Apple Horticulture/Postharvest Research Review
Yakima Convention Center

Wednesday, January 29, 2020

Time	Page	Presenter	Project Title	Yrs
8:00		Hanrahan	Welcome - Introductions and housekeeping	
			Continuing Projects: 8:15 - 12:00	
8:15	1	Kalcsits	How does fruit acclimation to sunburn affect sunburn management? ***Videoconference/NCE***	18-19
8:25	8	Schmidt	Crop load and canopy management of WA tree fruit	18-20
8:35	15	Schmidt	WTFRC apple pesticide residue study	18-20
8:45	18	DuPont	How do we measure and manage soil health for productive orchards? **Recorded presentation:** ***NCE***	17-19
8:55	24	Rudell	Non-destructive detection of sun stress compromised apples	19-21
9:05	31	Musacchi	Optimizing harvest time for WA38 **(O)**	19
9:15	39	Serra	Pollination, flower biology and fruit development in WA38 apples	19-20
9:25	46	Torres	Postharvest system optimization for organic apple storage **(O)**	19-21
9:35		Schmidt	WTFRC Technology projects (See Appendix)*	20
9:45			**Coffee break with scientists**	
10:15	50	Mendoza	Improving apple fruit quality & postharvest performance	19
10:25	53	Honaas	Apple genomes for postharvest fruit quality biomarkers	19-21
10:35	61	Rudell	Reducing carbon dioxide-related postharvest disorders	19-21
10:45	67	Critzer	Systems-based approach for improved packinghouse sanitation	18-20
10:55	74	Critzer	Critical limits for antimicrobials in dump tank systems	19-21
11:05	80	Critzer	Utility of rapid tools to assess cleanliness in apple packinghouses: **NCE**	18-19
11:15	88	Zhu	Fate of Listeria on apples at ozone and controlled atmosphere storage	18-20
11:25	96	Ganjyal	Complying with the FSMA preventative controls for human food rule: ***NCE***	17-18
11:35	103	Zhu	Control of *Listeria* on processing surfaces in apple packing facilities***: NCE***	17-19
11:45	111	Ganjyal	Increasing the efficacy of antimicrobial chemicals with surfactants	19-20
11:55			**Coffee break with scientists**	
12:30			**Committee lunch/Continuing Report Discussions**	
			Final Reports	
1:45	117	Hanrahan	Food safety update/Video presentation: 50th Anniversary of WTFRC	
2:00	121	Harper	Understanding decline on select apple scion-rootstock combinations ***(O)***	18-19
2:15	129	Ganjyal	Improving food safety by hot air impingement drying	15-17
2:30	137	Musacchi	WA38 fruit and dry matter for fruit quality/consumer preference	17-19
2:45	148	Kalcsits	Control of fruit size and bitterpit on Honeycrisp using irrigation; ***Videoconference***	17-19
3:00	161	Kalcsits	Optimizing light and water for orchards covered with netting ***Videoconference***	18-19
3:15	174	Lewis	WA38 demonstration trial block	18-19
3:30	183	Evans	Apple scion breeding program	18
3:45	189	Rudell	Reducing scald after long-term CA storage	16-18
4:00	200	Rudell	Risk assessment for delayed sunburn and sunscald	16-18

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

Project Title: How does fruit acclimation to sunburn affect sunburn management?

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Cooperators: Brenda Castaneda, Alexander Haase, Antoinette Avorgbedor, Orlando Howe

Total Project Request: **Year 1:** 86,621 **Year 2:** 87,846

Other funding sources

None

Budget: Kalcsits, Waliullah, Waite
Organization Name: WSU Contract Administrator: Katy Roberts/Kim Rains
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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, experiments were repeated to assess sunburn susceptibility, and additional experiments were run using placement and removal of netting throughout the season to assess the importance of timing and duration of heat priming stimuli in heat acclimation and to provide some assessment of sunburn risk from mid-season netting removal.

2. Link physiological and biochemical changes in the fruit to sunburn development.

In 2018 and 2019, we completed the major field experiments. Samples that were collected during the 2018 season were processed for gene expression analysis and pigment quantification. Samples collected in 2019 are currently being processed and data will be provided in the final report.

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. In addition, in 2019 we used placement and removal of netting to assess the importance of the timing of a below-sunburn threshold heat stimulus on acclimation to high temperatures later in the season.

SIGNIFICANT FINDINGS

1. **Exposure to near threshold temperatures in June induced important processes that led to increased sunburn resistance in July and August in 2018. 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.**

2. **Anthocyanins were found to increase in response to higher heat treatments in fruit that had received no priming stimulus, and did not respond to heat in fruit that had been previously primed.**

3. **Candidate genes were selected based on acclimation studied in a variety of plant species. At three days after fruit were heated, no differences were detected between treatments, suggesting either changes in gene expression occur earlier, or these genes are not involved in apple acclimation to sunburn.**

4. **In 2020, molecular analysis will be concluded and a replicated trial will be conducted where evaporative cooling cycles being at air temperatures of either 85°F or 90°F.**

METHODS

Objective 1: The 2019 season experiments took place at Sunrise research orchard. Eighty sample trees for each cultivar were selected early in 2019 for uniform size and crop load and thinned to appropriate commercial crop loads.

To manipulate fruit surface temperature (FST) on fruit in the field, fruit were bagged to increase the FST to 110-113 °F, 114-119 °F, or 120-124 °F for 20 minutes under full sunlight (40 fruit for each treatment plus an additional 160 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 both infrared thermal sensors and traditional thermocouples. Fruit were allowed to develop sunburn symptoms over 72 hours. Representative fruit samples were taken from each temperature treatment at 24, 48, and 72 hours post-treatment for physiological, molecular, biochemical, and non-destructive and destructive pigment analysis described in Objective 2 (10 fruit for non-destructive physiological analysis, 5 fruit for destructive molecular and biochemical analysis).

Four weeks later, the 160 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 and 2). Similar to the above experiment, representative fruit samples were taken at 24, 48, and 72 hours post-treatment for physiological, molecular, biochemical and non-destructive and destructive pigment analysis. However, during the 2019 season air temperatures, and therefore FSTs, did not reach sufficiently high enough levels to induce much sunburn in control populations (Fig 3). As a result, we have samples exposed to below-, at-, and above-FST threshold temperatures for sunburn in both early and late season timepoints that can be used to understand the dynamic physiological and molecular pathways underlying sunburn development in apple fruit. Samples from these experiments, where we obtained a time-course of material post-sunburn induction, will be used in an RNA-sequencing experiment to discover genes expressed under these conditions that may be involved in the development of both sunburn and acclimation to heat stress (Fig 4).

To understand the importance of the timing of a near-threshold heat stimulus for acclimation, experiments were designed using a combination of staggered stimuli and netting removal (Fig 3). In the first experiment, 30 fruit were treated with a below-threshold FST (110-113 F) stimulus 6 weeks, 5 weeks, and 4 weeks before a late-season measurement and collection date. Afterward, physiological measurements were taken and samples were collected for molecular and biochemical analysis. In the second experiment, sample trees were netted in the early season to protect against sunburn pressure. Fruit were treated with a below-threshold FST (110-113 F) stimulus under the nets, then nets were removed from treated and control trees 3 days, 7 days, and 14 days post-treatment, allowing trees to experience high, ambient sunburn conditions. Fruit were then measured and sampled 72 hours later for physiological, molecular and biochemical analysis.

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 (2018 season), (2) physiological changes that occur during sunburn development (2018 and 2019), and (3) physiological changes that occur that may be linked to acclimation to heat and light (2018 and 2019).

Peel tissue from the 2018 season was processed first by lyophilizing, followed by homogenization. For gene expression analyses, powdered peel tissue was used to extract RNA, following a modified CTAB protocol outlined by Honaas and Kahn, BMC Research Notes, 2017. Candidate genes were selected from literature focused on acquired thermotolerance in a variety of species, including both crop and model systems (Fig 5), and variety-specific primers were developed through collaboration with Loren Honaas and Heidi Hargarten, based on prior transcriptomic experiments in Honeycrisp and Granny Smith. Quantitative PCR was performed and analysed to assess gene expression in collected peel samples (Fig 6). Tissue from 2019 is currently being processed and will allow us to assess gene expression over time during the sunburn development

period. For pigment analysis, chlorophylls and anthocyanins were extracted using methanol-based extraction methods and read using a plate reader (Warren et al. *J Plant Nutr* 2008). Calculations based on the literature were used to determine pigment levels in the samples (Rayleigh's formula, Lichtenthaler et al. 2001) (Fig 7). These will be repeated with the tissue from 2019 over a time series.

Objective 3: Temperature activated solenoids were installed, tested, and working but failed at Sunrise research orchard during the 2019 season. Improved systems will be installed at Sunrise in 2020 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.

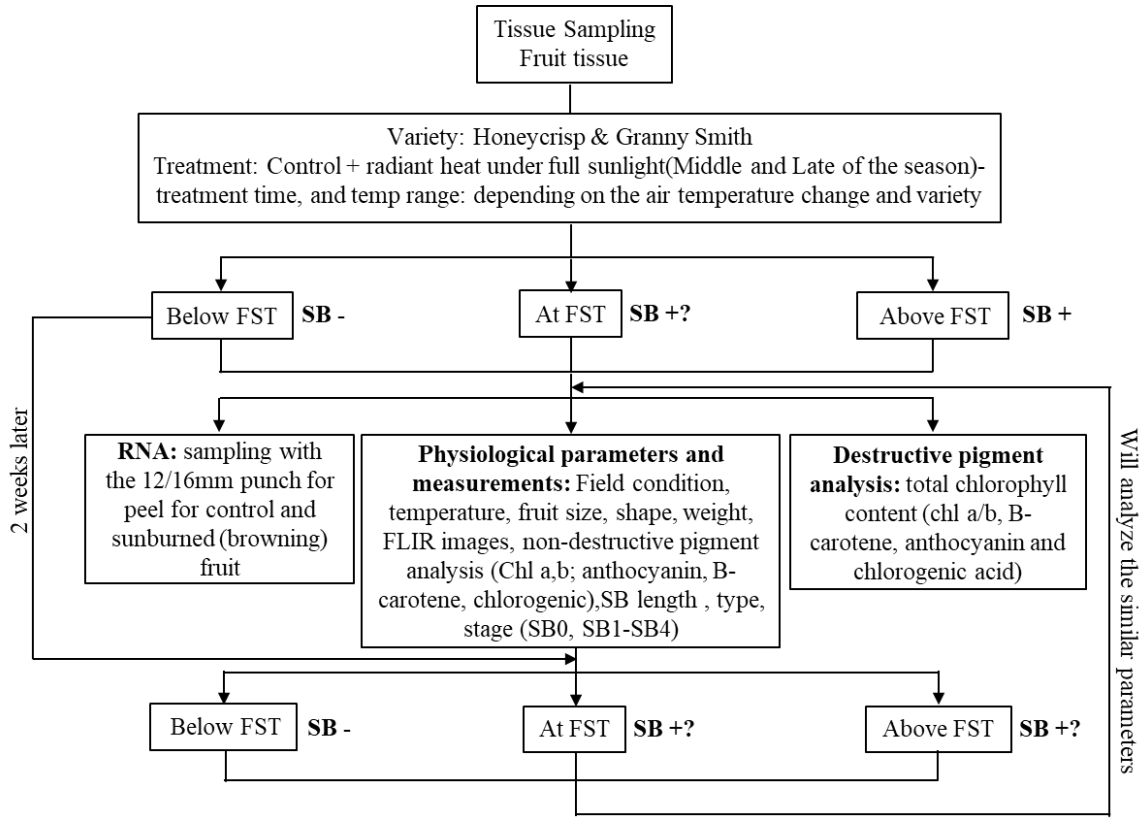

Fig. 1. Experimental layout for experiments conducted in 2018 (1) fruit acclimation to high temperature and (2) mechanisms underlying sunburn resistance and development in developing fruits. FST = Fruit surface temperature threshold.

RESULTS AND DISCUSSION

For experiments conducted to determine whether fruit can physiologically acclimate to elevated fruit surface temperatures, fruit was exposed to near threshold fruit surface temperatures in June of 2018 when temperatures were relatively cool. In addition to showing reduced sunburn compared to fruit that was exposed to normal conditions, peel tissue from these fruit also showed no increase in anthocyanin production, while fruit under normal conditions showed increased production with increased heat (Fig 7). To understand the genes involved in sunburn acclimation, we selected five candidate genes from the thermotolerance literature shown to be involved in

heat stress and acclimation (Fig. 5). Tissue from heat-primed fruit and untreated fruit showed no significant difference in gene expression of these candidate genes 3 days after heat treatments (Fig 6). This could suggest that these genes are either not involved in apple heat stress acclimation, or that we did not have enough temporal resolution to see changes in expression. These experiments were repeated in 2019, with the addition of collecting tissue 24, 48, and 72 hours post-treatment to capture the dynamics of pigment accumulation and gene expression, as changes in expression may occur sooner than 3 days. During the 2019 season however, with the exception of a few days in late July and early August, temperatures throughout much of the season were not sufficient to produce much sunburn pressure on fruit (Fig 3), thus acclimation could not take place and control fruit were not highly stressed as in the previous season. However, due to our experimental design (Fig 1 and 2A), we obtained samples from fruit that had been treated with near-threshold and above threshold temperatures, both at the beginning and late in the season, which can be used to perform an RNA-sequencing experiment in the winter of 2020 to address questions about the molecular

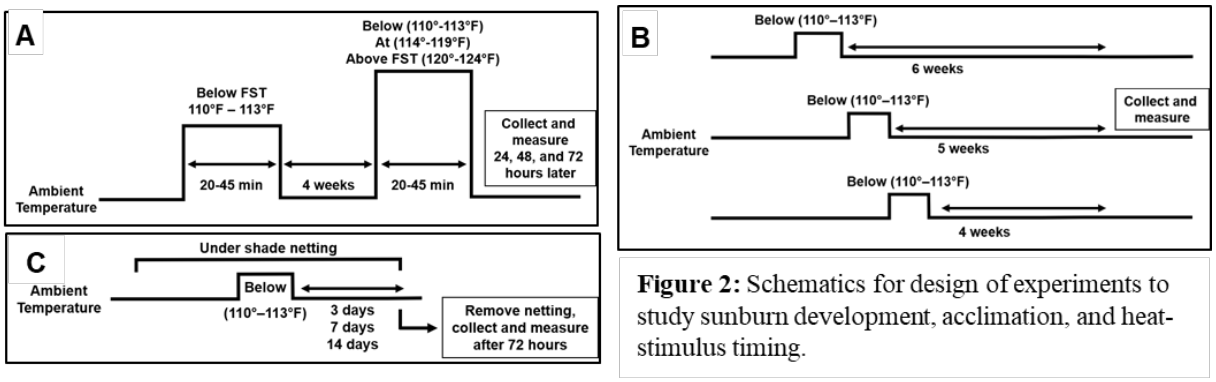

Figure 2: Schematics for design of experiments to study sunburn development, acclimation, and heat-stimulus timing.

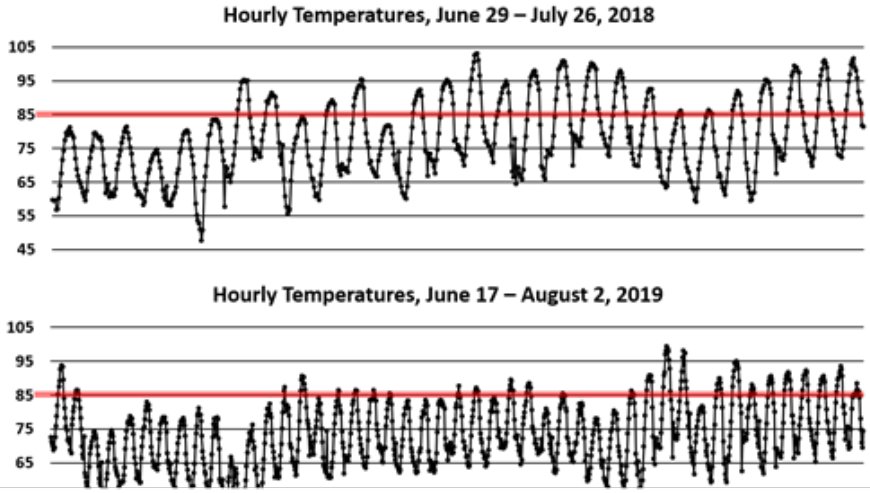

Figure 3: Hourly temperatures during the 2018 and 2019 season experiments. 85 degrees, the current recommended temperature for initiation of evaporative cooling, is marked. 2019 was significantly cooler during this experimental period.

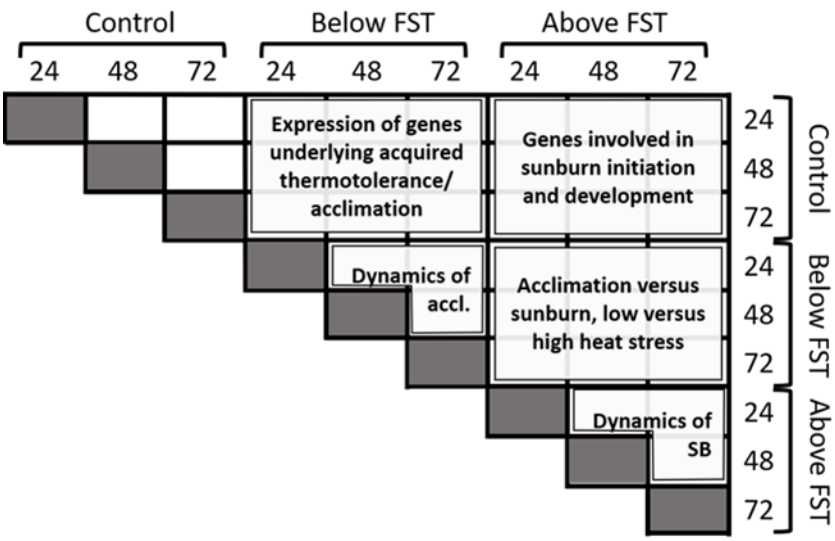

Figure 4: Potential comparisons to be made using RNA-sequencing. The outlined comparisons would highlight differentially expressed genes involved in acclimation, sunburn initiation and development, and useful information of the dynamics of these genes after heat treatments.

players and pathways underlying sunburn development in apple (Fig 4), which is a largely unanswered question in the field.

Additional experiments were designed in 2019 to understand the importance of the timing of the priming heat stimulus (Fig 2B and C). From the literature on acquired thermotolerance across plant species, the amount of time that a heat stimulus confers priming to heat stress can vary. In addition, mechanisms for short-term acquired thermotolerance (SAT), long-term acquired thermotolerance (LAT), and thermotolerance to moderately high temperatures (TMHT) involve distinct molecular pathways, and our initial experiments were not designed to tease apart which are involved in apple sunburn acclimation. The relatively low temperatures during the 2019 season unfortunately precluded obtaining useful data from these experiments, however, these would be valuable experiments to repeat in 2020.

Table 1. Candidate genes involved in heat stress and acquired thermotolerance

Gene name	Candidate Gene ID	Processes involved	References
LDOX	MD06G1071600	Phenylpropanoid/flavonoid pathway. Showed higher expression in sunned fruit peels.	Feng et al. Plant Phys Biochem 2013
MYB10	MD09G1278600	Anthocyanin biosynthesis. Peels of shaded fruit showed lower MYB10 expression and anthocyanin levels compared to sunned fruit.	Feng et al. Plant Phys Biochem 2013
APX2	MD12G1125600	Ascorbate peroxidase. Dependent on HSFA2, a gene required for heat shock memory after an acclimatizing stimulus.	Friedrich et al. Plant Cell Env 2018, Lamke et al. EMBO 2015, Charng et al. Plant Phys 2007.
DFR1	MD15G1024100	Phenylpropanoid/flavonoid pathway. Showed higher expression in sunned fruit peels.	Feng et al. Plant Phys Biochem 2013
HSP17.6/ HSFA2-Like	MD15G1209400	Heat shock protein, similar to HSFA2, a gene required for heat shock memory after an acclimatizing stimulus.	Friedrich et al. Plant Cell Env 2018, Lamke et al. EMBO 2015, Charng et al. Plant Phys 2007.
HK (reference)	MDP0000274900	Housekeeping gene, used as a reference for expression.	Perini et al. Mol Breed 2014.

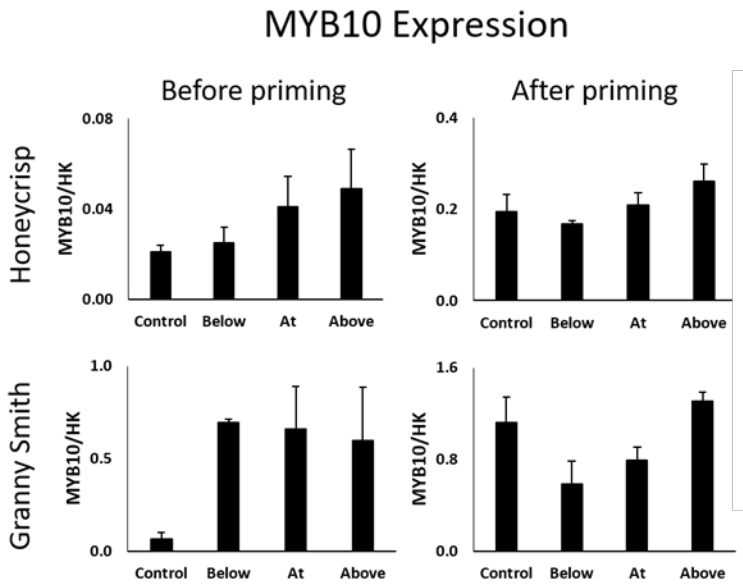

Figure 6: MYB10 expression in peel tissue from heat-primed fruit and fruit grown under normal conditions prior to being challenged with below-, at- and above-FST thresholds for sunburn. Similar to other genes observed, MYB10 showed no significant differences between treatment categories. This gene is involved in resistance to abiotic stress and anthocyanin production (red color)

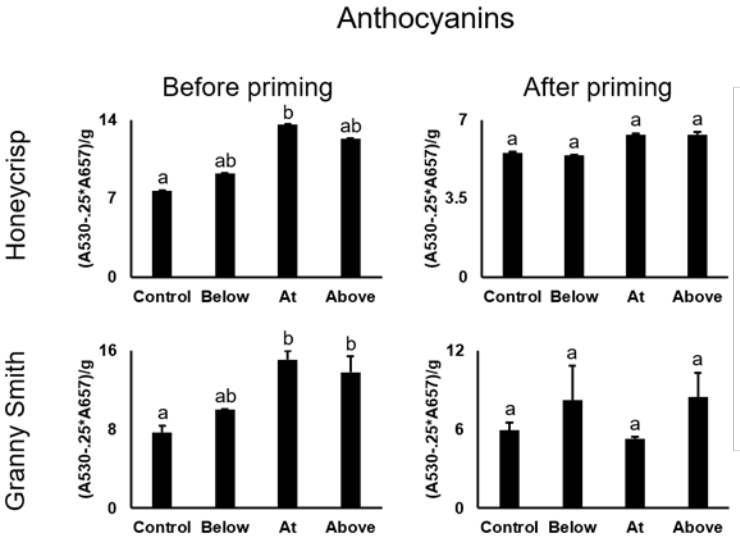

Figure 7: Anthocyanin levels in peel tissue from heat-primed fruit and fruit grown under normal conditions prior to being challenged with below-, at- and above-FST thresholds for sunburn. Non-primed fruit showed an increase in anthocyanins with increased heat treatments, while fruit that were primed did not.

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

Project Title: Crop load and canopy management of WA tree fruit

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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	na
Benefits	2440	2510	na
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	53,890
Anticipated Income (contracts and gift grants)	67,560	60,300	60,000?
Total net costs	(12,880)	(2180)	**(6110?)**

Footnotes:
Salaries: salary costs reflect time for Mendoza only in 2018 & 2019; no salary costs reflected in internal projects starting in 2020
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.

2019 SIGNIFICANT FINDINGS:

No treatments reduced fruit set in a chemical bloom thinning trial in our lone chemical bloom thinning trial, but fruit finish was improved by Regalia (Table 1)

The most efficacious options for chemical bloom thinning of apple continue to be spray oil + lime sulfur programs (Table 2)

Metamitron products continue to reduce fruit set, improve harvest fruit size, and increase return bloom more consistently than current industry standard thinning programs (Tables 3, 4)

Metamitron efficacy can be promoted by tank mixing with non-ionic surfactants, increasing rate, or use of multiple applications (Table 3)

2019-EXP-01 significantly boosts the performance of 6-BA as a chemical thinner (Table 3) and of GA_7 as an inhibitor of return bloom (Table 5)

2018 applications of GA_7 reduced 2019 return bloom in a single trial on Golden Delicious (Table 5); this new product is approved for organic use and should be available on a limited basis in 2020 for management of biennial apple blocks

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 began testing the material as a chemical bloom thinner in 2018. Results from that initial Gala trial did not demonstrate any significant treatment effects from Regalia on fruit set, fruit finish, or return bloom, but we did observe an increase in fruit size in one Regalia treatment, as well as the industry standard oil + lime sulfur program.

In 2019, we tried thinning with Regalia again, this time in a Jonagold block (Table 1). As with the 2018 Gala trial, no treatment significantly affected fruit set, but there was a clear improvement in fruit finish across most treatments, both from Regalia and oil + lime sulfur. While we were unable to document statistically significant improvements in fruit size in 2019, some Regalia treatments once again suggested a trend toward that effect. Return bloom will be assessed next spring.

While the lack of demonstrable thinning in our two Regalia trials has been disappointing thus far, it is worth recalling how infrequently our replicated field trials have documented significant treatment effects in other bloom thinning trials (Table 2). Regardless, improvements in fruit finish and size have been intriguing and we hope to continue to learn more about the potential benefits of applying Regalia during bloom in future studies.

Table 1. Crop load and fruit quality effects of bloom chemical thinning programs. WTFRC 2019.

Treatment	**Fruitlets/100 floral clusters**	**Blanked spurs**	**Singled spurs**	**Harvest fruit weight**	**Relative box size**	**Russet free fruit**
Jonagold / M.26 - Rock Island		*%*	*%*	*g*		*%*
2% Regalia	45 abc	63 ab	30 ab	247 ns	74	43 ab
4% Regalia	49 ab	57 b	37 a	221	82	60 a
1.5% CFO + 1% Regalia	40 abc	65 ab	31 ab	232	78	21 b
1% WES + 1% Regalia	52 a	57 b	35 ab	241	75	59 a
1% WES + 2% LS	36 c	69 a	27 b	231	79	51 ab
Control	37 bc	67 a	29 ab	215	84	16 b

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 1999-2019.

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 / 32 (47%)	5 / 31 (16%)	4 / 30 (13%)
ThinRite	7 / 22 (32%)	0 / 23 (0%)	0 / 12 (0%)

[1]Does not include data from 2019 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.

2019 conditions were generally favorable (warm temperatures, mostly cloudy skies) across Central WA for strong performances from postbloom chemical thinners (Table 3). In all 5 trial sites, metamitron products (ADA 46343 & ADA 46701) significantly reduced fruit set and improved harvest fruit size; as we have seen in the past, these effects were generally amplified with repeat applications, higher product rates, or the addition of a non-ionic surfactant, Regulaid. In seasons like 2019 with ideal conditions for chemical thinning, a single application of metamitron may be adequate to deliver the desired reduction in fruit set; in more "typical" Central WA springs (cool temperatures, mostly sunny skies), grower might need to be more aggressive in terms of rate, use of surfactant, or multiple applications to achieve the desired thinning effect.

For the third consecutive year, we observed greatly improved thinning from 6-benzyladenine (FAL 551) with the addition of a proprietary surfactant from Fine Americas (Table 3); this same surfactant also boosted the performance of Fine's GA_7 product for inhibiting return bloom (Table 5). After lackluster results in 2018, we documented some mild to moderate thinning with dilute postbloom applications of oil + lime sulfur (Orondo Gala); this use pattern is not currently covered by product labels, but the results may offer hope for organic growers needing to thin their crops after petal fall.

Table 3. Crop load and fruit quality effects of postbloom thinning programs. WTFRC 2019.

Treatment	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size	Russet free fruit
		%	*%*	*g*		*%*
Fuji / M.9 - Wapato						
FAL 551 25.6 fl oz PF & 10-12mm	121 b	34 cd	26 bc	200 c	91	78 ns
FAL 551 25.6 fl oz + 2019-EXP-01 16oz PF & 10-12mm	103 bcd	35 cd	36 ab	227 abc	80	86

FAL 551 25.6 fl oz + 2019-EXP-01 32oz PF & 10-12mm	84 cde	43 cd	36 ab	228 abc	80	90
FAL 551 25.6 fl oz + 2019-EXP-01 64oz PF & 10-12mm	32 f	74 a	22 c	261 a	70	86
ADA 46343 40 oz PF&10-12mm	108 bc	38 cd	30 abc	205 bc	89	93
ADA 46343 40 oz + Regulaid 32 oz PF & 10-12mm	49 ef	60 ab	31 abc	248 ab	73	85
Carbaryl 4L 36 oz + Fruitone L 2 oz PF & 10-12mm	67 def	49 bc	39 a	232 abc	78	91
Control	158 a	28 d	21 c	188 c	97	80
Gala / M.9 – Frenchman Hills, Quincy						
FAL 551 25.6 fl oz PF & 10-12mm	140 ab	28 c	28 ab	158 c	115	16 ns
FAL 551 25.6 fl oz + 2019-EXP-01 16oz PF & 10-12mm	168 a	19 c	28 ab	166 bc	109	16
FAL 551 25.6 fl oz + 2019-EXP-01 32oz PF & 10-12mm	144 ab	27 c	28 ab	165 bc	110	11
FAL 551 25.6 fl oz + 2019-EXP-01 64oz PF & 10-12mm	136 ab	31 bc	26 ab	164 bc	111	18
ADA 46343 40 oz PF&10-12mm	88 cd	46 ab	32 ab	180 ab	101	No data
ADA 46343 40 oz + Regulaid 32 oz PF & 10-12mm	58 d	56 a	32 ab	191 a	95	24
Carbaryl 4L 36 oz + Fruitone L 2 oz PF & 10-12mm	122 bc	30 bc	33 a	171 abc	106	6
Control	152 ab	29 bc	23 b	152 c	119	25
Gala / M.26 - Orondo						
ADA 46343 40 oz PF	66 d	56 c	27 b	155 cd	117	38 ns
ADA 46343 40 oz 10-12mm	71 d	52 c	32 ab	159 bc	114	43
ADA 46343 40 oz PF & 10-12mm	19 e	86 b	11 c	186 ab	98	44
ADA 46343 32 oz + Regulaid 32 oz	8 e	93 a	7 c	187 a	97	35
Carbaryl 4L 36 oz + Fruitone L 2 oz	20 e	82 b	16 c	188 a	97	36
CFO 1 gal + LS 1 gal @ 400 GPA 10-12mm	99 c	41 cd	33 ab	129 d	141	38
CFO 1 gal + LS 1 gal @ 400 GPA PF & 10-12mm	129 b	24 de	37 a	143 cd	127	33
Control	163 a	16 e	36 a	135 cd	135	40
Golden Delicious / Bud 9 – Rock Island						
ADA 46701 1.3 pt 12-14mm	25 b	77 e	21 ab	213 bc	85	45 b
ADA 46701 2 pt 12-14mm	20 bc	83 cde	15 bcd	239 abc	76	49 ab
ADA 46701 2.7 pt 12-14mm	12 cd	88 bc	11 de	259 ab	70	60 ab
ADA 46701 3.3 pt 12-14mm	8 de	92 ab	8 ef	259 ab	70	54 ab
ADA 46701 3.3 pt + Regulaid 32 oz 12-14mm	3 e	97 a	3 f	286 a	63	39 b
Carbaryl 4L 36 oz + Fruitone L 2.5 oz12-14mm	14 cd	87 bcd	13 cde	246 abc	74	51 ab

Exilis 9.5SC 25.6 oz + Fruitone L 2.5 oz 12-14mm	21 bc	81 de	18 bc	198cd	92	78 a
Control	41 a	66 f	28 a	154 d	118	58 ab
Granny Smith / M.9 – Rock Island						
ADA 46701 3.0 pt PF	59 a	48 b	45 a	214 ab	85	94 ns
ADA 46701 3.0 pt 8-11mm	32 b	72 a	25 b	220 ab	83	84
ADA 46701 3.0 pt 12-15mm	28 b	73 a	26 b	232 a	78	94
ADA 46701 3.0 pt 16-20mm	29 b	71 a	29 b	220 ab	83	94
Carbaryl 4L 36 oz + Fruitone L 2 oz	34 b	68 a	31 b	217 ab	84	89
Control	67 a	42 b	49 a	177 b	103	86

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. Due to especially impressive results in 2019 trials, we continue to see further separation of metamitron programs from current industry standards, suggesting that it may be a more consistent performer in WA than carbaryl, BA, and/or NAA products.

Table 4. Incidence and percentage of results significantly superior to untreated control. Apple chemical postbloom thinning trials. WTFRC 2002-2019.

Treatment	Fruitlets/100 blossom clusters	Harvested fruit size	Return bloom[1,2]
BA	7 / 29 (24%)	0 / 30 (0%)	0 / 26 (0%)
Carb + BA	33 / 91 (36%)	10 / 89 (11%)	13 / 86 (15%)
Carb + NAA	29 / 78 (37%)	20 / 76 (26%)	13 / 71 (18%)
BA + NAA	19 / 41 (46%)	9 / 40 (23%)	7 / 36 (19%)
Metamitron	18 / 29 (62%)	13 / 28 (46%)	6 / 22 (27%)

[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 2018 trial on biennial Golden Delicious with this GA_7 product (FAL 900) demonstrated dramatic reductions in 2019 return bloom when combined with a proprietary surfactant or partnered with a series of applications of GA_4 (Novagib).

According to Fine Americas, their GA_7 product should clear EPA registration shortly and be available for use in the spring of 2020; it was scheduled to be released for the 2019 season, but final approval by the EPA was delayed by the government shutdown in January of that year. The product will be called "Arrange" and has already been approved by OMRI for organic use. This material 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 2018.

Treatment	**2018 harvest fruit weight**	**2018 relative box size**	**2018 shoot growth**	**2019 return bloom**	**2019 return bloom per CSA**
	g		*cm*	*%*	*clusters/cm²*
Golden Delicious / M.9 – Rock Island					
4 x FAL 900 25ppm	245 ns	74	22.6 ns	2583 bc	1.2 a
FAL 900 100ppm @ petal fall	215	84	24.3	2398 bc	1.9 a
FAL 900 100ppm @ PF+14	216	84	24.2	1390 cd	1.2 a
FAL 900 100ppm + 2019-EXP-01 @ PF	216	84	24.9	154 d	0.2 b
FAL 900 50ppm; 4 x 20 oz Novagib	234	78	25.2	828 d	0.3 b
FAL 900 100ppm; 4 x 20 oz Novagib	246	74	16.8	650 d	0.2 b
4 x Falgro 4L 100ppm	211	86	22.5	3023 ab	1.6 a
Control	192	95	21.2	4399 a	1.9 a

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, Honeycrisp, and WA 38); help with outreach activities for new horticultural models (project funded through WTFRC technology committee)

Proposed: "Precision Crop Load Management for Apples" (USDA-NIFA Specialty Crop Research Initiative (SCRI) - PD: Terence Robinson, Cornell) – project has passed the initial screening and full proposal is currently being written; this project would include work to be done by collaborators in WA, NY, VA, MI, MA, and NC; objectives will focus on development of predictive models and horticultural strategies to develop/optimize crop load, as well as development of vision systems, robots, & other automated tools to assess and adjust crop load as various phenological stages

CROP YEAR: 2019

CONTINUING REPORT **PROJECT LENGTH (CROP YEARS)**: 2019-2021

Project Title: Pesticide Residues on WA Apples

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: Gerardo Garcia, Sandy Stone, Pacific Agricultural Labs, Northwest Hort Council, Doug Stockwell, Doyle Smith, various ag chemical companies

Item	2019	2020 (est.)	2021 (est.)
Salaries			
Benefits			
Wages[1]	1350	1400	1450
Benefits[1]	700	725	750
RCA Room Rental			
Shipping			
Supplies/Chemicals	250	275	300
Travel[2]	1000	1000	1000
Plot Fees			
Analytical lab fees	3500	3750	4000
Total gross costs	6,800	7,150	7,500
Anticipated Income (contracts and gift grants)	0	0	0
Total net costs	6,800	**7,150**	7,500

Footnotes: Schmidt estimates 10% of his time is dedicated to this project on an annual basis
Most pesticides tested are donated by their registrants or an ag chemical supply company
1 Wages & benefits primarily for Garcia (spray applications), crew help for Garcia, and Stone (data entry & review)
2 Travel costs include hauling equipment to & from plots & delivery of samples to Sherwood, OR

2019 WTFRC APPLE PESTICIDE RESIDUE STUDY

Since 2011, the Washington Tree Fruit Research Commission (WTFRC) has conducted annual trials to evaluate pesticide residues on 'Gala' apples. This year, we applied ten insecticide/acaricides and five fungicides (seventeen total active ingredients) with a Rears Pak-Blast sprayer in two different scenarios. SCENARIO A simulates typical industry use patterns for these products applied at 100 gal water/acre. SCENARIO B reflects 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) applied at 200 gal water/acre. We had intended to apply both standard and aggressive spray protocols at both carrier volumes, but sprayer error confounded the results. Fruit samples were collected at commercial maturity on August 28 and delivered the next day to Pacific Agricultural Labs (Sherwood, OR) for chemical residue analysis.

TRIAL DETAILS

- 12th 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 0.75" of rain on Aug 9 & 10 (19 & 18 days before harvest)

Measured residues vs. maximum residue levels (MRLs) for apple pesticide programs in SCENARIO A: typical industry rates, timings, and retreatment intervals applied in 100 gal water/acre. 'Gala'/M.9 Nic.29, Rock Island, WA. WTFRC 2019.

Chemical name	Trade name	Application rate	Application timing(s)	Measured residue	US MRL[1]	Lowest export MRL[1]
		oz per acre	*dbh*	*ppm*	*ppm*	*ppm*
Flutianil	Gatten	8	35	<0.01	0.15	0.01 (many)
Isofetamid	Kenja 400SC	12.5	35	0.019	0.6	0.01 (India)
Spinetoram	Delegate WG	7	35 & 21	<0.01	0.2	0.01 (India)
Cyantraniliprole	Exirel	13.5	35 & 21	0.097	1.5	0.01 (IND,TAI)
Spinosad	Entrust	3	35 & 21	<0.01	0.2	0.01 (India)
Tolfenpyrad	Bexar	27	35 & 21	0.20	1.0	0.01 (many)
Myclobutanil	Rally 40WSP	10	35 & 21	0.12	0.5	0.01 (UAE)
Novaluron	Rimon	32	35 & 21	0.22	3.0	0.01 (India)
Fluxapyroxad	Merivon	5.5	28	0.045	0.8	0.8 (CAN,MEX)
Pyraclostrobin	Merivon	5.5	28	0.029	1.5	0.5 (many)
Etoxazole	Zeal	2	28	0.026	0.2	0.01 (India)
Difenoconazole	Inspire Super	12	28	0.027	5.0	0.01 (India)
Cyprodinil	Inspire Super	12	28	0.052	1.7	0.01 (India)
Diazinon	Diazinon 50WS	16	28	0.016	0.5	0.01 (India)
Bifenazate	Acramite 50WS	16	28	0.032	0.7	0.01 (India)
Phosmet*	Imidan 70-W*	92	14	3.4	10.0	0.01 (India)
Fenpropathrin	Danitol	18	14	0.20	5.0	0.01 (IND,SAU)

[1] Top markets for WA apples; 26 Sep 2019. http://nwhort.org/export-manual/comparisonmrls/apple-mrls/, https://bcglobal.bryantchristie.com
* Imidan 70-W applications included 8 fl oz Tech-Spray/100 gal water to acidify spray tank

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

Measured residues vs. maximum residue levels (MRLs) for apple pesticide programs in SCENARIO B: aggressive use patterns (maximum rates with minimum retreatment and preharvest intervals) applied in 200 gal water/acre. 'Gala'/M.9 Nic.29, Rock Island, WA. WTFRC 2019.

Chemical name	Trade name	Application rate	Application timing(s)	Measured residue	US MRL[1]	Lowest export MRL[1]
		oz per acre	*dbh*	*ppm*	*ppm*	*ppm*
Isofetamid	Kenja 400SC	12.5	35 & 21	0.034	0.6	0.01 (India)
Diazinon	Diazinon 50WS	16	35 & 21	<0.01	0.5	0.01 (India)
Tolfenpyrad	Bexar	27	28 & 14	0.20	1.0	0.01 (many)
Novaluron	Rimon	32	28 & 14	0.20	3.0	0.01 (India)
Difenoconazole	Inspire Super	12	28 & 14	0.045	5.0	0.01 (India)
Cyprodinil	Inspire Super	12	28 & 14	0.081	1.7	0.01 (India)
Fenpropathrin	Danitol	18	28 & 14	0.26	5.0	0.01 (IND,SAU)
Myclobutanil	Rally 40WSP	10	21 & 14	0.065	0.5	0.01 (UAE)
Flutianil	Gatten	8	21 & 14	<0.01	0.15	0.01 (many)
Spinosad	Entrust	3	21 & 7	0.024	0.2	0.01 (India)
Phosmet*	Imidan 70-W*	92	21 & 7	3.7	10.0	0.01 (India)
Spinetoram	Delegate WG	7	14 & 7	0.014	0.2	0.01 (India)
Cyantraniliprole	Exirel	13.5	14 & 5	0.11	1.5	0.01 (IND,TAI)
Bifenazate	Acramite 50WS	16	7	0.027	0.2	0.01 (India)
Fluxapyroxad	Merivon	5.5	7 & 1	0.086	0.8	0.8 (CAN,MEX)
Pyraclostrobin	Merivon	5.5	7 & 1	0.072	1.5	0.5 (many)

[1] Top markets for WA apples; 26 Sep 2019. http://nwhort.org/export-manual/comparisonmrls/apple-mrls/, https://bcglobal.bryantchristie.com
* Imidan 70-W applications included 8 fl oz Tech-Spray/100 gal water to acidify spray tank

DISCUSSION

As with all previous WTFRC studies, no residue exceeded the US Environmental Protection Agency's tolerance, affirming that application of these materials following label guidelines produce residues determined to be safe for domestic US markets. Most of the products assayed in our 2019 study, however, generated residues which exceed MRLs for important export markets, particularly India. In most cases, those actual residue levels were relatively low, but could trigger potential problems because India has yet to publish MRLs on apples for most of these products; in the absence of a posted MRL, the *de facto* limit falls to the national default value, which is 0.01 ppm for India. Once India publishes more apple MRLs, most of those tolerances will be relaxed, allowing US growers a better chance of using a variety of pesticides while still meeting Indian standards.

Our intent this year was to apply both "standard" and "aggressive" spray programs at 100 and 200 gal water/acre, as we have done in the past. Unfortunately, application error confounded the results in two of our plots, leaving only proper application of the standard protocol at 100 gal/acre (Scenario A) and the aggressive protocol at 200 gal/acre (Scenario B). This error precludes valid comparison of spraying standard vs. aggressive protocols or concentrate vs. dilute applications. Nonetheless, the results reported here are valid and accurately mimic real-world spray programs for commercial Washington apple orchards.

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 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.

WASHINGTON TREE FRUIT RESEARCH COMMISSION

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**: No-Cost Extension

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

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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: $10,000 WSU Soil Health Initiative

WTFRC Collaborative Expenses: None

Budget 1
Organization Name: WSU-TFREC **Contract Administrator:** Katy Roberts/Shelli Tompkins
Telephone: 509-335-2885/509-293-8803 **Email address:** arcgrant@wsu.edu/ shelli.tompkins@wsu.edu

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

- Soil quality indicators measured fell over a wide range.
- 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 (64 bins per acre high, 38 bins per acre low; 1309 packs per acre high; 711 packs per acre low).
- 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 103 orchard plots were soil sampled. Of these plots 60 plots (30 matched pairs) were well matched with available/measured yield data. A subset of 32 plots (16 matched pairs) were 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).

RESULTS

Of 103 plots soil sampled to date, 60 were well matched sites (30 pairs) with grower available/measured yield data and 32 plots (16 pairs) 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

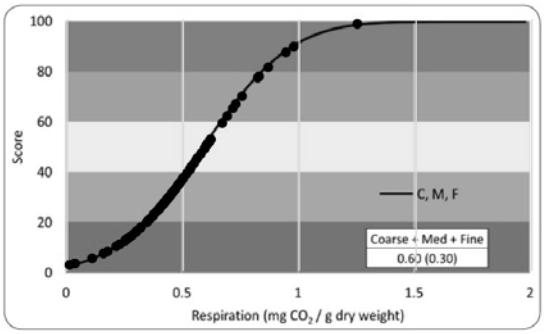
protein index (Figures 2&3), Washington Orchard soils have tended to score in the lower end of the scale compared to the scoring curve functions which were developed in the East. Conversely, subsurface hardness (Figure 4) tends to score high (low compaction levels). Root health indicators based on both 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 a 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 (64 bins per acre high, 38 bins per acre low; 1309 packs per acre high; 711 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 nearly a 50% reduction in gross yields and packed boxes per acre that was consistent across all cultivars (Figure 9). 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. The proportion of fruit that was culled due to external disorders and sunburn incidence was greater in sites with low productivity and poor health. These differences were consistent across cultivars.

Table 1. Crop load (fruit cm-2 TCSA), yield, cull proportion, and the number of packed boxes per acre for 16 paired orchards of Gala, Honeycrisp, and Granny Smith apple cultivars.

	Crop Load	**Yield**	**Cull proportion**	**Pack Out**
	Fruit cm^{-2} TCSA	Bins per acre	%	Boxes per acre
High Productivity	4.97	64.4	5.2	1310
Low Productivity	4.65	38.3	11.2	712

Figure 1. Organic Matter Indicators



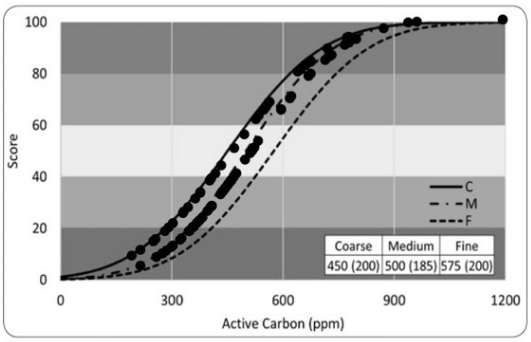
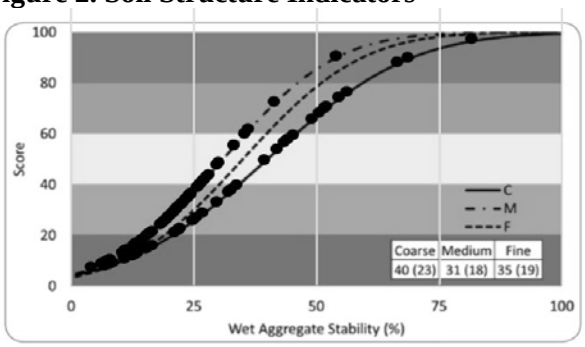


Figure 2. Soil Structure Indicators



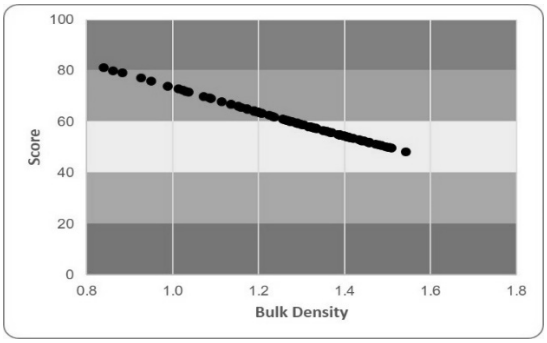
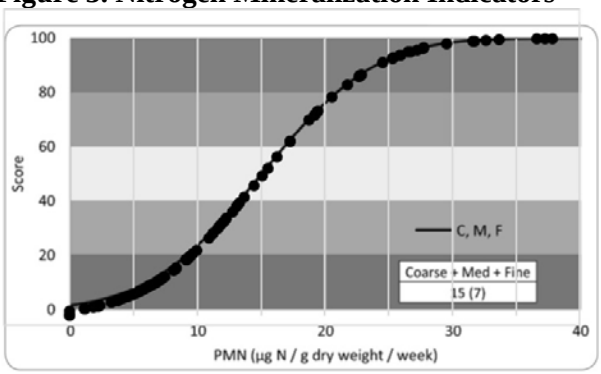


Figure 3. Nitrogen Mineralization Indicators



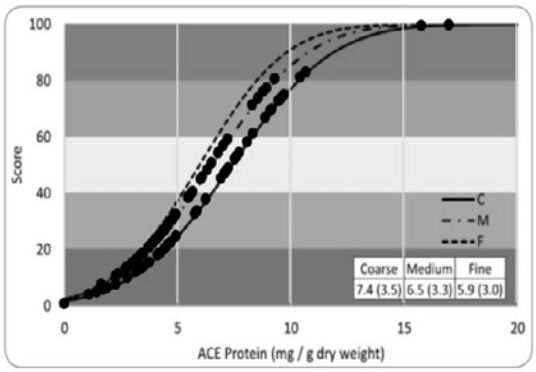
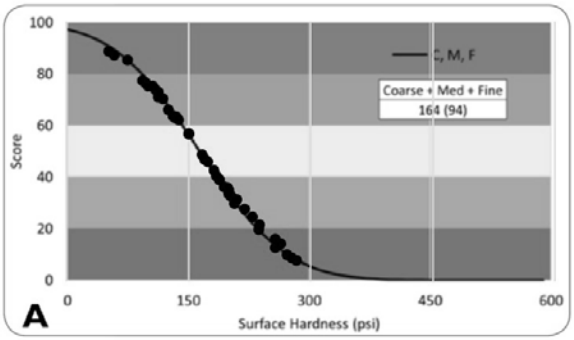


Figure 4. Soil Compaction Indicators



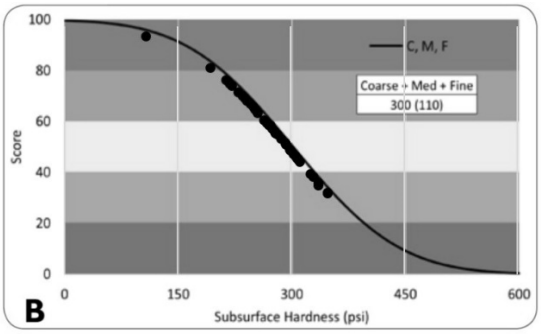
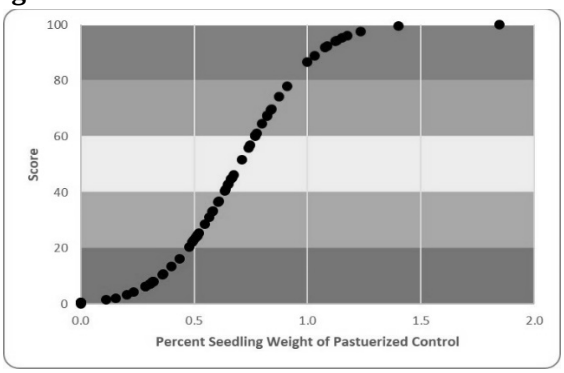


Figure 5. Root Health Indicators



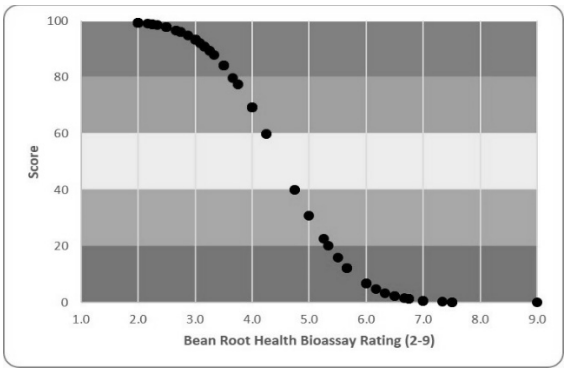
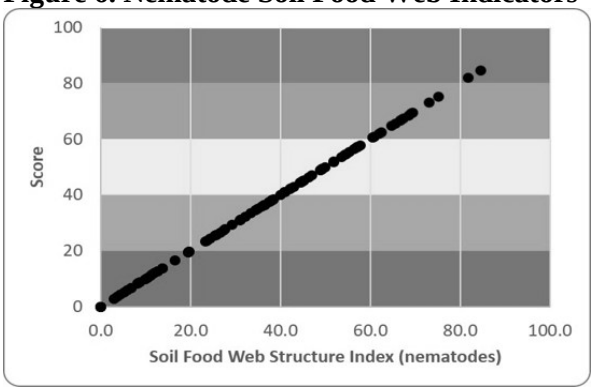


Figure 6. Nematode Soil Food Web Indicators



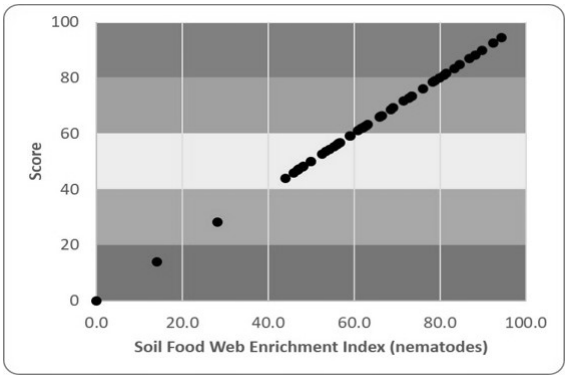
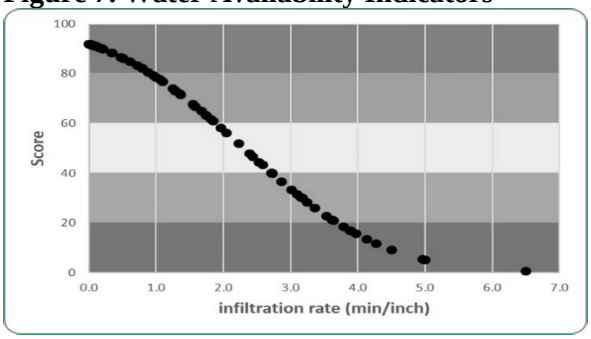


Figure 7. Water Availability Indicators



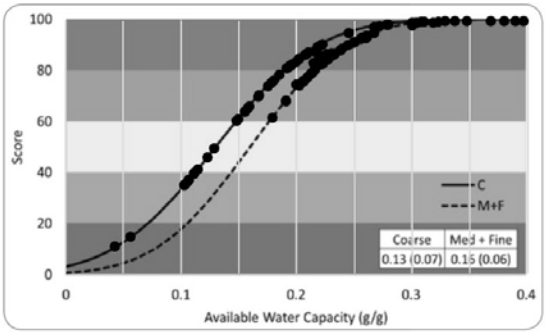
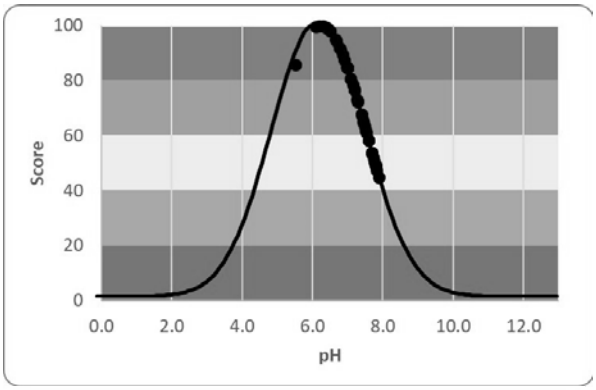
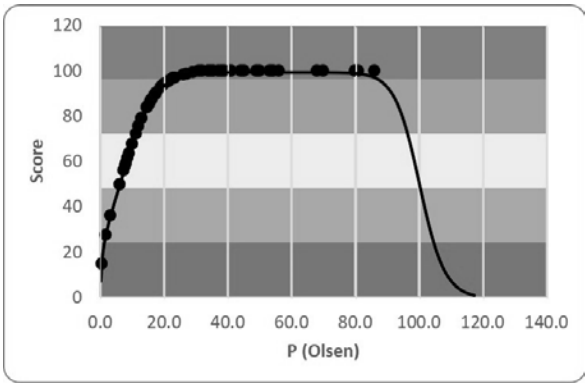


Figure 8. Nutrient Indicators





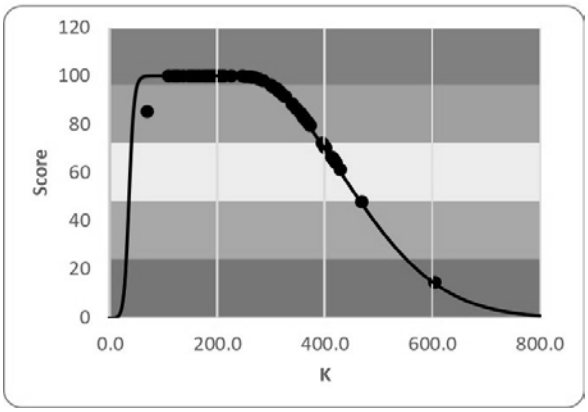


Figure 9. Estimated packed boxes per acre for Gala (N=8), Honeycrisp (N=5), and Granny Smith (N=3) paired orchards with either high or low productivity associated with soil-related problems (3 years).

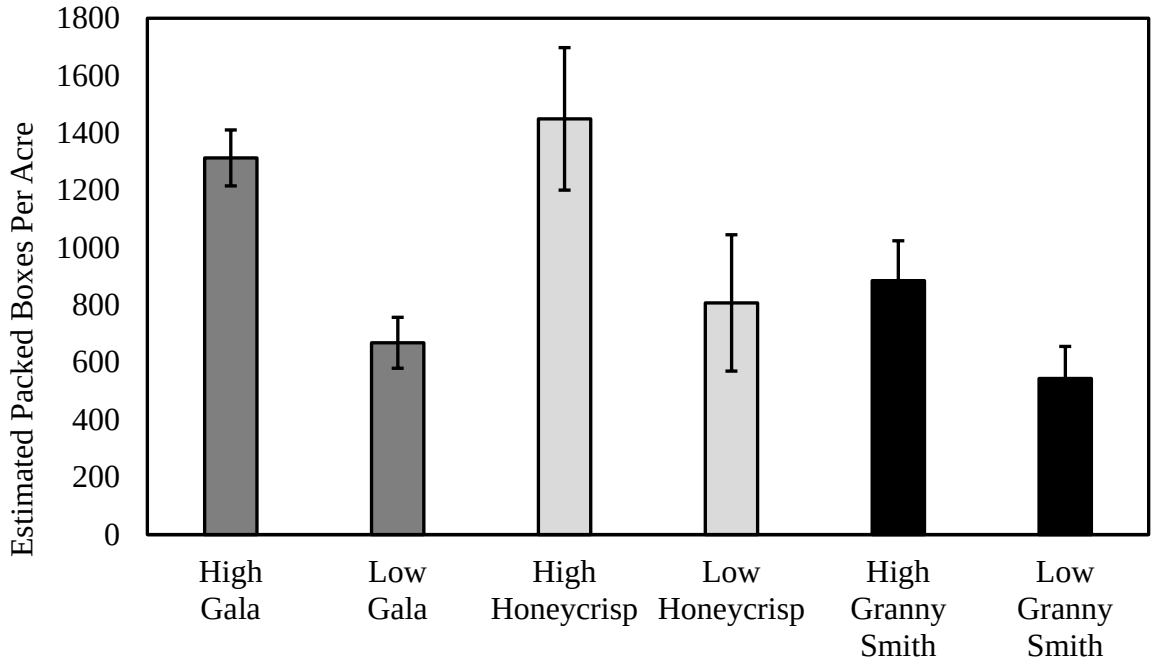
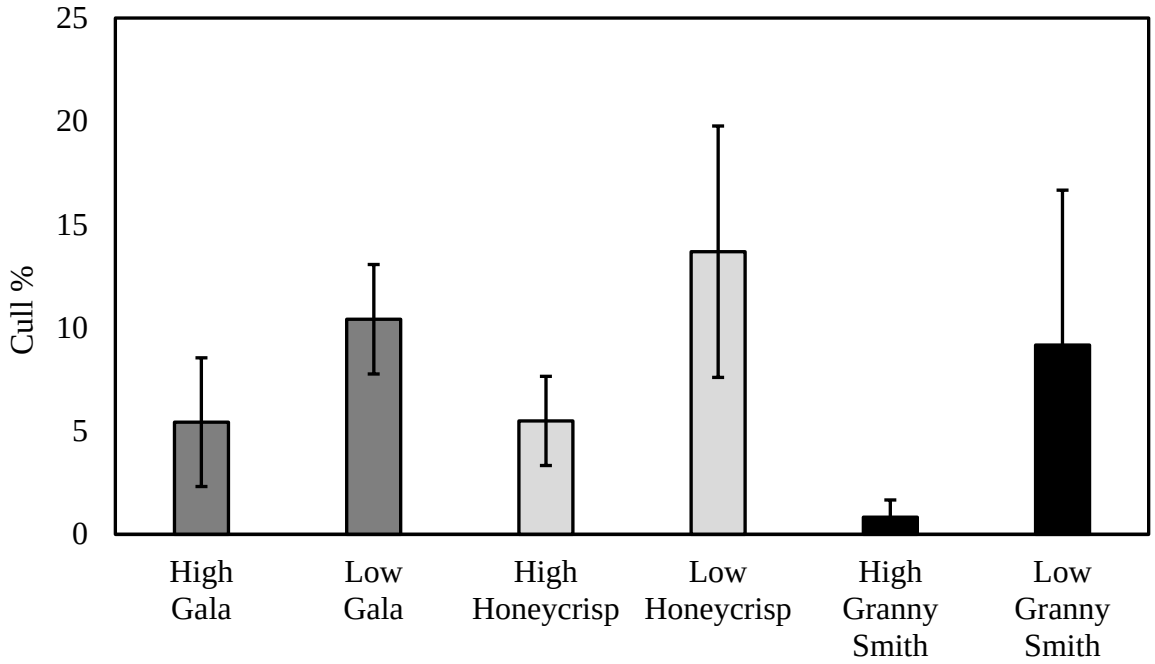


Figure 10. The proportion of fruit that was culled due to either external disorders (bitter pit, lenticel breakdown) or sunburn incidence for Gala (N=8), Honeycrisp (N=5), and Granny Smith (N=3) paired orchards with either high or low productivity associated with soil-related problems (3 years).



CONTINUING PROJECT REPORT **YEAR:** 1 of 3
WTFRC Project Number: AP-19-104

Project Title: Non-destructive detection of sun stress compromised apples

PI:	David Rudell	**Co-PI**:	Carolina Torres
Organization:	USDA-ARS, TFRL	**Organization**:	WSU/TFREC
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Email:	David.Rudell@usda.gov	**Email**:	ctorres@wsu.edu

Collaborators: Manoella Mendoza, Lorenzo León

Budget: **Year 1**: $88,947 **Year 2**: **$91,545** **Year 3**: $94,246

Other funding sources

Agency Name: USDA-ARS, In-house project
Amt. awarded/requested: $61,313/3 yrs.
Notes: In-house project with complimentary objectives. Funds for storage maintenance and costs ($8000/yr), supplies and materials ($3000/yr), travel ($5000/yr), and 0.1 FTE (co-PI).

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

Item	2019	2020	2021
Salaries (WSU post-doc)	47,500	49,400	51,376
Benefits (WSU)	17,447	18,145	18,870
Wages (ARS part time)	10,000	10,000	10,000
Benefits			
Equipment			
Supplies			
Travel (for Lorenzo León)	2500	2500	2500
Miscellaneous*	11,500	11,500	11,500
Plot Fees			
Total	88,947	**91,545**	94,246

Footnotes: One-third instrument service contract (TFRL, USDA-ARS)

Objectives:
1. Determine best non-destructive methods to segregate sun stress compromised fruit.
2. Validate accuracy of non-destructive method for detecting chemistries associated with solar stress.
3. Test if non-destructive sorting improves storage outcome for different sun stress related disorders.

Goals and Activities for the next year:
Repeat Year 1 sunscald prediction and sorting according to cumulative sun exposure. Additional experiments will focus on tissue dissection to confirm sun exposure assessment and sunscald prediction using hyperspectral imaging matches expected peel chemicals associated with spectral changes and cumulative sun exposure.

SIGNIFICANT FINDINGS:

At 4 months into the year 1 storage study, there are no significant findings to report.

METHODS

Sunscald prediction model development (Objective 1)
Granny Smith apples were harvested from a commercial block located in Mattawa, WA. Sun exposed fruit was selected from the periphery of the trees located at the beginning of the rows. Fruit were sorted into 3 categories according to sunburn severity: clean (no sunburn), mild and moderate yielding 324, 216 and 216 apples, respectively, for a total of 756. The sun exposed side of each fruit was marked with an indelible marker. Fuji apples was collected from Sunrise orchard in Rock Island, WA. The same harvest procedure was followed. A total of 273 Fuji apples were harvested and segregated in clean (144), mild (93) and moderate (36).

Cumulative sun exposure prediction (Objectives 1 and 2)
A bin of commercially harvested Granny Smith was also collected at the same day from a nearby block of the same orchard. Fruit were placed on pressed paper trays and numbered consecutively giving a total of numbered 1862 apples for repeated imaging. Harvest maturity (starch index, internal ethylene concentration, and D.A. index) and quality (color/defects, soluble solids, and titratable acidity) analysis were performed for both Granny Smith and Fuji.

Hyperspectral imaging
Apples were scanned monthly using a Nano-Hyperspec imager (400-1000 nm), tungsten light source, and scanning bed (Headwall Photonics, Bolton, MA) on all experiments. Composite hyperspectral images (data cubes) have been transmitted to Lorenzo Leon for Vis-NIR predictive model development based on multiple spectra. We will then test the model as a means to sort fruit according to cumulative sun exposure. This system will be compared and added to a model we expect to develop using targets in the ultraviolet (UV) bandwidth. We expect to have the UV-vis (Hyperspec UV-Vis, 250-500 nm, xenon light source) setup ready for method development by mid-February.

Objective 1: Determine best non-destructive methods to segregate sun stress compromised fruit.

We have linked specific natural peel chemicals with high sun exposure in the orchard that indicate elevated risk of sun-related postharvest disorders. Many of the changes may also provide potential

targets for non-destructive imaging devices which could be used to segregate fruit that received elevated light levels on this basis. Already, we have linked reflectance within the near ultraviolet and infrared spectral regions elevated light levels. Our prior results also indicate absorbed near UV light is ostensibly associated with quercetin glycosides, a class of compounds associated with high light while changes in absorbance of near IR absorbance remains unclear. We will use ultraviolet-visible hyperspectral imaging technology and NIR spectroscopy to determine spectral bands that best segregate fruit. **In years 1 and 2** of the project, we will image fruit from 2 different sun sensitive cultivars at harvest and then store them in air for up to 6 months and assessing fruit finish and appearance defects monthly. Hyperspectral images and spectra will be analyzed using targeted analysis based on expected associations with peel chemistry as well as interval spectral algorithms to find any additional spectral regions that could be used to segregate sun stress compromised apples. We will create predictive models from these results. Spectral interval analyses will be carried out as outlined by Grandon et al. (in press).

Objective 2: Validate accuracy of non-destructive method for detecting chemistries associated with solar stress.

In years 1- 3 of the project, peel from a subset of fruit will be peeled and analyzed (Rudell et al., 2009; Leisso et al., 2015) specifically to verify associations among peel chemicals associated with sun stress, peel condition, and hyperspectral images/ NIR reflectance spectra. Verification is required to indicate if links between chemicals with known associations with sun stress are the only basis for segregation using the non-destructive techniques. A full analysis, including cutin (Rudell, unpublished), may be necessary if the known markers do not account for spectral differences used to segregate fruit.

Objective 3: Test if non-destructive sorting improves storage outcome for different sun stress related disorders.

Once a method for non-destructive sorting is established, larger numbers of fruit from different orchards and stored in both air and CA will be sorted to determine how accurately and reliably sun stress fruit are segregated and if sorting fruit actually does improve storage outcome with respect to sun-stress associated postharvest disorders. **In years 2 and 3**, 300 apples from 4 Granny Smith (for sunscald) and Honeycrisp (for lenticel blotch) orchards will be sorted at harvest into 2 or more categories based on sun exposure using non-destructive sorting criteria established during years 1 and 2 of the project. Each category will be divided with half of the fruit stored for 6 months in air and the other half in CA (temperature and atmosphere depend upon cultivar) for 6 months plus up to 3 months in air. Fruit finish will be evaluated at 3 and 6 months for air stored fruit and monthly after 6 months CA. We expect to determine how effectively the non-destructive sorting system predicts incidence of defects related to sun stress.

RESULTS AND DISCUSSION

Sunscald was first noticeable on Granny Smith between 1 and 2 months of storage, primarily where moderate sun damage already existed at harvest. Hyperspectral images were taken of the front and back of every apple, and we are using these data to develop means to sort fruit according to sunscald risk and, also, cumulative sun exposure. The existing camera can detect 3 spectral regions that together have potential for sorting apples according to sun exposure. Two of these regions, representing anthocyanins (red color) and chlorophyll (green color) exist on the earlier spectrophotometric sunscald prediction model (Grandón et al., 2019; Fig. 1). The sun exposure pattern can clearly be seen when only looking at 677 nm (chlorophyll) as chlorophyll is reduced in sunburned peel and therefore the sunburn is lighter as less light is absorbed (Fig. 1). Anthocyanin

(550 nm) can be seen in spots in some of the sunburn, although this is not typical of Granny Smith. The spectrum of the carotenoids and chlorophylls overlap and chlorophyll absorption can interfere in

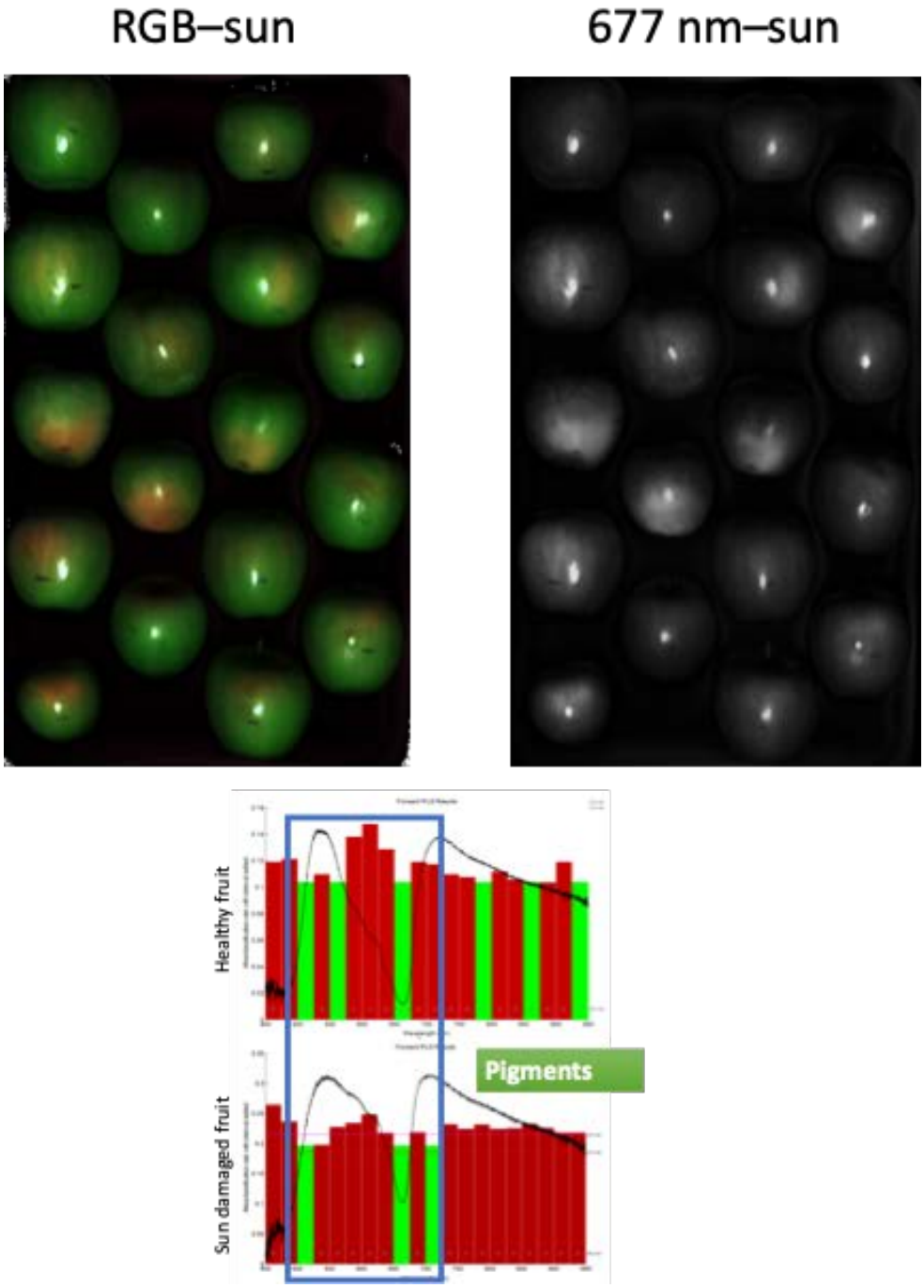

Fig. 1. Chlorphyll levels reveal sunburned areas and regions of high sun exposure on Granny Smith. Looking at only the band at 677 nm (Top right) or the region associated with chlorophyll absorption and sunscald risk by Grandón et al. (bottom), we eliminate interference from other wavelengths revealing lighter regions where light is not absorbed. Generally, the shaded side of the apple is darker in this band (more chlorophyll) than the light side (not shown). However, too little sunlight leads to less chlorophyll and a light patch on the shade side. This would lead to difficulty discriminating if only this band were considered.

these wavelengths (400-500 nm; Fig. 2). The key is to find compounds that accumulate with sun exposure and absorb in wavelengths with relatively little interference by other components. The flavonol glycosides have these properties (Fig. 3) and we have purchased and are setting up a special hyperspectral camera and light for this purpose.

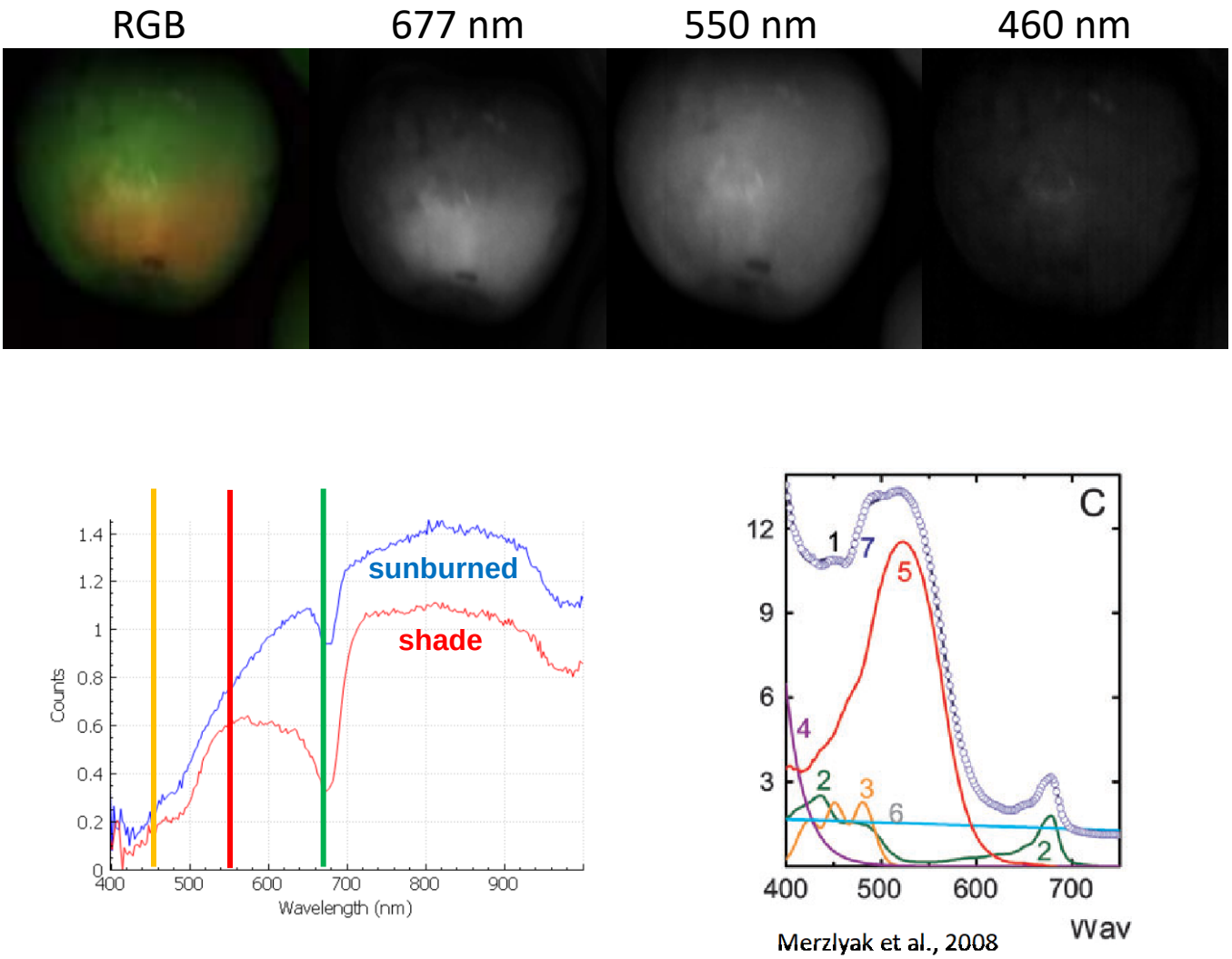

Fig. 2. Sun exposure assessment using wavelengths of interest in the UV-vis reflection spectrum can be interfered with by other pigments reducing the capacity to segregate according to cumulative sun exposure. The reflectance spectrum (bottom left) of sunburned (red line) and symptom-free peel (red line) reveal a prominent band at 677 nm where absorption (dip the line) indicates the presence of chlorophyll. The bottom right graph shows absorption curves of chlorophyll (green line), anthocyanin (red line), and carotenoids (orange lines). Whereas we can see sunburn at 677 nm (bottom right; green line, chlorphyll), we cannot at 550 nm (bottom right; red line, anthocyanins) or 460 nm (bottom right; orange line and green line, carotenoids and chlorophyll). We do not see a difference at 550 nm (lighter color, no absorption) because Granny Smith does not typically produce anthocyanins in this situation, especially early in the harvest season. In the 460 nm band, the increased levels of carotenoids with sun exposure counter the diminished chlorophyll levels leaving us with absorbance in both areas and a solid dark picture.

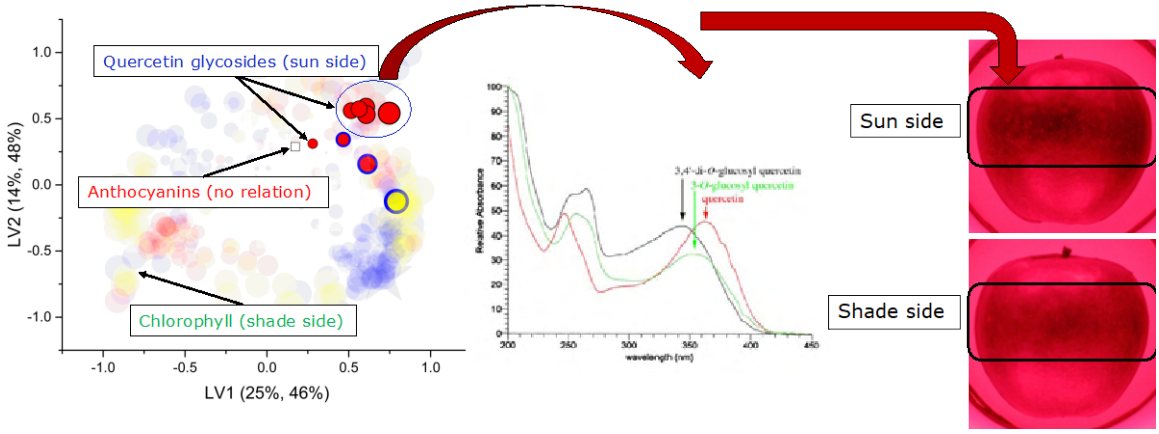

Fig. 3. The UV-vis camera and xenon lamp source will capitalize on a band in the near UV spectrum that can be used to detect a group of peel chemicals linked mostly with light exposure and light stress where we expect little interference from other peel chemicals. These are the quercetin glycosides as detected at higher levels in sun-facing and sunburned peel (far left) which adsorb light in the 350-400 nm range (middle). In our previous project, we were able to use a rudimentary camera/bandpass filter combination to image the darker sun-facing side (absorbing light in this band) compared to the shade side (far right).

Table 1. Proposed project milestones with anticipated products of "Non-destructive detection of sun stress compromised apples".

Objective		1: Determine best non-destructive methods to segregate sun stress compromised fruit		
Hypothesis		We can improve sun-related postharvest disorder non-destructive risk assessment by targeting specific metabolites associated with high sunlight exposure.		
Team	Months	Milestone	Anticipated Product	Progress/Changes
CT, DR	12	First year hyperspectral and spectrometric data to determine model for non-destructive detection of high sunlight exposure leading to sun-related disorders.		
	24	Finished models that non-destructively detect high sunlight exposure leading to sun-related disorders.	A non-destructive means for segregating fruit based on sunlight exposure.	
	36	No work planned		

Objective		2: Validate accuracy of non-destructive method for detecting chemistries associated with solar stress.		
Hypothesis		Risk assessment based on non-destructive means are supported by expected changes in levels of sun-stress related peel chemical targets.		
Team	Months	Milestone	Anticipated Product	Progress/Changes
DR,CT	12	Completed analysis of target compounds to compare with non-destructive data.	Verified mode of non-destructive sorting based on sunlight exposure.	
DR,CT	24	Completed analysis of target compounds to compare with non-destructive data.	Verified mode of non-destructive sorting based on sunlight exposure.	
DR,CT	36	Completed analysis of target compounds to compare with non-destructive data.	Verified mode of non-destructive sorting based on sunlight exposure.	

Objective		3: Test if non-destructive sorting improves storage outcome for different sun stress related disorders.		
Hypothesis		Non-destructive sorting based on hyperspectral imaging/spectroscopy will improve storage outcome with respect to sun-related postharvest disorders in the cold chain.		
Team	Months	Milestone	Anticipated Product	Progress/Changes
	12	No work planned		
CT, DR	24	Completed year 1 pilot test of non-destructive risk assessment for improving storage outcome.	Non-destructive sorting protocol that reduces sunscald and lenticel blotch in the cold chain.	
CT, DR	36	Completed year 1 pilot test of non-destructive risk assessment for improving storage outcome.	Non-destructive sorting protocol that reduces sunscald and lenticel blotch in the cold chain.	

CONTINUING PROJECT REPORT (off cycle) **YEAR:** No-Cost Extension (Funded on 05/07/19)

Project Title: Optimizing harvest time for WA38

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Co-PI: Carolyn Ross
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City/State/Zip: Pullman, WA 99164-6376

Cooperators: Ryan Sheick, Ines Hanrahan, Stemilt Growers (Quincy)

Other funding sources:
We will use an orchard realized with the support of the Washington State Department of Agriculture (WSDA) -Specialty Crop Block Grant Program. Cosmic Crisp: Training system and orchard management to optimize vigor control and quality. PI Stefano Musacchi. Total budget: $249,191. We can consider this project as matching funds for the new research.

Total project request (1 year): $95,419

WTFRC Budget:
Organization Name: WTFRC **Contract Administrator:** Kathy Coffey
Telephone: (509) 665-8271, ext. 2 **Email:** kathy@treefruitresearch.com

WFTRC	2019
Salaries[1] (include benefits)	$ 8,140
Wages[2]	$ 14,188
Benefits[3]	$ 7,565
Supplies	$ 1,500
Travel	$ 1,000
total	$ 32,393

[1]Salary and benefits for Manoella Mendoza and Tory Schmidt.
[2]Wages and [3]benefits for hourly employees.

Budget

Organization Name: Washington State Univ. Contract Administrator: Katy Roberts/Shelli Tompkins
Telephone: 509-335-2885/509-293-8803 Email address: arcgrants@wsu.edu/ shelli.tompkins@wsu.edu

Musacchi-Serra-Ross	**2019**
Salaries[1]	$ 28,800
Benefit[2]	$ 11,226
Plot fee[3]	$ 3,000
Sensory evaluation[4]	$ 9,000
Supplies[5]	$ 8,000
Travel[6]	$ 3,000
total	**$ 63,026**

Footnotes:

[1] Salary for 60% Research assistant ($4000/month) (Musacchi-Serra)

[2] Benefit on salary at 38.98%

[3] Plot fee for plots

[4] Sensory evaluation Ross lab.

[5] Labware/consumable, fruit sample reimbursement (Musacchi)

[6] 5,556 miles/year for domestic travel (0.54$/mile) to go to the orchard.

RECAP OBJECTIVES:

1. Determine optimum timing for WA38 harvest based on fruit production, pack out and quality.
2. Validate the new WA38 starch scale as tool to predict harvest time.
3. Assess consumers' acceptance of WA38 fruit harvested at different time (6 consecutively weekly picks[¥]).

[¥] *From the original project submitted and funded, the number of picks to study increased of the 50% from 4 to 6 picks for 6 weeks in a row. No budget modification was requested.*

SIGNIFICANT FINDINGS:

- Yield data in 2019 did not reveal differences in kg/tree between harvest dates, but significant differences in average fruit weights reporting 47 g heavier apples in Pick6 than in Pick1.
- The time of harvest had an impact on the pack-out: kg of cull fruit/tree increased significantly from Pick3 to Pick6 and consequently the amount of "good" fruit/tree decreased.
- With the latest pick, a significant increase of average fruit dropped per tree was noticed: about 1.7 Mton/Acre (4.4 bins/A) production could be lost if the harvest date is delayed to third week of October (Pick6), while at Pick1 the lost yield was only 0.5 Mton/A (1.3 bins/A).
- Pick3 and 4 showed a similar fruit size distribution; starting from Pick3 there was only a 16% of apples in the sizes below or equal to 70 mm (=113 apples/box) with 80-72 apples/box (80 mm) being the most representative sizes.
- The delay in harvesting is clearly influencing the grading quality of fruit: early harvest date (Pick1) had the lowest percentage of cull fruit (8.2%) versus a 34% six weeks later (Pick6).
- More delayed was the harvest date, higher was the incidence of defects like bird peck and split (Pick6: 27% of cull for bird peck and 40% cull for split).
- Across 6 picks, no significant differences appeared relatively to the color; all apples had similar Red color, Red intensity and background color.
- Apple flavor showed higher incidence of starchy/unripe flavor in Pick1 apples than in all the other 5 picks, while from Pick2, 80% of the tasted apples showed a ripe/good flavor.
- Parameters correlating the most with starch index were firmness and I_{AD} both at T0 and T1 quality assessment.

METHODS

Objective 1) Determine optimum timing for WA38 harvest based on fruit production, pack out and quality.

- Approach/method- year 1 (2019)

WA38 apples internal quality varies 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. The easiest way to implement a maturity assessment industry-wide is through starch degradation assessment. The iodine test is widely utilized from grower to packer and it could be a feasible tool to adopt. The starting date for WA38 picking was established when starch index was close to 2.5 (scale 1.0 to 6.0 with 0.5 increments). The original idea was harvesting weekly for 3 more weeks after the first starting date (Pick 1). For each pick, WA38 apples was sampled for 4 different purposes, to understand the variation of internal fruit quality on the basis of the harvest dates. Fruit were collected for quality analysis at harvest and one month after harvest (exactly 30 days in cold storage RA after each of the picks) as well as at the beginning of December 1st , date when fruit started to be sold in the retail stores for the first time in WA38's history). The plan changed during summer and the number of picks increased from 4 to 6 to have the opportunity to really understand the changes in fruit maturity from very early harvest (starch 1.5 out of 6.0 in the scale) to a late harvest, +6 weeks after that corresponding to third week of October.

Within WA38 P3 block in Quincy (trees planted in 2008 and grafted on M9337, 12 ft x 3 ft, 1210 trees/Acre and 1360ft of elevation), in August 2019, we selected 48 trees for this trial. Trees had similar TCSA (avr. 43.5 cm^2) with medium-high number of fruit per tree (range from 93 to 175 apples per tree) equal to crop load between 2.0 and 4.7 fruit/TCSA cm^2. For each of the 6 harvests (picks) we randomly choose 8 trees available as reps. To ensure each pick has the most homogenous possible selection of trees, we ranked them by ascending crop load and evenly distribute trees across the 6 picks in the way the average crop load in each pick was similar. Selected trees were labeled and color-coded in the field, each pick was accomplished when the 8 trees (reps) were harvested and brought back in to the lab for sorting purpose. Each pick day we sampled fruit for all the 3 project objectives.
The first harvest was planned at an average of starch index ≈ 1.5, that date set the beginning of the entire experiment. Here the dates of harvest in blk P3 and the corresponding average of starch index based on the WA38 fruit of T0 quality run immediately after harvest.
At harvest, each tree was independently picked and all apples labeled and boxed. Data collection was repeated in the same way for the 6 picks.
The following parameters were collected:

- Yield (kg/tree as net weight)
- Number of total fruit harvested from the tree
- Number of dropped fruit and their weight
- Size of all fruit/tree in mm (Musacchi's sizer 65 to 110 mm)
- Good (extra fancy and fancy) vs cull fruit (+ reason to cull) count (pack out by pick).

pick	dates	Starch index (1-6)
PICK1	09.17.2019	1.4
PICK2	09.24.2019	2.2
PICK3	10.01.2019	3.3
PICK4	10.08.2019	3.3
PICK5	10.15.2019	3.4
PICK6	10.22.2019	4.9

Here below a specific description for each of the fruit category utilized for sorting based on the newly released PVM grading criteria for bicolor apple (following WAC) with some more detailed specification of the sorting criteria utilized in the experiment.
Extra Fancy (XF) = apples > 65mm, with more than 50% red color, without green spot, bitter pit, sunburn, split, cracking, depression/sunken area, decay, limb rub, bruise, mechanical injury, misshapen/not round, bird peck, insect damage, russet or wandering sepal.
Fancy (F) = apples > 65mm, with 30% and greater red color, without bitter pit, split, cracking, decay, limb rub, mechanical injury, bird peck, insect damage (broken skin) and russet. Apples with leaf shade can be considered Fancy if the apple has at least 30% of red color. Green spot 1 or 2 and Sunburn categories 0 or 1 are allowed. Bruise, depression/sunken area smaller than a dime can also be considered Fancy. Limb rub can be considered fancy if there is no broken skin.
Culls = apples ≤ 65mm, less than 30% red color, with any of the following disorders: bitter pit, sunburn categories 2 or 3, split, cracking, decay, mechanical injury, bird peck or insect damage. Bruise, depression/sunken area bigger than a dime are considered cull.

Based on the most represented fruit size at Pick1 we sampled apples in a narrower range (70 to 80 for each date in 2019 harvest).
Apples were sorted as follow:

- Quality at harvest (T0q): 10 apples per tree per pick= 10X8= 80 per pick
- Quality after 30 days RA for each pick (T1q-30dd): 10 apples per tree per pick= 10X8= 80 per pick
- Quality "industry selling date" 1 day at room temp (T2q-1dSL): 8 apples per tree per pick= 8X8= 64 per pick
- Quality "industry selling date" 7 days at room temp (T2q-7dSL): 8 apples per tree per pick= 8X8= 64 per pick
- Consumer test 1 day at room temp (T2CT-1dSL): 4 apples per tree per pick =32 apples
- Consumer test 7 day at room temp (T2CT-7dSL): 4 apples per tree per pick =32 apples
- Starch degradation (obj.2): 10 apples per tree per pick = 80 apples per pick (16 apples/month).

For each pullout, after 24 h of re-equilibration at room temperature (or 7 days of shelf life in case of T2q-2dSL) fruit quality parameters assessed were: Weight, DA index (I_{AD}), Greasiness (only at T2q-1d and 7dSL), External Coloring (avr red intensity 1-5, max red intensity), Red color coverage (%, categories 1 – 4), Background color, Firmness, Predicted nondestructive Dry Matter % by Felix F750, Internal Disorders (bitter pit, water core, etc.), Starch index, SSC (Brix), Titratable acidity, and Flavor score (1-3 scale).

Objective 2) Validate the new WA38 starch scale as tool to predict harvest time.

- Approach/method- year 1 (2019)

At each pick date, a set of fruit was assigned to the "starch degradation" assessment until March 2019. In this report, we are not presenting any data about this because we need to have the entire destruction completed by March 2020.

Objective 3) Assess consumers' acceptance of WA38 fruit harvested at different time (4 consecutive weekly picks).

- Approach/method- year 1 (2019)

Fruit from the samples previously described were provided to Dr. Ross's lab at the WSU Sensory Evaluation Facility end of November 2019. Fruit from regular cold storage were brought up to room temperature 24 hours before analysis. Apples were evaluated by consumers (80-120) using an acceptance test with several attributes to be evaluated by a 1 to 9 hedonic scale on December 3rd and December 10th for shelf-life 1 day and shelf-life 7 days respectively. Results relative to the consumer preference will be presented in the final report.

RESULTS & DISCUSSION:

As expected yield data in 2019 did not reveal differences in kg/tree between picking times (trees have the same crop load level), but significant differences in average fruit weights of apples harvested at Pick6 and Pick1; with 47 grams more per fruit on average with the latest pick (Table 1). The time of harvest had an impact on the pack out of the fruit: kg of cull fruit increased significantly from Pick3 to Pick6 and consequently the amount of "good" fruit/tree decreased (presented as % in Table 1). I_{AD} index measured at harvest showed a consistent decrease with the delay of harvest and a significant drop from Pick3 to Pick6. With the latest pick, a significant increase of average fruit dropped per tree was noticed (from 1 apple/tree to 7apples/tree from Pick1 to Pick6; data not shown). Based on that, about 1.7 Mton/Acre (4.4 bins/A) production could be lost if the harvest date is delayed to third week of October (Pick6), while at Pick1 the lost yield was only 0.5 Mton/A (1.3 bins/A; data not shown).

Table 1: WA38 harvest data for 2019 in block P3 Quincy by picking dates from September 17th to October 22nd.

PICK	date	N=	August 2019 TCSA (cm^2)	Crop load (n frt/TCSA)	tot num frt/tree	yield 2019 (kg/tree)	avr apple weight (g)		%cull from n frt		%good from n frt		I_{AD} (10 apples/tree)		bins/A (1 bin=880lb)	Mton/A
1	9.17.19	8	45.1	3.2	139	28.6	206	b	8.2	c	91.8	a	0.73	a	86.8	34.7
2	9.24.19	8	44.2	2.9	125	27.0	219	ab	12.5	c	87.5	a	0.62	ab	81.9	32.7
3	10.1.19	8	43.7	3.1	134	30.3	226	ab	11.7	c	88.3	a	0.48	bc	91.7	36.6
4	10.8.19	8	43.2	3.4	145	32.6	225	ab	16.5	bc	83.5	ab	0.40	cd	98.8	39.5
5	10.15.19	8	42.6	3.1	129	30.9	239	a	23.9	ab	76.1	bc	0.31	d	93.8	37.4
6	10.22.19	8	42.3	2.9	120	28.5	243	a	33.4	a	66.6	c	0.28	d	86.4	34.5
	Significance		*NS*	*NS*	*NS*	*NS*	**		***		***		***		*NS*	*NS*

The size of WA38 fruit improved significantly more delayed was the pick (Figure 1); indeed, Pick6 had 21.8% more fruit belonging to the size 64 apples/box (=85 mm) than Pick1 (with only 4.7%). Pick3 and 4 showed a similar fruit size distribution; starting from Pick3 there was only a 16% of apples in the

sizes below or equal to 70 mm (=113 apples/box) with 80-72 apples/box (80 mm) being the most representative sizes (Figure 1).

The fruit grading carried out at each harvest showed significant differences in term of pack out (Figure 2). Pick1 had the lowest percentage of cull fruit (8.2%) versus a 34% six weeks later (Pick6); from Pick4 the amount of cull apples increased to 16.5% statistically similar to Pick5 with 24%. The proportion of extra fancy (XF) apples was the highest at Pick1 with 73.8%, while at Pick6 they were representing only the 39.3% of the harvested fruit. The delay in harvesting is clearly influencing the grading quality of fruit (Figure 2). Among all the possible reasons to cull the fruit, we observed that later was the harvest date higher was the incidence of defects like bird peck and split (data not shown). Of all the culled apples at Pick1, none was discarded for bird peck, but on the contrary, at Pick6, the 27% of the apples were culled for bird damage (data not shown). Similarly, split was reason to cull apples for the 4% at Pick1 while 6 weeks later the proportion got tenfold (40% culled for split, mainly stem split); after Pick4 in fact the incidence of split reached worrisome levels (data not shown). Other reasons to cull were significantly different across the picks, but for some of them there was not a clear relationship with maturity of the fruit on tree (i.e. mechanical damage, russet, insect, misshapenness and limb rub).

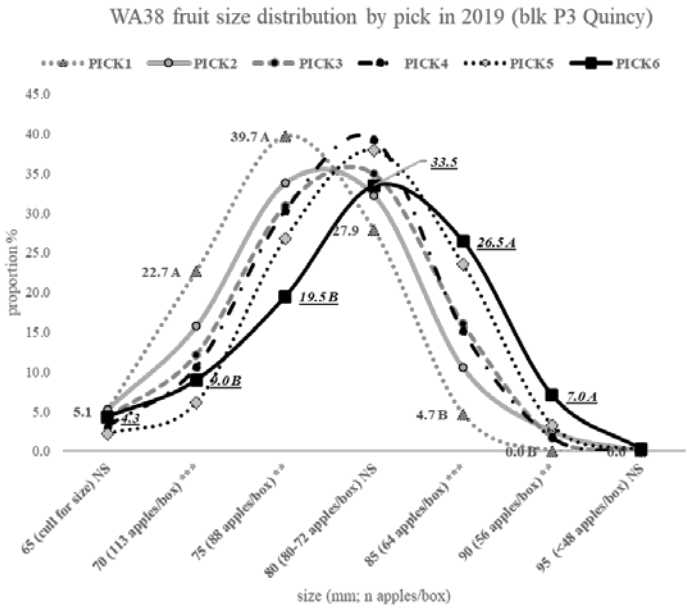

Figure 2: WA38 fruit size distribution by pick in 2019.

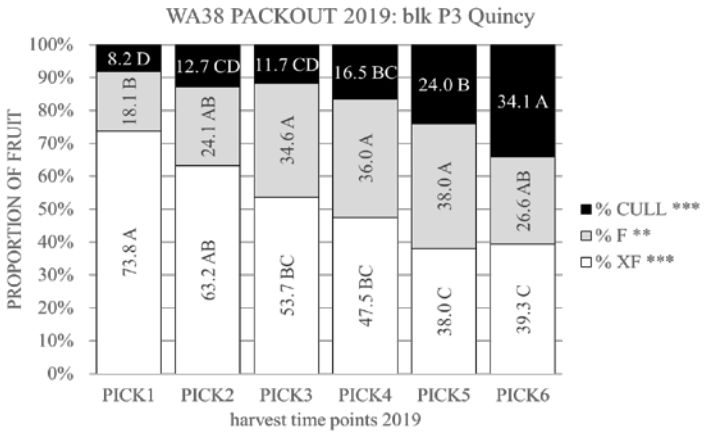

Figure 1: WA38 fruit grading 2019 by pick: XF= extra fancy apples, F=fancy, CULL=cull.

As a criterion of grading, all apples equal or below 65 mm size were assigned to "cull" and based on that Pick1 had the 53.5% of the culled apples discarded due to small size, while the proportion of those "cull for size" decreased significantly to 9-13% for Pick5 and 6. In this specific orchard, the color was always at the highest level (50 to 100% red colored surface) since Pick1 to Pick6, ranging from 94% to 100% (data not shown). Green spot did not affect significantly this production reaching a maximum of only 4.4% at Pick2, while all the other harvest dates were affected at lower incidence (data not shown). Among the types of green spot, the most frequent in this orchard in 2019 were green spot 1 and 6 that are the least detrimental types for this apple defect (data not shown).

Instrumental fruit quality assessment at each time of picking (T0=24h after harvest) revealed differences in apple physiology/quality related to delay in harvest (Table 2). Starch index increased significantly from Pick1 to Pick6 (1.4 to 4.9 out of a 1-6 scale, respectively) showing starch degradation of 0.8-0.9/week for the first 2 picks then from Pick 3 to Pick5 the index did not drop probably due to the important decrease in temperature registered in October in the area. Pick3 registered an average

starch index around 3.3 (across 80 apples), value already higher than the recommended 2.5, while Pick2 was 2.2 so closer to the recommended values. At T0, no significant differences appeared relative to the color, all apples across 6 picks had similar Red color, Red intensity and background color; while I_{AD} (DA index) showed a gradual decrease from Pick1 to Pick6 (0.73-0.28) indicating the advancing of maturity longer the apples are kept on trees (Table 2). A significant drop of the index was noticed between Pick2 and Pick3 reflecting also the decrease of firmness (from 19.4 lb to 17.9 lb, Table 2).

Table 2: WA38 quality at harvest 2019 (T0) by picking dates from September 17th to October 22nd.

pick	time	rep =trees (10apples /tree)	Starch index (1-6)	Avr frt weight (grams)	Red color (1-4)	Backgr. color (0.5-6.0)	Red intensity (1-5)	I_{AD} (DA index)	Firmness (lb.)	Soluble solids (SSC %Brix)	TA (% malic a.)
PICK1	T0	8 (10)	1.4 D	210 B	3.9	5.8	4.6	0.73 A	20.0 A	11.1 C	0.80 A
PICK2	T0	8 (10)	2.2 C	215 AB	3.8	5.5	4.2	0.65 A	19.4 A	11.2 C	0.56 C
PICK3	T0	8 (10)	3.3 B	228 AB	4.0	5.7	4.5	0.48 B	17.9 B	11.7 BC	0.52 C
PICK4	T0	8 (10)	3.3 B	224 AB	4.0	5.8	4.7	0.40 BC	16.8 C	11.6 BC	0.58 C
PICK5	T0	8 (10)	3.4 B	245 A	4.0	5.8	4.8	0.31 C	16.9 C	12.2 A	0.55 C
PICK6	T0	8 (10)	4.9 A	240 AB	4.0	5.7	4.6	0.28 C	16.7 C	12.1 AB	0.72 B
Significance			***	*	*NS*	*NS*	*NS*	***	***	***	***

SSC instead increased significantly only at Pick4 reaching 12.2 ° Brix, while at the same time titratable acidity (TA) showed similar values from Pick 2 to Pick5 (0.56 to 0.55%) with a spike at Pick6 difficult to explain.

Apple flavor was assessed at T0 and results revealed a higher incidence of starchy/unripe flavor in Pick1 apples significantly higher than in all the other 5 picks, while starting from Pick2, 80% of the tasted apples showed a ripe/good flavor. Only at Pick6 there was a small percentage of apples tasting bland/no flavor (2.5%, data not shown).

Same instrumental quality analyses were done exactly 30 days after each of the 6 harvest dates to see how the quality changed after one moth of regular air storage at 34°F. For Pick1, firmness decreased significantly of about 0.88 lb from T0 to T1 but no other significant differences were seen in the comparisons between T0 and T1 within each picking dates (data not shown). At T1 (+30d), starch index was already mostly degraded above 5 from Pick4 to Pick6. SSC showed an increase between T0 and T1 only at Pick1 and Pick4, while titratable acidity reported higher values at T1 (+30d) than at T0 for Pick 1, 3, 4 and 5.

Flavor rafter 30 days showed that apples from Pick1 decreased the proportion of starchy/unripe apples from 92.5% to only 32.5%, while some bland/no flavor apples appeared in Pick4 and 5 (but not statistically different across the 6 picks) and from Pick 4 to Pick6, at least the 88% of tasted apples were in the good/ripe flavor range (Table 3).

Pearson correlations were run across T0 and T1 in order to establish relations between quality parameters. At T0, the parameters correlating the most with starch index were firmness (r= -0.783) and I_{AD} (-0.673); the correlation coefficients with starch improved at T1 with firmness, r=-0.855, and I_{AD} r=-0.771.

Table 3: Flavor assessment on WA38 from P3 orchard Quincy by pick and comparison between T0 and T1 (at harvest vs after 30d of storage) in 2019.

pick	time	rep =trees (10apples/ tree)	Flavor 1 (unripe-sour/starchy) %	Flavor 2 (ripe/apple flavor) %	Flavor 3 (bland/no flavor) %
PICK1	T0	8 (5)	93 A	8 B	0
PICK1	T1-30d	8 (5)	33 B	68 A	0
Significance T0-T1			***	***	
PICK2	T0	8 (5)	20	80	0
PICK2	T1-30d	8 (5)	20	78	3
Significance T0-T1			**NS**	**NS**	**NS**
PICK3	T0	8 (5)	20 B	80 A	0
PICK3	T1-30d	8 (5)	58 A	43 B	0
Significance T0-T1			**	**	
PICK4	T0	8 (5)	38 A	63 B	0
PICK4	T1-30d	8 (5)	3 B	93 A	5
Significance T0-T1			**	*	**NS**
PICK5	T0	8 (5)	13	88	0
PICK5	T1-30d	8 (5)	5	88	8
Significance T0-T1			**NS**	**NS**	**NS**
PICK6	T0	8 (5)	10	88	3
PICK6	T1-30d	8 (5)	0	100	0
Significance T0-T1			**NS**	**NS**	**NS**

In general, non-significant correlations were reported between starch index and titratable acidity both at T0 and T1. DM% predicted by Felix F750 at harvest showed that the DM did not significantly changed keeping the fruit on trees for 6 weeks longer, values are ranging on average from 13.9% to 14.4% across the 6 picks (NS) and not significant differences after 30d of storage emerged.

All the remaining results regarding Objective 2 and 3 will be presented in the final report in 2020.

CONTINUING PROJECT REPORT Year: 1 of 2

Project Title: Pollination, flower biology and fruit development in 'WA38' apples

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Collaborators: Stefan Roeder, Ryan Sheick

Total Project Request: Year 1: $67,156 **Year 2: $69,262**

Matching funds/in-kind support:

- TASC (2014-2019): "Identifying and Managing Sources of Quarantine Significant Post-Harvest Diseases in Pacific Northwest Apple and Pear Orchards" total funds $ 1,913,832 provided several of the pollinizers we will use and contributed to develop expertise in flower biology.
- Project #AP14-103A: "WA38 rootstocks and training systems" (2014-2016+1yr NCE[¥]) total funds $ 242,519 provided the support to maintain the orchard for this project.

Budget: WSU

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Serra-Musacchi		
Costs	2019	**2020**
Salaries [1]	$ 36,000	$ 37,440
Benefit [2]	$ 14,033	$ 14,594
Wages [3]	$ 2,400	$ 2,496
Benefit [4]	$ 223	$ 232
Supplies [5]	$ 10,000	$ 10,000
Plot fee [6]	$ 1,500	$ 1,500
Travel [7]	$ 3,000	$ 3,000
Total	$ 67,156	**$ 69,262**

Footnotes:

[1] Salary for a 75% Research assistant/Research intern ($4,000/month)
[2] Benefit on salary at 38.98%
[3] One non-Student temporary for 4 wks: 40hrs/wk at $15/hr
[4] Benefits on temporary wage
[5] Labware/consumable, includes $ 1,200 membership for Franceschi Microscopy & Imaging Center (Pullman, WA) and $ 600 for electronic fruit sizer.
[6] Plot fee for the orchard
[7] 5,556 miles/year for domestic travel (0.54$/mile) to travel to orchard and to Pullman for microscopy work

¥: NCE= no cost extension

Recap objectives:

1. Assess the effective pollination period for 'WA38' and identify limiting factors (2019).
2. Evaluate pollen tube growth of different crabapples in 'WA38' flowers (2019-2020).
3. Analyze seed set, fruit drop, and fruit growth potential based on pollen source (2020).

Significant findings:

- Effective pollination period for 'WA38' apples was at least two days in 2019:
  - Stigmatic receptivity: 9 days
  - Pollen tube growth from stigma to ovule: 7 days
  - Ovule longevity: at least 9 days (preliminary results)
- Pollen tubes of 'Snowdrift', 'Granny Smith', and 'Evereste' grew faster inside 'WA38' styles than 'Frettingham', and 'Indian Summer'.

Methods

Plant material: The effective pollination period and pollen tube growth of different pollinizers were evaluated using seven-year-old 'WA38' trees on M9-Nic29 (V system) and six-year-old 'WA38' trees on M9-Nic29 (biaxis) respectively in spring 2019. All experiments were performed at the WSU Sunrise Research Orchard (Rock Island, WA). Pollen from all pollinizers were harvested from a previously established collection at the same location. Flowers were collected at a late balloon stage (unopened) to avoid cross-contamination with other pollen sources. Anthers were separated from the flower, dried for 24-48 hours at room temperature, and stored at 37.5 °F if not used immediately. An *in vitro* pollen germination test was performed to check the germinability of all pollen samples prior to use.

Objective 1: Assess the effective pollination period for 'WA38' and identify limiting factors

Stigmatic receptivity

Eighty 'WA38' king flowers on spurs at pink balloon stage (04/24/2019) were tagged, singled out (removed 5 laterals), emasculated, and isolated using Kleenguard™ A20 protective sleeves. Eight flowers were pollinated daily with Granny Smith pollen over a period of ten days at the same time each morning (Figure 1). Pollinated flowers were harvested 24-hours after pollination, fixed for 24 hours in a Formalin–acetic acid–alcohol (FAA) solution, and stored in 70 % ethanol at 37.5 °F until further microscopy analysis. Then, flowers were washed three times with distilled water, softened overnight in 8 M sodium hydroxide (NaOH), and cleared for 24-hours in 5 % potassium hydroxide (KOH) before transferring the samples to a 0.1 % (w/v) aniline blue solution. Styles were transferred to a microscope slide and the stigmas were evaluated for pollen adhesion and pollen germination (Figure 2A) using confocal laser scanning microscopy (Leica SP-5). This task was carried out at the Franceschi Microscopy and Imaging Center in Pullman, WA (WSU campus). Results were expressed as percent of stigmas with adhered or germinated pollen grains (Figure 2B).

Figure 3: 'WA38' flowers in 2019: emasculation of anthers, hand pollination with Granny Smith pollen and a brush, fixation of flowers for EPP study at the

Pollen tube growth

Eighty 'WA38' king flowers on spurs at pink balloon stage (04/24/2019) were tagged, singled out (removed 5 laterals), emasculated, cross-pollinated with 'Granny Smith' pollen by hand and isolated using Kleenguard™ A20 protective sleeves (Figure 1). Eight flowers were harvested at 24-hour intervals for ten days to track the pollen tube growth inside the styles. Samples were fixed in FAA for 24 hours and then transferred to 70 % ethanol for long-term storage at 37.5 °F. Prior to microscopy, samples were washed three times with distilled water, softened overnight in 8 M NaOH, and cleared in 5 % KOH for 48-hours before being transferred to a 0.1 % (w/v) aniline blue staining solution. Each flower was longitudinally cut in half with a scalpel and transferred to a microscope slide before being evaluated under an epi-fluorescence microscope at the WSU TFREC.

Ovule viability

Eighty 'WA38' king flowers on spurs at pink balloon stage (04/24/2019) were tagged, singled out (removed 5 laterals), emasculated, and were left unpollinated before covering with Kleenguard™ A20 protective sleeves to avoid uncontrolled pollination. Eight of the unpollinated flowers were sampled in 24-hour intervals over ten days. Sample fixation and storage methods were the same as described above for the pollen tube growth task. Samples were washed three times with distilled water, softened for six hours in 1 M NaOH, and cleared for five days in 1 M KOH. Ovules were extracted under a dissecting microscope, cleared for two days in 5 % KOH and stained in 0.1 Aniline blue for 24 hours. Accordingly, to literature, ovules that did not show any fluorescence were consider viable, while fluorescent ovules were classified as non-viable.

Objective 2: Evaluate pollen tube growth of different crabapples in 'WA38' flowers

Pollen tube growth

Thirty king flowers on spurs at pink balloon stage (04/24/2019) were tagged, emasculated, and cross-pollinated with one of the five tested pollinizers ('Evereste', 'Frettingham', 'Granny Smith', 'Indian Summer', 'Snowdrift'), for a total of 150 flowers. After hand-pollination, flowers were isolated with Kleenguard™ A20 protective sleeves. Five flowers were harvested in 24 h intervals for six days. Flowers were fixed and prepared following the protocol described above (see Objective 1, stigmatic receptivity for details). A razor blade was used to separate the style from the ovary. A cut was made directly at the base of the style. Afterwards, the styles were transferred to a microscope slide and the length of the longest pollen tube was measured using confocal laser scanning microscopy (Leica SP-5). This task was carried out at the Franceschi Microscopy and Imaging Center in Pullman, WA (WSU campus).

The five pollen sources used for this objective in 2019 corresponded to pollinizers with overlapping bloom windows with 'WA38'. 'Mt Blanc', which was previously listed as a pollen source to be tested in the original project proposal, was not available in 2019 because of alternate bearing and delay in phonological stage. For this reason, we used 'Frettingham' even if only 50% compatible with 'WA38' (S24 allele in common). In 2020, we aim to include 'Mt Blanc' as a pollinizer in this evaluation.

Objective 3: Analyze seed set, fruit drop, and fruit growth potential based on pollen source

This set of experiments designed to investigate Objective 3 will be carried out in 2020 accordingly to the original project proposal. However, in 2019, we established the protocols for each of the subtasks: A) stigma clipping and pollination intensity and seed set analysis, B) tracking natural shedding inside clusters from pre-bloom to harvest and C) fruit development with or without king flower in the cluster.

Results and Discussion

Objective 1: Effective pollination period

Stigmatic receptivity

The effective pollination period is defined as the time period in which a pollination event with compatible pollen can result in ovule fertilization (Sanzol and Herrero, 2001, *Scientia Horticulturae* 90(1):1-17). This period can be limited by the stigmatic receptivity, pollen tube growth, and ovule longevity. We observed a significant decrease in pollen germination on the stigmatic surface area on day 4; this is probably due to the use of less viable 'Granny Smith' pollen on this specific day, since the ability of the stigmas to support pollen adhesion was not affected (80% and not statistically different from Day 1 to Day 3. The stigmas almost lost the ability to support pollen adhesion (only 8% stigmas with adhered pollen) and pollen germination on day 10 (0% stigma with germinating pollen). Thus, in our 2019 study, stigmas of 'WA38' were considered receptive for nine days (Figure 2 A and B).

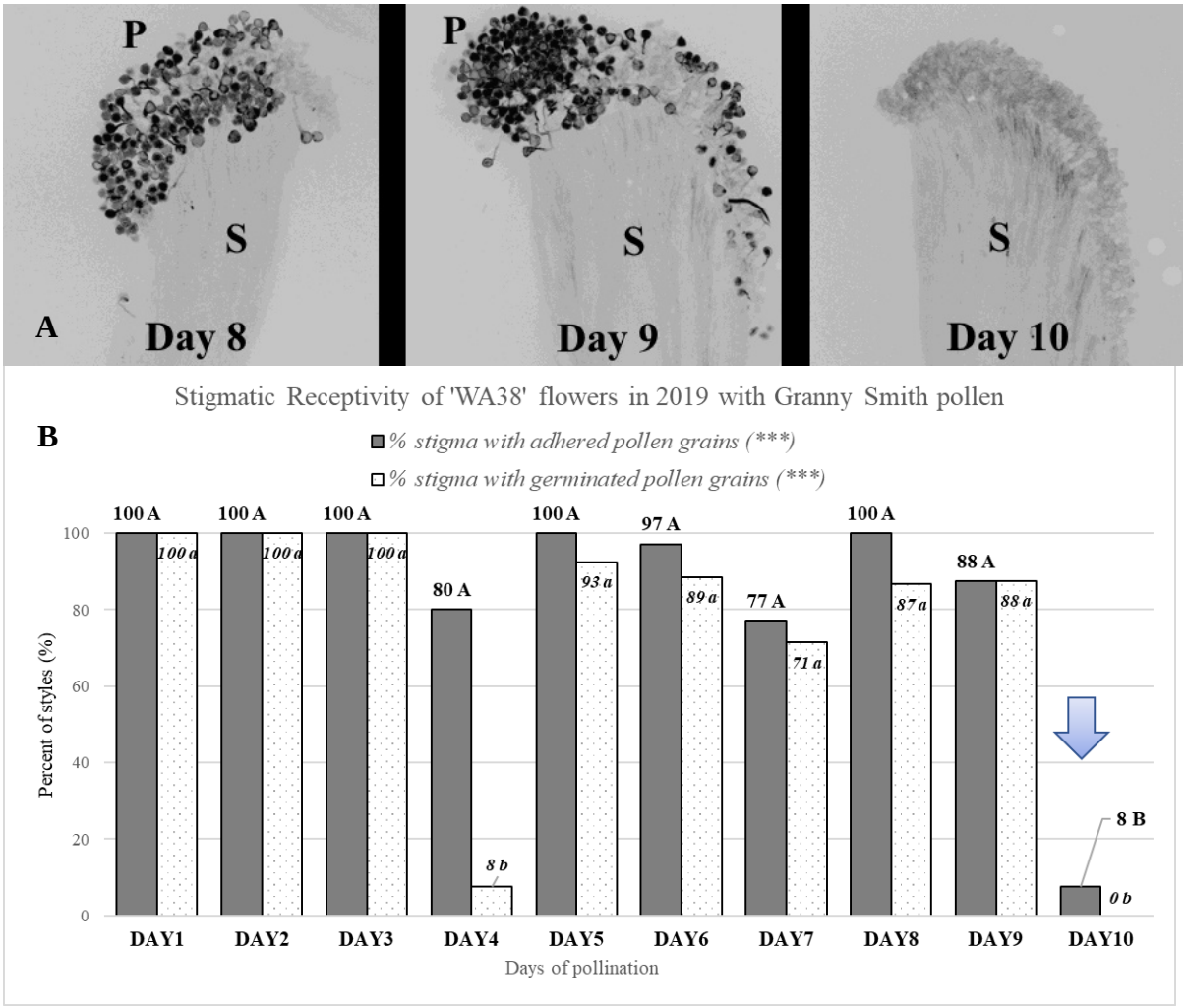

Figure 4: Stigmatic receptivity of 'WA38' flowers. Flowers were cross-pollinated daily for up to ten days with 'Granny Smith' pollen. Flowers were harvested 24-hours after pollination and analyzed for adhered and germinated pollen grains (expressed in % of stigma). A) WA38 stigmas (=S) at day 8, day 9 and day 10 with adhered and germinated pollen grains (P) in Day 8 and Day 9, no pollen in Day 10 (image was converted to a grayscale image for b/w printing); B) Proportion of stigmas with adhered and with germinated pollen for each of the time point (from Day 1 to Day 10).

Pollen Tube growth

'Granny Smith' pollen reached the ovules seven days after pollination (Figure 3). There was no variation between samples; however, pollen tubes from different pollinizers could potentially reach the ovules earlier or later (see Objective 2: Pollen tube growth of five different pollinizers for details). Because environmental factors also influence pollen tube growth, different locations could experience different results when it comes to pollen tube growth rates in 'WA38' pistils. In general, higher temperatures increase pollen tube growth rate.

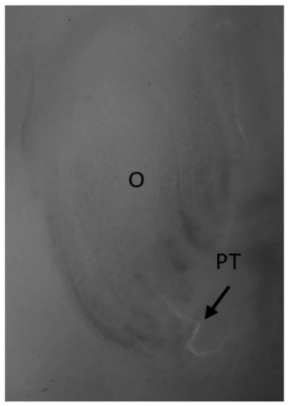

Figure 5: Example image of a pollen tube reaching an ovule seven days after pollination. Brightfield combined with fluorescence image. PT = Granny Smith pollen tube, O = 'WA38' ovule. Image was converted to a grayscale image for b/w printing. Light tissue on the right images represents callose-stained structures (pollen tube).

Ovule Viability (preliminary results)

We were able to analyze half of the samples collected for ovule longevity assessment in time for preparation of this report. Therefore, the results for the ovule longevity must be considered ***preliminary*** until we finish the analysis of the remaining samples. Ovules were classified either as viable (Figure 4 A) or senescent (Figure 4 B) based on absence/presence of the fluorescence signal. So far, the first fluorescent 'WA38' ovules were observed on Day 9 (out of 10 days of samples available). However, there was some variation between samples. Not all ovules from Day 9 and 10 showed fluorescence, which suggests there is some variability between flowers in the timing of ovule senescence.

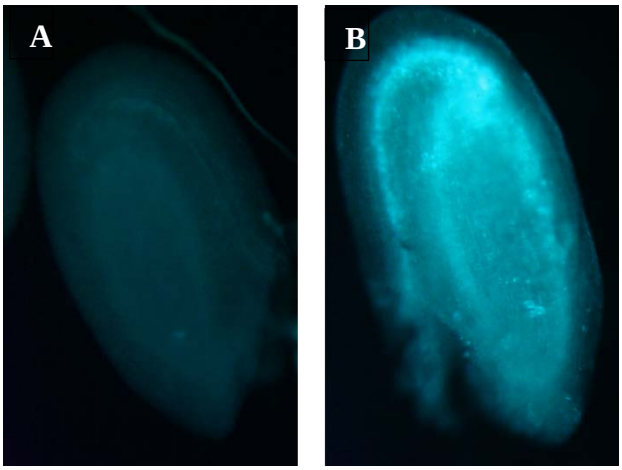

Figure 6: Example images of two 'WA38' ovule (O) developmental stages. Non-fluorescent ovule (A) was consider viable; while a fluorescent ovule (B) was classified as non-viable. Light tissue represents calluses stained structures.

Overall, based on the present data, the effective pollination period for 'WA38' in 2019 was two days (Figure 5). This window of time was calculated by subtracting the time the pollen tube took to reach the ovules (7 days) from the longevity of the ovules (9 days), knowing that the stigmatic receptivity was not a liming factor until Day 10.

Half of the samples for the ovule viability have yet to be analyzed. Therefore, this number represents a first preliminary estimate. The stigmatic receptivity, however, does not seem to be a limiting factor.

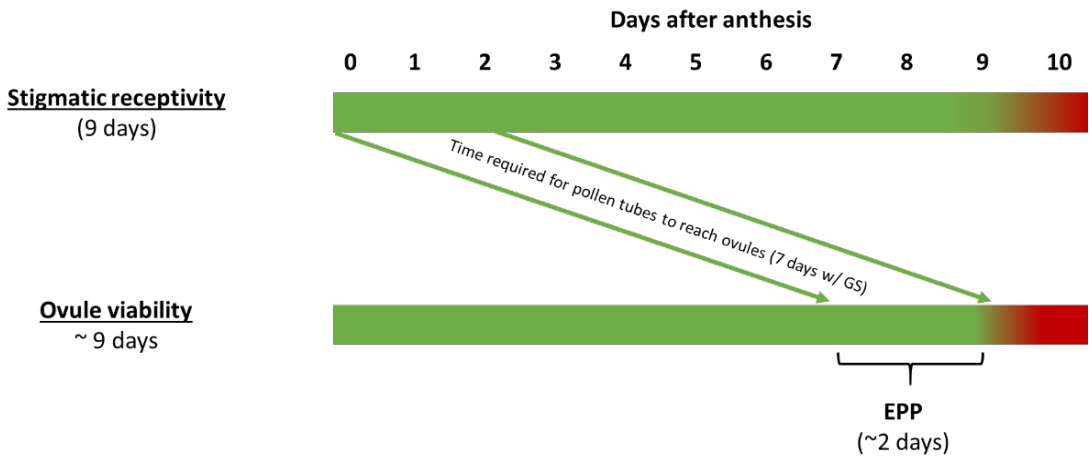

Figure 7: Effective pollination period (EPP) in 'WA38' flowers. EPP is based on duration of stigmatic receptivity, pollen tube kinetics, and ovule longevity. The bracket indicates the EPP approximately 2 days with the present experimental conditions in 2019.

All three components of the pollination period are temperature dependent. Usually, a higher temperature increases pollen tube growth but decreases ovule longevity. In this experiment, we decided to use 'Granny Smith' as a fully compatible pollen source based on bloom phenology, full compatibility, and availability; however, pollen tube kinetics may vary depending on the pollen source (see results Objective 2). Different growing regions can experience variations in the initial fruit set of 'WA38'. Research at Cornell University has shown that some apple cultivars with self-tinning attributes (e.g. 'Minneiska') produce a high ethylene peak during the first twelve days after full bloom (Robinson T., 2019 oral communication). Ethylene has shown to promote the development of the abscission zone and cause fruitlet abscission. Based on those observations and our results, applications of AVG (e.g. ReTain) should be investigated as a potential solution to extend the ovule longevity and reduce ethylene-induced fruitlet abscission.

Objective 2: Evaluate pollen tube growth of different crabapples in 'WA38' flowers

In 2019, 'WA38' flowers were cross-pollinated with pollen from 'Evereste', 'Indian Summer', 'Frettingham', 'Granny Smith', and 'Snowdrift'. 'Snowdrift' and 'Granny Smith' showed significantly higher pollen tube lengths than 'Evereste' (intermediate) and 'Frettingham' and 'Indian Summer' the shortest lengths at Day 3 (Figure 6). Overall, pollen tubes from 'Snowdrift' reached the base of the 'WA38' style four days after pollination, while the other four genotypes required one additional day (Day 5). 'Frettingham' pollen tube growth showed a fluctuating trend that could be possibly be explained by the independent nature of the replications. Effective pollinizers should show a high pollen tube growth rate in order to reach the ovary and fertilize the ovules in a short period of time. Pollinizers with a slow pollen tube growth can require one or two additional days to reach the ovules, which shortens the effective pollination period. However, pollen tube growth is highly affected by temperature. Research has shown that pollen sources may perform differently at various temperatures. Therefore, this experiment will be repeated in 2020 to account for a potential year-to-year variation.

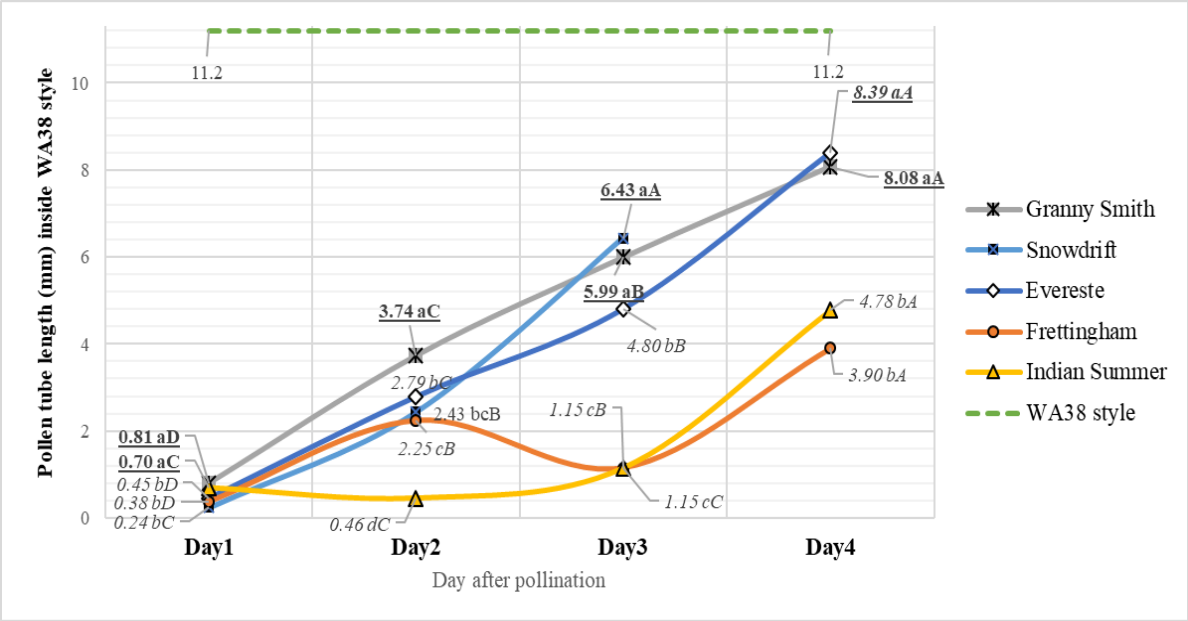

Figure 8: Pollen tube growth of five different pollen sources inside 'WA38' styles at 24 h intervals from hand pollination. At Day 4, Snowdrift pollen tubes had already reached the base of the style (average 'WA38' style length in 2019 was 11.2 mm, dashed line). At Day 5 all pollen tubes passed the base of the style. Capital letters discriminate means horizontally (significance across days for each pollen source) and small letters discriminate means vertically (significance across pollen sources within each day).

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

Project Title: Postharvest system optimization for organic apple storage

PI: Carolina Torres
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Co-PI: James Mattheis
Organization: USDA-ARS, TFRL
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Email: james.mattheis@ars.usda.gov
Address: 1100 N Western Av.
City/State/Zip: Wenatchee/WA/98801

Cooperators: David Granatstein (granats@wsu.edu), Lee Kalcsits (lee.kalcsits@wsu.edu), Stemilt.

Total project Request: Year 1: $50,000 **Year 2**: **$50,600** Year 3: $56,000

Other funding sources

Agency Name: Valent Biosciences, RipeLocker, WSU, USDA-ARS.
Cost-sharing: $150,000
Notes: Funds for technical support ($30,000/yr), travel expenses ($3,000/yr), and 0.1 FTE (P.I) from start-up funds.

Budget
Organization Name: Washington State University **Contract Administrator 1**: Katy Roberts
Telephone: 509 335-2885 **Email address:** arcgrants@wsu.edu
Contract Administrator 2 (TFREC): Shelli Tompkins
Telephone: 509 293-8803 **Email address:** shelli.tompkins@wsu.edu

Item	2019	**2020**	2021
Salaries			
Benefits			
Wages	20,000	16,000	16,000
Benefits	7,000	5,600	5,600
Equipment [1]	13,000	13,000	13,000
Supplies[2]	3,500	3,000	3,000
Travel			
RCA rental	6,500	13,000	13,000
Plot Fees			
Total	50,000	**50,600**	50,600

[1]Three LabPods (Storage Control Systems Inc) leasing for DCA-RQ.
[2]Fruit, laboratory consumables, boxes

Objectives:

1. **Evaluate the combination of DCA systems and RA storage on fruit quality postharvest.**

Activities 2019

1. Selection of Honeycrisp and Fuji organic orchards (2 warm sites/2 cool sites for each cv.)
2. Fruit from both cvs were harvested and placed in different dynamic storage regimes, monitored using chlorophyll fluorescence, ethanol concentration, and respiration quotient. Storage protocols are shown in Table 1.

Table 1.

Cultivar	Plot	Harvest date	Conditioning	DCA
Honeycrisp	Warm 1	8/31/2019	10 days/50°F	CF: (LOL≈ 0.3%O_2)- 3.0% O_2/ 0.5% CO_2 ILOS: 0.5% O_2/ 0.5% CO_2- 7-11d; 1.0% O_2/0.7% CO_2 RQ: 3.0% O_2/0.5% CO_2
	Warm 2	9/02/2019	10 days/50°F	
	Cool 1	9/10/2019	10 days/50°F	
	Cool 2	9/06/2019	10 days/50°F	
Fuji	Warm 1	10/03/2019	Delayed CA-34°F-20d	CF: (LOL< 0.4%O_2)- 5-2-0.8% O_2 in 7 days, 0.8% CO_2; 0.8% O_2/0.8% CO_2 ILOS: 0.6% O_2, 0.8% CO_2-10 d; 0.8% O_2/0.8% CO_2 RQ: 0.8% O_2/0.8% CO_2
	Warm 2	10/04/2019	Delayed CA-34°F-20d	
	Cool 1	10/04/2019	Delayed CA-34°F-20d	
	Cool 2	10/09/2019	Delayed CA-34°F-20d	

CF: Chlorophyll Fluorescence; RQ: Respiratory quotient

2. **Evaluate the effect of organic Retain in combination with different storage systems on fruit maturity and quality postharvest.**

Activities 2019

1. Two crop destruct plots were selected in a commercial orchard from the Hood River area, one Honeycrisp and one Gala. Applications of Retain OL were coordinated and carried out by Valent Biosciences personnel. The treatments were:

    1. Untreated Control
    2. Retain OL
        a. Application rate: 10 fl.oz/A (with an organo-silicone surfactant at 0.05-0.1 %v/v)
        b. Application timings: 4 weeks before harvest: 1 week before harvest
    3. Retain OL
        a. Application rate: 20 fl.oz/A (with an organo-silicone surfactant at 0.05-0.1 %v/v)
        b. Application timings: 1 week before harvest

2. Fruit samples were harvested at 2 times:
    - Untreated harvest maturity

        Honeycrisp: 14-15 lb, 4-5 starch index, 0.5 acidity, 13.5 ss, white-yellow color

        Royal Gala: 17-18.5 lb, 1.8-2.5 SI, 11.0 SS, >50% red color

• Retain-treated harvest maturity (similar maturity indexes)

3. Fruit was stored in CA for 9 months. Quality evaluations will be carried out after 3, 6 and 9 months. Honeycrisp apples were conditioned prior storage for 10 days at 50 °F.

3. Evaluate the performance of vacuum storage (RipeLocker) under different temperatures regimes on fruit quality and physiological disorder development.

Activities 2019

1. Selection of Honeycrisp and Fuji organic orchards (2 warm sites/2 cool sites for each cv.)
2. Fruit from both cvs and orchards were harvested and placed in vacuum storage bins (RipeLocker) at 34 °F and 36 °F for 9 months plus 3 or 6 weeks in air.

3. Fruit maturity and quality will be assessed after storage plus 1 and 7 days of shelf-life in all treatments.

Significant Findings 2019

Objectives 1 and 3:

Mineral Analyses

Mineral analysis of year 1 fruit is shown in Table 2. N and P analyses are still in progress. No discussion/conclusion can be drawn at this time.

Table 2. Mean values of % cations in fruit tissue (3 replicates/batch) at commercial harvest, 2019 season.

Cultivar	Plot	Ca	K	Mg	K/Ca	Mg/Ca	(K+Mg)/Ca
Fuji	Warm 1	0.19	1.00	0.15	5.98	5.21	0.77
	Warm 2	0.27	1.15	0.14	4.71	4.19	0.52
	Cool 1	0.13	1.12	0.11	9.16	8.34	0.82
	Cool 2	0.13	0.77	0.11	6.90	6.05	0.86
Honeycrisp	Warm 1	0.07	1.37	0.13	22.15	20.21	1.94
	Warm 2	0.09	0.78	0.09	9.67	8.67	1.00
	Cool 1	0.04	1.13	0.14	28.94	25.72	3.23
	Cool 2	0.11	1.03	0.09	10.02	9.21	0.80

Fruit maturity and quality at harvest

Fruit maturity at commercial harvest of year 1 is shown in Table 3.

Table 3. Fruit maturity indices at commercial harvest, 2019.

Cultivar	Plot	Weight (g)	Bkgd Color (1,green -4, yellow)	Red %	I_{AD}	Hue (°)	Firmness (lb)	SS (Brix)	SI (1-10 FU. 1-6 HC.)	IEC (ppm)
Fuji	Warm 1	503.1±52.2	-	-	0.9±0.3	28.0±7.5	16.6±1.4	13.1±0.8	6.1±0.7	0.21±0.02
	Warm 2	523.3±43.4	-	-	1.1±0.2	26.8±8.0	16.2±1.0	11.9±0.7	6.8±0.9	0.19±0.01
	Cool 1	237.3±23.4	-	-	1.1±0.1	26.8±5.9	17.8±1.7	14.0±0.5	6.6±0.9	0.18±0.01
	Cool 2	244.9±29.4	-	-	1.1±0.2	31.6±7.5	17.0±1.4	13.6±1.0	6.2±1.1	0.15±0.02
Honeycrisp	Warm 1	226.5±27.9	2.6±0.6	80.6±14.4	0.6±0.1	36.5±9.8	16.3±0.7	15.2±0.8	4.4±1.3	2.8±5.1
	Warm 2	219.3±22.1	2.2±0.7	65.7±15.1	0.8±0.2	55.1±15.2	15±0.9	11.8±0.7	4.3±1.3	10.4±18.3
	Cool 1	212.9±28.4	2.1±0.8	55.6±12.7	0.8±0.2	85.7±12.8	14.1±0.9	11.8±1.0	4.2±1.8	27.1±20.3
	Cool 2	265.4±29.0	3.4±0.5	87.8±9.3	0.3±0.1	32.9±9.4	15.6±1.2	13.4±1.1	5.1±0.8	0.0±0.0

Objective 2:
Fruit maturity data is being analyzed.

CONTINUING PROJECT REPORT **YEAR:** 2019

Project Title: Improving apple fruit quality and postharvest performance

PI: Manoella Mendoza
Organization: Washington Tree Fruit Research Commission
Telephone: 509-665 8271
Email: manoella@treefruitresearch.com
Address: 1719 Springwater Ave
City/State/Zip: Wenatchee, WA, 98801

Cooperators:

- WTFRC internal program: Mackenzie Perrault, Ines Hanrahan, Marcella Galeni, Federico Grignaffini
- Others: Rob Blakey, Corina Serban, Hannah Walters (Stemilt), misc. grower collaborators, WTFRC seasonal crew and interns
- Defect guide: TJ Mullinex (Good Fruit Grower), Darrel Kilgore, Matt Ziegler, Wendy Jones, Karen Lewis (WSU), Rob Blakey (Stemilt), Carolina Torres
- 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)
- Multistate FreshCloud technology validation: Chris Watkins & Al Shoffe (Cornell), Renae Moran (Main), Randy Beaudry (MSU), Jennifer De Ell (Ontario), Carolina Torres (WSU), Tara Baugher & Daniel Weber (PA)

Other funding sources

Majority of supplies and fruit donated by industry cooperators (approx. value: $5,000); WSU Postharvest Fruit School supplied $6,000 to complete initial draft of defect guide. All costs for layout and printing of the WA 38 starch scale are covered by Storage Control Systems.

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

Item	2019
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 an internal program report. Activities for 2020 will be based on need and are subject to WTFRC board approval.

OBJECTIVES

1. Develop postharvest outreach material
    a. Development of an apple defect guide.
    b. Development of a WA 38 starch scale (1-6).
2. Field test methods to induce bitter pit in Honeycrisp.
3. Multistate validation of Fresh Cloud technology to predict bitter pit and soft scald in Honeycrisp. (NEW)

SIGNIFICANT FINDINGS

Objective 1:

a) 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 (English and Spanish), a laminated booklet. New PI: Carolina Torres, WSU
b) A starch scale for WA 38 and a detailed description was developed, distributed and industry wide training was performed in 2019.

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

Objective 3: Evaluations are ongoing.

METHODS

Objective 3: Multistate validation of Fresh Cloud technology (project lead: Chris Watkins)

Within each state (or region within a state), a minimum six orchard blocks will be used, and horticultural information is obtained, including use of preharvest growth regulators. Preference is given to selecting, if historical information is known, for a range of bitter pit susceptibilities.
A minimum of 10 representative trees in each block are tagged.
Per orchard block:
- Sample of 100 fruit taken three weeks before anticipated first harvest and kept at 68 F for three weeks (bitter pit incidence evaluated). Ten fruit per tree (for 10 trees), average size, etc.
- At commercial harvest, for each orchard block, 40 fruit taken for genomic test* (standard protocol was provided by Agrofresh) plus normal maturity tests on an additional 20 fruit on day of harvest. Fruit weight, IEC, Firmness, SSC, TA, SPI (Cornell Chart and WA chart, photograph taken of the fruit), DA meter (blushed and unblushed sides) and F 750 (if available)

For storage evaluation:
3 bushels for storage
– one at 33 F
- one at 38 F
- one at 38 F without conditioning (7 d at 50 F)
Evaluation of storage disorders after two- and four-months storage plus seven days at 68 F.

RESULTS & DISCUSSION

In 2019, the fruit quality program has continued to focus part of its effort on Honeycrisp fruit quality. Due to the change in leadership at WTFRC, Manoella Mendoza took over the leadership

of this internal program area. We completed the development of the WA 38 starch scale. Dr. Torres assumed the lead of the WSU apple defect guide.

Defect Guide
Dr. Torres of WSU has resumed leadership of this project. To date a total of five posters have been finalized and are available for purchase ($35 each) through WSU Tree Fruit Extension.

Induced bitter pit
The project was completed in the 2018-19 storage season and a full report was submitted via Fruit Matters: http://treefruit.wsu.edu/article/the-utility-of-bitter-pit-prediction-models-for-honeycrisp-in-washington-state/

WA 38 Starch Scale
The project was completed in the 2018-19 storage season and a full report was submitted via Fruit Matters:

All printed material can be requested from WTFRC and will be provided to industry at no cost. Dr. Hanrahan led six field days in the fall organized by WSU Tree Fruit Extension to further disseminate the information.

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

Project Title: Apple genomes for postharvest fruit quality biomarkers AP-19-103

PI:	Dr. Loren Honaas	**Co-PI (2):**	Dr. Stephen Ficklin
Organization:	USDA ARS	**Organization**:	WSU Dep. of Hort.
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Address:	1104 North Western Ave	**Address**:	PO Box 646414
City/State/Zip:	Wenatchee, WA 98801	**City/State/Zip**:	Pullman, WA 99164

Co-PI (3): Dr. Jim Mattheis
Organization: USDA ARS
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Email: james.mattheis@ars.usda.gov
Address: 1104 North Western Ave
City/State/Zip: Wenatchee, WA 98801

Cooperators: Dr. Claude dePamphilis (Penn State Dep. of Biology), Dr. Dave Rudell (USDA ARS)

Total Project Request: **Year 1:** $87,142 **Year 2: $96,692** **Year 3:** $97,991

Other funding sources

Agency Name: USDA ARS base funding
Amount: $220,000
Notes: personnel $100,000, RNA-Seq $90,000, consumables $30,000

Agency Name: WSU Ficklin Start-Up Funds
Amt. awarded: $86,000
Notes: These funds were used to purchase high-performance computing resources on WSU's Kamiak computing cluster. These resources will be used to perform data analysis for this project.

Agency Name: US National Science Foundation (NSF) Award #1659300
Amt. awarded: $150,000
Notes: A portion of this award was used to fund almost 1 Petabyte of storage for execution of scientific workflows and storage of results. We will use that infrastructure for this project.

Budget 1
Organization: USDA-ARS **Contract Admin:** Chuck Meyers & Sharon Blanchard
Telephone: 510.559.5769, 509.664.2280 **Email address:** chuck.myers@ars.usda.gov, sharon.blanchard@ars.usda.gov

Item	2019	2020	2021
Salaries	33,000		
Benefits			
Wages			
Benefits			
Equipment			
Supplies	5,000	5,000	5,000
Travel			
Miscellaneous[1]	49,142		
Plot Fees			
Total	87,142	5,000	5,000

Footnotes: [1]Miscellaneous expenses category is genome sequencing for 3 apple varieties

Budget 2
Organization Name: WSU **Contract Administrator:** Ian McDonald, Katy Roberts
Telephone: 509-335-3943 **Email address:** grants.bc.johnson@wsu.edu

Item	2019	2020	2021
Salaries[1]		70,326	71,339
Benefits[1]		20,121	20,357
Wages[1]		1,245	1,295
Benefits			
Equipment			
Supplies			
Travel			
Plot Fees			
Miscellaneous			
Total		91,692	92,991

Footnotes: [1]Salaries, wages, and benefits will support a fulltime postdoc for 2 years and will provide partial support to a graduate student in Co-PI Ficklin's lab

Objectives:

1. Sequence genomes to build variety-specific genomes for 'Honeycrisp,' WA 38 ('Cosmic Crisp'), and 'Gala'
2. Refine biomarker discovery pipeline using machine learning algorithms, comparative network analyses, and comparative genomics
3. Begin validation of biomarkers via PCR gene tests in multi-lot, multi-year surveys

Year 2 goals:

In year 2 we will sequence, assemble, and begin annotation of the apple genomes. We will finish gathering gene activity data from 'Gala,' WA 38, and 'Honeycrisp' fruit samples. We will gather another set of maturity marker validation samples. We will begin the data analysis phase (step 2) with CO-PI Ficklin.

Significant findings:

- Obtained high granularity fruit quality data and ~500 fruit samples (replicated, cryopreserved)
- Year 1 validation fruit samples obtained – 11 cultivars across 7 orchard locations
- A new genome assembly strategy shows promise for pome fruit tree genomes
- New gene expression method improves efficiency of gene activity measurements

Methods:

Fruit quality data and tissue biobanking

We gathered fine-grained fruit texture data from 'Gala' fruit stored in various storage regimes (air at 68˚F, air at 50˚F, and air at 34˚F, plus CA, MCP, and MCP + CA each at 34˚F) for 'Gala' both at pull-out, and after a simulated supply chain period using a Mohr™ MDT-2. We collected corresponding cortex tissue (6 apples per biorep x 3 bioreps) for gene activity analysis that has been processed to a fine powder using a Spex® Cryogenic Grinder Mill and cryopreserved in -112˚F freezers. RNA was extracted according to Honaas' published protocols and submitted for analysis to PSU's Genomics Core Facility. Similarly, we gathered fruit quality data for WA 38 and 'Honeycrisp' from at least 10 weekly harvests of each cultivar from WSU's Sunrise research orchard. We harvested peel tissue at each harvest, plus peel tissues from fruit exposed to a brief cold treatment (48 hours at 34˚FC) and control (48 hours at room temperature). All cryopreserved peel tissues were processed and stored as described above. We collected year-1 validation samples which consisted of a 2 or 3 point sampling scheme centered on the approximate commercial harvest date. These samples were processed and cryopreserved as the above fruit peel samples for WA 38 and Honeycrisp. We collected samples from 11 cultivars across 7 orchard locations (See Table 1).

Genomic DNA for genome sequencing

Young leaf tissues from 'Gala', WA 38, and 'Honeycrisp' were harvested and cryopreserved as whole leaves, with no mixing of individuals (1 tree/individual = 1 container). Samples were sent to Penn State cooperator dePamphilis for cryogenic storage until extraction for High Molecular Weight DNA and cleanup prior to sequencing. It is preferable to store genomic DNA in frozen whole tissue, rather than as a frozen DNA extract.

Results and Discussion:

Fruit quality data, tissue biobanking and sample processing- Gala

We focused our fruit quality assessment on fruit firmness and texture of Gala fruit in various storage regimes. We observed the expected contrasts presented in Figure 1, with the most rapid loss of firmness in Gala fruit stored in air at 68ºF, and the best maintenance of fruit quality in fruit treated with MCP and stored in CA at 34ºF. While there were substantial differences in fruit stored in air, there were not dramatic quality differences between fruit in long term storage conditions (CA, MCP, CA+MCP, See

Figure 1). Although outcomes were not dramatically different, understanding how gene activity was different in fruit that had similar outcomes will help us learn about the mechanisms of fruit quality maintenance in the various storage conditions, especially between regimes that are conventional and ostensibly organic compliant. These samples are currently being analyzed for gene activity.

Fruit quality data, tissue biobanking and sample processing- WA 38 and Honeycrisp

Over the course of 10+ weeks we harvested WA 38 and Honeycrisp fruit at WSU's Sunrise Research Orchard from research blocks managed by Dr. Lee Kalcsits (Table 1). We aimed to capture transitions in numerous fruit quality aspects during fruit maturation. The fruit quality data for WA 38 and 'Honeycrisp' fruit are summarized in Figure 2. We captured transitions in many fruit quality aspects, suggesting we captured molecular processes that precede and/or accompany these changes during fruit maturation. In addition to using the fruit quality data to guide the gene activity analyses (i.e. correlate starch clearing with gene activity signatures) we also observed increases in variance that preceded shifts in fruit quality data measurements (i.e. starch and ethylene, Figure 2). Some of the earliest changes in fruit quality metrics are shifts that are sporadic across samples causing an increase in variance (noise indicated by larger error bars) - these signatures may also suggest timepoints to target for focused gene activity analysis.

Fruit quality data, tissue biobanking and sample processing- validation samples

We began to accumulate the validation sample set that will be used to test candidates in our preliminary biomarker list. We intend to bank these samples for repeated tests of gene candidates as we attempt to develop maturity biomarkers. In Table 1 the list of validation samples are presented (see those with 2-3 samples). Many of these samples are shared with in Dr. Dave Rudell's WTFRC project "Reducing carbon dioxide-related postharvest disorders" to explore CO_2 injury across multiple cultivars, adding another fruit quality factor to inform gene activity analysis.

Updates on genome assembly strategy

Based on promising results from cooperator dePamphilis, we evaluated genome data from 10x Genomics (10xgenomics.com) for apple and European pear. Cooperator dePamphilis' group has developed a sophisticated 9-step assembly strategy that leverages all genome data from our current projects, existing genome information from Rosaceae, including recently published assemblies and annotations of double haploid genotypes of 'Hanfu' apple (https://doi.org/10.1038/s41467-019-09518-x) and 'Bartlett' pear 2.0 (https://doi.org/10.1093/gigascience/giz138) to produce further improved assemblies. Our 'D'Anjou pear genome has been fully assembled and annotated. Based on our initial assessment the assembly is good, indeed preferred for gene activity analysis compared to the much improved 'Bartlett' 2.0. We have used the same approach to sequence and assemble the 'Granny Smith' genome achieving highly similar assembly results. The annotation step for 'Granny Smith' and other apple genomes will leverage highly similar, if not better, resources and tools. This expected to deliver similar or better results (for more details see the Final Report for Honaas' WTFRC Tech project "Enhancing reference genomes for cross-cultivar functional genomics").

Updates on gene activity measurements

In order to increase the efficiency of the costly transcriptome sequencing step (data generation step for gene activity measurements), we evaluated the performance of a new sample preparation scheme from Lexogen (lexogen.com) called QuantSeq that targets only a small portion of the gene (compared to targeting the whole gene). This effectively reduces the amount of information needed for reliable gene activity estimates by a factor of at least 4, but cultivar-specific genomes (which we are generating in this project) are required to access this technology. By combining this gene activity measurement approach with our custom, cultivar specific genomes, we can increase the efficiency of our ARS-funded sequencing efforts. The agreement between these two technologies is very good (R^2>0.8) and is expected to increase with our cultivar specific genomes and custom annotations (Figure 3).

Figures and Tables

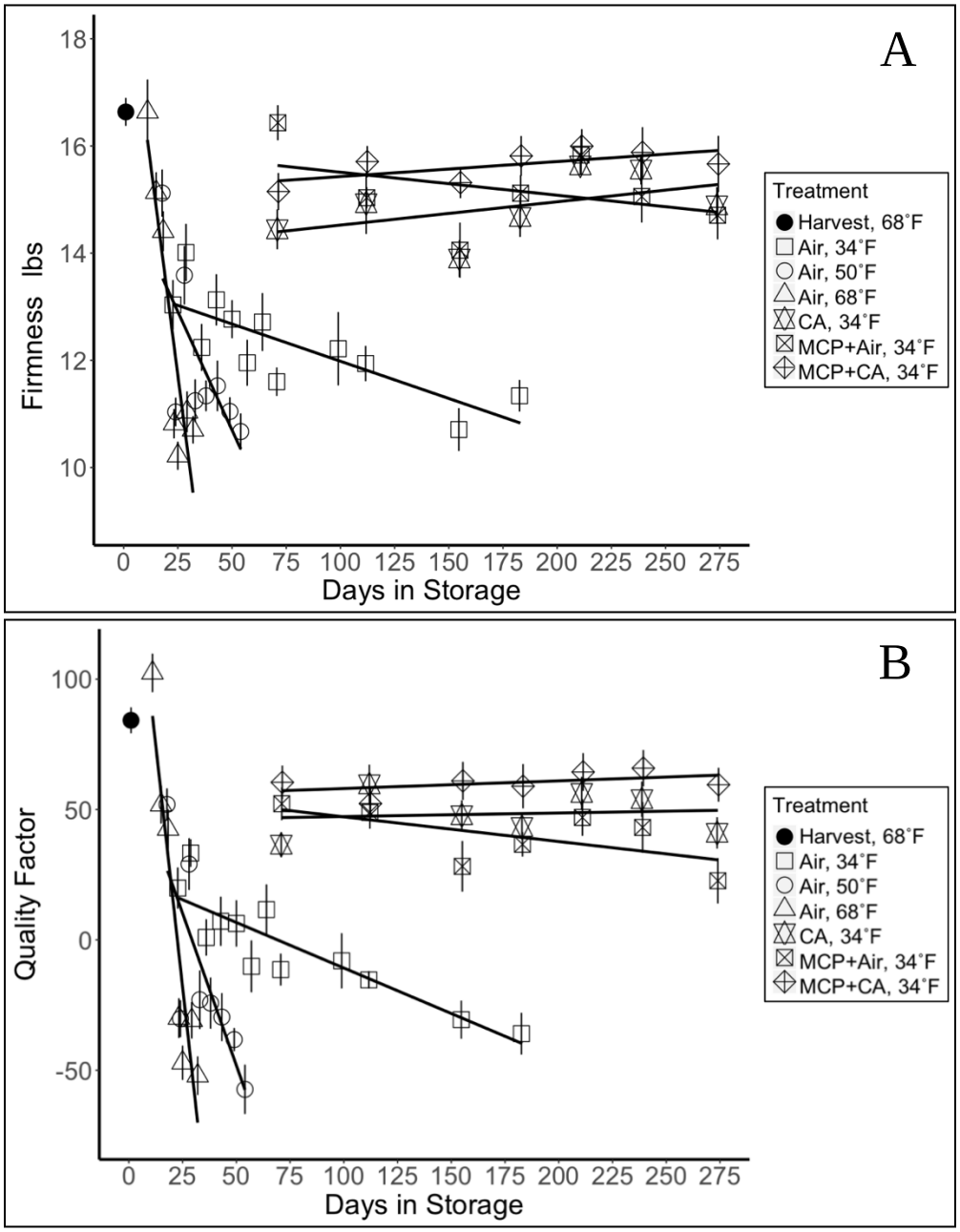

Figure 1: 'Gala' fruit firmness (M1) and integrated quality (Quality Factor) is maintained best when fruit are stored in CA+MCP at 34˚F – we observed the expected contrasts in loss of firmness across treatments. Fruit was assessed with Mohr MDT-2 for firmness (A) and over-all textural quality (B) following a simulated supply chain period after pull-out at the indicated day.

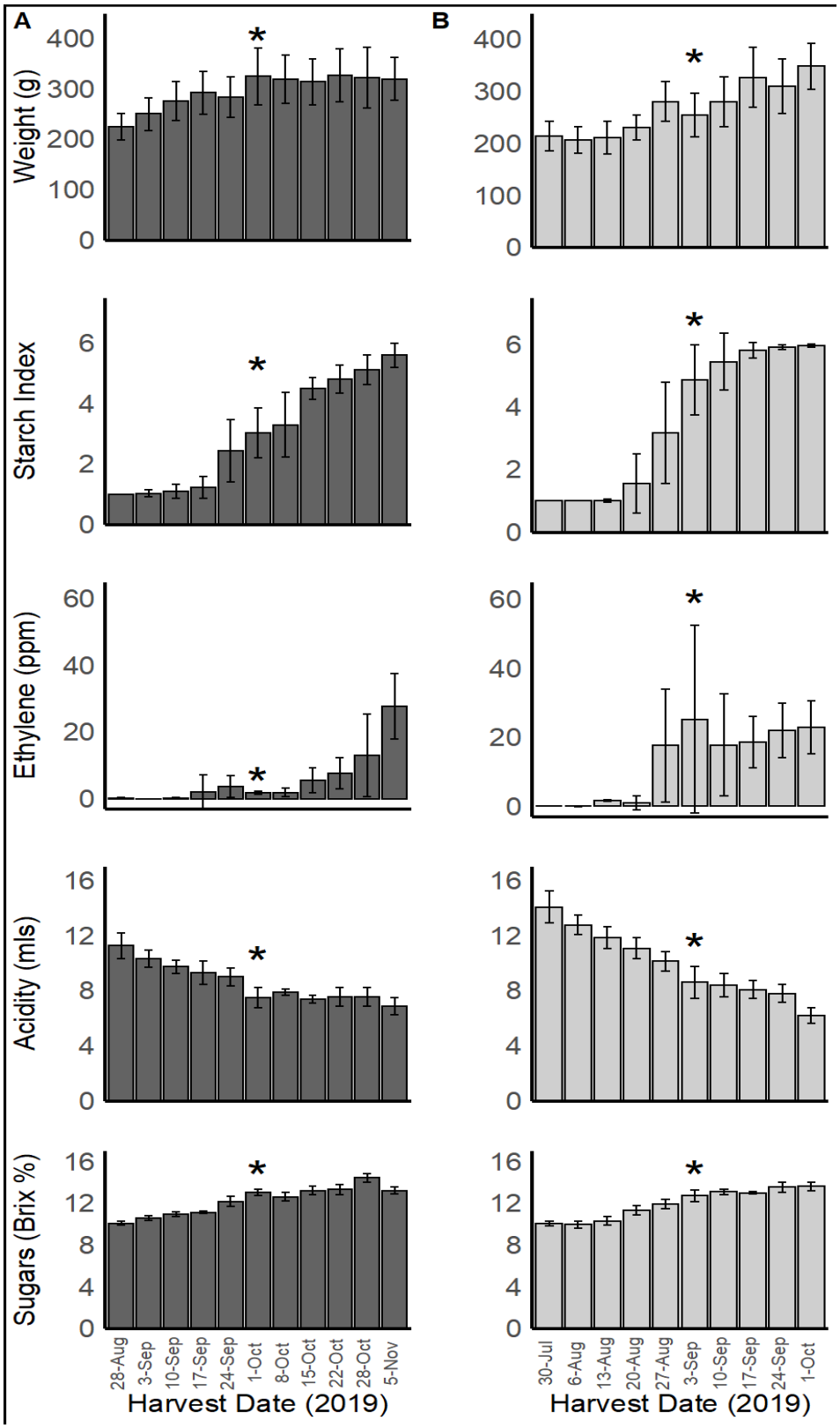

Figure 2 (cont'd next page): Fruit quality data gathered across harvest dates capture shifts that will inform gene activity analyses. WA 38 (column A) and Honeycrisp (column B). Fruit quality data are on the Y-axis, harvest date is on the X axis - approximate commercial harvest indicated with *.

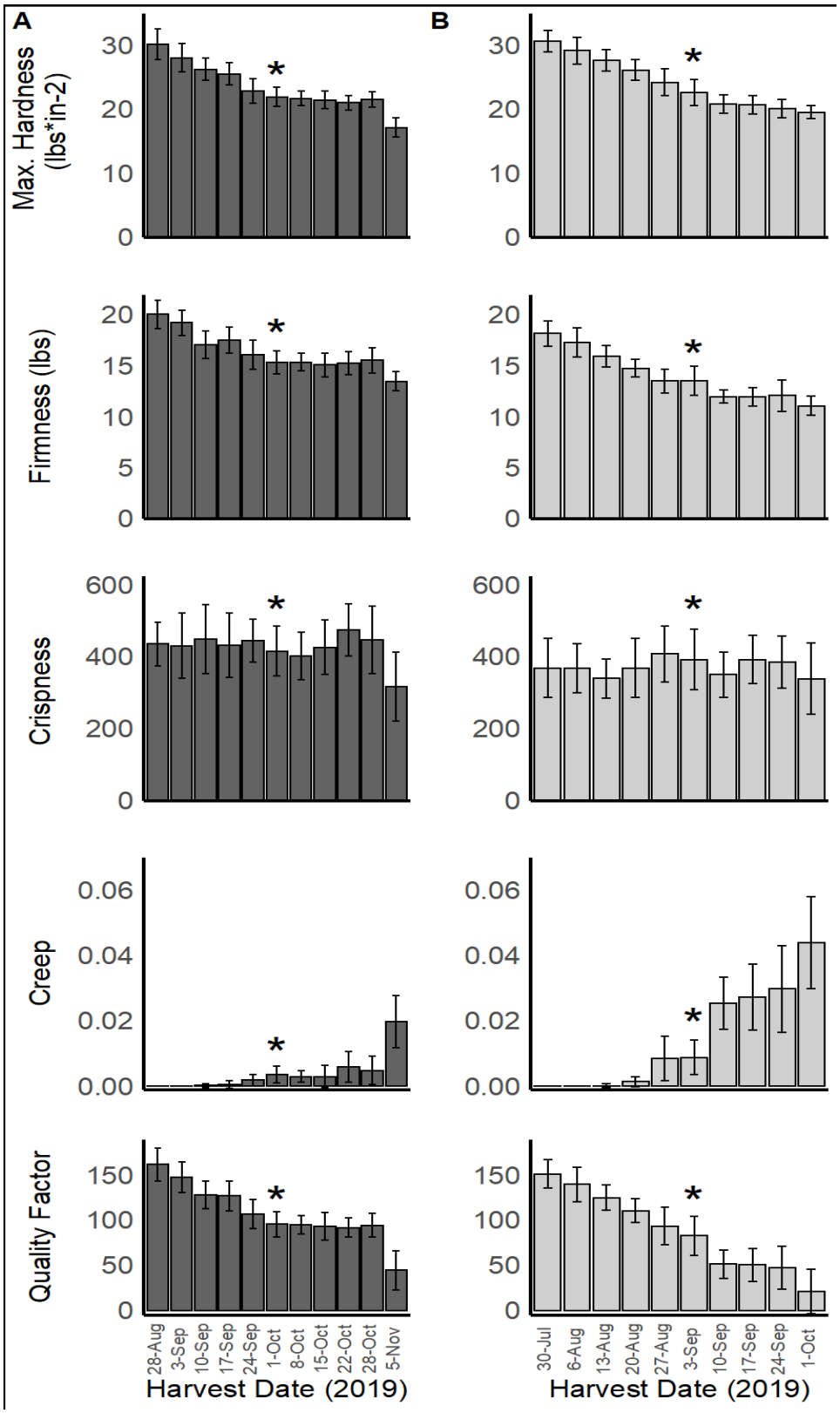

Figure 2 (cont'd): Fruit quality data gathered across harvest dates capture shifts that will inform gene activity analyses. WA 38 (column A) and Honeycrisp (column B). Fruit quality data are on the Y-axis, harvest date is on the X axis - approximate commercial harvest indicated with *.

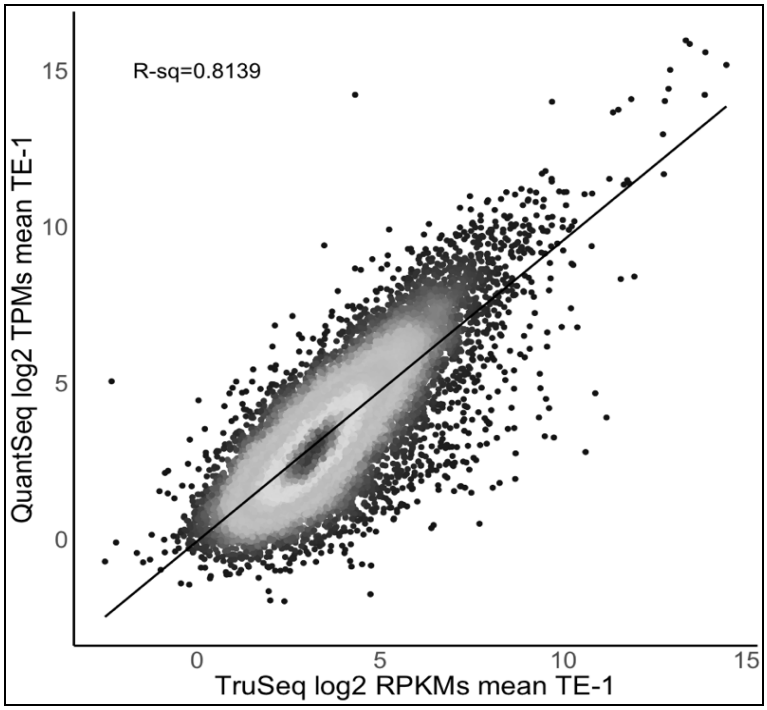

Figure 3. The correlation between gene activity data from Lexogen's QuantSeq and traditional TruSeq is good, and is expected to improve when using cultivar-specific genomes.

Table1. Summary of cryopreserved apple fruit peel samples. **High granularity samples for maturity biomarker discovery are bold**, validation samples are in normal font.

Cultivar	Location	Number of weeks
WA 38 (Cosmic Crisp)	**WSU Sunrise Orchard Block 9 (Rock Island, WA)**	**11**
Honey Crisp	**WSU Sunrise Orchard Block 9 (Rock Island, WA)**	**10**
Braeburn	Mattawa, WA	2
Cosmic Crisp	George, WA	2
Fuji	WSU Sunrise Orchard Block 10 (Rock Island, WA)	2
Gala	WSU Sunrise Orchard Block 5 (Rock Island, WA)	3
Golden Delicious	WSU Sunrise Orchard Block 5 (Rock Island, WA)	3
Granny Smith	WSU Sunrise Orchard Block 5 (Rock Island, WA)	3
Honeycrisp	Quincy, WA	2
Jonagold	WSU Sunrise Orchard Block 5 (Rock Island, WA)	3
Juici	Quincy, WA	2
Pazazz	Brewster, WA	2
Pink Lady	Mattawa, WA	2

CONTINUING PROJECT REPORT **YEAR:** 1 of 3
WTFRC Project Number: AP-19-100

Project Title: Reducing carbon dioxide-related postharvest disorders

PI: David Rudell
Organization: USDA-ARS, TFRL
Telephone: 509 664 2280 (ext. 245)
Email: David.Rudell@usda.gov

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

Collaborators: Dr. Ines Hanrahan, Christine McTavish

Budget: **Year 1**: $79,314 **Year 2**: **$92,893** **Year 3**: $95,036

Other funding sources

Agency Name: USDA-ARS, In-house project
Amt. awarded/requested: $174,719/3 yrs.
Notes: In-house project with complimentary objectives. Funds for storage maintenance and costs ($8000/yr), supplies and materials ($3000/yr), travel ($5000/yr), and 0.2 FTE (PI, co-PI) and 0.1 FTE (technical).

Agency Name: USDA-NIFA
Amt. requested: $540,888/4 yrs.
Notes: Pre-proposal with complimentary objectives submitted to SCRI program.

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

Item	2019	2020	2021
Salaries (GS-9 step 1)	52,116	53,679	55,290
Benefits (33.3%)	17,198	17,714	18,246
Wages (part-time employee)	10,000	10,000	10,000
Benefits			
Equipment			
Supplies			
Travel			
Miscellaneous*		11,500	11,500
Plot Fees			
Total	79,314	**92,893**	95,036

Footnotes: One-third instrument service contract

Objectives:
1. Develop methods to consistently identify CO_2 sensitivity.
2. Determine best cold chain practices when CO_2 sensitivity is indicated.
3. Identify fruit chemistry associated with CO_2 sensitivity.

SIGNIFICANT FINDINGS

At 4 months into the year 1 storage study, there are no significant findings to report.

METHODS

Equipment and Cooperative Summary: Storage experiments, fruit quality assessment, fruit chemistry analyses using analytical instrumentation (gas and liquid chromatography-mass spectrometry), and tissue cryopreservation will be performed using facilities currently in place at ARS-TFRL, Wenatchee. Storage experiments will be conducted in our in-house CA chambers capable of maintaining both O_2 and CO_2 CA environments accurately.

Outreach (Deliverables are summarized under "Anticipated Products" Table 1): Aside from reports to the WTFRC, new information will be disseminated through presentations at industry meetings and at professional conferences, and by publications in industry publications and peer-reviewed journals. We will cooperate with WTFRC (Lead: Ines Hanrahan) to document symptoms of injury not already covered by the new WSU Apple Defect guide. Symptomatic fruit will be photographed, defect rotations assembled, and associated descriptive text created. These updates will be incorporated into the existing guide as needed.

Objective 1: Develop methods to consistently identify CO_2 sensitivity

Year 1

Fifteen apple cultivars were harvested at approximately 2-4 weeks prior to commercial harvest and 1-2 days before commercial harvest (Table 1). Harvest maturity (starch index and internal ethylene concentration) and external/internal appearance were evaluated, and fruit was imaged with a digital camera. Two trays of apples were drenched with an emulsion containing DPA (2000 ppm), and 2 other trays were treated with a solution containing only the inactive ingredients. The DPA and control trays were put in separate CA chambers (to avoid DPA cross contamination) set at 0.6 % O_2: 5% CO_2. After 4 months, apples began to be evaluated for internal and external defects. Fruit and then external and internal defects were imaged. Damaged and healthy tissue are sampled, flash frozen, and cryo-preserved for chemical analysis where defects are found.

Table 1. Apple cultivars, orchard location, harvest dates, starch index and internal ethylene concentration for early and late harvests.

Cultivar	Location	early pick date	Late pick date	Starch index early	IEC early	Starch late	IEC late
Gala	46.754627, -119.855132	19-Aug	3-Sep	3.0	0.61	6.8	1.32
Ambrosia	46.7534697, -119.862191	19-Aug	12-Sep	1.0	nd	3.8	0.17
Smitten	46.4913458, -120.3500047	21-Aug	25-Sep	1.9	nd	6.2	0.03
Golden Delicious	47.306495, -120.066978	21-Aug	26-Sep	1.6	nd	6.1	0.02
Delicious	47.312363, -120.064973	28-Aug	7-Oct	1.7	nd	5.6	19.96
Juici	47.164138699999995, -119.9594429	11-Sep	10-Oct	0.6	0.18	5.5	0.86
Pazazz	48.121894, -119.711169	17-Sep	17-Oct	3.0	1.46	7.1	34.97
Plumac	46.5894027, -120.6827642	25-Sep	29-Oct	3.4	0.06	7.9	0.67
Scilate	47.421802,-120.184043	10-Sep	24-Oct	1.3	nd	3.4	0.62
Honeycrisp	47.2410682, -119.96313959999999	5-Sep	19-Sep	1.6	0.18	5.7	6.52
Braeburn	46.7565725, -119.6786515	12-Sep	15-Oct	1.4	0.08	5.2	0.23
Pink Lady	46.6928543, -119.75278499999999	1-Oct	28-Oct	1.9	nd	5.9	0.04
WA38*	47.0203596, -119.9239345	26-Sep	10-Oct	2.7	5.15	4.7	0.60
Autumn Glory	119.81166689999999	30-Sep	28-Oct	5.4	nd	7.7	0.22
Fuji	47.311816,-120.064926	30-Sep	24-Oct	5.0	0.06	6.9	2.64

*Early and late samples were harvested from different orchards

Activities in this objective focus on storage under conditions expected to cause internal and external symptoms to determine cultivar CO_2 sensitivity. Consequently, we are not using any temperature conditioning or atmospheres known to reduce CO_2 injury for this objective. DPA treatment, known to prevent external and internal CO_2 injury, is being used to determine if injuries result from CO_2 sensitivity. As relatively immature fruit are more likely to develop peel CO_2 injury and relatively mature fruit are more likely to develop internal CO_2 injury, we will use an early and late harvest for each of the cultivars screened.

All defects in every treatment are being photographically cataloged, etiology recorded, and will be sent for to Dr. Hanrahan for review for the WSU Apple Defect guide. This protocol effectively determined CO_2 sensitivity of these unnamed crosses while highlighting a variety of symptom presentations resulting from CO_2 injury. Cultivars showing no sensitivity in Year 1 or 2 will continue to be tested using these criteria until the third year of the project.

In year 3, we will evaluate the use of sealable plastic bags as a low-tech means to perform this test. The low O_2, high CO_2 modified atmosphere that develops when apples are sealed in plastic bags may be sufficient to develop high CO_2 and low O_2 to test CO_2 sensitivity at the warehouse level. Apples with or without DPA will be sealed in bags, O_2/CO_2 in the bag will be monitored, and duration needed for injury to develop will be evaluated.

Objective 2: Determine best cold chain practices when CO_2 sensitivity is indicated

In years 2 and 3, carbon dioxide thresholds will be evaluated under different storage O_2 concentrations for cultivars focusing on relative external or internal CO_2 sensitivity under low oxygen conditions. Fruit will be picked immature and/or mature depending if external or internal CO_2 sensitivity was determined as the result of objective 1 activities. One hundred fruit from each

harvest/cultivar will be stored at 2 different O_2 (0.6 and 1%) and 2 different CO_2 concentrations (1, and 5% CO_2). External CO_2 injury will be analyzed at 3 and 6 months. Internal CO_2 injury will be analyzed at 6 months. A more complete analysis of fruit chemical changes following multiple storage durations is outlined under objective 3 (methods section, see below).

In year 3, additional temperature acclimation, CA treatments, and high O_2 settings will be evaluated on cultivars determined to have high sensitivity. Strategies tested will depend on findings from previous activities but will be largely based on existing protocols effective for managing CO_2 sensitivity such as delayed CA pull down, temperature pull down, and/or room ventilation. This activity is to determine strategies for avoiding CO_2 injury on especially sensitive cultivars. Fruit quality will be evaluated as part of these activities.

Objective 3: Identify chemistry associated with CO_2 sensitivity

In year 2 and 3, peel and cortex chemistry will be analyzed on Honeycrisp and Fuji, two cultivars with known sensitivities to CO_2 leading to internal browning, to determine changes in chemistry related to high CO_2 levels, browning, or a combination of the two. Honeycrisp was chosen as it can develop both soggy breakdown and internal CO_2 injury. Fruit from each cultivar will be stored at 0.6% O_2 and 0, 1, 2.5, and 5% CO_2 for up to 6 months sampling cortex at 0, 0.5, 1, 2, 4, and 6 months. Half the fruit will be treated with 2000 ppm DPA to assure that injury and associated metabolism is caused by CO_2 sensitivity. We expect to indicate relationships between CA CO_2 and O_2 levels for CO_2 sensitive cultivars. Sampling, storage, processing, instrumental analysis will be performed as outlined by Leisso et al. (2016). We will use a combination of multi-variate (PCA, PLS-DA, and ASCA) and univariate (ANOVA) to find metabolites most associated with high CO_2 and/or disorder incidence.

To develop a system for diagnosing peel and cortex browning caused by CO_2 sensitivity, browned peel and cortex from activities outlined under objective 1 will be sampled regularly with adjacent healthy tissue and healthy tissue from DPA treated fruit as control. Any browned tissue in or on DPA treated fruit will be sampled as a control to reveal any similarities or differences of chemistry caused by non-CO_2 related browning. This is expected to our accuracy of discerning browning injuries caused by CO_2 sensitivity from other browning conditions.

Finally, we would like to investigate why apples can be sensitive to CO_2– What happens to the CO_2 that is different than apples that are not sensitive to CO_2? Does elevated CO_2 reduce energy production for tissue to stay alive? $^{13}CO_2$ labelling will be used to trace the fate of CO_2 under high CO_2 vs. low CO_2 environments and the rate of metabolism or how fast energy is produced. For this work we will sample Honeycrisp peel and flesh stored under the conditions and treated as above (Objective 3, first paragraph) with respect to CO_2. We will use a technique developed from our sunscald project (AP-16-102) where we expose whole fruit to a pulse of 2% $^{13}CO_2$ and sample periodically, first hourly and then daily up to three days. We will track the label using LC-MS (Leisso et al., 2016). We expect this will show us how metabolic pathways are changed and the rate of metabolism as impacted by atmospheric O_2 and CO_2 concentration. Most importantly, we expect this new approach will build on what we already know about why certain cultivars are far more sensitive to CO_2 than other cultivars.

RESULTS AND DISCUSSION

At 4 months into the year 1 storage study, only the early harvests of Gala, Golden Delicious, Ambrosia, Smitten, and Delicious have been evaluated. To date, minor peel browning symptoms

were recorded on Smitten in both control and DPA treated fruit. Delicious had some light browning in severely watercored flesh. These were imaged, sampled, and recorded although neither symptom could be attributed to CO_2 sensitivity as they were also found on/in DPA treated fruit.

Table 1. Proposed project milestones with anticipated products of "Risk assessment for delayed sunburn and sunscald".

Objective		1: Screen cultivars for CO_2 sensitivity		
Hypothesis		Phenotyping with elevated CO_2 in ultra-low oxygen will reveal CO_2 sensitivity and cultivar specific symptoms		
Team	Months	Milestone	Anticipated Product(s)	Progress/Changes
DR,JM, IH	12	Multi-cultivar harvest and storage experiment	A phenotyping protocol for high CO_2 stress, an assessment of CO_2 sensitivity of multiple cultivars, a catalog of CO_2 related postharvest peel and flesh disorders (Added to WSU defect guide when novel).	
DR,JM, IH	24	Re-test cultivars with no sensitivity	A phenotyping protocol for high CO_2 stress, an assessment of CO_2 sensitivity of multiple cultivars, a catalog of CO_2 related postharvest peel and flesh disorders (Added to WSU defect guide when novel).	
DR,JM, IH	36	Re-test cultivars with no sensitivity Evaluate use of sealable plastic bags to induce CO_2 injury.	A phenotyping protocol for high CO_2 stress, an assessment of CO_2 sensitivity of multiple cultivars, a catalog of CO_2 related postharvest peel and flesh disorders (Added to WSU defect guide when novel). An easy protocol using of modified atmospheres to induce injury to estimate CO_2 sensitivity will be written.	

Objective		2: Determine best cold chain practices when CO_2 sensitivity is indicated		
Hypothesis		Evaluation under multiple CO_2 and O_2 levels will determine relative sensitivity and acclimation experiments the means to avoid symptoms.		
Team	Months	Milestone	Anticipated Product(s)	Progress/Changes
	12	No work planned		
DR,JM	24	Evaluation of the extent of CO_2 sensitivity of sensitive cultivars.	Assessment of degree of CO_2 sensitivity of CO_2 sensitive cultivars.	
DR,JM	36	Evaluation of the extent of CO_2 sensitivity of sensitive cultivars. Tested protocols for reducing CO_2 injury in the most sensitive cultivars.	Assessment of degree of CO_2 sensitivity of CO_2 sensitive cultivars. Protocols for reducing CO_2 injury of sensitive cultivars.	

Objective		3: Indicate chemistry associated with CO_2 sensitivity.		
Hypothesis		Elevated CO_2 and existing CO_2 injury will have a diagnostic chemical "fingerprint" different from that of other disorders.		
Team	Months	Milestone	Anticipated Product(s)	Progress/Changes
	12	No work planned		
DR	24	Determine peel and flesh chemistry specifically linked with CO_2 sensitivity and CO_2 injury.		
DR	36	Determine peel and flesh chemistry specifically linked with CO_2 sensitivity and CO_2 injury.	A chemical "fingerprint" for CO_2 disorders that can be used to support a diagnosis.	

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

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	2020
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	0

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/Timothy Palacios
Telephone: (509) 335-2885/(509)786-9204
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 MS 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 MS 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, all five facilities enlisted in the study have been sampled once quarterly over the course of 2018-2019, representing the packing season for the 2018 crop. A total of 1,498 samples have been analyzed to date (749 after sanitation, prior to packing and 749 in within 4 hrs of beginning packing). We are currently in the process of gathering the second years' worth of data and should complete the project with n=2,996.
- 2.3% of samples have tested positive for *Listeria* spp., with the 0.5% coming post-sanitation and 1.8% isolated in-process (within 4 hrs of packing startup).
- The unit operations of sorting and oven drying were associated with 73% of *Listeria* spp. positives.
- Positives were only obtained in the 3rd and 4th quarter of sampling.

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. Five packinghouses have been enlisted into the study and have been sampled once quarterly during packing season for a total of eight data collection points per facility (Figure 1).

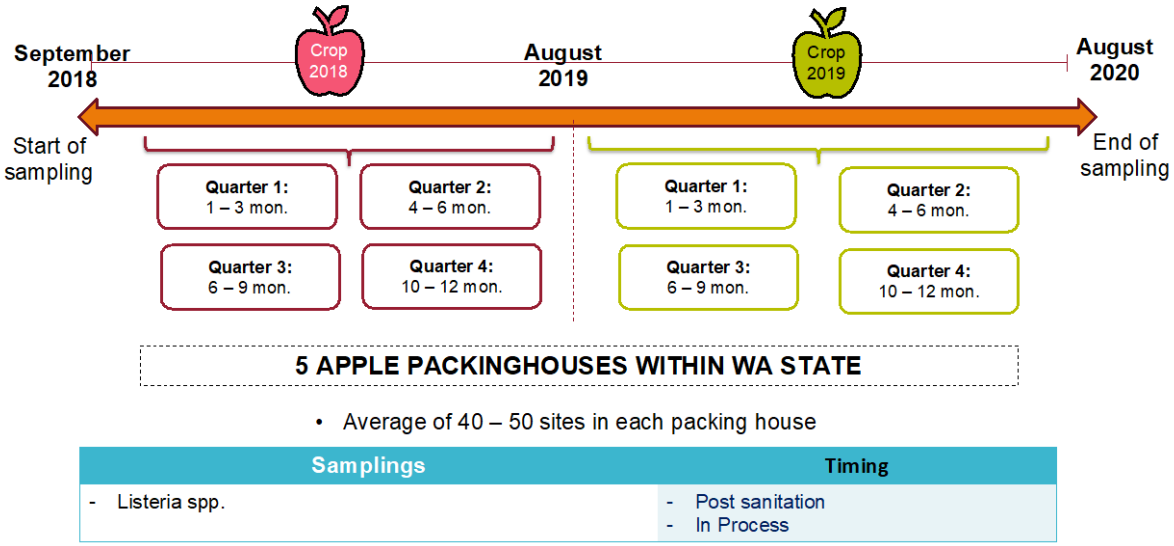


Figure 1. *Listeria* species sampling overview of apple packinghouses for the 2018 and 2019 apple crop.

Surface sampling methods. Sampling was coordinated to occur both after a sanitation (post sanitation) event and within 4 hrs of startup (in-process) 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.

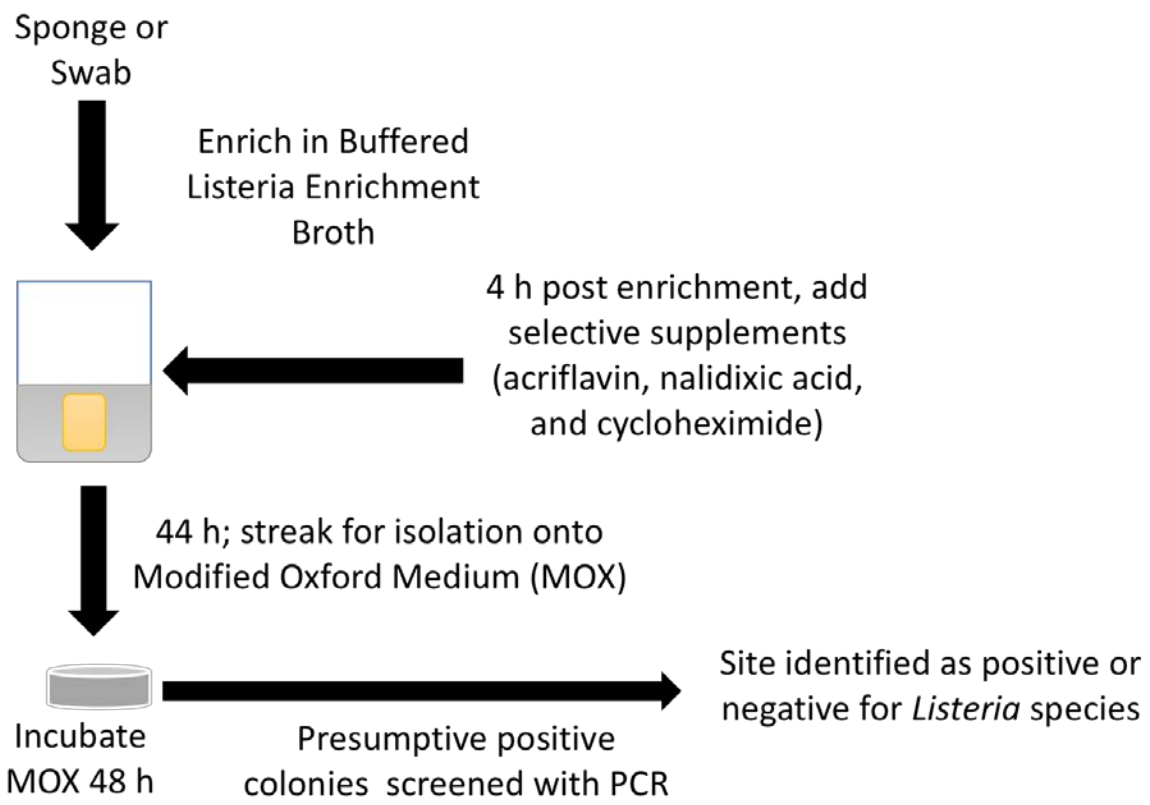


Figure 2**.** Sample processing to determine presence or absence of *Listeria* species (environmental indicator for *L. monocytogenes*).

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.

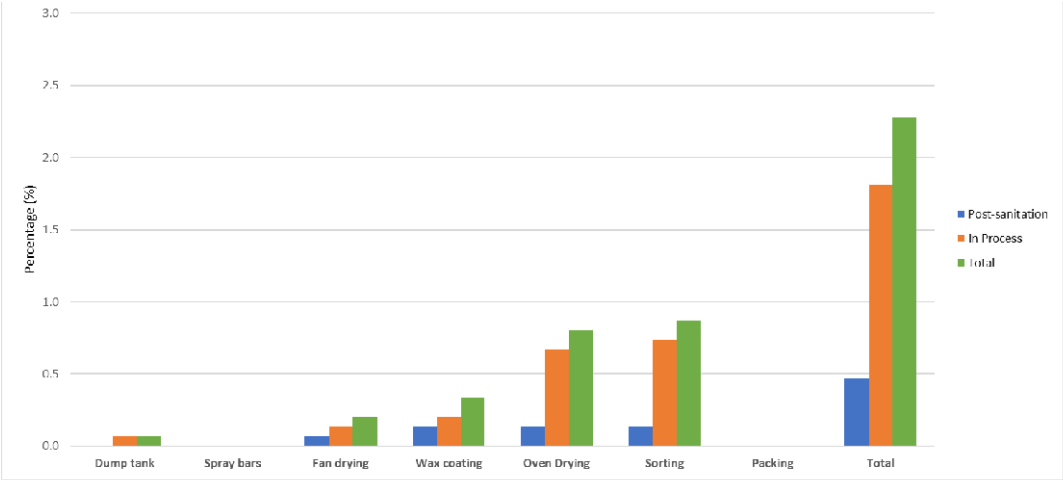
For instances where the population of attached *Listeria* species are below the level of detection, the remaining BPW Tween 20 rinsate will be enriched as described in objective one to determine the presence or absence of *Listeria* species.

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 34 samples tested positive for *Listeria* spp., seven post-sanitation and 27 in-process. This represents a prevalence of 2.3% amongst all samples, with 0.5% post- sanitation and 1.8% in-process (Figure 3). It is important to note that statistical analysis has not been conducted, but

thus far, but the surfaces associated with the sorting and oven drying unit operations tested positive most frequently, 38.2% and 35.3%, respectively, amongst all *Listeria* spp. positive samples. Sites associated with waxing comprised 14.7% of *Listeria* spp. positives, with fan drying (8.8%) and dump tank samples (2.9%) rounding out the breakdown of positive sites by unit operation. It is important to note that no sites associated with spray bars (primarily brush rollers) or packaging unit operations were positive in this first year; however, it is important to consider that the study is incomplete and trends may change across crop years.



	Dump tank	Spray bars	Fan drying	Wax coating	Oven Drying	Sorting	Packing	Total
Post-sanitation	0%	0%	0.1%	0.1%	0.1%	0.1%	0%	0.5%
In Process	0.1%	0%	0.1%	0.2%	0.7%	0.7%	0%	1.8%
Total	0.1%	0%	0.2%	0.3%	0.8%	0.9%	0%	2.3%

Figure 3. Percent of samples positive for *Listeria* spp. post-sanitation, in-process, and total.

The 3rd and 4th quarters represented all of the *Listeria* spp. positive sites in year one demonstrating a strong association with storage time (Figure 4). Once more, these data are preliminary, and it is important to see if similar trends are observed across both years of sampling.

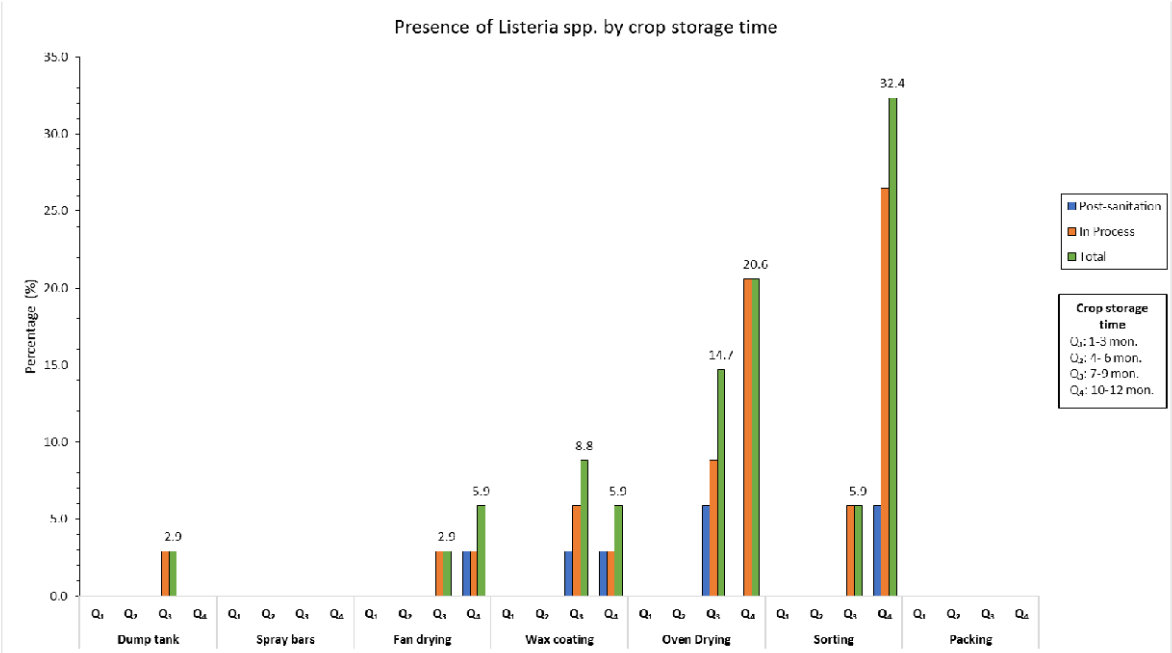


Figure 4. Percent of *Listeria* spp. positive samples by unit operation and quarter. of samples positive for *Listeria* spp. post-sanitation, in-process, and total. Value above bars represents the percent positive in the cumulative sum of post-sanitation and in-process samples.

Once data collection concludes for objective 1 (August 2020), statistical analysis will be conducted to determine if significant differences exist across unit operations, materials, and time of sampling and isolation of *Listeria* spp. This information will inform objectives 2 and 3.

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

Project Title: Critical limits for antimicrobials in dump tank systems

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Cooperators: WA packinghouses (TBD)

Total Project Request: Year 1: $86,183 **Year 2**: **$93,414** Year 3: $8,660

Other funding sources: None

Budget 1
Organization Name: Washington State University
Contract Administrator: Tim Palacios
Telephone: (509)786-9204
Email address: prosser.grants@wsu.edu

Item	2019	**2020**	2021
Salaries	38,245	39,775	
Benefits	2,538	2,639	
Supplies	42,000	50,000	8,660
Travel	3,400	1,000	
Miscellaneous			
Plot Fees			
Total	86,183	**93,414**	8,660

Footnotes:

Salaries: In year 1, $38,245, and year 2, $39,775, is requested for a Graduate Research Assistantship for a MS student to work on all objectives.

Benefits: $2,538 and $2,639 are requested for benefits tied to the Graduate Research Assistantship for a MS student to work on all objectives for years 1 and 2, respectively.

Supplies: Supply costs of $42,000 in year 1, $50,000 in year 2 and $8,660 in year 3 are requested to pay for disposable supplies such as glassware, microbiological media, pipettes, water attribute measurement instrumentation and calibration standards, and water makeup analysis.

Travel: $3,400 and $1,000 is requested in years 1 and 2, respectively, 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. Increased travel costs in year 1 are associated with cost of traveling to participating facilities to collect water samples associated with objective 1.

Objectives:

4. Establish the carbohydrate, protein, and mineral makeup of dump tank water during production in addition to the attributes of chemical oxygen demand (COD), temperature, pH, oxidation reduction potential (ORP), turbidity, and conductivity.
5. Determine the impact of free chlorine, peroxyacetic acid, chlorine dioxide or ozone concentration on the survival of Shiga toxigenic *E. coli, Salmonella*, or *Listeria monocytogenes* over time in water which has the similar composition as water evaluated in objective 1 and is representative of water chemistries observed throughout production in dump tank systems.

Significant Findings

- Chemical Oxygen Demand (COD) has been shown to be very effective parameter for predicting sanitizer efficacy in postharvest wash systems in other commodities.
- Mean COD value (preliminary data) was 586 mg/L, with considerable variation amongst sites and over time.

Methods

Objective 1. Establish the carbohydrate, protein, and mineral makeup of dump tank water during production in addition to the attributes of chemical oxygen demand (COD), temperature, pH, oxidation reduction potential (ORP), turbidity, and conductivity.

Packinghouse selection and descriptions. Three commercial apple packinghouses were recruited into the study which encompass different industry management practices for managing flumes. One packinghouse has a single flume which is used up to 68 hr or until water changeover is needed (e.g. conventional to organic break). There is no filtration within the system. The second packinghouse has a single flume up to 68 hr or until water changeover is needed (e.g. conventional to organic break). There is a flocculation system installed. The third packinghouse utilizes two flumes, the first is used for the same duration as the first two packinghouses and has a filtration system installed. The secondary downstream flume is utilized for up to 10 days and also has a filtration system installed. The initial plan of work scoped data collection for only the 24hr of production. To encompass the full period water will be used within the flumes, sampling periods were changed as shown in figure 1. Timing will be set to occur throughout the packing season to encompass natural differences which occur as apples are held in storage. The first sampling event for all packinghouses occurred November-December. Production variables such as additives to the flume system (e.g. acid, antimicrobials), flume capacity, varieites packed, storage conditions, % culls, line speed, will be provided by the packinghouse and noted for each data collection period.

Water sample collection. Two 500 mL water samples will be taken at time points shown in figure 1 at a consistent location from the dump tank/flume. These samples will be used to determine the carbohydrate, protein, and mineral content in addition to the real-time water quality parameters of chemical oxygen demand (COD), oxidation reduction potential (ORP), conductivity, pH, turbidity, and temperature. All samples will be held at 4°C (39.2°F) if not analyzed in real-time.

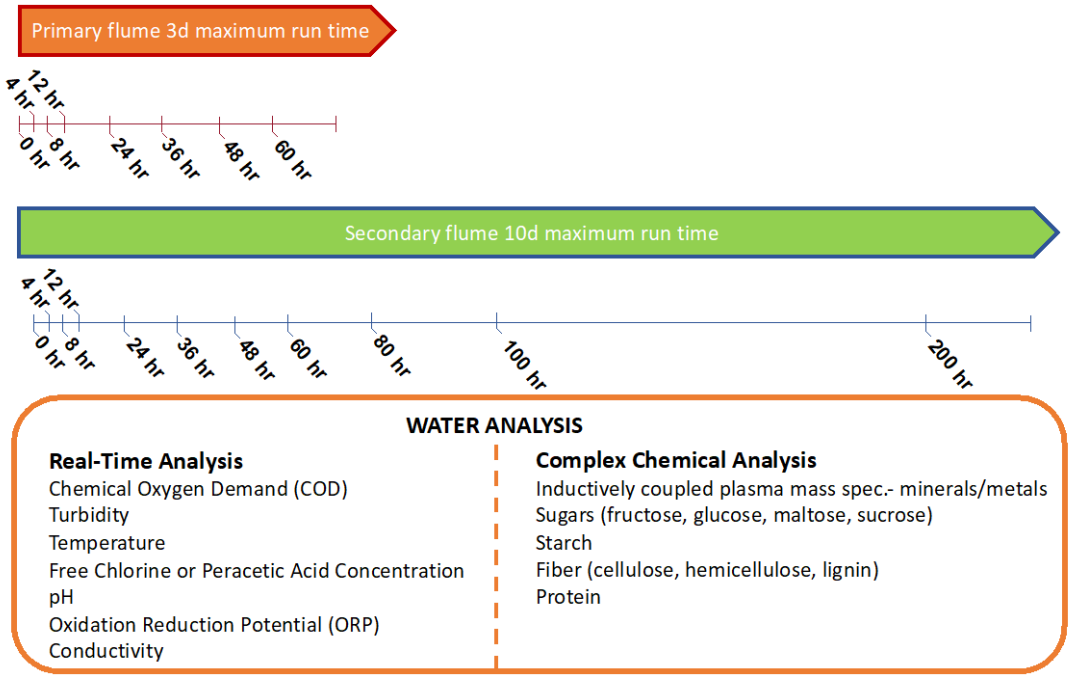

Figure 1. Timing of water sample collection and list of real-time and complex chemical analyses which will be evaluated.

Establishing carbohydrate, protein, and mineral makeup of dump tank. Samples will be shipped overnight for analysis with Merieux Nutrisciences. Target analytes will be as follows: carbohydrates [simple sugars (fructose, glucose, maltose, sucrose), starch, and fiber (pectin, cellulose, and hemicellulose)], protein, and minerals (calcium, iron, magnesium, phosphorus, potassium, and sodium). Based upon outcomes from the first replication, certain analytes may be discontinued if they consistently are below the limit of detection for the analyses.

Quantifying water chemistry attributes of dump tanks. Chemical oxygen demand is calculated using a reactor digestion method with colorimetric quantification *(4)* using the Hach DRB200 Reactor and DR900 multiparameter colorimeter. The colorimeter will also be used to measure sample turbidity. A multiparameter meter (Hach probe model 5048) will be used to determine pH, ORP, conductivity, and temperature in real time during collection.

Statistical analysis. A completely randomized design with repeated measures will be used to evaluate significant differences of water attributes and nutritional compounds.

Objective 2. Determine the impact of free chlorine, peroxyacetic acid, chlorine dioxide or ozone concentration on the survival of Shiga toxigenic *E. coli* (STEC), *Salmonella*, or *Listeria monocytogenes* over time in water which has the similar composition as water evaluated in objective 1 (year 2).

Water composition. Water quality measurements used in this part of the study will be developed to represent standard features of washwater used in packinghouses in Washington. Three variations of dump tank water quality will be used to represent postharvest water quality features

which will be inclusive of real-life conditions as determined by objective 1. The parameters described in objective one will also be determined for this objective.

Microbial cultures. A five-strain cocktail of STEC, *Salmonella*, and *L. monocytogenes* associated with an outbreak will be used for this objective. Bacterial strains to be used are as follows: STEC cocktail [O145 (Romaine outbreak), O104 (2011 European outbreak), O111 (apple juice outbreak), O103 (venison outbreak), and O157 (spinach outbreak)]; *Salmonella* cocktail [Agona (alfalfa sprout outbreak), Montevideo (tomato outbreak), Gaminara (orange juice outbreak), Michigan (cantaloupe outbreak), and Saint Paul (pepper outbreak)]; *L. monocytogenes* cocktail [390-1 (cantaloupe outbreak), 390-2 (cantaloupe outbreak), 1452 (caramel apple outbreak), 108 (hard salami outbreak), 310 (goat cheese outbreak)]. Each strain of Shiga-toxigenic *E. coli* and *Salmonella* will be individually grown in Tryptic Soy Broth (TSB) at 37°C (98.6°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 37°C (98.6°F) for 24 h to achieve a lawn of each strain. Each plate will be flooded with 10 ml of Buffered Peptone Water (BPW) to harvest cells. *E. coli* strains will be combined in equal volumes to create the five-species cocktail for inoculation. The same process will be used for *L. monocytogenes*, with the exception that each strain will be individually grown in Tryptic Soy Broth with Yeast Extract (TSBYE) at 32°C (89.6°F).

Sanitizer concentration. Four concentrations plus a no sanitizer control will be evaluated for each compound. The upper limit will be based upon EPA label (chlorine, PAA or chlorine dioxide) or 1 ppm for ozone (which does not have an EPA label as it is an EPA registered device). When determining the efficacy of chlorine, as per industry practice, the pH of the system will be maintained at 6.5 with addition of citric acid.

Determining impact of sanitizers on pathogen survival. Simulated washwater treatments will be inoculated and bacteria enumerated to estimate survival after 30 seconds of exposure. All samples will be neutralized with sodium thiosulphate to arrest sanitizer activity, after which they will be serially diluted and plated onto TSA or TSAYE and incubated at 37°C (98.6°F; STEC and *Salmonella*) and 32°C (89.6°F; *L. monocytogenes*) for 24 h to enumerate surviving bacteria.

Statistical analysis. The experiment will be independently replicated three times with four technical replicates (n=12) for reach sanitizer concentration evaluated. A completely randomized design with analysis of variance (ANOVA) will be conducted. Post-hoc analyses will also be conducted to determine significant differences between survival rates between and within treatments. Additionally, mean inactivation rates will be calculated using the formula shown below:

$$\%\, inactivation = \left(\frac{original\ population - population\ at\ 30\ s}{original\ population}\right) x\ 100$$

Expected outcomes. Concentrations for each sanitizer will be determined which result in rapid inactivation of pathogens in water which has similar properties to that observe during production. This will provide supporting documentation for apple packinghouses to substantiate minimum concentrations of each compound. This is especially important with the focus of HACCP-

approaches for managing food safety risks which require critical limits (minimum concentrations of sanitizers) to be specified for dump tank systems to mitigate the risk of cross-contamination.

Results and Discussion

Mean, minimum and maximum values obtained for real-time measurements for the first replicate of objective 1 are presented in Table 1. Given the natural variation amongst the dataset, it is important not to over analyze any values given that they may vary considerably, and thus require furture replication amongst sites to determine mean values for parameters such as COD, which can be utilized to determine parameters utilized in objective 2. The coming year will be focused on completing objective 1 (Feb-June) and initiating work associated with objective 2 (June-Dec).

Table 1. Preliminary results (mean, minimum value, maximum value) for first replicate.

	pH	**ORP (mV)**	**Conductivity (µS/cm)**	**Temperature °C (°F)**	**Turbdity (FAU)**	**COD (mg/L)**	**PAA (ppm)**	**Free Chlorine (ppm)**
mean	4.69	507.3	368.4	19.5 (67.2)	82.1	586.3	77.8	11.5
min	2.55	194.3	104.8	11.7 (53.0)	0.0	19.0	2.0	3.5
max	7.38	734.0	791.0	30.2 (86.0)	157.0	1950.0	150.0	18.2

Thus far, all complex chemical analyses have been returned below the limit of detection for the assay, with the exception of ICP-MS, which has several minerals above the limit of detection. Once the last dataset is received for the first replicate, the research team will analyze results and determine if it is most cost effective to just continue with ICP-MS and forgo carbohydrates [simple sugars (fructose, glucose, maltose, sucrose), starch, and fiber (pectin, cellulose, and hemicellulose)], and protein analysis.

Citations

1. Allende, A., M. V. Selma, F. Lopez-Galvez, R. Villaescusa, and M. I. Gil. 2008. Impact of wash water quality on sensory and microbial quality, including Escherichia coli cross-contamination, of fresh-cut escarole. *Journal of Food Protection*. 71:2514-2518.

2. Buchanan, R., S. Edelson, R. Miller, and G. Sapers. 1999. Contamination of intact apples after immersion in an aqueous environment containing Escherichia coli O157: H7. *Journal of Food Protection*. 62:444-450.

3. Goverd, K., F. Beech, R. Hobbs, and R. Shannon. 1979. The occurrence and survival of coliforms and salmonellas in apple juice and cider. *Journal of Applied Bacteriology*. 46:521-530.

4. Jirka, A. M., and M. J. Carter. 1975. Micro semiautomated analysis of surface and waste waters for chemical oxygen demand. *Analytical chemistry*. 47:1397-1402.

CONTINUING PROJECT REPORT **YEAR**: No-cost Extension

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

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

WTFRC Budget:

Item	2018	2019	2020
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	**0**

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: Tim Palacios
Telephone: (509)786-9204 **Email address:** prosser.grants@wsu.edu

Item	2018	2019	2020
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	**0**

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

- Rapid tests are not suitable for predicting microbial loads on food contact surfaces.
- Rapid tests are useful to assess residual matter and allow for re-cleaning of equipment.
- Cleaning and sanitation practices should focus on both wet and dry areas of apple packinghouses.
- To validate sanitation practices, traditional microbiological methods are still needed.

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. Five packinghouses were enlisted into the study and were sampled once a quarter during packing season (October 2018-August 2019). The facility was diagramed, and sampling points have been predetermined in all six facilities. Table 1 describes the types of surfaces sampled within each unit operation.

Table 1. Examples of food contact surfaces tested at each unit operation

Area	Unit operation	Sample sites (Food contact surfaces)
Wet	Washing (Dump tank)	Dump tank, rollers, traction belting, brushes under the rot blaster
	Washing/Sanitizing/Rinsing (Brush beds)	Brush rollers, bristle rollers, Teflon tapes, plastic flaps
	First drying (Fan and/or blower)	Brush rollers, metal dividers, plastic flaps
	Wax coating	Brush rollers, rubber flaps
	Second drying (Tunnel drier)	Foam rollers, bristle rollers, Teflon tapes, rubber flaps
Dry	Sorting	Rollers, foam rollers, bristle rollers, brush rollers, sorter cups, cup-droppers, rubber flaps, interlocking belts, belts, Teflon tapes, guide rails
	Packing	Packing tables, belts, rubber flaps, plastic flaps, Teflon tape, guide rails

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).

Statistical analysis. Data analysis was carried out using Minitab software (version 19). APC, *Enterobacteriaceae,* coliforms, *E. Coli,* and ATP values were normalized using log transformation. To identify the correlation between populations of indicator organisms (APC, *Enterobacteriaceae,* coliforms , and *E. Coli*) with RLU values, Pearson correlation coefficient (r) was determined. A Student's *t* test was performed for pairwise mean comparisons of the different populations of indicator organisms with the scores of Glucose/Lactose residue swabs (Pass or Fail); populations of indicator organisms with the detection of *Listeria* spp. (Positive or Negative), and rapid tests with the detection of *Listeria* spp. (Positive or Negative). Tukey test was used for multiple mean comparisons of populations of indicator organisms (APC, *Enterobacteriaceae,* coliforms and *E. Coli*) and RLU values throughout unit operations with $\alpha = 0.05$.

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.

Alterations to original design of experiments. Unfortunately, no significant correlations were obtained for any indicator and rapid test, highlighting the fact that rapid tests cannot be utilized to supplant microbiological testing.

Results and Discussion

Populations of indicator organisms throughout unit operations. As shown in Table 2, the highest populations recovered were from APC, followed by, in order of population size, *Enterobacteriaceae*, coliforms, and *E.coli*. APC, *Enterobacteriaceae* and coliforms populations were statistically different at the different unit operations. For APC, the wax coating and tunnel drying unit operations showed significantly higher mean values than the washing step. However, regarding *Enterobacteriaceae* and coliform populations, the highest mean values were obtained only in the wet area (Table 2).

Association between RLU values of the ATP test with CFU values of populations of indicator organisms. Table 3 summarizes the Pearson correlation coefficients (r) of RLU values between the different populations of indicator organisms ($r < 0.01$). No statistically significant association was found.

Table 2. Mean of populations of indicator organisms at each unit operation

Unit operation	n[A]	**Mean ± Std Dev of indicator organism populations** (Log CFU/100 cm^2)			
		APC	*Enterobacteriaceae*	Coliforms	*E. coli*
Washing	70	2.74 ± 1.18 (b)[B]	1.59 ± 1.48 (a)	1.36 ± 1.32 (ab)	0.23 ± 0.46 (a)
Washing/sanitizing /rinsing	79	2.83 ± 1.19 (ab)	1.56 ± 1.29(a)	1.41 ± 1.27 (a)	0.19 ± 0.35 (a)
Fan drying	75	2.94 ± 1.08 (ab)	1.31 ± 1.22 (ab)	0.91 ± 1.09 (bcd)	0.27 ± 0.51 (a)
Wax coating	50	3.29 ± 0.88 (a)	1.34 ± 1.26 (ab)	0.96 ± 1.14 (abcd)	0.17 ± 0.39 (a)
Tunnel drying	85	3.21 ± 0.83 (a)	1.45 ± 1.18 (a)	1.05 ± 1.13 (abc)	0.19 ± 0.40 (a)
Sorting	302	2.98 ± 0.80 (ab)	0.90 ± 0.98 (b)	0.61 ± 0.90 (d)	0.18 ± 0.43(a)
Packing	80	2.99 ± 0.74 (ab)	0.85 ± 0.99 (b)	0.82 ± 0.94 (cd)	0.31 ± 0.60 (a)

[A] Number of samples
[B] Means within a column followed by different letters are significantly different ($p \leq 0.05$)

Table 3. Pearson coefficient correlation between populations of indicator organisms (Log CFU/100 cm^2) with ATP test (Log RLU/100 cm^2)

Indicator Organism	**R^2 (Pearson coefficient)**	**p-value**
Aerobic Plate Count	0.010	0.011
Enterobacteriaceae	0.003	0.158
Coliforms	0.001	0.373
E. coli	0.011	0.009

ATP and Glucose/Lactose residue swab readings throughout unit operations. The obtained readings for ATP and glucose/lactose residue swabs on the different food contact surfaces are described by unit operation in Table 4. Concerning the ATP rapid test, the sorting and packing steps, both part of the dry area, showed the lowest and highest RLU mean values respectively. The results for the glucose/lactose residue tests were expressed as percentages of “fail” or “pass” for hygiene surfaces. The unit operations that presented the greatest percentage of “failed” surface hygiene were sorting and packing. Unlike the ATP test, the wet area showed more “pass” results when Glucose/lactose swabs were tested.

Association of the Glucose/Lactose residue test with different populations of indicator organisms. The APC population was significantly higher when the test for surface hygiene failed. The population dropped significantly to reach a passing level on this test (Table 5). However, the test did not detect significant differences in the populations of *Enterobacteriaceae*, coliforms, and *E. coli* populations with failing and passing scores.

Table 4. Rapid test readings at each unit operation

Unit operation	**n**[A]	**ATP test**	**Glucose/ Lactose residue test**	
		Mean ± Std Dev (Log RLU/100 cm^2)	**% Pass**	**% Fail**
Washing	59	2.28 ± 0.83 (ab)[B]	66.1	33.9
Washing/sanitizing /rinsing	83	2.27 ± 0.70 (ab)	63.9	36.1
Fan drying	75	2.09 ± 0.69 (ab)	60.0	40.0
Wax coating	51	2.38 ± 0.81 (ab)	52.9	47.1
Tunnel drying	78	2.19 ± 0.78 (ab)	38.5	61.5
Sorting	236	2.08 ± 0.97 (b)	22.9	77.1
Packing	77	2.48 ± 0.86 (a)	27.3	72.7

[A] Number of samples
[B] Means within a column followed by different letters are significantly different ($p \leq 0.05$)

Table 5. Association between indicator organism populations with Glucose/Lactose residue test

Mean ± Std Dev of indicator organism populations (Log CFU/100 cm^2)			
Indicator organisms	Pass (n=269)	Fail (n=390)	p-value
Aerobic Plate Count	2.91 ± 1.06	3.08 ± 0.84	0.031*
Enterobacteriaceae	1.25 ± 1.26	1.13 ± 1.13	0.219
Coliforms	0.98 ± 1.15	0.89 ± 1.08	0.341
E. coli	0.20 ± 0.42	0.19 ± 0.42	0.865

*Significant difference ($\alpha < 0.05$)

Association between traditional detection of Listeria spp. and rapid tests. Table 6 shows that ATP test readings were not statistically different when comparing both positive and negative detections of *Listeria* spp. ($p > 0.05$). Regarding Glucose/Lactose swabs, the percentage of sites that presented a "pass" result was higher (66.7%) than the percentage of sites with a "failed" result (33.3%), where *Listeria* spp. were detected as positive. However, it is important to consider that the number of positive samples for *Listeria* spp. was low (n=7).

Association between traditional detection of Listeria spp. and populations of indicator organisms. Table 6 also shows that mean populations of APC, *Enterobacteriaceae*, coliforms, and *E. coli*, were not statistically different when comparing both positive and negative detections of *Listeria* spp. ($p > 0.05$). However, it is important to consider that the number of positive samples for *Listeria* spp. was low (n=7).

Table 6. Association between indicator organism populations, and ATP test with the detection of *Listeria* spp.

Detection of Listeria spp.	**n[A]**	**Mean ± Std Dev**				
		Indicator organisms (Log CFU/100 cm^2)				**ATP (Log RLU/100cm^2)**
		APC	*Enterobacteriaceae*	Coliforms	*E.coli*	
Positive	7	3.08 ± 1.39	1.41 ± 1.35	1.18 ± 1.10	0.08 ± 0.00	2.64 ± 0.72
Negative	740	2.99 ± 0.92	1.16 ± 1.17	0.88 ± 1.09	0.21 ± 0.44	2.21 ± 0.85
p-value		0.867	0.568	0.466	0.443	0.222

[A] Number of samples

One of the objectives of this study was to evaluate the prevalence of APC, *Enterobacteriaceae*, coliforms, and *E. coli* at the different unit operations within an apple packinghouse after sanitation procedures. For APC populations, means varied from 2.74 to 3.29 Log CFU/100 cm^2. Unit operations in both wet and dry areas showed significant higher counts of this indicator organism. In previous studies, where food contact surfaces were evaluated after cleaning methods, similar values of APC mean populations were found. APC mean counts of 3.48 Log CFU/100 cm^2 and 2.82 Log CFU/100 cm^2 were obtained in a facility that processes fresh-cut carrots and lettuce (Lehto et al., 2011) and raw vegetable preparation surfaces in a university canteen (Osimani et al., 2014), respectively.

The lower mean values obtained after the washing/sanitizing/rinsing step for *Enterobacteriaceae* populations, except for the tunnel drying unit operation, could be explained by the fact that bacteria belonging to the *Enterobacteriaceae* family, which are part of the regular microflora on apples (Wassermann et al., 2019), are easily inactivated by chemicals used for sanitation purposes (Kornacki et al., 2015). Because coliforms and *E.coli* populations represent sub-populations of the larger *Enterobacteriaceae* population, the total *Enterobacteriaceae* population is expected to be higher than either of the sub-populations (Baylis et al, 2011). This result was also found in this study.

The total coliforms test included the detection of generic *E.coli* on the same petrifilm plate as an indicator of fecal contamination. The interpretation of lower coliform populations after the second drying might be attributed to the removal, within the wet area, of potential sources of coliforms that come with the fruit from the orchards. Regarding *E.coli*, population means were meager throughout all the unit operations (0.17 to 0.31 Log CFU/100 cm^2). *E. coli* is highly related to and used as an indicator for water contamination, and in spite of all the tested packinghouses using recirculated water in the dump tank, no higher population was found at this unit operation (the washing step). The use of sanitizers such as Chlorine and PAA explains this result (Pietrysiak et al., 2019). Similar results were obtained by Ailes et al., (2008), who evaluated microbial concentrations on different types of produce during post-harvest processing.

Moore (2003) conducted a review of microbiological limits for acceptable general microbial indicator counts on food contact surfaces. Results for an “appropriate” hygienic surface ranged from < 2.3 to 5 Log CFU/100 cm^2 for different types of industries. No specifications for the fresh produce industry were available. It is important to consider that to date, no institution or regulatory agency provides specific standards to define acceptable levels of microbial loads on food contact surfaces. Results of counting colonies techniques could vary depending on the sampling method employed, type of product that has been processed, and the stage at which the samples have been taken. Therefore, counts of indicator organisms are suggested for trend analysis for samples that are taken

under the same conditions. It is recommended that each facility construct its own thresholds for accepting or rejecting the cleanliness of a surface based upon target standards obtained after a validated sanitation procedure that has been duly and fully performed (Blackburn, 2003; Forsythe, 2000).

Another objective of this research was to evaluate the correlation between rapid tests with populations of indicator organisms and the detection of *Listeria* spp. The lack of association observed between detection of indicator organisms via the ATP test and the actual populations could be attributed to different factors. ATP is very sensitive to low levels of residual matter on a surface. However, it is not capable of distinguishing if the contamination on the surface originates from microbial or non-microbial sources (Moore, 2003). The amount of ATP varies based upon the type of microorganisms present on the surface. From different studies, bacteria, yeast and fungal spores have shown different amount of ATP (Shama and Malik, 2013). Furthermore, other factors such as nutrient level, environmental stress level, and the stage of growth have shown to influence on the amount of ATP present (Betts and Blackburn, 2009; Shama and Malik, 2013). Additionally, ATP amount differs depending on the type of product. Raw fruits and vegetables usually contain a higher amount of ATP compared to dry products (Griffith, 2005). Other factors affecting ATP readings include the use of sanitizers and cleansers, the state of the surface (wet or dry) (Davidson et al., 1999; Green et al., 1999), pH, salts and metal ions that affect the stability of the enzyme luciferase within the reagent of the ATP test (Moore, 2003).In order to establish acceptance limit levels for ATP values, similar factors, as discussed for populations of indicator organisms need to be considered.

The higher results for both rapid tests at the packing unit operation, ATP of 2.48 Log RLU/100 cm^2 and 77% of “failed” hygienic surface for the glucose/lactose swab, may be caused by stickers and labels that get stuck in belts and packing tables, making cleaning procedures harder to perform. Furthermore, the dry area was not cleaned and sanitized as often as the wet area in order to avoid water residues on the dry side of the plant.

Lastly, the lack of correlation between both rapid tests and populations of indicator organisms with the positive detection of *Listeria* spp. is supported by previous data. APC is not considered an indicator of food safety because it does not specify the presence of any pathogen (Ryser and Schuman, 2015). The presence of organisms from the *Enterobacteriaceae* family including coliforms and generic *E. coli* are not suitable to assess the presence of *Listeria* spp., since these species are more resistant to environmental factors than enteric pathogens such as *Salmonellae, Shigella dysenteriae,* or pathogenic *E. coli* (Baylis et al., 2011; Tortorello, 2003).

The results of this study suggest that apple packinghouses use both rapid tests and traditional microbiological methods for indicator organism populations when assessing cleaning and sanitation practices. Rapid tests are valuable for monitoring residual matter on a surface, thus validating the efficacy of cleaning procedures. However, to validate sanitation practices, traditional microbiological methods are still needed. Future studies may include the improvement of dry cleaning and sanitation methods for the dry area.

Citations

Due to page limitations citations have been omitted, but are available upon request from Dr. Critzer

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: AP-18-104

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

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City/State/Zip:	Pullman/WA/99164	**City/State/Zip**:	Yakima, WA 98903

Cooperators: Allan Brothers. Inc., Stemilt Growers LLC., 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[1]	15,340	15,953	16,592
Benefits[1]	1,094	1,137	1,183
Equipment	/	/	/
Supplies[2]	25,500	25,500	25,500
Travel[3]	2,000	2,000	2,000
Miscellaneous [4]	5,000	5,000	5,000
Plot Fees	/	/	/
Total	98,470	101,108	**103,853**

Footnotes:
[1]Researchers'salaries plus benefits.
[2]Bacteria culture media, reagents and consumable supply cost
[3]Travel funds for industrial sampling and experiments.
[4]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. There were 2.9-3.5 and 2.2-2.7 Log reduction of *Listeria innocua* on Granny Smith apples (GSA) after 36 weeks of cold storage under a commercial RA and CA storage environment, respectively.
2. Continuous low dose ozone gas application in CA storage provided an additional 2-Log reduction of *L. innocua* on GSA. Different doses of ozone gas within the tested range (51-87ppb) demonstrated similar bactericidal effects against *Listeria*. However, for GSA, MCP-1 application in CA room slightly decreased antimicrobial efficacy of ozone gas.
3. The resident bacteria on GSA apples remained stable (3.5 – 4.0 log CFU/apple) during the 36-week storage at RA or CA at 33°F. Continuous low dose ozone gas application in CA room significantly decreased resident bacteria in GSA after 24 weeks of storage.
4. The initial population level of indigenous yeast/mold (Y/M) counts of un-inoculated GSA apples was 4.5-5.0 log CFU/apple. The Y/M counts of GSA remained stable during the first 12 weeks of RA and CA. By 24-week of storage and beyond, the Y/M count of GSA stored under RA was significantly more than that of CA room. The Y/M count in GSA of CA with different doses of ozone gas decreased during the first 24 weeks of storage. Nevertheless, the inhibitory effect of ozone was attenuated with prolonged storage time.
5. During 36-week CA storage, continuous low dose ozone gas at 50-87 ppb had no negative influence on fruit firmness, total soluble solids and titratable acidity, as well as internal disorders. However, prolonged continuous low dose ozone gas in CA storage had some impacts on the visual quality of GSA pretreated with MCP-1 in year 2. An additional batch of GSA is underway for 2019-2020 season for additional quality evaluation.

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, innoculation and establishment on apple surface

A 3-strain *L. innocua* cocktail was prepared **via** mixing equal numbers of each respective strain into a 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 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 whirl-pak bags with 10 ml of 0.1% buffered peptone water, hand rubbed to release attached microorganisms, then serial diluted. Appropriate dilutions were plated on agar plates. Plates were incubated at 35˚C (95˚F) for 24 -48h and enumerated manually. Enrichments were done when *L. innocua* levels were 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, apples were sampled and transferred to a sterile Whirl-Pak bag with 10 ml of 0.1% buffered peptone water bag, rubbed to release attached microorganisms, 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.

Fates of *L. innocua* inoculated on GSA apples at 6.0-6.5 Log_{10} CFU/apple level under RA, CA, and CA with different doses of O_3 gas (51-87 ppb) were studied for 2 years.

During 3 weeks of cold storage, *L. innocua* was reduced by 1.0-1.5 Log_{10} CFU/apple on GSA stored in RA, CA, and CA plus different doses of O_3 with a die-off rate of 0.35-0.45 Log_{10} CFU/apple/week (Figure 1). There were 2.9-3.5 and 2.2-2.7 Log reduction of *Listeria innocua* on Granny Smith apples (GSA) over 36 weeks of cold storage under a commercial RA and CA storage environment, respectively (Figure 1). Continuous low dose ozone gas application in CA storage generated about additional 2-Log reduction of *L. innocua* on GSA. Different doses of ozone gas at the tested range (51-87ppb) demonstrated similar bactericidal effects against *Listeria* (Figure 1B). However, MCP-1 application in CA room slightly decreased antimicrobial efficacy of ozone gas ; the population of Listeria in CA+MCP+ High O3 group at 12-24 weeks of storage was significantly higher than those in the CA+ High O3 group (Figure 1B).

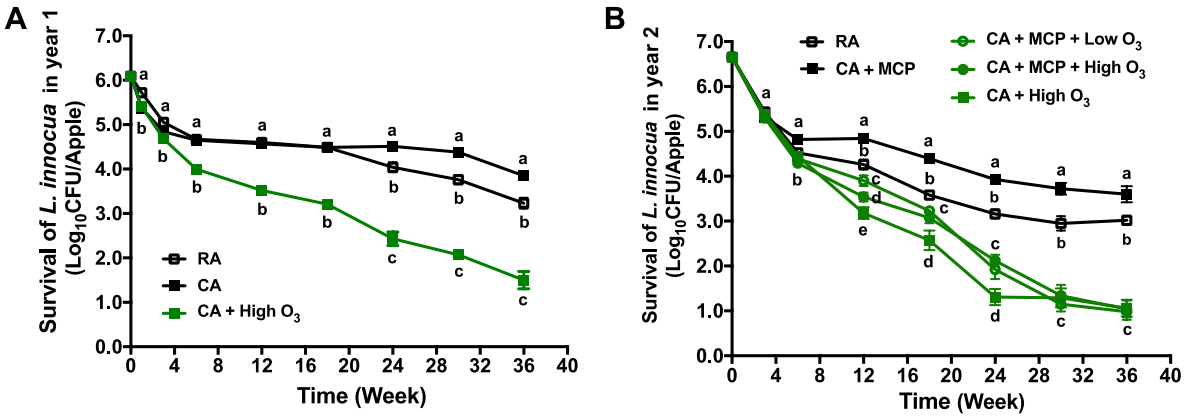

Figure 1. Survival of *Listeria* on Granny Smith apple under commercial cold storages. RA: refrigerated atmosphere (33°F); CA: controlled atmosphere (33°F, 2% O2, 1% CO2). Year1, CA + High O_3: CA with 87 ± 38.8 ppb ozone gas; Year 2, CA + Low O_3; Ozone gas concentration at 51 ± 5 ppb; CA + High O_3: Ozone gas concentration at 68 ± 7 ppb. [a-d]Means within a column within the same sampling point with no common letter differ significantly ($P < 0.05$). Mean ± SD; n=40.

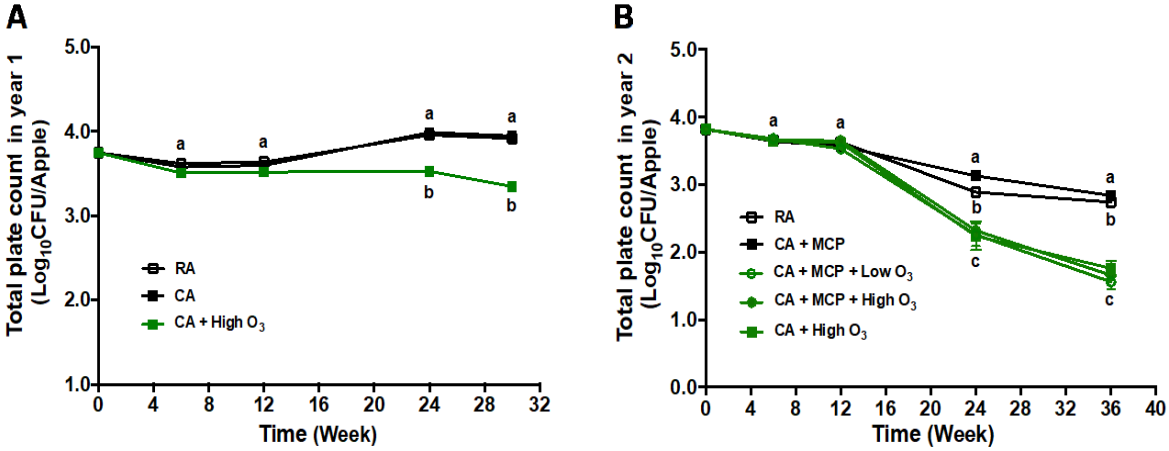

Figure 2. Survival of resident bacteria on Granny Smith apple under commercial cold storages. RA: refrigerated atmosphere (33°F); CA: controlled atmosphere (33°F, 2% O2, 1% CO2). Year 1, CA + High O_3: CA with 87 ± 38.8 ppb ozone gas; Year 2, CA + Low O_3; Ozone gas concentration at 51 ± 5 ppb; CA + High O_3: Ozone gas concentration at 68 ± 7 ppb. [a-d]Means within a column within the same sampling point with no common letter differ significantly ($P < 0.05$). Mean ± SD; n=40.

2. Fates of resident microbiota on GSA apples stored in refrigerated air, controlled atmosphere, and controlled atmosphere with different doses of ozone gas.

Resident bacteria, mold and yeast cause postharvest decay of apples (Janisiewicz and Korsten, 2002), which were assessed during storage. Non-waxed and uninoculated Granny Smith apples were subjected to different storage conditions (RA, CA and CA with different dose of O_3 gas) in the same condition as inoculated apples. Total plate count (TPC) and yeasts/molds (Y/M) count were evaluated at the selected storage durations. The initial background bacterial level of GSA apples was 3.5-4.0 Log CFU/apple, which was relatively stable during 36-week storage at RA or CA (Figure 2). Continuous low dose ozone gas application in CA room significantly decreased resident bacteria in GSA after 24 weeks of storage with more reduction observed in the year 2 study (Figure 2).

The initial level of indigenous yeast/mold (Y/M) counts of un-inoculated GSA apples was 4.5-5.0 log CFU/apple, which remained relatively stable during the first 12 weeks of RA and CA, then gradually increased in apples under RA storage (Figure 3). By 24-week of storage or after, the Y/M count of GSA stored under RA was significantly more than that of CA room (Figure 3). The Y/M count in GSA under CA storage with different doses of ozone gas decreased during first 24 weeks of storage. Nevertheless, the inhibitory effect of ozone was compromised with prolonged storage time (Figure 3).

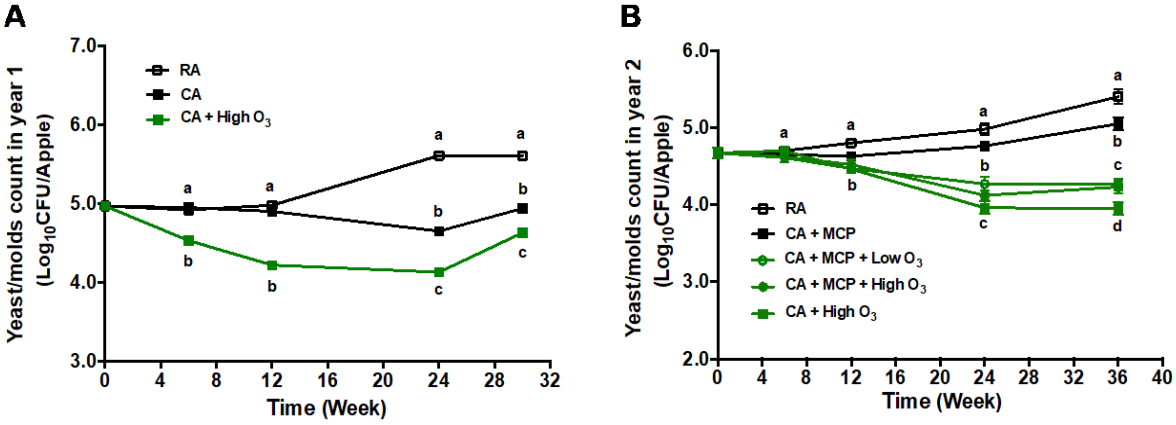

Figure 3. The yeast/mold counts of Granny Smith apple under commercial cold storages. RA: refrigerated atmosphere (33°F); CA: controlled atmosphere (33°F, 2% O2, 1% CO2). Year 1, CA + High O_3: CA with 87 ± 38.8 ppb ozone gas; Year 2, CA + Low O_3; Ozone gas concentration at 51 ± 5 ppb; CA + High O_3: Ozone gas concentration at 68 ± 7 ppb. [a-d]Means within a column within the same sampling point with no common letter differ significantly ($P < 0.05$). Mean ± SD; n=40.

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. During 36-week CA storage, continuous low doses of O_3 gas application at 50-87 ppb had no negative influence on fruit firmness, total soluble solids and titratable acidity (Table 1). Granny Smith apples subjected to RA storage had a significantly lower firmness, total soluble solids and titratable acidity compared with CA with or without gaseous ozone storages (Table 1). The incidence of external and internal disorders was visually evaluated at the end of each storage treatment. Overall, the parameters evaluated for internal disorders were not significantly different among apples stored under RA, CA, or CA with different levels of O_3 gas concentration (Table 2). Continuous low-dose ozone gas application at 87 ± 38.8 ppb in 6-month cold storage did not have a negative influence on external visual quality of Granny Smith apples (Table 3).

Table 1. Fruit quality parameters at harvest and after respective cold storages

Treatment	Weight (g)	Diameter (cm)	Firmness (lbs)	TSS	TA
Year 1 (6 months)					
All (0m)	228.0 ± 14.0	8.08 ± 0.10	16.0 ± 0.3	11.5 ± 0.3	0.710 ± 0.05
RA (6m)	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 (6m)	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+HO_3 (6m)	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]
Year 2 (9 months)					
All (0m)	213.0 ± 33.1	NA	17.9 ± 0.9	11.2 ± 0.2	0.9 ± 0.02
RA (9m)	196.1 ± 32.4[a]	7.87 ± 0.20[a]	8.0 ± 0.5[a]	10.6 ± 0.2[a]	0.4 ± 0.03[a]
CA+MCP (9m)	202.3 ± 30.0[a]	7.62 ± 0.20[a]	18.0 ± 0.2[b]	11.9 ± 0.3[b]	0.8 ± 0.09[b]
CA+MCP+LO_3 (9m)	201.9 ± 21.8[a]	7.62 ± 0.20[a]	17.7 ± 0.1[b]	12.1 ± 0.2[b]	0.8 ± 0.04[b]
CA+MCP+HO_3 (9m)	213.4 ± 33.1[a]	7.87 ± 0.20[a]	18.1 ± 0.1[b]	12.4 ± 0.2[b]	0.9 ± 0.02[c]

TSS: total soluble solids; TA: titratable acidity; Year 1 CA+HO_3: CA with 87 ± 38.8 ppb ozone gas; Year 2 CA+LO_3; Ozone gas concentration at 51 ± 5 ppb; CA+HO_3: Ozone gas concentration at 68 ± 7 ppb. 0 m: at harvest; 6m/9m: after 6/9 months of cold storage. [ab]Means within a column with no common letter differ significantly ($P < 0.05$). Mean ± SD; n=200.

Table 2. Internal disorder analysis for Granny Smith apples

Treatment	Watercore (%)	Internal browning (%)	Cavity (%)
Year 1 (6 months)			
All (0m)	0[a]	0[a]	0[a]
RA (6m)	0[a]	70.2 ± 1.2[a]	0[a]
CA (6m)	5.0 ± 0.5[a]	0[b]	0[a]
CA+HO_3 (6m)	10.0 ± 1.2[a]	0[b]	0[a]
Year 2 (9 months)			
All (0m)	0[a]	0[a]	0[a]
RA (9m)	0[a]	100 ± 0.0[a]	0[a]
CA+MCP (9m)	0[a]	0[b]	0[a]
CA+MCP+LO_3 (9m)	0[a]	0[b]	0[a]
CA+MCP+HO_3 (9m)	0[a]	23 ± 39.0[b]	0[a]
CA+HO_3 (9m)	0[a]	20 ± 24.0[b]	0[a]

Year1 CA+HO_3: CA with 87 ± 38.8 ppb ozone gas; Year 2 CA+LO_3; Ozone gas concentration at 51 ± 5 ppb; CA+HO_3: Ozone gas concentration at 68 ± 7 ppb. 0m: at harvest; 6m/9m: after 6/9 months of cold storage. [ab]Means within a column with no common letter differ significantly ($P < 0.05$). Mean ± SD; n=200.

Table 3. External disorder 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.

However, year 2 study further indicated that 9-month of continuous low dose ozone gas application CA storage had no negative impacts on all external visual quality attributes of GSA pretreated with MCP-1 except ozone burn (Data not shown). An additional batch of GSA is underway during 2019-2020 season for additional quality evaluation. In addition, we are conducting additional studies to discern interactions among apple varieties, storage time, low ozone gas and MCP-1 application using different apple varieties.

3. Conclusion

Continuous low doses of ozone gas application delayed decay microbial growth and provided additional antimicrobial efficacy against Listeria on fresh GSA surfaces over 9-month of CA storage and had no negative influence on the apple fruit quality. Ozone gas at test concentration ranges had similar antimicrobial efficacy against *Listeria* as well as resident microbiota.

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**: No-cost extension

Project Title: Complying with the FMSA preventive controls for human food rule

PI: Girish M. Ganjyal
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Email: girish.ganjyal@wsu.edu
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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 (Stemilt Growers, Double Diamond Fruit, Borton Fruit, Crane and Crane, Allan Brothers, Kershaw, Washington Fruits, Cowiche, Blue Bird, McDougall & Sons Inc, Columbia Reach)

Total Project Request: **Year 1:** $48,711 Year 2: $50,260
No-Cost Extension Requested

Other funding sources: WSDA-SCBG program
Amount:
Agency Name: WSDA
Notes: 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.

WTFRC Budget: ***(If no WTFRC expenses are anticipated, type none and delete table)***

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

Item	2017	2018
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

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 the 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.

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 a model food safety plan for the apple packing process.

The specific objectives of the proposal are as detailed below:

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

SIGNIFICANT FINDINGS

Assessment of current packing facility practices:

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

Food safety model plan development:

- **The model food safety plan for the apple packing house was developed.**
- The observations of the current practices from the first objective were incorporated into this plan.
- The model food safety plan was shared, explained and discussed with attendees of 1-day special FSMA-PCHF class (November 2nd, 2018, and May 6th, 2019).

Summary of literature on different interventions:

- *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, 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 facilitate 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.

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 a 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 the 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 the mode of action and effectiveness of technologies used in the fresh produce industry was conducted.
- Reviewed literature was discussed and presented in the form of a table.
- More than 50 scientific publications were reviewed.

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 the 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 the food safety plan model, draft of literature review, and presentation slides.

RESULTS & DISCUSSION

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

The food safety practices vary significantly across the industry. Apple packing houses across the WA State are very motivated to improve food safety in their facilities. Substantial investment in food safety systems was observed in recent years, and it continues to be one of the priorities in management and development of apple packing facilities.

The cleaning and sanitation of the packing line are of crucial importance; however, it is often difficult because of difficult to clean equipment, an insufficient amount of time allowed for cleaning and sanitation, and problems with rotation of employees. Other challenges reported by food safety managers are a limited amount of water used to conduct proper sanitation of the flume piping and pump systems, recycled systems are not designed to perform adequate sanitation. restricted capacities of wastewater allowed for municipal sewage systems.

Currently used and potential solutions for improving food safety during the apple packing process, based on the assessment of the current apple packing process and literature review are summarized in Table 1.

Table 1. Currently used and potential solutions for improving food safety during the apple packing process.

	Potential solution for reducing microbial load
Post-harvest fungicide treatment (drenching/fogging)	● Use of fogging method rather than drenching to avoid reuse of fungicide solution and minimize the possibility of cross-contamination of apples. Alternatively, the use of a fungicide solution does not support pathogen growth (Gomba et al., 2017; Guan et al., 2001; Ng, Fleet, and Heard, 2005).
Dump tank and flumes water treatments	● Use of double dump tank. The role of the first tank is to remove most of the debris and organic matter from the surface of the bins. It will greatly decrease the amount of organic matter, which causes a significant decline in sanitizer concentration. Thus, the concentration of sanitizer in the second tank and flumes will be more stable and easier to control (Luo et al., 2011). ● Aeration in dump tank to help with total apple saturation while in the dump tank ● Separation of dump tank from flumes. ● Maintaining the quality of the water by use of sanitizing agents such as

	chlorine, PAA, or EOW combined with surfactant. ● Proper monitoring system (Suslow, 2004).
Brush bed: Fruit may be treated with soaps and/or sanitizers	● Application of surfactant combined with sanitizing solution. Brushing and rotating apples can help evenly cover apples with cleaning solutions, increase detachment of microorganisms. The use of sanitizer to ensure bacteria deactivation and prevent contamination of brushes and cross-contamination of subsequently washed apples. ● Steam cleaning of wax brushes.
Wax coating: Fruit coated with food grade wax	● Application of wax with antimicrobial treatment (Jo et al. 2014)
Drying	● Automated dryer cleaning system to allow for more frequent dryer cleaning.
Personnel	● Frequent personnel training on understanding the basics of food safety and personal hygiene.
Cleaning and sanitation	● Allowing enough time for sanitation crew to perform adequate cleaning, especially in Zone 1 and 2. ● Reward system for sanitation crew, recognition of the importance of their work.
Packing plant environment	● Automatic door foamers. ● Forklifts designed only to the specific areas (i.e. forklifts used in the cold room do not enter the packing area, forklifts in the wet area are not used in the dry area). ● Drain system accessible for cleaning
Other	Traceability system - geolocation system, room identification, specific lot, and grower tagging. Efficient environmental monitoring program - seek and destroy approach. Support from chemicals and sanitation systems suppliers (often they provide trainings, ensure calibration and maintenance of the equipment, and provide information about new food safety interventions)

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

Most apple packing houses fall under the FSMA **Produce Safety Rule,** which does not require the implementation of food safety plans; however, the customers often expect them to comply with **Preventive Control for Human Food Rule,** which in turn requires food safety plan.
Required or not, a food safety plan can help facilities in managing the food safety system and ensuring the safety of the final product.

In this objective we aimed to develop model food safety plan that can be used by industry as an example, guide in developing their proper food safety plans. Food safety plan is based on hazard

analysis for each step of apple packing process. It is crucial to recognize all potential risks that can lead to contamination of final product and identify the appropriate preventive controls for managing these hazards. In case of apple packing process majority of the hazards can be addressed by good manufacturing practices (GMPs) and sanitation preventive controls. Drafted model food safety plan was reviewed by industry and by regulators (FSPCA) and based on obtained comments the final version of model food safety plan was developed.

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

Current FSMA-PCHF regulations require 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 provides the fresh apple packing industry with peer-reviewed literature on the effectiveness of these technologies. Based on the presented information, apple packers can make decisions on the use of different interventions. It can also aid in developing food safety plans.

The review includes 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. This information can help to better understand the food safety risks, how cleaning treatments work, and why bacteria removal is so important.

Current methods of produce decontamination can be divided into chemical, physical, and biological methods that can be used individually or in combination. Scientific investigations on the efficacy of various decontamination methods have been conducted by numerous research groups. However, there is still a need for studies that will evaluate the suitability of a given method for application in the packing process of apples, or other types of produce.

Lack of standard methodology for evaluating the efficacy of antimicrobial agents on fresh produce, laboratory-scale experiments, as well as differences between fresh produce morphologies, makes it difficult to compare the results between studies, and hard to predict their effectiveness in the industrial settings. A standardized methodology for the validation of the antimicrobial potential of sanitizing agents would facilitate more objective and standardized evaluation.

Manuscript titled, "*Food Safety Interventions to Control Listeria Monocytogenes in Fresh Apple Packing Industry: A Review,"* has been published in *Comprehensive Reviews in Food Science and Food Safety* with the open access.

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 training, focused on the implementation of the FSMA-PCHF rule.

We offered 2.5-day training as a part of the WSDA-SCBG in 2017 and two 1-day trainings on implementation of the FSMA-PCHF rule with an emphasis on the apple packing process (November 2nd in Yakima, and May 17th in Wenatchee). The 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 the food safety plan model, draft of literature review, and presentation slides. The training was well received with full attendance and great feedback from attendees. During the training, we were able to assist some of the packers with their food safety plans and answer questions related to a different aspect of food safety.

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Luo, Y., Nou, X., Yang, Y., Alegre, I., Turner, E., Feng, H., Abadias, M., and Conway, W. (2011). Determination of free chlorine concentrations needed to prevent Escherichia coli O157: H7 cross-contamination during fresh-cut produce wash. Journal of Food Protection. 74: 352-358.

Ng, P. J., Fleet, G. H., and Heard, G. M. (2005). Pesticides as a source of microbial contamination of salad vegetables. International Journal of Food Microbiology. 101: 237-250.

Suslow, T. V. (2004). Oxidation-reduction potential (ORP) for water disinfection monitoring, control, and documentation. University of California. Division of Agriculture and Natural Resources (2004) Publication 8149.

CONTINUING PROJECT REPORT **YEAR**: No-Cost Extension
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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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

Year 3: No request

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

4. Assess antimicrobial efficacies of different commonly used chemical sanitizers against *L. monocytogenes* biofilm on the main food-contact surfaces.
5. Examine antimicrobial efficacies of steam alone and in combination with selected sanitizers against *Listeria* biofilm on different food-contact surfaces.
6. Validate antimicrobial efficacies of steam and/or selected sanitizers in apple packing lines using parameters selected based on laboratory testing.

SIGNIFICANT FINDINGS

6. Efficacies of all tested sanitizers against aged (7-day-old) *Listeria* biofilm were reduced when compared 2-day-old biofilm.
7. In general, efficacies against *L. monocytogenes* biofilms on major food-contact surfaces including stainless steel (SS), low-density polyethylene (LDPE), polyvinyl chloride (PVC), polyester (PET) and rubber were enhanced by increasing concentrations of quaternary ammonium compound (QAC), chlorine, and chlorine dioxide, or extending treatment time from 1 min to 5 min.
8. A 5 min treatment of 400 ppm QAC, 5.0 ppm chlorine dioxide, or 200 ppm chlorine reduced 3.0-3.7, 2.4-2.7, and 2.6-3.8 $\log_{10}$ CFU/coupon *L. monocytogenes* biofilms depending on surfaces.
9. Peroxyacetic acid (PAA) at 160 ppm and 200 ppm showed similar antimicrobial efficacies against *L. monocytogenes* biofilms either at 1 min- or 5 min- contact time for all tested food-contact surfaces (SS, LDPE, PVC and PET). A 5 min treatment of 200 ppm PAA caused 4.0-4.6 $\log_{10}$ CFU/coupon reduction of *L. monocytogenes* biofilms on tested surfaces.
10. Food-contact surfaces had more impact on the efficacies of QAC and chlorine, less influence on those of PAA and chlorine dioxide.
11. Organic matter soiling, regardless of sources, impaired sanitizer efficacies against *L. monocytogenes* biofilms independent of food-contact surfaces.
12. PAA was the most effective sanitizer against aged multi-strain *L. monocytogenes* biofilms on different surfaces (polyester, SS, LDPE, PVC and PET).

METHODS

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

Objective 1: Assess antimicrobial efficacies commonly used chemical sanitizers against *L. monocytogenes* biofilm on the main food-contact surfaces.

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 stored at -80°C until used.

2. Selection and preparation of food-contact 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 diluted apple juice or whole milk before being 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 nutrient broth to achieve the target population density.

Biofilm formation on different surfaces: All four surface coupons (conditioned with/without organic matter) were transferred to *Listeria* suspension in culture media 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. QAC, chlorine dioxide and 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 coupons: 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 antimicrobial efficacies of steam alone and in combination with selected sanitizers against *L. monocytogenes* biofilm on different food-contact surfaces

1. Strain selection

L. innocua, a widely used surrogate for *L. monocytegenes*, were used for the Objective 2 studies. To avoid the impact of strain variability, three *L. innocua* isolates from produce packing facility/ processing plants were used to prepare 3-strain cocktail *Listeria* inoculum per our well-established method.

2. Food-contact surface selection and conditioning

Stainless steel, PVC, PET and PE were selected representing most commonly used surface materials. The selected food-contact surfaces will be prepared and conditioned as described in Objective 1.

3. Biofilm formation

Biofilm formation on different food-contact surfaces were conducted as described in the Objective 1 studies. To mimic harsh conditions in the apple packing facilities, the aged (7-day-old) multi-strain *L. innocua* biofilm were used in the Objective 2 studies.

4. Steam generator and temperature monitoring

The steam generator was located at Washington State University pilot plant due to power requirements. A stainless steel (SS) chamber with three steam pipes and 25 steam nozzles was used to treat *L. innocua* biofilms formed on different food-contact surfaces. The temperature profile of food-contact coupons inside the steam chamber was monitored using a T-type self-adhesive thermocouple (OMEGA, Norwalk, USA). Three-wire thermocouples were used to monitor the temperature profiles of steam at three different sites of the chamber.

5. Steam intervention against biofilms

The 7-day-old *L. innocua* biofilms on respective food-contact surfaces were treated with steam (~100°C) for 0-180 seconds. The treated surface coupons were transferred to 50 ml Falcon tubes containing 5~6 glass beads and 2 ml sterile PBS immediately after treatments.

6. Efficacy of steam in combination with sanitizers against Listeria biofilm on different surfaces

To determine efficacies of the steam in conjunction with sanitizer treatments, the surface coupons were prepared and inoculated as previously described. Surfaces bearing *Listeria* biofilm cells were sequentially subjected to steam and the selected sanitizer treatments.

The Objective 1 studies indicated that PAA was the most effective sanitizer against aged multi-strain *L. monocytogenes* biofilms on different surfaces (polyester, SS, LDPE, PVC and PET). QAC is one of the most commonly used sanitizers for surface disinfections. Therefore, PAA or QAC at their optimized conditions were used to treat *Listeria* biofilm following steam treatment.

7. Microbiological analysis.

Detachment of biofilm and enumeration of *Listeria* biofilm of food-contact surfaces treated with steam alone or steam in combination with sanitizers were analyzed as described in Objective 1.

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.

RESULTS AND DISCUSSION

Our previous years' studies on polystyrene surfaces indicated that antimicrobial efficacies of sanitizers against *L. monocytogenes* biofilms were dramatically impacted by biofilm stage, strains present and cleaniess of surfaces. In this study, we evaluated efficacies of four commonly used chemical sanitizers at practical concentrations against *L. monocytogenes* biofilms on major food-contact surfaces including SS, LDPE, PVC, PET and rubber using the aged multi-strain *Listeria* biofilm.

1. Efficacy of QAC against *L. monocytogenes* biofilms on food-contact surfaces

In general, increasing QAC concentration from 200 ppm to 400 ppm improved its efficacy against *L. monocytogenes* biofilms on different food-contact surfaces except LDPE surface for both 1

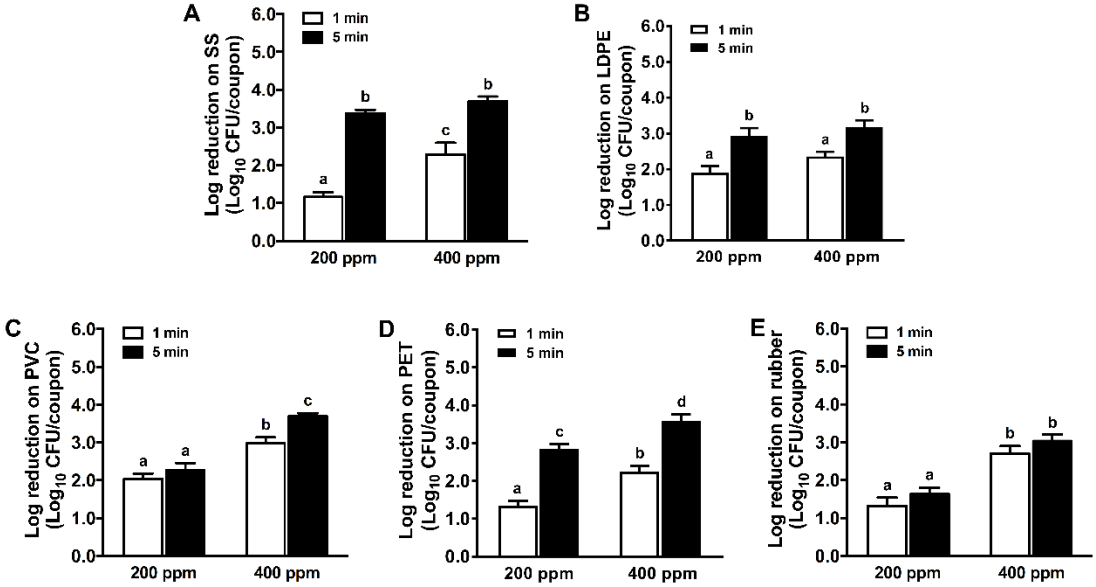

Fig. 1. Antimicrobial efficacy of quaternary ammonium compound (QAC) against *L. monocytogenes* biofilm on food-contact surfaces. (A) SS: stainless steel; **(B)** LDPE: low-density polyethylene; **(C)** PVC: polyvinyl chloride; **(D)** PET: polyester; **(E)** Rubber. The surviving bacteria were shown as means ± SEMs. [a-d] Bars topped with the different letters are significantly different at $P \leq 0.05$. Experiments were conducted independently three times.

min and 5 min exposures (Fig. 1). A 5 min exposure of QAC at 200 or 400 ppm showed a similar efficacy against *L. monocytogenes* biofilms on SS coupons (Fig. 1A). Except for rubber surface, the efficacy of QAC against *L. monocytogenes* biofilms on different surfaces was enhanced when exposure time increased from 1 min to 5 min (Fig. 1). Among all surfaces, QAC at 5 min exposure was the most effective against *L. monocytogenes* biofilms on SS (Fig. 1A), least effective against *L. monocytogenes* biofilms on rubber (Fig. 1E), while exhibiting a comparable efficacy against *L. monocytogenes* biofilms on LDPE and PET (Fig.1 B and D). For *L. monocytogenes* biofilms on PVC surface, the 5 min exposure of 400 ppm QAC showed a similar efficacy as those of LDPE and PET; however, 200 ppm QAC for 5 min exposure was less effective on PVC surface than those of LDPE and PET (Fig. 1 B-D). QAC at the FDA-approved concentration of 400 ppm for 5 min caused 3.7, 3.2, 3.7, 3.6, and 3.0 $\log_{10}$ CFU/coupon reductions of *L. monocytogenes* biofilms on SS, LDPE, PVC, PET and rubber surface, respectively (Fig. 1).

2. Efficacies of chlorine and chlorine dioxide against *Listeria* biofilms on food-contact surfaces

Chlorine dioxide solution at 2.5 ppm exhibited a limited efficacy against *L. monocytogenes* biofilms on all surfaces tested; 1 min treatments only reduced ~ 1.1, 0.6, 0.9, 1.1, and 0.9 $\log_{10}$ CFU/coupon *L. monocytogenes* biofilms on SS, LDPE, PVC, PET and rubber surfaces, respectively (Fig. 2). Though the efficacy of chlorine dioxide was enhanced with increased concentration and contact time, it displayed limited potency to inactivate *L. monocytogenes* biofilms on food-contact surfaces. A 5 min treatment of 5.0 ppm

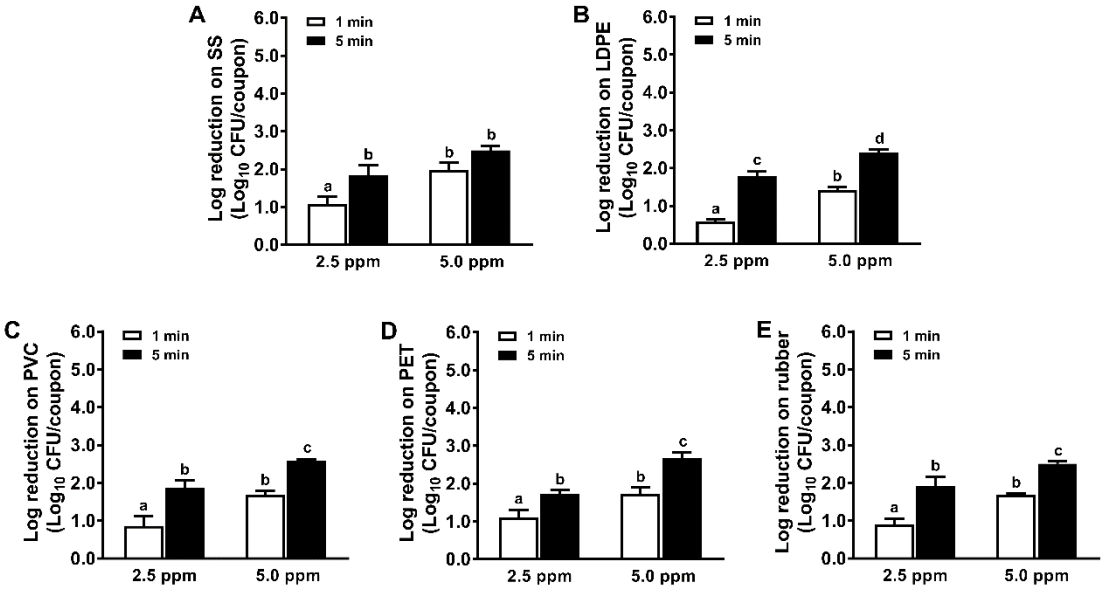

Fig. 2. Antimicrobial efficacy of chlorine dioxide against *L. monocytogenes* biofilm on food-contact surfaces. (A) SS: stainless steel; **(B)** LDPE: low-density polyethylene; **(C)** PVC: polyvinyl chloride; **(D)** PET: polyester; **(E)** Rubber. The surviving bacteria were shown as means ± SEMs. [a-d] Bars topped with the different letters are significantly different at $P \leq 0.05$. Experiments were conducted independently three times.

chlorine dioxide caused similar bactericidal efficacy against *L. monocytogenes* biofilms on all surfaces with 2.4-2.7 $\log_{10}$ CFU/coupon reductions (Fig. 2). The efficacy of chlorine against *L. monocytogenes* biofilms on the tested surfaces was enhanced at increased concentration and extended contact time except LDPE surface (Fig 3). A 1 min treatment of 100 ppm chlorine showed a similar efficacy against *L. monocytogenes* biofilms as 1 min exposure of 200 ppm QAC (Fig. 1) and was more effective than 1 min treatment of 2.5 ppm chlorine dioxide (Fig. 2), causing 1.0 - 2.0 $\log_{10}$ CFU/coupon reductions of biofilms on all surfaces tested. Chlorine at 200 ppm for 5.0 min exposure caused 3.8, 2.7, 3.3, 3.6, and 3.0 $\log_{10}$ CFU/coupon reductions of *L. monocytogenes* biofilms on SS, LDPE, PVC, PET and rubber surfaces, respectively (Fig. 3).

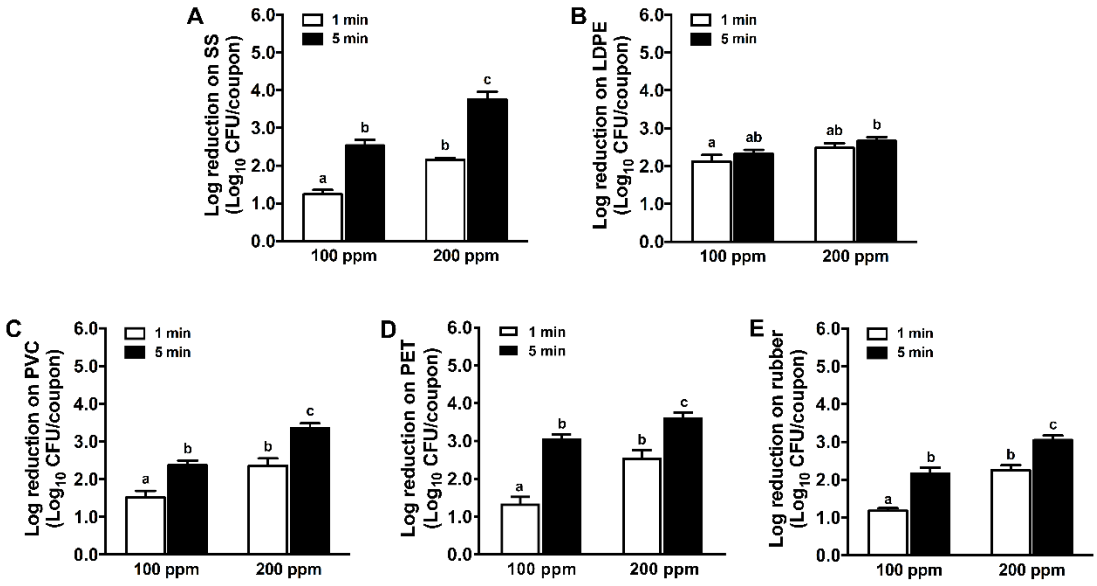

Fig. 3. Antimicrobial efficacy of chlorine against *L. monocytogenes* biofilm on food-contact surfaces. (A) SS: stainless steel; **(B)** LDPE: low-density polyethylene; **(C)** PVC: polyvinyl chloride; **(D)** PET: polyester; **(E)** Rubber. The surviving bacteria were shown as means ± SEMs. [a-d] Bars topped with the different letters are significantly different at $P \leq 0.05$. Experiments were conducted independently three times.

3. Efficacies of PAA against *L. monocytogenes* biofilms on food-contact surfaces

Among all selected sanitizers, PAA was the most effective against *L. monocytogenes* biofilms on all food-contact surfaces (Fig. 4). One min treatment of 160 ppm PAA reduced ~ 4.3, 3.5, 3.8, 4.1, and 3.7 $\log_{10}$ CFU/coupon *L. monocytogenes* biofilms on SS, LDPE, PVC, PET and rubber surfaces, respectively (Fig. 4). In general, the bactericidal effects of PAA against *L. monocytogenes* biofilms on all surfaces was not improved when the PAA concentration increased from 160 ppm to 200 ppm or when the treatment time

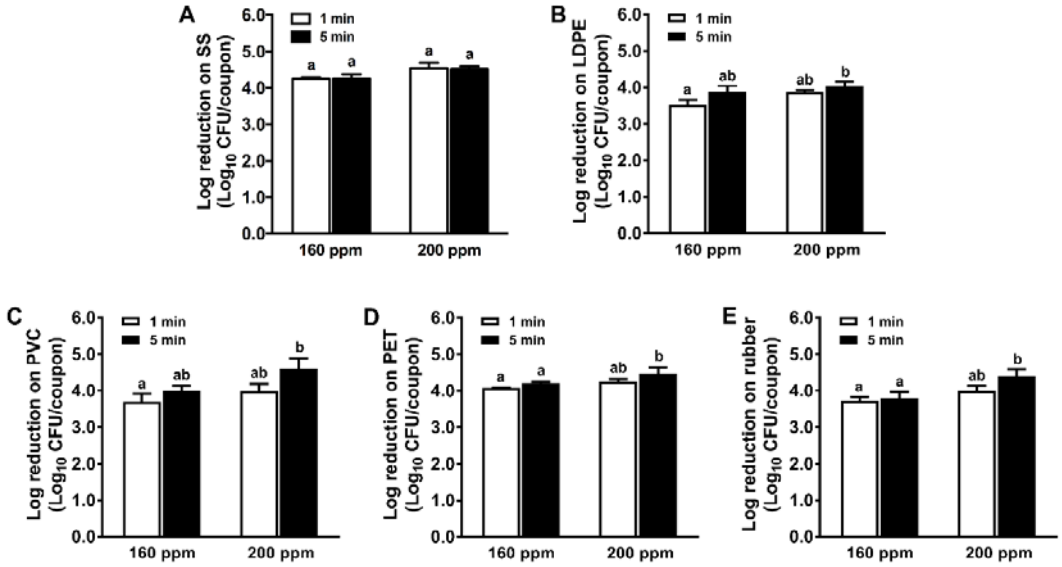

Fig. 4. Antimicrobial efficacy of PAA against *L. monocytogenes* biofilm on food-contact surfaces. (A) SS: stainless steel; **(B)** LDPE: low-density polyethylene; **(C)** PVC: polyvinyl chloride; **(D)** PET: polyester; **(E)** Rubber. The surviving bacteria were shown as means ± SEMs. [a-d] Bars topped with the different letters are significantly different at $P \leq 0.05$. Experiments were conducted independently three times, there are 6 replicated per treatment in a selected independent study.

increased from 1 min to 5 min (Fig. 4). The 5 min treatment of 200 ppm PAA caused 4.5, 4.0, 4.4, 4.3, and 4.4 $\log_{10}$ CFU/coupon reductions of *L. monocytogenes* biofilms on SS, PET, PVC, LDPE and rubber, respectively (Fig. 4).

4. Effects of organic matter on sanitizer's efficacy.

The anti-*Listeria* efficacies of all sanitizers were compromised by organic matter regardless of surfaces tested; food residues from apple juice or milk comparably impacted QAC efficacy (Fig. 5). Though the PAA efficacy against *L. monocytogenes* biofilms on all surfaces was impaired by organic soiling as much as other sanitizers, it was still the most effective sanitizer, which caused 3.0-3.7 $\log_{10}$ CFU/coupon reductions of *L. monocytogenes* biofilms on different surfaces (Fig. 5D).

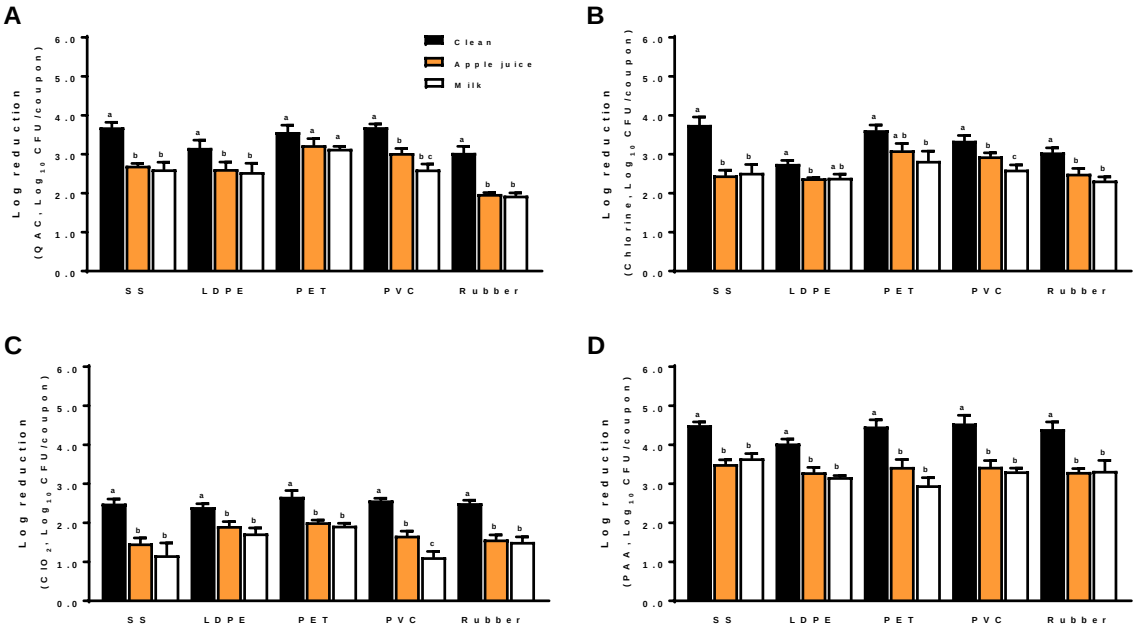

Fig. 5. Efficacy of four commonly used sanitizers against *L. monocytogenes* biofilm on food-contact surfaces conditioned with organic matters. (A) QAC (400 ppm); **(B)** Chlorine (200 ppm); **(C)** Chlorine dioxide (ClO_2, 5.0 ppm); **(D)** PAA, 200 ppm; **SS**: stainless steel; **LDPE**: low-density polyethylene; **PET**: polyester; **PVC**: polyvinyl chloride. Means ± SEMs. Experiments were conducted independently three times, [a-c] Bars topped with the different letters are not significantly different at $P \leq 0.05$.

CONCLUSIONS

Eradication of *L. monocytogenes* biofilm in food-contact surfaces is challenging. Type of surface material has more dramatic effects on anti-*Listeria* efficacy of QAC and chlorine than those treated with chlorine dioxide and PAA. Food residue soiling, regardless of sources, reduced anti-*Listeria* efficacies of all sanitizers against biofilms on surfaces in general. Among all sanitizers, PAA was the most effective sanitizer against *L. monocytogenes* biofilms on different surfaces. A 5-min treatment of 200 ppm PAA resulted in 3.0-3.7 $\log_{10}$ reductions of aged multi-strain *L. monocytogenes* biofilms on different food-contact surfaces in the presence of organic matter. Data once again highlight the importance of thorough cleaning food-contact surfaces prior to sanitizer interventions. More studies are ongoing to evaluate efficacies of steam alone and in combination with the selected sanitizers against biofilms formed on different food contact surfaces.

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

Project Title: Increasing the efficacy of antimicrobial chemicals with surfactants

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City/State/Zip: Pullman, WA, 99164

Cooperators: Various apple packing house in Yakima and Wenatchee, Wesmar, Inc., CleanLogix, Inc.

Total Project Request: Year 1: $47,148 **Year 2: 53,575**

Other funding sources: **None**
Amount:
Agency Name:
Notes: Donation of apples, sanitizer, and surfactants will be requested to decrease the cost of this research.

WTFRC Budget: ***None***

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

Item	2019	**2020**
Salaries	$17,098	$17,782
Benefits[1]	$2,172	$2,270
Wages[1]	$14,880	$15,475
Benefits[1]	$1,488	$1,548
RCA Room Rental		
Shipping		
Supplies[2]	$10,000	$15,000
Travel[3]	$1,500	$1,500
Plot Fees		
Miscellaneous		
Total	$47,148	**$53,575**

Footnotes:
[1] Salaries, Wages and Benefits for technical and student support
[2] Supplies and analysis fees, including for microbial testing. In the 2nd year, we will be conducting extensive microbiological tests due to the testing needed for numerous surfactants being tested.
[3] Travel costs of trips to the packing facilities in Wenatchee and Yakima.

OBJECTIVES

In this project, we proposed to evaluate the efficiency of surfactants (with different chemical properties), combined with sanitizers, for the removal of *Listeria* from fresh apples. So far, we have evaluated one of the sanitizers combined with three different surfactants. Based on the outcomes of our research, we prepared a manuscript titled: "*Efficacy of surfactant combined with peracetic acid in removing Listeria innocua from fresh apples"*, which was published in a *Journal of Food Protection.*

In this report, we compressed version of this manuscript. For full version please see the publication. (*Pietrysiak, E., Kummer, J. M., Hanrahan, I., & Ganjyal, G. M. (2019). Efficacy of Surfactant Combined with Peracetic Acid in Removing Listeria innocua from Fresh Apples. Journal of Food Protection, 82(11), 1965-1972*.).

The specific objectives for the next year are:

1. Examine the efficacy of additional types of surfactants combined with the standard sanitizers for the removal of *Listeria* from fresh apples during the packing process.
2. Assess the impact of optimum treatment on quality of the most significant apple varieties, such as Gala, Fuji, Granny Smith, Honeycrisp, and Cosmic Crisp.

SIGNIFICANT FINDINGS

- Combining surfactants with PAA decreased the population of *L. innocua* on apples.
- Treating apples with PAA-T20 reduced the load of *L. innocua* by 2.2 log.
- Stem bowl and calyx cavity are difficult to reach areas during the cleaning operation.
- Cleaning treatments were not completely effective in removing all *L. innocua* from apples.

METHODS

Inoculum preparation. A non-pathogenic isolate of *L. innocua* 51742 (ATCC) was used as a surrogate strain to *L. monocytogenes*. The stock inoculum was diluted appropriately with water at room temperature to prepare an inoculum of the desired concentration.

Apple inoculation. Apples were inoculated with *L. innocua* by submerging 15 apples in 5 L of inoculum suspension (with an approximate concentration of 10^7 CFU/mL) for 10 min and then air-dried at room temperature for a minimum of one hour before wash treatment.

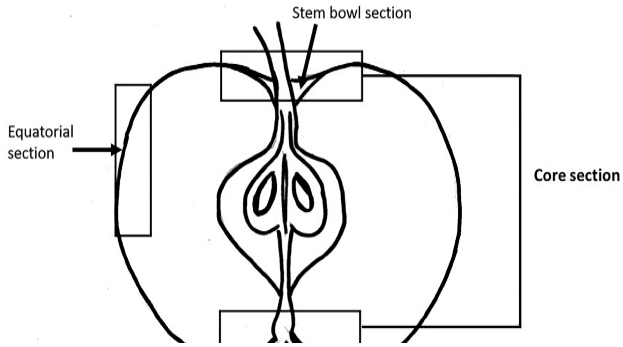

Figure 9. Apple sections examined for attachment of L. innocua (Pietrysiak et al., 2019).

Enumeration of *L. innocua* on different sections of the apple. Apple samples (approximately 3 x 3 x 1 cm cubes) were cut using a sterilized knife from either core (calyx and stem bowl cavities) or equatorial sections, as shown in Figure 1.

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.), anionic sodium dodecyl sulfate (SDS) (Sigma-Aldrich, St. Louis, MO, U.S.) and nonionic Tween 20 (T20) (Sigma-Aldrich), alone and combined with peracetic acid

(PAA) (Pace International, Wapato, WA, U.S.). The cleaning 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 a titration kit (LaMotte, Chestertown, MD, U.S.).

Contact angle measurements. The contact angles of cleaning solutions and apple surfaces were measured using the VCA Optima Video Contact Angle System (Ast Products, Inc., Billerica, MA, U.S.).

Antimicrobial activity of cleaning solutions. To examine the antimicrobial activity of the wash treatments, 0.2 mL of *L. innocua* stock solution was mixed with 9.8 mL of appropriate wash treatment (final concentration of 7 log CFU/mL), gently vortexed and kept for one minute at room temperature before plating on TSAYE plates.

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

Enumeration of *L. innocua* after cleaning. *L. innocua* was enumerated from the apples as described previously in the section describing the enumeration of *L. innocua* on different sections of the apple. Only the apple core section (stem bowl and calyx cavities) was analyzed since bacteria attach primarily in this section.

Scanning electron microscopy. Apple peel pieces observed with an SEM (Model Quanta 200F, FEI Company, Hillsboro, OR, U.S.) Representative images were recorded and analyzed.

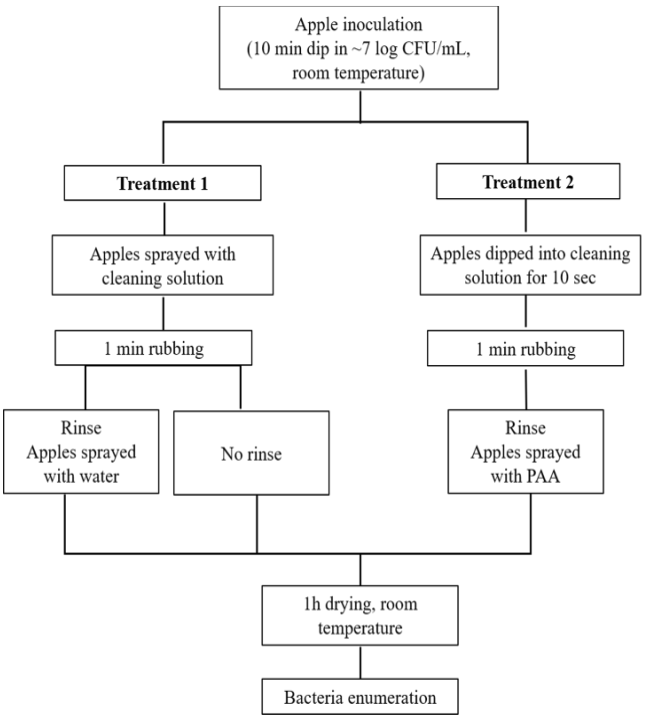

Figure 10. Cleaning treatments procedures. (Pietrysiak et al., 2019).

Statistical analysis. Results were expressed as means with standard deviation. Data were analyzed using a one-way analysis of variance (ANOVA). The least significant difference test, LSD Fisher, was performed using Minitab 18 (Minitab Inc., State College, PA, U.S.). *P* values of 0.01 or less were considered statistically significant.

RESULTS & DISCUSSION

Enumeration of *L. innocua* on different sections of the apple. The core section of the apple harbored relatively high number of bacteria (~4.82 log CFU/apple), compared to the equatorial section (~2.66 log CFU/apple. We have previously shown that stem bowl and calyx sections of the apples are heavily covered with different types of microstructures, such as microcracks, lenticels, and trichomes, which serve as a harbor sites for bacteria attached by physical entrapment. Microstructures may also protect bacteria from cleaning treatments, by preventing the chemicals from reaching the harbored cells, due to the surface tension. Additionally, apples are characterized by irregular shape and presence of calyx and stem bowl cavities. The stem bowl cavity of Gala apple can be up to 2 cm (0.8 inches) deep. Calyx cavity is not usually as deep as a stem bowl cavity; however, some apple varieties have open channels into the

core region, allowing for penetration of inoculum to the inside of the apple. The micro and macro structures of the apples are hard to reach into during a typical industrial cleaning process.

Contact angle measurements. The surface of apple peel is covered with a natural layer of wax which governs hydrophobic character and water-repelling properties. The high surface tension of the water prevents it from spreading on the apple surface. PAA, commonly used sanitizer in the apple packing industry, at a concentration of 80 ppm (maximum allowed concentration for use in fresh produce cleaning, did not change the surface tension of the water (Figure 2). The addition of surfactants led to a significant decrease in surface tension of cleaning solutions, which was confirmed by contact angle measurements on the apple surface. The addition of 0.1% of selected surfactants, allows for better spreading of cleaning solution on the surface of the apple and covering a larger surface with the same amount of liquid. SDS alone and combined with PAA exhibited the highest potential in reducing the surface tension of the solution. Lower surface tension and a better spread of the cleaning solution on the apple surface may help reach microstructures present on the surface of the apple and leading to more effective decontamination.

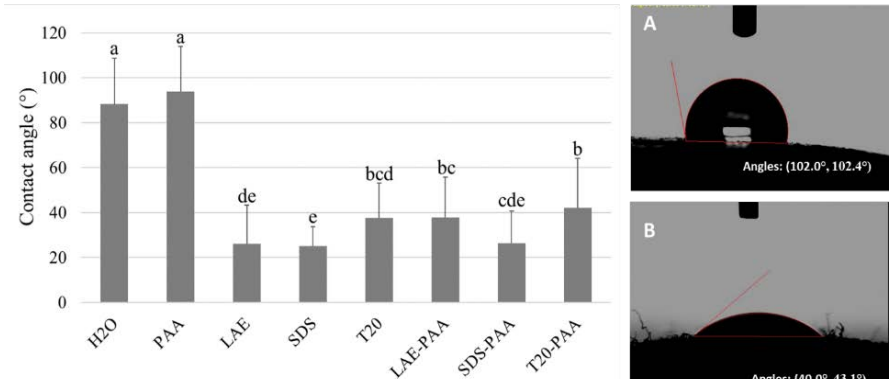

Figure 11. Contact angle of selected cleaning solutions on the apple surface (Pietrysiak et al., 2019).

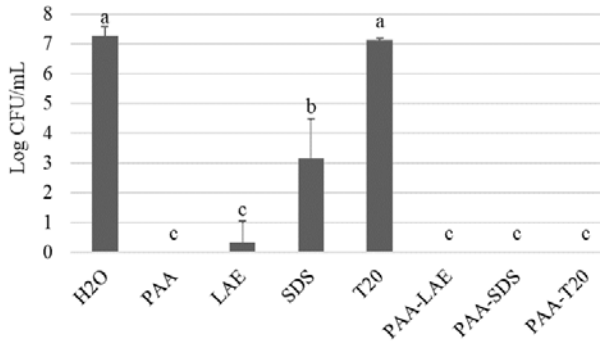

Figure 12. Antimicrobial activity of cleaning solutions against L. innocua. The initial populations of L. innocua used in this study was 7.28±0.29 log CFU/mL (Pietrysiak et al., 2019).

Antimicrobial activity of cleaning solutions. To determine the antimicrobial potential of the selected cleaning solutions against *L. innocua*, the antimicrobial activity was examined (Figure 3). All cleaning solutions containing PAA completely deactivated *L. innocua* after 1 min of contact time. Surfactants LAE and SDS also showed antimicrobial activity. LAE (0.1%) and SDS (0.1%) reduced the population of *L. innocua* by 6.9 log and 4.1 log, respectively. LAE, a compound derived from L-arginine, lauric acid, and ethanol, can damage the bacteria cell membrane and result in cell lysis. Combining selected surfactants with PAA dramatically increased their antimicrobial potential. Cleaning solutions that contained both surfactant and PAA showed high antimicrobial efficacy with very short contact time (1 min). Additionally, these cleaning solutions s presented a much lower contact angle when in contact with the apple surface compared to the PAA solution (Figure 2).

Enumeration of *L. innocua* after cleaning. All treatments used in this study were designed to simulate as much as possible, with the real conditions in the apple packing house. Therefore, in this study, we evaluated two different treatment settings (Figure 1). Treatment 1 consisted of cleaning apples with cleaning solutions, with or without water rinse. Cleaning apples with water or with LAE, SDS, T20

solutions, did not significantly decrease the concentration of bacteria on the apple surface. Application of PAA, with or without a rinse, reduced concentration of *L. innocua* on the surface of the apples by 1.1 and 1.3 log. PAA-LAE (with and without rinse) and PAA-T20 (rinse) were the most effective among all cleaning solutions used in this treatment. However, the maximum reduction observed was only up to 1.6 log, recorded after cleaning apples with PAA-T20 R.

Combining surfactants with PAA increased the efficacy of cleaning treatments. Use of surfactants decreased the surface tension and facilitated the spreading of the solution on the apple surface, which might lead to the increased reduction of bacteria.

Our results showed that surfactants combined with sanitizer enhanced *L. innocua* reduction, however, their efficacy was limited, and intact bacteria cells were still detected on the apple surface.

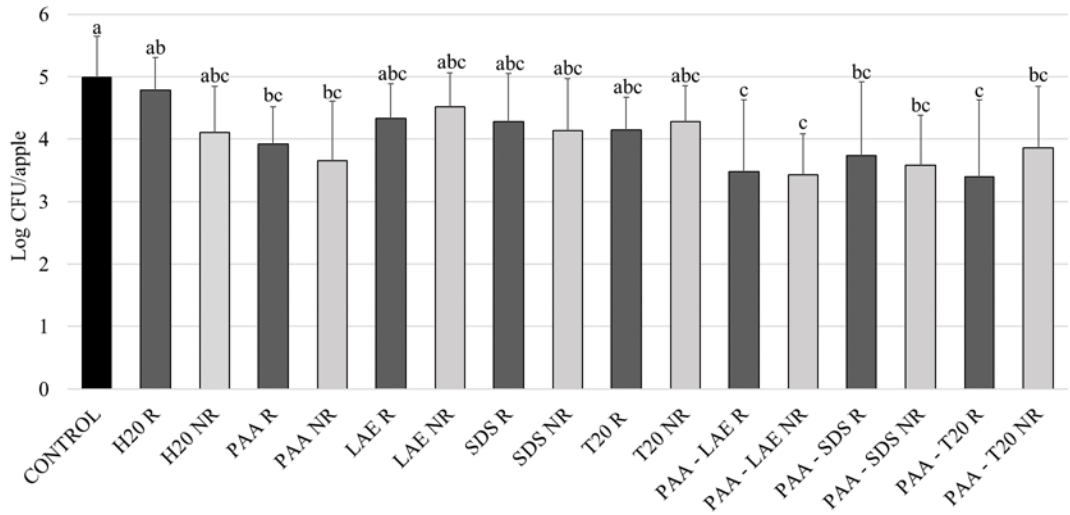

Figure 13. Treatment 1. Antimicrobial activity of cleaning solutions against L. innocua on apple (Pietrysiak et al., 2019).

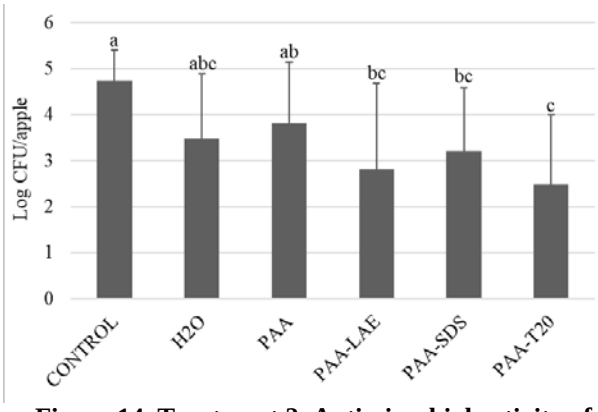

Figure 14. Treatment 2. Antimicrobial activity of cleaning solutions against L. innocua on apple (Pietrysiak et al., 2019).

Based on the results of Treatment 1, in Treatment 2, only solutions that were a combination of surfactant and sanitizer were used. In this treatment, apples were first dipped into the cleaning solution, rubbed, and sprayed with sanitizer. Sanitizer spray was applied to inactivate the detached bacteria. Treatment 2 resulted in greater bacteria reduction when compared with Treatment 1 (Figure 6). Cleaning apples with water or PAA followed by PAA spray did not significantly reduce the concentration of bacteria. Probably, these cleaning solutions were not able to reach and detach or deactivate the bacteria cells. The addition of a surfactant to PAA increased bacterial reduction. All treatments containing surfactant (PAA-LAE, PAA-SDS, PAA-T20) were more effective than water or PAA solution. Surfactants such as LAE, SDS, T20, reduced the surface tension of water by adsorption at the liquid-solid interface, which helped to reach bacteria harborage sites and increase bacteria reduction. However, applied treatment did not remove all the bacteria. The maximum bacteria reduction, ~2.2 log, was recorded for samples treated with PAA-T20.

Scanning electron microscopy (SEM). SEM imaging confirmed that applied treatments were not entirely effective in removing *L. innocua* from the surface of the apple peel. Bacteria cells were easy to locate in microcracks. *L. innocua* cells on untreated apples had regular rod-shape with a smooth surface (Figure 7A). In apples treated with cleaning solutions containing PAA alone or in combination with surfactants, *L. innocua* cells were deformed, exhibited collapsed contour, and damaged walls (Figure 7B, and C). Furthermore, in apples treated with PAA-LAE bacteria, clumping was observed (Figure 7D). Nevertheless, not all bacteria cells were affected by cleaning treatments. A portion of the bacterial cells found in microcracks seemed to be unaffected (Figure 7E, F), suggesting that the cleaning treatment did not reach the inside of all the microcracks. The results of SEM analysis confirmed the protective role of microcracks and emphasizes the importance to consider apple morphology in removal bacteria from the apple surface.

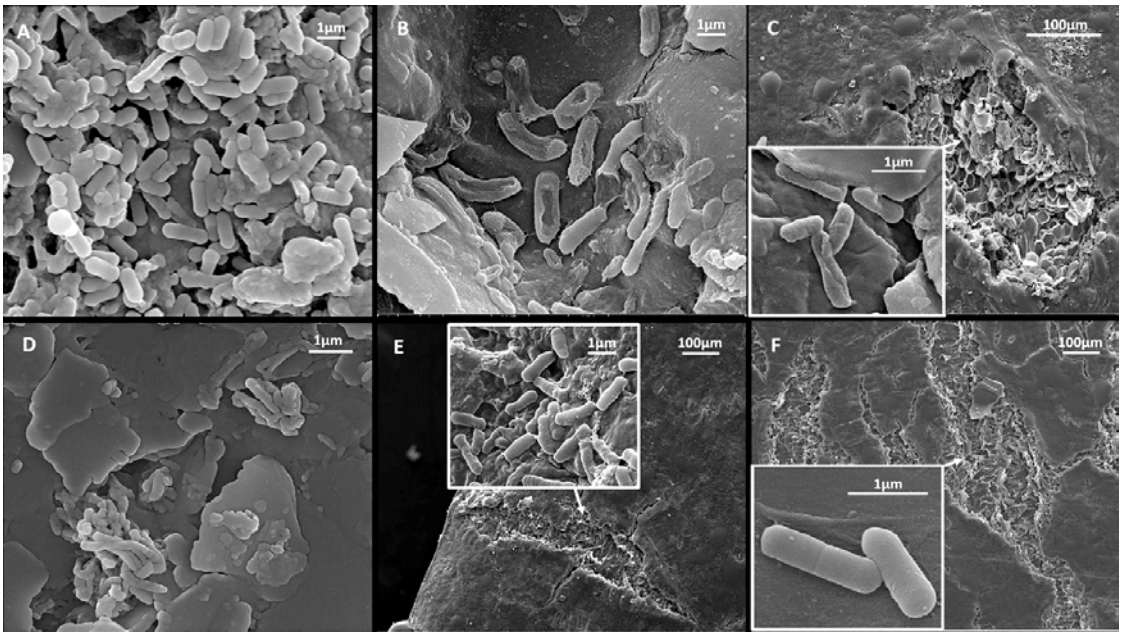

Figure 15. Treatment 2. SEM images of L. innocua on the Gala apple surface A) Untreated apple - regular rod-shaped cells with a smooth surface. B) Apple treated with PAA-SDS – bacteria cells located inside the microcrack, deformed and damaged. C) Apple treated with PAA-T20 – bacteria cells located inside the lenticel, deformed and damaged. D) Clumping of bacteria cells after treatment with PAA-LAE. (E, F) Unaffected bacteria cells located inside the microcracks after treatment with PAA-LAE (E) and PAA-SDS (F) (Pietrysiak et al., 2019).

In summary, this study demonstrated the potential of the use of PAA combined with surfactant in the apple cleaning process and emphasizes the importance of apple morphology in bacteria attachment and decontamination. *L. innocua* attached primarily to stem bowl and calyx cavities and further settled mainly in the surface microstructures such as lenticels and microcracks. The use of surfactant decreased the surface tension of the cleaning solutions, improved their spread on the hydrophobic surface of the apple, and increased bacterial reduction. However, part of bacteria, settled in hard to reach sites, was protected from cleaning operations and disinfection. Future research is needed to optimize the cleaning treatment of apples during the commercial apple packing process. In such future studies, additional surfactants and, in combination with other antimicrobial treatment should be evaluated.

REFERENCES:

Pietrysiak, E., Kummer, J. M., Hanrahan, I., & Ganjyal, G. M. (2019). Efficacy of Surfactant Combined with Peracetic Acid in Removing Listeria innocua from Fresh Apples. Journal of Food Protection, 82(11), 1965-1972

CONTINUING REPORT **Year: 2019**

Project Title: WTFRC Internal Program – Food Safety Efforts

PI: Ines Hanrahan
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Telephone: 509 669 0267
Email: hanrahan@treefruitresearch.com
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City/State/Zip: Union Gap, WA, 98903

Cooperators: Jacqui Gordon (WSTFA), Faith Critzer, Meijun Zhu & Girish Ganjyal (WSU), Manoella Mendoza and Mackenzie Perrault (WTFRC), Rob Atwill & Ronny Bond (UC Davis), Bonnie Fernandez-Fenaroli (CPS)

Other funding sources

Agency Name: WA SCBGP
Amt. awarded: $ 216,682 Title: Enhanced food safety education and training for tree fruit producers (awarded to WSTFA with Jacqui Gordon as PI)
Agency Name: FDA
Amt. awarded: $243,651 for FY19 awarded to WCFSS (Atwill et al.) Title: Facilitating implementation of FSMA regulations for agricultural water quality
Agency Name: CPS
Amt. awarded: $290,000 to Zhu and Suslow; Title: Control of Listeria monocytogenes on apple through spray manifold-applied antimicrobial intervention
Agency Name: WA SCBGP
Amt. /awarded: $ 248,227 to Zhu; Title: *E. faecium* as a surrogate for *L. monocytogenes* intervention in apple dump tank systems (additional $80,000 of matching funds)

WTFRC internal program budget:

Item	2019
Salaries[1]	3,100
Benefits	1,325
Wages[2]	7,500
Benefits	3,230
Supplies[3]	350
Travel[4]	3,100
Total	**18,605**

Footnotes:
[1]Salaries: 5% of Mendoza (with 41% benefits), not included in salaries: 15% of Hanrahan time, 1% Schmidt
[2]Wages: 53% benefit rate
[3]Supplies include 1 poster for ASHS and misc.
[4]Travel includes: CPS annual meeting, 3 trips to WSU in Pullman, in state day travel to attend trainings, Annual NW Food Safety and Sanitation Conference in Portland

OBJECTIVES

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

SIGNIFICANT ACCOMPLISHMENTS IN 2019

Food safety remains one of the highest priority items within the industry. As some compliance dates of FSMA have been effective, it is of utmost importance to continue to provide the Washington growers with timely assistance. Further, in order to interest and engage microbiologists to work on problems related to food safety for tree fruit, a strong collaboration from scientists with a horticulture background is of great advantage to ensure that project goals and outcomes reflect immediately actionable items. Lastly, translating research into layman's terms and providing a bridge between science, politics and farming is another important goal of this project.

Research:

We participated in several on-going and new collaborative projects, funded by WTFRC, CPS, SCBG and FDA (see Table 1). Notably, WTFRC is increasingly sought as collaborator in national grant applications to NIFA or SCRI.

The WTFRC, under leadership of Ines Hanrahan, continued to serve as a partner in research for the Center for Produce Safety (CPS) and she attended the annual meeting in Austin, TX. Tree fruit specific research priorities were developed and integrated into the annual CPS call for proposals, with the help of the NHC Food Safety Committee. During the proposal process Dr. Hanrahan frequently serves as subject matter specialist to answer questions asked by scientists preparing to propose new research projects. In 2019, one project involving local scientists has been brought to completion: 'Control of Listeria monocytogenes on apple through spray manifold-applied antimicrobial intervention' (Zhu/Suslow; $290,000). WTFRC has developed and executed a packing line survey for this project in 2017 to determine the current industry practices related to spray manifold interventions and has been assisting Dr. Zhu's team to set-up packingline validation studies with industry collaborators in 2018-19. The team has also been sourcing fruit for the experiments.

Our team collaborated with Dr. Zhu on another project in 2019: "*E. faecium* as a surrogate for L. monocytogenes intervention in apple dump tank systems", funded through the SCBGP. WTFRC developed and conducted a dump tank survey to assist the project team in developing industry relevant experimental methods.

In 2018 and 2019 Hanrahan has participated in SCRI industry relevance reviews of proposals submitted to USDA-NIFA in the area of food safety.

Table 1: Summary of WTFRC collaborations* in food safety research in 2019 and pending research for 2020

Keyword	PI's	Affiliation(s)	Funding Source	Amount
Continuing/finishing/new in 2019				
Listeria storage*	Zhu/Amiri/Hanrahan	WSU, WTFRC	WTFRC	195,414
Imp. Dryer	Ganjyal/Zhu	WSU	WTFRC	57,000
Water safety	Atwill/Bond	UC Davis, WTFRC	FDA	243,651

Food Safety Training	Gordon	WSTFA	SCBG	216,682
List. Cleaning*	Zhu/Hanrahan	WSU, WTFRC	WTFRC	306,285
FMSA PCHF	Ganjyal	WSU, WTFRC	WTFRC	98,971
Brush bed sanitation*	Critzer et al.	WSU, WTFRC	WTFRC	51,967
Listeria monitoring	Kovacevic et al.	OSU	ODA SCBG	174,540
Packing sanitation*	Critzer et al.	WSU, WTFRC	WTFRC	203,000
Rapid detection tools*	Critzer	WSU, WTFRC	WTFRC	112,000
Ozone in storage*	Zhu	WSU, WTFRC	WTFRC	300,000
E.Faecium as surrogate	Zhu et al.	WSU	WA-SCBG	250,000
Water treatment	Critzer	WSU	WA-SCBG	194,000
Listeria growth/survival	Strawn	Virginia Tech	CPS	185,052
Pending for 2020				
Lm apple waxing*	Zhu	WSU	WTFRC	TBD
Microbiome*	Zhu	WSU	WTFRC	TBD
Sensing platforms	Critzer et.al.	WSU	USDA	TBD
Effective mgt. for FSMA	Danyluk	U. of Florida	USDA-CAP	TBD
Listeria HUB	Kovacevic	OSU	USDA-NIFA	TBD
Wax supplements	Wang	UC Davis	USDA-NIFA	TBD

*collaborations involve a separate WTFRC internal budget

FSMA implementation: In order to best serve the needs for timely information distribution related to new laws pertaining to food safety, WTFRC staff (Hanrahan, Mendoza) leads efforts to coordinate outreach activities by the various industry organizations. Specifically, NHC (policy), WSTFA (education) and WTFRC (research) efforts have continued to be combined and talking points were coordinated to assure clarity of messaging, when stakeholders are learning how to implement the already complicated laws. Further, the WSTFA continued to host numerous PSA training sessions in 2019. WTFRC staff assisted in meeting logistics and Hanrahan frequently serves as expert to help field questions.

Development of industry training modules: Under leadership of the WSTFA and in collaboration with UC Davis, a workshop named: "FSMA water quality testing" was held in two locations in 2019. This workshop built on a curriculum originally developed in 2016. At the time it was the first of its kind in the nation to address practical considerations for water testing under FSMA. Workshops are designed to give participants theoretical background knowledge of water testing, in combination with outdoor activities geared towards learning based on examples, coupled with hands on training. The entire workshop was video taped and is available at no cost to industry members through the WSTFA portal.

Food safety outreach: For the Annual WSTFA meeting in Wenatchee, Drs. Critzer (WSU) and Hanrahan lead a session named: "Fruit Maturity & Precision Harvest Management". This session included several food safety related items such as: "Risk management within the harvest environment" (review of OFRR's, panel discussion) and "Produce Safety Rule On-farm Inspections" (panel discussion on implementation of on farm inspections).

In addition, Ines Hanrahan is serving as an adjunct faculty member for the WSU/UI School of Food Science. She is currently participating as a committee member on two Ph.D. and two MSc. committees in the Food Science Department. All students work in the general area of food safety on very relevant tree fruit industry topics and are interested in a career in tree fruit upon graduation.

Further, Dr. Hanrahan is serving on the PNW Food Safety Committee, which is housed by NHC. She contributes to the annual in-person meeting program development and serves as presenter. Other outreach activities covered by WTFRC staff, may include, but are not limited to posters at national/international meetings, invited talks, lectures for WSU classes. The following is a list of the most important invited talks/posters/lectures given in 2019:

Ines Hanrahan, 2019, Food Safety Research Priorities: Looking into the Future, talk at PNW Food Safety Committee annual meeting

Ines Hanrahan, 2019, Food Safety Research Roundup, talk at PNW Food Safety Committee annual meeting

Ines Hanrahan, 2019, Food Safety for the Tree Fruit Industry, 75-minute lecture for FS 220 students at WSU/UI

Manoella Mendoza, Ines Hanrahan, Lina Sheng, Xiaoye Shen, Meijun Zhu, 2019, Fate of Listeria on Granny Smith Apples Treated with Continuous Ozone During Storage, talk at ASHS presented by Mendoza

Alexis Hamilton, Ines Hanrahan, Marcela Galeni, Victor Villegas, Martin Blackburn, Monique Aguilar Borba, Cecilia Yiu, Daniel Gleason and Faith Critzer, 2019, Assessment of the Efficacy of Rapid Tests on Predicting Bacterial Growth on Apple Packinghouse Equipment Surfaces, poster at IAFP presented by Hamilton

Ronald F. Bond, Melissa L Partyka, Jennifer A. Chase, Ines Hanrahan, Justin Harter and Edward R. Atwill, 2019, The Whole Is Greater Than the Sum of Its Parts: Building Cooperative Monitoring Programs Among Farms, talk presented by Bond at IAFP

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

Project Title: Understanding decline on select apple scion-rootstock combinations

PI: Dr. Scott Harper
Organization: Washington State University
Telephone: 509-786-9230
Email: scott.harper@wsu.edu
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City/State/Zip: Prosser WA 99350

Co-PI (2): Dr. Alice Wright
Organization: Washington State University
Telephone: 509-786-9206
Email: alice.wright@wsu.edu
Address: 24106 N. Bunn Rd.
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 was 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 proposed to take a systematic approach to clearly identifying what pathogens are present in declining plants. This project looked 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.

- 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.

- 17 new viral species have been identified from some of the symptomatic trees.

- While many viruses have been identified as being present in symptomatic trees, there is no clear association between virus species and the onset of decline symptoms. This does not exclude a secondary role for viruses in this disease, perhaps weakening the plant for subsequent infection/damage by an unknown agent.

METHODS

Determine whether a virus or viral-like pathogens are associated with decline and dieback on G.935

The goal of this project was 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 underwent 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 were first screened by RT-PCR for common endemic and recently discovered viruses, then representative samples submitted for high-throughput sequencing. The resulting reads were passed through a data analysis pipeline, and candidate viruses identified. Non-diseased trees, and trees on other apple rootstocks, were 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 2018 and 2019, trees exhibiting decline symptoms were collected from growers and nurseries in north-central Washington State. Honeycrisp cultivars and/or cultivars with Honeycrisp parentage 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).

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).

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). Several plants 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 variants on G.935, although interestingly milder necrosis and poor root development was observed on one Honeycrisp on Nic-29 and three on Pajam2 rootstock, as well as on one Fuji cultivar on M.9 rootstock. In Washington State the pathology appears to be consistent, although it should be noted that in Pennsylvania symptoms were observed above the graft union rather than below, with sucker formation on the rootstock; indicative perhaps, of a different causal agent.

Screening of 52 symptomatic Honeycrisp cultivars on G.935 rootstocks by RT-PCR and/or high throughput sequencing (HTS) revealed the presence of nine endemic and newly-reported apple-infecting viruses (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. Individual symptomatic plants had between one to eight distinct viral species infecting them, with an average of 3-4 viruses per plant. In only one diseased plant were no viruses detected. In contrast, the 18 asymptomatic Honeycrisp cultivars on G.935 tested had fewer viruses infecting them, with an average of 1-2 viruses per plant; seven had no viruses, although there was one asymptomatic outlier with a total of six viruses detected (Table 2).

While one could propose that symptomatic Honeycrisp cultivars on G.935 are more heavily infected than asymptomatic plants, there is no obvious correlation between the viral species present and the onset of disease. Examination of the frequency of viruses identified (Table 3) indicated that 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 in over 50% of the symptomatic samples, but were also at high frequencies in asymptomatic plants. The only virus detected that showed significant differences in frequency between symptomatic and asymptomatic plants was Apple rubbery wood-associated virus-2 (ARWaV-2), however this was only in 54% of symptomatic plants so no clear conclusion can be drawn.

Table 1. Pooled results of the RT-PCR and HTS screening of disease-expressing samples of Honeycrisp cultivars on 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 (ARWaV-1 and ARWaV-2), 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	ARWaV-1	ARWaV-2	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	-	+	+	-	+	+	+	+	+
32	-	-	-	-	-	+	-	-	-
33	-	-	-	-	-	+	+	-	-
34	-	-	-	-	-	+	+	-	-
35	-	-	-	-	-	+	+	-	-
36	-	-	+	+	+	+	+	-	+
37	+	-	+	-	+	+	-	-	-
38	+	-	-	-	+	+	-	-	-
39	+	-	-	-	+	+	+	-	-
40	-	-	-	-	+	-	-	-	-
41	-	-	-	-		-	+	-	+
42	+	-	-	-	+	+	+	-	-
43	+	-	-	-	+	+	+	-	-
44	+	-	-	-	+	+	+	+	-
45	+	-	-	-	-	-	-	-	-
46	+	-	-	-	-	-	-	-	-
47	-	-	-	-	-	+	-	+	-
48	-	-	-	-	-	+	-	-	-
49	-	-	-	-	-	+	-	-	+
50	-	-	-	-	-	-	-	-	-
51	-	-	-	-	-	+	-	-	+
52	-	-	-	-	-	-	-	-	+

Table 2. Pooled results of the RT-PCR and HTS screening of asymptomatic samples of Honeycrisp cultivars on G.935 rootstock from Washington State.

Sample	ACLSV	AGCaV	ApMV	ARWaV-1	ARWaV-2	ASGV	ASPV	CCGaV	AhVd
1	+	-	+	-	+	+	+	+	-
2	-	-	+	-	-	+	-	+	+
3	-	-	+	-	-	+	-	-	+
4	-	-	+	-	-	+	-	-	-
5	-	-	+	-	-	+	-	-	-
6	-	-	+	-	-	+	-	-	+
7	-	-	-	-	-	-	+	-	-
8	-	-	-	-	-	+	+	+	-
9	-	-	-	-	-	+	+	+	-
10	-	-	-	-	-	-	+	-	+
11	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	+	-	-
17	-	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-	-

It should be noted here that nearly all of these viruses, with the possible exception of Apple rubbery wood-associated virus-1 (AWRaV-1) are endemic and widespread in commercial apple orchards in Washington State, and indeed, across the country (Harper, unpublished data). 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 3. Frequency table for viruses detected in symptomatic and asymptomatic Honeycrisp cultivars grown on G.935 rootstock.

Disease	ACLSV	AGCaV	ApMV	ARWaV-1	ARWaV-2	ASGV	ASPV	CCGaV	AhVd
Symptomatic	48%	13%	54%	2%	54%	62%	62%	21%	38%
Asymptomatic	6%	0%	33%	0%	6%	44%	33%	22%	22%

Next, we compared these detection rates to viruses present in Honeycrisp on other rootstocks, both symptomatic and asymptomatic, as well as one symptomatic Fuji on M.9 and two G.935 samples (Table 4). Again, there was no correlation between the expression of disease and virus presence and/or load; indeed, symptomatic Honeycrisp on Pajam2 were less infected that asymptomatic plants. Cumulatively, these data suggest that there is no obvious correlation between presence of known, named viruses, and the expression of Apple Decline disease.

Table 4. Results of the RT-PCR screening of asymptomatic samples of Honeycrisp cultivars on different rootstocks from Washington State

Sample	Cultivar/Rootstock	Symptomatic	ACLSV	AGCaV	ApMV	ARWaV-1	ARWaV-2	ASGV	ASPV	CCGaV	AhVd
1	Honeycrisp / Nic29	Yes	+	-	+	-	+	+	+	-	-
2	Honeycrisp / Pajam2	Yes	+	-	-	-	-	-	-	+	-
3	Honeycrisp / Pajam2	Yes	-	-	-	-	-	-	-	+	-
4	Honeycrisp / Pajam2	Yes	+	-	-	-	-	-	-	-	-
5	Honeycrisp / Pajam2	No	-	-	+	-	-	+	+	+	-
6	Honeycrisp / Pajam2	No	+	-	-	-		+	+	+	-
7	Honeycrisp / Pajam2	No	+	-	+	-	+	+	+	+	-
8	Honeycrisp / G.41	No	-	-	-	-	-	+	+	-	+
9	Fuji / M.9 (T337)	Yes	+	+	-	-	+	+	+	+	+
10	G.935 Rootstock	No	-	-	+	-	-	-	-	-	+
11	G.935 Rootstock	No	-	-	-	+	-	+	+	-	+

Finally, we sequenced six disease expressing samples using either root or shoot tissue, as well as two asymptomatic samples by HTS and searched for novel or putatively new viruses. From these plants we found seventeen putative novel virus-like sequences (Table 5). Each one of the contigs has a low percentage coverage and amino acid identity (25% to 61%) to named viruses, indicating that these are distinct and novel viral species. These viruses included an ilarvirus, two tombus-like viruses, a barna-like virus, a picorna-like virus, three ourmia-like viruses, three partiti-like viruses, and two narna-like viruses; four additional viruses could not be classified. The presence of these viruses in these samples was confirmed by RT-PCR using primers designed against the detected sequences. These data further indicate that while novel, these viruses are also rare, being found in only a small number of samples and so cannot be correlated presence with disease; it is likely that they are incidental and not related to the onset of apple decline.

Table 5. Putative novel viruses detected in Honeycrisp cultivars on G. 935 rootstock exhibiting apple decline from Washington State.

Name	Sample No.	Sequence Coverage	Contig length	BLASTx results
Apple barna-like virus 1	4	40.78	4099	Riboviria sp RdRp (QDH90348)
Apple ilarvirus 1 RNA2	5	10.71	1058	Blackberry chlorotic ringspot virus replicase P2a (ARS65724.1)
Apple ilarvirus 1 RNA3	5	91.49	2124	Parietaria mottle virus movement protein (CAJ58667.1)
Apple narna-like virus 1	3	12.26	2511	Wenzhou narna-like virus 1 RdRp (APG77283.1)
Apple narna-like virus 2	3	29.05	2668	Wenzhou narna-like virus 1 RdRp (APG77283.1)
Apple ourmia-like virus 1	3	42.71	1856	Pyricularia oryzae ourmia-like virus 2 RdRp (BBF90577.1)
Apple ourmia-like virus 2	5	15.56	2570	Phomopsis longicolla RNA virus 1 RdRp (YP_009345044.1)
Apple ourmia-like virus 3	3, 6	93.05	3067	Cladosporium cladosporioides ourmia-like virus 1 RdRp (QDB74999)
Apple partiti-like virus 1	1, 2, 3	234.3	2010	Partitiviridae sp. RdRp (QDH87388)
Apple partiti-like virus 2	1, 2, 3	374.44	1825	Partitiviridae sp. RdRp (QDH87090)
Apple partiti-like virus 3	2, 3	783.33	1930	Partitiviridae sp. RdRp (QDH87090)
Apple picorna-like virus 1	4	39.05	11885	Polycipiviridae sp. RdRp (AZL87720.1)
Apple tombus-like virus 1	3, 5	36.81	2971	Sanxia tombus-like virus 3 hypothetical protein 1 (YP_009337434.1)
Apple tombus-like virus 2	6	914.6	4340	Cowpea tombusvirid 1 RdRp (APA23091.1)
Apple virus A	3	18.32	5751	Rhizoctonia solani putative virus 1 hypthetical protein (QDW81310)
Apple virus B	5	41.19	9234	Penicillium glabrum negative-stranded RNA virus 1 RdRp (QDB75014)
Apple virus C	1, 3	73.9	4242	Hubei narna-like virus 8 RdRp (APG77202.1)
Apple virus D	2	26.87	7966	Riboviria sp RdRp (QDH87729)

Of the putatively novel viruses detected, the ilarvirus and two tombusviruses are likely plant infecting, whilst the barna, ourmia, and narnaviruses may be. The partitiviruses and the picornavirus could be plant, fungal or insect infecting, as members from those genera are found in all three types of host. Given how distinct apple viruses A through D are, it is not possible to give an assessment of their putative host range. This illustrates one of the limitations of HTS as a diagnostic tool, for while it is effective at indicating whether viruses are present, it provides no information on their biological

activity or relevance. Although the hosts of these viruses, apple or fungal, are suspected, they cannot be confirmed with the existing data. How the viruses are transmitted is also unknown. Lastly, it is unknown if any of these viruses are pathogenic in any apple cultivar or if they have a synergistic effect when co-infecting. With 9 known viruses and viroids just in these trees and seventeen additional putative viruses, the number of combinations when addressing synergistic effects becomes very large (and even greater if multiple cultivars or rootstock/scion combinations are included). It should also be considered that the viruses themselves are not causing 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 also 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. Furthermore, during the 2019 season we observed several trees that showed symptoms of back cracking and necrosis above the graft union. After further study we found that this was likely due to winter injury given the severe weather in February, combined with an opportunistic infection by *Valsa ceratospora*. Growers should be cautioned that there are many pathogens and agents that could cause decline symptoms on a tree.

In conclusion, until Koch's postulates are performed, identifying whether any virus or group of viruses are responsible for the decline and death of trees, notably Honeycrisp, on G.395 rootstock will require further research. However, based on the data collected in this study, there is no one virus or group of viruses that we can say with any degree of confidence to be associated with apple decline. This syndrome has, from reports and observations across the country, potentially multiple causes and variable pathology, such as necrosis above versus below the graft union depending on location and cultivar / rootstock combination. Our observations in this study do indicate however, that cultivars with a Honeycrisp genetic parentage do seem to be susceptible to whatever the causal agent is, when grown on G.935 rootstock.

EXECUTIVE SUMMARY

Project title: Understanding decline on select apple scion-rootstock combinations

Key words: Apple decline, Virus, Disease

Abstract: The causal agent(s) of apple decline, particularly of Honeycrisp variants on G.935 rootstock are unknown. This project explored the possibility that one or more viruses could be the cause. While diseased plants were heavily infected with viruses, no clear association was found between specific species and disease.

Executive Summary: This project examined whether viruses are a potential cause of apple decline, specifically that of Honeycrisp variants on G.935 rootstock, a combination that has exhibited the most severe decline-like symptoms in Washington State. Our first step in this project was to examine the physiology of the disease, and determine what apple decline of Honeycrisp on G.935 really was. We found that the most obvious symptom was dieback of the limbs and upper parts of the tree, usually at the second leaf stage, and visible necrosis of the trunk below the graft union. The rootstock was more severely affected, with necrosis of the cortex and phloem. Necrotic streaking was also observed throughout the primary and feeder roots. Our conclusion is that decline of Honeycrisp on G.935 is due to dieback of the rootstock, rather than direct damage to the scion.

Next we examined whether viruses were the causal organism of this disease. We collected and tested a total of 52 symptomatic and 18 asymptomatic trees over the course of the 2018 and 2019 growing seasons, and found that both disease and healthy trees were infected with known, named viral species. As a general observation, diseased trees had more viruses than the healthy, although there was no correlation between the presence of specific viral species and disease, nor groups of viruses and disease. From this we suggest that while infection with known viruses may be generally detrimental to tree health, there is no link between these apple-infecting viruses, and no specific management recommendation can be made at this time. We further examined the potential viruses present by high-throughput sequencing (HTS), and found the named viruses detected by PCR, as well as a total of 17 new viral species in some of the symptomatic trees. These new viruses are not common or widespread in the diseased plants, and are not likely to be involved in pathogenesis but are endemic or environmental viruses.

In summary, while many viruses have been identified as being present in symptomatic trees, there is no clear association between virus species and the onset of decline symptoms. This does not exclude a secondary role for viruses in this disease, perhaps weakening the plant for subsequent infection/damage by an unknown agent. However, the data does not support active management of these viruses for the purpose of preventing apple decline, rather management to promote general plant health, and the prevention of the diseases that they do cause.

FINAL PROJECT REPORT

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 other packing houses.

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

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

Organization Name: WSU **Contract Administrator:** Carrie Johnston/Ben Weller

Telephone: 335-4564 509-335-0052 **Email address:** carriej@wsu.edu / wellerb@wsu.edu

Item	2015-16	2016-17	2017-18
Salaries[1]	40,000	40,000	42,000
Benefits[1]	11,960	11,960	12,558
Wages[1]	3,750	6,000	4,500
Benefits	368	132	441
Equipment[3]	4,000	2,000	0
Supplies[2]	6,873	12,706	11,399
Travel[4]	7,000	2,000	5,000
Miscellaneous	0	0	0
Plot Fees	0	0	0
Total	$73,951	$74,798	$75,898

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

OBJECTIVES

The objective of this proposal was 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 a broad range of wax-coated apples and the impact on the quality of the apples with in-plant testing.
2) Study the effectiveness of impingent drying in reducing the microbial levels in apples.
3) Develop scale-up strategies for commercial packing lines and complete the energy efficiency analysis.

SIGNIFICANT FINDINGS

Objective 1:

- Two types of wax (shellac and carnauba) behave differently under higher heat conditions.
- The viscosity of both waxes reduces significantly with the increase in temperature.
- Both Carnauba and Shellac waxes can be effectively dried at higher temperatures (up to 200°F), in a regular convection air dryer.
- The Shellac wax tends to have flaking issues around 100°F. However, with an increase in temperature beyond 150°F, it provides a high level of gloss on apples.
- Carnauba wax provides good performance in the temperature range of 100 to 200°F.
- In general, at a higher temperature, shorter drying time was better for maintaining gloss.
- The overall quality of the apples (as determined by measurement of total solids, moisture loss, and pH) were comparable with the control apples (without waxing, but similar drying treatment) over the 3-week storage period.
- Longer drying times at higher temperatures had negative effects on the wax quality on the apples.

Objective 2:

- Microscopy analysis revealed that apple peel surface is covered with numerous microstructures, such as lenticels, and microcracks, present mostly in calyx and stem bowl sections.
- Calyx and stem bowl sections harbored approximately 150 times more bacteria than the equatorial section of the apple.
- Drying along could not provide the microbial load reduction of more than 1 log.
- Application of surfactants combined with sanitizer followed by drying with hot temperature air helped to decrease bacterial count on the apple surface. However, bacteria are attached to the microcracks in the stem bowl cavity, which may protect them from contact with a cleaning solution for a time long.

Objective 3:

- Impingement drying method can be used effectively to reduce the current dryer footprint and provides the opportunity to use additional food safety interventions on the packing line.
- Drying times can be reduced significantly by using higher drying air temperatures for shorter times and thus increasing the production capacity.
- Overall, this drying technique can provide economic benefits to the packing house.

RESULTS & DISCUSSION

Objective 1:

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

From the plant trials related to the testing for quality of the apples and the wax, we concluded that the best temperatures for the red delicious apple variety is 250°F or lower. The "golden delicious" variety of apples is not negatively impacted by high temperatures (up to 300°F).

Objective 2:

In 2016 microbial testing was performed. The maximum of 1 log CFU/apple was observed (Fig. 1 and 2).

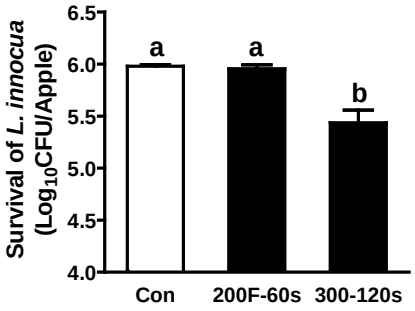

Fig. 1. Survival of L. innocua on Granny Smith apples.
Mean ± SEM, n=12, bars with different letters on the top are statistically different at P < 0.05.

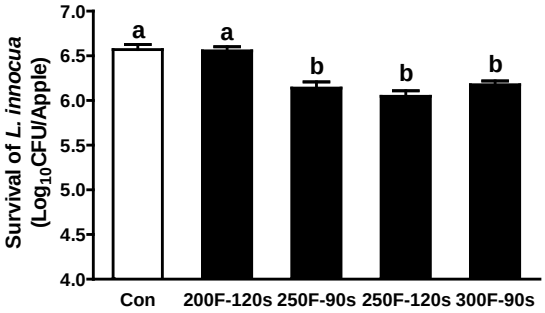

Fig. 2. Survival of L. innocua on Granny Smith apples.
Mean ± SEM, n=12, bars with different letters on the top are statistically different at P < 0.05.

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.
This made us try to understand why we did not see the reduction even though we know from the literature that the *L. monocytogenes* cannot survive at high temperatures.

To better understand how bacteria can survive on the surface of the apple during drying at high temperature, the apple peel surface was analyzed under scanning electron microscopy. Microscopy analysis revealed that the apple peel surface is covered with numerous microstructures, such as lenticels and microcracks. Three common apple varieties were analyzed, Gala, Granny Smith, and Golden Delicious. In all varieties, microcracks were mostly present in the calyx and the stem bowl

areas. These microcracks are actually big size for the *Listeria* spp. and other pathogens, but probably 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.

Microscopy observations were followed by microbial enumeration, which confirmed that bacteria attached to the calyx and stem bowl sections primarily. Calyx and stem bowl sections harbored approximately 150 times more bacteria than the equatorial section of the apple. The *L. innocua* was well hidden in the cracks, which are hard to reach by many of the interventions typically used, including the hot air (Fig 3).

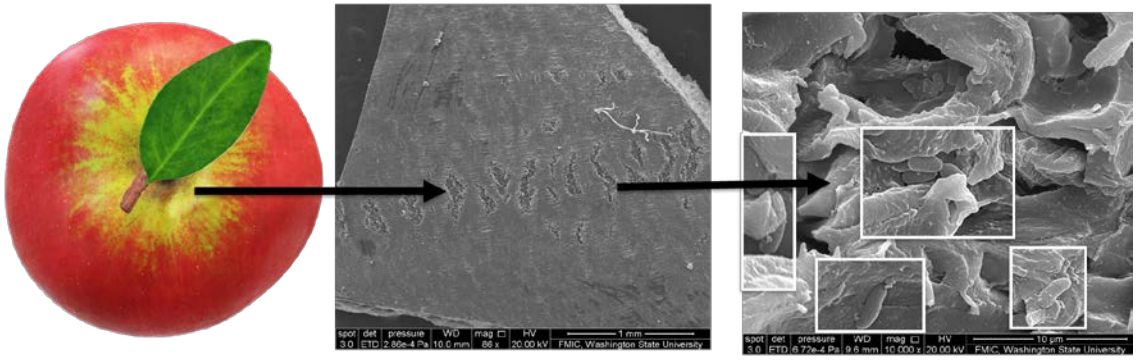

Fig. 3. Scanning electron microscopy analysis of apple peel surface. *Apple peel surface was covered with microcracks which harbored and protect bacteria.*

Furthermore, the surface of apple peel is covered with a natural layer of wax that governs hydrophobic character and water-repelling properties. The high surface tension of the water and water solution of commonly used sanitizers such as chlorine and peracetic acid prevent it from spreading on the apple surface. We found that the water or other chemicals used with water have a very high contact angle, which can suggest they don't adhere to the apple skin closely and thus may not reach the hidden *L. innocua* in microcracks.

To decrease the surface tension, we decided to add a small concentration of surfactant to water and examine if it will help to reach bacteria. Lower surface tension and a better spread of the cleaning solution on the apple surface may help reach microstructures present on the surface of the apple and aid in their decontamination. Three different food-grade surfactants at concentration 0.1%, combined with peracetic acid at concentration 80 ppm, were used to clean two different varieties of apple, Gala and Granny Smith, which were then dried at two different time/temperature conditions. **The use of a surfactant helped to reduce bacterial count (Fig. 4).**

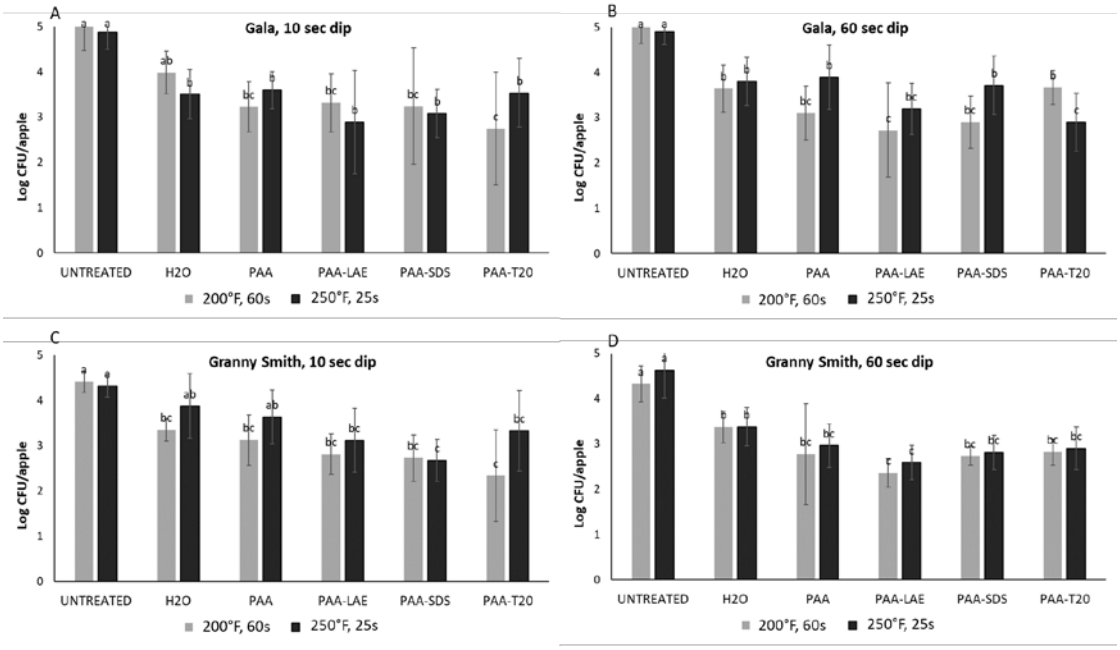

Fig. 4. Reduction of L. innocua on Gala and Granny Smith apples after surfactant dip followed by hot air drying. *Inoculated apples were dipped into cleaning solutions (H2O, PAA, PAA-LAE, PAA-SDS, PAA-T20), washed for 60 sec and hot air-dried at 93°C for 60 sec or 121°C for 25 sec. H_2O – tap water, PAA – Peracetic acid (80 ppm); LAE - lauric arginate (0.1%); SDS - sodium dodecyl sulfate (0.1%); T20 – Tween 20 (0.1%). Mean ± standard deviation; n = 9. Bars labeled with different letters indicate a significant difference (P<0.05) between treatments.* ***(Confidential data).***

Additionally, the application of surfactants resulted in enhanced drying of the solutions from the 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 to the apples, such as heat burn or skin discoloration. 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.

We also examined the influence of surfactant wash followed by hot air drying on apple texture over the 21-day storage (at 70°F and 40°F). Applied treatment had no significant effect on the apple texture of Gala and Granny Smith apples (Fig. 5), showing that surfactant wash combined with hot air drying did not affect apple quality.

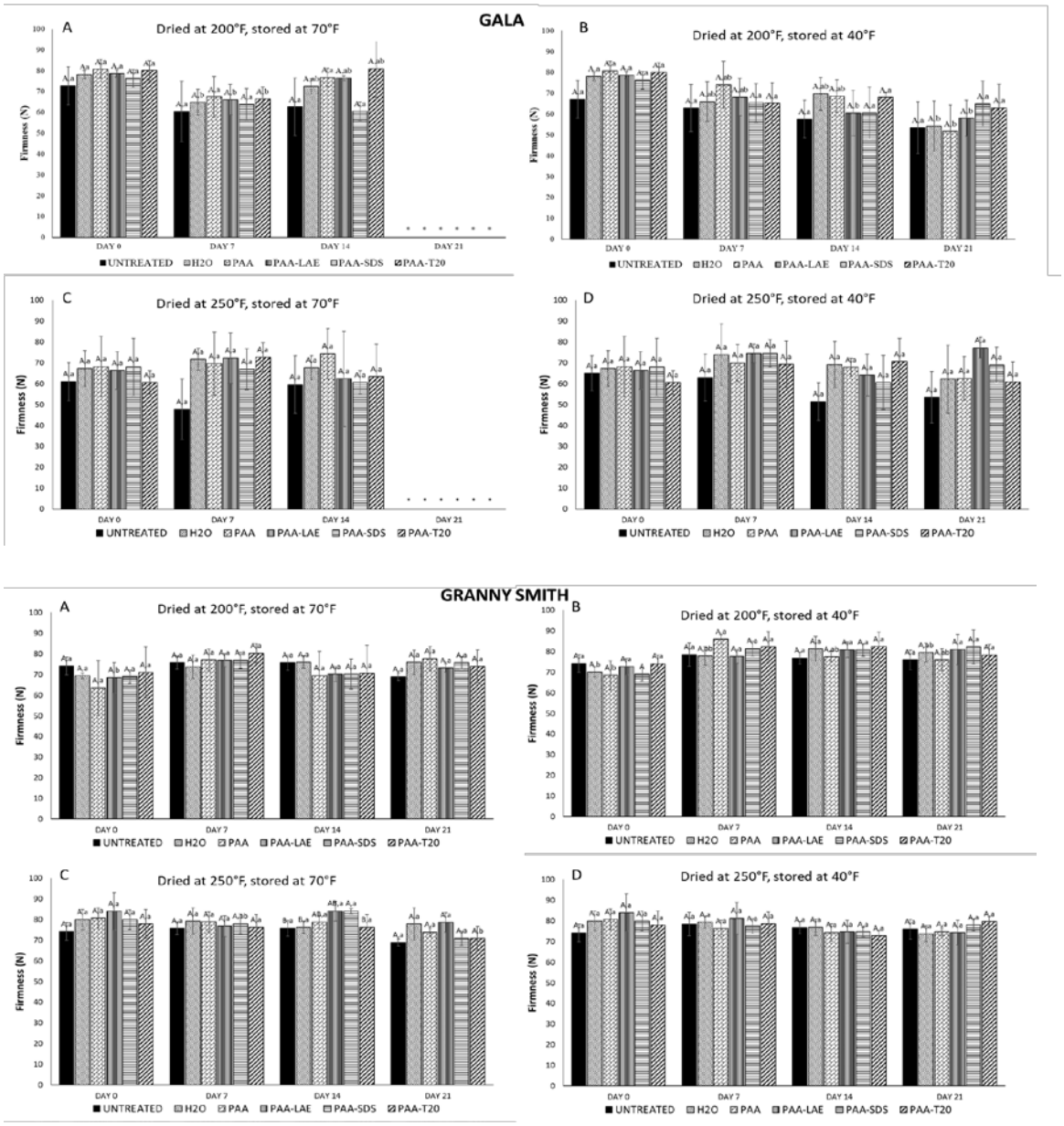

Fig. 5. Effect of the hot air drying on the firmness of the Gala and Granny Smith apples over 21-day storage. *Apples were cleaned with various cleaning solutions (H2O, PAA, PAA-LAE, PAA-SDS, PAA-T20) for 60 sec and hot air-dried at 93°C for 1 min (A, B) or 121°C for 25 sec (C, D). The firmness of the apples was measured at 0, 7, 14, and 21 days of storage at room temperature and compared with untreated apples. For each treatment and time point, 5 apples (n=5) were analyzed.*
** Apple texture was too springy, the probe could not penetrate the flesh.*
H2O – tap water, PAA – Peracetic acid (80 ppm); LAE - lauric arginate (0.1%); SDS - sodium dodecyl sulfate (0.1%); T20 – Tween 20 (0.1%).
Bars represent mean and standard deviation. Bars labeled with different uppercase letters (A, B) indicate a significant difference between apples after the same treatment at different time points (P<0.01). Bars labeled with different lowercase letters (a, b) indicate a significant difference between apples after the same treatment at different time points (P<0.01). ***(Confidential data, please do not publish this online for at least 12 months).***

Objective 3

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

We surveyed the current drying systems in various packing houses. Some of the key observations include:

- The current drying temperatures range from 70 to 140°F for two to three minutes.
- Often much heat is lost to the surroundings.
- The dryer footprint is generally significant, relative to the packing line.
- Most packing houses do not keep track of the energy spent on this unit operation.
- From our interviews with the packing houses, they are aware that this unit operation does take a significant amount of space and money to operate.
- Most packing houses expressed that if the dryer footprint is reduced, then that will enable them to use the saved space for other interventions.

Key points from our assessments related to the scale-up of this unit operation:

- For the small scale system that we modified using off the shelf pizza oven, we were able to dry the apples with an actual dryer bed of 16 inches x 16 inches.
- The maximum drying temperature that can be used for all apples would be 225°F with the residence time of less than 25 seconds, without compromising the quality of the apples.
- Even if we are more conservative and use a temperature of less than 200°F, the residence time in the machine can be below one minute.
- This can increase the throughput through the dryer by more than double.
- From our calculations, in the scaled-up version, we can have a drying bed length of less than 3 feet, keeping the same bed width as we currently have. This will reduce the footprint by at least 50%.
- Further, the quantity of the hot air required will also be reduced significantly, as the air will be directed very close to the fruit.
- It will also be easier to maintain the environment inside the dryer at a higher temperature due to less volume inside the dryer.

We will be very happy to work with any of the packing houses if they would like to experiment with this, especially if they are planning on installing new lines or at a state where they would be replacing the existing dryer.

EXECUTIVE SUMMARY

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

Keywords: food safety, impingement drying, *Listeria innocua*, wax

Abstract: This project explored the potential of the use of impingement drying technique in the apple packing process. The impingement drying can reduce the current dryer footprint and provide the opportunity to use as additional food safety interventions on the packing line.
The primary goal of this project was to understand the impacts of the higher drying temperatures on the quality of the wax and the fruit over the standard storage period. Tests were conducted in the laboratory using an impingement drying oven to assess the impacts of the higher drying temperature on the wax and fruit quality. Temperatures of 100, 150, and 200˚F were tested at different drying times of 1, 2, and 3 min. The wax quality (glossiness) and the fruit quality (weight loss, soluble solids, and pH) were tested on a weekly basis for three weeks of cold storage. It was found that higher temperatures resulted in higher gloss values. In other words, higher drying temperatures led to more shiny apples for both Carnauba and Shellac waxes. Although, for the Shellac wax in specific, the wax quality was negatively impacted when the temperature of the drying was increased from 100 to 150ºF. However, at 200ºF, the wax quality got better with increased glossiness compared to 100ºF drying temperature. Although, for the Carnauba wax, the glossiness increased with any increase in drying temperature.

Based on this, we conducted additional studies to assess the impact of the higher drying temperatures on the microbiological load of the apples. From the results from the initial tests, 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. To better understand how bacteria can survive on the surface of the apple during drying in high temperature, the apple peel surface was analyzed under scanning electron microscopy, which revealed that the apple peel surface is covered with numerous microstructures, such as lenticels and microcracks that can protect the bacteria.
Results of the microscopy analysis were summarized in the manuscript titled "*Apple Peel Morphology and Attachment of Listeria Innocua through Aqueous Environment as shown by Scanning Electron Microscopy"* published in the Food Control journal.

To enhance the bacterial reduction, apples were first cleaned with sanitizer combined with food-grade surfactants and then dried at temperatures below 250ºF. The use of surfactant helped to reduce bacterial count, with maximum reduction at approximately 2 log CFU/apple. Additionally, the application of surfactants resulted in enhanced drying of the solutions from the surface of the apples when compared with water and peracetic acid. Applied treatments did not cause damage to the apples, such as heat burn or skin discoloration, and had no significant effect on the apple texture over the 21-day storage (at 70°F and 40°F) of analyzed apples.

FINAL PROJECT REPORT **YEAR**: 18 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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Total Project Funding: $170,198

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Item	2017	2018
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**

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).

RECAP ORIGINAL 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. 2018 production showed a tendency toward higher dry matter classes than the 2017 fruit distribution.
    - Younger orchards generally produced larger proportions of higher dry matter fruits than the more mature orchard.
    - WA38 non-destructive dry matter prediction model (created in 2017 and adopted across the whole project) reported an increase in mean absolute error of its performance when utilized in 2018 on young orchard with very high dry matter apples.
2. ***Correlate fruit quality parameters of selected fruit categories (by size and dry matter) to consumer preference.***
    - Mature crop (5th crop) produced apples with lower firmness, soluble solid content (SSC), titratable acidity (TA), I_{AD} and lower starch index than 1st and 2nd cropping orchards (young) both at +1.5 M and + 5.5 months of after harvest 2018.
    - Firmness, I_{AD} and starch index decreased linearly with the increase of apple size (from Small to Extra-large) with the larger apples being softer, with lower I_{AD} and starch index.
    - The four different apple sizes at +1.5M after harvest 2018 did not statistically differ in terms of soluble solid content (SSC) nor for titratable acidity.
    - The top 3 WA38 attributes that contribute the most to the overall liking are, in order, apple flavor, sweetness and sourness
    - WA38 apples with dry matter between 14.00% and 16.99% were always preferred by consumers if compared to dry matter classes 17.00% to 18.99% in the Medium and Large sizes.
    - Consumers are more inclined to pay higher prices for WA38 apples coming from mature orchards and Large in size.

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 (2017-2018)

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 with WA38 in 2016 (2018 being its 1st crop). Additionally, two new commercial WA38 orchards trained to spindle– one budded on G41 (Freepons-Prosser) and the other on M9-NIC29 (Quincy) – were harvested as their 1st

cropping in 2018. 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 65 mm or smaller (163/case) were not considered marketable fruit for the purpose of this study.

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 (carried out contemporarily).

Dry matter predicted at 2018 harvest was higher than dry matter 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. Not all class combinations were available in sufficient amounts 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 both at +1.5 month after harvest (November 2018) and + 5.5 month of storage (March 2019). Additionally, at-harvest sorting classes used for 2017 harvest of Sunrise Orchard (low, mid, high dry matter) needed to be enlarges to accommodate 2018 harvests as substantial portions of fruit belonged to groups outside these classes. Complete data analysis was finalized after the last consumer test in March 2019 and reported below in the results session.

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), I_{AD} (Sinteleia, Italy) 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 both in 2017 and 2018.

Consumer Panels

For the first year, 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.

In the second year (2018) we ended up with 29 combinations of rootstock-orchard age-fruit size and DM class to compare after 1.5 M after harvest and offer to the panelists across 6 days of sensory analysis. We worked with 3 teams of panelists, each team was coming to taste fruit for two days in a row then the second team was taking over and then the third one (panelists number in each team: 102, 101, 99). Each panel team tasted apples coming from young and mature orchards within one rootstock. To verified that the judgmental capacity of each team was not significant different across days, we introduced an internal control (not done in 2017 panel test) of Honeycrisp apples harvested in 2018 from the same farm where WA38 mature (5th crop) block is planted (Sunrise Rock, Island) to be tasted every other day across the 6 days of panel. Another reason to introduce a highly appreciated variety in the trail was to see how the different combinations of WA38 sorted by DM and size scored in comparison to Honeycrisp in terms of overall liking. Statistical analysis of the responses for Honeycrisp samples across the 3 teams presented only slice appearance as the only one attribute barely significant; given the lack of differences across the other attributes, we felt comfortable compiling the data and making comparisons across all apples across 6 days (29 combinations and 3 controls). In addition, also the ballot was modified in 2018 integrating a series of 3 "willingness to pay" dichotomous questions in order to identify which price is the most appropriate for the consumers based on the eating experience ($1.21/lb, $2.23/lb or $3.25/lb). In March 2019, the same procedure was repeated offering to the panelist WA38 apples from 23 combinations after 5.5 M of RA cold storage plus 6M stored organic Honeycrisp as internal control across the 6 days. Numbers of panelists in the three teams in March 2019 were 96, 98, 101 for a total of 2,486 responses (including Honeycrisp control).

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).*

At-Harvest Size and Dry Matter Distribution of Young and Mature Orchards (2017-2018)

Figure 1 illustrates the predicted 2018 dry matter distribution among dry matter classes (each DM class covers 1% DM increase). We sampled 4477 apples in 2018 and 1360 apples in 2017 (mature 4th crop SRO for trees budded on G41 and Nic29). Younger orchards 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 despite the high vigor of this cultivar. The large proportion of high dry matter fruits in the first cropping year is likely transitory and will even out as the orchard matures. 2018 production showed a tendency toward higher dry matter classes than the 2017 fruit distribution. Dry matter shifts among fruit sizes and rootstocks and

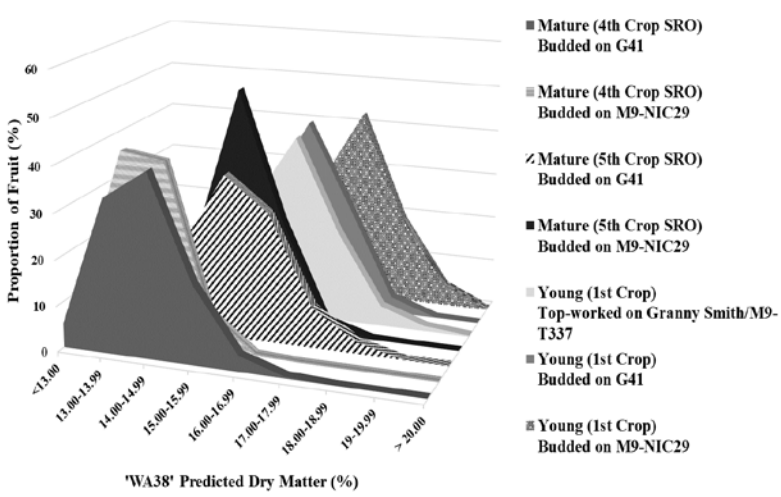


Figure 1: *Predicted dry matter (%) distribution among 'WA38' orchards both mature (SRO) and young as determined at-harvest using Felix F-750 Produce Quality Meter at harvest 2017 (4th crop SRO) and 2018 (5th crop SRO and 1st Crop elsewhere).*

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 (data shown in previous report).

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

Instrumental Fruit Quality

Comparing WA38 apples for fruit quality based on orchard and cropping age revealed significant differences both at +1.5 M after harvest 2018 (corresponding to the same period when the first WA38 apples sale hit the markets in 2019) and at +5.5 M after storage in March 2019.

Mature vs Young orchards

Mature crop (5th crop in SRO) produced apples with lower firmness, soluble solid content (SSC), titratable acidity (TA), I_{AD} and lower starch index than 1st and 2nd cropping orchards both +1.5 M after harvest (Figure 2) and after 5.5 months of storage (data not shown). First and second cropping of young orchards were characterized by apples with high firmness, high acidity and SSC > 16% with an average starch index of 5.3 in the 1-to-6 scale.

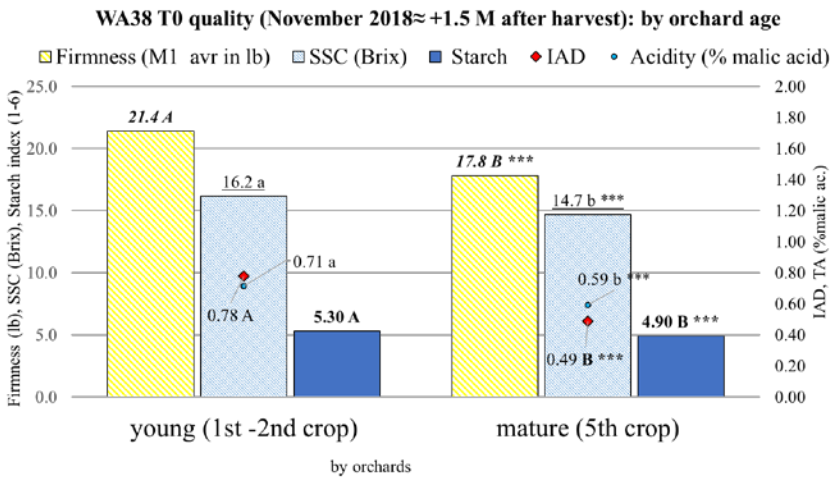


*Figure 2: Average values for instrumental quality +1.5 month post-harvest including average firmness (lb), soluble solids content (°Brix), I_{AD}, Starch index and titratable acidity among fruit from young (1st and 2nd crop) and mature (5th crop) orchards. Significant difference in means indicated by different letters via SNK. * = P < 0.05, ** = P < 0.01, *** = P < 0.001.*

Fruit size

Another way to look at the instrumental fruit quality data is by apple size. In Figure 3, the main quality differences between apples of the different sizes - from Small (70-75mm=113-88apples/box) to Extra-Large (100-105+=<48 apples/box) - are highlighted for T0 assessment (+1.5 M after harvest). Firmness, I_{AD} and starch index decreased linearly from Small to Extra-Large with the larger apples being softer (> 3 lb softer in firmness than size Small), higher level of chlorophyll degreening (lower I_{AD}) and lower starch index (avr. 4.7 out of 6). It is worth noting that the four different apple sizes at +1.5M after harvest did not statistically differ for soluble solid content (SSC) ranging from 15.5 to 14.9 °Brix, nor for titratable acidity (0.67 to 0.61 % malic acid; Figure 3). After 5.5 months of storage (data not shown), while all four fruit sizes reached already the complete starch degradation (starch index 6 out of 6), firmness was measured higher in Small fruit than in larger fruit and Extra-Large apples showed the lowest SSC (statistically different from Small and Medium fruit SSC) and lowest titratable acidity. Interesting to report that the dry matter (by destructive method) at T0 and T1 was not significantly different between the four sizes ranging from 16.2 to 16.5% and from 15.3 to 16.5%, respectively in the two time points of assessment. When analyzing dry matter values from the non-destructive readings by Felix F750 on all fruit available (other than a subset of fruit assessed by the destructive method) - after 5.5 months of storage- it emerged that the Extra-Large

fruit had a significant lower dry matter average value than the Small, Medium and Large fruit probably due to a dilution effect (data not shown).

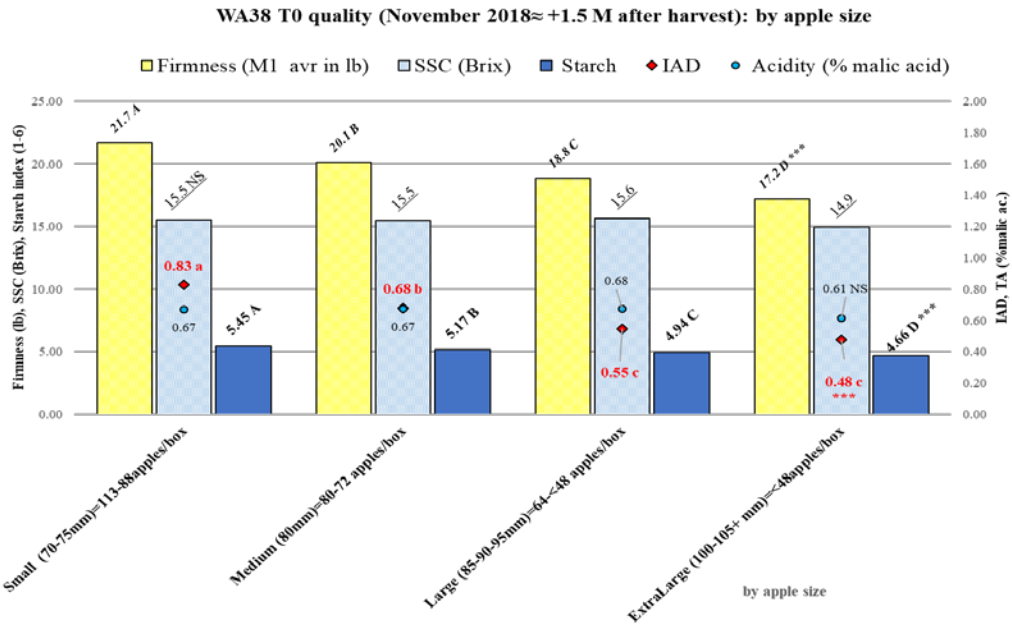


*Figure 3: Average values for instrumental quality +1.5 month post-harvest including average firmness (lb), soluble solids content (°Brix), I_{AD}, Starch index and titratable acidity: comparison among apples of four different sizes from Small to Extra-large accordingly to the sorting method described above. Significant difference in means indicated by different letters via SNK. * = P < 0.05, ** = P < 0.01, *** = P < 0.001.*

Dry matter categories

In the comparison between dry matter levels (from 12.00-12.99% to 19.00-19.99%, with 0.99% increment per each class) within each of the orchard age groups (interaction orchard age x dry matter classes, regardless of apple sizes; Table 1), relevant differences in fruit quality emerged at T0 and T1 assessments. After 1.5 M after harvest, apples harvested from both mature and young orchards showed higher firmness with dry matter increase [ranging in the young crop from 21 lb for low DM (13.00-13.99%) to 24.2 lb for very-high DM (19.00-19.99%)]. Regarding SSC in mature orchard (1.5 M after harvest), fruit in the classes between 13.00-13.99% to 18.00-18.99% reported fluctuating but statistically comparable values, while apples in class 12.00-12.99% showed lower SSC (SSC range across the 7 dry matter classes from 11.7°Brix to 16.8°Brix). On the other hand, within the young orchard production (+1.5 M after harvest), the SSC range was shifted toward higher values with apples belonging to 19.00-19.99% DM class reaching 18.2°Brix, while apples from 13.00-13.99% reported 13.1°Brix (Table 1). The difference in SSC between those extreme classes is 5°Brix and consumers can clearly perceive it. In both mature and young crops (Table 1), apples with higher dry matter (>16.00%) tend also to have higher titratable acidity and lower starch index in comparison to low DM classes (<13.99%).

Fruit quality assessment at T1 (+5.5 M after storage in March 2019) reported the same trend described above for T0. The only differences are that Red overcolor and starch index did not show statistically significant difference across the DM classes within each of the crop age while firmness, SSC and TA increased with the increase of DM% of the apple (data not shown). We believe that the fruit quality data related to +1.5 M after harvest are more interesting for the reader since they represent the status of the apples approximately around the period of the year when WA38 will be sold.

*Table 1: Comparison between dry matter classes of WA38 apples within each cropping age: average values for main parameters of instrumental quality +1.5 month post-harvest (T0 assessment). Parameters listed are average fruit weight (g), I_{AD}, Red overcolor %, firmness (lb), soluble solids content (°Brix), Starch index, dry matter % and titratable acidity. Significant difference in means indicated by different letters via SNK. * = P < 0.05, ** = P < 0.01, *** = P < 0.001. Areas shaded in grey represent combinations of cropping age x DM classes not present in the experiment.*

T0 quality (harvest 2018)-cropping (age)	WA38 Dry matter (DM) classes sorted by NIR spectroscopy	Weight (g)		I_{AD}		Red overcolor (%)		Firmness (lb)		SSC (°Brix)		Starch (1-6 scale)		destructive DM (%)		Titratable Acidity (% malic acid)	
young (1st -2nd crop)	12.00-12.99 %																
	13.00-13.99 %	161	B	1.15	A	96		21.05	C	13.08	G	6.00	A	13.77	G	0.63	CD
	14.00-14.99 %	225	A	0.62	C	94		19.26	D	13.89	F	5.90	A	14.85	F	0.56	D
	15.00-15.99 %	255	A	0.75	BC	92		20.21	CD	14.90	E	5.59	B	16.08	E	0.66	CD
	16.00-16.99 %	261	A	0.73	BC	93		21.19	C	16.12	D	5.28	C	17.01	D	0.71	BC
	17.00-17.99 %	260	A	0.81	BC	93		21.82	BC	16.89	C	5.20	C	17.78	C	0.74	ABC
	18.00-18.99 %	244	A	0.84	BC	93		22.88	B	17.44	B	4.79	D	18.64	B	0.83	A
	19.00-19.99 %	230	A	1.02	AB	90		24.19	A	18.18	A	5.01	CD	19.62	A	0.80	AB
	Sign.	***		***		NS		***		***		***		***		***	
mature (5th crop)	12.00-12.99 %	172	C	0.72	A	83	B	17.75	C	11.74	B	5.94	A	12.37	G	0.56	B
	13.00-13.99 %	245	B	0.62	AB	91	A	17.38	C	13.03	AB	5.79	A	13.68	F	0.54	B
	14.00-14.99 %	281	B	0.48	BC	94	A	17.11	C	13.52	AB	5.23	B	14.22	E	0.55	B
	15.00-15.99 %	328	A	0.44	C	94	A	17.26	C	14.16	AB	4.83	C	15.10	D	0.57	B
	16.00-16.99 %	341	A	0.44	C	95	A	18.07	C	16.37	A	4.58	D	16.35	C	0.61	B
	17.00-17.99 %	337	A	0.48	BC	95	A	19.06	B	15.77	AB	4.43	D	17.40	B	0.67	AB
	18.00-18.99 %	344	A	0.65	A	95	A	21.15	A	16.76	A	4.07	E	18.37	A	0.74	A
	19.00-19.99 %																
	Sign.	***		***		***		***		***		***		***		***	

Consumer Panels (+1.5 M and 5.5 M after harvest)

Sensory analyses carried out both at T0 and T1 time points, contemporarily to the instrumental fruit quality assessments, revealed meaningful differences in consumer perception of WA38 apples in comparison to Honeycrisp, introduced in year 2 only as internal control.

Mature vs young orchards

Comparing WA38 apples from mature orchard to young orchard (age of cropping) and to Honeycrisp control at +1.5 M after harvest, it appeared clear that apples produced from mature orchard scored the highest values (in a hedonistic scale 1 to 9) for many of the attributes tested, such as slice appearance, firmness, crunchiness, juiciness, sweetness, sourness, apple flavor and overall liking (score 7.2 out of 9.0). Mature WA38 apples were different from WA38 apples cropped in young orchard for the overall liking score (for young cropping 6.5 out of 9.0), while Honeycrisp with an overall liking of 6.9 placed in between mature and young cropping (data not shown). Honeycrisp control apples scored lower than WA38 mature apples for slice appearance, firmness, crunchiness, juiciness, sweetness, sourness, apple flavor and overall liking at +1.5 M after harvest, while they resulted similar for sweetness and sourness. In general, aroma, whole apple appearance (size and shape) and overall apple color did not reported significant differences in the way consumers perceived WA38 mature, young and Honeycrisp apples (data not shown). In addition, the consumer test carried out in March 2019 (+5.5 M after harvest) confirmed the previous results where WA38 apples from mature orchard were preferred in comparison to WA38 from young orchard and Honeycrisp control. After long storage, Honeycrisp apples reported the lowest overall liking and showed to be statistically different from WA38 from mature blocks with scores equal to 5.9 and 7.0 respectively; apples from young orchard registered an intermediate liking between mature crop of WA38 and Honeycrisp, equal to 6.3 (data not shown). Honeycrisp at the same time of tasting (March 2019) showed lowest scores for sweetness, sourness, apple flavor and overall appearance in comparison to mature and young WA38 apples.

Fruit size

Analyzing sensory results (at +1.5 M after harvest) by comparing fruit by size we can highlight other relevant differences (Figure 4). The only attribute among the three sizes provided for panel test (Small, Medium and Large) and Honeycrisp control that was not differently perceived by consumers in November 2018 was aroma (Figure 4), with scores always higher than 6.5 (out of 9.0). Same

results reported for March 2019 consumer test; aroma was not perceived differently across sizes and control. The smallest WA38 apples (113-88 apples/box) emerged as the least preferred in November 2018, firstly for their appearances, considering together whole apple appearance (size and shape) and overall apple color, and then for all the other attributes (Figure 4). The overall liking by consumers scored WA38 Large (64-<48 apples/box) apples as the first preferred followed by Honeycrisp (control), WA38 Medium apples and as least favorite WA38 Small fruit (Figure 4).

After 5.5 M of storage (March 2019), the least preferred for the whole fruit appearance and apple flavor was Honeycrisp control. Small WA38 fruit scored low also at this time point and in particular resulted similar to Honeycrisp as overall liking, sweetness and sourness. Once again, Large WA38 apples were the favorite overall due to high scores for apple flavor, sourness, sweetness and juiciness (data not shown). In terms of whole fruit appearance, the Medium WA38 apples were endorsed, reaching scores above 7 (and significantly higher than the Large WA38 fruit).

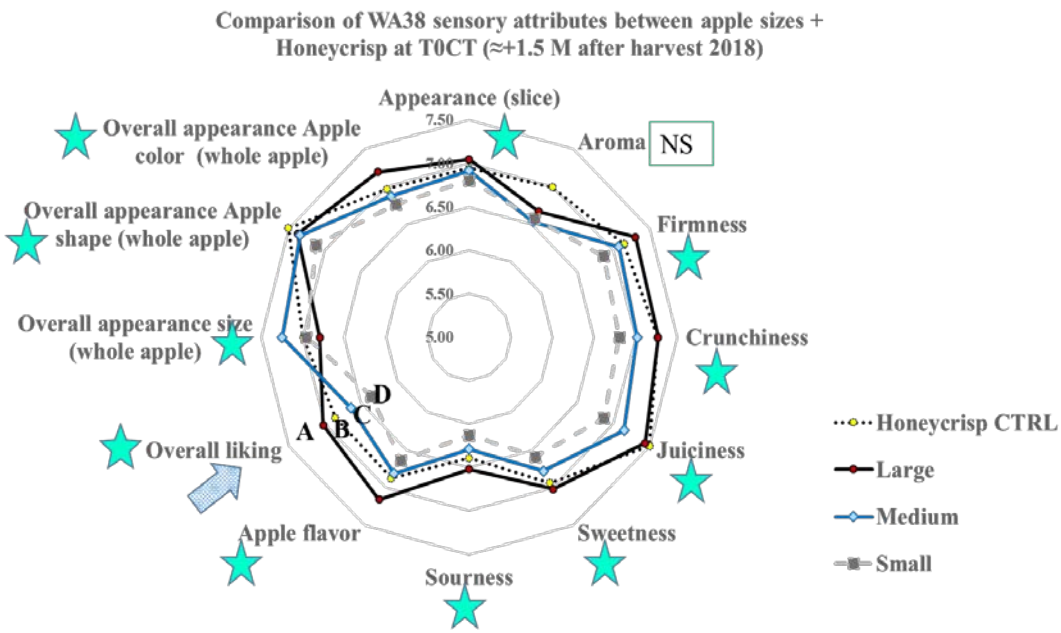


Figure 4: Spider net chart representing the scores collected from sensory analyses from consumers approximately +1.5 month post-harvest 2018 (November 2018) when comparing the three apple sizes (Small, Medium and large) with Honeycrisp control. Stars close to attributes (11 stars out of 12 attributes) identify significant difference from statistical analysis (for P < 0.01 or P < 0.001). Only overall liking presents SNK discrimination letters. Size categories: Small (70-75 mm) =113-88 apples/box, Medium (80 mm) =80-72 apples/box, Large (85-90-95+ mm)= 64-<48 apples/box.

Dry matter categories

Comparing the five dry matter levels in trial regardless of the cropping age, size and rootstock just fewer differences appeared at +1.5 M after harvest. While apples sorted in to DM categories did not differ for whole apple color and size, nor aroma, sweetness, sourness and apple flavor, though they were diversely perceived for firmness, crunchiness, juiciness, whole apple shape and overall liking. WA38 apples above 17% DM reported a lower overall liking than WA38 apples from 14.00 to 15.99%. This judgement reflected the lower scores recorded for firmness, crunchiness, juiciness preferences in the WA38 apples > 17% DM (data not shown). Consumer's opinion data during March 2019 panel test reported a decrease in overall liking with the increase in DM in WA38 apples (ranging from 6.9 to 5.9), in particular the highest scores were assigned to WA38 apples in 14.00-14.99%, 15.00-15.99% and 16.00-16.99% and the lowest to Honeycrisp, while WA38 17.00-17.99% and 18.00-18.99% scored in between them (6.2-6.3). Juiciness, crunchiness and sweetness preferences decreased with the increase of dry matter in the fruit (data not shown).

Focusing on the overall consumer liking at +1.5 M after harvest as interaction of apple sizes x dry matter categories, a clear discrimination about consumer preference between combinations under evaluation appeared clear (Figure 5). The average overall liking for control Honeycrisp apples resulted 6.85 (out of a 9-point scale) and Small size WA38 apples (from 14.00% to 17.9% DM) scored lower than the control value (average 6.34) with no difference across the four DM categories within the small size, while medium size WA38 apples reported values very similar to the Honeycrisp control in the 14.00 to 16.99% DM categories (with a maximum avr. score of 6.8). Furthermore, Large size WA39 fruit overpassed the control value reaching avr scores above 7 for 14.00% to 16.99% DM classes (Figure 5). We can conclude that at 1.5 M after harvest, the consumer preferred Large apples with a dry matter between 14.00% and 16.99% and that higher dry matter categories (>17.00%) are less desired (Figure 5), probably due to the high firmness and lower juiciness in the bite.

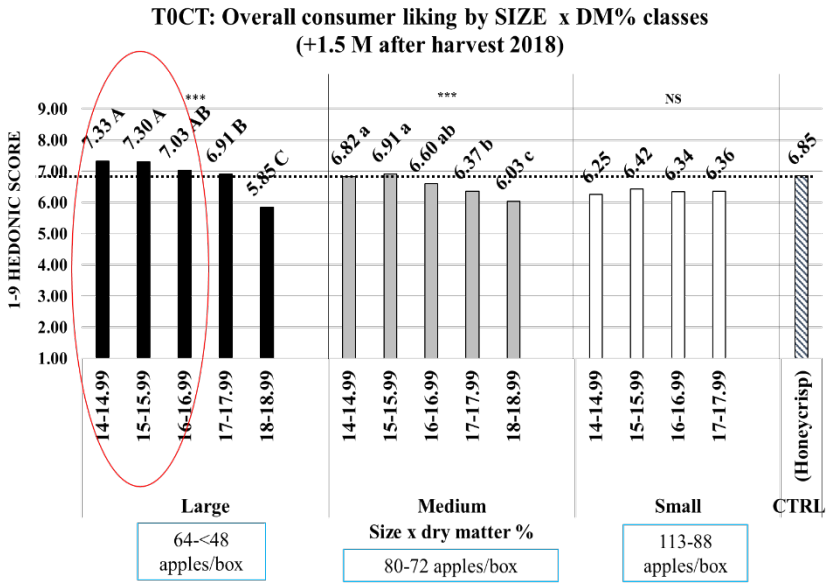


*Figure 5: Column chart representing the consumer overall liking scores from sensory analyses at +1.5 month post-harvest 2018 (November 2018) when comparing the dry matter classes within each of the three apple sizes (Small, Medium and large) and Honeycrisp control. Significant difference in means with each size class are indicated by different letters via SNK. * = P < 0.05, ** = P < 0.01, *** = P < 0.001.*

After long storage (about 5.5 M after harvest 2018), a lot more combinations of WA38 size and dry matter classes showed to be superior in overall liking than Honeycrisp control (data not shown). Small WA38 fruit, regardless of the dry matter class, scored 6 (average) for overall liking, while Medium and Large WA38 apples reported average scores equal to 6.4 and 6.7 respectively (HC scored 5.9). Moreover, WA38 apples with dry matter between 14.00% and 16.99% were always preferred if compared to dry matter classes 17.00% to 18.99% in the Medium and Large sizes (data not shown).

Correlation analysis

For both consumer panels run on fruit harvested in 2018 (at +1.5 and +5.5 M after harvest), a correlation analysis showed that the top 3 WA38 attributes that contribute the most to the overall liking of this new variety are, in order: apple flavor, sweetness and sourness (Table 2). On the other hand, whole apple size, whole apple color and aroma seemed to contribute the least to the overall consumer liking of WA38 apples (Table 2).

Willingness-to-pay (WTP)

Regarding the consumers' willingness to pay to purchase WA38 fruit after 1.5M from harvest, we noticed that consumers tend to be more inclined to buy mature WA38 apples with a higher price ≥ $2.23/lb (45.5%) versus young fruit (35.0%; data not shown). Moreover, the Large WA38 fruit reported the same proportion of consumer willing to pay a higher price as for Honeycrisp; 42% of the consumers are prone to pay ≥ $2.23/lb to buy those fruit (while 58% will buy for prices < $2.23/lb). A lower percentage of consumers (only 20%) are willing to pay higher tiers of price (≥ $2.23/lb) for WA38 apples in the highest dry matter category (18.00-18.99% DM), while this

proportion doubled (42%) when WA38 apples judged belonged to the 14-14.99% DM category (data not shown). In general, after long storage (+5.5 M after harvest 2018, March 2019), a slight decrease in the proportion of consumer willing to pay premium prices was noticed, but the same trends as at +1.5 M after harvest were confirmed (Figure 6). Consumers are more inclined to pay higher prices for WA38 apples coming from mature orchards and Large in size.

*Table 2: Correlation analysis between all the sensory attributes tested on WA38 apples for consumer preference and the overall liking on November 2018 and March 2019 respectively +1.5 M after harvest 2018 and +5.5 M after harvest in storage. Higher is the correlation coefficient, stronger is the correlation between the two parameters. Significant *** = P < 0.001.*

Pearson Correlation Coefficients, N = 3223										
T0CT November 2018										
Prob > |r| under H0: Rho=0										
	Appearance	**Aroma**	**Firmness**	**Crunchiness**	**Juiciness**	**Sweetness**	**Sourness**	**Apple Flavor**	**WholeSize**	**WholeColor**
Overall	0.40823	0.35741	0.59576	0.62261	0.67533	0.78884	0.72414	0.82871	0.23492	0.33545
Overall liking	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Pearson Correlation Coefficients, N = 2486										
T1CT March2019										
Prob > |r| under H0: Rho=0										
	Appearance	**Aroma**	**Firmness**	**Crunchiness**	**Juiciness**	**Sweetness**	**Sourness**	**Apple Flavor**	**WholeSize**	**WholeColor**
Overall	0.42308	0.3875	0.62548	0.65292	0.67674	0.77721	0.7101	0.8515	0.28345	0.36527
Overall liking	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

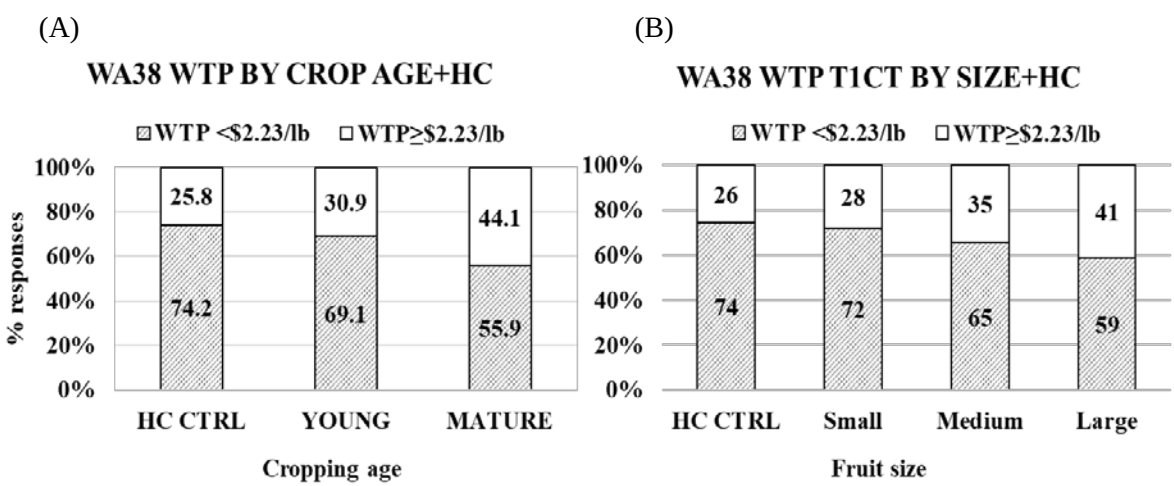


Figure 6: Willingness to pay (WTP) WA38 fruit based on cropping age (A) and fruit size (B) at +5.5 month post-harvest 2018 (March 2019): results are presented as proportion (%) of answers for the lower tier of prices (<$2.23/lb) and the highest (≥$2.23/lb). Inside each comparison Honeycrisp control (HC CTRL) is reported as reference.

EXECUTIVE SUMMARY

Project title: 'WA38' fruit size and dry matter for fruit quality/consumer preference
Key words: at-harvest sorting, sensory analysis, overall liking

Abstract:
Presorting WA38 apples at harvest by size and predicted dry matter allowed to identify differences in fruit quality and consumer preferences. Consumers overall preferred Large and Medium apples with dry matter <17%. Higher proportion of consumers is willing to pay higher prices (≥$2.23/lb) for WA38 apples from mature orchard.

PROJECT OUTCOMES (Presentations):

- **Musacchi S.:** "Report activity year 2017". Oral presentation by Musacchi S. at the Endowment Advisory Committee meeting (EAC) on 03/13/2018.
- **Musacchi S., Evans K., Ross C. and Serra S.:** "WA38' fruit size and dry matter for fruit quality/consumer preference". Oral presentation by Musacchi S. at the Cosmic Crisp® Quality Standards Sub-committee meeting on 07/24/2018.
- **Musacchi S., Evans K., Ross C. and Serra S.:** "WA38' fruit size and dry matter for fruit quality/consumer preference". Oral presentation by Musacchi S. at the Cosmic Crisp® Quality Standards Sub-committee meeting on 12/10/2018.
- **Musacchi S., Evans K., Ross C. and Serra S.:** "WA38' fruit size and dry matter for fruit quality/consumer preference". Oral presentation by Musacchi S. at the Cosmic Crisp® Quality Standards Sub-committee meeting on 02/28/2019.
- **Musacchi S.:** "Report activity year 2018". Oral presentation by Musacchi S. at the Endowment Advisory Committee meeting (EAC) on 03/11/2019.
- **Musacchi S., Evans K., Ross C. and Serra S.:** "WA38' fruit size and dry matter for fruit quality/consumer preference". Oral presentation by Musacchi S. at the Washington State Tree Fruit Association (WSTFA) annual meeting 2019 on 12/11/2019.

SUMMERY OF FINDINGS

- In 2018, production showed a tendency toward higher dry matter classes than the 2017 fruit distribution. Younger orchards generally produced larger proportions of higher dry matter fruits relative to the more mature orchard.
- Mature orchards (5th crop) produced apples with lower firmness, soluble solid content, titratable acidity, I_{AD} and lower starch index than 1st and 2nd cropping orchards (young) both at +1.5 M and + 5.5 months of after harvest 2018.
- Firmness, I_{AD} and starch index decreased linearly with the increase of apple size with the larger apples being softer, with lower I_{AD} and starch index. At +1.5M after harvest 2018, no statistical differences for SSC and titratable acidity were found across the four apple sizes.
- The top three WA38 attributes that contributed the most to the overall liking were: apple flavor, sweetness and sourness.
- WA38 DM 14.00% -16.99% apples were always preferred by consumers if compared to dry matter classes >17.00% in the Medium and Large sizes.
- Consumers are more inclined to pay higher prices for WA38 apples coming from mature orchards and Large in size.

FUTURE DIRECTIONS

- Further explore consumer preference in relation to optimal harvest time for WA38 to maximize internal quality and minimize production losses.

FINAL PROJECT REPORT

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

Cooperators: Michelle Reid, Columbia Fruit Packers, Columbia Reach Orchards, Zirkle Fruit Company, McDougall and Sons, and Washington Fruit Co.

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 2019.

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: WSU Contract Administrator: Katy Roberts/Shelli Tompkins
Telephone: 509-335-2885/509-293-8803
Email: arcgrants@wsu.edu/shelli.tompkins@wsu.edu

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

- When water limitations were applied, fruit size was reduced in all treatments but timing affected 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 summer (45-105 DAFB).
- Red color was the greatest when water was limited later in the season (75-105 DAFB)
- 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.
- Vigor was reduced by more than 25% by any of the deficit irrigation treatments

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, August 30th in 2018, and September 7th in 2019. The total amount of fruit from each tree was weighed in the field, categorized by diameter, 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 stored in regular atmosphere (RA) for 3 months at 33°F. 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 each of 2018 and 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. 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 AND DISCUSSION

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. 2018 had significantly less bloom than 2017 or 2019. There were no significant differences in return bloom between deficit treatments and the normally watered control (Figure 1). Crop load was standardized as much as possible using bloom

thinners, post-bloom thinners and hand thinning to target fruit and crop load differences between years were much lower than bloom differences. There were no treatment effects on crop load with the exception where trees that had early season deficit irrigation applied in the first year were over thinned by approximately 4 fruit per tree.

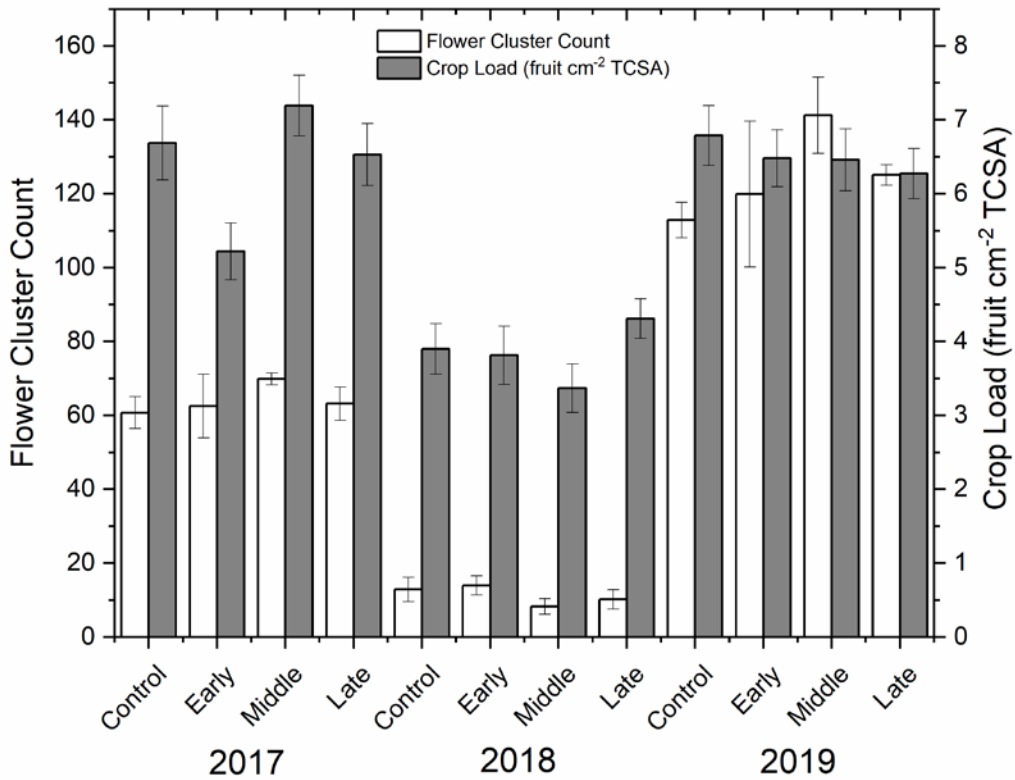
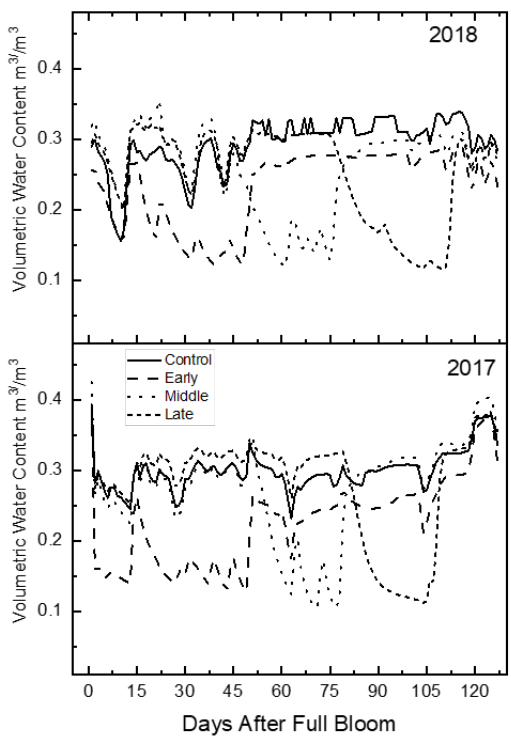


Figure 1. Mean flower cluster count and crop load in 2017, 2018, and 2019 for treess exposed to early, middle, or late summer water limitations compared to a well-watered control.

At WSU Sunrise which has a shallow sandy loam soil, the maximum volumetric soil water content was approximately 33% 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 based on evapotranspirative demand or 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 resuming irrigation, 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).

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 considered when deciding on watering patterns, whether your operation is deficit irrigating or not.



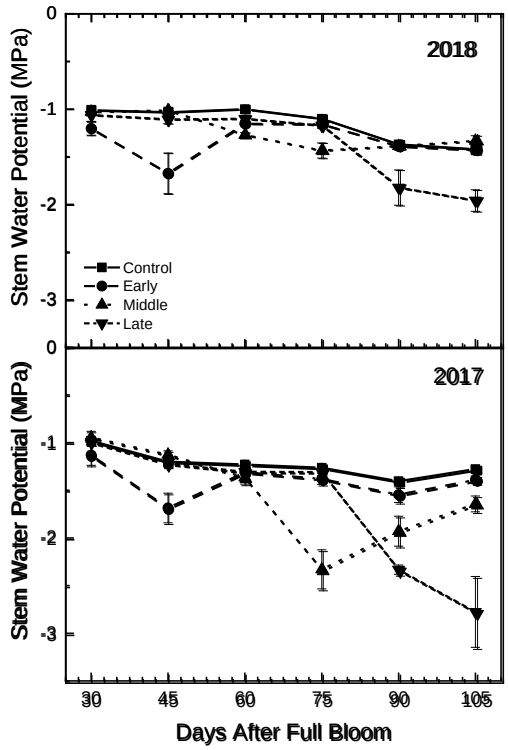


Figure 2 (left). *Volumetric water content (m^3/m^3) during the 2017 growing season (N=3) for trees within a well-watered control (solid grey line), early water limitation (dashed line; 15-45 DAFB), middle water limitation (solid black line; 45-75 DAFB), and later water limitation (dotted black line;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

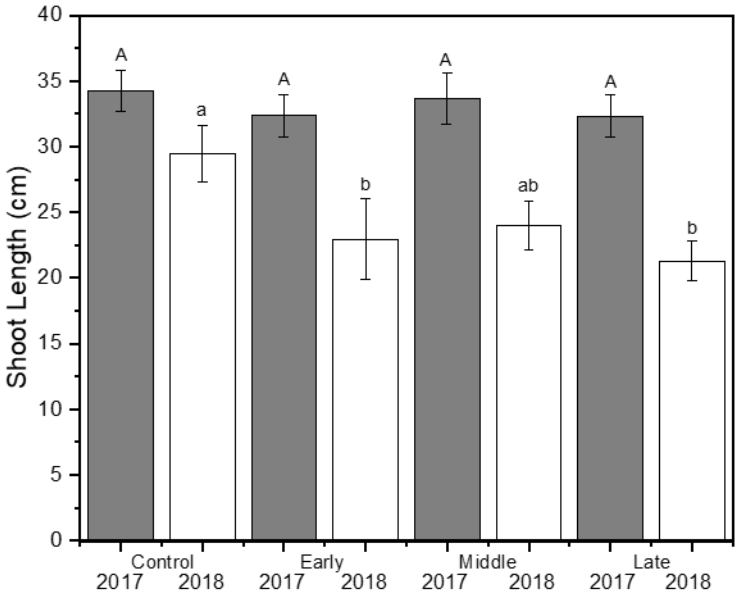


Figure 4. Mean shoot length (cm) for trees exposed to early, middle, or late summer water limitations compared to a well-watered control. Letters denote significant differences determined using Fisher's LSD test (α=0.05).

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, 3.04 in 2018, and 3.59 in 2019. 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.

Mean fruit weight was always the greatest for the fully watered control. However, differences were only significantly different in 2017. In 2018, differences among treatments in fruit weight were different than 2017, particularly under the early deficit irrigation treatment. Because crop load was lower in 2018 than in 2017, overall fruit weight was greater in 2018. In 2019, when crop load was higher again, fruit size was more similar to that observed in 2017. Despite showing similar patterns in stem water potential during the early period, fruit weight 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.

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

Treatment	Weight (g)	Color Class (1-4)	Firm (lb)	Brix (%)
		2017		
Control	255 a	2.6 b	17.1 a	13.8 a
Early	247 ab	2.7 ab	17.4 a	15.3 c
Middle	226 b	2.3 b	18.1 a	14.5 b
Late	209 c	2.9 a	19.6 b	15.6 c
		2018		
Control	326 a	2.7 a	16.0 a	14.9 a
Early	269 a	2.9 a	16.4 ab	15.7 a
Middle	299 a	2.8 a	17.4 b	15.4 a
Late	305 a	3.0 a	16.3 ab	15.2 a
		2019		
Control	290 a	3.6 a	14.1 a	13.5 a
Early	276 a	3.2 a	14.0 a	13.6 a
Middle	278 a	3.6 a	14.1 a	13.2 a
Late	274 a	3.8 a	14.1 a	14.1 a

Overall, fruit size profiles were similarly reduced in both middle and late summer deficit periods compared to the control. Bitter pit incidence was higher immediately after harvest in fruit from trees that were water-limited early in the summer where 23% of the fruit had bitter pit. This was consistent across all three years of the project. Bitter pit incidence at harvest was lowest 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 13%, 9%, and 14%, respectively. After 3 months of RA storage at 33°F, bitter pit incidence increased in all treatments. Periodic water limitations had a significant impact on bitter pit incidence. Overall, middle and late-summer deficits reduced bitter pit incidence compared to the control. Bitter pit averaged approximately 51% for the well-watered control in all years. Late summer water limitations limited bitter pit to approximately 42%. Fruit from trees exposed to middle summer irrigation deficits had the lowest bitter pit incidence with 35% of the fruit affected. Bitter pit was the highest in 2017 and decreased on average from 2017-2019 (Figure 5), Despite calcium sprays in 2018, the low crop load strongly stimulated bitter pit development. 2019had the lowest bitter pit incidence when the crop load was full again,. 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. In 2019, conditions were cooler, particularly late in the summer and fruit size and bitter pit incidence were lower as a result.

Fruit size affected bitter pit incidence and bitter pit was the greatest in 2017 all fruit diameter classes (Figure 6). Fruit that had a diameter of less than 80 mm had 38, 30, and 14% of fruit affected by bitter pit for 2017, 2018, and 2019, respectively. Fruit that had a diameter of greater than 90 mm had 77, 52, and 48% of fruit affected by bitter pit for 2017, 2018, and 2019, respectively. Fruit that had a diameter between 80 and 90 mm had bitter pit incidence between the smallest and largest fruit categories. Trees that were treated with either middle or late summer deficit irrigation had a larger proportion of fruit with diameters less than 80 mm (Figure 7) which largely accounts for differences in bitter pit between the irrigation treatments.

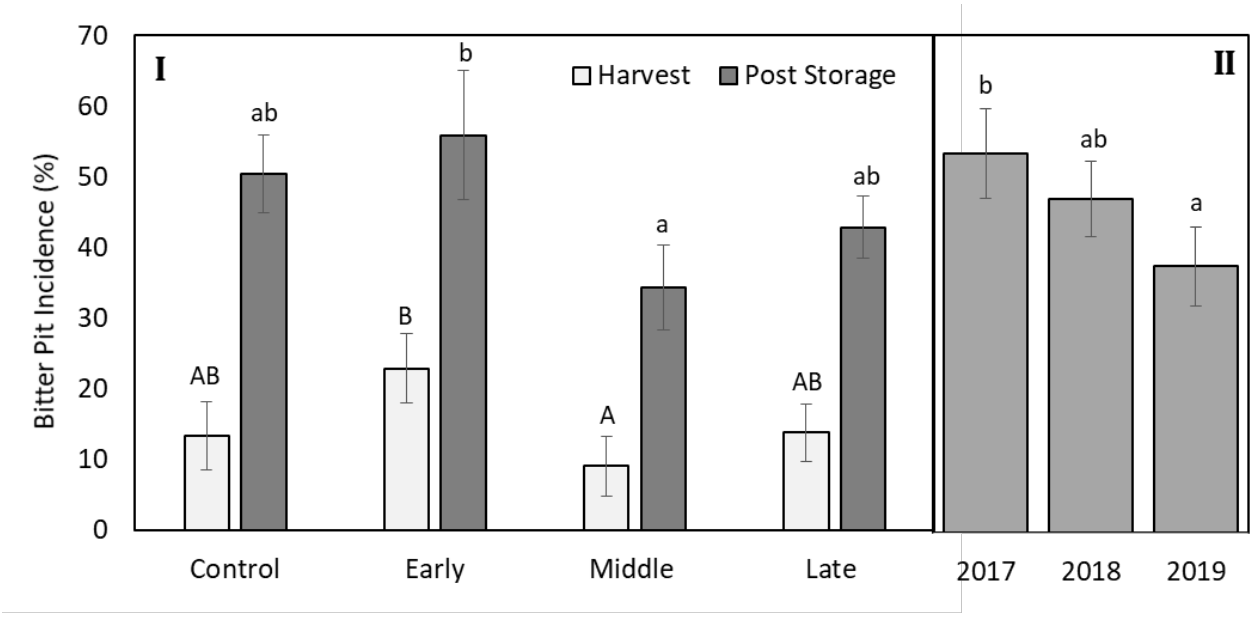


Figure 5. I. Mean bitter pit incidence (%) for 2017, 2018, and 2019 at harvest and after three months storage for fruit sampled from trees exposed to early, middle, or late summer water limitations compared to a well-watered control. II. Mean bitter pit incidence for 2017, 2018, and 2019 after three months of regular atmosphere storage. Vertical bars represent the SE of the total (N=12). Letters denote significant differences determined using Fisher's LSD test (α=0.05). There were no significant interactions between treatment and year (P=0.51).

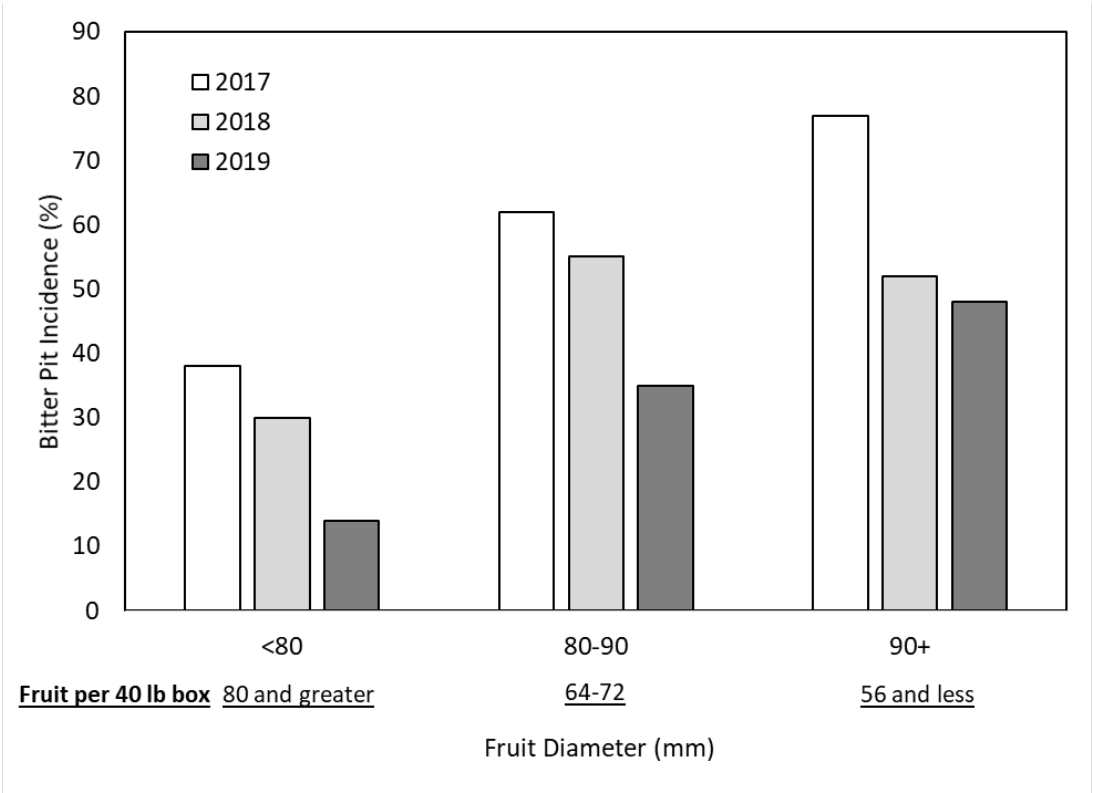


Figure 6. Bitter pit incidence for each size category in 2017, 2018, and 2019. Fruit per 40 lb box is presented below each diameter category

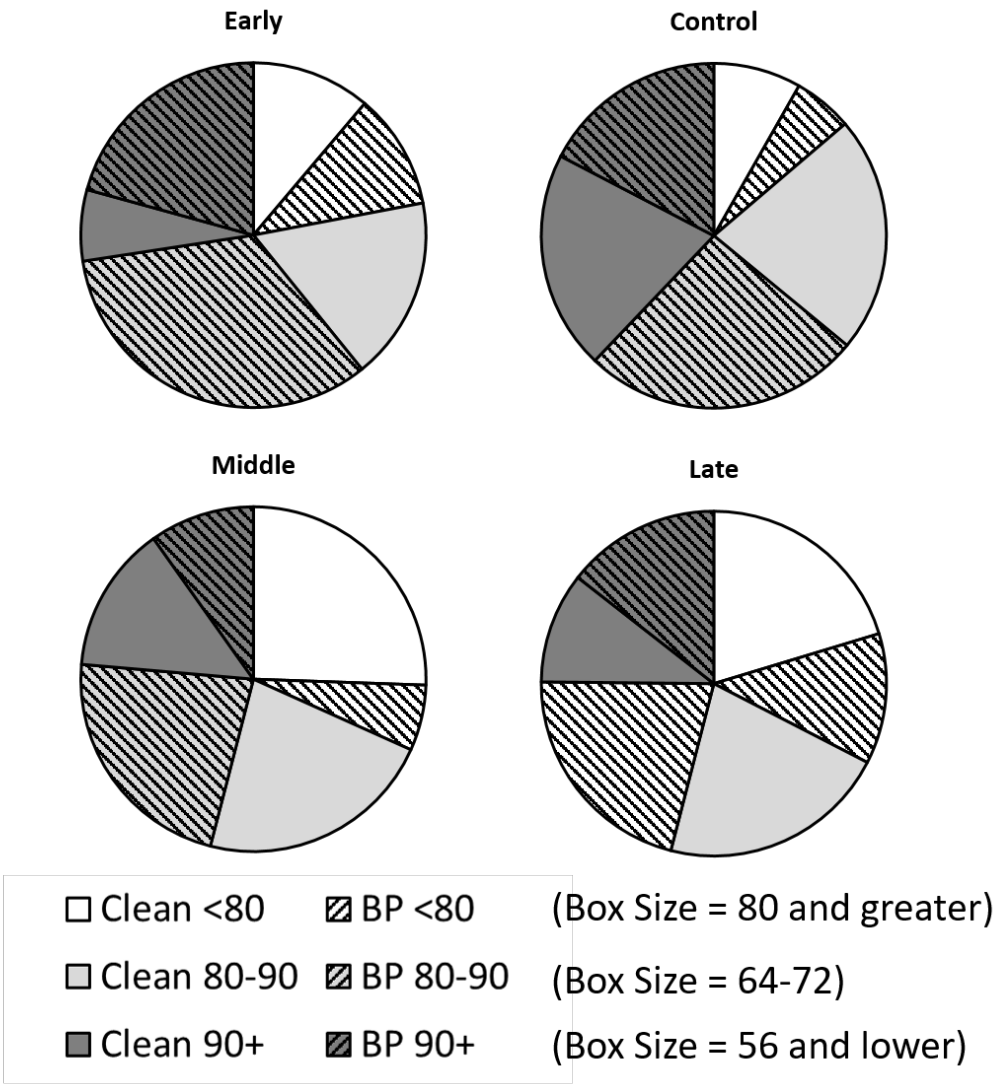


Figure 7. Mean proportions of fruit with a diameter of <80 mm (white), 80-90mm (light grey), or 90+ mm (dark grey) that is either healthy (solid color) or with bitter pit (diagonal lines) harvested from trees exposed to either early, middle, or late summer water limitations compared to a fully watered control.

COMMERCIAL ORCHARDS

7 out of 10 orchards deploying deficit irrigation had bitter pit incidence lower than 10% (Figure 8). Fruit weight mostly fell within the size category of 72-88s in these orchards with few fruit belonging to larger size categories. For the three orchards with bitter pit incidence above 10%, the K:Ca ratio was above 25, 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 tool to control bitter pit when nutrients are not correctly balanced. Potassium: calcium ratios for the three orchards with bitter pit incidence greater than 10% were greater than 25:1 indicating nutrient imbalances increasing bitter pit risk.

Table 2. Fruit calcium, potassium, and magnesium concentrations and associated ratios for ten commercial orchard sites deploying deficit irrigation in 2018 and 2019

Year	Orchard	Ca (%)	K (%)	Mg (%)	K/Ca	K+Mg/Ca	Fruit Size (g)
2018	A	0.045	0.611	0.033	13.6	14.3	177
2018	B	0.018	0.817	0.042	45.4	47.7	271
2018	C	0.042	0.625	0.03	14.9	15.6	277
2018	D	0.036	0.708	0.039	19.7	20.8	213
2018	E	0.027	0.674	0.033	25.0	26.2	263
2019	F	0.106	0.916	0.042	8.6	9.0	226
2019	G	0.046	0.834	0.040	18.2	19.0	219
2019	H	0.058	1.019	0.046	17.6	18.4	249
2019	I	0.030	0.989	0.044	33.1	34.5	226
2019	J	0.042	0.998	0.044	23.8	24.8	216

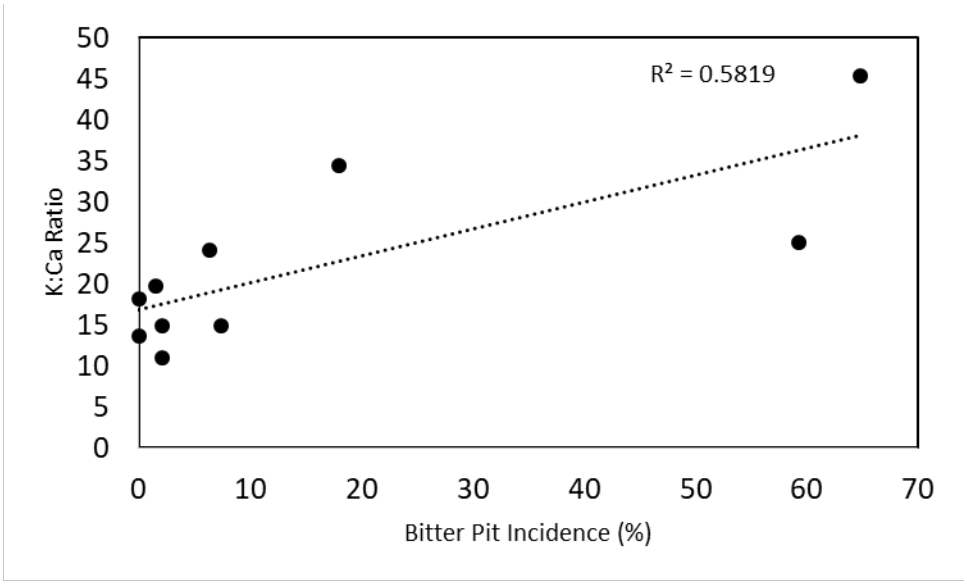


Figure 8. The relationship between bitter pit incidence (x-axis) and the potassium: calcium (K:Ca) ratio in the peel and cortex for fruit from 10 commercial orchards using calcium sprays, careful crop load management, and deficit irrigation to control bitter pit.

Industry Outreach

Since the start of this project, Lee Kalcsits has given 19 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. In 2019, WSU Wenatchee Tree Fruit Research and Extension Center hosted a field day to more than 100 industry members and this research was showcased here. Michelle Reid completed her M.S. working on this project and has presented this research at national and international and also provided 10 talks in 2018 and 2019 to industry members. At the completion of this project, the team will continue to work with the industry including producers and irrigation service providers to provide unbiased information on soil moisture and plant water status monitoring. This will include working with extension to increase the output of online and personally-delivered information to the industry.

EXECUTIVE SUMMARY

Project title: Control of fruit size and bitter pit in Honeycrisp using irrigation
Key words: Bitter pit, Honeycrisp, Irrigation
Abstract: *Malus* x *domestica* cv. 'Honeycrisp' produces large fruit that is susceptible to bitter pit. This project tested the use deficit irrigation for limiting fruit growth and bitter pit. Middle to late summer deficit irrigation during fruit expansion reduced bitter pit by decreasing the proportion of large fruit.

As the acreage of planted 'Honeycrisp' apples continues to expand across the entire United States, the market will become more selective in size and quality. 'Honeycrisp' apples have a propensity to grow oversized fruit. Fruit with larger diameters are more susceptible to the development of bitter pit. To remain competitive, the Washington apple industry must find ways to limit bitter pit occurrence and increase the amount of premium packed fruit per bin. One approach that can be used is controlling the water supply to the orchard to limit fruit size and subsequently, limit bitter pit occurrence. Washington State has the advantage of relying on irrigation for water supply and therefore has much tighter control over water delivery than other growing regions.

Deficit irrigation has been shown to decrease fruit size and subsequently, increase fruit quality. For many varieties, a decrease in fruit size would be a limitation of deficit irrigation. However, for 'Honeycrisp', where oversized fruit is common, particularly in young orchards, controlled deficit irrigation has the potential to allow growers in irrigated environments to control fruit size in accordance to their projected crop load and market demand. However, there are risks with deficit irrigation. Over stressing the tree or applying stress at the wrong time could lead to early fruit drop or losses in final fruit quality. There is a strong potential to develop advanced water management strategies as a tool to improve quality and help growers reach higher value size classes than would be possible using normal irrigation strategies.

The objectives of this project were to test how irrigation timing affects fruit size and bitter pit incidence in 'Honeycrisp' and to develop indicators to make irrigation decisions. Over three years, we show that reducing irrigation during middle and late summer during cell expansion can limit fruit size and shift the proportion of fruit in smaller, more marketable size categories. These smaller fruit were also much less susceptible to bitter pit. However, to adopt these strategies, growers need soil and plant based indicators that allow them to accurately deliver water at the right developmental stages to ensure proper sizing, development and quality. Soil moisture and stem water potential remain the two best indicators of stress in apple and closely followed the drying cycles in the orchard. It is key to make sure soil moisture sensors are placed within the root zone each orchard. Fruit quality was largely unaffected by summer water restrictions except red color development was greater when late summer water limitations were applied. Middle and late summer water limitations are effective at reducing bitter pit by 10-15% compared to non-limiting irrigation applications. However, in commercial orchards that have adopted these irrigation strategies, bitter pit incidence remained high in orchards that had not achieved optimal nutrient balance. In orchards where nutrient and crop load balance were achieved, bitter pit incidence was below 10% when irrigation deficits were used in 2018 and 2019.

FINAL PROJECT REPORT

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 visit to identify trial sites and consultancy services at 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: WSU **Contract Administrator: Katy Roberts/**Shelli Tompkins

Telephone: (509) 663-8181 **Email address:** shelli.tompkins@wsu.edu

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

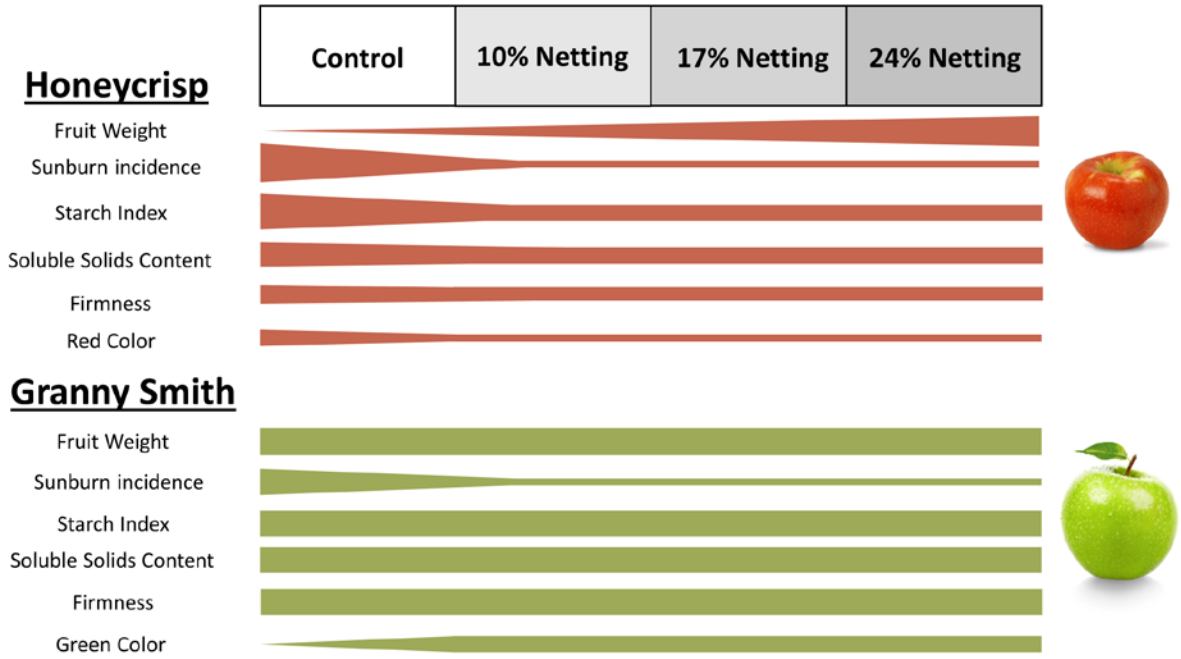
OBJECTIVES

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

- All objectives were completed as planned with two years of data acquired for each experiment
- In 'Honeycrisp', 10%, 17%, 24% shade factor significantly reduced sunburn incidence compared to an uncovered control. 10% protective netting had slightly higher proportions of fruit with severe sunburn symptoms compared to higher shading factors.
- In 'Granny Smith', 17% and 24% had higher sunburn reduction compared to 10% shade which performed significantly better than the control
- In 'Honeycrisp', 10%, 17%, 24% shade factor significantly reduced red color compared to an uncovered control, with no differences being observed between the different shade factors.
- In 'Granny Smith' 17% and 24% shade factor had lower incidence of red blush compared to 10% shade which performed significantly better than the control
- In 'Granny Smith', Extenday deployed in early summer improved light penetration and return bloom in Granny Smith directly contributing to higher yields in 2019.
- In Honeycrisp, Mylar and Extenday improved red coloration significantly under 17% protective net compared to protective netting without reflective ground cover as a control
- Under protective net, mylar had significantly higher sunburn incidence compared to protective netting without reflective ground cover as a control which was not significantly different from the control
- Despite increased leaf area under 17% protective netting compared to an uncovered control, overall water use was approximately 20% lower in 'Honeycrisp' because of reduced tree transpiration and soil evaporation.

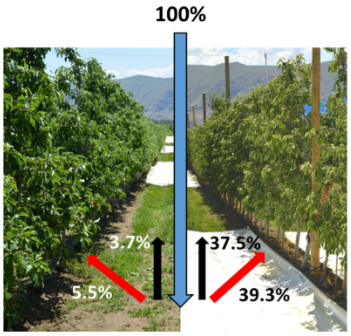
SHADING FACTORS AND ITS EFFECT ON FRUIT QUALITY



REFLECTIVE FABRICS UNDER PROTECTIVE NETTING

- No changes to photosynthetic rates or light use efficiency
- Return bloom increased in 2019 when reflective deployed for Granny Smith
  - 112 clusters for Extenday from 98 clusters for control
- Stem water potential lower when reflective was used
  - -0.9 MPa for the control and -1.2 Mpa when Extenday was used for Granny Smith
  - -1.4 MPa for the control and -1.48 and -1.52 MPa for Extenday and mylar, respectively for Honeycrisp

- No changes to fruit color for Granny Smith
- Both Extenday and Mylar increased red color equally for Honeycrisp under 17% netting
- Color development with reflective under 17% netting (2.8) was greater than an uncovered control with no reflective fabric (2.5)
- Reflective fabrics did not change fruit quality or maturity



METHODS

Site 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. Trees were managed commercially including crop load management that included bloom and hand thinning. Fruit was harvested on September 4, 2018 and September 6, 2019 at full maturity in a single pick. Full bloom was on May 5, 2018 and May 3, 2019 for Honeycrisp. The reflective ground cover was installed on 30th July 2018 and 2nd August 2019 (Extenday), 20th August 2018 and 19th August 2019 (Mylar) and removed immediately after harvest. The netting was deployed the third week of June in 2018 and the middle of May for 2019. Installation of weather equipment for monitoring environmental conditions was done on 8th of May 2018.

Site 2: McDougall & Sons, Inc., Mattawa, WA.

12th leaf 'Granny Smith' on M9 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 19 April 2018 and April 29, 2019. The reflective ground cover (Extenday) was installed after bloom and was removed the last two weeks of June in 2018 and 2019. The netting was deployed 5th of May for the shade% trial and 2nd of July for the reflective. Installation of weather equipment for monitoring environmental conditions was done on the last week of May 2018.

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' respectively. Ecophysiological measurements comprising of leaf gas exchange, leaf spectral reflectance, leaf chlorophyll fluorescence and plant water status were done 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, including standard metrics, was assessed at harvest and after 3 months of regular cold storage. This objective helped determine whether different cultivars might have different optimum shade requirements under protective netting in Washington.

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 ≈5 weeks (Extenday®) and ≈2 weeks (Mylar®) before harvest. 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’ to better understand how improved light penetration affected return bloom, photosynthesis, and tree growth in a mature orchard under netting. For Honeycrisp, the entire focus was on improving red color development under netting.

Objective 3: Quantify changes in water needs for orchards under protective netting in ‘Honeycrisp’ apple.

The trial was 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 Exo Stem gage is based on heat balance method for sap flow measurement. 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 performed 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. This work helped to determine the change in irrigation requirements of trees under protective netting.

RESULTS & DISCUSSION

OBJECTIVE 1 - Determine the optimal shade percentage for the most common cultivars under protective netting in WA (Honeycrisp and Granny Smith)

There were no differences in the probability of sunburn incidence between the different shade factors ‘Honeycrisp’ (Figure 1). Sunburn was much more likely to occur on fruit without netting. In ‘Granny Smith’, the two highest shade factors (17% and 24%) reduced sunburn incidence probability compared to 10% shade factor. Sunburn was likely to occur in 20% of the fruit for 10% shade netting but only about 15% of the fruit for higher shade factors in ‘Granny Smith’. 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 with sunburn reductions. Honeycrisp has significantly less fruit without any sunburn symptoms that ‘Granny Smith’ (Table 1). Patterns in sunburn development were similar across both cultivars with netting being the main effect and shade factors having little influence over sunburn incidence. However, 10% netting had more severely damaged apples (Y3 or Tan) for Honeycrisp than the two other shade factors (17% and 24%).

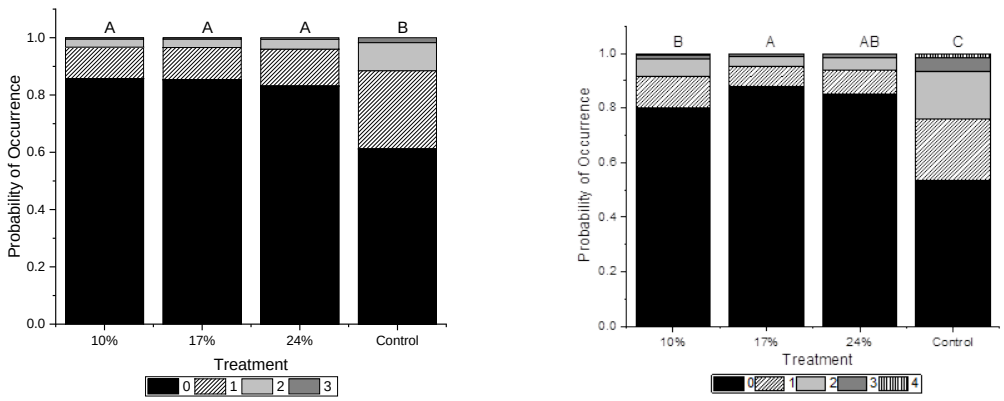


Figure 1. Overall probability of sunburn occurrence in 'Honeycrisp' (left) and 'Granny Smith' (right) apple grown under 10%, 17% and 24% protective netting compared to an uncovered control. 0 is equal to no sunburn incidence and 1, 2, 3, and 4 are increasing severities of sunburn incidence. Letters denote significant differences between treatments.

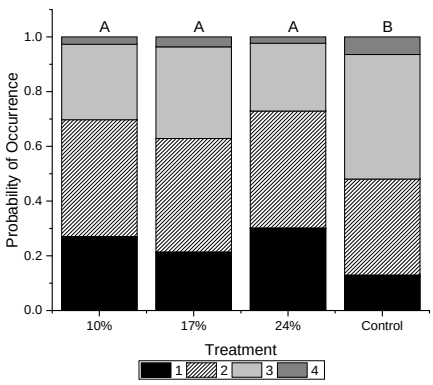
Table 1. Mean percentage of fruit belonging to each sunburn category following a modified sunburn scale for bi-color cultivars (Mendoza and Hanrahan 2012). Letters denote significant differences between treatments determined using a Fisher's LSD test (α=0.05).

	Clean	**Y1**	**Y2**	**Y3**	**Tan**
			Honeycrisp		
Control	48.7 a	23.4	15.9 b	8.1 b	3.8 b
10%	69.7 b	20.9	7.5 a	0.6 a	1.3 a
17%	69.3 b	21.2	8.1 a	1.4 a	0.0 a
24%	74.7 b	15.9	7.8 a	1.3 a	0.3 a
			Granny Smith		
Control	55.8 a	23.2	13.1 b	5.0 b	2.9 b
10%	81.2 b	12.7	4.8 a	1.0 a	0.2 a
17%	83.3 b	11.7	4.6 a	0.4 a	0.0 a
24%	78.8 b	16.5	4.6 a	0.2 a	0.0 a
			Significance		
Cultivar	0.03	0.08	0.14	0.26	0.41
Treatment	<0.001	0.13	0.009	<0.01	<0.01
Cultivar x Treatment	0.83	0.35	0.99	0.69	0.94

All shade factors had lower probability of occurrence for 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. In 'Granny Smith', shade factor played an important part in 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 and may be a key factor in decisions on shading factor for protective netting for Granny Smith.

Fruit weight was significantly larger in 2019 than 2018 for both Honeycrisp and Granny Smith (Table 2 and 3). Fruit was the smallest for Honeycrisp grown without netting compared to any

of the netting treatments. These results were consistent with previous results reporting increased fruit size under 20% netting (Kalcsits et al, unpublished). Fruit firmness was not affected by netting treatment. However, consistent with the observed reductions in fruit size and corresponding increases in carbohydrate pools in the fruit, soluble solids content was greater for fruit from the control compared to fruit that was grown under protective netting.



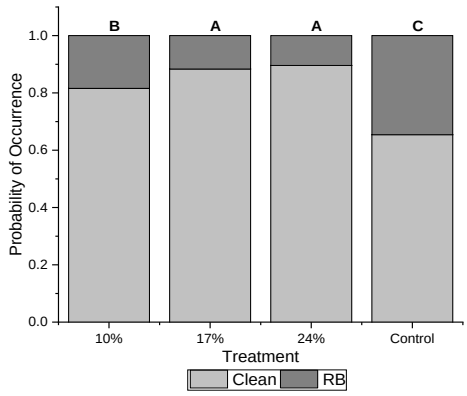


Figure 2 (left). Probability analysis of red color coverage in 'Honeycrisp' apple grown under 10%, 17% and 24% protective netting compared to an uncovered control

Figure 3 (right). Probability analysis of red blush occurrence in 'Granny Smith' apple grown under 10%, 17% and 24% protective netting compared to an uncovered control

Table 2. The effect of protective netting with 10%, 17% or 24% on fruit weight, fruit firmness, total soluble solids, and titratable acidity of 'Honeycrisp' apple at harvest at Quincy, WA in 2018 and 2019

Treatment	Fruit weight (g)	Fruit firmness (lb)	Total Soluble solids (°Brix)	Titratable acidity (% MA)
		2018		
Control	260.7	15.8	13.6 a	0.67
10% Shade	246.2	15.7	13.0 b	0.66
17% Shade	271.1	15.4	13.0 b	0.67
24% Shade	282.6	15.5	12.9 b	0.68
		2019		
Control	298.5	14.0	15.3 a	0.67
10% Shade	312.7	14.1	14.7 ab	0.71
17% Shade	310.9	13.6	15.0 ab	0.72
24% Shade	324.7	13.4	14.3 b	0.71
		Significance		
Treatment	0.046	0.63	0.02	0.22
Year	<0.001	<0.0001	<0.0001	<0.01
Treatment x Year	0.68	0.74	0.71	0.18

Table 3. The effect of 10%, 17% and 24% protective net on fruit quality of 'Granny Smith' apple at harvest at Mattawa, WA in 2018 and 2019

Treatment	Fruit weight (g)	Fruit firmness (lb)	Total Soluble solids (°Brix)	Titratable acidity (% MA)
		2018		
Control	228.8	17.4	13.14	1.03
10% Shade	235.3	17.6	13.40	1.06
17% Shade	228.5	16.8	13.05	1.08
24% Shade	226.5	16.3	12.91	1.01
		2019		
Control	243.3	17.1	11.65	1.07
10% Shade	247.5	16.9	11.50	1.06
17% Shade	243.8	16.5	11.31	1.09
24% Shade	241.7	17.2	12.61	0.98
		Significance		
Treatment	0.56	0.38	0.62	0.11
Year	<0.01	0.73	<0.001	0.56
Treatment x Year	0.73	0.33	0.28	0.81

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.

Light quality was significantly affected by the use of reflective material either immediately following bloom (Granny Smith) or near harvest (Honeycrisp) (Table 4). More than 35% of the incoming light was scattered into the lower canopy of the trees for both cultivars. Even with different ages and training systems the estimates of reflected and scattered light were similar between the two cultivars. There was less reflected and diffuse light in the tree canopy for the Honeycrisp location. This may be, in part, due to a narrow v-trellis training system that intercepted more light than the simple upright training system for Granny Smith.

Table 4. 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 for Honeycrisp (near harvest) or Granny Smith (post bloom) apple orchards

Treatment	Center of drive row			Tree Canopy	
	Incoming PAR	Reflected PAR	Diffuse PAR	Reflected PAR	Diffuse PAR
		Honeycrisp			
Control	1296	50.0 c	60.8 c	15.7 c	22.3 b
Extenday®	1289	418.8 b	415.0 b	148.4 b	79.8 a
Mylar®	1307	449.3 a	530.1 a	206.2 a	99.3 a
		Granny Smith			
Control	1765	65.3 b	97.1 b	28.2 b	31.8 b

Extenday®	1786	669.8 a	701.9 a	355.4 a	346.5 a

Reflective fabrics deployed in early summer did not affect fruit size or overall yield in the first year of deployment (2018) (Table 4). However, the number of flower clusters was greater in 2019 and trees where Extenday was deployed in 2018 had a greater yield in 2019 for Granny Smith. Deployment of either Mylar or Extenday in August did not affect fruit size or overall yield in either year for Honeycrisp.

Table 5. Yield, fruit weight for Granny Smith and Honeycrisp apple and flower cluster counts for Granny Smith (±SEM; N=4-5) when reflective fabrics were used either in early summer for Granny Smith or in August for Honeycrisp apple. Letters denote significant differences among treatments determined using a Fisher's LSD test (α=0.05).

	Granny Smith			**Honeycrisp**	
	Yield (lb)	**Fruit weight (g)**	**Flower Clusters**	**Yield (lb)**	**Fruit weight (g)**
2018					
Control	57.9 ± 3.25 a	232 ± 4.8 a		33.9 ± 1.72 a	299 ± 12.5 a
Extenday	60.3 ±3.17 a	233 ±18.9 a		34.8 ± 1.74 a	301 ± 12.7 a
Mylar				36.7 ± 1.70 a	297 ± 12.2 a
2019					
Control	33.9 ± 3.89 a	231 ± 9.1 a	98 ± 4.2 a	32.6 ± 2.00 a	245 ± 14.7 a
Extenday	43.8 ± 4.49 b	232 ± 7.1 a	112 ± 5.3 b	28.6 ± 2.20 a	246 ± 12.7 a
Mylar				31.9 ± 1.69 a	254 ± 8.7 a

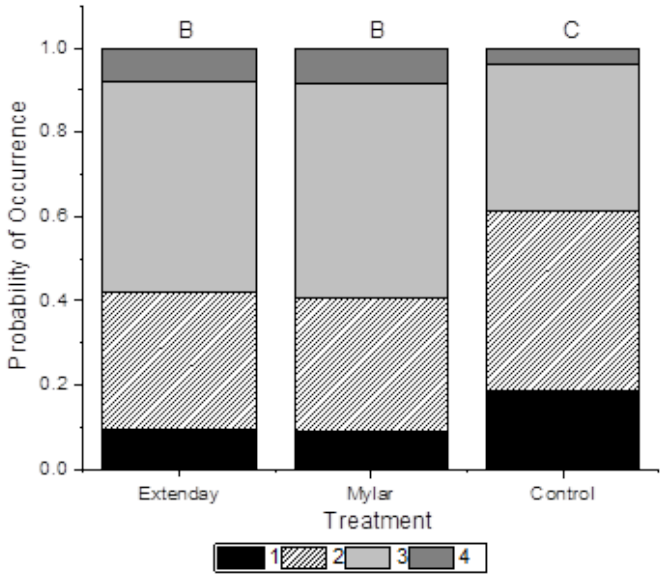


Figure 4. Probability analysis of red color coverage in 'Honeycrisp' apple grown under 17% protective netting with Extenday® and Mylar® reflective ground covers compared to a control with grass cover. 1 = 0-25% red coverage, 2 = 25-50% coverage, 3 = 50-75% coverage, and 4 = 75-100% coverage.

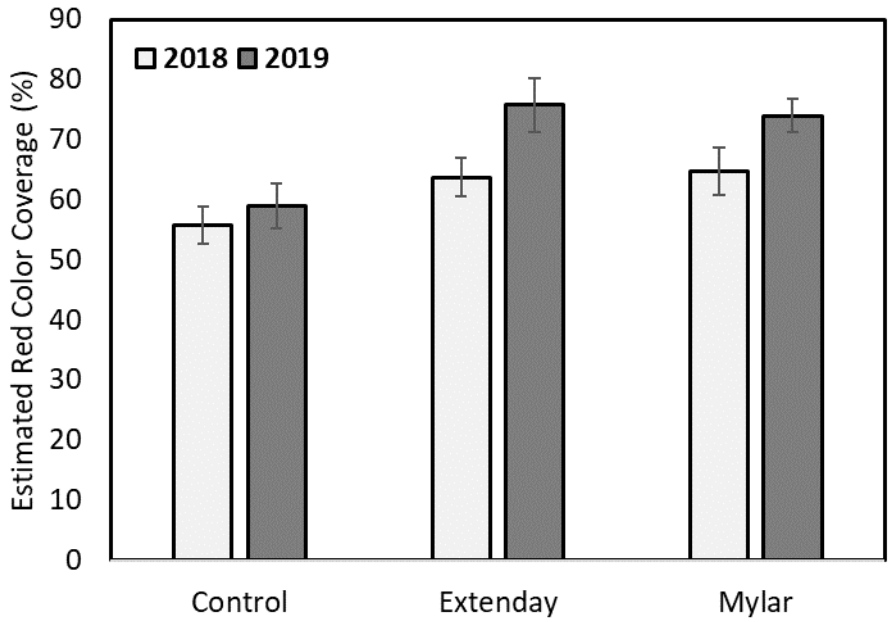


Figure 5. Estimated red color coverage (%) for Honeycrisp with either Extenday or Mylar deployed prior to harvest in 2018 and 2019. Error bars indicate SEM (N = 5 Honeycrisp).

Red color development was greater when reflective fabrics were used under netting. The probability of having fruit with low color development (<50%) was much greater in the control than either of the reflective fabric treatments (Figure 4). Extenday was deployed earlier but did not have significantly better red color development compared to Mylar that was deployed two weeks before harvest. Differences in red color development between the control and reflective fabrics was greater in 2019 than 2018 (Figure 5).

OBJECTIVE 3 - Quantify changes in water needs for orchards under protective netting in 'Honeycrisp' apple.

Protective netting reduced whole tree transpiration by approximately 20% 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. In a year with water restrictions, growers using protective netting will be able to protect their trees better in addition to the sunburn protection afforded by netting. When averaged over 70 days of water-use measurements, cumulative water use was approximately 20% lower where mean tree water use was approximately 4 L (1.06 Gal) per day in the uncovered control compared to 3.2 L (0.85 Gal) per day under protective netting. Furthermore, evapotranspiration was also lower between rows reducing the need to microsprinkler use.

Table 5. Mean water-use, leaf number and leaf number (±SEM; N=4) for trees under protective netting compared to an uncovered control. Letters denote significant differences between means determined using a Fisher's LSD test (α=0.05).

	Water Use	**Leaf Area**	**Leaf Number**
	(mL H_2O m^{-2} day^{-1})	**m^2**	
Control	1400 ± 201.2 b	3.23 ± 0.22 a	1310 ± 85 a
Netting	911 ± 182.2 a	3.76 ± 0.37 a	1622 ± 109 b

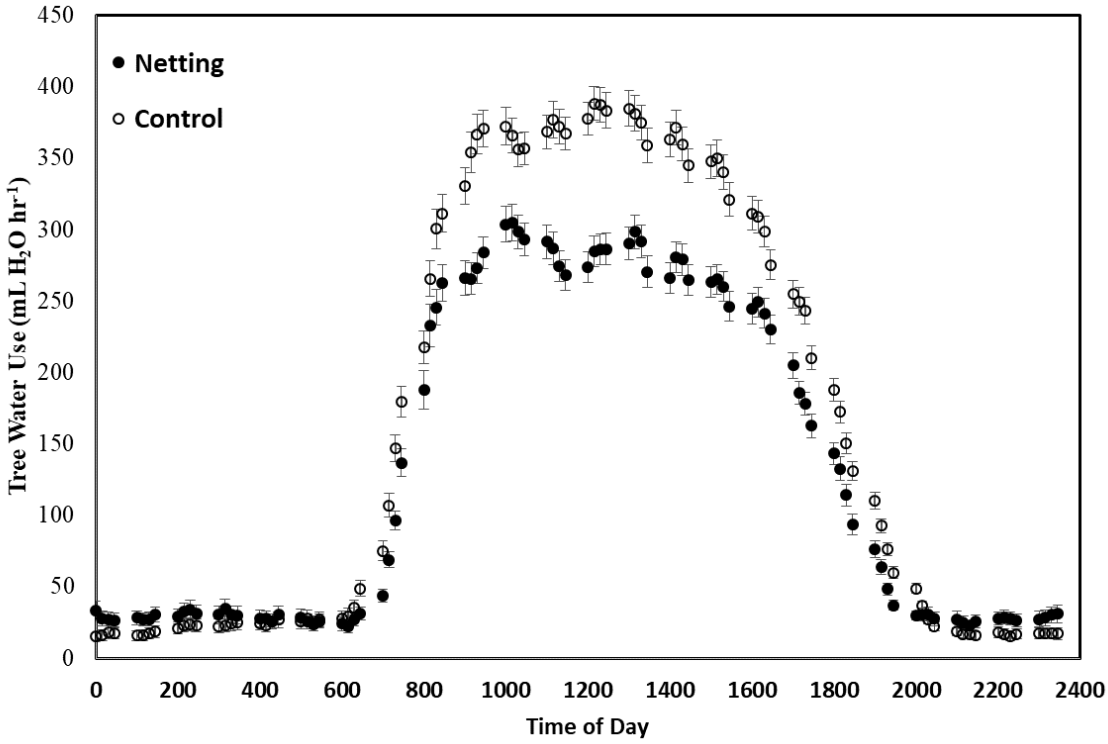


Figure 6. Influence of protective netting on whole tree transpiration (mL H_2O hr^{-1}) for 40 recorded days in 2018 and 30 recorded days in 2019.

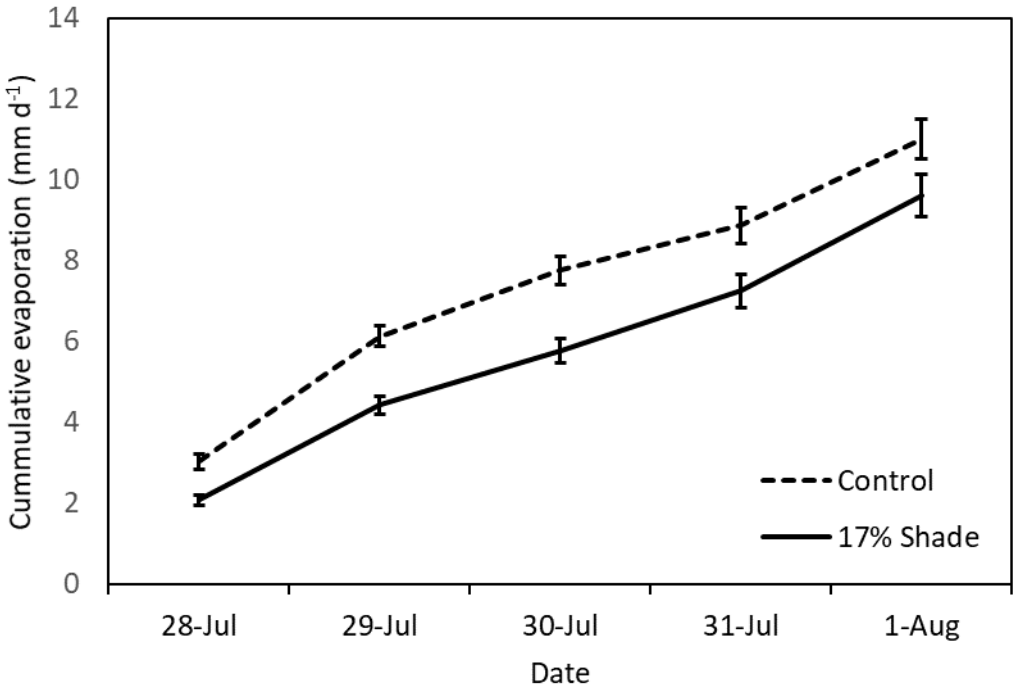


Figure 7. Mean evapotranspiration from the orchard grass between rows for a five-day period under protective netting compared to an uncovered control area (N=4).

EXECUTIVE SUMMARY

Project title: Optimizing light and water for orchards covered with netting
Key words: Protective Netting, Sunburn, Color, Reflective Fabric
Abstract: Netting is used for apple sunburn and hail protection. This project determined the effect of shading factor and reflective fabric on apple fruit quality. 10-17% and 17-24% shade is sufficient for Honeycrisp and Granny Smith apple, respectively. Reflective fabric improved fruit color under netting. Water-use is 20% lower under nets.

The apple growing season in Washington State is characterized by high winds, light intensities, and temperatures which can all negatively impact both the tree and fruit. The adoption of protective netting is increasing as a way to reduce environmental stress in apple production by growers. Most of the research on protective netting use in apple production in WA was focused on microclimatic changes in the orchard environment, sunburn reduction and fruit quality, impact of netting on tree stress and light use efficiency, and evaluating different colors of photoselective protective netting. The optimum shading percentage still needs to be determined for 'Honeycrisp' and 'Granny Smith' which make up most of the acres under protective netting. Identifying the optimum shade factors for Honeycrisp, a bi-color cultivar, as well as Granny Smith will also enable more specific recommendations to be made in future for other cultivars. The optimal shading factor was determined by studying the response of 'Honeycrisp' and 'Granny Smith' at 10%, 17%, or 24% shade factors. The effect of reflective ground fabric on fruit quality in 'Honeycrisp' and 'Granny Smith' was tested by comparing plots under protective netting either with or without the reflective ground fabric to better understand its effect on light penetration and fruit quality. The reduction in incident solar radiation and wind under protective netting has implications for tree water use. This was measured in a Honeycrisp orchard covered in 17% netting.

Shade factor affected sunburn incidence. In 'Honeycrisp', 10%, 17%, 24% shade factor significantly reduced sunburn incidence compared to an uncovered control. For both 'Honeycrisp' and 'Granny Smith', 10% protective netting had slightly higher proportions of fruit with severe sunburn symptoms compared to higher shading factors. In 'Honeycrisp', 10%, 17%, 24% shade factor significantly reduced red color compared to an uncovered control, with no differences being observed between the different shade factors. The incidence of red blushing was lower in 'Granny Smith' under 17% and 24% shade compared to 10% shade which was also lower than the uncovered control. In 'Honeycrisp', Mylar and Extenday improved red coloration significantly under 17% protective net compared to protective netting without reflective ground cover. In 'Granny Smith', Extenday deployed in early summer improved light penetration and return bloom in 'Granny Smith', directly contributing to higher yields in 2019. Under protective net, mylar had significantly higher sunburn incidence compared to protective netting without reflective ground cover as a control which was not significantly different from the control.
Despite increased leaf area under 17% protective netting compared to an uncovered control, overall water use was approximately 20% lower in 'Honeycrisp' because of reduced tree transpiration and soil evaporation.

10 or 17% were optimal shade factors for 'Honeycrisp' but 10% would need additional sunburn protection measures if used. 17 or 24% netting is recommended for Granny Smith to limit red blush development. Reflective fabric installed prior to harvest can improve red color development under net equal to an uncovered control. Reflective fabric used earlier in the season improved return bloom and yield in a mature Granny Smith orchard. The reductions in water use under netting are important for water conservation measures and for cultivars like Honeycrisp that require more careful water management to limit disorder development.

FINAL PROJECT REPORT

Project Title: WA 38 demonstration trial block

PI: Karen Lewis
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Co-PI (3): Ines Hanrahan
Organization: WTFRC
Telephone: 509.669.0267
Email: hanrahan@treefruitresearch.com
Address: 1719 Springwater Ave
City: Wenatchee
State/Zip: WA 98801

Cooperators: **Bleyhl Farm Service, Burrow Tractor – Sunnyside, DrapeNet, G.S. Long**

Total Project Funding: 44,258

Budget History:

Item	2018	2019
WTFRC expenses	16,858	0
Salaries		
Benefits		
Wages	1,000	1,000
Benefits		
Equipment	2,200	1,000
Supplies	1,200	1,200
Travel	2,500	2,500
Plot Fees	2,400	2,400
Miscellaneous	5,000	5,000
Total	**31,158**	**13,100**

Footnotes: WTFRC

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

Footnotes: WSU

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

JUSTIFICATION

The WSU WA 38 block was established at the WSU Roza Farm in 2013. The purpose of the trial was to evaluate rootstock and training systems. The 0.8-acre block was divided in 4 rows on a Spindle 3x10 using the "bending" technique, 4 rows on V trellis 1.5x10 with individual trees facing opposite sides, and 3 rows on Bi-axis on a 3x10 spacing. Within each row, G41 and M9-nic 29 rootstocks were randomized in blocks of 11 trees for Spindle and Bi-axis system and 22 trees on the V trellis. In addition, different pruning techniques were established later in a project lead by Stefano Musacchi and Karen Lewis, applying hand pruning versus mechanical pruning. The most significant findings from this project have been published and shared in detail on the WSU Tree fruit webpage – WA 38 section and in the WTFRC final report. Once the funding ended for that project in 2016, the block was not managed and was slated to be removed. In 2017 the Roza Farm was affected by a hail event during bloom accompanied by favorable conditions for fire blight development. This situation resulted in tree infections and a buildup of inoculum for 2018.

This proposal offered the opportunity to investigate and demonstrate several cultural practices specific to WA 38 under the Roza Farm growing conditions and historic circumstances. The benefit of having a mature orchard allowed us to understand challenges and demonstrate practical management techniques including but not limited to canopy and crop load management, fruit maturity and fruit quality, green spot, root growth, and more. The block also served as a gathering space to bring growers together and develop a collaborative community. Finally, this block provided fruit to PVM and WSU for marketing purposes in 2015-2018.

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 spray-able 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 health 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

FINDINGS

1. Under the Roza Farm growing conditions, M9 rootstock was the most productive (approx. 80 bins/acre) in both V trellis and bi-axis system, with no difference between those two training system, despite having double number of trees in the V trellis.
2. Under the Roza Farm growing conditions, the spindle system trees were the most vigorous, had the most blind wood and had the lowest productivity. However, the trees trained to this system were previously managed by bending branches, and have been in transition to a traditional spindle since 2017.

3. G41 rootstock showed reduced productivity in spindle and V trellis systems when compared with M9.
4. Drape net improved fruit weight and size distribution. In 2019 the block had less than 1 % sunburn, thus the protective effect was not assessed.
5. G41 and M9-Nic 29 showed significant differences in root growth, length and volume. These differences did not translate to significant differences in nutrient uptake, however, green spot incidence was 56% in G41 compared to 14% on M9 with p= 0.12.

RESULTS & DISCUSSION

Objective # 1 Canopy Management and Crop load

In 2018 we recruited industry cooperators to prune the entire block on April 4th. Mechanical hedging was applied on July 13th when temperatures reached 100°F to reduce potential fire blight infection, leading to sunburn in the most exposed fruit. Thus, late hedging (20+ leaf stage) can lead to sunburn if temperatures are expected to be above 90 F.

Trees trained using "bending" have been pruned intensively during winter and summer to transition trees to "narrow" robot ready architectures. After two years of winter and summer pruning, tress still have high vigor and blind wood. This process has given us a good example of the importance of adequate training and pruning during the orchard establishment are fundamental for the profitability, quality and long term performance of the orchard. These rows have also served for detailed analysis of green spot and its relation to tree vigor and nutrient imbalance.

In 2018, full bloom was between April 25th to 28th, while in 2019 full bloom was recorded on May 3rd. In 2018, the pruning and crop load management strategy was to leave as much fruit as possible to control tree vigor with crop load. However, crop load was low with approximately 12 bins in the entire block, equivalent to 25 bins/acre at a 2420 trees/acre. Low production can be attributed to several factors including; lack of pollen and poor pollinator activity, unfavorable weather during bloom with 0.21 inches of rain between the 27th and 28th of April and wind speeds up to 32.5 mph.

In 2019, nine teams of industry cooperators selected post to post sections in V trellis and bi-axis rows and using varying strategies, pruned to bud count of 80-85 buds/tree. At harvest, yield/acre was estimated by harvesting 10 complete trees for each rootstock, weighing each fruit and multiplying by the trees per acre. Yield improvement from 2018 to 2019 can be attributed to; adequate pruning 2019, better conditions during bloom and overall good conditions during the growing season. (Table 1). A third year of evaluation would be required to determine the biennial potential in this block.

Table 1. Production indicators for each training system and rootstock.

System	Spindle		V Trellis		Bi Axis	
Trees/acre	1210		2420		1210	
Rootstock	M9	G41	M9	G41	M9	G41
Fruit weight (g)	290	298	221	240	240	328
Yield/tree (lbs)	43	35	33	25	62	66
Yield (ton/acre)	26	21	40	30	37	40
Yield (bins/acre)	56	45	86	65	81	86

Trees have gained adequate growth balance after two years of training, spring and summer pruning, and spring and dormant hedging.
Because we only have one year of evaluated pruning strategies, we cannot fully develop pruning rules that are transferable and executable by pruning crews with an acceptable level of confidence.

Objective # 2 Sunburn / Fruit Finish
Due to the need for intensive fire blight scouting in 2018, we did not install any netting. We did deploy Drape Net in 2019. In 2018, sunburn protectants were applied 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.
On June 20, 2019 a drape net was deployed in 4 post to post sections of G41 and M9 on the bi-axis system. There were no differences in sunburn incidence in 2019 between the netted and none netted treatments, however, fruit from netted trees weighed more than fruit from non- netted trees (Figure 1). The distribution of starch content as indicator of fruit maturity and fruit size indicated that netting can potentially delay maturity, but increase size homogeneity in the canopy (Figure 2).

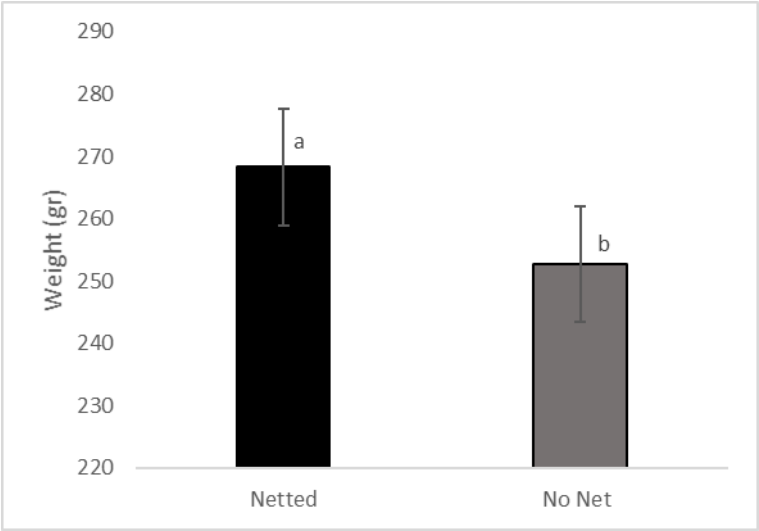

Figure 1. Netting effect on fruit weight. Bars indicate standard error; different letters indicate statistical significance (Tukey test p=0.06)

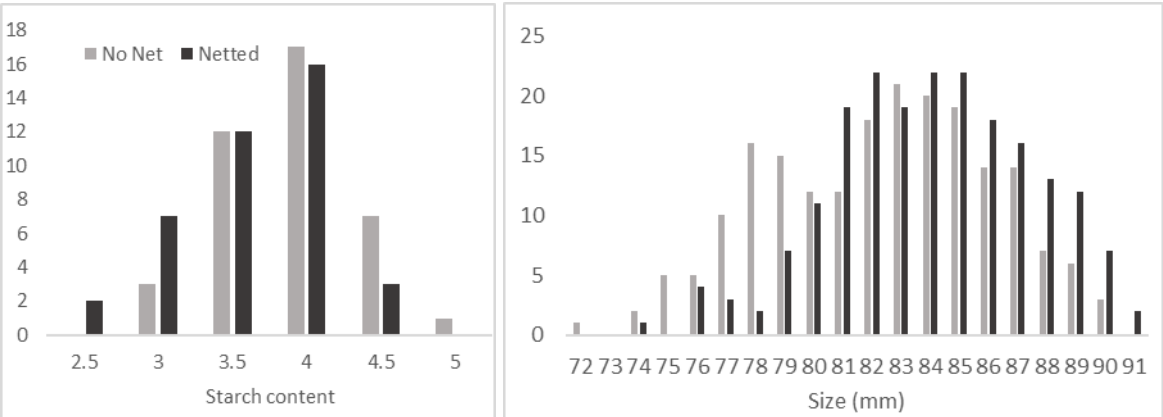

Figure 2. Netting effect on distribution of fruit starch content (left) and size (right).

Objective # 3 Mechanization
In cooperation with vendors, platforms and hedging were utilized in the block for demonstration purposes. The entire block was hedged in both 2018 and 2019 at dormant and spring (12 and 20 leaf stages) . Hedging process was demonstrated and discussed at several field days and was recorded for social media. Platforms were used and demonstrated in pruning, net deployment and harvest.

Objective # 4 Fruit Quality and Storage Potential
Mid-project, Drs. Sara Serra and Stefano Musacchi assumed leadership for this project aim. Protocols were developed to reflect industry needs expressed through the PVM Quality Standards Committee and previous experience in both WTFRC and WSU labs. The results from this objective contributed to the development of a starch scale for WA 38 distributed to the industry by WTFRC and WSU Extension. A fruit maturity field day was conducted at the Roza Farm block in both 2018 and 2019.

Objective # 5 and 6 Evaluate and Demonstrate Nutrient Status and Root Development
Soils samples were obtained in spring 2018 and 2019 for chemical analysis following the standard methods recommended for western soils (Miller et al 2013). Mineral deficiencies of phosphorous (10 mg/kg), sulphur (8 mg/kg), zinc (0.50 mg/kg) and boron (0.12 mg/kg) were identified in 2018. 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/).
To bring the soil back to adequate nutrient levels, the complete block was treated with 100 lbs of mono ammonium phosphate (MAP)/acre, 25 lbs of $ZnSO_4$/acre and 2 lbs of B/acre in 2018 and 2019. Leaf tissue analyses during the sampling season (August) showed adequate levels of all nutrients indicating adequate nutrient absorption. In 2018 Ca levels were slightly low in the V trellis, thus in 2019 additional Ca sprays were applied in spring. In 2019 tissue samples indicated adequate nutrient levels across all systems and rootstocks.

Irrigation management was done utilizing a moisture and temperature sensor located at two depths placed on G41 row (identified as the most demanding section in the block). Moisture was maintained between 0.33 and 0.19% (equivalent to 100 – 50% field capacity in a silt loam soil), which led to an average of 6 hours of irrigation per week during the growing season. Irrigation was stopped 3 weeks before harvest and we did not observe moisture levels below 0.19 during that period.

Root growth between rootstocks was evaluated by placing 3 root windows on each rootstock (replicates) in the V trellis system. A detailed explanation of how to develop the root window was shared with the Good Fruit Grower and published in April, 2019 (https://www.goodfruit.com/a-window-to-the-roots/) (Figure 3). In 2018 root growth showed no differences between rootstocks with temperatures above 59 F and at approximately 30 DAFB. In 2019, G41 initiated its root growth approximately 10 days before M9-nic 29, also with temperatures above 59 F, however in 2019, root growth happened simultaneously with the bloom period in G41 and 10 DAFB in M9-nic 29 (Figure 4).

Figure 3. Root windows (3 ft^2) built with 2x4 pine wood, plexiglass window drilled in one side of the window and placed between 5 to 7 inches from the tree trunk.

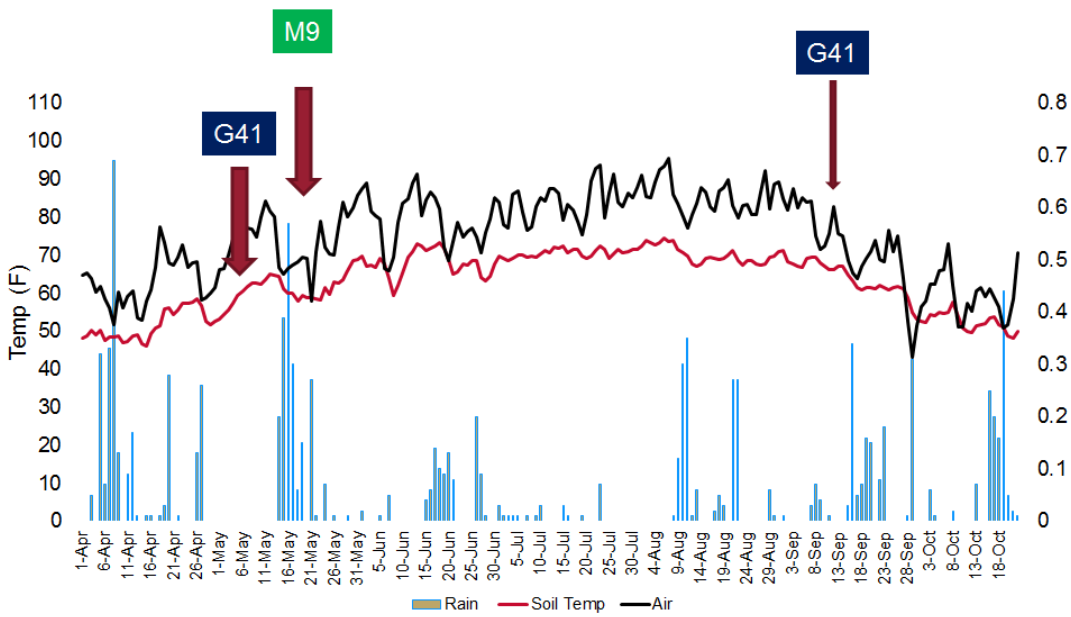

Figure 4. Root growth start date for G41 and M9 during 2019 and its relation with soil and air temperature (principal axis) and rain (inches secondary access) during the growing season.

Root growth in G41 started earlier in the season than root growth in M9 (May 3 and May 16 respectively). Both roots had the greatest rate of growth between start date and 40 DAFB, coinciding with the period of cell division in apples. While M9 stop growing after June 7, G41 continue growing until June 27. The volume of roots was also significantly different between rootstocks. G41 had 3X the volume of roots when compared to M9 (Figure 5). This root growth differences can explain the vigor differences observed in both rootstocks and the potential to develop Ca deficiency disorders.

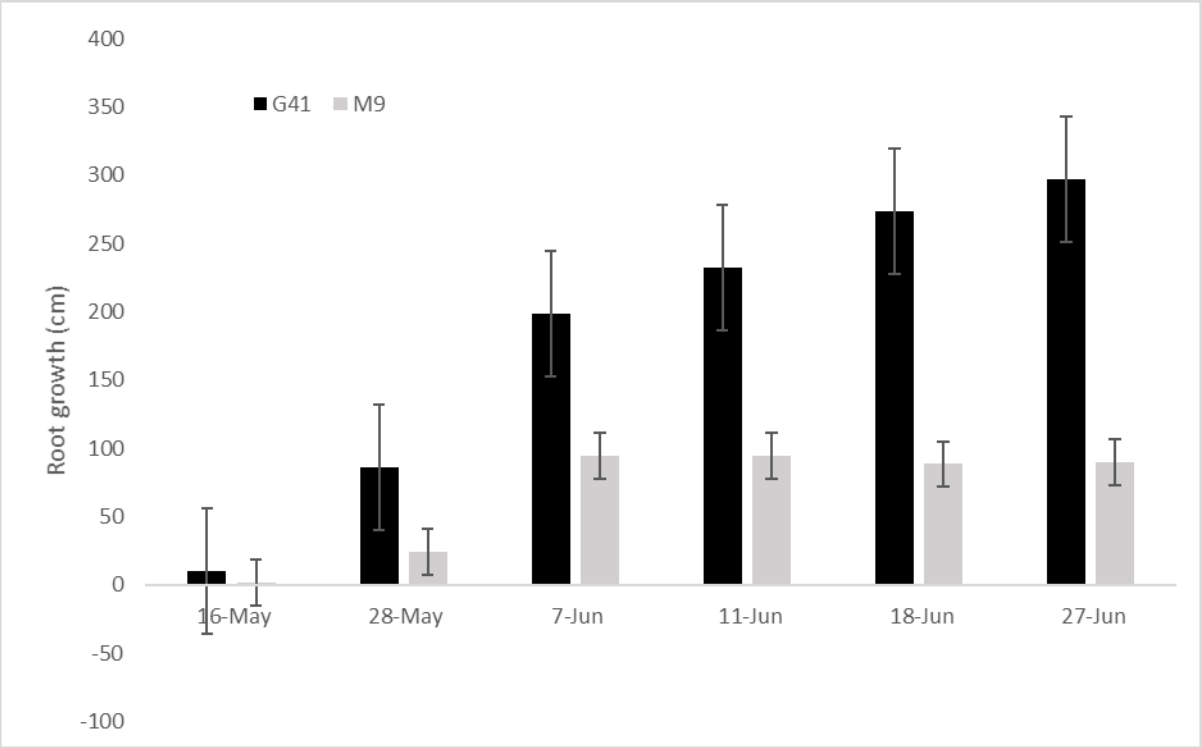

Figure 5. Root growth length for G41 and M9 in 2019.

Despite the differences in root growth pattern, nutrient content in fruit showed no differences between rootstocks. The incidence of green spot was significantly different between the M9 and G41 rootstock (Table 1). However, internal variability within the tree could be masking the effect of root growth on nutrient uptake and its relation with green spot incidence.

Table 1. Macronutrient levels and green spot incidence in G41 and M9 on V trellis.

Root	**N**	**P**	**K**	**Ca**	**Green Spot %**
G41	2.16 a	0.35 a	2.25 a	1.6 a	**56**
M9	2.30 a	0.32 a	1.99 a	1.5 a	**14**
Pr > F(Model)	0.30	0.69	0.28	0.43	**0.12**

Objective #7 Conduct field days and document best management practices. Contribute to body of knowledge using all methods and informational platforms.

Field days and Orchard Tours – Over 200 people toured or attended a field day at Roza Farm block in both 2018 and 2019. Information / lessons learned were shared in presentations, in WSU Fruit Matters, on social media and in the Good Fruit Grower Magazine.

Reference

Miller, R.O, R. Gavlak, D. Horneck. 2013. Soil, Plant and Water Reference Methods for the Western Region. WREP 125

Project Title: WA 38 Demonstration Trial Block
Key words: WA38, Cosmic Crisp®, Root growth, Netting
Abstract:
Approximately 400 people toured the block, attended field days and / or collaborated in pruning events. G41 and M9-Nic 29 showed significant differences in root growth, length and volume. M9 rootstock was the most productive in V trellis and bi-axis systems, with no difference between training systems.

EXECUTIVE SUMMARY

The WSU WA 38 block was established at the Roza Farm in 2013. The purpose of the trial was to evaluate rootstock and training systems. The 1-acre block was established with 4 rows on a spindle 3x10 using the "bending" technique, 4 rows on V trellis 1.5x10 with individual trees facing opposite sides, and 3 rows on bi-axis on a 3x10 spacing.

After a few seasons of minimum management in the block plus fire blight outbreaks and a hail event the PI's of this project developed a plan to rehabilitate the block by replacing fire blight infected trees, replacing and increasing diversity of pollinizers, transitioning trees trained using the bending technique to the click technique and improving the uniformity of the irrigation system. Moisture sensors were deployed that allowed for data driven irrigation strategies to be executed and a scheme for soil and plant nutrient testing was developed. The process opened up the opportunity to investigate and demonstrate several cultural practices specific to WA 38 under the Roza Farm growing conditions and historic circumstances. The benefit of having a mature orchard allowed us to understand challenges and demonstrate practical management techniques including but not limited to canopy and crop load management, fruit maturity and fruit quality, green spot, root growth, and more. The block also served as a gathering space to bring growers together and develop a collaborative community. Finally, this block provided fruit to PVM and WSU for marketing purposes in 2015-2018.

Significant finds include:

1. Under the Roza Farm growing conditions, M9 rootstock was the most productive (approx. 80 bins/acre) in both V trellis and bi-axis system, with no difference between those two training system, despite having double number of trees in the V trellis.
2. Under the Roza Farm growing conditions, the spindle system trees were the most vigorous, had the most blind wood and had the lowest productivity. However, the trees trained to this system were previously managed by bending branches, and have been in transition to a traditional spindle since 2017.
3. G41 rootstock showed reduced productivity in spindle and V trellis systems when compared with M9.
4. Drape net improved fruit weight and size distribution. In 2019 the block had less than 1 % sunburn, thus the protective effect was not assessed.
5. G41 and M9-Nic 29 showed significant differences in root growth, length and volume.

   These differences did not translate to significant differences in nutrient uptake, however, green spot incidence was 56% in G41 compared to 14% on M9 with p= 0.12.

Approximately 400 people toured the block, attended field days and / or collaborated in pruning events over the 2-year period. This mature block of apples is a valuable asset for WSU Extension and specifically for our programming efforts in WA 38 production, management and fruit handling.

FINAL PROJECT REPORT AP-18-105

Project Title: Apple scion breeding program

PI:	Kate Evans	**Co-PI (2):**	Cameron Peace
Organization:	WSU TFREC	**Organization**:	WSU
Telephone:	509-293-8760	**Telephone**:	509-335-6899
Email:	kate_evans@wsu.edu	**Email**:	cpeace@wsu.edu
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; Manoella Mendoza, 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		
Salaries[1]	10,935		
Benefits	3,609		
Wages[2]	15,000		
Benefits	5,000		
RCA Room Rental[3]	12,600		
Shipping	---		
Supplies[4]	500		
Travel	500		
Total	**48,144**		

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: TFREC-WSU **Contract Administrator:** Shelli Tompkins
Telephone: 509 293 8803 **Email address:** shelli.tompkins@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 and evaluated through storage for three Phase 3 selections.
11. One Phase 3 selection was discontinued, and one is advancing within Phase 3 in accordance to BPAC advice to the program.

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 from four elite selections in Phase 3 from the Quincy site (three from the Prosser site).

Fruit evaluations in Phase 3 include field observation and horticultural management. All activities are guided by the BPAC. Apples harvested from Phase 3 plantings were 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 collected at harvest was tested in the WTFRC lab as well as the TFREC lab; WTFRC evaluates long term storage.

- The two selections planted in 2017 will continue in Phase 3 for further evaluation
- One of the selections planted in 2015 was discontinued due to major flaws in its long-term storage performance (high incidence of internal browning, softening, inconsistent flavor)
- The second selection planted in 2015 was advanced within P3, due to its consistent flavor profile, annual bearing, fruit size profile, and high-performance during storage (fruit maintains its quality for up to ten months, potentially one year). In addition to field evaluations and quality parameters analysis already mentioned, this apple will be evaluated on at the packing line (bruising incidence and wax quality) and in a consumer taste panel during the upcoming storage season (2019-20).

Thanks to Dave Allan and Sarah Franco in Prosser, Scott Driscoll and Dale Goldy in Quincy for horticultural management assistance and Ray Fuller for maintaining 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.

<u>WABP Publicity (March 2018-March 2019)</u>
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:
Feb 2018 - Omak Horticulture Day (*Hanrahan*): WA 38 fruit quality and starch scale.
Mar 2018 – Washington Farm Bureau visit. (*Evans*): 'WSU apple breeding program'.
Mar 2018 - Postharvest Fruit School (*Hanrahan*): WA 38 fruit quality and starch scale.
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.'
May 2018 – Pace Academy (*Hanrahan*): Harvest and storage management of WA 38.
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.'
Dec 2018 - Washington State Tree Fruit Association (WSTFA) 114th annual meeting (*Hanrahan*): Fruit quality of WA 38.
Dec 2018 - Washington State Tree Fruit Association (WSTFA) 114th annual meeting (*Mendoza*): 'WA 38 -Resumen de actividades cosecha y postcosecha'

<u>Peer-reviewed publications</u>

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

EXECUTIVE SUMMARY

Project title: Apple scion breeding program

Key words: apple breeding, new apple varieties, phenotypic evaluation

Abstract: This one-year project describes in detail the progress of the Washington State University apple breeding program for the field season 2018/19.

This one-year project describes in detail the progress of the Washington State University apple breeding program for the field season 2018/19 and the no-cost extension to enable completion of the postharvest fruit evaluation. Twenty-two new families were made in 2018 with approximately 70,000 seeds produced in the WSU Apple Breeding Program (WABP), with 8,000 seedlings screened with DNA markers for fruit quality. 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. The final count of new Phase 1 trees planted in 2018 was approximately 2,250.

Twenty-two new promising selections (on Geneva 41 rootstock) were planted at three evaluation sites in Phase 2 trials in 2018; and fifteen promising selections made in 2017 were propagated in 2018 for planting in 2020 Phase 2 trials at three diverse sites in Central Washington.

Fruit was harvested from four elite selections in Phase 3 from the Quincy site (three from the Prosser site). Fruit evaluation data was presented in further detail to the apple breeding program advisory committee in a meeting on May 24th, 2019 with follow up orchard visit to the Phase 3 planting in Quincy (repeated July 30th). A further orchard visits to both Quincy and Prosser were offered September 6th, 2019.

FINAL PROJECT REPORT
WTFRC Project Number: AP-16-101

Project Title: Reducing scald after long-term CA storage

PI: David Rudell
Organization: USDA-ARS, TFRL
Telephone: 509 664 2280 (ext. 245)
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Co-PI: James Mattheis
Organization: USDA-ARS, TFRL
Telephone: 509 664 2280 (ext. 249)
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
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

Footnotes: Costs for 1 RCA room

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

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

Footnotes: One-third of instrument service contract

OBJECTIVES:

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

SIGNIFICANT FINDINGS:

1. Scald induction is cumulative and rapidly imposed, effective (below 0.8% O_2) CA reduces the rate of induction. Induction resumes following CA storage.
2. At-harvest delayed cold storage (2 d), intermittent warming, or hot water treatment reduces scald better on more mature fruit but only following air storage.
3. Hot water treatment following effective CA storage (3-4 months 0.5% O_2), reduces scald during the post-CA storage cold chain.
4. 1-MCP treatment following effective CA storage (3 or 6 months 0.5-0.8% O_2) reduces scald in the subsequent cold chain.
5. The CTOL test (see Blakey and Rudell, 2017) may be used to indicate whether post-storage scald treatments will be effective.
6. 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.

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.

<u>Year 1 (includes activities outlined for Objectives 1, 2, and 3)</u>

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.

<u>Year 2 (includes objectives 1, 2, and 3)</u>

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. Apples were stored in air for 6 months or CA (0.5%O2: 0.5% CO2). Scald incidence was evaluated on air stored fruit at 3 and 6 months and in CA stored fruit at 10 months (6 months post-CA).

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. Scald incidence was evaluated on CA stored fruit at 10 months (6 months post-CA).
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.

<u>Year 3 (includes objectives 2, 3, and 4)</u>

Delayed post-storage 1-MCP and DPA treatments following delayed CA storage and scald risk assessment
Granny Smith apples were harvested from Sunrise research orchard and placed in in-house CA (0.5% O_2:0.5% CO_2; 33°F) chambers immediately or after 2 or 4 weeks in 33°F air. Apples were removed from storage after 4 months and transferred to 37°F air storage. At 0, 14, and 28 days, 8 trays of apples from each CA regime were drenched with 2000 ppm DPA or treated for 12 h at 33°F with 1 ppm 1-MCP. Treated apples were then placed back into 37°F air storage. Scald incidence was evaluated monthly during the post-CA storage simulated cold chain.

For scald risk assessment, peel was sampled from 1 tray of control apples from each storage delay treatment at harvest, upon CA establishment, following removal from CA (4 months), and monthly thereafter until 8 months (4 months 37°F air storage). Peel (3 composite samples containing 6 apples) were processed and analyzed for CTOLs and other natural peel chemicals associated with superficial scald risk using HPLC-MS as outlined by Rudell et al. (2009).
Delayed DPA treatment during CA storage at different O_2 percentages
Granny Smith apples were harvested from Sunrise research orchard and placed in are storage (33°F) or in-house CA chambers (1% or 2% O_2:0.5% CO_2; 33°F) for up 6 months. At 0 weeks and after 2, 8, 12, 16, and 24 weeks 3 trays (18 fruit/tray) were removed from each storage, drenched with 2000 ppm DPA, and immediately placed back into storage. Apples were transferred to 37°F air storage at 6 months and stored until 8 months (4 months post-CA storage) at which scald incidence was evaluated.
Post-storage hot water or 1-MCP treatment of Granny Smith stored in commercial CA
Granny Smith apples were sampled after 4 months from 1 lot stored in a commercial CA room (800 bin; 0.8% O_2:0.6% CO_2; 33°F). Upon removal, 8 trays apples were treated for 12 h at 33°F with 1 ppm 1-MCP, treated by submerging in 118°F water for 3 min or left untreated. Temperature of hot water treated apples was evaluated prior to and following treatment. Apples were moved to 37°F air storage where scald incidence was evaluated at 8 months (4 months post-CA storage).

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 and DPA 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 in our storage chambers. In Year 2, again scald was reduced following 3 months ultra-low O_2 CA storage as well as in multiple lots of commercially CA stored Granny Smith (0.6-0.8% O_2). Year 3 results confirmed that post-storage 1-MCP treatment is nearly as effective as pre-storage 1-MCP treatment only if less than 0.8 % O_2 CA storage (commercial rooms and in-house chambers) is established immediately following harvest. Likewise, delaying CA storage establishment for up to 4 weeks reduced scald control by post-storage 1-MCP treatment (Fig. 1). Year 3 results also demonstrated a similar effectiveness of post-storage DPA treatment. This indicates that postharvest scald control, as with immediately after harvest, is not merely based on controlling ripening but is also a function of oxidative stress.

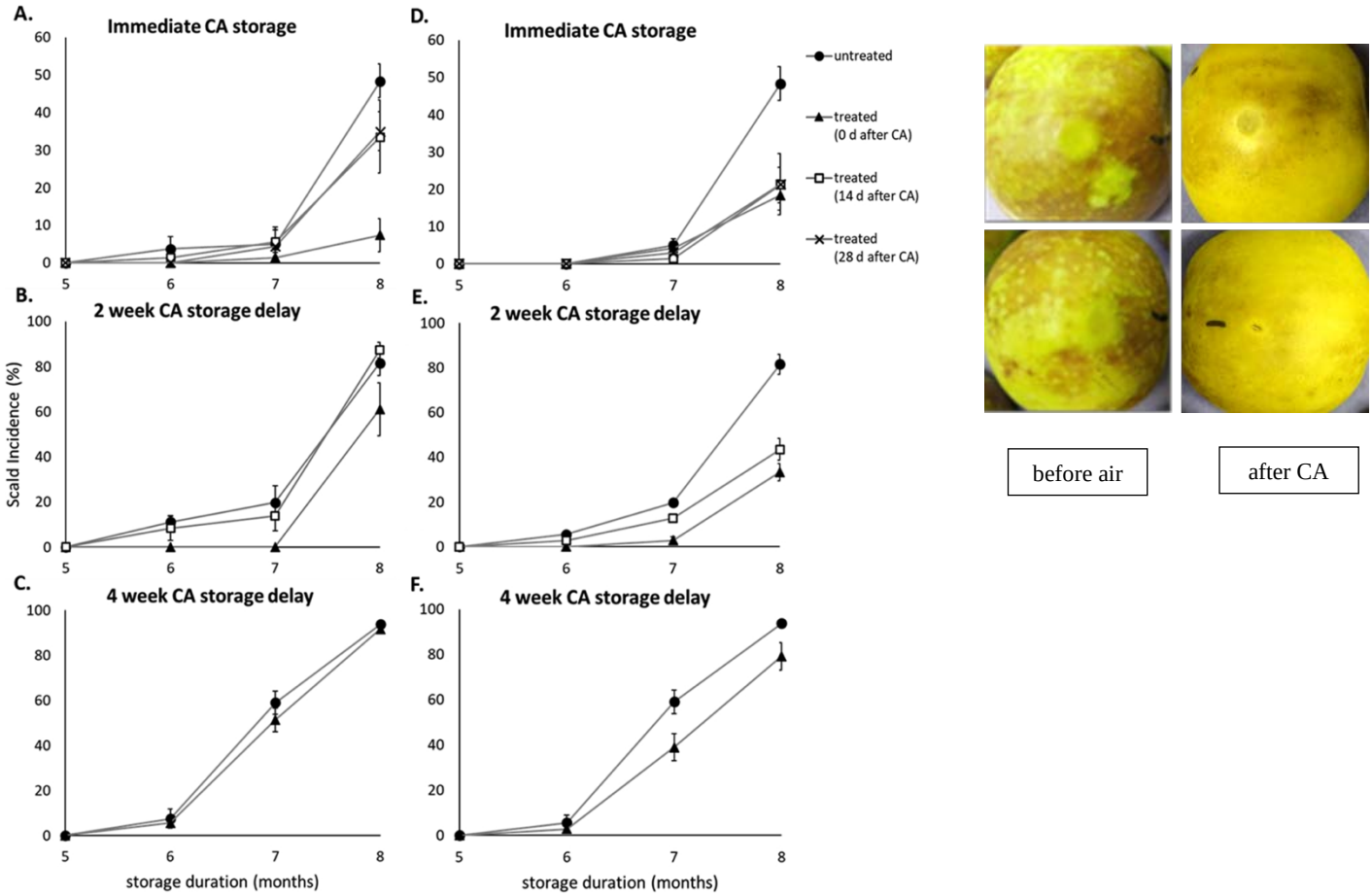

Fig. 1. Delayed (0, 7, and 14 d) CA storage (0.6 % O_2; 0.5% CO_2) establishment combined with delayed post-storage 1-MCP (left) and DPA (right) treatment demonstrates reveals that the fruit's response to chilling that results in scald reduced or stopped by ULO-CA. Error bars represent standard error (n=8 trays). 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.

Scald induction period is prolonged by ultra-low controlled atmosphere storage. Our results demonstrating the effectiveness 1-MCP and DPA 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-

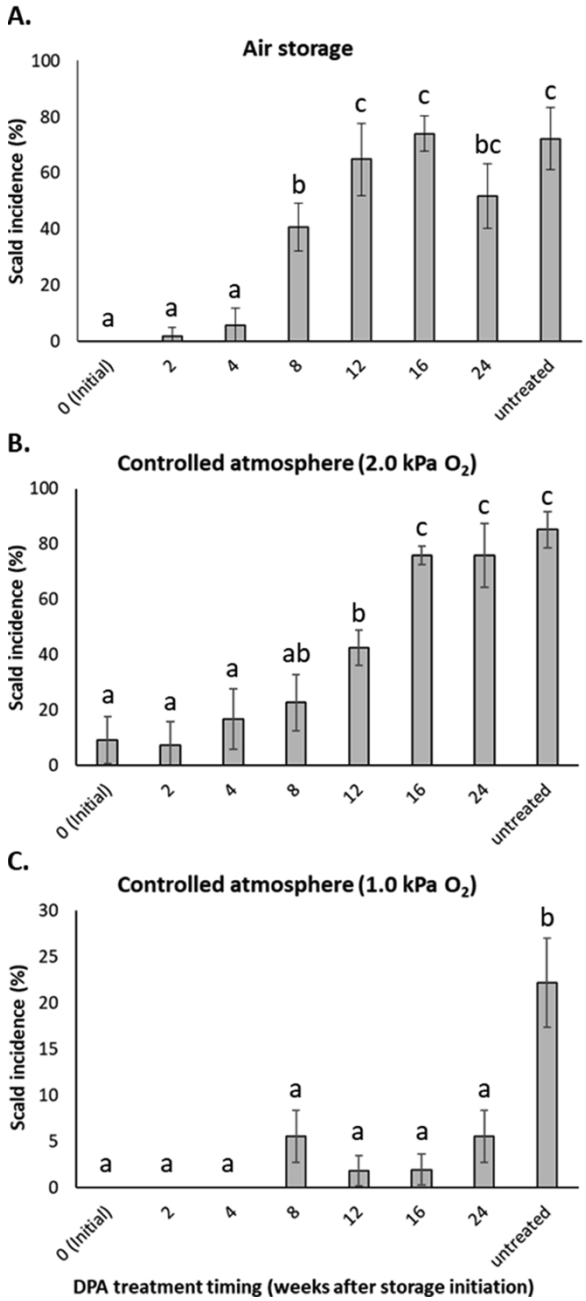

Fig. 2. Storage O_2 setpoints of below 1 % reduce scald induction and work best for controlling scald during the post-storage supply chain and providing fruit that will be receptive to post-storage scald control treatments. Reduced CA storage O_2 % increases the storage duration at which delayed DPA treatment to 'Granny Smith' remains effective at controlling scald. Apples stored under different atmospheric compositions, including air (A), 2.0 kPa O_2: 0.5 kPa CO_2 (B) and 1.0 kPa O_2: 0.5 kPa CO_2 (C) were treated with DPA throughout storage, moved to 37 °F air storage at 6 months and evaluated for scald incidence (%) at 8 months. (n=3 trays of 18 apples each, ±standard error, means with same letter are not significantly different according to Tukey HSD test at $p \leq 0.05$).

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 stored without use of these crop protectants, scald control relies on 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 manifested by scalded apples as fruit approach 6 months storage. Our results show that delayed DPA application loses its effectiveness when drenching is successively delayed during storages set at sequentially higher (1.0%, 2.0%, and air storage; 33°F) O_2 % indicating that the scald induction period occurs more rapidly at higher O_2 percentages (Fig. 2). This clearly indicates that 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 and scald induction is slowed, can scald induction begin again when controls are no longer in place?

Scald induction takes a set amount of time both before and after effective CA storage.

Successively delaying 1-MCP or treatment (0, 7, and 14 d) following 4 months of 0.6% O_2 CA established at 0, 14, or 28 d revealed that the scald induction period indeed resumed once apples were removed from storage (Fig. 1). If the CA environment was established immediately and 1-MCP was applied immediately following removal from storage, scald control continued in the cold chain for up to 8 months (<10% incidence). When these fruit were treated at 14 and 28 d, they developed over 30% between 7 and 8 months (incidence) scald. Post-storage DPA similarly controlled scald, albeit slightly less efficiently. The duration of post-storage scald control diminished to 2 and 1 month if CA was established at 2 and 4 weeks, respectively. One particularly useful result was that the combination of time was about 2 months regardless of whether it was before or following CA storage. Interestingly, this is the same amount of induction time indicated using delayed DPA and 1-MCP treatment during the first months of air storage. This result reveals that scald induction is a cumulative process that, once susceptible apple cultivars are harvested, requires a set amount of time

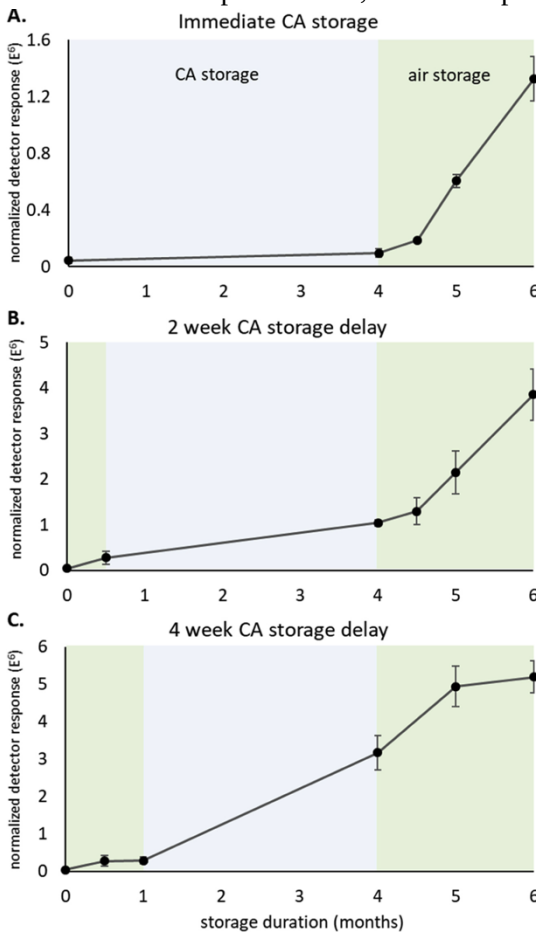

Fig. 3. Scald risk assessment using the CTOL test reveals if post-storage scald control treatments will be successful. Levels of CTOL during and following 4 months CA (0.6% O_2, 33°F) storage established immediately (A), 14 d (B), or 28 d (C) following harvest. Scald incidence during the subsequent cold chain at 37°F is presented in Fig. 1. These results show how CTOL level reflect how well the storage protocol controlled scald as well as estimated how well post-storage 1-MCP and DPA treatments would work. CTOL levels at 4 month indicate how much of the scald induction period has occurred up to that point. CTOL levels can be evaluated using the test outlined by Blakey and Rudell (2017).

under conditions that promote the process. Ultimately, these results establish some loose guidelines by which we can expect post-storage scald mitigation treatments to work. However, our results also indicate that the same tool we to determine if storage conditions are controlling scald induction may also be a means to indicate whether an apple will be receptive to post-storage scald mitigation.

Monitoring CTOL levels can indicate whether post-storage scald treatments will be effective (Fig. 3). The protocol for monitoring conjugated trienol (CTOL) levels in apple peel (Blakey and Rudell, 2017) is a direct way to monitor in scald-susceptible cultivars whether a storage environment is controlling scald given many other factors that influence disorder incidence. Our current results indicate the same test, when performed immediately following removal from storage, may be used to

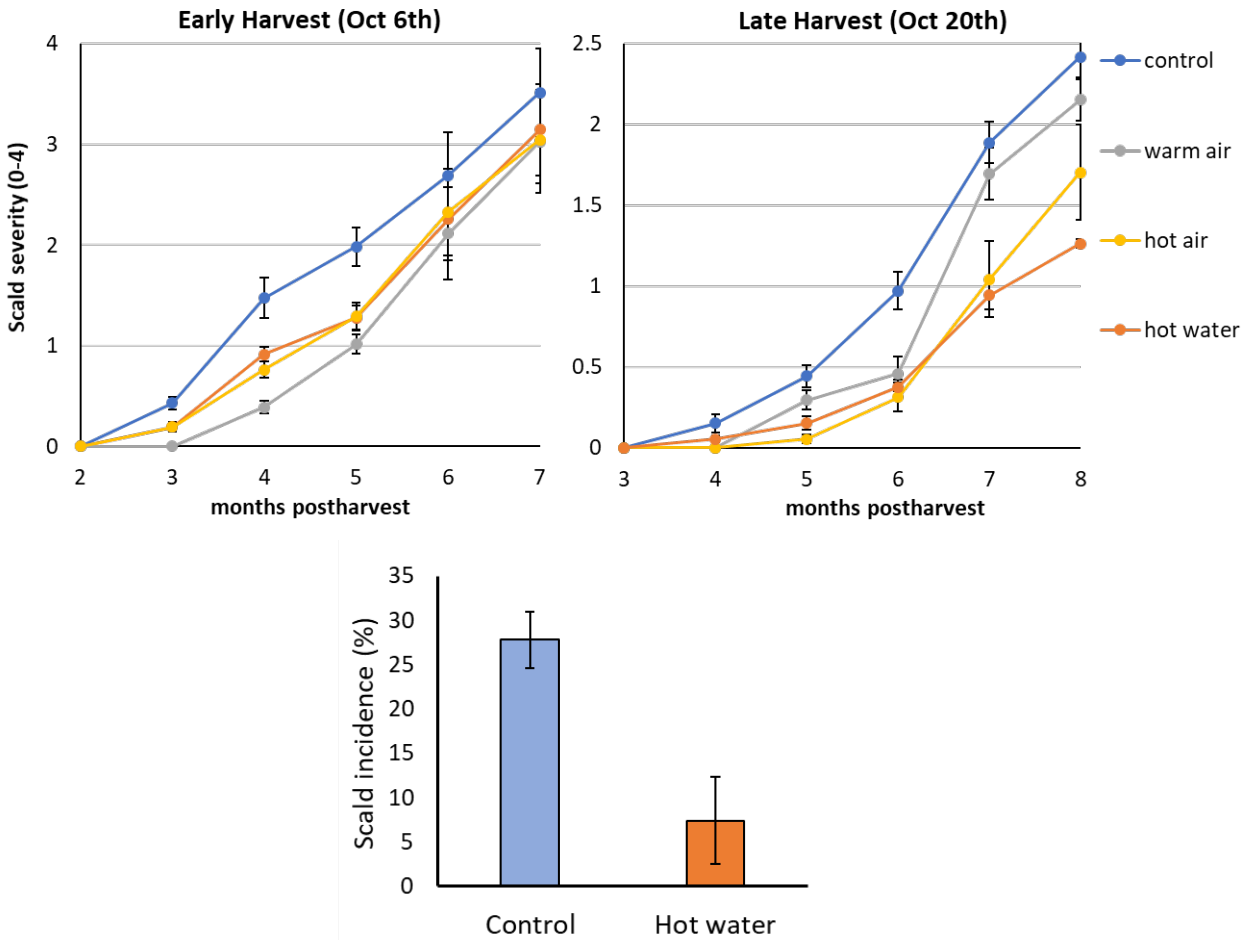

Fig. 4. At-harvest hot water, hot air and warm air treatments reduce scald during air storage but not following CA storage (0.5% O_2: 0.5% CO_2). Scald reduction using these strategies was most effective on mature fruit (Top left and top right). Post-CA-storage hot water treatment reduced scald up to 5 months following 3 months CA storage (bottom). Error bars = standard error.

indicate whether post-storage scald mitigation treatments will be effective. Samples taken from our experiment combining delayed CA storage establishment and delayed post-storage 1-MCP and DPA treatment were tested for CTOL and other natural peel chemicals associated with superficial scald risk. CTOL (Fig. 3) and ASG (not shown) levels reflected eventual scald incidence. CTOL levels increased very little by the end of storage where effective (0.6% O_2) CA conditions were established immediately.

Post-storage (0.6% O_2–4 months) heat treatment reduces scald development. While post-storage 1-MCP treatment provides a scald mitigation tool for apples produced for a conventional market, other solutions are required for stock that is to remain in an organic or crop protectant-restricted cold chain. The consensus of our at-harvest stress and wounding treatments intended to reduce scald during a prolonged cold chain indicates 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 "softer" at-harvest treatments was lost over a long period of ultra-low oxygen CA as opposed to 1-MCP and DPA which have a considerable residual impact that can control scald throughout a long cold chain. 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 hot air, or hot water treatments was more effective for controlling scald on relatively more mature (at harvest) apples (Fig. 4). Warm air did not provide any appreciable control and hot air damaged the peel. As in Year 1, hot

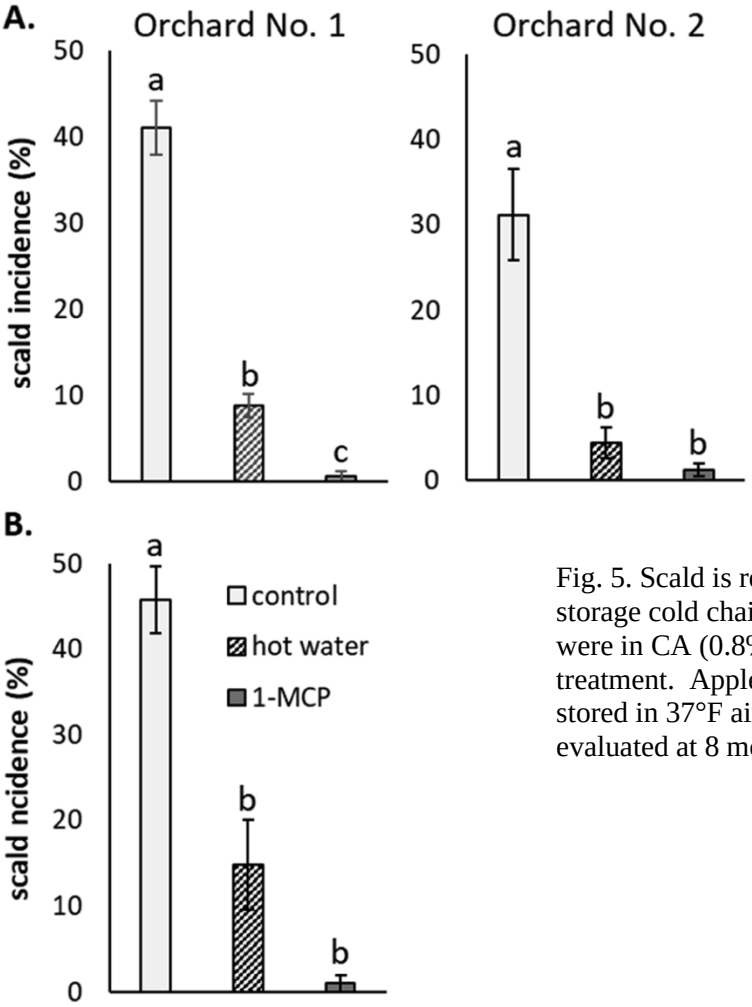

Fig. 5. Scald is reduced over a 4 month post-commercial CA storage cold chain by both 1-MCP and hot water treatment. Apples were in CA (0.8% O_2: 0.6% CO_2, 33°F) for 4 hot water or 1-MCP treatment. Apples were stored for 4 months in CA, treated, then stored in 37°F air for an additional 4 months. Scald incidence was evaluated at 8 months.

water impacted scald incidence during air storage and not following

CA storage during a prolonged cold chain. However, applied hot water after removal from 3 months CA, reduced scald beyond 5 months in air at 33°F (Fig. 4). In Year 3, we sought to confirm that hot water treatment immediately following 4 months commercial and in-house CA effectively controlled scald. Hot water controlled scald up to 8 months at 33°F in air (Fig. 5). Fruit firmness and titratable

acidity were no different among controls and treatments (not shown). Scald control by post-storage hot water treatment was equal to that of post-storage 1-MCP treatment on 1 of 2 commercially stored lots and the in-house stored lot. Results indicate that post-storage hot water treatment may be a viable scald control that could be adapted for controlling scald over a long post-storage cold chain for apples produced for a crop protectant restricted market.

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. 6, 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. 6, right). A moderate decrease in scald was observed when temperatures were decreased from 68°F to 55°F after only one week, and 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.

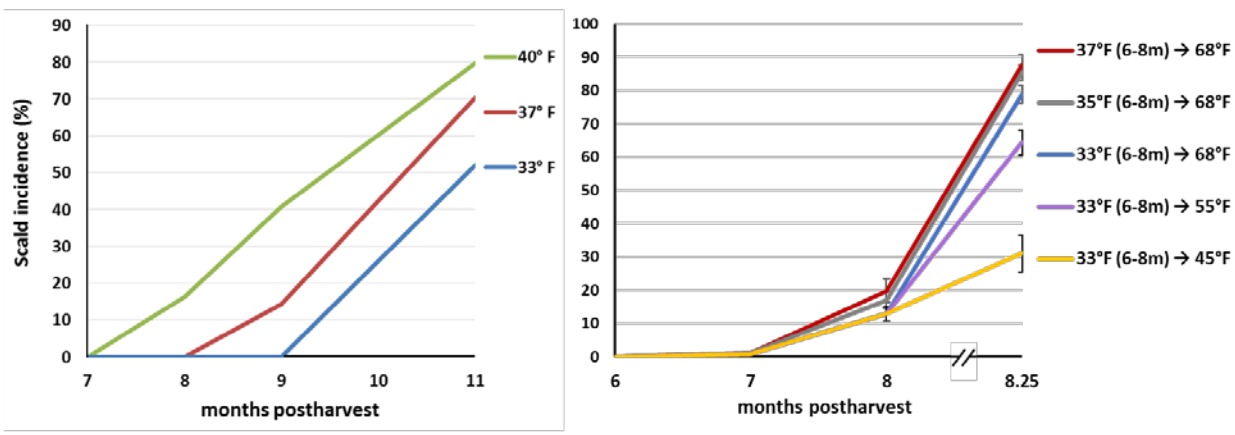

Fig. 6. Cooler temperatures reduce scald on commercial Granny Smith lots following storage at 0.6-0.8% O_2:1% CO_2 for 6 months. During year 1, 33, 37, and 40 °F was tested (left). In Year 2, we focused on determining the optimum temperature between 33 and 37 °F for 2 months post-storage and, then, best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

Conclusions

Where control options are limited by regulation or customer requirement and cold chains can last months beyond removal from storage, scald control strategies rely on advanced CA storage technologies coupled non-crop protectant-based controls such as acclimation and hot water. When scald is controlled using 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 after removal from CA. A critical step 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 O_2 settings, or extended storage period may negatively influence any post-storage scald treatment. Our results indicate that scald induction is

cumulative and only occurs where crop protectants are not used or CA conditions are not optimal (not less than 1%). Given our current information, we expect that the entire cumulative scald induction to be approximately 2 months sub-optimal conditions before and/or after storage. While this could be used as a general guideline to estimate scald-free life in the post-storage cold chain, we do not know of how pre-harvest conditions, such as chilling hours received, may influence this factor. A more exact approach may be using the protocol for monitoring scald risk (Blakey and Rudell, 2017) following CA storage to assess how much scald induction has occurred prior to and during storage. Finally, cold chain temperature, including retail storage is especially critical for maintaining scald free fruit where crop protectants are not used.

Publications

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

Poirier, B.C., Mattheis, J.P., and D.R. Rudell. 2020. Extending 'Granny Smith' apple superficial scald control following long-term oxygen controlled atmosphere storage. Postharv. Biol. Technol. 161 https://doi.org/10.1016/j.postharvbio.2019.111062

Project Title: Reducing scald after long-term CA storage (AP-16-101)

Executive Summary

Keywords: Superficial scald, apple, cold chain, hot water, scald risk assessment, post-storage scald mitigation

Abstract: Combinations of CA and other novel and established treatments were used to find protocols that reduce or eliminate scald following long-term CA where crop protectants are restricted. Hot water treatment following CA (below 0.8% O_2), immediately established after harvest worked best. Likely effectiveness of post-storage treatment could be indicated.

Project outcomes:

1. A protocol for reducing superficial scald up to 4 months following effective CA storage.
2. New evidence that shows superficial scald induction is cumulative and continues following CA storage.
3. New evidence that post-storage superficial scald treatments including both crop protectants and hot water treatment are effective.
4. A method for estimating the degree to which scald induction has occurred and the potential effectiveness post-storage scald mitigation treatments.
5. Temperature recommendations for the post-CA storage cold chain.

Significant Findings:

1. Scald induction is cumulative and rapidly imposed, effective (below 0.8% O_2) CA reduces the rate of induction. Induction resumes following CA storage.
2. At-harvest delayed cold storage (2 d), intermittent warming, or hot water treatment reduces scald better on more mature fruit but only following air storage.
3. Hot water treatment following effective CA storage (3-4 months 0.5% O_2 CA storage), reduces scald during the post-CA storage cold chain.
4. 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.
5. The CTOL test (see Blakey and Rudell, 2017) may be used to indicate whether post-storage scald treatments will be effective.
6. 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.

Future Directions:

1. Test hot water treatment on packing line for non-crop protectant post-storage scald control
2. Improve existing and develop new post-storage scald risk assessment tests that accurately indicate the degree to which scald induction has occurred.
3. Develop at-harvest superficial scald risk assessment tests that accurately reflect chilling hours and other factors impacting susceptibility.

FINAL PROJECT REPORT
WTFRC Project Number: AP-16-102

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

Footnotes: One-third instrument service contract

Objectives:

1. Identify changes in apple peel chemistry associated with response to light prior to and during cold storage.
2. Determine if changes in peel chemistry are specifically indicative of delayed sunscald and other sun-related postharvest peel disorder risk prior to symptom development.
3. Develop protocols to establish tissue viability before and during cold storage.

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, possibly, cutin structure and, thereby, epidermal cell protection.
4. Heating in the orchard can lead to symptoms with similar appearance and etiology to sunscald.
5. Heating in the orchard can inhibit sunscald development.
6. Sunscald severity can be altered after harvest.
7. Simple UV reflectance imaging targeting metabolites associated with light exposure can non-destructively detect relative sun exposure.
8. 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 and/or cumulative sun exposure.

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) and stored at 33 ºF for up to 6 months. 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 was repeated a second time in year 3 using stainless steel chambers linked together for a 15 min $^{13}CO_2$ treatment. Apples were sampled until 72 h for this experiment.

A subset of sunburned Granny Smith were selected 2 weeks after harvest and treated with combinations different test active ingredients dissolved in carrier and surfactant to form emulsions when added to water. Controls only contained carrier and surfactant. One tray (per treatment) was treated with each combination. Additional apples were treated with half active ingredient and half untreated.

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.

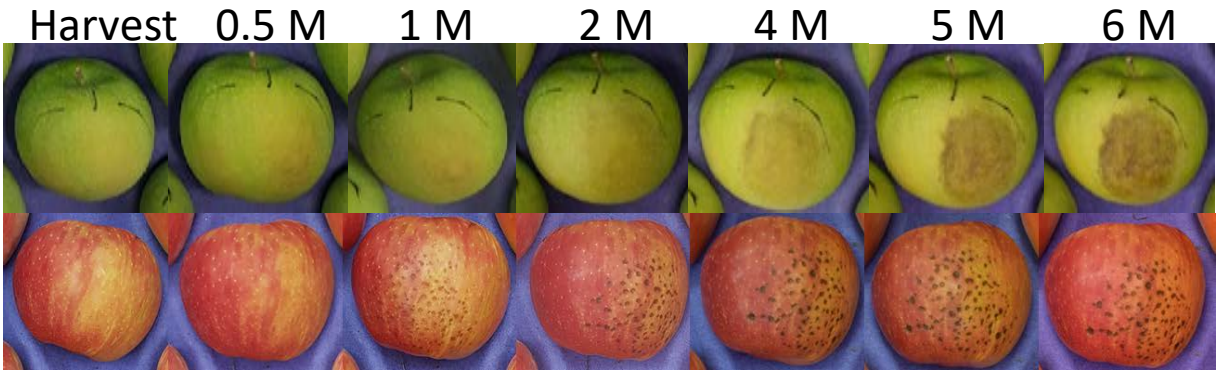

Fig. 1. Progression of peel disorders occurring on the sun side of 'Granny Smith' (sunscald) and 'Honeycrisp' (lenticel blotch) apples. In each case, the injury was not present at harvest and began to develop after 1 month in 33 ºF air storage.

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 (which was?) 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.

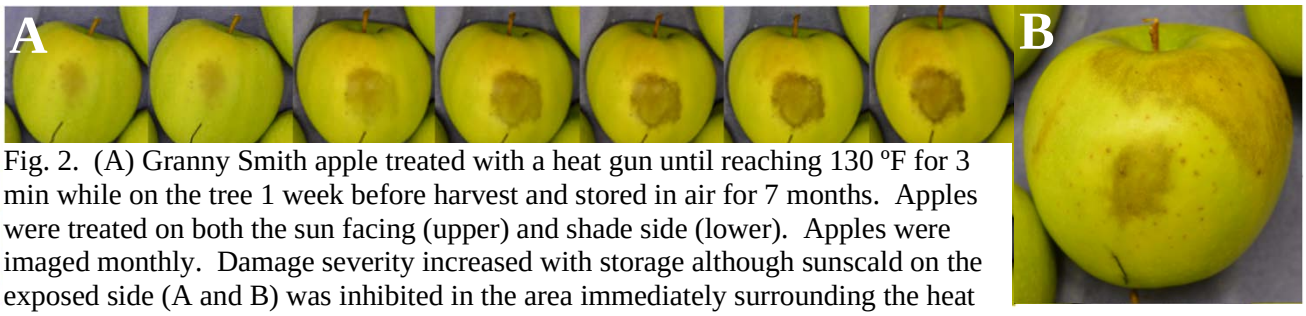

Fig. 2. (A) Granny Smith apple treated with a heat gun until reaching 130 ºF for 3 min while on the tree 1 week before harvest and stored in air for 7 months. Apples were treated on both the sun facing (upper) and shade side (lower). Apples were imaged monthly. Damage severity increased with storage 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 disorder progression. As in the previous season, heating provoked an either physical and/or chemical response that renders a portion of the peel of some apples to be 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.

Fig 3. Drenching with soap and water can impact sunscald development on Granny Smith. Apples were selected for sun exposure, treated with carrier and surfactant in water (right) or left untreated (left). While we do not suggest treating apples with high rates of carrier and surfactant, this is one instance indicating sunscald outcome can be altered by postharvest factors and may be worth further examination in future research.

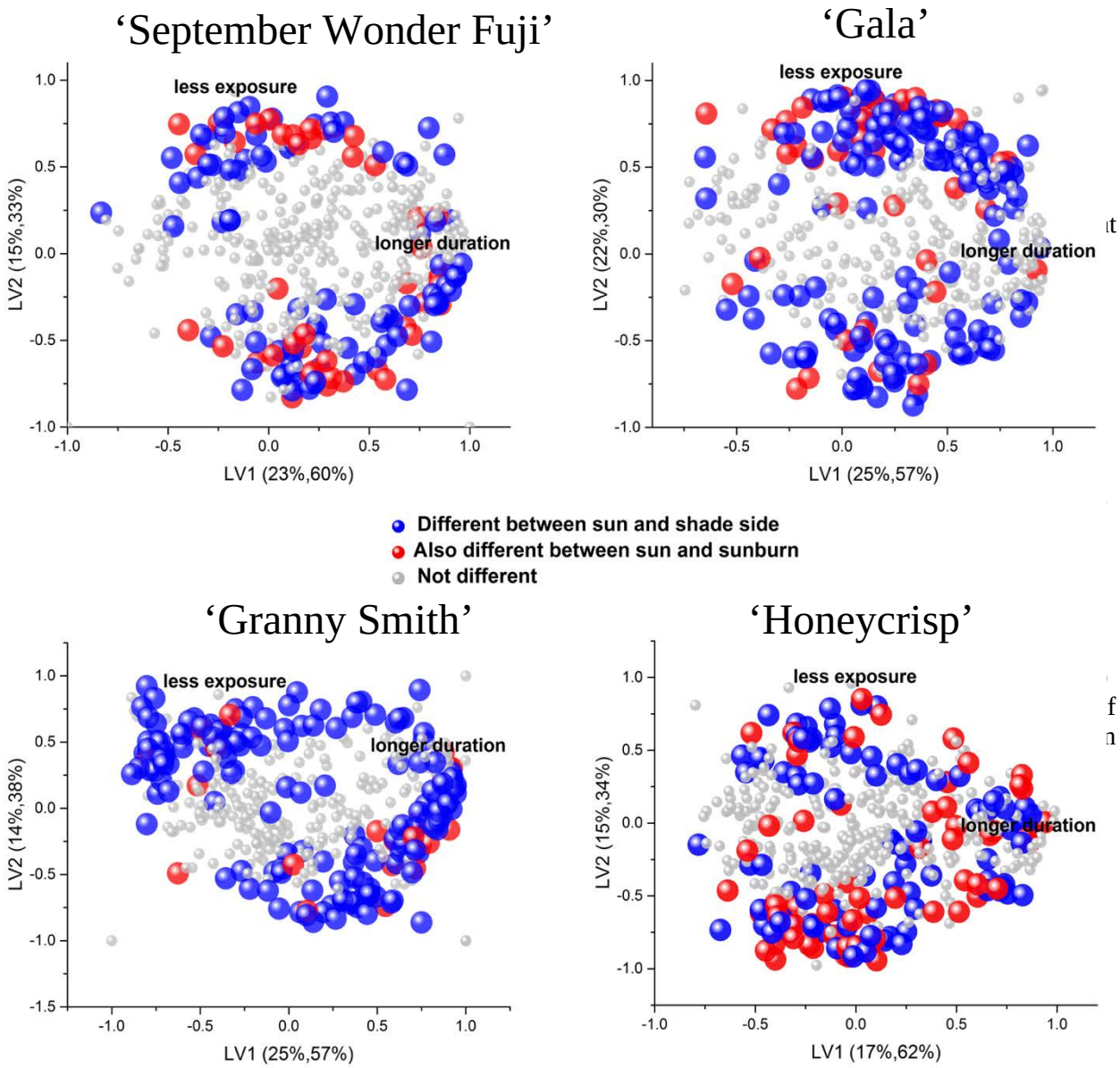

Fig. 4. Peel chemistry during 6 months air storage at 33 F is different depending upon prior sun exposure. This analysis (Partial Least Squares-Discriminate Analysis) of sun-facing and shaded peel of 'September Wonder Fuji', 'Gala', 'Granny Smith', and 'Honeycrisp' indicates how different sun facing, shaded, and sunburned tissue reacts to cold storage. Small gray bubbles indicate metabolites that had the same level in sun-facing and shaded peel. Larger bubbles indicate metabolites with levels different in sun-facing and shaded peel (blue and red) as well as metabolites different in sun-facing peel with and without sunburn (red only). The position of the bubbles on this graph indicate which sun exposure chemicals are associated with and whether levels increase or decrease during storage. We can use this to find chemistries associated with structure and physiological processes. We can also determine targets by which we can sort fruit according to cumulative sun exposure.

Sunscald development was reduced by drenching with a solution containing carrier and surfactant. In a separate experiment aimed at altering sunscald development by attempting to alter cuticle composition after harvest using natural chemicals, we found that the carrier and surfactant we used for formulation reduced sunscald formation (Fig. 3, right) compared with the untreated control (Fig. 3, left). These preliminary results indicate that sunscald is more likely related to a chemical changes that alter the physical properties of the surface of the epidermis. More importantly, it emphasized that sunscald development can be altered after harvest. Far more evidence is required to validate the utility of this finding as many more factors are likely impacting this result.

Differences in natural peel chemistry during storage are linked with preharvest sun exposure
To investigate any difference in chemical composition during the first 6 months of storage caused by the combination of cold stress and preharvest sun exposure, peel from either side of September Fuji, Gala, Granny Smith, and Honeycrisp 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, chemicals that act as signals to muster defensive responses, and aroma differed depending upon sun exposure (Fig. 4). 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. Some of these metabolites, the quercetin glycosides, may make good targets for non-destructive sorting.

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 (Fig. 5). 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 coniferol, an alcohol derived from 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.

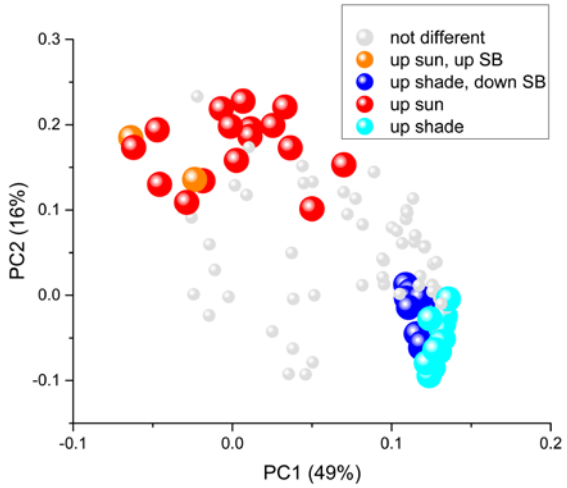

Fig. 5. The chemical composition cutin, the natural plastic plant coating, is different depending upon sun exposure and sunburn. In this analysis, Granny Smith peel was sampled from sun facing and shaded sides and stored in 33ºF air for 6 months. Sunburned peel was also analyzed at 0 and 6 months only. Cutin isolated over 2 weeks then prepared by hydrolyzing it so that our instruments could analyze is chemical structure. The figure on the left shows that sun facing, shaded, and sunburned peel were different over the entire storage period (the storage duration is included beside each bubble). The right-hand figure indicates individual chemicals that make up cutin associated with cutin coating sun-facing peel (red and orange) or cutin coating shaded peel (blue and turquoise). These differences could impact the physical properties of cutin such as elasticity or porosity that could impact liquid and gas exchange.

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. 6). 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.

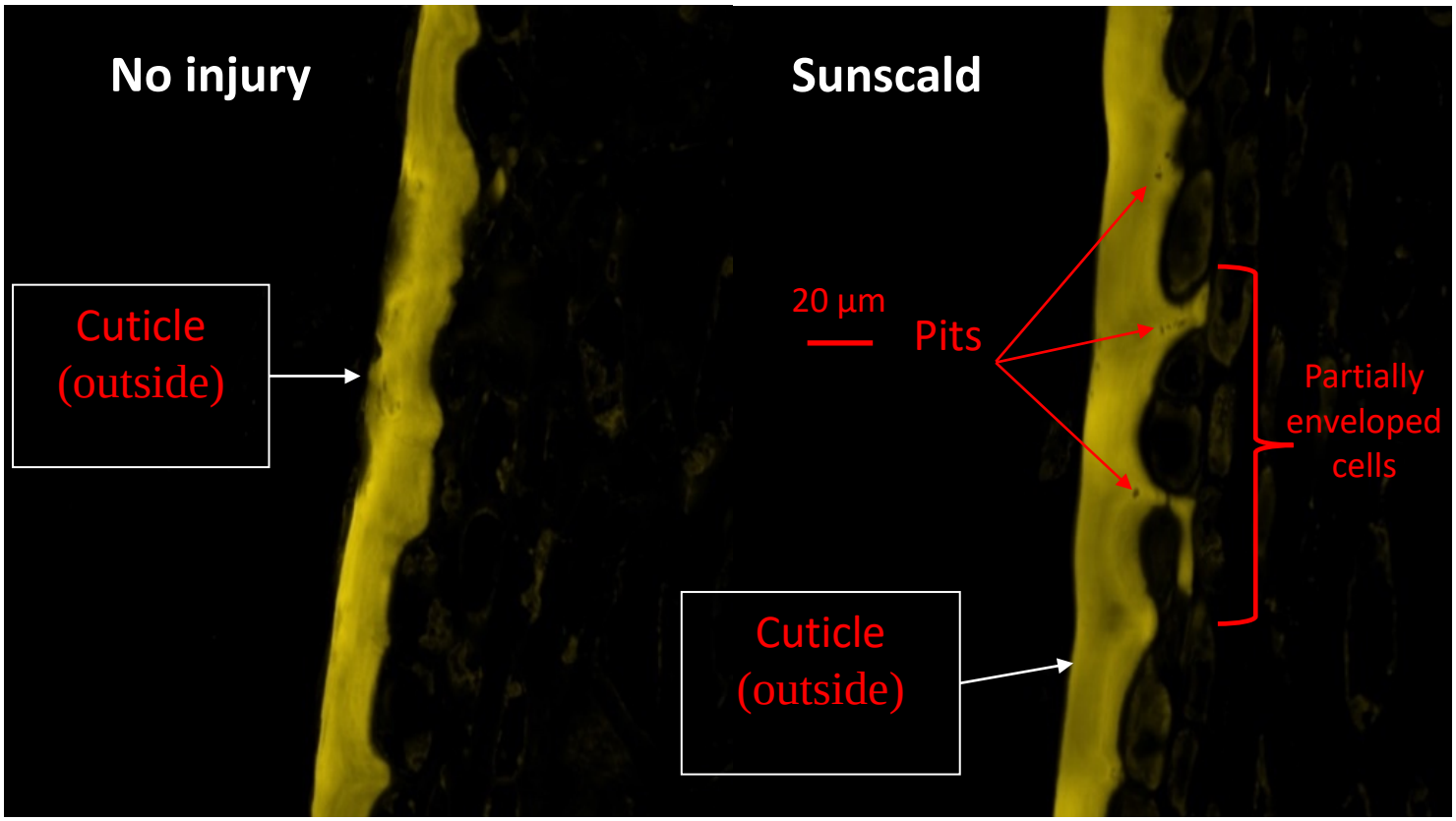

Fig. 6. (A and insets) Examples of natural peel chemicals with higher levels in the sun facing side of Granny Smith peel. These include quercetin glycosides which are a potential target for non-destructive detection as well as hydroxycinnamoyl esters that are likely building blocks of cutin, the external "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.

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. 7). 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.

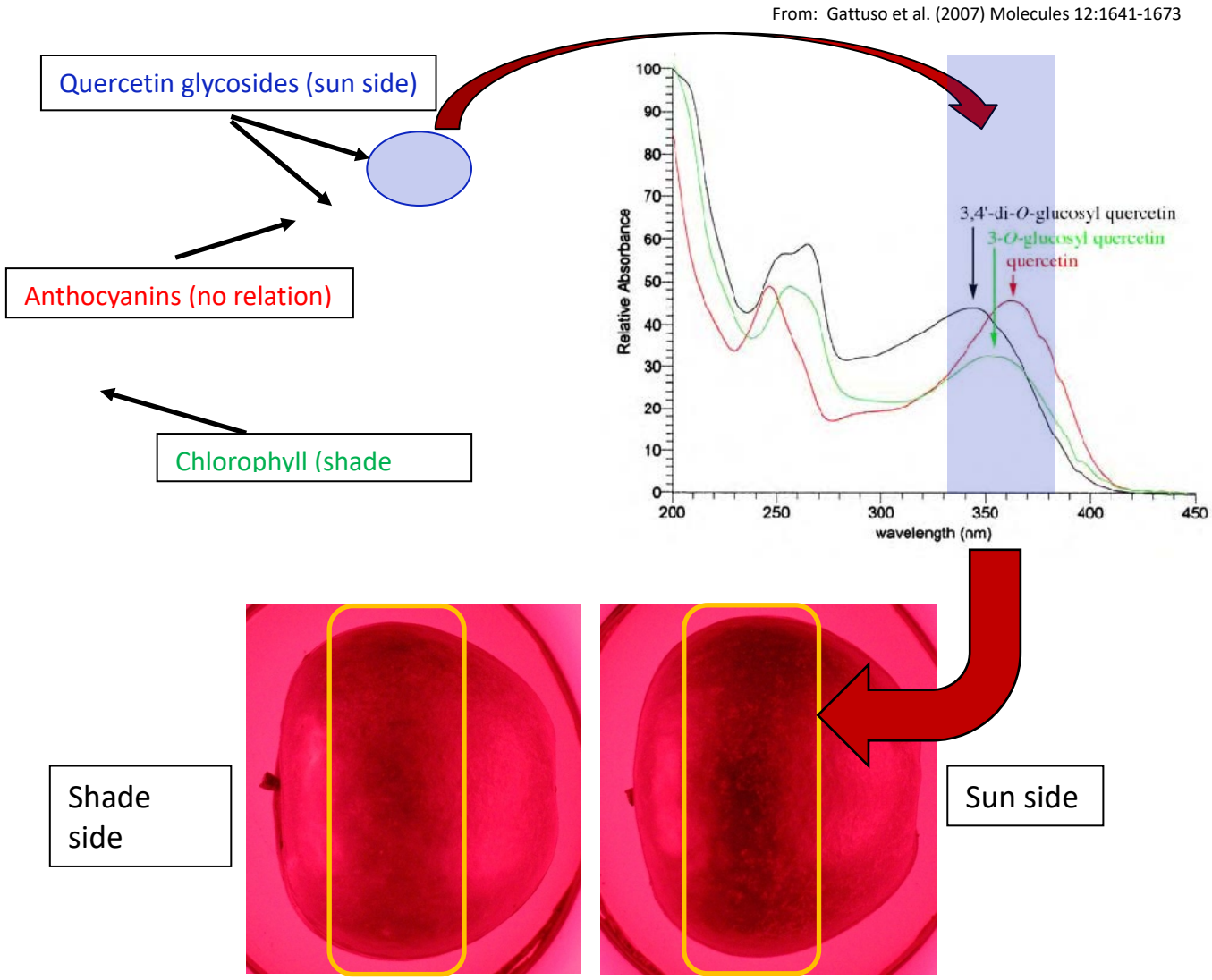

Fig. 7. Levels of apple peel chemicals associated with cumulative sun exposure can be assessed non-destructively. Quercetin glycosides accumulate in peel exposed to sun (upper left-hand figure). These natural peel chemicals absorb light at wavelengths to low to see by the human eye. The wavelengths that absorb the most energy are unique in that few other compounds in apple peel absorb light in the same wavelengths so any light absorbed in this region would be highly related to quercetin glycoside content (upper right-hand figure). A modified camera with a filter that images mostly light from these wavelengths can preferencially image these chemicals in Granny Smith peel (lower figure). Because these chemicals absorb more light at these wavelengths, the image appears darker in the region where higher concentrations are present as on the sun-facing side (lower right) as opposed to the shaded side (lower left) which is lighter.

Project Title: Risk assessment for delayed sunburn and sunscald (AP-16-102)

Executive Summary

Keywords: cold chain, sun stress, fruit finish

Abstract: Apple peel appearance and chemical changes in response to orchard sun exposure were evaluated to develop solutions that reduce sun-related cold-chain losses. Our results reveal residual impact of sun exposure during the cold chain and potential strategies for reducing losses based on altering fruit surface, and disorder avoidance using non-destructive sorting.

Project outcomes:

6. Identification of continued impacts sun exposure has on fruit quality, appearance, and stress during the cold chain.
7. Identified factors related to acclimation and peel surface chemistry that could be useful to develop treatments that reduce sunscald at-harvest.
8. Chemical targets linked with cumulative sun exposure that could be used as targets for non-destructive detection of and, potentially sorting for, sunscald and other sun-related defect risk.

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, possibly, cutin structure and, thereby, epidermal cell protection.
4. Heating in the orchard can lead to symptoms with similar appearance and etiology to sunscald.
5. Heating in the orchard can inhibit sunscald development.
6. Sunscald severity can be altered after harvest.
7. Simple UV reflectance imaging targeting metabolites associated with light exposure can non-destructively detect relative sun exposure.
8. 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 and/or cumulative sun exposure.

Future Directions:

4. Evaluation of at-harvest drenches and coatings to reduce sunscald.
5. Develop non-destructive techniques to detect sun using chemical targets identified in this study.
6. Determine if at-harvest sorting according to cumulative sun exposure could be used to improve appearance and quality.