tombusvirus,* and *Ourmiavirus* genera all have to potential, based on analogy with other characterized members of their respective genera, to infect plants. The putative *Tombusvirus* and *Ourmiavirus* were found at high titer with almost complete coverage of their genomes, suggesting that they are unique and not misidentified extant viruses.

It is possible that one of these new viruses, either by itself or in conjunction with the named viruses identified earlier, causes the disease observed. It should also be considered that the viruses themselves are not caused the symptoms observed directly, but are instead weakening the tree by downregulating host defenses or interfering with signaling pathways, and a secondary pathogen such as a bacteria or fungus is actively killing the tree. To this end, sequencing of tissue from the roots of infected trees revealed the presence of *Fusarium oxysporum*, *Leptosphairea biglobosa*, *Leptosphairea macculans*,

Nectria haematococca, and *Rhizoctonia solani*, pathogens that are known to cause root rot in other species. At this time, it is not known if these pathogens are simply present or if they are responsible to some degree for the damage observed in the roots

Table 3. New or uncharacterized viruses identified in disease-expressing apple trees by High-throughput sequencing.

Virus Genus	No. Diseased Plants +	Description
Ourmiavirus	6/6	Plant infecting virus, may be new/undescribed species
Sobemovirus	1/6	Plant infecting virus, may be new/undescribed species
Tombusvirus	5/6	Plant infecting virus, may be new/undescribed species
Carlavirus	3/6	Plant infecting virus, may be new/undescribed species
Goravirus	1/6	Plant infecting virus, may be new/undescribed species
Ilarvirus	1/6	Plant infecting virus, may be new/undescribed species
Partitivirus	5/6	Viruses of this genus infect both plants and fungi, may be new/undescribed species
Potexvirus	1/6	Viruses of this genus infect both plants and fungi, may be new/undescribed species
Picornavirus	1/6	Animal virus, environmental
Mycovirus	1/6	Fungal virus, environmental
Levivirus	6/6	Bacterial virus, environmental
Mitovirus	5/6	Insect virus, environmental
Narnavirus	2/6	Fungal virus, environmental

In conclusion, until Koch's postulates are performed, identifying which virus or viruses are responsible for the decline and death of trees, notably Honeycrisp, on G.395 rootstock will require a larger sample set and further research. Therefore we propose to expand the search next year to sample more diseased and asymptomatic trees and identify which viruses are present – narrowing the list of pathogens such that Koch's postulates can be performed in the future. Furthermore, the appearance of similar disease symptoms on other rootstocks such as Nic-29 should serve as a warning to apple growers: this is not an isolated issue, and our data from this year alone suggests that there are many, many viruses present in apples in Washington State – and there is little to no information about what they do.

PROJECT REPORT **YEAR**: 3

Project Title: 'Honeycrisp' apple rootstock evaluation
PI: WTFRC Staff
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WTFRC Staff cooperators: Mike Willett, Ines Hanrahan, Mano Mendoza, Tory Schmidt

Cooperators: Scott McDougall, Dave Taber. Tianna DuPont (WSU Extension)

Total Project Request: Year 1: 57,900 Year 2: 110,165 Year 3: 50,200

WTFRC Expenses:

Item	2016	2017[3,4]	**2018[4]**
Salaries[2,3]	30,500	40,500	14,000
Benefits[2,3]	10,000	13,365	4,800
Crew Wages[3,4]	5,000	33,000	12,000
Crew Benefits[3,4]	1,000	4,900	5,000
Stemilt RCA room	8,400	8,400	8,400
Shipping			
Supplies			
Travel[1,4]	3,000	10,000	6,000
Miscellaneous			
Total	57,900	110,165	50,200

[1]Fuel and maintenance plus hours for time slip to travel to plots
[2]Salaries and benefits for Hanrahan, Schmidt, and Mendoza apportioned to this project
[3]2017 will see large increase in field activity, fruit harvest, storage and lab activity.
[4]Minimum wage increase

Note: WTFRC work on Phase 3 trials of the apple breeding program is available in the apple breeding program report along with the WTFRC collaborative budget for the scion project.

OBJECTIVES:

1. Evaluate performance of replant tolerant Geneva apple rootstocks in new ground and replant sites compared to commercial standards, in commercial settings in Washington State.
2. Conduct outreach activities to provide opportunity for nurseries and industry to see new commercially available rootstocks in the Geneva family as the trees grow canopy and become productive.

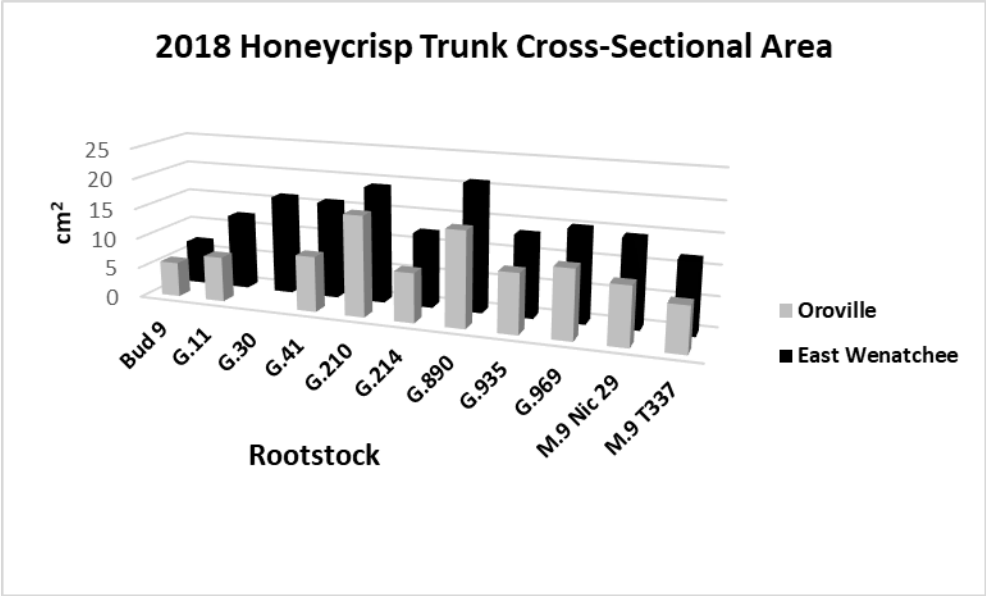
The two 'Honeycrisp' rootstock trials were planted in 2015 in grower/cooperator orchards in East Wenatchee and Oroville, Washington. The plots were planted in border rows of the grower/cooperator's orchards. In the case of the Oroville site, there are four replicates trained in a vertical axis system in four rows on the outer eastern edge of the planting with equal numbers of trees on each rootstock in each replicate, although the number of trees on each rootstock varied by rootstock from 4 to 12 trees depending on rootstock availability. In this planting the main cultivar is a proprietary red 'Honeycrisp' cultivar. The east Wenatchee site was entirely 'Honeycrisp' planted to a 'V' system. The plots are planted in 4 replicates on the eastern edge of the block with replicate 1 the eastern-leaning trees in row 1 and replicate 2 the western leaning trees in row 1 with replicates 3 and 4 planted similarly in row 2.

Tree size (trunk cross-sectional area was measured at planting, again in Fall 2015 and in each fall each year after harvest thereafter. Fruit was first harvested from each of the plots in 2017. No fruit was harvested from the East Wenatchee site in 2018 due to inadequate communication between the WTFRC staff and the orchard management staff regarding the thinning regime in 2017, resulting in almost no return bloom in the plot during the 2018 season.

All of the tree and crop load measurements taken thus far are represented in the figures below, designated by year. Although, the layout of the plots likely affects the performance of tree in certain replicates at each location, there is substantial interest in continuing to maintain these plots into the future, given that both of these sites have a fairly large number of trees per rootstock, facilitating visual comparison between rootstocks.

Many of the rootstocks planted at these sites are new to the Washington apple industry. Growers are cautioned against assuming that the performance data reported here will be the same when planting other variety/rootstock combinations. If the performance of other varieties on any of these rootstocks is unknown, growers and nurserymen should be cautious when considering propagating trees for extensive plantings.

2018 Honeycrisp Average Tree Size for Eleven Geneva and Malling Series Rootstocks Planted in 2015 at Two Sites in Northcentral Washington.



2018 Honeycrisp Average Yield per Tree (kg) (Figure 1) and Yield Efficiency (Figure 2) for Ten Geneva and Malling Series Rootstocks Planted in 2015 at Oroville, Washington.

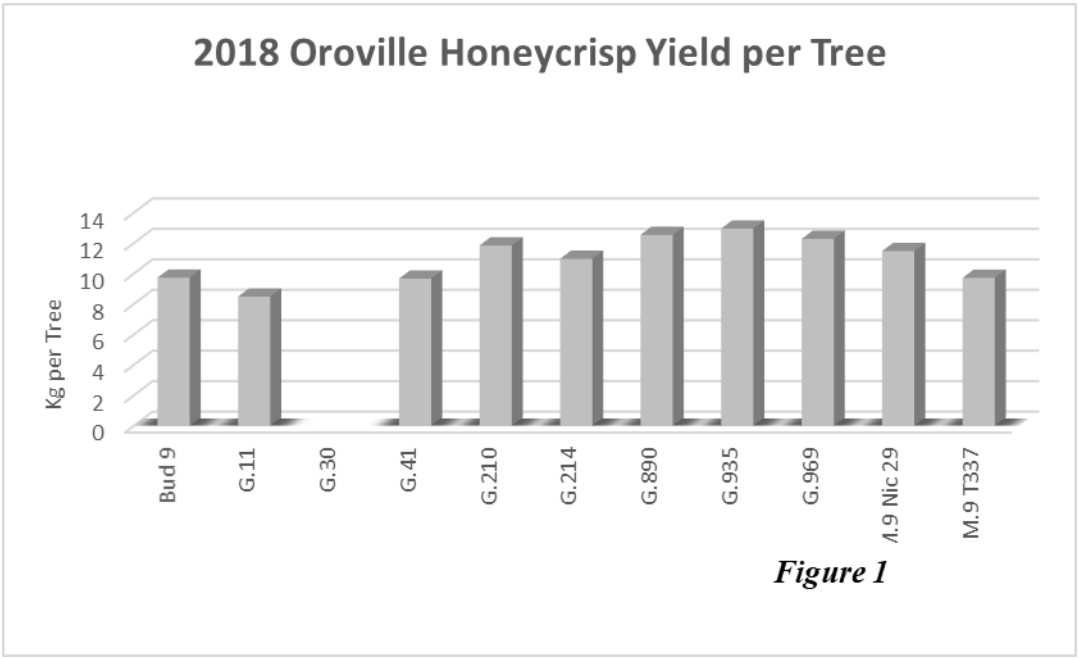


Figure 1

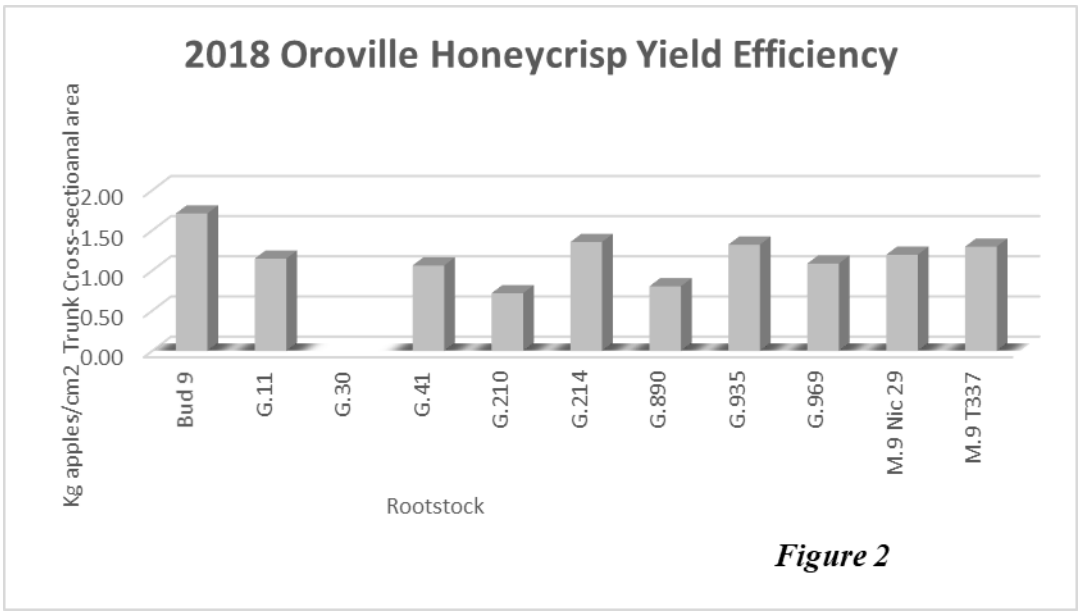


Figure 2

2017 Honeycrisp Average Yield per Tree (Figure 1) and Yield Efficiency (kg/cm^2) (Figure 2) for Eleven Geneva and Malling Series Rootstocks Planted in 2015 at Two Sites in Northcentral Washington.

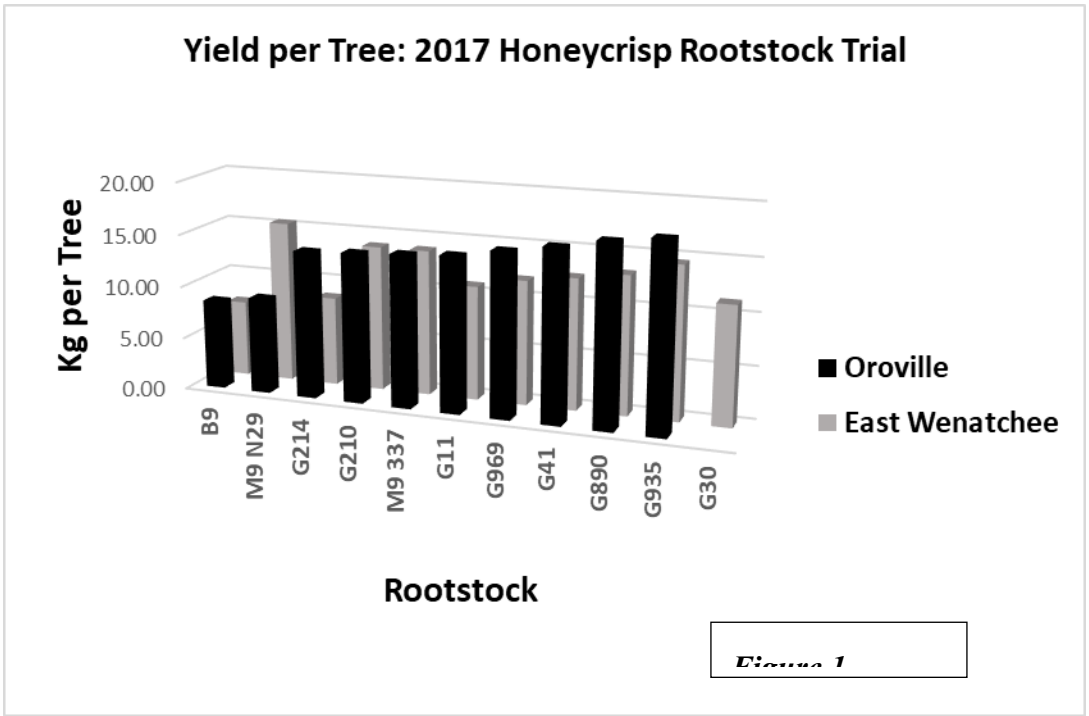


Figure 1

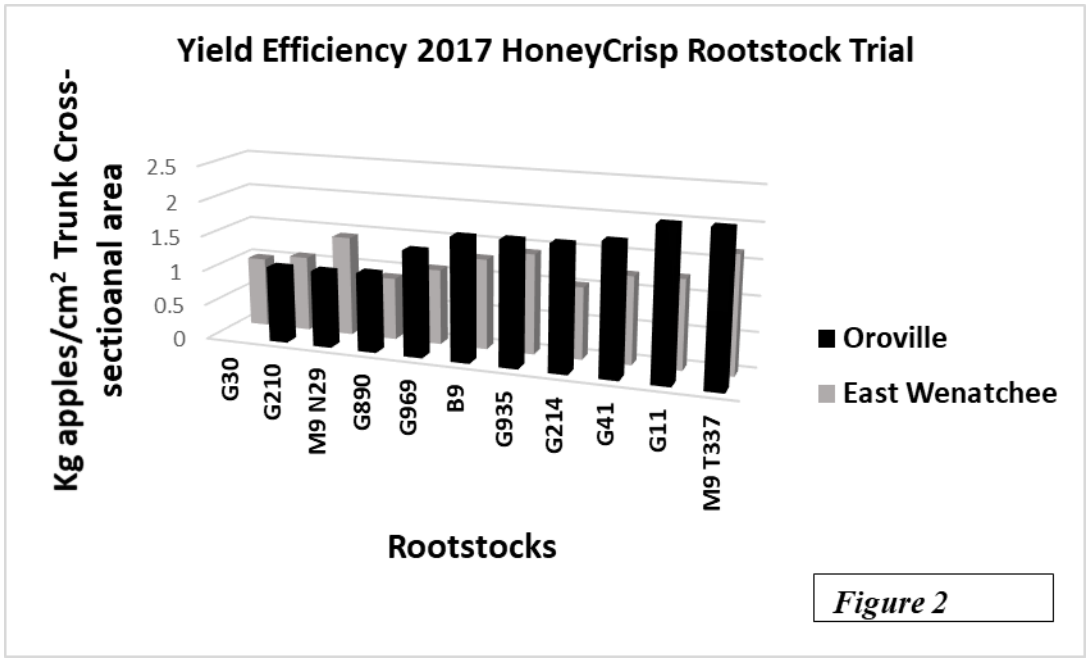


Figure 3: 2016 Oroville Honeycrisp trunk cross sectional area (TCSA)

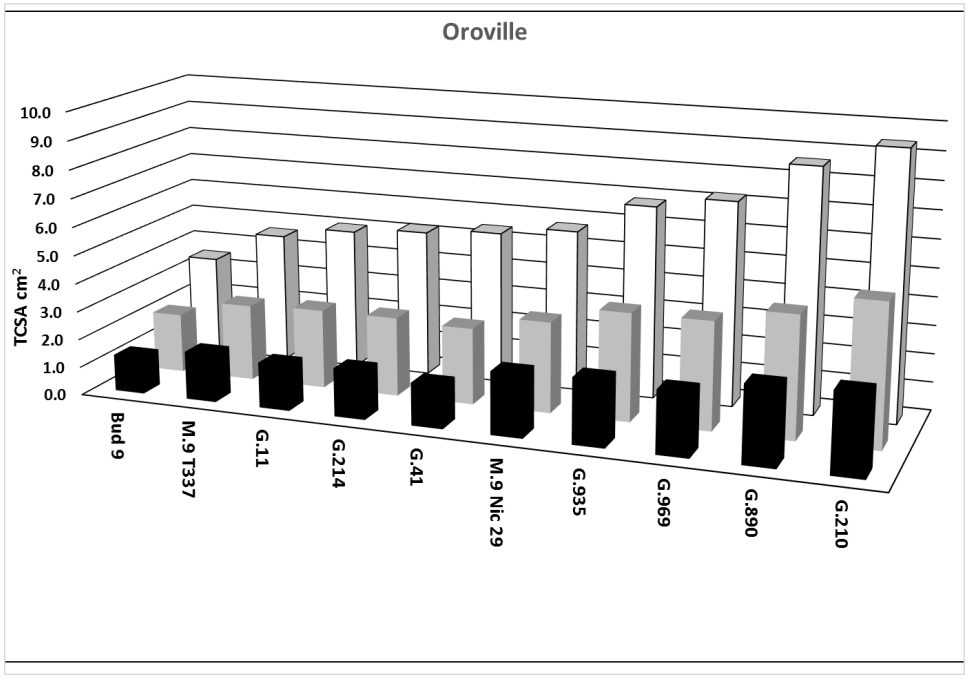
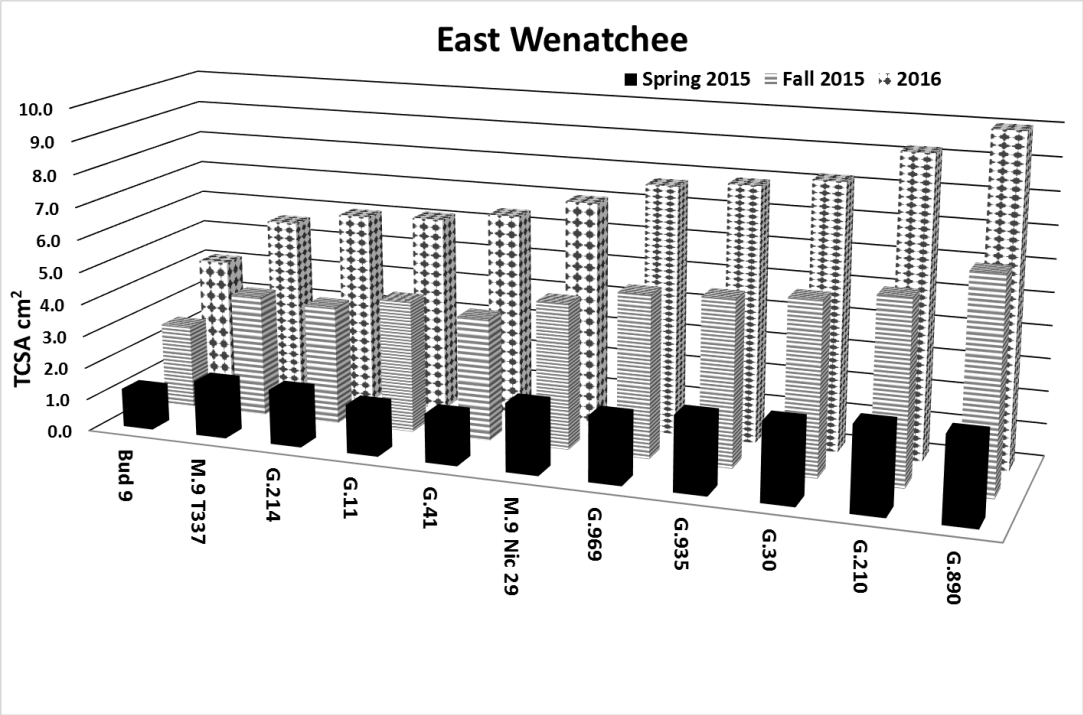


Figure 4: 2016 East Wenatchee Honeycrisp trunk cross sectional area (TCSA)



Rootstock findings and activities:

- A grower field days have been held on once or twice each year in cooperation with WSU Extension-Tianna DuPont
- Rootstock information is updated annually and is on the treefruit.wsu.edu website.
- G.969 continues to look very promising in many aspects including nursery propagation, yield, woolly aphid resistance and replant tolerance.
- G.935, G.30, G.11 and CG. 4011 are NOT woolly aphid resistant. Growers and nurseries are encouraged to pursue the woolly aphid resistant genotypes.
- Availability of G.30 is declining due to its unreliable propagation performance.
- G.41 has encountered broken unions especially with large caliper trees from some, but not all nurseries. ½" and smaller caliper trees have very minimal union breakage. ¾" caliper and larger trees on G.41 can have serious losses.
- G.41 has issues in transplanting which may be related to the number of roots on the plant being transplanted (fewer roots = less transplant success). Tissue culture sourced liners seem to have more consistent and higher root count.
- G.969 has good to excellent finished tree propagation traits. Yield data indicates G.969 will be similar to other members of the replant tolerant Geneva's in productivity.