2024 Apple Horticulture and Postharvest Research Review

Apple Breeding Program field day in Quincy, WA.

Photo Source: Ines Hanrahan

January 24, 2024

Hybrid Format

Wenatchee, WA

Project/Proposal Title: Retraction of netting near harvest: risks vs. rewards

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Report Type: Continuing Project Report

Project Duration: 2-Year

Total Project Request for Year 1 Funding: $ 37,761
Total Project Request for Year 2 Funding: $ 39,107

Other related/associated funding sources: Requested
Funding Duration: 2023 - 2028
Amount: $6.5 million
Agency Name: USDA SCRI
Notes: This was our third time submitting this proposal that is focused on mitigating the impacts of temperature extremes on pome fruit. While not funded, it was, again, ranked highly and will be resubmitted in 2024.

WTFRC Collaborative Costs: none

Budget 1
Primary PI: Lee Kalcsits
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Station Manager/Supervisor: Chad Kruger Email Address: cekruger@wsu.edu

Item	2021	2022	2023
Salaries	17,514[1]	18,215[1]	
Benefits	6,548[1]	6,811[1]	
Wages	7,800[2]	8,112[2]	
Benefits	1,749[2]	1,819[2]	
Equipment			
Supplies	3,000[3]	3,000[3]	
Travel	1,150[4]	1,150[4]	
Miscellaneous			
Plot Fees			
Total	37,761	39,107	0

Footnotes:
[1]Funding is requested for a scientific assistant at 35% during August to November of each year of the project. Benefits rates for the scientific assistant are equal to 37.4%
[2]Funding is requested for a summer staff member to work on netting set up at Sunrise research orchard, fruit thinning and horticultural management, and experimental set up in August. Benefits for this position are equal to 22.4%
[3]Supplies are for netting set up and consumables for field and lab experiments that may include new data loggers, solar panel hardware, as well as lab supplies for fruit quality analysis.
[4]Funding for travel is requested for weekly trips to Sunrise research orchard as well as twice-weekly trips to Quincy in August and September for conducting retraction experiments.

OBJECTIVES

This project had two objectives aimed at quantifying risks and rewards of using retractable netting systems for high-value apple cultivars.

1. Test the timing of retraction of netting across two growing seasons to determine how much netting retraction enhances red color development and how close to harvest deployment should occur.
2. Determine whether fruit under netting is at a greater risk of developing sunburn when netting is retracted.

SIGNIFICANT FINDINGS

- **Netting had the greatest benefit to reducing sunburn and EC reduced severe sunburn when used in conjunction with retractable netting systems. Evaporative cooling alone was not sufficient to limit sunburn development on fruit in 2021.**
- **After two seasons, retraction 7 days before harvest had equal red color as when netting was retracted 14 days before harvest. While netting retraction had a significant benefit in 2021, it did not in 2022 for the commercial trial. However, color development was extremely poor in 2022 for Honeycrisp across the state and as such, differences between treatments were not as great.**
- **When comparing additional losses from sunburn to gains in red color in 2021 and all other things being equal, these changes translated to an additional 1.5 packed boxes per bin when retraction was used compared to leaving netting in place. These differences were mostly consistent between the commercial and research orchard locations. In 2022, the commercial orchard only had an additional 0.25 packed boxes per bin.**
- **When these differences are calculated for a 60 bin/acre crop and a box price of $56/box, it translates to an additional $5040/acre in revenue in 2021 and only $840/acre in 2022 for the commercial orchard site. Note: Fruit prices are variable, please price out benefits based on current market pricing and color and sunburn thresholds for those markets.**
- **There was no evidence of the development of photo oxidative sunburn from removing netting prior to harvest even when netting was retracted at higher temperatures (above 100 °F in 2022).**
- **No increases in postharvest disorders were observed after three months of storage at 35 °F in regular atmosphere.**

METHODS

<u>Experiment 1: Removal timing for netting retraction</u>

This experiment was performed in a Honeycrisp orchard that was planted in 2018. It consisted of Honeycrisp on G890 rootstocks planted to a tall spindle training system. Netting was installed and covered the orchard in 2020 consisting of a panel and cable system that extends over the entire orchard. Each panel was 55' wide and covers 4 rows. In August, 14 days before harvest, in 2021 and 2022, netting was removed from a 55' section within the block. Then, 7 days before harvest another 55' wide section will be retracted. These two treatments were compared against a control that was left covered until after harvest. These treatments helped determine the impact of duration of retraction before harvest on color development for previously netted trees.

Measurements (Summarized in Table 1):

Fruit surface temperatures were continuously measured for 8 days to determine if there were differences in fruit surface temperatures of fruit between treatments. To assess fruit quality for each treatment, 100 fruit were harvested from the upper canopy area of each replicate to look at sunburn incidence and fruit color development. After harvest, fruit was run on an AWETA sorting line that can measure fruit diameter, weight, red color coverage and intensity as well as background color. Sunburn incidence and severity was graded on all fruit using a six-point scale adapted from Schraeder et al. (2003).

Table 1. Measurements made on fruit in the orchard and at harvest for experiment 1 which is focused on identifying optimum timing of net retractions near harvest for Honeycrisp apple.

Measurement	What	When	Where	Why
Fruit surface temperature	Thermocouples	Entire duration of the experiment in 2021 and 2022	Two trees per replication and four fruit per tree	Assessing sunburn risk and differences in acclimation between treatments
Fruit sizing	AWETA Sorting Line	Within one week of harvest	WSU TFREC	Grading for size, color area, and color intensity
Sunburn incidence and severity	Graduate student and technician	One week after harvest	WSU TFREC	Assessing the impact of netting retraction on sunburn risk
Postharvest disorders	Graduate student and technician	January 2022	WSU TFREC	Assessment of postharvest sunburn development along with other external and internal disorders that might emerge from retracting netting near harvest

Experiment 2: Combining netting retraction with evaporative cooling

This second experiment was conducted at the Sunrise Research Orchard in Wenatchee, WA in a top-worked Firestorm® Honeycrisp orchard that was regrafted in 2016. The experimental design had six treatments arranged in a split plot design with evaporative cooling treatments as a main plot and then retraction as a secondary plot. There were three replications for each treatment. Netting was deployed in early June using a modified retracted netting setup from Extenday (See Figure 2). Evaporative cooling was available from June 15 to harvest with automated sprinklers that were triggered when air temperatures reached 85 °F. Cycling was set to be 15 minutes on and 30 minutes off during those times. Netting was retracted two weeks prior to harvest for replications with either evaporative cooling or no cooling and there was a completely uncovered control to compare all sunburn mitigation treatments against to look at effect on red color and sunburn.

Experiment 2 Measurements (Summarized in Table 1):

Thermocouples that measure fruit surface temperatures were installed on the day of retraction on four fruit on each of one tree per replicate. There was a total of 15 dataloggers used for the entire experiment in 2021 and 2022. Fruit surface temperatures were monitored for the whole 10 days to determine differences in fruit surface temperatures among treatments. Environmental conditions were pulled from the WSU AgWeatherNetwork (Sunrise Weather Station). Like experiment 1, fruit quality was assessed for each treatment. Approximately 100 fruit were harvested from the upper canopy area of each replicate to look at sunburn incidence and fruit color development. After harvest, fruit was run on an AWETA sorting line that can measure fruit sizing, weight, red color coverage and intensity as well as background color. Sunburn incidence and severity was graded using a six-point scale adapted from Schraeder et al. (2003). In 2022, fruit was also stored at 33 °F in regular atmosphere for three months to assess fruit quality after storage.

RESULTS AND DISCUSSION

Experiment 1: Retraction timing

For both years in the commercial trial, retraction produced higher proportions of fruit with premium red color coverage (>33%) but were not statistically significant ($\alpha = 0.05$). Whether retraction was done 14 days or 7 days before harvest had no difference in red color coverage or the proportion of fruit with premium red color (>33% coverage). Unsurprisingly, fruit weight was not affected by retraction timing. However, despite low statistical confidence that red color coverage was greater, Figure 3 shows that there was a higher visible red color presence when netting was retracted prior to harvest.

Table 2. Fruit weight, red color coverage, and sunburn for netting treatments in 2021. P-values were found using ANOVA tests in RStudio at alpha=0.05.

Treatment	Weight (g)	Red coverage	sunburn incidence	SB1	SB2	SB3	SB4
Retracted 14 days before harvest	300	56.7%	31.8%	15.7%	9.3%	4.4%	2.3%
Retracted 7 days before harvest	292	62.9%	28.1%	12.8%	7.9%	4.9%	2.5%
No retraction	285	53.5%	18.6%	9.5%	5.6%	2.7%	0.7%
P-value	0.7016	0.1722	0.1604	0.3152	0.3765	0.3597	0.3077

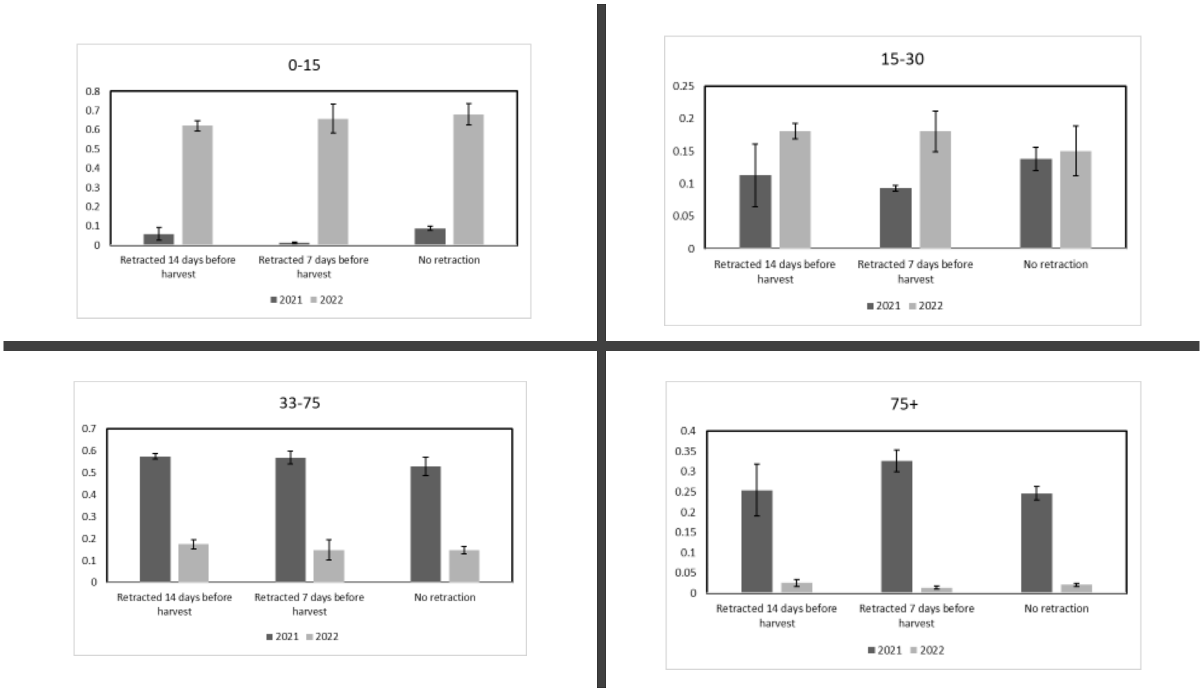

Figure 1. Mean proportions of total fruit (N=3) with red color at four different ranges of color coverage for Honeycrisp apple; 0-15% (top left), 15-30% (top right), 33-75% (bottom left), and 75%+ (bottom right) in 2021 and 2022 netting retracted 14 days before harvest, 7 days before harvest, or at harvest. No significance was found using ANOVA tests in RStudio at α=0.05.

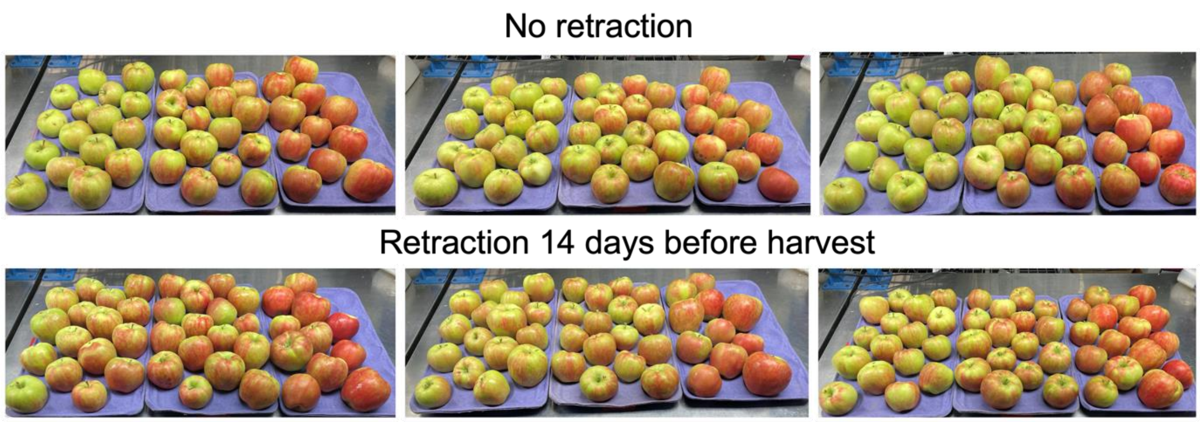

Figure 2. Fruit faced with sun-exposed portion of fruit in order from least colored (left) to most-colored (right) for Honeycrisp with either netting left in place until after harvest (top) or retracted 14 days before harvest (bottom).

Table 0. Fruit weight, red color coverage, and sunburn for netting treatments in 2022. P-values were found using ANOVA tests in RStudio at alpha=0.05.

Treatment	**Weight (g)**	**Red coverage %**	**Sunburn incidence %**	**SB1**	**SB2**	**SB3**	**SB4**
Retracted 14 days before harvest	265	17.3%	18.5%	9.3%	7.2%	2.1%	0.0%
Retracted 7 days before harvest	255	14.9%	18.3%	7.9%	6.3%	3.9%	0.2%
No retraction	269	14.2%	17.8%	6.3%	6.7%	3.5%	1.4%
P-value	0.2850	0.5558	0.9808	0.0154	0.8836	0.5277	0.0217

Table 4. Proportion of fruit belonging to five red color categories (0-20, 20-40, 40-60, 60-80, 80-100) for netting retracted 14 days before harvest, 7 days before harvest, or at harvest in 2021. P-values were found using ANOVA tests in RStudio at alpha=0.05.

Treatment	% 0-20 red	% 20-40 red	% 40-60 red	% 60-80 red	% 80-100 red
Retracted 14 days before harvest	9.0	13.6	27.5	33.4	16.7
Retracted 7 days before harvest	3.2	11.1	26.5	35.7	23.5
No retraction	13.3	16.7	25.4	25.8	18.8
P-value	0.1483	0.6208	0.7833	0.1472	0.4957

Table 5. Proportion of fruit belonging to five red color categories (0-20, 20-40, 40-60, 60-80, 80-100) for netting retracted 14 days before harvest, 7 days before harvest, or at harvest in 2022. P-values were found using ANOVA tests in RStudio at alpha=0.05.

Treatment	% 0-20 red	% 20-40 red	% 40-60 red	% 60-80 red	% 80-100 red
Retracted 14 days before harvest	68.8	16.2	9.3	4.2	1.6
Retracted 7 days before harvest	72.2	16.0	7.2	3.7	0.9
No retraction	75.2	11.8	7.4	3.9	1.6
P-value	0.5707	0.4219	0.5819	0.9725	0.5787

Retraction increased the proportion of fruit culled from sunburn, even in 2021 when sunburn pressure was lower during retraction (daily maximum temperatures were approximately 85 °F during this period) (Figure 4). In 2022, retraction was delayed until after September 5 to limit the risk of fruit sunburn in the commercial orchards as daytime maximum temperatures neared 100 °F. In 2021, 7% of fruit had severe sunburn whether it was retracted 7 days or 14 days before harvest. However, less than 4% of fruit had severe sunburn when netting was left in place until after harvest. Trends were similar in 2022 between treatments but sunburn incidence was lower. Between 4 and 5% of fruit was culled from sunburn for both retraction treatments compared to only 2% when netting was left in place until after harvest.

Experiment 2: Combining evaporative cooling and netting retraction at harvest

Table 6. Mean fruit weight, red color coverage, and sunburn of 'Honeycrisp' apple fruit with netting applied all season until harvest, netting applied all season and then retracted 10 days before harvest, or no netting used all season (Factor A) or with evaporative cooling (EC) or not (Factor B) in 2022.

Treatment	Fruit weight (g)	Red color coverage (%)	Sunburn incidence (%)	Sunburn rating (% fruit)			
				SB1	SB2	SB3	SB4
Factor *A*							
No retraction	244 a	12.5 a	13.7 a	8.4 a	4.2 a	0.2 a	0.9 a
Retracted	225 a	22.4 a	18.9 a	11.8 a	6.0 a	0.4 ab	0.8 a
No netting	236 a	18.3 a	24.9 a	12.4 a	7.5 a	3.2 b	1.8 a
p-value *A*	0.671	0.151	0.162	0.440	0.456	0.028	0.413
Factor *B*							
EC	232 A	17.6 A	15.8 A	9.1 A	4.7 A	0.8 A	1.2 A
No EC	238 A	17.9 A	22.5 A	12.7 A	7.1 A	1.7 A	1.1 A
p-value *B*	0.718	0.958	0.156	0.197	0.271	0.372	0.861
p-value *A* x *B*	0.600	0.643	0.386	0.361	0.866	0.310	0.194

Significant differences were determined using ANOVA tests performed in RStudio using the general linear model function. Letters indicate significant differences at α =0.05 according to a Tukey HSD test. Sunburn was rated on a scale of SB0-SB4 adapted for Honeycrisp from Schraeder et al. 2003 and shown in Willsea et al. 2023.

Since maturity was delayed in 2022 compared to 2021, the retraction period occurred 11 days later in 2022 (Table 2). However, the daily maximum temperature was approximately 8 °F greater in 2022 than 2021 during the retraction period. Fruit color development was poor, even in red Honeycrisp strains like Firestorm. Although color development was so poor, the mean starch rating was 3.5-4 for all fruit harvested at Sunrise and the background color was breaking from green to yellow indicating maturity of fruit on the tree. Delaying harvest longer would have resulted in excessive fruit drop and poor storability.

Table 7. Comparisons of the average red color coverage, retraction period, and average maximum temperature for 2021 and 2022.

	2021	2022
Average red color coverage (%)	58.3	17.7
Retraction period	August 18-August 30	August 29-September 8
Average daily maximum temperature during retraction (°F)	83.8	91.5

Unsurprisingly, uncovered fruit had the highest proportion of fruit with severe sunburn compared to netted fruit (Figure 5). Evaporative cooling only reduced the proportion of fruit with severe sunburn when it was used for uncovered or retracted trees. In 2021, when trees were left covered until after harvest, evaporative cooling did not significantly reduce the proportion of fruit with severe sunburn. We did not observe this same pattern in 2021. Looking at the main effects, evaporative cooling decreased losses from severe sunburn and netting, whether retracted or not, was effective at reducing severe sunburn. Interestingly, red color coverage (%) was improved when evaporative cooling was used in 2021 but while also higher in 2022, there was low statistical confidence in those differences. Overall, there were 10% more fruit with >33% red color coverage when EC was used in 2021 and 2.5% more fruit with >33% red color coverage when EC was used in 2022.

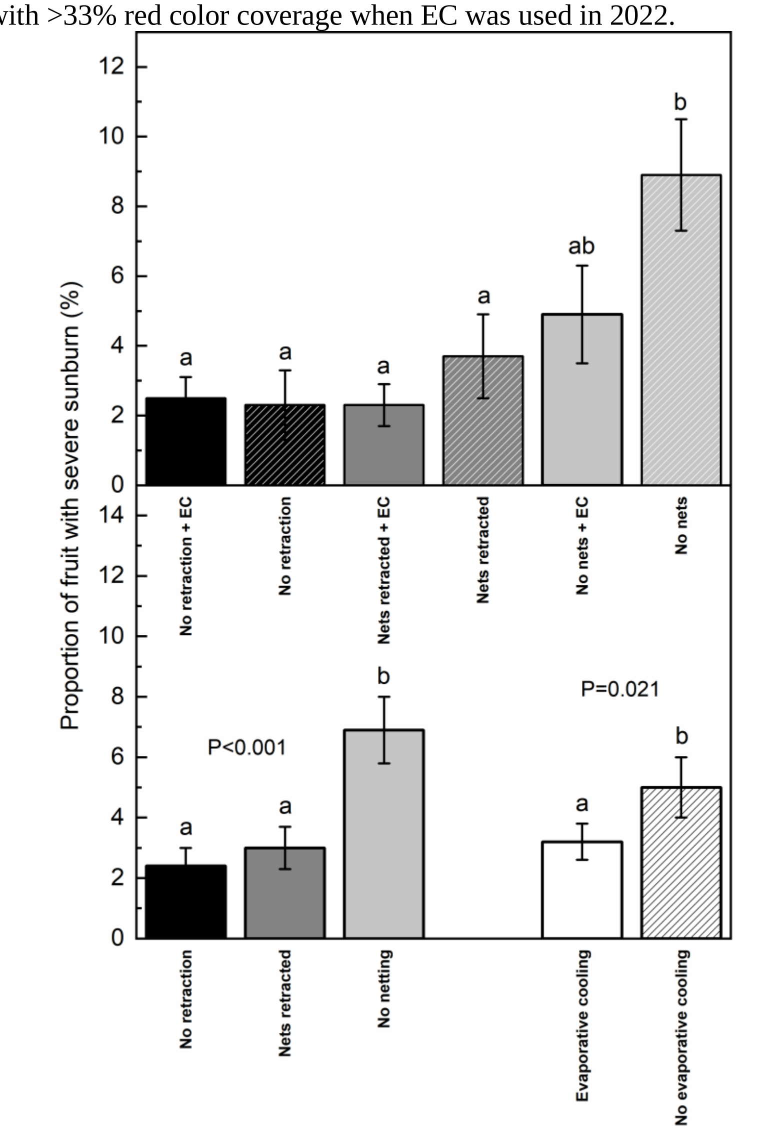

Figure 3. Mean proportions of total fruit (N=3) with sunburn exceeding SB2 based on the scale developed by Schrader et al. (2003), which would result in cullage in a commercial setting in 2021 and 2022 with no netting, netting deployed until harvest, or nets retracted 10 days before harvest and then either evaporative cooling (EC) or no EC applied. Letters indicate significant differences among means determined using a Tukey's HSD test (α = 0.05).

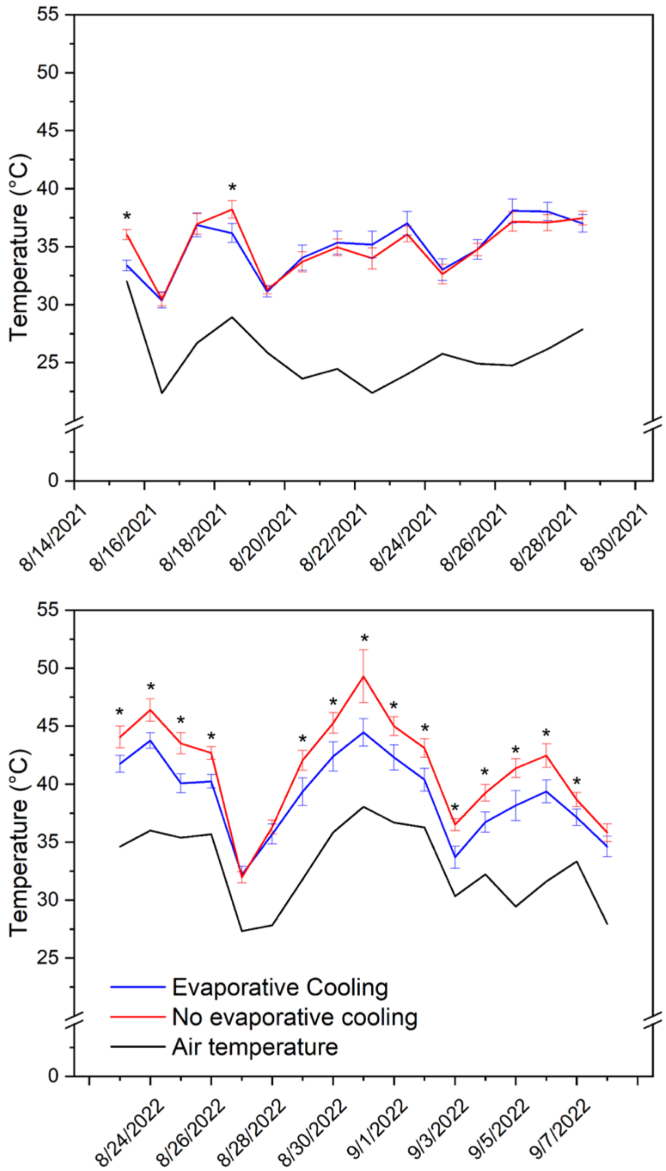

Figure 4. Average maximum daily temperature for the retraction periods in 2021 and 2022 for air temperature, treatments with evaporative cooling, and treatments without evaporative cooling. (N=3)

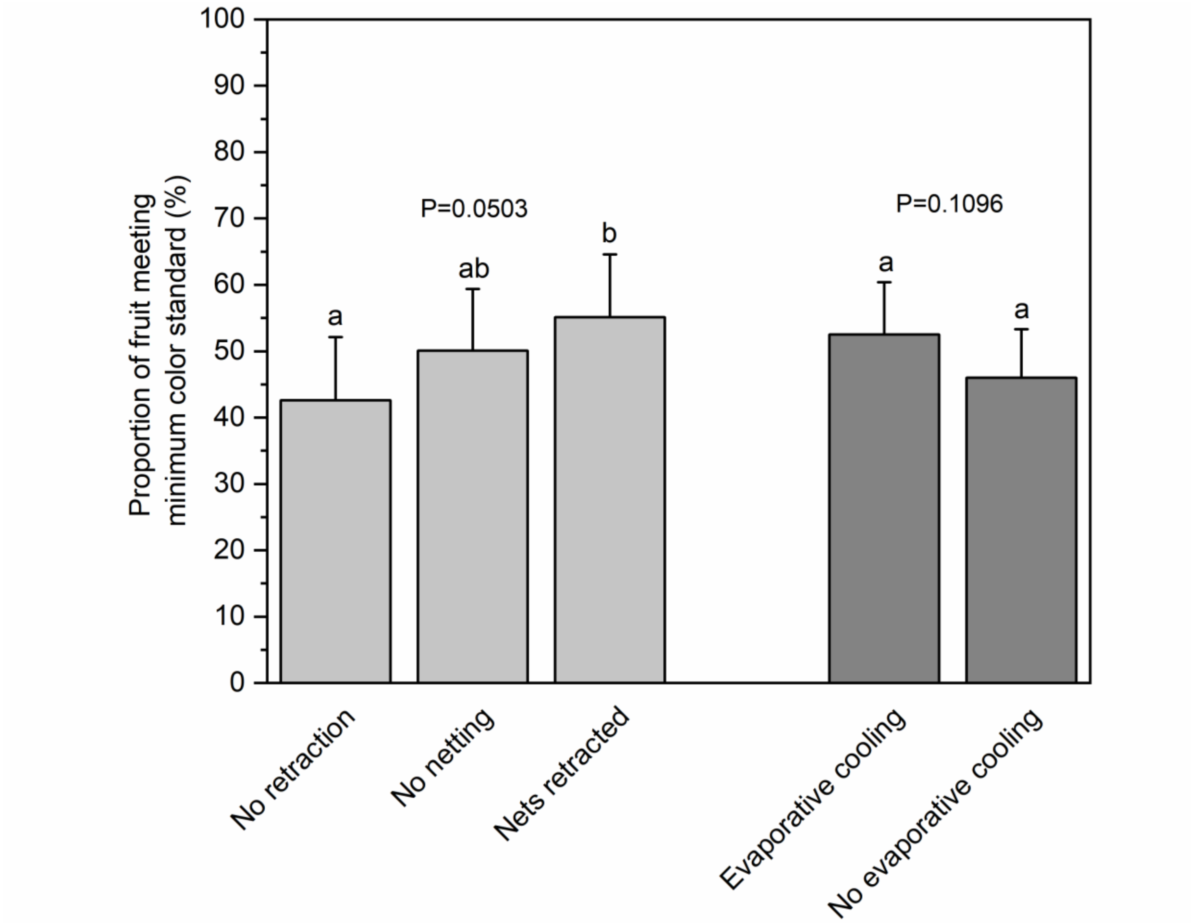

Figure 5. Mean proportions of 'Honeycrisp' fruit meeting Washington Extra Fancy standards for red color coverage thresholds for Honeycrisp apple in 2021 and 2022 with no netting, netting deployed until harvest, or nets retracted 10 days before harvest and then either evaporative cooling (EC) or no EC applied (N=3). Letters indicate significant differences among means determined using a Tukey's HSD test (alpha = 0.05).

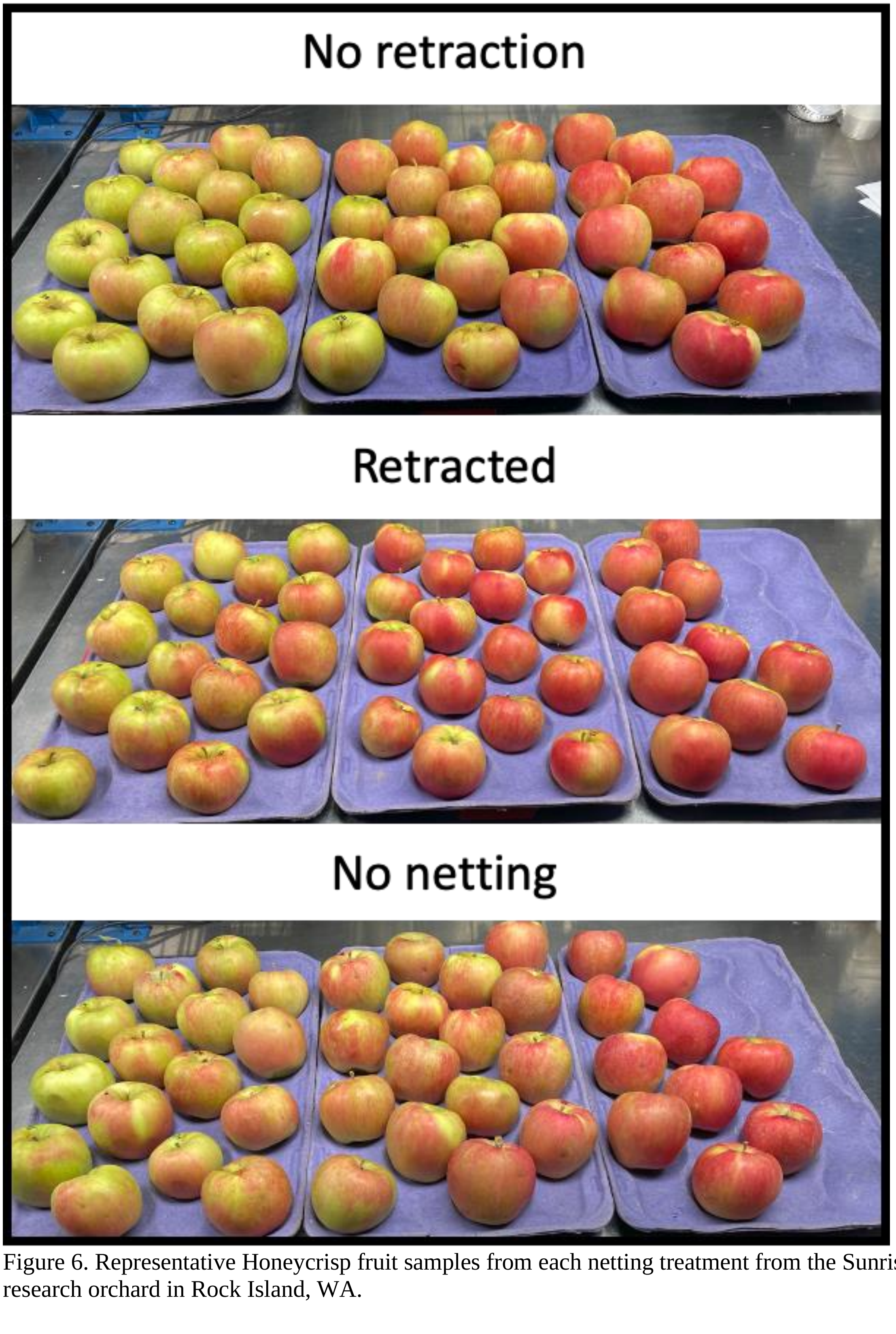

Figure 6. Representative Honeycrisp fruit samples from each netting treatment from the Sunrise research orchard in Rock Island, WA.

PROJECT OUTPUTS

Publications (open access)

Willsea N‡, Blanco V‡, Howe O‡, Campbell T‡, Kalcsits L. **2023**. Retractable netting and evaporative cooling to control sunburn and increase red color development in 'Honeycrisp' apple. HortScience, 58(11), 1341-1347.

Willsea N, Blanco V, Rajagopalan K, Campbell T, Howe O, Kalcsits L. **2023**. Reviewing the tradeoffs between sunburn mitigation and red color in apple under a changing climate. *Horticulturae*, *9*(4), 492

Presentations

Kalcsits L. **2022.** Tradeoffs between red color and sunburn in apple and management strategies to find this balance. Northwest Wholesale Annual Meeting. February 11, 2022.

Kalcsits L. **2021**. Physiology of Heat Stress and Mitigation Technologies. WSTFA Annual Meeting, Yakima, WA. December 7, 2021.

Lee Kalcsits and Noah Willsea. **2022**. Netting retraction focused discussion at monthly meeting for the Apple Horticulture and Protection metting. Yakima, WA. May 14, 2022.

Noah Willsea and Victor Blanco. **2022**. Heat impacts and management. Columbia Growers Club meeting. Pasco, WA. June 30, 2022.

Noah Willsea and Lee Kalcsits. **2022**. Netting retraction as a tool to improve red color in apple. American Society for Horticultural Sciences Annual Meeting. Chicago, Illinois. August 1, 2022.

Noah Willsea and Lee Kalcsits. **2022**. Netting Retraction to Improve Red Color in Apple. WSTFA Annual Meeting, Wenatchee, WA. December 7, 2022.

Willsea N, Kalcsits L. **2023.** Netting retraction to improve red color. Wenatchee Tree Fruit Days. January 19, 2023.

Kalcsits L. and Willsea N. **2023**. Fruit sunburn management. International Fruit Tree Association Annual Meeting. Grand Rapids, MI. February 15, 2023.

Kalcsits L. 2023. Heat mitigation. **2023** Virtual Meet Ups. July 23, 2023.

Extension publications

Sunburn in apple and strategies to mitigate it. **2021**. Jenny Bolivar-Medina and Lee Kalcsits. http://treefruit.wsu.edu/sunburn-in-apple-and-strategies-to-mitigate-it/

Cooling Mechanisms for a Tree Fruit Orchard. **2021.** Bolivar-Medina J, Kalcsits L. Fruit Matters July 2021. http://treefruit.wsu.edu/cooling-mechanisms-for-a-tree-fruit-orchard/

Netting retraction for enhancing red color development for Honeycrisp. **2024**. Lee Kalcsits, Victor Blanco, Noah Willsea. Nearing submission.

Executive Summary

Project title: Retraction of netting near harvest: risks vs. rewards

Key words: Netting, sunburn, red color, retraction

Abstract: Protective netting and evaporative cooling are commonly used in apple (*Malus domestica* Borkh.) orchards to protect apple fruit from sunburn in semi-arid environments like Central Washington. Sunburn is a physiological disorder caused by the combination of solar radiation and heat, which causes up to 10% or $100 million in yearly crop damages in Washington state. While protective netting and evaporative cooling can be effective for preventing apple sunburn, netting can also introduce new risks, especially the limitation of red color development on the apple peel. This study evaluated whether the retraction of netting before harvest improves red color development and/or changes sunburn risk compared to leaving netting in place until harvest. The first experiment compared six different treatments of 'Honeycrisp' apples in a research orchard consisting of combinations of netting either retracted ten days before harvest or not retracted and the presence or absence of evaporative cooling. The second experiment was performed on 'Honeycrisp' apples in a commercial orchard in Quincy, WA. Netting that had been in place during the growing season was removed fourteen days before harvest, seven days before harvest, or not at all. Fruit from both experiments were harvested and evaluated for sunburn incidence and external quality characteristics. Over the two years of experiments, netting reduced the levels of severe sunburn compared to the un-netted control. Meanwhile, the retraction of netting even up to 14 days before harvest did not increase sunburn risk in a younger orchard but in an older orchard with less vigor, sunburn was slightly greater when netting was retracted. Additionally, the retraction of netting before harvest increased the proportion of fruit with good and excellent red color. Overall, the use of retractable netting provided sunburn protection during the summer while avoiding red color penalties that come from netting deployed through harvest.

Project Title: Efficient heat stress management for improved apple fruit quality

Report Type: Final Project Report.

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Contributing Researchers (supported in part by this grant): Basavaraj Amogi, Dr. Rakesh Ranjan, Dr. Rene Mogollon, Nisit Pukrongta, Freddy Jimenez, Nelson Goosman, Juan Munguia, Prasanna Medarametla.

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $ 68,717
Total Project Request for Year 2 Funding: $ 66,232
Total Project Request for Year 3 Funding: $ 68,035

Other related/associated funding sources: Awarded
Funding Duration: 2019 - 2023
Amount: $450,000
Agency Name: USDA NIFA/ NSF Cyber Physical System
Notes: Funded in 2018 to develop localized orchard climate and crop physiology sensing system for apple fruit surface temperature and heat stress monitoring.

WTFRC Collaborative Costs:

Item	**2021**	**2022**	**2023**
Salaries	40,500	42,120	43,804
Benefits	14,875	15,470	16,089
Wages			
Benefits			
Equipment			
Supplies	10,210	5,510	5,010
Travel	3,132	3,132	3,132
Miscellaneous			
Plot Fees			
Total	68,717	66,232	68,035

Footnotes: Year 1 -- Salaries of $20,000 will support 5-months at 100% FTE of postdoc jointly supervised by Khot & Peters; $14,000 to support 7-months research associate at 50% FTE supervised by PI-Torres and $6,500 to support lab technician for 4-months at 50% FTE supervised by PI-Sallato. Pertinent HR benefits for these three personnel will be $14,875.Supplies include procurement of material to integrate crop physiology sensing nodes (8 nodes, $700/unit), telemetry (Wi-Fi router, cellular subscription, $620), pressure transducers w/ data logging capability ($250 × 4 units), misc. hardware, harness & related costs ($150) and orchard diagnostics/testing supplies for Soil test, Tissue samples, Fruitlets and Fruit ($1,350). Travel include 60 trips (× 90 miles/round × 0.58/mile) for members of team to travel to field sites for research and extension activities. **Year-2 and -3 –** Salaries are inflated by 4% respectively and pertinent benefits. Supplies include $2,670 to upkeep the sensing nodes and $1,350 for orchard diagnostics/testing supplies. Travel costs will remain unchanged from year-1.

Objectives

1. Evaluate the impact of three different heat stress management techniques on fruit quality at harvest and after storage.
2. Assess the effectiveness of sensing technology for automated stress monitoring and management.
3. Estimate the economic cost-benefits of each technology.
4. Deliver new knowledge to the apple industry through extension and outreach.

Key findings

1. Conventional overhead evaporative cooling (hereafter referred as ‘conventional’), fogging, and a combination of fogging and netting (fognet), were reliable in regulating air (T_{air}) and fruit surface temperature (FST) below the critical threshold of 113 °F. However, seasonal variability was observed for each of these techniques' efficacy with respect to control (without heat mitigation) and netting.
2. Conventional cooling and fogging can be effective in mitigating heat stress with desired modification. Although effective, the conventional (25 min ON/OFF) cooling cycle can fail to keep FST below the threshold during the late afternoon (15:00 – 17:00 p.m. pacific) periods of hotter days. Thus, it is recommended to use variable cycle frequency tied with changes in either or both T_{air} and FST. Manual cyclic operation often results in considerably higher amount of water use (up to 63%). Automation would help in saving such excess water (/energy) usage and operational labor costs.
3. FST thresholds ranges for automated fogging were identified to be between 86 and 95 °F. Fogging did not cause FST to exceed the threshold during the study, its effectiveness however can be compromised if T_{air} > 95 °F for prolonged period. Reducing spacing between foggers, their diagonal placement in adjacent rows, and using high flow rate foggers could be potential solution to remove additional heat load on fruits.
4. For wider adoption of automation using FST thresholds, a feasible technology is needed to estimate FST. CPSS is not readily available or scalable due to commercialization challenges. Hence, the project explored a broadly useable machine learning model to estimate FST using in-orchard — open field weather, fruit size, and ground truth FST. A more comprehensive model is being developed on similar lines, incorporating high temporal FST data collected using CPSS through this project. The developed model is being planned to be ready by 2024.
5. In Honeycrisp, netting (in 2022 and 2023) led to smaller and lighter fruits, with delayed coloration across three seasons. In 2021, fruit size under netting was comparable to other treatments and potentially contributed to increased storage losses to bitterpit and softscald. Compared to netting, conventional, fogging, and fognet treatments with larger fruits, caused more storage losses to bitterpit and softscald. Such variations corresponded to changes in T_{air} and FST. Adoption of these treatments shall be considered in relation to crop load, tree vigor, and fruit size.
6. For WA38, netting delayed fruit coloration. Weight and fruit size was comparable to fogging and control. No soft scald and bitter pit disorders losses were observed in any treatment. Overall, treatment effect on fruit quality in WA38 were minimal compared to Honeycrisp.

Objective 1: Evaluate the impact of three different heat stress management techniques on fruit quality at harvest and after storage.

Experiment design.

The project was conducted at two independent sites: 1. Honeycrisp block (of Farmland Services commercial orchard near Prosser, WA); and 2. WA-38 research block (WSU Roza farm, Prosser, WA). Honeycrisp trees were on M9-339 rootstock planted in 2016 on vertical system with three leaders per trees planted at 10’×4’ tree spacing. WA-38 trees were on M9-Nic 29 rootstock planted in

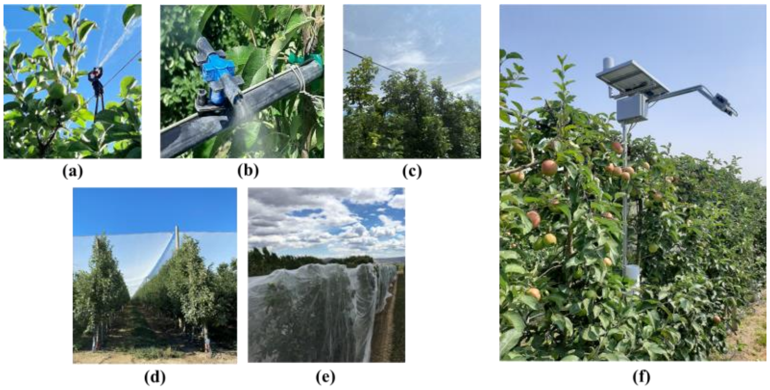

Figure 1. Heat stress mitigation treatments: (a) Conventional, (b) Fogging, (c) Over-the-top netting, (d) Fogging + Over-the-top netting (fognet), (e) Drape net. A (f) crop physiology sensing system (CPSS) was installed to monitor heat stress on fruits.

2013 on a vertical system with a bi-axis training system at 10'× 3' spacing. Evaporative cooling using conventional overhead sprinklers (hereafter referred as 'conventional'), fogging, netting, and fognet (foggers installed underneath netting) [Figure 1a, 1b, 1d, and 1c, respectively] were the mitigation techniques under evaluation. Effectiveness of conventional and fognet treatments was studied only in Honeycrisp block. Fogging treatments were established at both sites in similar manner by installing foggers perpendicular (East-West) to the tree row (South-North). However, netting treatment was established using over-the-top type nets in Honeycrisp (Figure 1d) and drape net (Figure 1e) in WA38.

Heat stress monitoring.

Within each heat mitigation treatment, several soil, plant, and weather processes were monitored during the growing season. Heat stress in each treatment was monitored using automated crop physiology sensing system (CPSS). CPSS nodes installed in each treatment utilized thermal-RGB imager (Teledyne FLIR LLC., OR) and an all-in-one weather station (Meters Group, Pullman, WA) to estimate apple FST. Thermal-RGB imagery data help derive the mean measured FST of 20% hottest part of the fruit surface (FST_{20}). The weather data helps derive weather-model-predicted FST (FST_w). Detailed methods on FST_i and FST_w estimation are in Ranjan et al. (2020) and Amogi et al. (2023). The FST_w has been found to be highly sensitive to variables as fruit size, color, and part of fruit exposed to sun. These variables are either difficult to measure or cannot be measured in real time. Hence assumptions are made for real time FST_w estimation, leading to less accurate FST values compared to imagery based FST. Therefore, this study used imagery based FST (FST_{20}) estimates in data comparison stage. In 2023, preliminary studies were also carried out to improve weather data based FST modeling using advanced machine learning algorithms. Developed model has shown some promising results over previously available energy balance-based methods for weather based FST estimation (Goosman et al., 2023) and our team is further refining these models.

Honeycrisp: Investigation into the effects of heat stress on fruits involved assessing the variations in T_{air} and FST across different treatments. This analysis focused specifically on the hottest days of the season. Selected dates for Honeycrisp were 19, 20, 24, 26, and 28 July in 2021; 12, 13, 14, 25, and 29 July in 2022; and 14, 15, 16, 27, and 28 August in 2023. Statistical evaluations of the differences were conducted using appropriate methods, with a significance set at 5% level. The comparisons were based on mean values and standard deviations. Additionally, a time-series analysis was performed on T_{air} and FST data collected in 2022 using CPSS at one-minute and five-minute intervals, respectively.

W38: Similar to Honeycrisp, effectiveness of heat stress mitigation techniques in WA38 were studied for, 25, 28, 30 July and 02, 04 Auguust in 2021; 24, 26, 27 July and 14, 24 August in 2022.

Fruit quality.

At commercial harvest, five to ten trees per replicated sub-block were selected based on uniform trunk cross-sectional area and crop load for at harvest and post harvest fruit quality analysis. In 2021 for both cultivars, trees underwent one time strip harvesting, followed by field assessment of sunburn damage, categorized into four levels: 1) no external symptoms, 2) browning, 3) photooxidative, and 4) necrosis. The 2022 and 2023 seasons, for the Honeycrisp block due to notable color and maturity disparities among treatments, three trees per replicated unit were evaluated at two distinct harvest timings: the first when 60% of the fruits in the most advanced treatment met commercial harvest criteria (over 50% red coloration), and the second coinciding with the least advanced treatment reaching these guidelines.

Moreover, in 2022 and 2023, the Honeycrisp trees were strip harvested by section (top, middle, and bottom) and transported to the WSU IAREC fruit laboratory. There, assessments for sunburn, bitter pit, and other defects, along with fruit color and size distribution, were conducted at harvest. For the WA38 block, one time strip harvest was continued from 2021 through 2023 without sectional distinction due to smaller study area, low crop load, and uniform maturity. From the total harvest, 110 representative fruits from each of the three replicated sub-blocks per treatment were transported to WSU-TFREC Wenatchee (PI-Torres lab) for post harvest quality evaluation over six months. Treatment-specific fruit quality was determined using a commercial sorting line (Aweta Inc., The Netherlands). Additionally, standard lab procedures were employed to measure maturity indexes, including flesh firmness (lb), soluble solids (ºBrix), titratable acidity (% malic acid), and starch index (1-6), using 10 fruits per replicate per treatment. Post harvest storage evaluation for the 2023 dataset is still in progress.

RESULTS (Objective 1)

cv. Honeycrisp

Air temperature. Distinct variations were observed in effectiveness of heat mitigation techniques, over three seasons. In 2021, T_{air} distribution across all treatments were closely aligned, indicating generally consistent effectiveness (Figure 3a). The 2022 data showed contrary trends with varying effects of mitigation techniques impacting T_{air} (Figure 3b). Control treatment recorded a significantly higher mean T_{air} (M [mean] = 89.24 °F, SD [standard deviation] = 5.4 °F). Netting, while better than the control, exhibited relatively more frequent higher temperatures around 95 °F. Fognet treatment showed highly effective cooling, achieving the lowest mean T_{air} (M = 84.2 °F, SD = 3.96 °F). Compared to 2021, T_{air} in 2022 exhibited increased variability and extreme temperature fluctuations. No significant difference was observed between the fogging (M = 85.1 °F, SD = 3.96 °F) and conventional (M = 84.74 °F, SD = 4.14 °F). In 2023, T_{air} in conventional (M = 90.1 °F, SD = 4.68 °F) was significantly lower than all others. There was no difference between control, fogging, fognet, and netting.

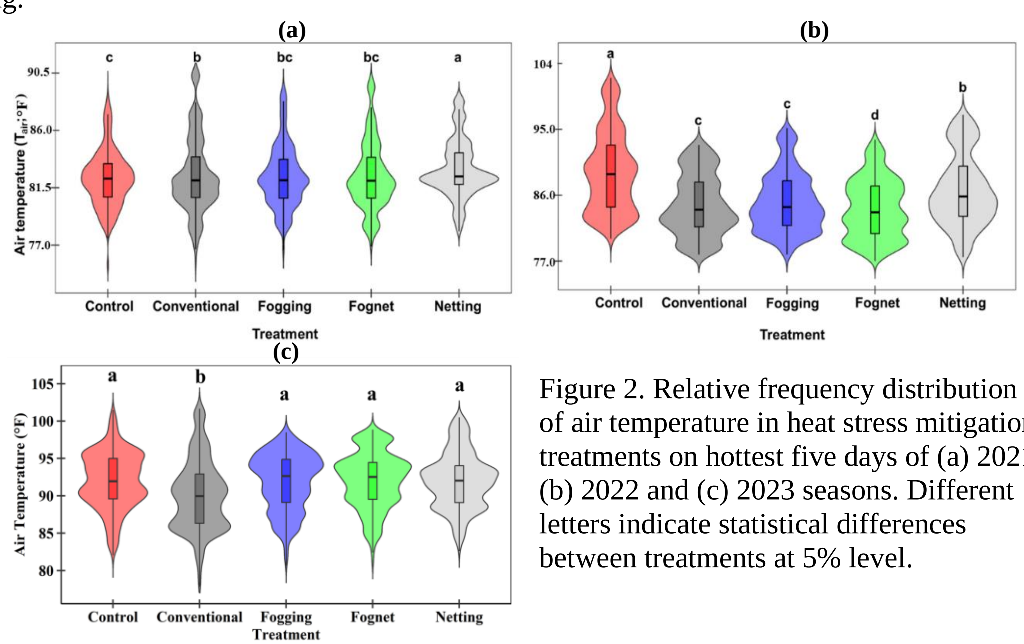

Figure 2. Relative frequency distribution of air temperature in heat stress mitigation treatments on hottest five days of (a) 2021 (b) 2022 and (c) 2023 seasons. Different letters indicate statistical differences between treatments at 5% level.

Comparing the three seasons, while the netting had some effect in regulating T_{air}, its overall impact was relatively modest in 2021 and 2022. There was no difference between conventional, fogging, and fognet in terms average air temperature measured during hottest days of all three seasons.

Fruit surface temperature. Heat stress mitigation treatment effects on FST were highly significant in 2021 and 2022. In 2021, the control treatment had the highest FST (M = 115.88 °F, SD = 5.22 °F), followed by netting (M = 111.92 °F, SD = 3.78 °F), fogging (M = 104.72 °F, SD = 4.14 °F), fognet (M = 100.4 °F, SD = 4.5 °F), and conventional (M = 98.96 °F, SD = 3.24 °F). Similarly, in 2022, the control and netting treatments exhibited significantly higher means (M = 110.66 °F, SD = 7.38 °F; M = 109.94 °F, SD = 7.2 °F, respectively) of FST compared to the conventional, fognet, and fogging treatments (M = 104.36 °F, SD = 6.84 °F; M = 104.18 °F, SD = 5.22 °F; M = 102.38 °F, SD = 7.02 °F, respectively). Latter three treatments were not different from each other. Control and netting had interesting results, where both were significantly different from each other in 2021 but not in 2022. This can be explained from corresponding T_{air} (Figure 2). A more detailed analysis was hence conducted for 2022 using timeseries analysis of T_{air} and FST.

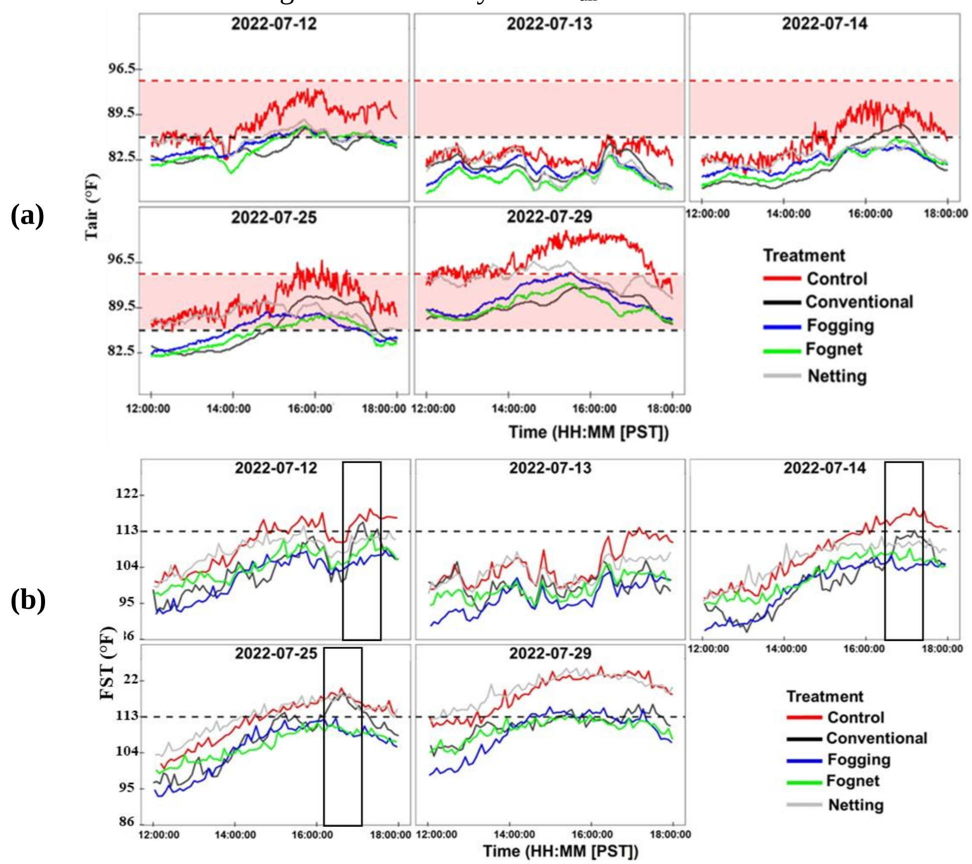

Figure 3. (a) Changes in air (T_{air}) and (b) apple fruit surface temperature (FST) during heat stress hours (12 h to 18 h) under four heat stress mitigation techniques and control. The dotted black line at 113 °F FST is a typical threshold for sunburn damage. 89 – 95 °F range (shaded in red) in T_{air} is where FST can ross 113 °F threshold.

Timeseries data from the five hottest days in 2022 (noon to 18:00 p.m.) (Figure 3), showed that netting and control treatments had higher FST than fogging, fognet, and conventional treatments. T_{air} between 86 – 95 °F (Figure 3a) could lead FST to exceed 113 °F (Figure 3b) if no mitigation measures were in place. For instance, on the 12th, 14th, 25th, and 29th of July 2022, FST in control treatment exceeded 113 °F when T_{air} exceeded 90 °F. However, fogging, fognet, and conventional methods kept FST below the critical threshold even when T_{air} neared 100 °F. On the July 13, 2022, a cloudy day with reduced solar radiation, the FST in the control treatment still reached 113 °F in the afternoon with T_{air} at 86 °F. This scenario provided a crucial insight into how T_{air} alone, even in the absence of intense solar radiation, can significantly influence FST.

Fruits absorb heat from surrounding air (environment). This heat is not instantaneous but rather accumulative. Solar radiation and T_{air} are the key drivers of this rate of change. Direct solar radiation can raise FST almost instantaneously through radiative heating of fruits. Whereas convective heat transfer from surrounding air to the fruit gets compounded over time, increasing the thermal load on fruit. Unlike evaporative cooling under conventional methods of heat mitigation, fogging removes heat from fruits and surrounding environment mainly through convection (thermal energy exchange with surrounding air) and evaporation of small droplets. Hence cooling in fogging is not immediate due to thermal inertia of the fruits, meaning retained heat inside fruits takes time to cool down. Considering this lag time between initiation of fogging and its actual effect on FST, it is crucial to start fogging before fruits reaches the FST threshold. From the time series data, it was interpreted that fogging actuation when FST is in the ranges of 86 (13th July) to 95 °F (29th July), typically earlier in the day, can help regulate FST at or below 113 °F in the late afternoon hours.

Conventional method occasionally failed to keep FST below the threshold, particularly from 15:00 – 16:00 p.m., likely due to inadequate cooling from its 25-minute ON-OFF cycle. Here, water evaporated from the fruit surface and exposed fruits to intense solar radiation and elevated T_{air} until the next cycle. This lead to quick changes in FST on July 12th, 14th, and 25th, corresponding to fluctuations in T_{air}. In contrast, fogging and fognet treatments were consistently effective throughout the day. Data from July 29, 2022, suggests that FST is reflects cumulative heat exposure and doesn't show immediate decline with T_{air} drops, which is critical for understanding the lag in heat dissipation within the fruits. Therefore, adjustments to the conventional method, in response to FST inputs from the CPSS may enhance its performance. Meanwhile, for fogging treatments, optimizing the system by increasing flow rates, adjusting spacing, or rearranging fogger positions could enhance its capacity to mitigate heat effectively.

Fruit size. In 2021, heat mitigation techniques mildly affected fruit diameter. Average fruit diameter of fruits under conventional (82 mm) highest whereas lowest under control (79 mm). In 2022, however, differences in fruit diameter were more pronounced. Average diameter of fruits under fogging, fognet, and conventional differed from control and netting with significantly smaller fruits. A significant decrease in the performance of netting and control treatments from 2021 to 2022 could be attributed to the higher T_{air} and FST, which may have negatively impacted fruit growth under these treatments.

The growth pattern in 2021 was characterized by uniformity and consistency, with fruits developing steadily over the season. In contrast, during the period from the 12th of July to the 9th of August in 2022 — a span marked by episodes of extreme heat stress — the growth of fruits in the control and netting treatments was notably hindered and virtually halted. This stagnation in growth during the peak heat stress weeks highlights the limitation of the no mitigation (control) and netting treatments during extreme heat events. However, further validation is necessary as other factors may also influence these outcomes.

In 2023, unlike previous years, conventional resulted in significantly smaller fruits (M = 53.6 mm, SD = 7.82 mm) compared to other treatment. The observed difference was primarily due to the non-uniform crop load management. Average crop load in control (78 fruits/ tree) was about 30% lower than conventional (113 fruits/tree). Similarly, the crop load in fogging, fognet, and netting was 92, 100, and 126 per tree, respectively.

Sunburn. In the 2021 and 2022 seasons, control treatment fruits experienced the highest sunburn, with 30% losses in 2021 and 8% in 2022, a threefold decrease. Conventional evaporative cooling, netting, and fognet significantly reduced sunburn. In 2021, netting was most effective, reducing sunburn by 81% compared to the control. In 2022, there was less than 2.5% damage across treatments. More direct sunlight exposure led to higher damages on canopy top than in middle and bottom layers. While this pattern was consistent across all treatments, there were slight variations. In 2023, sunburn losses were lowest with no damage under netting and fognet, and only 2-3% in control, conventional, and fogging.

Post harvest fruit quality.

Soft scald. Soft scald incidence in storage in 2021 and 2022, ranged between 0 – 9.4%. There were no differences in soft scald levels among the treatment groups in 2021. In 2022, however, differences were significant after six months of storage. Fruits under conventional treatment had 7.5 % soft scald compared to 0% in the control and netting, with no differences among the other treatments.

Bitter pit. Bitter pit incidence was higher in 2021 ranging between 35.4% and 60.6%, and much lower in 2022, ranging from 1.4% to 9%. In 2021, after three months of storage, the incidence had already reached over 30%. Bitter pit incidence in fogging (M = 51.9%), fognet (M = 60.6%), and netting (M = 53.1%) treatments were significantly higher than in the control (M = 35.4%) and conventional (M = 38.2%). In 2022, the incidence of bitter pit was significantly lower in the control (M =1.8%) and in netting (M = 1.4%) than others. There was no difference between conventional (M =8.7%), fogging (M = 5.6%), and fognet (M = 9%).

Higher bitter pit incidence in 2021 can be attributed to larger fruits where the fruit size was > 80 mm. Previous studies have found that fruits with > 80 mm diameter can cause more than 50% of bitter pit after storage (Reid & Kalcsits, 2020). This exceptional growth in fruit size can be attributed to bienniality with lower crop load (Total fruits/ tree) in 2021. In 2021, netting has the lowest number of fruits whereas, fogging with 73 fruits per tree represented highest crop load. However, in 2022, the crop load varied between 200 (SD = 60) under fognet to 265 (SD = 45) under control. Average fruit count per tree under conventional, fogging, and netting was 254 (SD = 72), 219 (SD = 50), and 235 (SD = 35), respectively. Therefore, chosen mitigation techniques should consider fruit size, crop load, and tree vigor. This might help in avoiding any excessive growth in fruit size and post storage bitter pit losses. Treatments that lead to higher bitter pit losses reportedly decreased soft scald incidences, similar to observation made by Tong et al. (2003). No sufficient reasoning can be made with available data to explain this association between soft scald and bitter pit.

Fruit maturity. Heat stress mitigation treatment effects on quality was analyzed over six months at Initial (after harvest) and 1st and 3rd day after three and six months of storage (five evaluation points). Results for each of the five maturity indices (2023 analysis is ongoing), i.e., color (% Red), weight (g), firmness (N), Chlorophyll degradation (IAD index), and SSC (°Brix) are described below.

Weight (g): Fruit weight in control and netting were lower than other treatments in both seasons. There was no significant difference between fogging, fognet, and conventional treatments in 2021.

Color (% Red): In 2021, at initial evaluation point after harvest, fruit color was also lower under netting. There was no consistent difference between netting and other treatments after three and six months of storage. In 2022, after storage, no consistent difference was observed in fruit color under netting and other treatments. For both years, after storage, fruit color under conventional, fogging, and fognet was advanced than or equal to control and netting.

Firmness (N): In 2021, fruit firmness in netting was about 2 N higher after six months of storage. In 2022, fruit firmness in control followed by netting were higher compared to all other treatments, starting after three month and seven days post-storage.

SSC (°Brix): In 2021 and 2022, treatment significantly affected SSC at most evaluation points, with moderate to high differences, but differences between treatments were inconsistent. Average SSC was highest in control and netting, followed by fogging, conventional, and fognet.

IAD index: In 2021 season data, the only consistent difference over six months of storage was IAD for fruits under netting. It was significantly higher than all other treatments. In 2022, however, IAD in netting (except at initial evaluation after harvest) and control were significantly higher than all other treatments. This again corresponded to T_{air} being significantly higher under netting only in 2021 (Figure 2a), whereas in 2022 (Figure 2b), control and netting both exhibited higher T_{air}. This signified that heat accumulations under netting might have delayed maturity.

Overall, post-storage analysis revealed that fruits under netting were less ripe in 2021; whereas in 2022, both netting and control had less ripened fruits. Such results are most possibly due to higher T_{air} (Figure 2a,b) and FST (Figure 3a,b).

cv. WA38

Air and fruit surface temperature. For 2021, the analysis revealed a significant effect of treatment on T_{air}, with fogging resulting in the highest temperature (M = 91.76 °F, SD = 4.79 °F), followed by control (M = 90.32 °F, SD = 4.37 °F), and netting being the lowest (M = 88.16 °F, SD = 4.00 °F). In 2022, netting recorded the highest temperature (M = 96.8 °F, SD = 8.01 °F), while the control (M = 94.46 °F, SD = 7.65 °F) and fogging (M = 94.46 °F, SD = 7.24 °F) treatments were not different from each other. In terms of FST, results in WA38 were similar to Honeycrisp for both years. In 2021, the control had the highest FST (M = 124 °F, SD = 5.68 °F), followed by netting (M = 120 °F, SD = 5.26 °F), and fogging (M = 114 °F, SD = 5.31 °F). Similar results were observed in 2022. T_{air} and FST analysis for 2023 data is in progress.

Sunburn. External sunburn symptoms in 2021 varied between 5 and 9%, with no difference between treatments. The sunburn was associated mostly to necrosis and cracking (averaging 11%), while browning averaged only 1%. In 2022, sunburn damage was considerably lower in both netting (0.6%) and fogging (1.8%) compared to control (7.6%). Overall, browning was prominent compared to other types of sunburn. Lower sunburn % trend continued in 2023, which varied between 0.8% in fogging and 2.4% under netting. There was no difference between treatments, however, browning was prominent under netting.

Post harvest fruit quality. No significant losses to bitter pit and soft scald were observed.
Weight (g): In 2021, no significant treatment effects were observed at initial evaluation stages under storage. However, after 3 months and 1 day, fruit weight in fogging (M = 229.69 g, SD = 26.54 g) and netting treatments (M = 232.51 g, SD = 29.46 g) were significantly lower than control. In contrast, average fruit weight in 2022 under fogging (M = 262.91 g, SD = 40.14 g) and netting (M = 209.66 g, SD = 38.88 g) were significantly higher when compared to the control (M = 195.59 g, SD = 44.32 g).
Color (% Red): No consistent differences were observed in 2021 as well as in 2022.
Firmness (N): In 2021, the netting had lower firmness (M = 67.33 N, SD = 3.91 N) compared to the control (M = 70.36 N, SD = 4.45 N). However, subsequent evaluations showed no differences. In 2022, inconsistent differences in firmness have been observed in the later stages of storage.
SSC (°Brix): In 2021, at initial and 3 months and 1 day of storage, higher SSC was observed in fruits under the netting (M = 13.19, SD = 0.77) compared fogging (M = 12.45, SD = 0.98). However, no differences were observed at later stages of storage. In contrast, in 2022, SSC in the control remained significantly higher than fogging at all stages of storage.
IAD index: In 2021, the IAD score indicated significant differences, only after six months of storage. After six months of storage, netting had lower IAD score than control and fogging. No differences in IAD score were observed in 2022.

Overall, across both years, treatment effects became more evident with increased storage time, particularly for weight, IAD, and SSC. The weight differences were slightly reversed between the two years, with fogging and netting resulting in lighter fruits in 2021 but heavier fruits under fogging in 2022. The color retention was better under treatments than control in the 2022. Firmness showed the least variation due to treatments, while SSC and IAD indicated some treatment-related effects, suggesting these parameters as sensitive indicators of post harvest changes due to different heat stress mitigation techniques.

Objective 2. Assess the effectiveness of sensing technology for automated stress monitoring and management.

In years 2022 and 2023, automated fogging was tested in WA38 research block. Instead of using FST, T_{air} was used as an input. Figure 4 below represents the schematic workflow of the automation.

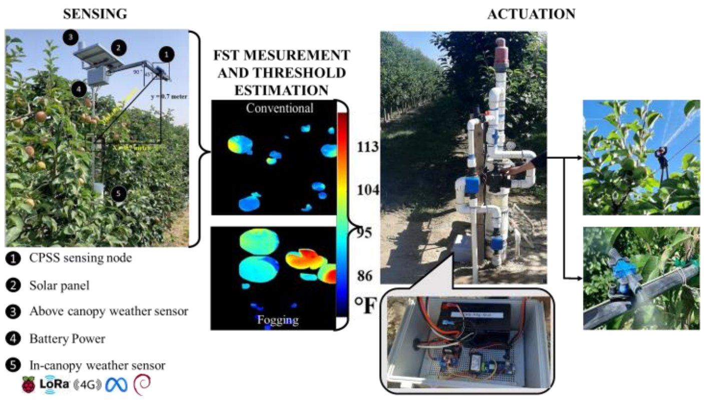

Figure 4. Automation system installed in Honeycrisp block to actuate overhead sprinklers and foggers.

Additional details of the automation and technology know-how are in the PhD dissertation (Amogi, 2023). Ideally, actuation of foggers based on FST would provide a more precise approach to manage heat stress. The current industry practice is to initiate fogging when T_{air} hits 80 ºF, but exact FST thresholds for activation were not well defined. Objective 1 outcomes helped our team to identify a critical FST range (between 86 – 95 ºF) for fogging activation. With this new understanding, one can better assess and optimize the effectiveness of CPSS-driven automated fogging against these established FST thresholds.

In contrast to fogging, conventional cooling treatments involve the direct application of water to the fruit and its surrounding canopy. This method effectively removes heat from the air and the fruit predominantly through evaporation and conduction, leading to an immediate heat stress mitigation and reduction in FST. Therefore, 108 ºF of FST (with buffer from actual 113 ºF threshold) could be an input to actuate overhead sprinklers.

To enable season long automation, significant work was done in 2022 by developing advanced machine learning (Convolutional Neural Network) algorithm to segment canopy from green fruits and reliably estimate FST on CPSS, independent of cultivar and fruit color (Amogi et al., 2023).

Objective 3. Estimate the economic cost – benefits of each technology.

Please note: Cost-benefit analysis has been done with key assumptions listed in following paragraphs. Though the numbers might differ, the relative significance should remain same amongst the different heat stress mitigation techniques.

The study assessed the costs of conventional, fogging, netting, and fognet treatments ‘Honeycrisp’ block. We measured water usage in conventional and fogging methods, excluding energy costs under the assumption of comparable water usage through automation (7% or less; Table 1). Cost comparisons focused on initial and operational expenses. This included hardware purchase, installation, operation, and maintenance of each treatment over a season.

Volume of water used. To estimate the total water usage by conventional and fogging treatments, flow rate per treatment rows was measured using ultrasonic flow sensors (Sonata Ultrasonic Water meter, Master Meter, TX). At the end of the hottest days, water flow (Gallons/day, GPD) was recorded from these flow sensors for 20 days in 2022 and 8 days in 2023 season. This daily water usage was a single digit value in GPD. In 2023, this daily water usage was cross validated by monitoring per minute flow rate (Gallons per minute; GPM) in a continuous manner for multiple times a day. The recorded gallons/day data was used to quantify actual percent difference between volume of water used in fogging and conventional treatment. This data was then scaled for an acre.

Table 1. Estimated water usage per acre in conventional and fogging technique.

Treatment	Nozzle configuration (ft.)	sprinkler or fogger rows/acre	*Flow rate, GPM/row	Flow rate, GPM/A	Operation time (min)	#Water use (gallons/acre)
Fogging	10 × 10	8	2.12	16.96	360	6108
Conventional	20 × 20	4	9.37	37.48	180	6559

*Each 650-foot row contained 60 sprinklers (conventional) and 30 foggers (fogging), based on the specified nozzle spacing. #The estimations are calculated for 6 hours (12.00 p.m.– 18.00 p.m.) of usage.

Over 28 (20 in 2022 + 8 in 2023) days, maximum T_{air} were compared for conventional and fogging treatments. No significant difference was found in recorded maximum T_{air} between conventional (M = 88.39 °F) and fogging (M = 89.31 °F). However, water usage differed significantly. Daily water use in conventional averaged 10,605 gallons/acre, about 63% higher than fogging (6496 gallons/acre). Actual water use in fogging matched estimates based on average flow rate (GPM) (Table 1). However, actual daily water use in conventional (10,605 gallons/acre) treatment was higher than estimates (6559 gallons/acre). Possibly, conventional might be running more than expected duration leading to overutilization of water (Figure 5). Thus, study emphasize the need of an automated cooling based on T_{air} or FST, as per the availability of technology and resources, which can help save significant volume of water.

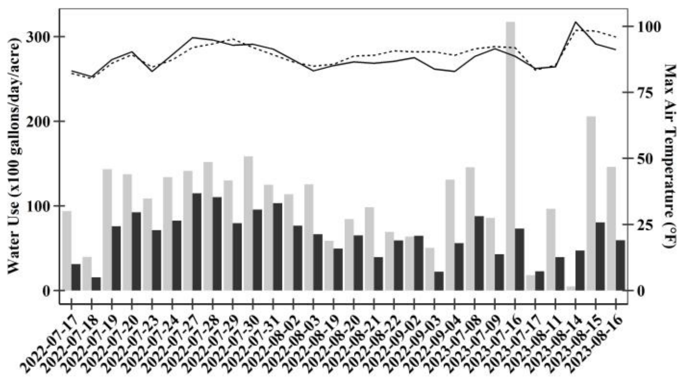

Figure 5. Per acre daily water usage in conventional overhead cooling and fogging for selected hottest days in 2022 and 2023. 'maxT_Conventional' and 'maxT_Fogging' are maximum air temperature recorded for that day.

Fixed and operational costs of mitigation techniques.

Assumptions: Per acre budgeting was based on retail equipment prices, excluding wholesale discounts. Fixed costs, including land and irrigation infrastructure, were considered equal for all treatments. Since all treatments used drip irrigation, their costs weren't compared. Installation labor charges were uniform across treatments, ranging from $250 to $300. Operational labor costs differed, with sprinklers costing about $50/day and fogging, assumed at 50% of sprinkler costs, at $25/day. These operations often coincided with other farm tasks. Seasonal maintenance, applicable only to sprinklers and foggers, focused on checking for clogs and leaks. The analysis was based on an average usage of 35 days for fogging and overhead evaporative cooling.

Costing: To set up conventional overhead sprinkler and fogging treatments per acre, 120 sprinklers and 480 foggers are needed, with 10 × 10 feet spacing for sprinklers and 20 × 20 feet for foggers. With a 650 ft row, an acre comprises about eight rows. Fogging lines are required for each row, while sprinklers are placed in alternate rows due to the spacing differences.

Table 2. Cost per acre of establishing and operating different heat stress mitigation techniques

Cost ($/acre)	Cost component	Conventional	Fogging	Netting	Fognet
Fixed	No. of rows/acre*	4	8	8	8
	1" sprinkler poly tube ($95.26/500 ft. role) / ¼" drip tape for fogger ($13.28/100 ft.)	476	637		800
	Sprinkler FT2 feed tube assembly with R10 rotator + stake [Total ~$10.23]	1227			
	Two-way fogger with 2 way cross + raiser ($2 + $1.86)		1852		1852

	Over the top shade net (shade cloth + cables + wood posts)			4000	4000
	Initial installation	250	250	250	250
	Total Fixed	1953	2739	4250	6902
Operational					
	General Farm Labor 1. Operating sprinklers in cycles ($50/day) 2. Actuating foggers ($25/day) 3. Netting retraction before harvest + Seasonal maintenance	1750 + 250	875 + 250	250	875 + 250 + 250
	Total (Fixed+ operational)	3953	3864	4500	8277

Labor rate is considered as $17.56/hour (Source: Employment Security Department/DATA; NGTS, UI Wage File).
* Row length was assumed to be 650 ft. Hence in one acre, there will be 8 tree rows.

Comparing the costs, fogging appears to be the most economical option at $3,864 per acre (Table 2), with lower fixed and operational costs. Labor accounts for 44% and 22% of total cost in conventional and fogging methods, respectively. Both can benefit from automation. Netting costs around $4500, 16.5 % more than fogging. While offering comprehensive mitigation, fognet is significantly expensive. Conventional cooling, slightly expensive than fogging, can also be used for supplementary irrigation and frost protection, an advantage not offered by fogging. Fogging may require supplemental irrigation for water deficit on hotter days. Similarly, irrigation scheduling frequency would need to be adjusted on days the conventional overhead sprinklers are operational as it adds considerable surplus moisture to the soil. In summary, automation of both water-based cooling systems and actuation based on air or fruit surface temperature, especially in conventional evaporative cooling, would help realize reliable heat stress mitigation in peak heat hours and help growers in saving considerable amount of water (& energy) usage.

Objective 4. Deliver new knowledge to apple industry through extension and outreach.

Field days. Throughout the project, approximately ten field days were organized, coordinated by Co-PI B. Sallato, PI Lav Khot, Co-PI Carolina Torres, and J. Bolivar. These events consistently attracted 16 to 35 participants each. Industry collaborators like Jain USA, along with other professionals in tree fruit crop production management, actively participated in these field days. In addition to these regular events, the project outcomes were also showcased at Smart Orchard 2022 & 2023 Field Day (Grandview, WA), and Smart Orchard + AgAID Field Day. The latter event was held on September 15, 2023, at Sunrise Orchard in Wenatchee and was well-attended by over 50 attendees, including USDA NIFA and NSF national program leaders.

Presentations, Meeting, Media, and Peer reviewed publications. Study outcomes were showcased at the WTFA annual meetings from 2021–2023, reaching over 200 individuals, including growers, and professionals. Results were also presented at the ASABE meetings, and IEEE conference, reaching out to wider range of academic experts in the US, and Europe. Findings were also discussed with growers at the Columbia Tree Fruit Club meeting, for research feedback. The team authored an article for ‘Irrigation Today’ magazine, distributed to over 12,000 growers. The project gained visibility through coverage in ‘Good Fruit Growers’, ‘Fruit Growers News’, and WSU CAHNRS News. Additionally, five research articles related to the project were submitted for peer review in international journals to get feedback from academic community.

FINAL PROJECT REPORT **YEAR**: 3 of 3

Project Title: Apple Crop Load Management

PI: Tory Schmidt
Organization: WA Tree Fruit Research Commission
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Email: tory@treefruitresearch.com
Address: 1719 Springwater Ave.
City/State/Zip: Wenatchee, WA 98801

Cooperators: Stefano Musacchi (WSU), Sara Serra (WSU), Karen Lewis (WSU), Gerardo Garcia, Manoella Mendoza, private chemical companies

Total Project Request: **Year 1:** $0 **Year 2:** $0 **Year 3:** $0

Other funding sources: **Awarded**
Amount: $127,283 (4 year total)
Agency Name: NIFA – SCRI: Precision Crop Load Management for Apples (PD: Terence Robinson, Cornell University)
Notes: funding primarily supports 2 research assistants for 3 months/year to be shared with co-PIs Musacchi and Lewis; selected trial sites will be jointly utilized for WTFRC and SCRI projects

Other funding sources: **Requested**
Amount: Unknown
Agency Name: Contract work with private chemical companies (i.e. Adama, Fine Americas, Valent)
Notes: amount requested & awarded typically offsets all costs (excluding PI salary) associated with execution of trial protocols; annual total contributions from registrants (typically $30-50K) vary depending on trial number and complexity of protocols

WTFRC Budget

Item	**2021**	**2022**	**2023**
Salaries	na	na	na
Benefits	na	na	na
Wages	28,000	28,000	28,000
Benefits	15,000	15,000	15,000
Travel	1000	1000	1000
Plot Fees	4600	4600	4600
Miscellaneous	400	400	400
SCRI funding	*(20,000)*	*(20,000)*	*(20,000)*
Contract funding	*(29,000)*	*(29,000)*	*(29,000)*
Total net cost	**$0**	**$0**	**$0**

Footnotes:
All budget figures are rough estimates and will change depending on the number of trial sites and complexity of individual trial protocols in any given year; regardless of costs incurred, external funding should likewise adjust to offset cost totals
NOTE: Budget for informational purposes only; research is funded through WTFRC internal program

OBJECTIVES:

1. Ongoing screening of novel thinning chemistries (i.e. metamitron) for bloom and post-bloom thinning of apple including development of best practices regarding rates, timings, and use of adjuvants.

2. Ongoing screening of plant growth regulators (i.e. gibberellins) with potential to influence shoot growth, flowering, fruit set, fruit growth, fruit quality, etc. to the benefit of commercial apple production.

3. Collaborate with state and national research team on SCRI grant "Precision Crop Load Management for Apples."

SIGNIFICANT PROGRESS & FINDINGS:

No thinning treatment produced significant reductions in fruit set or increases in harvest fruit size vs. untreated controls in 2023 chemical thinning trials on Honeycrisp and Cripps Pink (Table 2)

Despite generally favorable conditions for chemical thinning in 2023, WTFRC field trials were sprayed during cooler temperatures, perhaps leading to more modest thinning results

The most efficacious options for chemical bloom thinning of apple continue to be spray oil + lime sulfur programs (Table 1)

Despite more moderate performance in recent years, metamitron continues to consistently reduce fruit set, improve harvest fruit size, and increase return bloom (Tables 2 & 3)

ACC and ABA thinning programs have yet to show clear efficacy in WTFRC trials (Table 2), but are often reported to be successful in other settings

GA_7 (Arrange) inhibited return bloom in a 2022 Golden Delicious trial (Table 4), demonstrating the product's potential to mitigate biennial bearing for conventional and organic apple growers

Collaborative research efforts improve our understanding of cropping physiology and help develop new models, strategies, 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. Reduced fruit set and need for 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:

Much of our early work in chemical thinning (1998-2010) focused on screening of dozens of potential bloom thinners including various formulations of salts, sulfur compounds, oils, weak acids, and bioregulators. Very few of those products proved to be sufficiently efficacious, whether alone or in combination with other products, to offer viable options for commercial use. Over time, programs featuring the use of lime sulfur, whether applied by itself at higher concentrations (6-8%) or partnered with various spray oils at lower concentrations (2-3%) emerged as relatively consistent performers effective at achieving the three primary goals for chemical thinning described above.

With a lack of novel blossom thinning chemistries emerging in recent years, we have conducted relatively few bloom thinning trials in the last decade. In 2023, however, we did execute two very basic experiments at the WSU Sunrise Research Orchard near Rock Island in support of the Precision Apple Crop Load Management (PACMan) project. The intent of the trials was to develop field data for new versions of the Pollen Tube Growth Model (PTGM) being developed by Brent Arnoldussen at Cornell University. Trials were conducted on Gala and Jonagold with protocols only featuring a standard rate of JMS Stylet Oil + lime sulfur vs. an untreated control. Spray timings were determined by the experimental pollen tube growth models being investigated by Arnoldussen and did not necessarily align with timings that would have been suggested by the standard PTGM. The spray programs did not demonstrate any thinning or increases in fruit size in either variety (data not shown), but hopefully the data generated by detailed counts of flowers and fruit set will prove to be helpful in the potential development of an improved PTGM.

Table 1 summarizes the results of more than 200 chemical bloom thinning trials conducted by the WTFRC since 1999 including the 2023 PTGM trials, indicating how frequently various thinning chemistries produced results in fruit set, harvest fruit size, and return bloom that were statistically superior to untreated control treatments in those field trials.

Table 1. Incidence and percentage of results significantly superior to untreated control. Apple chemical bloom thinning trials. WTFRC 1999-2023.

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 / 26 (54%)	8 / 25 (32%)	4 / 22 (18%)
WES + LS	15 / 32 (47%)	5 / 31 (16%)	4 / 31 (13%)
ThinRite	7 / 22 (32%)	0 / 23 (0%)	0 / 12 (0%)

[1]Does not include data from 2023 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. Even though WTFRC pesticide residue studies have been unable to detect any trace of carbaryl at harvest when used as a chemical thinner, some retail grocers have already established policies prohibiting the sales of produce which has been treated with specific pesticides, including carbaryl.

Fortunately for apple growers, there are multiple alternatives that are now or will soon be available for postbloom chemical thinning. Our ongoing trials seek to evaluate several of those products:

Metamitron – this chemistry was initially developed as an herbicide for use in sugar beets and is currently being developed by Adama. It is already registered as a postbloom thinner of apple in several countries including Italy, France, Spain, South Africa, Chile, and New Zealand under the trade name "Brevis." Metamitron has been shown to induce temporary reductions in carbon fixation by inhibiting Photosystem II; this effect tends to be more pronounced during weather conditions associated with increased carbohydrate stress in apple trees, namely when days are hot and cloudy and nighttime temperatures are warm.

We have been fortunate to work with metamitron since 2011 and have found it to be very effective under Washington field conditions. Our early metamitron studies explored various chemical formulations, application rates and timings, use of adjuvants, and combinations with other thinning chemistries. Results from these trials have been key in helping develop best use patterns for metamitron and will help guide the development of a product label when the commercial product is finally registered. Unfortunately for both the registrant and US apple industry, the registration process at the US EPA has been delayed several times, including a recent request that more work be done regarding protection of off-target animal species. Considering ongoing delays, it is most likely that a commercial product will be available to US apple growers for the 2025 growing season.

Much of our early work with metamitron utilized high product rates (64+ ounces/acre) and aggressive timings to establish its efficacy and to determine a red line of what would be "too much" for our conditions in WA. After several instances of over-thinning when the product was applied during hot conditions (85+ F), we concluded that more modest rates of 24-28 ounces/acre would be more appropriate for most chemical thinning scenarios, especially when the product would be tank-mixed with a non-ionic surfactant such as Regulaid, which consistently has improved thinning efficacy. Use of these lower rates in recent years has reduced the incidence of phytotoxicity as well as the degree of thinning.

Even though the 2023 chemical thinning season featured several hot days, they did not coincide well with the actual spray days for our field trials and likely led to some disappointing results. Table 2 reveals that metamitron treatments (ADA 46701) did not affect fruit set or size on Cripps Pink in Monitor or Honeycrisp in East Wenatchee, although the high rate of metamitron did reduce fruit set by 35%.

ABA (abscisic acid) – ABA has been sold by Valent under the trade name "ProTone" for a few years. It was initially registered to enhance color in table grapes but now also has a label for postbloom thinning of apples and pears. ABA is known to boost ethylene biosynthesis, causing increased abortion of developing fruit. It is generally considered to be a mild thinner of apples, but has been approved by OMRI, making it a welcome option for organic growers.

As with all other products tested, ProTone failed to provide significant thinning in our 2023 Honeycrisp trial (Table 2). This result was especially disappointing given that weather conditions were nearly ideal for ABA efficacy (85°F +) according to colleagues with extensive experience with the product. Our first-hand experience with ABA is still relatively limited and we look forward to the opportunity to use it across more cultivars, locations, and growing seasons.

ACC (1-aminocyclopropanecarboxylic acid) – ACC is a metabolic precursor of ethylene, which promotes fruitlet abscission in apples. Unlike ethephon which produces a sudden burst of ambient ethylene gas, ACC is taken up by the plant and subsequently metabolized, resulting in a more steady, controlled production of ethylene in the plant tissue. Research trials in the Eastern US have proven it to be an effective chemical thinner of apples, especially when applied late in the spring (15-20mm fruitlet size). Due to its efficacy at the tail end of chemical thinning season, ACC may offer some potential as a "rescue" thinner in circumstances when apple growers may feel they need additional thinning after assessing early fruit set. ACC was available for commercial use under the trade name "Accede" for the first time in the 2022 thinning season.

While Accede did not provide significant thinning in either 2023 trial, the 10-12 mm application timing did reduce fruit set numerically on Cripps Pink (Table 2). Interestingly, this result conflicts with reports from other research and demonstration trials which have suggested that ACC is more efficacious either prior to petal fall or after 15 mm fruitlet size. These reports of successful thinning with ACC come from credible sources and we will continue our field testing of Accede in hopes of finding similar results.

BA (6-benzyladenine) – BA is a cytokinin which can induce some fruitlet abortion and increase fruit size by promoting cell division. Previous WTFRC trials with BA have shown it to be a relatively weak thinner of apples in WA conditions and typically requires tank mixing with other chemistries like NAA or carbaryl to provide adequate reductions in fruit set. Many BA products including MaxCel and Exilis have been available to industry for several years, but in 2023 we had the opportunity to screen several new formulations (FAL 567, FAL 571, FAL 581) on Honeycrisp in East Wenatchee. Once again, our BA treatments did not produce any significant thinning effects (Table 2), but neither did any other thinning programs in this trial.

Table 2. Crop load and fruit quality effects of postbloom thinning programs. WTFRC 2022.

Treatment	Fruitlets/100 floral clusters	Blanked spurs	Singled spurs	Harvest fruit weight	Relative box size	Russet free fruit
		%	*%*	*g*		*%*
Cripps Pink / M.26 - Monitor						
Accede 34oz + Reg 16oz Petal fall	94 bc	45 ab	29	189	96	94
Accede 34oz + Reg 16oz 10-12 mm	56 ab	66 bc	20	193	94	86
Accede 34oz + Reg 16oz 16-18 mm	100 c	38 a	34	182	100	94
Accede 34oz + Reg 16oz 22-24 mm	77 abc	53 abc	26	178	102	88
ADA 46701 32oz + Reg 16oz PF	104 c	38 a	34	186	98	84
ADA 46701 32oz + Reg 16oz 10-12 mm	74 abc	51 abc	32	188	97	81
ADA 46701 32oz + Reg 16oz 16-18 mm	84 abc	49 abc	28	183	99	83
ADA 46701 32oz + Reg 16oz 22-24 mm	82 abc	48 abc	30	180	101	95

Carbaryl 4L 36oz + PoMaxa 3oz PF & 10-12 mm	42 a	70 c	21	205	89	*85*
Control	80 abc	48 abc	31	186	98	96
Significance (p value)	*0.000*	*0.001*	*0.097*	*0.153*		0.012
Gale Honeycrisp / G.935 – East Wenatchee						
Accede 46 oz + Reg 16 oz	109 ab	49 ab	15	193 ab	94	0
ADA 46701 24oz + Reg 16oz - Low	89 ab	56 ab	14	187 ab	97	4
ADA 46701 30oz + Reg 16oz- Med	105 ab	47 ab	20	214 b	85	14
ADA 46701 36oz + Reg 16oz - High	67 a	63 b	18	203 ab	89	4
Exilis 9.5 25.6oz + Reg 16oz	88 ab	55 ab	17	188 ab	97	4
FAL 567 51oz + Reg 16oz	96 ab	52 ab	18	193 ab	94	0
FAL 571 124oz + Reg 16oz	99 ab	51 ab	19	189 ab	96	3
FAL 581 12.8oz + Reg 16oz	93 ab	53 ab	18	163 a	111	0
ProTone 33.1oz+ Reg 1 oz	114 b	44 a	18	185 ab	98	8
Control	102 ab	49 ab	19	182 ab	100	1
Significance (p value)	*0.055*	*0.117*	*0.877*	*0.110*		*0.527*
SRO Gala / M.9 Nic 29 - Rock Island						
JMS Stylet Oil 1.5 gal + LS 2.5 gal	72	54	25	136	134	9
Control	71	58	21	143	127	9
Significance (p value)	*0.787*	*0.268*	*0.149*	*0.100*		*1.000*
SRO Jonagold / M.26 - Rock Island						
JMS Stylet Oil 1.0 gal + LS 2.5 gal	64	57	26	192	95	33
Control	68	57	24	192	95	45
Significance (p value)	*0.627*	*0.951*	*0.676*	*0.980*		*0.278*

Given the variability in results from one chemical thinning trial to the next, it is important to look at the "big picture" of research data. Similar to an earlier table which demonstrated chemical bloom thinning results, Table 3 summarizes the results of every chemical postbloom thinning trial conducted by the WTFRC over the last 20 years. These findings confirm that apple growers can use thinning programs based on BA and NAA (naphthaleneacetic acid) and reasonably expect results comparable to those produced with thinning programs based on carbaryl. Further, Table 3 reveals the steady performance of metamitron, suggesting that when that chemistry is finally registered for commercial use, it may offer a more consistently efficacious option for postbloom thinning than any other program that is currently available to WA apple growers.

Table 3. Incidence and percentage of results significantly superior to untreated control. Apple chemical postbloom thinning trials. WTFRC 2002-2023.

Treatment	Fruitlets/100 blossom clusters	Harvested fruit size	Return bloom[1,2]
BA	7 / 32 (22%)	0 / 33 (0%)	0 / 32 (0%)
Carb + BA	33 / 91 (36%)	10 / 89 (11%)	13 / 86 (15%)
Carb + NAA	30 / 87 (34%)	23 / 86 (27%)	19 / 84 (23%)
BA + NAA	20 / 42 (48%)	9 / 41 (22%)	9 / 38 (24%)

Metamitron	20 / 36 (56%)	16 / 35 (46%)	10 / 32 (31%)

[1]Does not include data from 2023 trials.
[2] (no. blossom clusters year 2/sample area) / (no. blossom clusters year 1/sample area)

GIBBERELLIC ACID FOR BLOOM INHIBITION:

Our interest in using gibberellins to help promote annual cropping in apple grew out of several years of unsuccessful trials trying to promote return bloom with flowering promotors like auxins (i.e., NAA) and ethylene (i.e., ethephon). Despite enthusiastic testimonials from several prominent industry figures, we were simply unable to demonstrate any increase in flowering from summer applications of NAA or ethephon. We decided to instead, explore a strategy of attacking biennial bearing from the opposite direction by applying a flowering inhibitor like gibberellic acid (GA) in the "off" year of a biennial cycle in hopes of reducing the return bloom in the "on" year and ultimately producing more flowers in the subsequent "off" year approximately 23 months after the GA application.

This strategy has proven much more successful, and over 15+ years of testing, we have demonstrated the efficacy of several GA products at reducing return bloom and ultimately mitigating the amplitude of year-to-year swings in apple flowering. Most of our early work focused on GA_3 products like Falgro and ProGibb which are primarily used to delay harvest and promote fruit firmness in cherry. While these programs were effective and relatively inexpensive, the registrants of these products were reluctant to pursue expanded labels for chemistries whose patents had already expired. More recently, Fine Americas developed a formulation of GA_7 that has proven to be effective at lower concentrations than GA_3 products; that product is now sold as "Arrange" and is approved for use by OMRI, providing a valuable tool to organic growers who have limited chemical options for managing crop load.

As with GA_3 products, our work has shown Arrange to be most effective around 10mm fruitlet size timing. Generally speaking, most bioregulator spray programs benefit from multiple applications of lower doses but in prior trials, as was the case in a trial sprayed in the spring of 2022 on severely biennial Golden Delicious at WSU's Sunrise Research Orchard near Rock Island (Table 4). In that trial, all treatments with Arrange reduced flowering in 2023, but as is often the case in return bloom studies, the wide variability in the data precluded statistical significance for most treatment effects.

Arrange can be reasonably efficacious in a single dose, especially when partnered with an effective adjuvant. Based on our work with Arrange and other GA formulations, we feel that the best use pattern would be to make 2-4 weekly applications of reduced rates of the product starting around petal fall in a block with uniformly lightly cropped (but not blank) apple trees. Obviously, application of a GA product to the occasional heavily cropped tree would only further inhibit return bloom and increase the severity of its alternation. We look forward to a future with smart spray technology that allows prescriptive application of chemical thinners and plant growth regulators to individual trees based on their respective crop loads, but until then, growers with blocks that are mixed with heavily and lightly bloomed trees should consider spraying individual light trees with a handgun to bring the entire block into more synchronous and consistent cropping.

Table 4. Effects on tree vigor, fruit size, and return bloom of GA applications. WTFRC 2022.

Treatment	2022 harvest fruit weight	2022 relative box size	2023 return bloom	2023 return bloom/CSA
	g		*%*	*clusters/cm²*

SRO 1B Golden Delicious / Bud 9 - Rock Island				
Arrange 100 ppm Petal Fall	218	83	3925 ab	2.5
Arrange 200 ppm Petal Fall	199	91	2955 ab	2.4
Arrange 100 ppm 10 mm	184	99	4341 ab	2.7
Arrange 200 ppm 10 mm	199	91	3277 ab	2.1
Arrange 100 ppm Petal Fall & 10 mm	201	90	2103 a	2.0
Control	198	92	5015 b	3.0
Significance (p value)	*0.604*	*na*	*0.012*	*0.382*

COLLABORATIVE CROP LOAD MANAGEMENT RESEARCH:

"Precision Crop Load Management for Apples" (USDA-NIFA Specialty Crop Research Initiative (SCRI) - PD: Terence Robinson, Cornell) – field work for project initiated in 2021 and includes trials in WA, NY, VA, MI, MA, and NC; objectives 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; WTFRC efforts have focused on:

- support for Musacchi group (WSU) including data collection and plot spraying to investigate effects of pruning severity and floral density on cropping in Gala and Honeycrisp
- facilitating evaluation of digital technology (Farm Vision/Pometa, Fruit Scout, Green Atlas) to count and measure buds, flowers, and fruit on the tree throughout the growing season
- execution of chemical bloom thinning field trials to help evaluate novel pollen tube growth models
- multiple outreach efforts including organization of field days, surveys, written reports, and oral presentations in several regional meetings

"Maximize pollination window to improve fruit set in WA38" (PI: Serra) – helped coordinate field activities including trial layout, data collection, spray application, reflective material deployment, sample collection, and harvest analysis; field trials showed few significant effects of application of ethylene-inhibiting materials (ReTain, Harvista) on WA38 yields and fruit quality, but deployment of a reflective material (Extenday) throughout the growing season did increase cumulative fruit yields and quality; see Serra final report for more detail

"Smart Orchards Year 4 + Connectivity" (PI: Mantle) – worked with Mantle, Hoheisel, Khot, and Washington Fruit to develop a differential chemical thinning spray strategy for the Grandview Smart Orchard (Honeycrisp) based on heat maps generated from digital scans of flower density and crop load in previous seasons; spray programs were executed by a variable rate sprayer with Smart Apply technology to deliver higher doses of chemical thinners to portions of the orchard with relatively higher bloom density; see Mantle report (WTFRC Technology Committee) for more detail

Project Title: Apple Crop Load Management (2023)
PI: Tory Schmidt, WTFRC

Executive Summary

Keywords: chemical thinning, PGR, return bloom

Abstract: The primary key to profitability in apple production is the ability to generate consistently high yields of quality fruit. Spiraling costs for labor and other inputs have put a premium on less expensive strategies to manage crop load including the use of chemical thinners and plant growth regulators. In this ongoing research, we sought to develop practical best use patterns for emerging chemistries for thinning and regulation of fruit set, fruit size, fruit quality, and flowering through a series of field trials. Further, we collaborated with other scientists and commercial interests in the development of new chemistries, models, and technologies to improve precision and reliability of commercial crop load management.

Project outcomes:

1. Identification of novel efficacious chemical thinners (i.e. metamitron) and PGRs (i.e. GA for floral inhibition) with practical commercial relevance.
2. Development of best use practices (timings, rates, use of adjuvants, etc.) for these products.
3. New collaborative working relationships with a broad range of scientists, chemical registrants, technology providers, and other allied industry vendors working in crop load management.

Significant Findings:

1. Metamitron shows great promise as a postbloom chemical thinner of apple in WA conditions, providing consistent results across multiple years, locations, and cultivars.
2. ABA and ACC have demonstrated efficacy as chemical thinners in other settings, but not in preliminary WTFRC trials.
3. GA_7 is effective at inhibiting floral initiation in apple and offers a new tool for organic and conventional growers to manage annual cropping.
4. Emerging digital imaging and sprayer technologies offer potential to manage crop load on an individual-tree basis in the coming years.

Future Directions:

1. Provide outreach/guidance to industry when the new metamitron product is labeled and released.
2. Ongoing screening and refinement of new chemistries for crop load management.
3. Investigation of developing technologies with implications for crop load management (i.e. digital vision/crop mapping, smart sprays, robotic pruning/thinning/picking machines).
4. Further collaboration with other scientists to improve knowledge of physiology of apple cropping and predictive models to help manage it effectively.

Project/Proposal Title: Measuring the impact of leaf removal on spur and tree health

Report Type: Continuing Project Report

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Cooperators: Thiago Campbell, Orlando Howe, McDougall and Sons, Gebbers Farms,

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $ 60,344
Total Project Request for Year 2 Funding: $ 66,377
Total Project Request for Year 3 Funding: $ 52,580
Other related/associated funding sources: None

Budget 1
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Item	2022	2023	2024
Salaries[1]	$40,777	$43,826	$31,460
Benefits[2]	$6,637	$7,393	$10,895
Wages[3]	$5,187	$5,394	$0
Benefits[4]	$518	$539	$0
Equipment	$0	$0	$0
Supplies[5]	$3,000	$5,000	$5,500
Travel[6]	$4,225	$4,225	$4,225
Miscellaneous[7]	$0	$0	$500
Plot Fees	$0	$0	$0
Total	$60,344	$66,377	$52,580

Footnotes:

[1] Salary is requested for a 25% post-doc in years 1 and 2 and then 50% in year 3 as well as a graduate assistant in year 1 and 2 to complete the applied physiology experiments.

[2] Benefits are calculated at 34.6% for the post-doc and 12.6% for the graduate assistant.

[3] Wages are for covering summer salary for the graduate assistant

[4] Benefits are calculated at 10% for summer graduate students

[5] Supplies are for field and lab consumables to conduct applied experiments for objective 1 and 2 and then Extension material for objective 3.

[6] Travel funds are requested for frequent travel to the Sunrise research orchard for PIs and personnel and to commercial orchards to conduct deleafing trials.

[7] Funding is requested for a small personal service contract for a videographer to capture some of the applied experiments being conducted for this project.

Objectives

1. Quantify improvements in leaf color and changes to sunburn incidence from leaf removal for an early and late-season bicolor apple cultivar.

2. Determine whether differences in leaf removal severity and timing before harvest impacts energy and nutrient storage and subsequent spur health the following season or an early and late-season bicolor apple cultivar.

3. Develop practical operating guidelines and economic cost-benefit thresholds for leaf removal based on commercial trials in WA.

Significant Findings

- In 2022, color development was poor for earlier cultivars but was much improved in 2023 for both cultivars. Leaf removal did not affect red color for WA 38 in a good coloring year like 2023.
- Leaf removal greater than 50% reduced return bloom, yields but did not affect vegetative vigor.
- Leaf removal significantly enhanced color development but also increased sunburn damage for Honeycrisp but not for WA 38. Benefits were observed as low as 25% leaf removal. Unsurprisingly, above 75% leaf removal increased sunburn damage in unprotected fruit.
- Leaf removal had limited benefit for a high coloring cultivar like WA 38, but also had limited sunburn risk. In a poor coloring year, leaf removal would likely have benefits for bicolored cultivars with high color requirements.
- Leaf removal timing had little impact on red color development in 2022.
- Carbohydrate content in storage tissues were relatively unaffected by deleafing treatments.
- Timing of leaf removal had little impact on red color development. Deleafing can be done in as little as 7 days before harvest with improved color still observed.

Methods

1. Leaf removal timing

An experiment was started in 2022 to answer when the optimum timing is for defoliation to maximize fruit red color development and decrease risks of sunburn of previously shaded fruit. Treatments included early defoliation (14 days before harvest) and defoliation closer to harvest (7 days before harvest). Weather conditions during this period are presented in Figure 1 below for both experiments with Honeycrisp and WA 38. 50% of the leaves will be removed for both defoliation treatments. We will also have an undefoliated control to compare fruit quality with no interventions. Five trees will be selected for each treatment selecting for uniformity of fruit distribution in the canopy and vigor for both Honeycrisp and Fuji. This experiment will be continued in 2023 by Orlando Howe (MS student). Whole tree fruit samples were single picked at commercial harvest timing to assess fruit color coverage. 48 fruit per tree were used for each tree to capture a full assessment of fruit quality. Fruit was run on a commercial sorting line at WSU TFREC (AWETA) to measure fruit weight and diameter, red color coverage, intensity, and background color. Sunburn incidence was also evaluated in harvest fruit using the Schraeder and McFerson (2003) sunburn scale.

1. Leaf removal severity - part I

This experiment was also conducted by Orlando Howe (MS Student). There are five treatments with five single-tree replications for each treatment. The five treatments were: 0% removal, 25% removal, 50% removal, 75% removal or 100% removal of foliage. Both Honeycrisp and WA 38 were used as the two cultivars for these experiments. These experiments were conducted in single-axis tall spindle plantings at a density of 3’ x 12’ that are entering their sixth leaf. Defoliation was conducted 14 days before harvest for all severity treatments.

For each treatment, leaf number was counted per tree (5 trees per treatment). Defoliation was evenly applied to the entire tree. Whole tree fruit samples was taken from all five trees for each treatment. Fruit was hand-graded for sunburn and then run on a commercial sorting line to measure fruit weight and diameter, red color coverage, intensity, background color. Then, we will sample spur and non-spur reproductive buds on January 1, and March 1 to analyze nutrient and non-structural carbohydrate concentrations. Nutrient concentrations will be analyzed for all macro and micronutrients at a commercial analysis lab. To measure sugar concentrations (adapted from Chow & Landhausser, 2004), 10 mg of previously freeze-dried and ground tissue will be weighed and then extracted with 80% hot ethanol followed by colorimetric analysis with phenolsulfuric acid. The resulting bulk sugar extract will be read at 490 nm on a microplate reader (Epoch Microplate Spectrophotometer; Bio-Tek Instruments, Winooski, VT, USA) or a spectrophotometer (Thermo Fisher Scientific GENESYS 10S UV-Vis, Waltham, MA, USA). Sugar concentrations (expressed as mg sugar per g dry wood) will be calculated from a 1:1 :1 glucosefructosegalactose (Sigma Chemicals, St Louis, MO, USA) standard curve. To determine starch concentrations, the remaining tissue will be solubilized in NaOH and then digested with an a-amylase/amyloglucosidase digestive enzyme solution. Glucose hydrolysate will be determined using a PGO-colour reagent solution (Sigma Chemicals) and read at 525 nm. Starch concentrations (expressed as mg starch per g dry material) will be calculated based on a glucose (Sigma Chemicals) standard curve.
The same trees will also be monitored for return bloom using two approaches: 1. Dissecting five spur buds per tree in late March, and 2. Counting flower clusters per tree at king-bloom stage.

Results and Discussion

Although 50% deleafing increased red color, it didn’t matter whether it was done 7 or 14 days before harvest for WA 38 (Figure 1). However, for Honeycrisp, fruit color coverage was higher when deleafing was done 14 days before harvest compared to 7 days before harvest (Figure 2). Sunburn incidence was less consistent. 7 days before harvest, temperatures exceeded 100 °F and there was overall little color development for Honeycrisp for any treatment. Treatments did not impact sunburn development for WA 38. In 2023, there was almost no sunburn pressure after deleafing. Even then, there were increases in sunburn incidence for both timings for Honeycrisp. In 2022, deleafing led to 16% more fruit having more than 40% red color coverage. Then, in 2023, approximately 20% more fruit had more than 40% red color coverage for both deleafed treatments compared to the control and there were no differences between the two times.

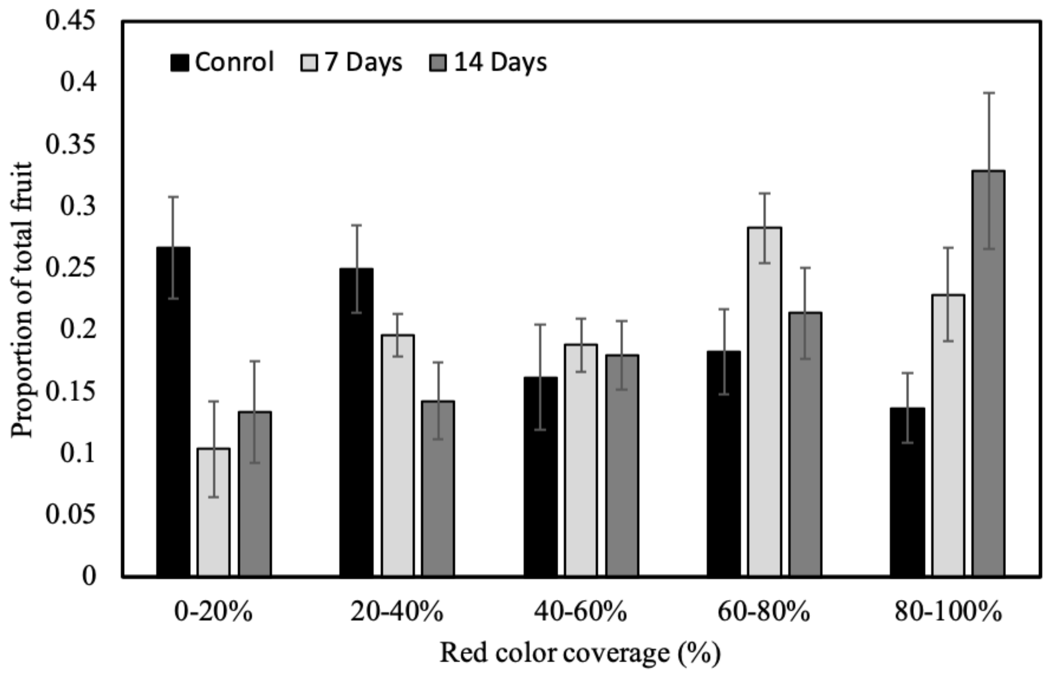

Figure 1. The proportion of fruit (%) with 0-20%, 20-40%, 40-60%, 60-80%, or 80-100% red color for 'Honeycrisp' trees where 50% of leaves were removed either 7 or 14 days before harvest compared to an untreated control in 2023.

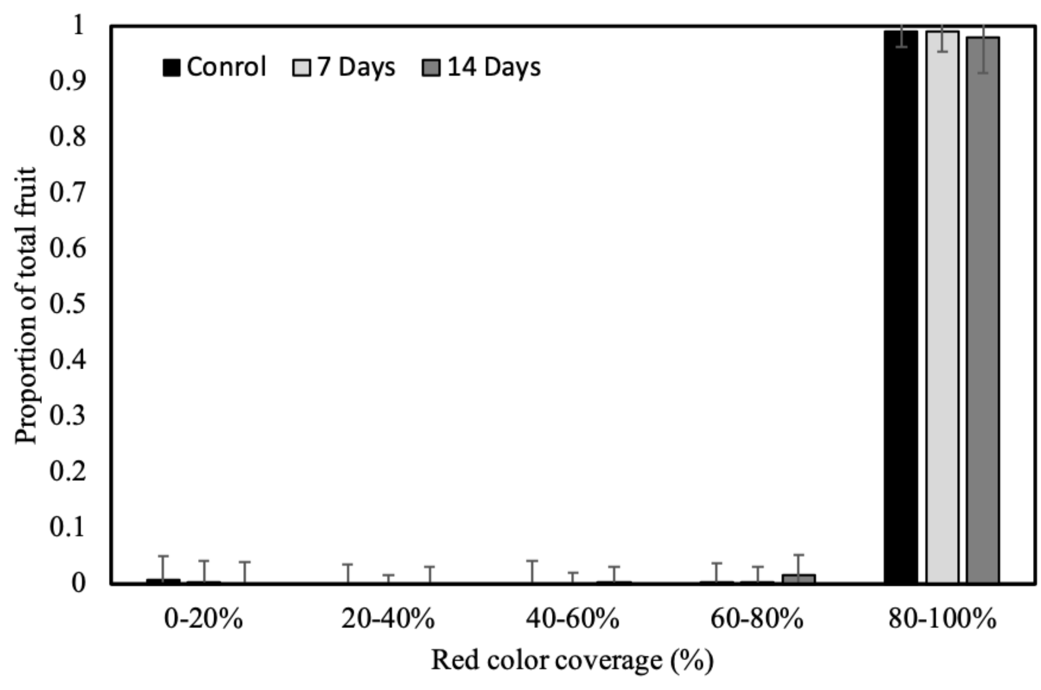

Figure 2. The proportion of fruit (%) with 0-20%, 20-40%, 40-60%, 60-80%, or 80-100% red color for 'WA 38' trees where 50% of leaves were removed either 7 or 14 days before harvest compared to an untreated control in 2023.

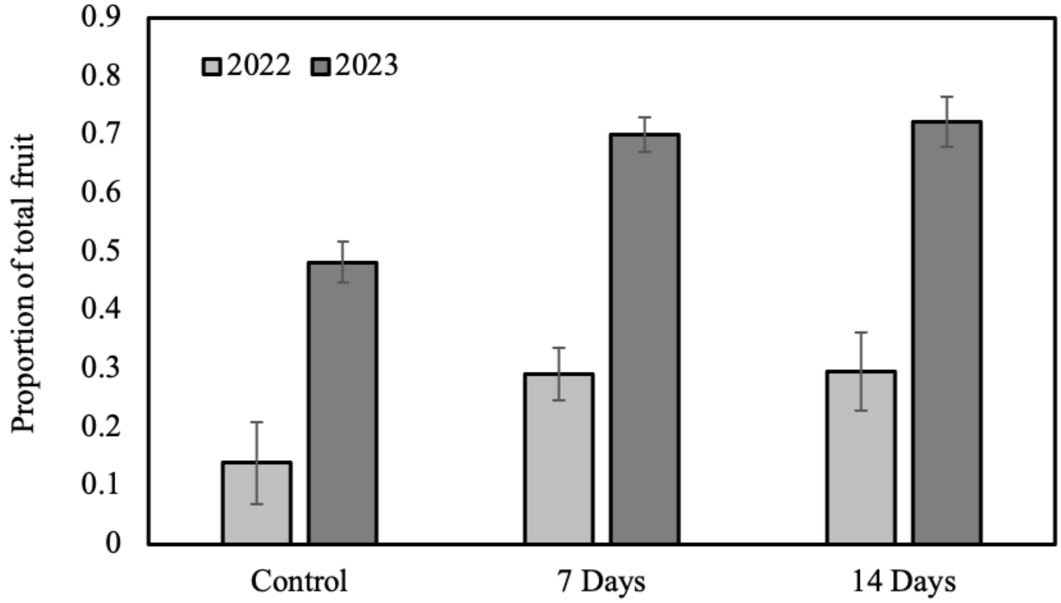

Figure 3. The proportion of fruit with >40% red color coverage for Honeycrisp in 2022 (poor color year) and 2023 (good color year) with no leaf removal or 50% leaf removal 7 or 14 days before harvest.

As the proportion of leaves removed increased, there was greater red color and sunburn. However, the results were less consistent across years. The impacts of deleafing on the incidence of sunburn and red color coverage were greater for the earlier cultivar, ‘Honeycrisp’, than the later cultivar, ‘WA 38’, which naturally developed color easier than ‘Honeycrisp’. For ‘Honeycrisp’, more than 50% leaf removal led to significant losses to sunburn. Complete leaf removal led to sunburn incidence of about 5% more losses than the control for both years in WA 38. However, 75% leaf removal did not lead to higher incidences of sunburn.

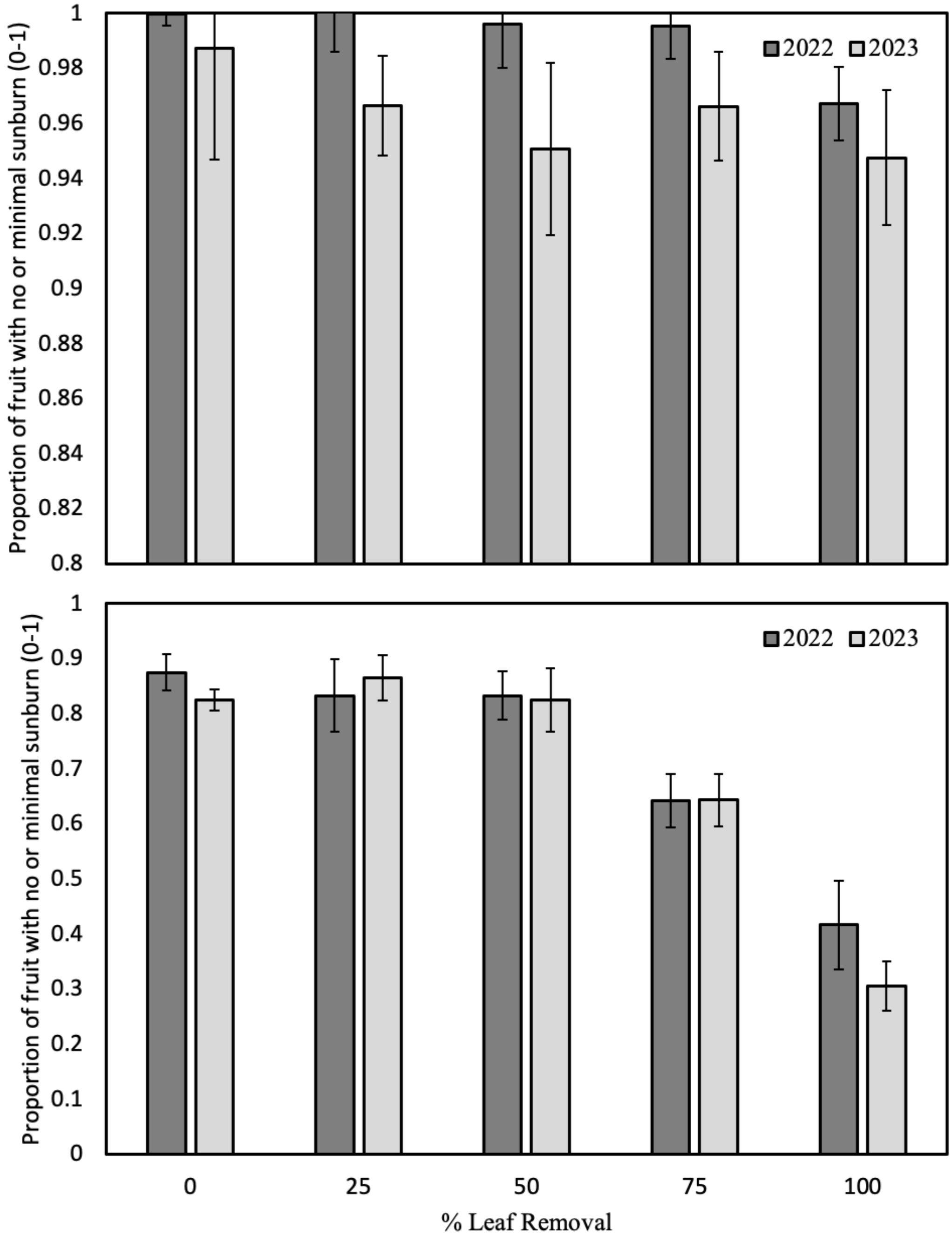

Figure 4. Sunburn incidence for ‘Honeycrisp’ (bottom) and ‘WA 38’ (top) in 2022 (dark grey bars) or 2023 (light grey bars) for fruit from trees with 25%, 50%, 75%, or 100%* leaf removal 14 days before harvest compared to an untreated control. Error bars denote standard error (N=5). *Fruit assessment of sunburn will be completed for this treatment after storage along with evaluation of other treatments.

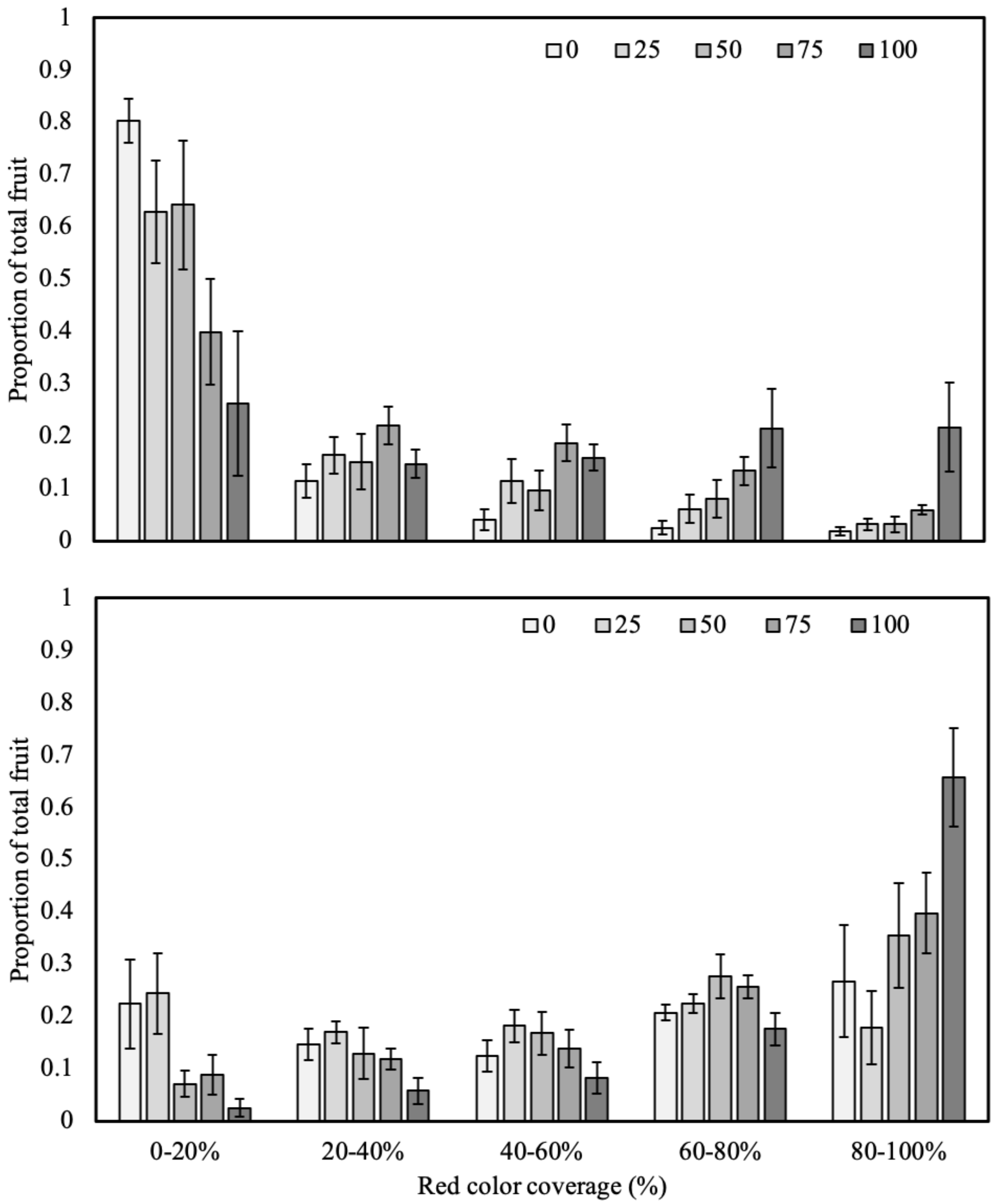

Figure 5. Percentage of fruit with 0-20%, 20-40%, 40-60%, 60-80%, 0r 80-100% red color coverage for ‘Honeycrisp’ fruit treated with five defoliation severities (control, 25% removal, 50% removal, 75% removal, or 100% leaf removal) in 2022 (top) or 2023 (bottom).

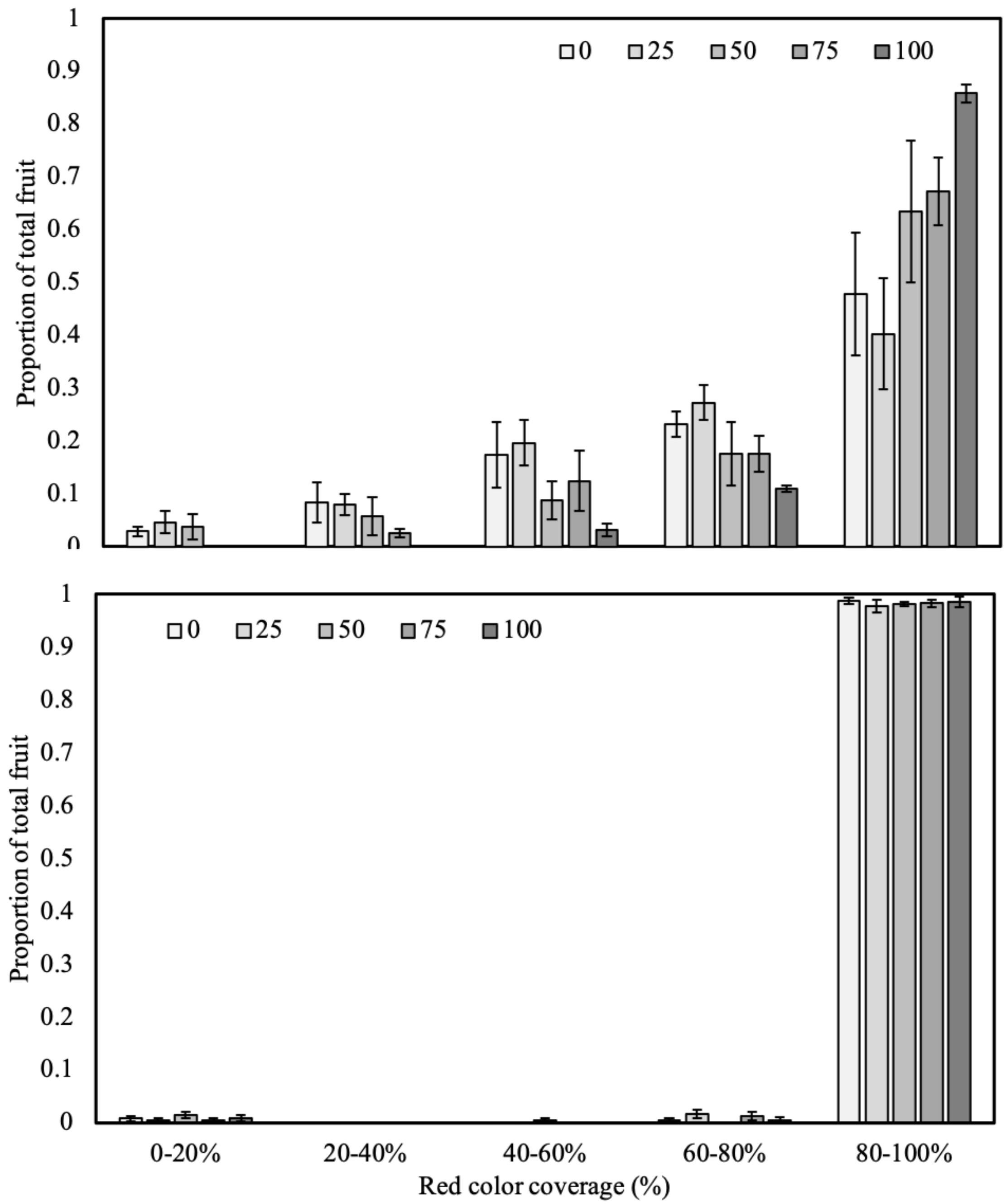

Figure 6. Percentage of fruit with 0-20%, 20-40%, 40-60%, 60-80%, 0r 80-100% red color coverage for ‘WA 38’ fruit treated with five defoliation severities (control, 25% removal, 50% removal, 75% removal, or 100% leaf removal) in 2022 (top) or 2023 (bottom).

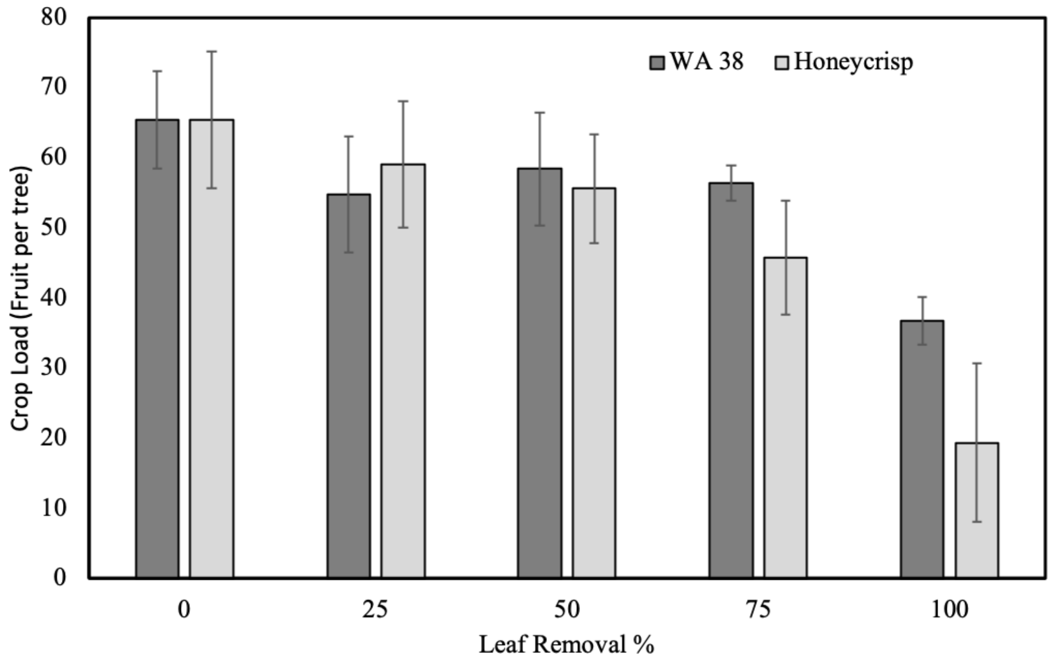

Figure 7. Return yields in 2023 for differing severity treatments imposed in 2022.

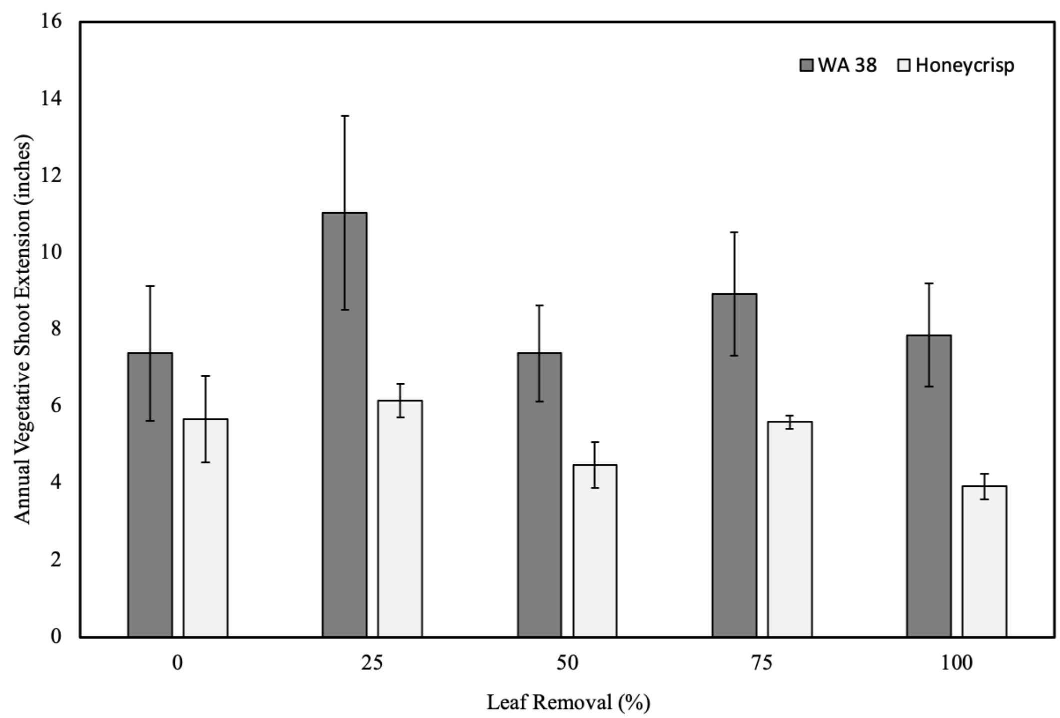

Figure 8. Vegetative vigor in 2023 for differing severity treatments imposed in 2022.

Table 1. Commercial trials and plans for Extension case study material

Cultivar	Location
Gala	Pateros/Chelan
Honeycrisp	Pateros/Chelan
WA 38	Quincy
Fuji	Quincy
Envy	Mattawa
Cripps Pink	Mattawa

Figure 9. Control (left) and deleafed (right) for ‘Envy’ (Top) and Fuji (Bottom) in commercial orchards using pneumatic deleafing machines.

Table 2. Project timeline for the completion of objectives 1-3

	2022				2023				2024			
	Spring	**Summer**	**Fall**	**Winter**	**Spring**	**Summer**	**Fall**	**Winter**	**Spring**	**Summer**	**Fall**	**Winter**
Objective 1												
Commercial defoliation trials on four cultivars and fruit quality evaluation		■	■			■	■			■	■	
Evaluation of changes in nutrient and carbohydrate reserves				■				■				■
Assessment of changes to return bloom and shoot vigor					■	■			■	■		
Objective 2												
Defoliation timing experiment												
Treatments			■				■					
Fruit quality evaluation			■	■			■	■			■	■
Defoliation severity experiment part I												
Treatments			■				■					
Evaluation of changes in nutrient and carbohydrate reserves				■				■				
Assessment of changes to return bloom and shoot vigor					■	■			■	■		
Defoliation severity experiment part II												
Treatments and monitoring of leaf health and longevity			■				■					
Evaluation of changes in nutrient and carbohydrate reserves				■				■				
Assessment of changes to return bloom and shoot vigor					■	■			■	■		
Objective 3												
Field Days							■					
Fruit Matter Publications								■	■	■	■	■
Share updates at winter meetings				■				■				■
Extension publications								■	■	■	■	■
Economic Analysis				■				■			■	■

Plans for 2024

Developing extension material including case studies, Fruit Matters postings and presentations to industry.

Finishing off evaluations of return bloom, yields, and carbohydrate and N content for buds and branches.

ROI analysis on using deleafing in a range of cultivars in commercial orchards

Project/Proposal Title: Measuring storage reserves to assess severity of biennial bearing

Report Type: Continuing Report

Primary PI: **Lee Kalcsits**
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Cooperators:

Project Duration: 1 Year

Total Project Request for Year 1 Funding: $ 58,927
Other related/associated funding sources: None

Budget 1
Primary PI: Lee Kalcsits
Organization Name: Washington State University
Contract Administrator: Anastasia Mondy
Telephone: 509-335-4563
Contract administrator email address: arcgrants@wsu.edu
Station Manager/Supervisor: Chad Kruger
Station manager/supervisor email address: cekruger@wsu.edu

Item	2023	2024
Salaries[1]	$29,315	
Benefits[2]	$13,062	
Wages[3]	$6,500	
Benefits[4]	$650	
Equipment	$0	

Supplies[5]	$8,900	
Travel[6]	$500	
Miscellaneous[7]	$0	
Plot Fees	$0	
Total	$58,927	0

Footnotes:

[1] Salary is requested for 16.7% of a post-doc and 50% of a research intern (technician).

[2] Benefits are calculated at 39.9% for the post-doc and 46.5% for the research intern (technician).

[3] Wages are for covering summer salary for the graduate assistant

[4] Benefits are calculated at 10% for summer graduate students

[5] Supplies are for field and lab consumables to conduct applied experiments for objective 1 and 2.

[6] Travel funds are requested for frequent travel to the Sunrise research orchard for PIs and personnel.

OBJECTIVES

1. To establish baseline levels of carbohydrate concentrations in storage organs of apple trees that are in different biennial bearing cycles.
2. To establish cost-effective and industry-adoptable methods for measuring non-structural carbohydrates in apple trees.

SIGNIFICANT FINDINGS

Starch and total non-structural carbohydrate contents were higher for all plant tissues of trees with high crop loads.

Rootstocks vary in their capacity to store non-structural carbohydrates in spurs, apical buds and terminal shoots.

For 'Honeycrisp', there was a positive relationship between crop loads and non-structural carbohydrates in dormant aboveground tissues. This means that trees had lower crop loads had less carbohydrates and higher crop loads had more stored carbohydrates.

We are trying to understand whether these patterns are Honeycrisp specific or whether other cultivars like 'Gala' also still have these patterns. This analysis will be done on samples collected during the 2023/2024 fall/winter season.

METHODS

The trees used for this study were located at the WSU-Sunrise experimental orchard in Rock Island, WA (47°18′ 35.6″ N 120°03′ 59.5″ W). Trees of 'Honeycrisp' grafted on 4 different rootstocks (G.41, G.890, M.9-T337, and B.9) were planted in 2016 at a spacing of 0.9m between trees and 3.6m between rows trained as a slender spindle system. The tree rows were 55m long and oriented north to south. The site is characterized by shallow sandy loam soil. Due to previous experimental treatments on these trees (Valverdi & Kalcsits, 2021), the orchard is variable and has a high incidence of biennial bearing.

The experiment was a completely randomized design with rootstock as a factor. There were four rootstocks and five plots per rootstock, each plot consisting of ten trees. In 2022, trees in their "on" year and "off" year were identified. One "on" tree and one "off" tree was selected per plot, resulting in five "on" and five "off" trees per rootstock, or twenty "on" and twenty "off" trees total. Five branches, ranging from the bottom to the top of the canopy and representative of the overall tree, were tagged for measurements. Fruits were harvested on 12 September 2022. Crop load was determined for each flagged branch with yield and fruit number. Total yield and crop load for each tree were also measured.

Trunk cross-sectional area (TCSA, cm^2) was calculated from two diameter measurements perpendicular to one another on 25 October 2022 approximately 10cm above the graft union with calipers (Mitutoyo, Sakado, Japan). Two perpendicular measurements were taken per tree to account for irregularities in trunk size. Branch cross-sectional area was measured approximately 15mm from the point of origin on the main trunk. A subsample of leaves were taken from each branch and then total number of leaves were counted to estimate total leaf area per branch.

On 7 December 2022, one spur borne on 2021-wood and one apical bud was collected per branch per tree. One terminal shoot (2022 growth) was also collected per tree. On 12 January 2023, one bourse shoot was collected from each tree. The five spurs, five buds, one bourse shoots and one terminal shoot from each tree were analyzed separately for non-structural carbohydrate content. There was a total of 40 spur samples (1 sample per tree), 40 apical bud samples, 40 terminal shoot samples and 40 bourse shoot samples for the 2022 sampling. Samples were microwaved for 180 seconds at 800W within two hours of collection to deactivate NSC-modifying enzymes (Quentin et al., 2015). Protocols from Landhäusser et al. (2018) were followed for non-structural carbohydrate extraction. Soluble sugars were determined analyzed using an anthrone-sulfuric acid assay (Leyva et al., 2008) and starch was determined using a glucose hexokinase-6-phosphate (GHK) enzymatic assay (Landhäusser et al., 2018). Samples were dried in a freeze-dryer (Labconco, Kansas City, MO). Samples were then homogenized with a Powergen High Throughput Homogenizer (Fisher Scientific, Waltham, MA). Samples were placed in 80% hot ethanol (EtOH) at 90°C for 10 minutes. Quantification of soluble sugars and starch was performed by enzyme with absorbance in a multi-detection microplate reader (Bio-Tek Instruments, Winooski, VT).

In 2023, 'Gala' was added to sampling procedures. Unexpected results in 2022 resulted in another cultivar being added to monitor leaf functionality and carbohydrate assimilation throughout the season. Ten leaves were sampled per tree on 26 May 2023 and repeated weekly until 8 November, when leaf senescence occurred. Five "on" trees and five "off" trees were sampled per cultivar. Starting on 6 July, an LI-600 Poromoter/Fluorometer (LI-COR, Lincoln, NE) was used to measure stomatal conductance, fluorescence, and leaf temperature each week. Spurs were collected on 24 August and repeated weekly until 8 November, when sampling frequency switched to monthly. Five representative spurs of varying ages were collected per tree. Total yield and trunk cross-sectional area were used to calculate crop load in 2023 for all trees included in the sampling. Sampling methods from 2022 were replicated on 30 November 2023 for the 'Honeycrisp' and 'Gala' trees used in 2023.

RESULTS AND DISCUSSION

Starch content was significantly higher in all sampled tissues for trees following their "on" year when crop loads were high (Figure 1). When nonstructural carbohydrates were studied as a whole, starch and sugar contents were still significantly greater for trees with high crop loads (Figure 2). This is opposite to what was found in previous work, with "on" trees (high crop loads) expected to have lower carbohydrate content in buds and tissues. Spur and terminal shoots corresponded more closely to return bloom the following year than apical buds.

Table 1. Starch content (% d.w.) (±SEM; N=5) in spurs, apical buds, and terminal shoots sampled during Winter 2022/2023 for Honeycrisp trees grafted onto Bud.9, G.41, M.9 T337, and G.890 rootstocks.

Rootstock	Biennial	Spur (% d.w)	Apical (% d.w)	Terminal (% d.w)
Bud.9	Off	1.9 ± 0.52	1.8 ± 0.26	4.5 ± 0.19
	On	4.7 ± 0.69	3.8 ± 0.22	6.3 ± 0.37
G.41	Off	1.3 ± 0.34	2.2 ± 0.20	4.8 ± 0.31
	On	3.3 ± 0.70	3.3 ± 0.57	5.4 ± 0.51
M.9T337	Off	2.3 ± 0.63	2.7 ± 0.39	4.9 ± 0.36
	On	4.5 ± 0.51	4.4 ± 0.24	6.6 ± 0.53
G.890	Off	2.7 ± 0.29	2.1 ± 0.18	4.6 ± 0.18
	On	3.7 ± 0.45	2.7 ± 0.40	6.0 ± 0.40
Rootstock		0.16	0.01	0.39
Biennial		<0.0001	<0.0001	<0.0001
R x B		0.46	0.18	0.45

Table 2. Total non-structural carbohydrate content (% d.w.) (±SEM; N=5) in spurs, apical buds, and terminal shoots sampled during Winter 2022/2023 for Honeycrisp trees grafted onto Bud.9, G.41, M.9 T337, and G.890 rootstocks.

Rootstock	Biennial	Spur (% d.w)	Apical (% d.w)	Terminal (% d.w)
Bud.9	Off	6.8 ± 0.38	6.6 ± 0.74	5.8 ± 0.47
	On	7.5 ± 0.20	6.9 ± 0.74	6.9 ± 0.50
G.41	Off	7.2 ± 0.25	6.7 ± 1.00	5.8 ± 0.15
	On	7.2 ± 0.44	6.4 ± 0.90	6.2 ± 0.31
M.9T337	Off	6.9 ± 0.25	6.5 ± 0.85	6.4 ± 0.58
	On	7.8 ± 0.43	7.1 ± 0.83	7.5 ± 0.87
G.890	Off	6.8 ± 0.27	6.4 ± 1.04	5.8 ± 0.18
	On	6.6 ± 0.21	6.6 ± 0.59	5.4 ± 0.48
Rootstock		0.23	0.98	0.05
Biennial		0.12	0.74	0.13
R x B		0.26	0.95	0.36

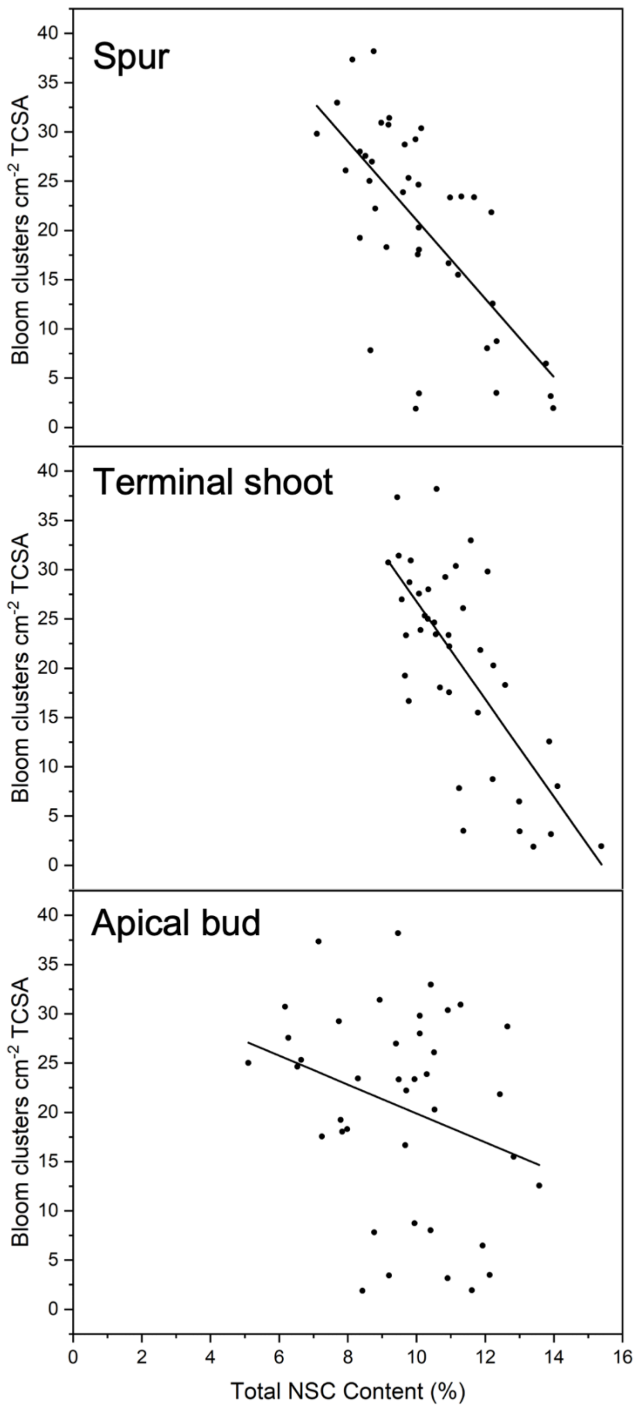

Figure 1. Relationship between 2023 blossom counts (blossom clusters cm^{-2} TCSA) and total non-structural carbohydrate content (NSC) for spurs (top), terminal shoots (middle), and apical buds (bottom) for Honeycrisp trees sampled during the 2022/2023 winter (N=40).

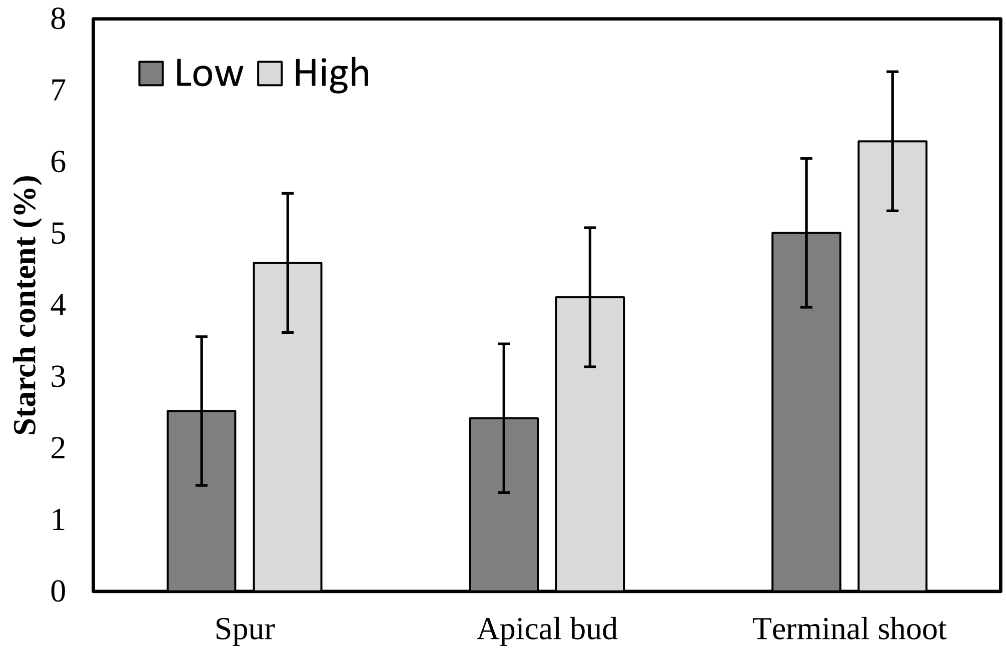

Fig. 2. Starch content (%) of spur, apical bud, and terminal shoots for 'Honeycrisp' trees with low (<4 fruit cm^{-2} TCSA) and high crop loads (>7 fruit cm^{-2} TCSA).

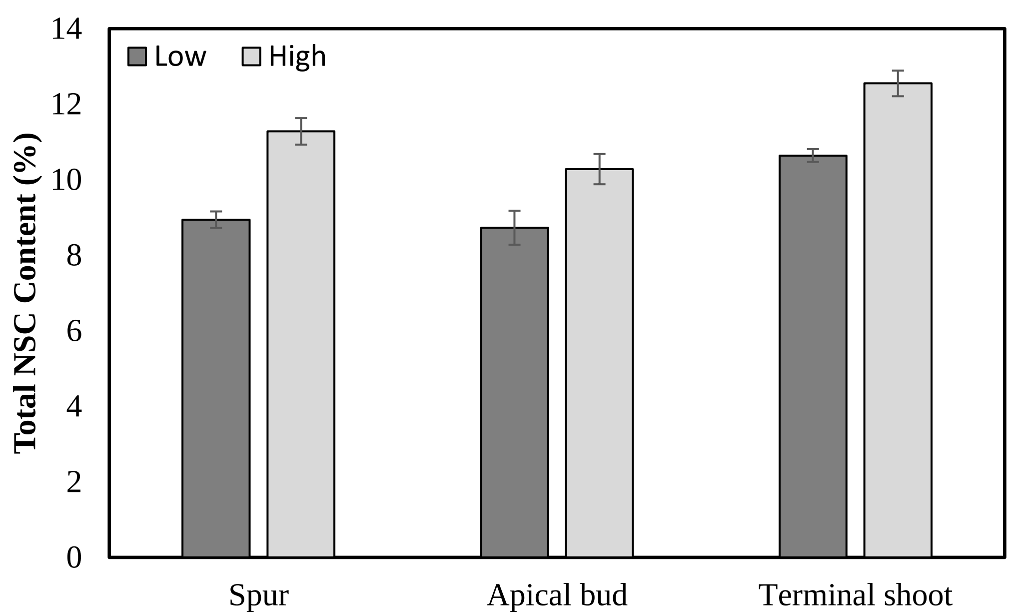

Fig. 3. Total non-structural carbohydrate (NSC) content (%) of spur, apical bud, and terminal shoots for 'Honeycrisp' trees with low (<4 fruit cm^{-2} TCSA) and high crop loads (>7 fruit cm^{-2} TCSA).

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Crop load and subsequent bloom often form an inverse relationship, with a high crop load leading to less bloom the following year. Our data reflects this trend well (Fig. 3), although some outliers did occur, with trees having two years of heavy fruiting or two years of little to no fruiting. Crop load is not the sole factor in return bloom, although Fig. 3 indicates it has a significant effect. Carbohydrate concentrations in buds and plant tissues may also play a role in flower bud initiation. A comparison of these two factors could help understand flower bud initiation and carbohydrate assimilation in trees with more clarity.

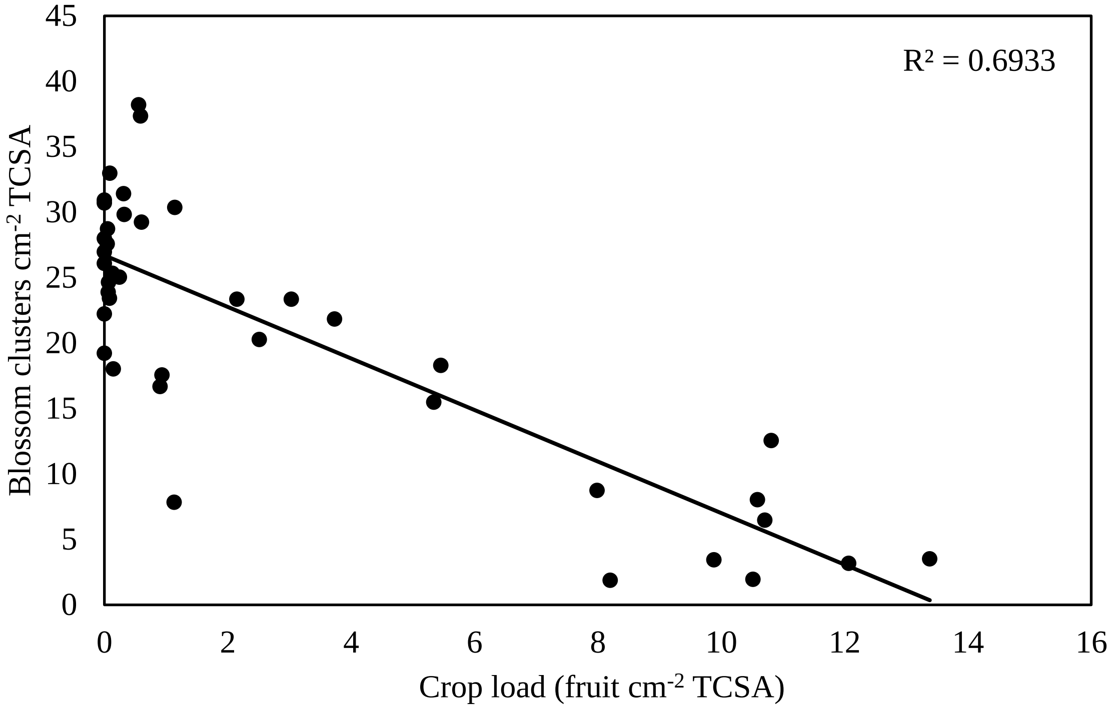

Fig. 4. Linear relationship between 2022 crop load (fruit cm^{-2} TCSA) and 2023 bloom (blossom cluster cm^{-2} TCSA) for 'Honeycrisp'.

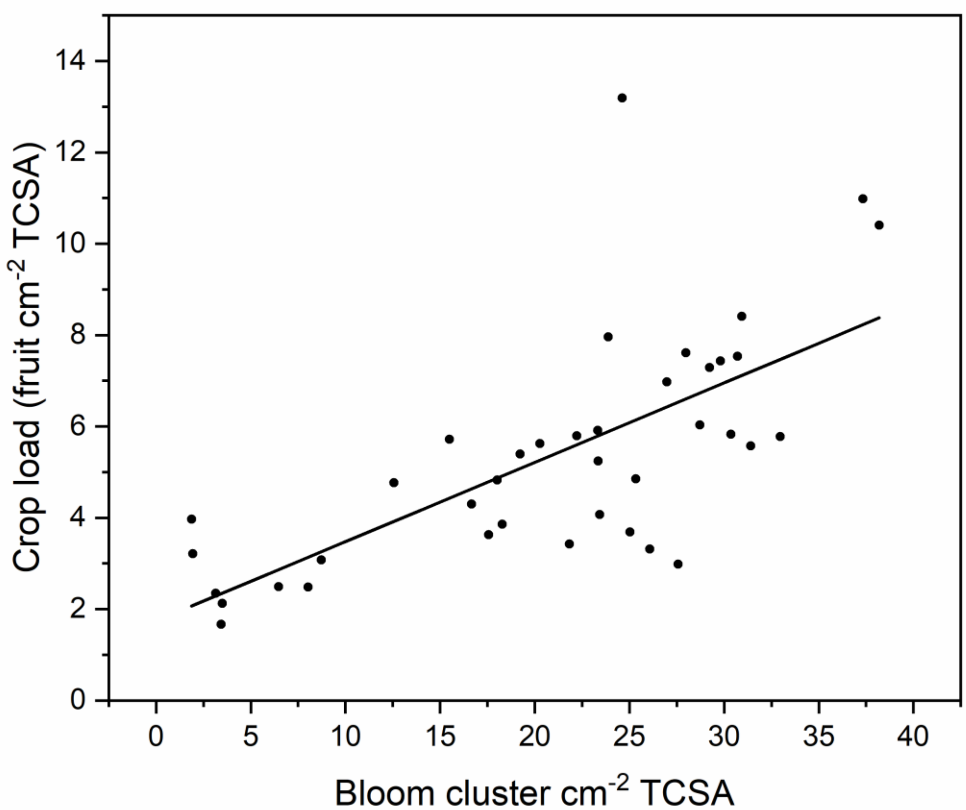

Figure 5. Linear relationship between bloom cluster counts per cm^2 TCSA compared to final fruit counts per cm^2 TCSA for Honeycrisp trees in 2023.

CONTINUING PLANS

We sampled buds from two cultivars this year (‘Honeycrisp’ and ‘Gala’) to test for variation in trees with high or low crop loads. We also expanded our sampling times to look at leaf storage and in-season spur storage as well as during the winter as originally described in the proposal.

Treatments have been applied to trees to alter biennial bearing effects and trees will be monitored for effects, especially with a return bloom next year. This research is part of a grant proposal submitted to the USDA by Thiago Campbell.

Thiago Campbell will work to develop Extension material that can be utilized by growers affected by biennial bearing.

We anticipate a peer-reviewed publication from this research in addition to presenting this research at meetings.

Project Title: Phase 3 Evaluation of WSU Apple Breeding Selections

Report Type: Continuing Project Report

Primary PI: **Manoella Mendoza**
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Cooperators: Agrofresh Inc., Legacy Fruit, Columbia Fruit. Growers: Stemilt Inc. and Allan Brothers. Apple Breeding Program Advisory Committee: Aylin Moreno (Taggares Fruit), , Paul Cathcart (Columbia Reach), Dale Goldy (Gold Crown), Dave Gleason (Kershaw), Dena Ybarra (WTFRC commissioner), Jeff Cleveringa (Columbia Fruit), Jeff LaPorte (Chelan Fruit), Lauren Gonzalez (GS Long), Sarah Franco (Allan Bros.), Suzanne Bishop (Allan Bros.), Tim Welsh (Columbia Fruit), Rob Blakey (Stemilt), Hannah Walters (Stemilt), Anne Morrell (Columbia Fruit), Erick Smith (Taggares Fruit Company), Craig Anderson (Gilbert Orchards), Matt Miles (WTFRC commissioner), Technical consultants: Stefano Musacchi, Carolina Torres, Bernardita Sallato, Lee Kalcsits

Project Duration: 3 Years

Total Project Request for Year 1 Funding: $ 53,478.00
Total Project Request for Year 2 Funding: $ 56,127.00
Total Project Request for Year 3 Funding: $ 59,791.30

Other related/associated funding sources: Stemilt and Allan Brothers provide farm crew assistance for pruning, thinning, and field maintenance, Agrofresh donates Smartfresh and Stemilt assists with SmartFresh and postharvest fungicide application. Columbia Fruit assists with packing line assessment.

Agency: WSU apple breeding program royalties
Amount awarded: ~$500,000 per year (2023-2026)
Notes: Funding supports all other aspects of the apple breeding program (Phase 0 to Phase 2), including all program staff, a full-time farmworker position at WSU Columbia View orchard, and graduate student assistantships. Funds to supplement Phase 3 evaluations are provided as necessary for consumer tastings, equipment, and consumables.

Funding Duration: 2021-2024

Amount awarded: $220,045
Agency Name: WSDA Specialty Crop Block Grant Program
Notes: Establishing rootstock and production system recommendations for new Washington apple selection (WSU 'L') Evans, Musacchi, Sallato. This project will collect complementary information for an elite P3 selection that will be released.

Primary PI: Manoella Mendoza
Organization Name: WA Tree Fruit Research Commission
Contract Administrator: Paige Beuhler
Telephone: (509) 665-8271
Contract administrator email address: paigeb@treefruitresearch.com
Station Manager/Supervisor: Ines Hanrahan
Station manager/supervisor email address: hanrahan@treefruitresearch.com

Item	(Type year of project start date here)	(Type year start date of year 2 here if relevant)	(Type year start date of year 3 here if relevant)
Salaries			
Benefits			
Wages	$28,523.00	$30,079.00	$32,259.00
Benefits	$11,409.00	$12,120.00	$13,204.00
RCA Room Rental	$12,746.00	$13,128.00	$13,528.00
Shipping			
Supplies	$500.00	$500.00	$500.00
Travel	$300.00	$300.00	$300.00
Plot Fees			
Miscellaneous			
Total	$53,478.00	$56,127.00	$59,791.00

Footnotes:

Wages/Benefits: calculated based on expected staff wage adjustments
RCA room rentals: 2 rooms, including room operation costs and warehouse fees, adjusted yearly.
Supplies: consumables for fruit quality analysis (KOH, distilled water, iodine, etc.)
Travel: in-state travel

Justification

New and improved apple varieties are essential to enhance a successful Washington apple industry. The goal of the WSU apple breeding program (WABP) is to produce a portfolio of new, improved, unique varieties specially selected for the environment of central Washington and available to Washington's growers. The development of improved apple varieties leads to *sustainable production and enhanced postharvest efficiency to promote sustainability and long-term economic viability by increasing apple packouts*.

Currently, five selections are planted in three grower-collaborator sites. The advantage of this arrangement is the ability to observe the growth habits and characteristics of advanced selections in a commercial production setting. Having the WTFRC manage P3 provides an independent and industry-oriented evaluation that, with the input of industry representatives in the apple breeding program advisory committee (BPAC), ensures that the data collected and information provided align with stakeholders' interests. The project results, including single pick potential, harvest window, storability, and resistance to biotic and abiotic stress, are presented to the BPAC annually. Field visit opportunities are included throughout each season.

Objectives

1. Evaluate and determine the commercial potential of advanced selections of the WABP

Significant Findings

1. Q, R, and S grew to reach the top wire within the first year on both sites
2. Selection Q has good firmness retention, losing only about 2 lb. after long-term storage
3. Selection P has good shelf-life potential, but bi-annual bearing and fruit size are a concern
4. The clusters of WA 64, also known as selection L, are mostly singles and doubles, but there are differences between sites
5. WA 64 performed well in packing line assessments, presenting high to medium gloss and low disorder incidence

Methods

<u>Bud and Bloom observation</u>: Field observations start as the trees begin to bloom, occurring at least twice a week, considering the weather pattern and its influence on blooming. Full bloom date is determined for each Phase 3 (P3) selection and the standard varieties near the P3 plots. Starting at this stage, every field visit includes general observations on disease incidence, tree growth habits, and health. Standard management practices (rodent activity monitoring, powdery mildew sprays, row mowing, etc.) are conducted and discussed with field managers. Pest and disease incidence and monitoring are documented during the entire season.

<u>Fruitlet development and pre-harvest:</u> Field activities for this stage start after June drop. Orchard visits occur at least every other week until a month before the predicted harvest. Observations on fruit sets

and self-thinning are documented. The orchard crew will perform hand-thinning and summer pruning when appropriate, as if the selections were being produced commercially. A specific plan will be made for each selection by the PI, with consultation from the grower, BPAC members, and other specialists (i.e., Stefano Musacchi).

Harvest: Starch degradation, color, and background color development are assessed to determine the harvest date. Once the harvest date is established, the harvest is conducted in one to three picks, depending on selection and crop load. The selections are typically strip-picked. Apples are harvested using picking bags and placed in blue crates (30 lb.). The apples with cracks, insect damage, chemical damage, splits, severe sunburn damage, bitter pit, and bird peck are classified as culls in the field. These apples are collected during harvest and weighed separately; the reason for cullage is assessed on individual fruit, and the data is used to calculate the percentage of fruit loss in the field.

The storage samples are weighed in the field and separated into two or three storage conditions: Refrigerated air (RA, 33°F), RA 37°F, and controlled atmosphere (CA, 34°F 1% CO_2, 2% O_2), with and without 1-MCP treatment. This fruit is drenched with postharvest fungicide at a Stemilt drencher location and stored at the Research CA rooms at Stemilt. Stemilt administers the 1-MCP treatment within one week of harvest.

Quality at harvest is assessed within 48 hours of harvest. Evaluation includes starch degradation (Cornell 1-8), firmness (lb.), soluble solids (% Brix), titratable acidity (% m.a.), color (% of red coverage), background color, size (in.), weight (gr.), DA index, and presence/absence of internal and external defects/disorders.

Post-harvest: Quality assessment takes place after 3, 6, and 8 months of storage for apples in RA and 6 and 9 months for apples in CA. Apples with and without 1-MCP treatment are evaluated at the same time points. Quality analysis assessment is conducted after seven days at room temperature to determine the potential quality for consumers after shipping, handling, and purchase. Box size distribution data will be generated from individual fruit weights. Fruit will be distributed at meetings and events as available; industry taste panel and informal consumer acceptance evaluation data will be collected.

Advanced Phase 3

When a selection is considered a good contender for commercialization (typically after at least four years in P3), it will receive the following additional evaluations:

- commercial packing line handling: glossiness and bruising will be evaluated on the same day, after 7 days in RA storage, and 7 days in RA + 7 days at room temperature.
- formal consumer taste panels: coordinated with Kate Evans (co-PI and WSU apple breeder) and performed in locations or events with diverse consumer demographics (i.e., Spokane mall, Apple Blossom Festival). The protocol utilized was generated by Carolyn Ross (Professor and Director of the Sensory Evaluation Facility, WSU Pullman).

Results and Discussion

Selections Q, R, and S

Q: Cripps Pink x Honeycrisp

R: Cripps Pink x WSU 3

S: Honeycrisp x WA 2

These three selections were top worked in Quincy and Sagemoor in 2020 and 2021, respectively. Most of the trees reached the top wire within one year. Tree growth is similar on both sites in the first year, with some blind wood in the middle section and heavily cropped treetop. Both locations were defruited in the first year and hand-thinned in the following year. At the Quincy site in 2023, the farm crew performed hedging and summer pruning in accordance with Stefano Musacchi's recommendations.

Fruit was harvested in 2022 from Quincy and from both sites in 2023. Information provided in the following section is based on the 2022 season only. All ABP selections and apple varieties harvested by the WTFRC crew generally had less color and higher bruising incidence during this season. Also, we observed stagnation of starch degradation for a few weeks followed by rapid depletion, which might have resulted in the fruit being picked at advanced maturity. Evaluations from the 2023 season are ongoing.

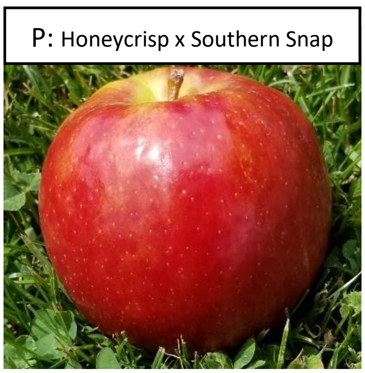
Specific characteristics

Q: Large to medium size fruit, with box size peaking at 72 (range 72-88). Fruit typically has a short stem, and it was considered easy to pick. This selection was harvested in three consecutive picks, with starch averages at 4.3, 4.7, and 5.8. In 2022, it bruised relatively easily. Little variation was observed for firmness over time. At harvest it ranged between 15.3 and 16.8 lb, with a maximum loss averaging 2lb. Incidences of bitter pit, soft scald, superficial scald, and split were below 1% per pick. Internal browning incidence was only observed in fruit stored in CA.

R: This selection's top three box sizes were 150, 113, and 125, respectively. The elongated stems made the fruit more difficult to detach during harvest. Fruit presented high color variability during harvest. No pre-harvest drop was observed. Internal browning was observed on all three picks after 6 months in storage. Split incidence increased with maturity. Bitter pit and soft scald incidence were below 2%. No superficial scald was found. Greasiness was high, especially for fruit stored for 6 and 8 months in RA storage.

S: The box size distribution peaked at 113. There was a wide variation in starch degradation at harvest (Cornell 2 to 7). This selection bruised relatively easily. Overall, greasiness was low, with a higher incidence on fruit stored for 6 months in RA. Most lots had internal browning and internal cavities. Stem puncture and soft scald were below 2%. Split incidence was higher in the last pick (2.5%). No bitter pit or superficial scald was found.

Selection P

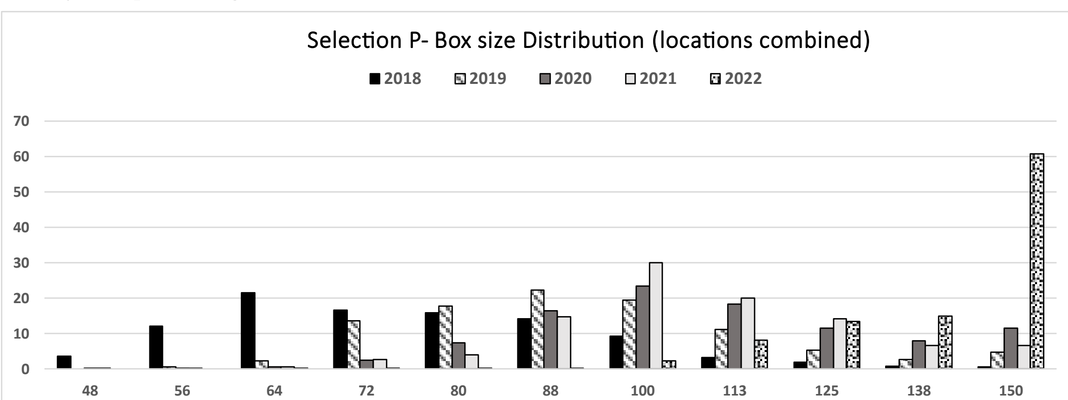


This selection was grafted in Quincy and Prosser in 2017 and 2018, respectively. It is a bicolored apple that develops good red color coverage on a fruiting wall (Prosser) or spindle system (Quincy). The apples are crisp and juicy and have a unique tart-sweet flavor. It has low field cullage and a long shelf-life.

Fruit is typically harvested in two or three weekly consecutive picks from mid-September to early October. Firmness at harvest is around 18 lb. with good firmness retention throughout storage. Soluble solids concentration and titratable acidity at harvest are 14 (%brix) and 0.9 (% m.a.), respectively.

Size distribution is variable and affected by crop load. Most apples in the first year belonged to the 64 to 80 box size (Figure 1). When combining years and locations, 62% of the fruit was classified in the 88 to 113 size distribution range. Trees with moderate crop load typically produce apples peaking in the 80 to 100 box size range.

Figure 1. Box size distribution of selection P for Quincy and Prosser combined from 2019 to 2022. In 2018, only Quincy was producing fruit.



Over the past three years, hand-thinning alone has not been aggressive enough to adjust high crop load, resulting in small fruit (box size 100-150) on heavy-cropped trees. Poor thinning in Quincy in 2022 resulted in the majority of the fruit being classified as size 150. Although alternate bearing is more prominent in Prosser (Table 1.), it can be observed on both sites, with heavy-cropped trees producing very little crop in the following year.

Table 1. The total number of apples evaluated for fruit size distribution by location and year.

Location	2018	2019	2020	2021	2022
Prosser	0	202	1264	377	2906
Quincy	1289	1758	3768	2860	7118
Total	1289	1960	5032	3237	10024

WA 64

L: Honeycrisp x Cripps Pink

Formerly known as selection L, WA 64 is in the commercial release process. Commercial planting availability and the first commercial harvest are predicted for 2026 and 2029, respectively.

This selection was grafted in 2015 on both Prosser and Quincy locations, on M9.337 and G.41 rootstocks, respectively. Tree structure (type III) and harvest timing are similar to cv. Golden Delicious, with bloom time similar to cv. Gala in Quincy.

WA 64 is a bicolored symmetrical apple that colors well when exposed to sunlight, typically achieving 50% to 70% red/pink blush with a yellow background. It is slow to brown, easy to pick, and pre-harvest drops have not been observed. Its unique trait is high firmness retention during storage, which, combined with the low incidence of disorders and diseases in the field and during storage, grants this selection a long shelf-life potential.

This selection self-thinned to singles and doubles in the first few years of production, but in the past two years developed more triples and quadruples. Following BPAC advice, quantification of the fruit set at the bloom and fruitlet stages to assess self-thinning potential, was initiated in 2023. Sections of 30 trees in each location were marked with ribbons during bloom, and the number of flower clusters was recorded. The number of fruitlet clusters and cluster classification (singles, doubles, triples, and quadruples or higher) were recorded after the June drop but before hand-thinning.

The tree sections were selected to have 50 to 60 bloom clusters (table 2.). From bloom to fruitlet, Prosser and Quincy had an average loss of 12 and 33 clusters, respectively. Quincy typically has heavier-cropped trees in comparison to Prosser. The incidence of singles and doubles was higher than the other categories in both locations. While Quincy had more singles than Prosser, the latter had a more evenly distributed crop load between categories. The data collection will be repeated in both sites in the Spring of 2024 to account for year seasonality.

Table 2. Average (avg.) and standard deviation (sd) of flower and fruitlet cluster count for WA 64 in Prosser and Quincy. Fruitlet count was performed after June drop before hand-thinning. Cluster loss was calculated as a decrease in the number of fruitlet clusters related to bloom clusters. Fruit clusters were classified in accordance with the total number of fruitlets. Clusters with more than four fruitlets are included in the quadruples category. Fruitlet classification is presented as a percentage of the total number of fruitlet clusters.

	Flower cluster	Fruitlet cluster	Cluster loss	Fruitlet classification (avg. ± sd)			
Location	**(avg. ± sd)**	**(avg. ± sd)**	**(avg. ± sd)**	**single**	**double**	**triples**	**quadruples +**
Prosser	53 ± 3	41 ± 5	12 ± 4	40% ± 12	27% ± 8	21% ± 7	12% ± 7
Quincy	56 ± 5	23 ± 5	33 ± 7	68% ± 16	22% ± 13	8% ± 6	2% ± 3

In addition to fruit collected for quality analysis, in 2022, two bins of WA 64 were harvested from Quincy for packing line handling evaluations, including glossiness, bruising, stem puncture, decay, storage disorders, and fruit flavor. The evaluations occurred in March and October of 2023 using fruit stored in RA and CA, respectively.

Glossiness classification was similar to the preliminary study conducted in 2020, with most fruits presenting medium to high gloss and a decrease in glossiness over time, especially after 1 week at room temperature. There was a high incidence of carnauba stain and scuffing on both packing line runs. Changes in the procedures were discussed with the collaborator and will be adopted in the next packing line assessment with fruit harvested in the 2023 season.

Incidence of disorder and decay were below 5% on both packing line assessments. Lenticel breakdown was observed in fruit stored in CA the day after the packing line run (2%) and slightly increased during the three weeks of evaluation (2.7%). Fruit stored in RA did not develop lenticel breakdown.

The packing line assessment will be repeated with fruit harvested in 2023 to investigate further bruising susceptibility, glossiness retention, lenticel breakdown, and the need for stem clipping. Two bins of apples were harvested from each site, and one bin per site was stem clipped. One set (stem clip vs. non-stem clipped) was stored in RA, and the other in CA. In addition, we partnered with Storage Control Systems to determine the low oxygen limit of WA 64, which could be a good storage alternative for organic growers. We are also determining CO_2 sensitivity with the assistance of the USDA-ARS.

WSDA Specialty Crop Block Grant funding was awarded to Co-PI Evans (with Musacchi and Sallato) to establish rootstock and production system recommendations for this selection. A research block (Musacchi) and a demonstration block (Sallato) were planted in the spring of 2022. Each includes four different rootstocks (G.41, G. 969, G. 890, and B. 9) and both spindle and bi-axis trees. WABP BPAC members have already visited these plantings and will continue to be invited to future grower field events.

Title: Towards next generation maturity indices: apple biomarker discovery AP-22-101A

Report Type: Continuing Project Report - Year 1

Primary PI: Dr. Loren Honaas
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Cooperators: AllanBrothers Inc., Stemilt LLC.

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $ 266,267
Total Project Request for Year 2 Funding: $ 155,303
Total Project Request for Year 3 Funding: $ 160,510

Other related/associated funding sources:
Funding Duration: annual congressional appropriation
Amount: $85,000
Agency Name: USDA ARS
Notes: 3-year total = $255,000: Personnel $180,000, Consumables/Supplies $30,000, Equipment (including computational resources): $45,000

Funding Duration: The funding source has expired but resources are still available.
Amount: $86,000
Agency Name: WSU Ficklin Start-Up Funds
Notes: These funds were used to purchase high-performance computing resources on WSU's Kamiak computing cluster. These resources will provide the computing power necessary for model development.

Funding Duration: 2017-2022
Agency Name: US National Science Foundation (NSF) Award #1659300
Amount: $150,000
Notes: A portion of this award was used to fund 600 Terabytes of storage for execution of scientific workflows and storage of results. We will use that infrastructure for this project.

Funding Duration: 2020-2025.
Amount: $100,000
Agency Name: Auburn Harkess Start-Up Funds
Notes: These funds are being used to purchase molecular genomics reagents and equipment for apple DNA and RNA isolation and sequencing.

Budget 1
Primary PI: Dr. Loren Honaas
Organization Name: USDA ARS TFRL
Contract Administrator: Chuck Meyers & Sharon Blanchard
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Contract administrator email address: chuck.myers@ars.usda.gov, sharon.blanchard@ars.usda.gov
Station Manager/Supervisor: N/A
Station manager/supervisor email address: N/A

Item	**2022**	**2023**	**2024**
Salaries	44,775	45,894	47,042
Benefits	18,806	19,276	19,758
Wages			
Benefits			
Equipment			
Supplies			
Travel			
Miscellaneous			
Plot Fees	5500	5700	5900
Total	69,081	71,410	72,700

Footnotes: Plot fees for WSU SRO blocks that provide validation samples.

Budget 2
Co PI 2: Dr. Stephen Ficklin
Organization Name: WSU Department of Horticulture
Contract Administrator: Anastasia Mondy
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Station Manager/Supervisor: N/A
Station manager/supervisor email address: N/A

Item	2022	2023	2024
Salaries	61509	63969	66528
Benefits	19677	20464	21282
Wages			
Benefits			
Equipment	46,000		
Supplies			
Travel			
Miscellaneous			
Plot Fees			
Total	127,186	84433	87810

Footnotes: Postdoc will be co-advised by all project PIs. Salaries and benefits are estimated to be inflated by 4% per year per WSU guidelines.

Budget 3
Co-PI 3: Dr. Alex Harkess
Organization Name: Auburn University and HudsonAlpha Institute for Biotechnology
Contract Administrator: Mercedes McKoy
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Contract administrator email address: MLF0015@auburn.edu
Station Manager/Supervisor: Optional
Station manager/supervisor email address: Optional

Item	2022	2023	2024
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel			
Miscellaneous	70,000		
Plot Fees			
Total	70,000	0	0

Footnotes: Miscellaneous funds are for RNA-Seq: this is global gene activity analysis of a majority of the total validation samples, estimate analysis of 350 total samples from 45 cultivar/years.

Budget 4 N/A
Co-PI 4: Dr. James Mattheis
Co-PI requests no funding.

Objective:

Develop and improve methods for biomarker discovery.

A. Use novel analytics and modeling approaches to strengthen biomarker discovery approach
B. Generate new global-scale gene activity data from current and new multi-year samples for rapid validation
C. Investigate disagreement between technologies for gene activity estimates to enhance translation to NGMIs

Year 3 goals:

1. **Complete gene activity analysis of additional validation sample sets**
2. **Build prototype NGMI model that:**
    a. **integrates information from other fruit traits**
    b. **uses qPCR data (instead of RNA Seq data) towards a point-of-contact test**
    c. **utilizes new projects data**
3. **Summarize outcomes of commercial prototype NGMI trials emphasizing storage outcomes**

Significant findings/results in the first 18 months:

1. Preliminary analysis of new RNA-Seq data from 276 validation samples
2. Models work with new validation data (includes new cultivars)
3. 2022 and 2023 validation samples in hand, including commercial lots
4. New model concept anchored on physiological maturity
5. Modeling experiment suggests data requirements: 2,500 - 3,000 datasets
6. Model stability analysis reveals model performance differences
7. Gradient boosted trees improve predictions
8. Models built with multiple variables reveal shared genetic factors
9. New genomes improve targeted gene tests

Methods (Significant findings indicated in parentheses)

Analysis of validation samples (Significant findings #1-3)

Starting in 2018, the team has been collecting apple fruit samples near harvest time in order to build a catalog of mini time course test samples - hereafter referred to as the **validation samples**. The purpose is to test our prototype maturity models in real world samples consisting of new years, new orchards, and/or new cultivars. The team had >300 samples in cryogenic storage - 276 were sent to the HudsonAlpha core facility for gene activity analysis (done as described in Hadish *et al., in press*).

Raw data were processed in our custom pipeline (GEMmaker, Hadish *et al.*, 2022). Project meta data was harmonized in order to facilitate testing, setting approximate commercial harvest to T_0. All samples taken before T_0 were assigned negative integers and all those following T_0 were assigned positive integers. This allowed direct comparisons with regard to commercial harvest across the entire project. Gene activity profiles in the validation samples were used to predict harvest date using our prototype maturity index model. Predicted harvest dates were plotted against the actual harvest dates. Root Mean Square Error (RMSE) and Pearson's R^2 were used to assess model performance.

Additional validation samples were obtained, including samples from research blocks and commercial samples from industry partner AllenBros Inc. **Validation sample sets** are collections of fruit from unique cultivar/orchard/years. These sets include fruit for gene activity analysis, full fruit quality analysis, and postharvest storage trials; typically over 600 fruit are analyzed per set, yielding a running total of data for >30,000 individual apple fruits across >50 sample sets. In 2022 we collected 10 new validation sample sets and in 2023 we collected 6 new validation sample sets. The latter includes 3 door sample sets from commercial packing houses that were picked at ostensibly similar maturity, providing us with a testing framework for very fine differences in maturity.

NGMI concept model (Significant finding #4)
Towards an eventual product, we have created an NGMI scale concept that is anchored by fruit physiology. The scale concept is anchored by fruit ripening capacity with "0" set as a milestone for the earliest stages of ethylene perception as a ripening cue. Fruit that has not passed this milestone would be assigned a negative value, and fruit senescence will mark the end of the scale at 100.

Evaluation of model performance as a function of input data (Significant finding #5)
Recent work by the team (Hadish, Honaas and Ficklin, 2023) has explored how plant trait prediction model performance changes as large-scale genomics data is added. To do this, we fetched a massive gene activity data set from the National Center for Biotechnology Information (NCBI): ~75,000 files consisting of millions of data points each for a model plant called *Arabidopsis*. This is perhaps the largest such public data set for a plant and orders of magnitude larger than what we currently have for apple. This plant has a short life cycle of 8-12 weeks, so we decided to model plant age as a proxy for a trait like fruit maturity. We filtered the data files for the most reliable plant age information, leaving just over 5,000 data files. Using a machine learning strategy similar to our apple harvest date prediction models described above, we then built models to estimate plant age based only on gene activity. The predictions were compared to the plant age reported for each of the data files. Root Mean Square Error (RMSE) and Pearson's R^2 were used to assess model performance. This analysis provides information about how more data will impact our NGMI prototype model performance.

In silico *tests of model performance (Significant finding #6)*
We can evaluate our models with computational methods as well, one such test is for stability. This is done by taking random samples of the data over-and-over (called boot-strapping) and re-creating the models with those replicate random data samples. Comparisons of the resulting models can reveal how often the model gives a similar result, that is, we can see how often the models select the same gene activity signatures and whether these signatures are assigned similar weights (i.e. a measure of model importance). In a recent paper (Hadish et al. *in press*) we applied this technique to models that predict fruit firmness. We have applied similar tests to our unpublished harvest date prediction models.

Improved prototype NGMIs (Significant findings #7 & 8)
A key goal of this work is to improve our prototype models by exploring new modeling strategies. One of these approaches builds on what we have by adding steps that boost model performance iteratively - the use of gradient boosted trees. We have applied this approach to our harvest date prediction models, and also to starch clearing prediction models in an effort to integrate these data towards a starch index validation test. That is, a test that leverages prototype NGMIs to check starch ratings and then assign a confidence or trust score.

This approach is rooted in the notion that models for starch clearing prediction and harvest date should look similar, or at least have common gene activity signatures because starch clearing ***is*** a maturity index. We examined our various apple models in a new way based on gene family classifications - this provides a very sensitive approach to find genes that have similar functions for a trait (like fruit maturity), but that may have been shuffled around during evolution and domestication such that a simple 1:1 match is not possible. We applied this strategy to find gene activity signatures for suites of apple genes with similar functions to test if our harvest date prediction models and starch clearing prediction models identified similar sets of genes.

Reproducing gene activity profiles with qPCR (Significant finding #9)
A recently published paper (Waite *et al.*, 2023) included work from the team that advanced our understanding of various gene activity measurement techniques, and importantly, the potential causes of disagreement between them. As reported therein, we made careful comparisons that considered scion genotypes ('Golden Delicious' vs 'Gala' vs 'Honeycrisp') and the quality of reference genome resources ('Golden Delicious' vs 'Gala'). We also explored the way gene activity signals are influenced

by other members of gene families - focusing on how the signal can be amplified (signal combination) or attenuated (signal split between genes).

Results and Discussion

Do prototype NGMIs work? (Significant findings #1-4)

The NGMI prototypes are built around known differences in maturity - we picked fruit multiple times along a time course imposing a contrast of maturity that was centered on commercial harvest. So, if we can predict harvest dates accurately, or order picks in time accurately, we can use prototype NGMIs to estimate fruit maturity. An initial goal was to analyze samples from our cryogenic storage - this is complete: 276 samples were analyzed, producing roughly 50M gene activity measurements per sample. Tests of this "real-world" at-harvest apple sample set are summarized in Figure 1. This analysis includes new years and orchards for cultivars that were used to build the models, and also cultivars that were *not* used to build the models. This indicates that the eventual NGMIs may work across cultivars, and even for new varieties. Importantly, the predictions were often imprecise by a small margin; previous work by the team that explored cultivar-specific correction factors might be useful to account for model imprecision of roughly this scale.

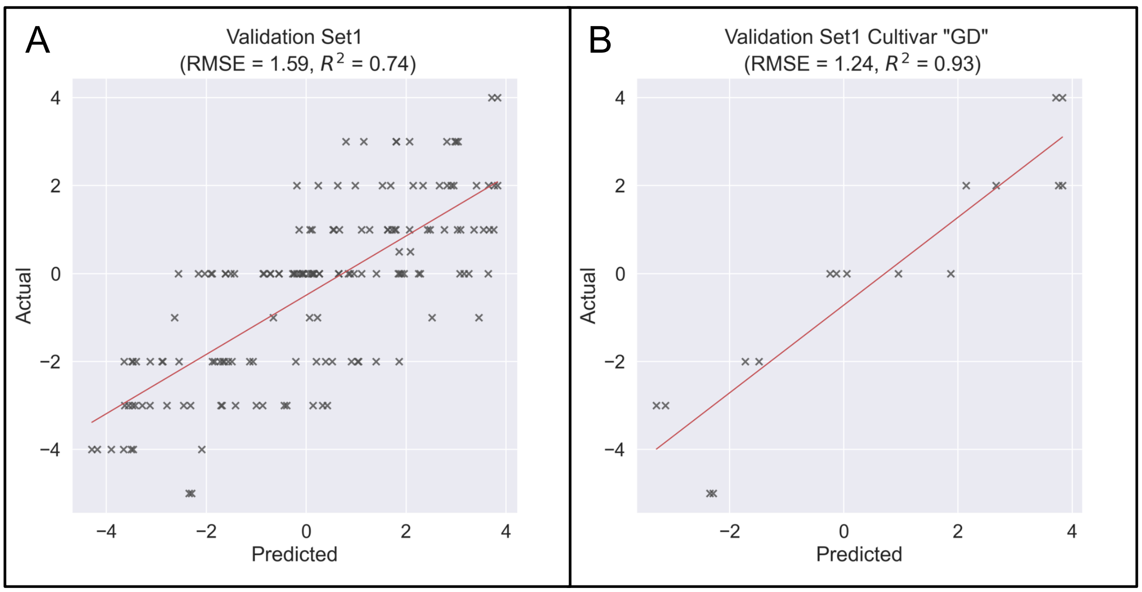


Figure 1. Our initial tests of the validation sample set are reassuringly good. Harvest intervals are weeks, with commercial harvest at T_0. **A** - Harvest date predictions vs actual harvest dates in the full, multi-cultivar validation sample set; these data were *not* used to train the model, and without any cultivar-specific adjustments or other changes, the preliminary model error average is ~11.2 days. **B** – A similar plot, but with a subset of 'Golden Delicious' samples (a cultivar that was not used in model development) showing higher precision with an average error of <9 days. Model development is iterative, and as we add more data and leverage new modeling techniques and strategies for model improvement, the accuracy of harvest time prediction will improve - these results should be seen as the first real world tests of a preliminary model.

Part of our previous validation work (see final report for AP-19-103) was focused on individual genes in our harvest date prediction model. Via manual assessments of test samples we found that we could order picks in time (e.g. early vs late) about 70% of the time, providing a concordant estimate of model performance. These tests provide two important functions: 1) they identify features in the model that need to be pruned or adjusted in order to improve model accuracy, and 2) show us that these tests likely have broad applicability and utility for estimating maturity in pome fruit. As we progress towards a commercial test, we are building concept models like the one shown below (Figure 2). This shows a conceptual scale where we would assign an NGMI value based on physiological milestones, like ethylene production, ripening capacity, and/or senescence. This will provide a scale that accounts for cultivar specific differences, but has wide applicability. This scale could be used in much the same way as starch clearing or pressures – for instance, the NGMI numbers 20-25 could indicate commercial maturity for 'Granny Smith,' while 'WA 38' would have an NGMI of ~50. Despite reliability issues with starch clearing, we could ostensibly classify such a scale into bins that match up with a starch scale of choice.

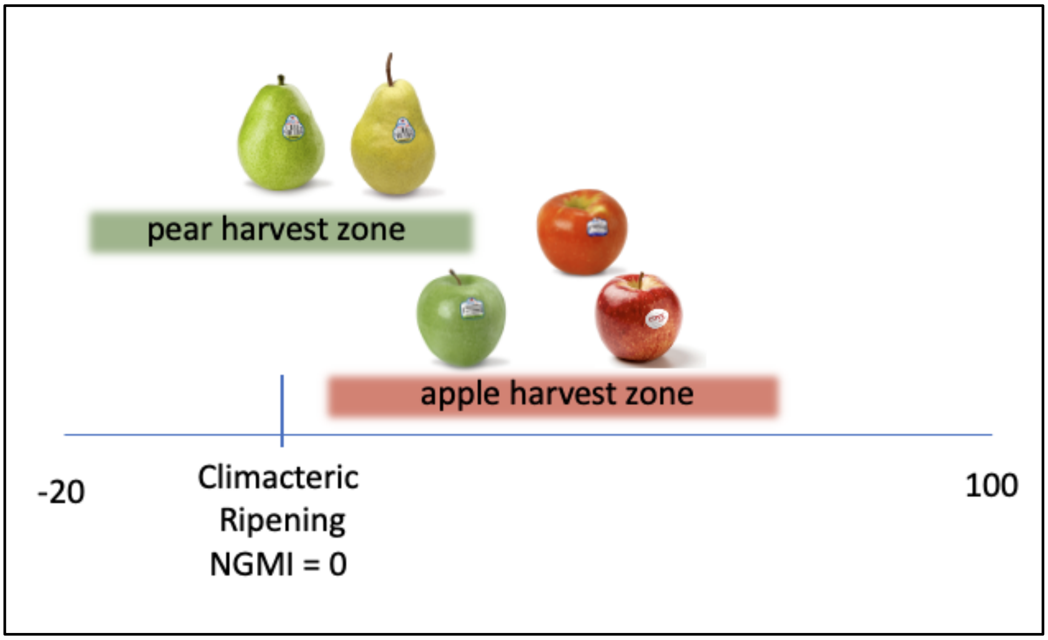


Figure 2. Our NGMI concept anchors the fine-grained scale on physiological milestones. We aim to provide information that can indicate if fruit has passed key milestones, and also one that is useful to make relative comparisons. Eventually, it may be possible to use NGMIs across cultivars and, combined with other tools, make more specific predictions about postharvest quality.

How much more work is needed? (Significant finding #5)

The team is taking a parallel approach on multiple fronts of the NGMI project. With regard to model performance, our recent work has established some rough estimates of the required data for models to stabilize, that is, the point where adding more data provides diminishing returns. These estimates were made based on ~75,000 publicly available data sets (which are technically similar to our apple data sets) from a small annual weed called *Arabidopsis* (Hadish, Honaas and Ficklin, 2023). Among the findings are that for a trait like plant age (conceptually similar to fruit maturity), *Arabidopsis* models are reassuringly good (Figure 3A & B). This level of performance is achieved at ~2,500 - 3,000 genome scale data sets (Figure 3C), which provides us with a rough estimate to maximize model performance while balancing efficient use of resources.

Are models better, and how do we know? (Significant finding #6)

In addition to the labor-intensive tests of our validation samples (>30,000 individually tracked and analyzed fruit over 5 years), we can run *in silico* tests of models that help us understand how well models work after various tweaks and adjustments. Initial tests are based on subsets of the data that are set aside for testing, and that are *not* included in model development. That way, we can take some of the original data and test drive it in the latest model. Typically, in our plots one will see a "train" designation and "test" designation in the plot title; for example see Figure 3 that shows *Arabidopsis* plant age prediction models. The model accuracy is typically better when the data used for training the model is used to make predictions, and then usually worse when we test the model with the data that was set aside for testing (note Root Mean Square Error- RMSE in Figure 3A vs B). Another way is through a random sampling test that is conceptually similar to train/test splits, but instead probes models based on many random samples. In this test, called bootstrapping, we can ask the question "How consistent is the modeling approach when we take random subsamples?" We can visualize this stability in a plot that shows the scores and relative ranks of gene activity signatures across all the replications of this test (Figure 4). Better models select more of the same signatures every time, or conversely, poorer models just model noise and give highly variable results (Figure 4). As we change the data recipe, base modeling functions, and even ways to boost model performance, we run tests such as these. These tests help us build more accurate models, thus making the most out of the data we have; it is another major way to improve model performance, after the addition of data.

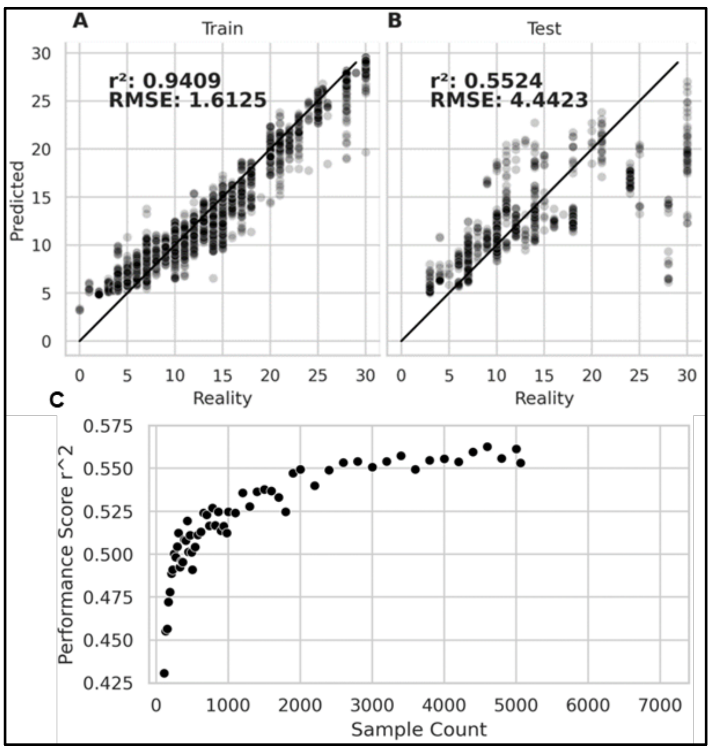


Figure 3. From (Hadish, Honaas and Ficklin, 2023). **Gene activity profiles predict plant age with high accuracy.** Regression of plant age model prediction vs real age. Panel A shows model performance with the training data, Panel B shows model performance with the test data. Panel C shows how the plant age model performance test changes as more data are added. Fruit maturity is a complex trait like plant age, specified by many genetic factors. We hypothesize that we can extrapolate from the results of this experiment to predict how our apple models will perform as a function of input data. The plant age model stabilizes at ~3,000 samples. This does not mean that this many samples are required for the model to work, but instead that adding more data will likely not increase model performance. We think this pattern is generalizable and can be used as a guide regarding target data amounts.

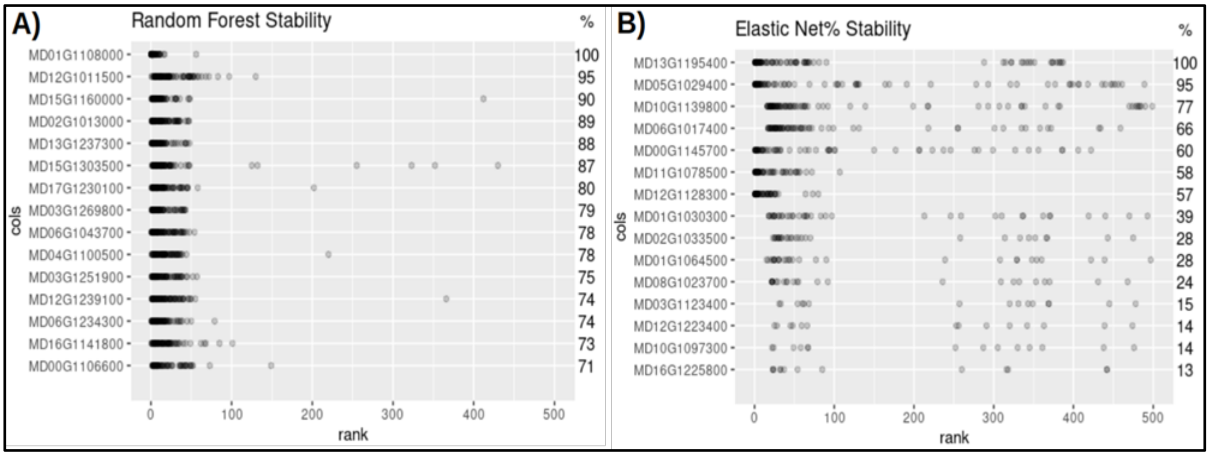


Figure 4: Model stability analyses are done *in silico*, revealing important performance differences. This plot shows such an analysis from recently published work (Hadish et al. *in press*), where we took samples over-and-over, then built a model with each sample. Each time the model selected different sets of genes as being the most important for predicting 'Gala' fruit texture in our dataset - importance was categorized by rank, with 1 being the most important gene. The stability plot shows the importance rank (x-axis) for the top 15 genes in each respective model (y-axis) for each of 100 bootstrapped runs. **A)** In the Random Forest Model each gene was consistently ranked within the top 50 in almost every bootstrapped run. These same 15 genes were identified in ~70% of the bootstrapped runs, the other 30% contained some other genes. **B)** However, in the Elastic Net Model, rankings for each gene varied considerably across each bootstrapped run, most being ranked somewhere within the top 50 to top 500. The top 15 genes identified in the Elastic Net Model were only identified in ~10% of the bootstrapped runs. The stability analysis indicates that, with all other parameters being equal, the Elastic Net Model was not consistently identifying genes predictive of fruit texture.

New and improved models validate our approach (Significant findings #7 & 8)

The latest models have been improved with an additional step called gradient boosting, and we have built several models that predict harvest date and starch clearing patterns. The two key findings are 1) that gradient boosted trees outperform our original random forest trees (with an average of 4.3% higher testing accuracy from the new models), and 2) that predictive gene activity signature panels include similar genes across models and experimental variables (Figure 5). These results support our hypothesis that predictive signatures will consist of signals from similar genomic features across varieties, and it is an endorsement of the comparative genomics approach we are using.

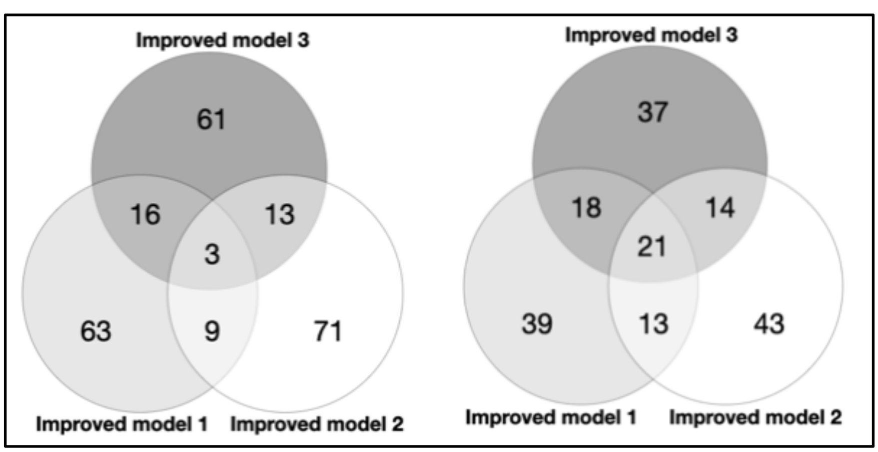


Figure 5. Models that aim to predict starch clearing and harvest week select overlapping sets of genes. Orthogroups are approximate gene family groups. The classification into these groups across cultivar genomes is done with software and genome resources from the team. Left: overlapping orthogroups from Boosted Tree models; Right: overlapping orthogroups from Random Forest models.

Point of contact tests - updates (Significant finding #9)

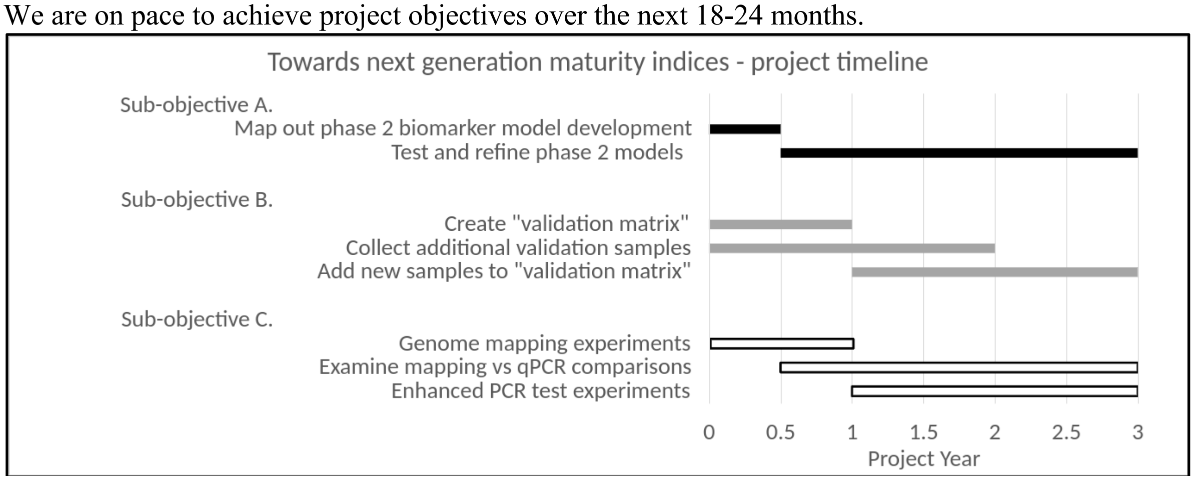
A point of contact test will likely be based on PCR, or a derivative like a loop-mediated isothermal amplification (LAMP) assay. This is a fundamentally different data type from our digital gene activity data (RNA Seq) from which we build models. Previous work form the team (Hargarten *et al.*, 2018; Honaas *et al.*, 2021) and work related to this project (Wafula *et al.*, 2022; Zhang *et al.*, 2022; Waite *et al.*, 2023) has improved our ability to understand variations, and then accurately reproduce gene activity profiles from RNA Seq using qPCR. Almost exclusively the improvements relate the amount of knowledge we have about the genes of interest, not to technological limitations of the respective technologies. We have built 4 top quality reference genomes (3 already published) using previous funding from the WTFRC and PRSC that substantially improves our knowledge of genes within and across cultivars. Other recently published work (Zhang *et al.* 2022) has allowed us to classify genes into family groups at scale (e.g. all current apple genomes and many other Rosaceae), helping us understand signal variations across highly similar genes. Altogether, this moves us closer to models that work on different data types than were used to build the models. *That is, this is a necessary step for commercializable tests for NGMIs that use PCR or LAMP technology.*

Project resources update

We hired Alex Haase as a biological science technician who has handled the logistics of fruit sampling and storage trials for USDA apple fruit samples, with an emphasis on the commercial samples from industry partners. Despite the difficulty we have had to recruit a postdoc to the project, we have made progress by tapping personnel from various places to work on project objectives, including two graduate students from WSU, and postdocs on other projects who have taken on various roles for AP-22-101A. ARS funds were used to purchase a replacement for Honaas' high performance minicluster (which miraculously is still working after 8 years!).

Figure 5. Project Timeline.

We are on pace to achieve project objectives over the next 18-24 months.



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Project Title: Life Cycle Assessment for Apple Production in the Pacific Northwest.

Report Type: Continuing Project Report

Primary PI: **Greg Thoma**
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Other funding sources (see attachment)

Cooperators: Dave Epstein (NHC), Chad Kruger (WSU), Andrew Bierer (USDA ARS), Brent Milne (McDougall & Sons, Inc.), Derek Tweedy (Domex Superfresh), Greg Pickel (G.S. Long), Suzanne Bishop (Allan Brothers F) Marty Matlock (Stakeholder meeting facilitation, Nokose, Inc.)

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $ 144,465
Total Project Request for Year 2 Funding: $ 201,346
Total Project Request for Year 3 Funding: $ 154,000

Budget 1
Primary PI: Greg Thoma
Organization Name: Resilience Services, PLLC.
Contract Administrator: Greg Thoma
Telephone: 479-445-5277
Contract administrator email address: gjthoma@gmail.com

	2023	2024	2025
Salaries (fully burdened) Thoma & Matlock	$32,250	$48,500	$55,000
Benefits			
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies			
Travel	$4,800	$1,000	$1,000
Plot Fees			
Miscellaneous			
Total	$37,050	$49,500	$56,000

2023 expenses to be invoiced:
Matlock salary: $7000
Thoma salary: $20,000
Travel: $0 (we may request re-budgeting of the travel budget to cover some anticipated costs associated with survey deployment expected to be incurred by WSU)

Budget 2

Co PI 2: Hans Blonk
Organization Name: Blonk Sustainability Tools
Contract Administrator: Hans Blonk
Telephone: 0031628848241
Contract administrator email address: hans@blonksustainability.nl

	2023	2024	2025
Salaries	$65,200	$92,400	$92,400
Benefits			
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies			
Travel	$2,500	$2,500	$2,500
Survey Dissemination	$22,000		
Miscellaneous			
Total	$87,200	$94,900	$94,900

Actual costs 2023

Salaries and survey dissemination: € 84,749.75 or $92,377 based on an average exchange rate of 1.09 for 2023

Travel: $0

Budget 3

Co-PI 3: Georgina Yorgey
Organization Name: Washington State University
Contract Administrator:
Telephone:
Contract administrator email address:
Email Address: yorgey@wsu.edu

	2023	**2024**	**2025**
Salaries	$7,641	$7,947	
Benefits	$2680	$2788	
Wages	$28,800	$9318	
Benefits	$2,938	$951	
RCA Room Rental			
Shipping			
Supplies			
Travel	$1123		
Plot Fees			
Survey Distribution			
Total	$43,182	$21,004	$ 0

Total Spent, 2023: $21,843.56

Salaries and Wages: $21,843.56

Benefits: $6780.79

Purchased Services (David Granatstein Services Contract): $3145.00

Travel: $378.59

Budget 4
Co-PI 4: Suzette Galinato
Organization Name: Washington State University
Contract Administrator:
Telephone:
Contract administrator email address:
Email Address:

	2023	**2024**	**2025**
Salaries	$7260	$1888	
Benefits	$2292	$596	
Wages			
Benefits			
RCA Room Rental			
Shipping			
Services	$1920		
Travel	$1869		
Plot Fees			
Focus Group			
Total	$13,341	$2,484	$ 0

Total Spent, 2023:

$0

Objectives

We will perform a lifecycle assessment to evaluate the environmental impacts of apple production from orchard establishment through harvest and cold storage (or alternate supply chain stage where the apples are ready for delivery to the consumption stage). The analyses will include the upstream (e.g., suppliers) and downstream (e.g., waste management) processes associated with apple orchard and warehouse operations (e.g., production of raw, auxiliary, and operating materials), including all relevant inputs, emissions into the air, water, and soil, and disposal of all elements of production (e.g., pruning wood and end-of-life trees). This, in turn, will enable the apple industry to respond with cost-effective adaptive strategies to sustain production and profitability into the future, address buyer concerns, take advantage of government programs, and prepare for potential federal regulatory oversight (e.g., reduction in GHG emissions) being developed. The results of an apple LCA would also offer insights into the entire production system from which the sector could construct a public policy or public relations narrative regarding the impacts of tree fruit production on climate change and other environmental impacts.

The primary project goals are to provide a baseline assessment of the environmental sustainability of Northwest apple production and to ***develop a scenario analysis tool*** that will support the evaluation of management decisions over the orchard life cycle and provide the standard against which future improvements can be documented. These objectives will be achieved through stakeholder-engaged efforts to define the sector's most relevant data and sustainability metrics.
At scale, we envision a continuum of orchard stages. As new practices and technologies emerge, this tool can inform decisions regarding the next establishment phase's management. Since environmental sustainability metrics are vital components of the scenario analysis tool, baseline life cycle impact assessment results will be a key deliverable from this project.
Specific objectives of this project are:

- Design and test a comprehensive life cycle data collection survey to provide data for a baseline sustainability assessment (e.g., Carbon and water footprint, energy consumption, eutrophication, etc.) and the development of a tool for the evaluation of alternate management scenarios (e. g., biomass to energy versus composting of end-of-life trees)
- Provide an evaluation of current sustainability metrics of a range of management alternatives of NW apple production – that is, ***a baseline suite of metrics against which future progress can be evaluated***.
- Develop an LCA model for environmental impact assessment and scenario testing.
- Engage stakeholders in the development of a scenario analysis tool with which producers can simulate alternate management practice effects on environmental sustainability metrics that can be used to identify strengths and weaknesses of alternate management systems to identify environmental hotspots as opportunities for improvement.

Significant Findings

Workflow 1: Survey development

- An extensive list of over 100 questions for apple orchard growers was created based on a literature review, expert judgment and previous LCA experience. This list formed the basis for focused, in-depth interviews. This process of developing potential questions also involved evaluating potential modeling platforms and approaches to determine necessary input parameters.
- Focus interviews with 9 growers held between July and December 2023 provided an understanding of the apple orchard system in terms of common management practices,

variability in practices across orchards and blocks, availability of data, and ease of answering questions or retrieving records about different elements of production.

- Insights from the 9 focus interviews, in combination with insights from the preliminary LCA and sensitivity analyses (described below) supported an iterative process of simplifying the survey, to enhance participation, by reducing the original number of questions from over 100 questions to about 40 questions. The selection of questions was based on the contributions of the parameter to the LCA results, and the estimated variability of the parameter between blocks. The main categories of questions are:
  - Overall block characteristics (size, location, spacing, trellis type, etc.)
  - Nutrient application
  - Fuel and material (netting, etc.) consumption
  - Water use and irrigation infrastructure
  - Planting/grafting and tear-out procedures.
  - Varietal yield (block level)
- The shortened survey has been submitted for review by WSU's Internal Review Board for Human Subjects Review.
- The shortened survey has been developed in Qualtrix and pre-testing is ongoing. The survey will be sent widely to growers in Jan/Feb 2024.
- To encourage producers to respond to the survey, Dan Langager led (with team input) the development of a 2-page white paper r summarizing the effort (included at the end of this document). Working together, the team also submitted articles to Fruit Matters and Good Fruit Grower, and presented at a Columbia Basin Tree Fruit Club Meeting, and the Washington State Tree Fruit Association Meetings.

Workflow 2: LCA study and report

- A parameterized lifecycle inventory (LCI) model has been created in the SimaPro software platform. The model includes an accounting of inputs (including upstream processes), outputs, and emissions of the establishment and production phases of apple cultivation. The model has been used for the preliminary LCA and sensitivity analyses, which has been crucial in the iterative refinement of the survey to ensure that the most important operational characteristics have been captured. It will be further used to perform LCA calculations using data collected from the Qualtrix survey. A schematic overview of the production activities within the scope of the model, and the associated environmental impacts, are depicted in Figure 1.
- A preliminary LCA and several sensitivity analyses have been performed for the first six focus interviews conducted between September and December 2023. Aggregated preliminary results are presented in Figure 2 and discussed in the results section. As mentioned, feedback from this process has been instrumental in streamlining and developing the Qualtrix survey.
- An LCA methodology report is in development. The report describes the LCA process and methodologies employed (including alternative considerations) in detail.

Workflow 3: LCA tool developments

- All learnings regarding management practices and LCA calculations form a basis for the development of the scenario analysis tool in year 3 of the project. Detailed user input on tool functionality and the types of alternative scenarios that are of interest to producers will be forthcoming. A specific list of requirements is scheduled to be finalized this year (2024).

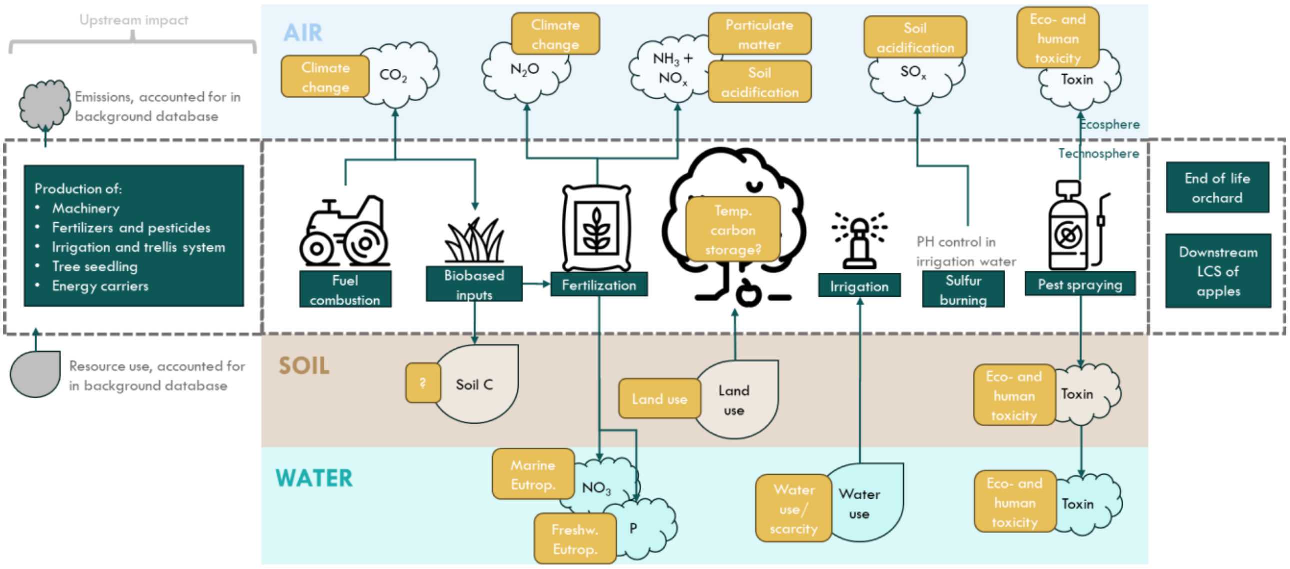

Figure 1. Schematic overview of processes considered in the LCI and associated environmental impacts.

Methods

Quantifying the impacts of current Pacific Northwest apple production practices on the environment is ongoing to understand the environmental impacts associated with apple production and supply chains in the region, to position the apple industry to be in compliance with buyer demands, and to engage with the USDA Climate-Smart Commodities Program. ***The LCA from this project will provide a baseline environmental profile and can assist in identifying opportunities for greenhouse gas mitigation and other sustainability efforts, in turn allowing the apple industry to make impactful, data-driven decisions. Further, the effort will support communication to educate the Pacific Northwest apple industry, retail partners, and consumers about the sustainability characteristics of apple production.*** Finally, the research will assist the industry in generating a credible, science-based narrative showcasing its efforts as good caretakers of the land and resources.

An LCA relies on the concept of a functional unit, which allows comparison across different production systems. In this assessment, the functional unit will be reported in terms of 40 lb boxes and on a 1000 lb basis of apples for the fresh market, ready for delivery to the retail sector. Apples may be directly sold after harvest or stored in cold storage for up to several months. Differences in the storage period will be averaged for the LCA; however, in the sustainability assessment tool to be developed, the length of storage will be parameterized to enable an understanding of the potential effects on the sustainability characteristics.

Broadly, this project will rely on stakeholder-engaged life cycle inventory data collection, which can be used in standard LCA software to calculate carbon and water footprints and other sustainability indicators. A lifecycle inventory model is constructed as a set of linked unit processes. Each unit process accounts for a specific activity in the supply chain (e.g., drip or other irrigation systems, or application of crop protection chemicals) and captures the full production chain of the system under study.

Figure 2 shows the workflows for the project. The overarching structure is intended to be highly integrated from the outset. The gradient shading is intended to indicate the degree of completeness of the activity. There is, of course, a linear flow that is depicted by the three workflow columns. Close coordination between the three workflow columns has been achieved through weekly or biweekly team meetings and is contributing to the overall project's success. Initial efforts focused on defining the current best state of knowledge regarding the environmental sustainability of apple production were used to inform the surveys developed in conjunction with stakeholders through in-depth interviews. Surveys have been refined interactively with lifecycle assessment modeling.

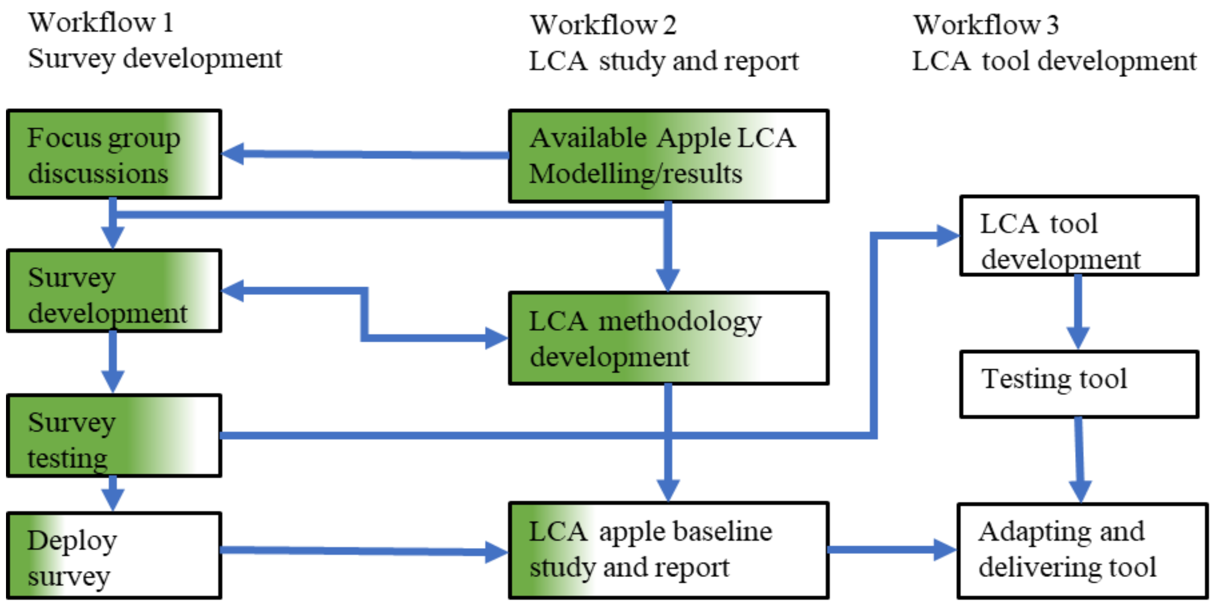

Figure 2. Workflow for the project. Highlight indicates approximate level of completion of the task.

Results and discussion

The work done this past year has been preparatory for the LCA and has provided a good understanding of the most important data points to collect via the survey and insight into common management practices to ensure the LCA model is complete and accurate. The current model has also provided some preliminary LCA results. The in-depth interviews and external communications (2-page summary, articles, presentations) helped to grow understanding and support for the LCA study among growers and other stakeholders.

The development and use of the parameterized LCI model is an important step in automating large-scale survey data assessment and LCA calculations. The LCI model is an important foundation for the LCA and will enable rapid LCA calculations after survey data are received. It has also informed our survey development, allowing shortening of the survey to focus on the questions most relevant to LCA outcomes.

Initial LCA impact results

Figure 3 presents the preliminary results from the carbon footprint assessment of the focus group survey data sets. Because these are preliminary results, we intentionally have not reported numerical values associated with the carbon footprint. We anticipate that the numerical values will change as we continue to collect data during 2024 from the survey. An interesting result of this preliminary evaluation is the relatively large contribution of the trellis system to the overall carbon footprint.

- The main contributing processes to the carbon footprint (CFP) have been identified:
  - Diesel use (field operations and irrigation), electricity (irrigation), and propane (frost protection).
  - Synthetic and biobased fertilizer application (impacts due to direct and indirect nitrous oxide emissions from nitrogen application).
  - Production impact of capital goods, mainly the trellis system (the impact depends on the type of trellis system and main material (wood vs. steel)).
- Milder weather conditions in 2021 appear to be a main reason for the year-to-year variations in CFP. This reduced the consumption of propane required for frost protection. The higher footprint for Honeycrisp is mainly related to a rough estimate of increased fuel use related to more trips through the orchard at harvest (color picking). The higher footprint for organic apples compared to conventional apples is related to the slightly lower yield per acre. These results and observations are preliminary and should thus not be used in (external) communication.

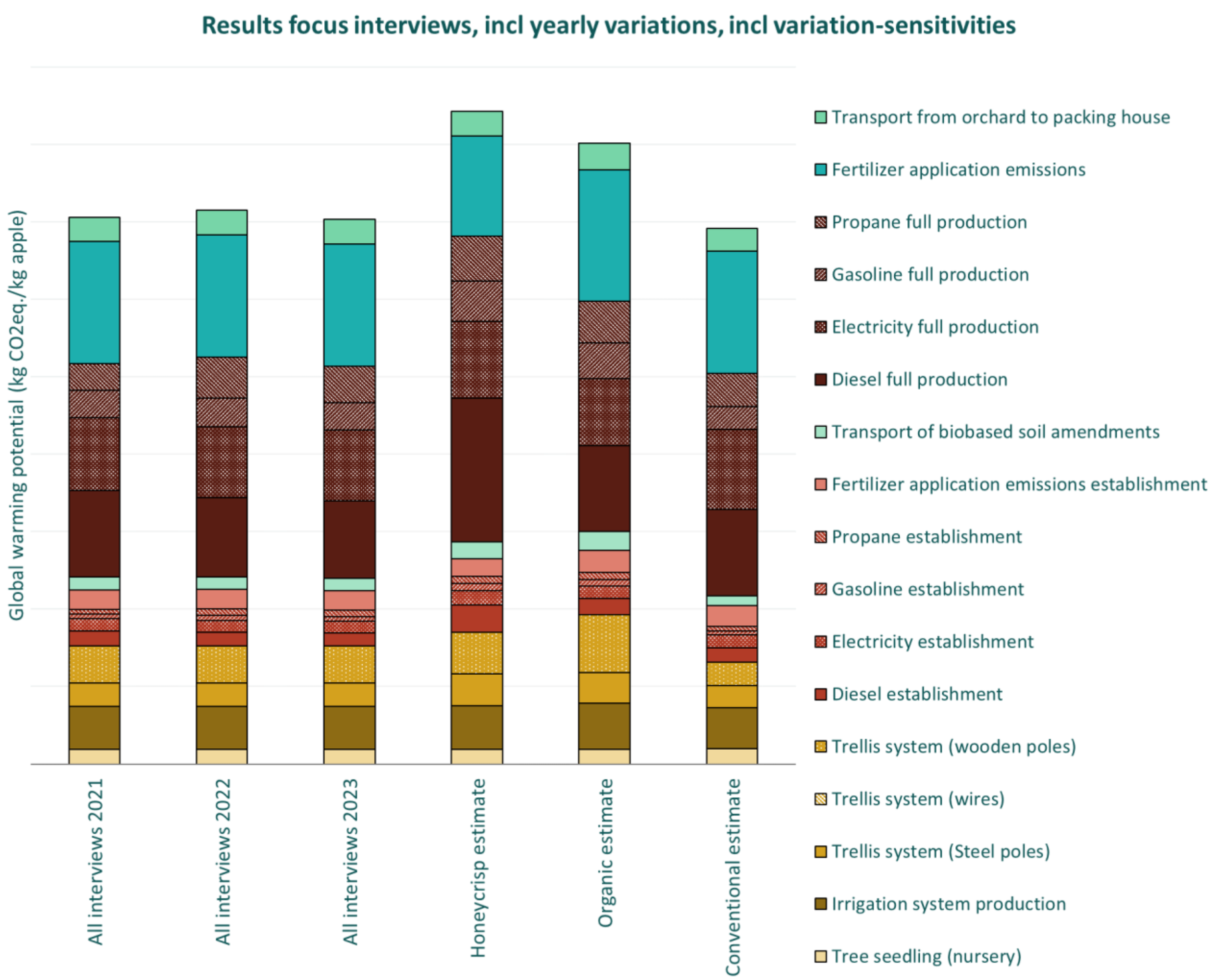

Figure 3. Preliminary carbon footprint results for PNW apple production of focus group interviews

- Calculations of Carbon Opportunity Cost were also performed. This is a metric showing the difference between the current carbon stock in the soil and vegetation of a system and the potential under natural (unmanaged) land use. A preliminary analysis for two focus interviews indicated a higher current carbon stock (mainly in vegetation) compared to the potential natural carbon stock. This indicator implies that having orchards in these locations results in more carbon sequestered than if the native ecosystem had been undisturbed, likely driven largely by irrigation of the orchards.

Continuing efforts

Focus points for next year (year 2 out of 3 of this project) are the following:

- Sending out planned communications to encourage survey responses and fielding the survey with apple growers in the Pacific Northwest in early 2024 will be a major milestone and is one that we are on track to meet. We are optimally using the 'slow season' for growers to obtain as many survey responses as possible.
- Further developing the LCA methodology and LCI model, mainly to include ecotoxicity assessment, location-specific characterization of water use impact (water scarcity), more refined carbon stock calculations related to biobased inputs and chipped wood at end-of-life (e.g. through carbon opportunity cost metric).
- Efficiently (automatically) processing the survey responses to match the data to the parameters in the LCI model. This will enable large-scale data processing to generate block specific LCA results.
- Sensitivity and uncertainty analysis based on the survey results, using the LCI model.
- Finalizing the LCA report, which will explain the goal and scope, methodology, data inventory, results, conclusions, and discussion regarding the environmental impact of Pacific Northwest apple production.
- Finalizing the list of requirements for the LCA tool, which is to be developed in year 3.

2 page project description for communication with industry stakeholders regarding the why and how of this project.

The ABCs of LCAs

With grower input, a life cycle assessment, or LCA, of Washington's apple production will establish a baseline of environmental data.

Why has industry identified the LCA as a research priority?

As demand for agriculture to reduce its climate impacts continues to build from regulators, retailers, and consumers, tree fruit growers are committed to quantifying those impacts in order to find realistic, actionable solutions for their industry.

This is a grassroots effort by industry, for industry. The Washington Tree Fruit Research Commission funded the LCA study to develop a scientifically defensible benchmark of apple production's climate impacts, ensuring industry control of the data.

Now the research team needs growers' input to build the LCA's confidential apple production and storage data.

What metrics will the LCA analyze?

Growers need climate information specific to their crop in their growing region. This type of LCA research serves as a foundation for quantifying environmental indicators associated with a single production system in a distinct geographic area by establishing an accurate baseline.

In addition to an industry baseline of fuel, fertilizer, land, and water use, the project will provide a scenario analysis tool. The tool will enable producers to customize inputs specific to their growing and/or packing operation, calculate climate metrics (such as carbon footprint), and identify hot-spots to inform changes in management.

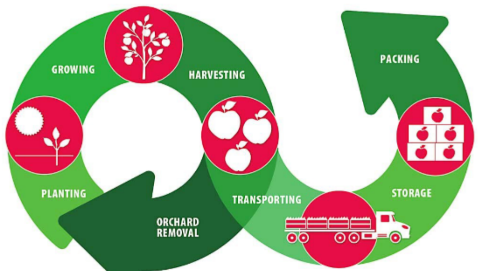

Illustration: Jarod Johnson, Good Fruit Grower

The LCA will encompass conventional and organic apple production from planting to packing.

The ABCs of LCAs

Grower input and feedback is essential for the success of the LCA.

Who is involved in the LCA?

The Washington Tree Fruit Research Commission (WTFRC) funded the three-year study. The project lead is Dr. Greg Thoma, a nationally recognized expert in agricultural LCAs, with assistance from the Northwest Horticultural Council, Washington State Tree Fruit Association, Washington State University, Blonk Sustainability, industry consultants, and you! Grower feedback is critical to accurately assess activity and inputs across an orchard's life.

What are the benefits to industry?

With the project funded by the WTFRC, the industry can own and manage its climate data. Significant safeguards have been put in place to protect growers' privacy and confidentiality, including "blind aggregation," which means anonymizing any identifying information in the data.

"The LCA can tell the real story of apple production in Washington and the benefits of having a robust apple industry."

- Brent Milne, McDougall & Sons

By identifying practices that may negatively impact climate, alternatives and mitigations specific to the Pacific Northwest can be developed. This may include opportunities for:

- Greenhouse gas reduction
- Farm input optimization
- Water and soil conservation
- Transportation efficiencies

In addition to helping identify areas that need improvement, LCA data can substantiate climate-smart practices already in place.

The LCA baseline data and subsequent analysis tool will help apple growers:

- meet climate-related retailer directives and government mandates.
- be better prepared to participate in voluntary government incentive programs.
- develop and fund future industry research priorities.
- inform the public of their work to contribute to climate change solutions.
- demonstrate a collaborative and forward-thinking approach to difficult issues.

How can growers support this effort?

The LCA depends on broad industry participation via the collection of orchard input data. In addition to discussions with orchard managers, an online survey will ask apple growers to use their records to answer general questions about one block of their operation, such as variety, infrastructure, and water, fuel, and fertilizer use. All data provided will remain confidential and anonymized.

Grower Survey
Early 2024
Online
1 Block

Questions?

Contact Dan Langager at the Northwest Horticultural Council:
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Project/Proposal Title: Evaluation of an alternative postharvest fungicide applicator

Report Type: Continuing Project Report Year 3 NCE

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Project Duration: 3 Years
Total Project Request for Year 1 Funding: $132,793
Total Project Request for Year 2 Funding: $110,993
Total Project Request for Year 3 Funding: $4,500

Other related/associated funding sources: None

WTFRC Collaborative Costs: None

Budget 1
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Item	2021	2022	2023	2024
Salaries	69,064	63,989	0	
Benefits	21,563	18,520	0	
Wages	6,483	5,411	0	
Benefits	807	541	0	
Equipment	0	0	0	
Supplies	29,160	21,060	4,500	
Travel	5,716	1,472	0	
Miscellaneous	0	0	0	
Plot Fees	0	0	0	
Total	132,793	110,993	4,500	0

OBJECTIVES

1. Optimize coverage of fruit in alternative sprayer with fluorescent tracer and water sensitive paper (Hoheisel; yr 1).
2. Comparison of efficacy against postharvest decay organisms between drench and alternative fungicide application (Amiri; yr 1and 2).
3. Quantification of indicator organisms (*E. coli* and coliforms) in water and on fruit treated with fungicides applied in drench and alternative applications (Hoheisel; yr 1 and 2).
4. Communication of findings with the apple and allied industries and engage regulatory bodies for approaches for implementation of alternative fungicide application on farm (Amiri, Hoheisel; yr 1, 2 and 3).

Significant Findings

- Field drencher optimized for spray coverage.
- Coliform counts were higher in the field sprayer whereas *E. coli* recovery was higher in the warehouse drencher.
- Residue levels of thiabendazole (TBZ) were similar between the field sprayer and warehouse drencher but levels of fludioxonil (FDL) were higher on apples treated with the field sprayer.
- Spores of *Penicillium* spp. (blue mold) were neither detected on apples nor in fungicide solutions of field sprayer or warehouse drencher
- Total microflora recovered from apples treated with fungicides through the field sprayer was significantly reduced compared to the control and fruit treated via warehouse drencher.
- Overall, decay incidence after 8 months of storage was lower in apples treated the field drencher compared to those treated with warehouse drencher.

METHODS

OBJECTIVE 1. Optimize coverage of fruit in alternative sprayer with fluorescent tracer and water sensitive paper. (Hoheisel; Year 1).

<u>Alternative fungicide applicator</u>. As previously mentioned, Matson Fruit has been field testing an alternative fungicide applicator which utilizes single-pass water to deliver fungicides given risk from cross-contamination from decay causing pathogens. Their initial work has resulted in spray systems which were utilized for the 2019-2020 growing seasons to treat in their operation as shown in Figure 1. A video depicting the sprayer in operation can be seen at https://www.youtube.com/watch?v=q685NrigZfw. Others in the industry are also interested in evaluating this applicator during the 2021 harvest. Jason and Jordan Matson are interested in partnering with WSU through this proposed work to further evaluate and optimize this system so that best efficacy can be achieved. Helping advance the number of alternative systems growers have at their disposal for controlling postharvest decays.

Figure 1. Novel fungicide spray applicator shown treating crop in 2020. The system is composed of a mix tank, pump, spray nozzles and catch basin to collect run-off with tank storage for disposal.

Expected outcomes. Deposition will be statistically compared for 27 different zones in a bin for both a new and traditional system and will be paired with biological efficacy and food safety (obj 2 and 3). Combining all three creates an extremely robust assessment of an alternative drenching system that may provide better control.

Potential pitfalls and limitations. We were very successful in this objective and believe it is completed unless results from fluorometry or efficacy study show large differences between top and bottom zones. Aside from that, in an engineering project constructing a novel system can lead to delays for the completion to the project. Biological efficacy studies suggested in Obj 2 and 3 should only be completed once a functional well-developed prototype is developed. In this study, prototype development has already been developed by Matson and therefore minimizes that pitfall and advances the total project. Also, because of COVID and tariffs, our lab has found difficulty ordering supplies that are no longer available in the US. For other projects, we have been able to order well in advance to ship internationally.

OBJECTIVE 2. Comparison of efficacy against postharvest decay organisms between drench and alternative fungicide application. (Amiri; Year 1 & 2)

Alternative sprayer application (Field sprayer). The optimized configuration for coverage as determined in objective 1 will be utilized for all subsequent studies. We will use Penbotec (pyrimethanil) and Scholar (fludioxonil) in coordination with Matson Fruit and will rotate fungicides from year 1 to year 2. The cultivars Honeycrisp and Gala were used for these trials.

Drench application. Matson Fruit will drench fruit, from the same cultivar used in alternative sprayer, following standard industry practices. The same antifungal compound evaluated in the alternative applicator will be utilized in the drench application.

Quantification of spores of fungal pathogens in fungicide water for alternative and drench applications. For the alternative sprayer, three-100mL fungicide solution samples will be aseptically captured during each spray. For drench application, fungicide solution samples will be collected at 0, 100, 200, and 500 bins treated with the same fungicide solution (same tank). Samples will be held at 4°C until further processed on agar media amended with triton x 100 (Amiri and Bompeix, 2005). Plates will be incubated for 5 days at 20°C (68°F) and fungal colonies will be counted and identified to the genus level. The concentration of fungal spores in fungicides solutions will be expressed as colony-forming unit (CFU). This experiment will be repeated in Year 2.

Quantification of fungal colonies on apples before and after fungicide application through alternative and drench approaches. Twelve individual apples will be sampled before and 30 min after treatment per replicate in a way to collect 4 apples from each bin (4 bins make one replicate) treated at the same time. Apples will be placed in a sterile plastic bag and held at 4°C until further analysis. An individual apple will be immersed in 100ml buffered peptone water with 0.1% Tween 80 and placed on rotary shaker for 30 minutes to suspend fungal spores in the buffer. Samples will be serially diluted and plated in duplicate on agar medium amended with triton x 100 and plates will be incubated for 5 days at 20°C (68°F) and fungal colonies will be counted and identified to the genus level. The size of each fruit will recorder to estimate the area and the concentration of fungal spores/cm^2 of each fruit will be expressed as colony-forming unit (CFU). This experiment will be repeated in Year 2.

Determination of decay incidence and decay types in cold storage on fruit treated at harvest with fungicides through alternative and drench approaches. Four replicates of 100 apples each will be collected from different bins before fungicides are applied and four other replicates will be collected after the fungicides are applied via each method. Fruit will be placed in separate (each 100 fruit rep) labeled crates and stored at WSU-TFREC in regular atmosphere at 1°C (34°F). Fruit will be inspected every two months for decay incidence and decay type for up to 8 months. We will work with Matson fruit to conduct a second efficacy trial in commercial settings. At the time of harvest, 10 bins will be left untreated, 10 bins will be treated via alternative sprayer, 10 bins will be treated via traditional drencher. All bins will be labeled and stored in CA room at Matson's storage facilities for a period to be determined. At the end of the storage period, the bin will be run through the packing line to separate decayed from healthy fruit. Decayed fruit will be collected by Amiri' team to determine decay incidence and types in each bin set. Packout from each bin set will be obtained from storage facility manager.

Fungicide residue levels. In addition to the work outlined in Objective 1, we will evaluate fungicide residue levels generated by the alternative sprayed and traditional drencher. Two samples of 10 apples each will be sampled from individual bins, one sample on the top of the bin and the other sample will be from one foot deep from the top of the bin. A total of 4 replicate samples will be collected from each application methods and fruit will subjected to fungicide residue analyses.

Expected outcomes. We will determine if the alternative method of fungicide application has reduced risk for carrying-over fungal spores and is more effective in reducing fungal spores on fruit surface prior to storage. We will also assess the efficacy of this new alternative method in reducing decays in long-term storage. We should also obtain data on the fungicide residue level provided by this new alternative method and if those levels are adequate to provide protection against postharvest pathogens.

Potential pitfalls and limitations**.** Disease pressure may vary between seasons to obtain adequate or comparable data from the presence of the fungal spores on the fruit surface at the time of harvest. Some fungi may consist of endophyte (infections) that may not be detected by plating. Comparing the alternative method to the traditional spray method should take into consideration the number of bins treated via drencher to assess efficacy of a "clean" versus "dirty" tank. At the end of Year 2 (2023), we'll have 2 years of data and will be able to better compare the efficacy of the two sprayer models.

OBJECTIVE 3. Quantification of indicator organisms (*E. coli* and coliforms) in water and on fruit treated with fungicides applied in truck and alternative applications. (Hoheisel, Years 1 & 2)
Experimental design. A completely randomized design will be used for both water and apple analysis. There are four water samples per replicate and all treatments will be independently replicated eight times. There are twelve apple samples per replicate and all treatments will be independently replicated eight times. Populations of *E. coli* and coliforms will be the independent factor which will be evaluated to determine significant differences

Quantification of *E. coli* and coliforms in fungicide water for novel and drench applications. Three-100mL water samples will be aseptically captured during each spray or drench application. Samples will be held at 4°C until further processed utilizing the Colilert Quanti-Tray 2000 (Idexx, Wesbrook, ME). Samples will be incubated for 24h at 36°C. The wells in the Quanti Tray will be observed for their change in color from colorless to yellow (coliform detection) and presence of fluorescence (*E. coli* detection) using a fluorescence analysis cabinet Model CM-10A (Spectroline, Westbury, NY). Positive wells for *E. coli* and coliforms will be recorded and equivalent populations of Most Probable Number (MPN) for each organism per 100mL will be determined.

Quantification of *E. coli* and coliforms on apples before and after fungicide application through novel and drench approaches. Twelve individual apples will be sampled before and after treatment per replicate. Apples will be placed in a sterile plastic bag and held at 4°C until further processed. An individual apple will be immersed in 100ml buffered peptone water with 0.1% Tween 80 and rubbed by hand for 30 seconds to suspend bacteria in the buffer. Samples will be serially diluted and plated in duplicate on Petrifilm E. coli/Coliform Count Plates. Samples will be incubated for 24h at 35°C, after which colonies showing typical characteristics for *E. coli* and coliforms will be enumerated and used to calculate Colony Forming Units (CFU) per apple.

Expected outcomes. We will determine if there are differences in water quality and populations of indicator organisms on fruit in the novel, single-pass fungicide spray system compared to that of a traditional drench system. It is anticipated that the novel single-pass applicator will have improved water quality based upon populations of *E. coli* and coliforms compared to a recirculated drench system. If true, we would also anticipate a significant increase in cross-contamination from drench systems onto fruit. Ultimately, this information will help growers managing risk within their operation make informed decisions about the food safety benefits, if any, from this alternative fungicide application system.

Potential pitfalls and limitations. The authors do not foresee any significant pitfalls given past experiences enumerating *E. coli* and coliforms from postharvest water and on apples. Limitations to this approach are that the team is quantifying differences in indicator organisms and not foodborne pathogens. Therefore, any inferences will be with respect to indicator organism behavior and not that of foodborne pathogens (*Listeria monocytogenes*, Shiga-toxigenic *E. coli,* and *Salmonella*) directly. However, *E. coli* and coliforms are commonly used indicators and the most appropriate selection for this approach.

OBJECTIVE 4. Communication of findings with the apple and allied industries and engage regulatory bodies for approaches for implementation of alternative fungicide application on farm (Amiri, Hoheisel; yr 1, 2 and 3).

Communication with the Washington Department of Ecology. The team will also work with Marsha Porter at the WA Dept. of Ecology to outline specific criteria which must be adhered to when utilizing the novel applicator. This will help clearly communicate expectations to growers during outreach.

Communication with the apple industry. Each member of the WSU team has an extension appointment and regularly communicates with the Washington apple industry. Findings from this work will be communicated to the industry through grower meetings, newsletter articles, and factsheets to further disseminate knowledge gained. A detailed explanation of the sprayer parameters will be given for others to construct. Factsheets will be printed in both English and Spanish.

Results and Discussion

Objective 1. Optimize coverage of fruit in alternative sprayer with fluorescent tracer and water sensitive paper.

Optimization. Examination of the field drencher (FD) and prior residue analysis by Matsons showed that very little improvements needed to be made to the FD design. There are three nozzles (QCTF-VS20 Quick Turbo FloodJet Wide Angle Flat Spray Tip) across the spray bar and to reduce drift plastic guards have been installed on the side. The FD is run at 15psi to ensure large droplets and reduce drift. Time for each bin to be sprayed in the field drencher was 12 sec (n=16) and the packing house (PH) is standard 30 sec. The gallons per bin based on spray time and gpm of nozzle (FD) or water collected (PH) is FD=0.5g/bin and PH=1.5g/bin stack.

Apple bins were modified to have 4 slits in the top and bottom and rebar was inserted to form a rectangle that kept an 'apple-free zone' in which water sensitive paper (WSP) could be inserted on a pole. There were four collection zones in the top and four in the bottom. The WSP in both the single layer FD and stacked bin PH were complete coverage. This was expected and desired result of this type of chemical application (drench).

Evaluation. Fluorescent tracer (pyranine) was used to assess deposition of FD and PH. Pipe cleaners that are absorbent in their cotton fibers were placed in bins in the 'apple-free zones' so that 4 samples were on the top and bottom (n=8/bin). Based on the high volume of liquid applied by drenchers compared to standard field sprayers, we reduced the pyranine rate from 1000mg/L to 83mg/L=FD and 328mg/L. Tank samples were collected and differences in initial pyranine will be adjusted for in the calculations of pyranine parts per billion (ppb). Pipe cleaners were bagged, labeled, and stored in a dark cooler at 4°C (39°F) until laboratory analysis (currently being done). To each sample, deionized water will be added, and bags will be vigorously shaken for 30 seconds and allowed to settle. An aliquot of wash from each sample bag will be extracted and analyzed with a 10-AU fluorometer.

A linear mixed effects model was fit to characterize the tracer concentration (ng/cm^2) by zone and location for the packing house. Zone, location and the interaction between zone and location are included in the model as fixed effects, while the truck (or rep) is included as a random effect. An analysis of variance is performed to assess the effects of zone, location, and the interaction with Kenward-Rodger degrees of freedom. Least squares means and 95% confidence intervals are extracted for each zone x location combination. Pairwise differences with 95% confidence intervals are extracted between the top and bottom for each zone, with no family-wise adjustment for multiplicity. Pairwise differences between zones are extracted with a family-wise adjustment for multiplicity using the Tukey method.

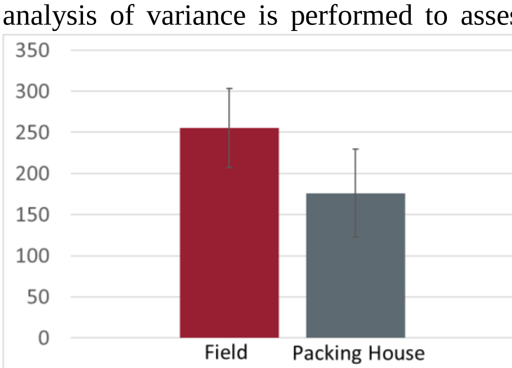

Figure 2. There is no significant difference (P-value: 0.2696) between the mean of the tracer concentration from the field and packing house, averaging across the experiment.

Overall, there was no significant difference in coverage between packing house and field samples (Figure 2).

Deposition within the packing house was fairly uniform except for the upper most collection zone and location receiving more (Figure 3a). This is obviously due to the shower-down nature of the application. Nonetheless, it is positive that the lowest collection area (Lower, bottom zone) had similar deposition to other areas and is likely due to the extremely high flow rate in the packing house.

The field drencher (Figure 3b) is not stacked but goes under the spray bar with bin 1 going in first. After the last bin is sprayed, the driver waits 30 seconds and backs out with the bin 4 being the first under the spray bar. In this analysis there was a difference in deposition with the third bin receiving slightly less. We need to inspect possible differences in driving or patterns that could explain this difference. It contrasts with the regularity of time sprayed per bin (12 sec) which showed no significant difference in spray time among bins. Additional differences can be seen between the top and bottom zone of the bin, however, the impact of this would need to be assessed with efficacy data from storage rots. Meaning, there may be adequate deposition in the lower portion to control , but if not, rate should be increased to achieve more deposition in the bottom.

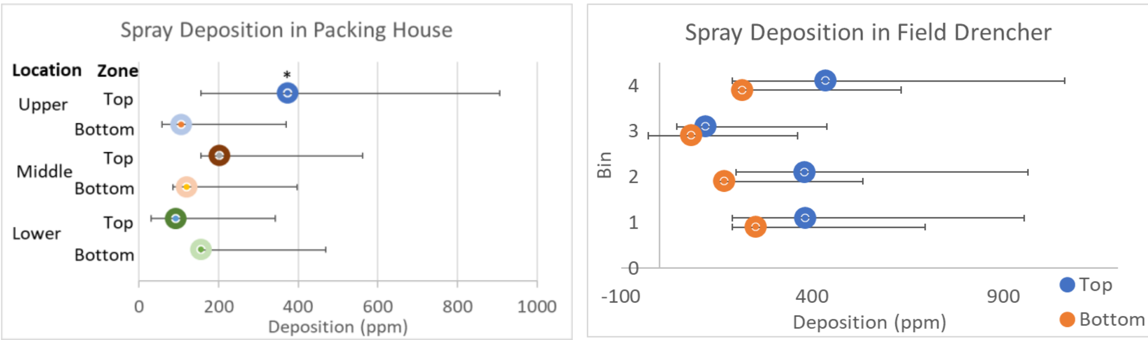

Figure 3. Spray deposition in the packing house and field drencher. In the packing house, bins are stacked (location) and there are two zones within a bin. Only the upper top collection area showed significant difference ($p<0.001$). In contrast, the field drencher is not stacked, but goes under the spray bar from bin 1 to 4. Significant differences were seen between the top and bottom of the bin ($p>0.0197$) and bin order ($p>0.047$).

Objective 2. Comparison of efficacy against postharvest decay organisms between drench and alternative fungicide application

2.b. Quantification of spores of fungal pathogens on fruit treated via two drench applicators

On Average, very a few *Penicillium* spores were recovered from the surface of the fruit and were generally less found in fruit drenched in the field than in the warehouse especially for Gala lots 1124 and 1113 (Table 1). Other minor fungi and often not pathogenic were recovered from fruit treated through both drenchers although slightly higher in the field-drenched fruit.

Table 1. Number of colonies of *Penicillium* spp. and other fungi recovered from the surface of the fruit treated through field (FD) and warehouse (WH) drenchers in September 2022.

		Penicillium			Other fungi		
Cultivar	**Lot**	**Control**	**FD**	**WD**	**Control**	**FD**	**WD**
Honeycrisp	1136	0.04	0.3	0.4	17.2	14.8	31.8
Gala	901	0.2	0.2	0.2	5.4	12.1	9.3
Gala	1124	0.04	0	0.3	2.4	6	4.4
Gala	1113	0.08	0	1.2	27.7	42	13

2.c. Fungicide residue levels

Residue levels of pyrimethanil on Honeycrisp apples collected from the top and the middle of the bins were equal between in the field and the warehouse drenchers, but residue levels were lower at the bottom of the bins drenched in the field (Figure 4right). Residue levels of pyrimethanil on Gala apples collected from the top, middle and the bottom of the bins were equal, for the same bin position, between in the field and the warehouse drenchers (Figure 4left). However, in the field drenched-bins, significantly lower residue levels were found at the bottom of bin compared to the top. This should not have an impact on decay management, since the minimum levels recommended for pyrimethanil are met (>1 ppm).

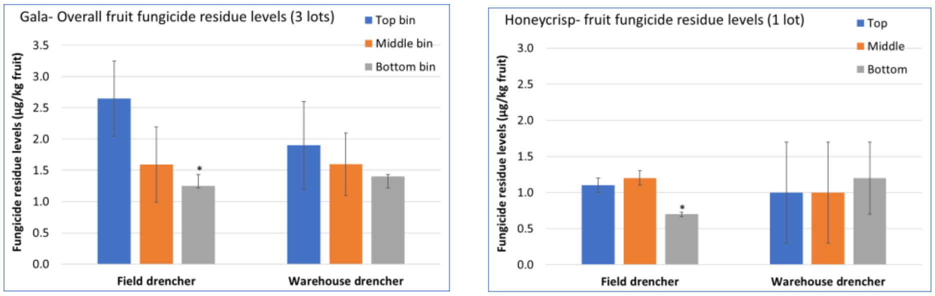

Figure 4. Residue levels of pyrimethanil on Gala apples (left) and Honeycrisp apples (right)

Concentration of pyrimethanil levels in solutions of the field sprayer were slightly bigger than those found in the solutions applied in the warehouse drencher (Figure 5). In the warehouse drencher, pyrimethanil concentrations were all above 200 ppm regardless of the number of bins treated with the same tank.

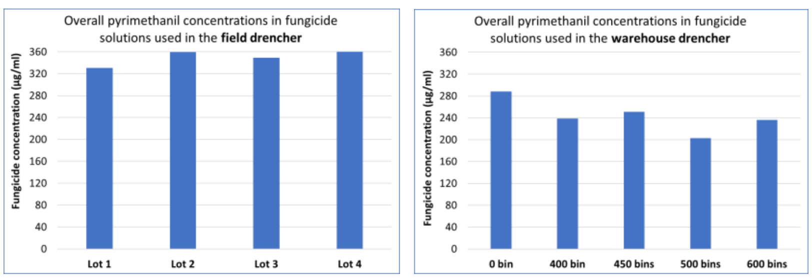

Figure 5. Concentrations of pyrimethanil in fungicides solutions of field (left) and warehouse (right) drenchers

<u>2.d. Determination of decay incidence and decay types in cold storage on fruit treated at harvest with fungicides through alternative and drench approaches</u>

Three hundred apples (100 apples/treatment) were collected from each lot and stored at 55°F for 2 weeks, then at 37°F in RA. Overall decay varied between lots and was either lower in field drencher after 9 months or equal to incidence recorded in warehouse drenched-fruit except in lot 1139 (Figure 6).

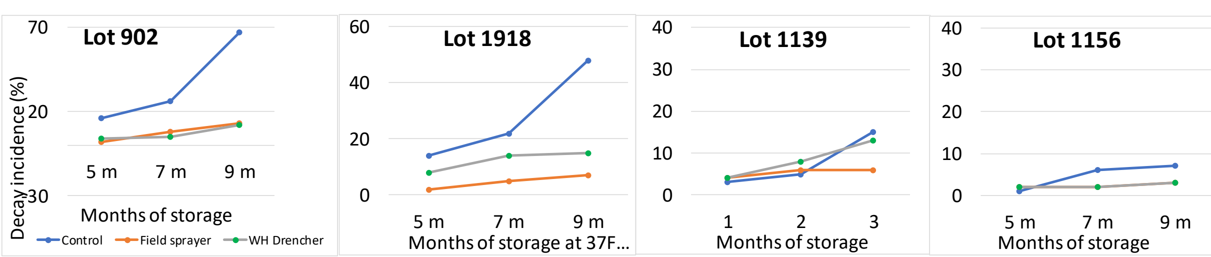

Figure 6. Overall decay incidence in four Honeycrisp lots untreated (control) or treated via field or warehouse drenchers in 2021-22 season and stored in regular atmosphere at 37°F.

During the 2022-23 season, five lots of Honeycrisp and three lots of Gala that were not treated with any fungicide preharvest were treated at harvest with Penbotec. Four bins of each lot were treated using the field sprayer and 4 other bins from each lot were treated with the warehouse sprayer. Bins were stored at the collaborating were house in CA. The overall decay incidence after 8 months for Honeycrisp lots was significantly lower in two lots (1162 and 1918) and was numerically lower in the 3 other lots when treated with the field drencher (Table 2). For the Gala lots after 9 months of storage, incidence was equal in two lots and was significantly higher in apples treated with the warehouse drencher for lot 1124 (Table 2)

Table 2. Overall decay incidence on Honeycrisp and Gala apples stored at the commercial storage cold room during the 2022-23 season under controlled atmosphere for 8 months.

		Decay incidence (%)	
Cultivar	Lot	Field sprayer	Warehouse Drencher
Honeycrisp	903	1.5	1.83
	1156	2.5	2.5
	1162	0.9	1.4
	1918	7.1	15.2
	1136	3.2	3.4
Gala			
	901	0.3	0.24
	1113	0.8	0.6
	1124	0.4	2.1

Objective 3. Quantification of indicator organisms (*E. coli* and coliforms) in water and on fruit treated with fungicides applied in truck and alternative applications

Water samples were collected in the harvest of 2021 and 2022, while apple samples were collected in 2022. Approximately 94% (85-98%) of the apple samples in the packing house and 84% (70-93%) in the field are coliform free before any drench treatment (Figure 7a). However, post drench treatment, 6% (2-17%) of the apple samples in the packing house and 94% (84-98%) of the apple samples in the field are coliform free after treatment. This is a significant ($p>0.001$) decrease for the packing house with a 87% (75-94%) decrease. Although there is a 9% difference (0.8- 20.5%) for the field drencher, pre and post treatments are not significantly different to each other.

Apple samples: Of the samples that tested positive for Coliform, some also showed *E. coli* populations. Nearly 100% (96-100%) of the packing house apple samples and 96% (86-99%) of the field apple samples were *E. coli* free on arrival. After the drench treatment, an estimated 93% (79-98%) of the packing house apple samples and 98% (92-99%) of the field apple samples were *E. coli* free. There was no significant different between pre- and post-spray application for either Drencher. For the subset of apples that did have contamination, the colony forming units (CFU) were compared pre and post spray applications. The mean CFUs for Coliform contaminated post application apple samples for field and packing house drenchers was 548 (127-2371) and 23899 (8255-69190), respectively (Fig 8a). For the field drencher, there is a non-significant 0.9-fold decrease in the CFUs for contaminated apples. In contrast, there is a 36.9-fold increase in the coliform CFUs for apples that tested positive for coliform. The mean CFUs for *E. coli* contaminated post application apple samples for field and packing house was 254 (51-1278) and 2288 (706-7417), respectively (Fig 8b). For apples from the field drencher, that is only 1.0 fold non-significant change in *E. coli* CFUs. Whereas apples from the packing house were nearly 100% free of coliform before treatment, the drench application introduces on average 2288 *E. coli* CFUs.

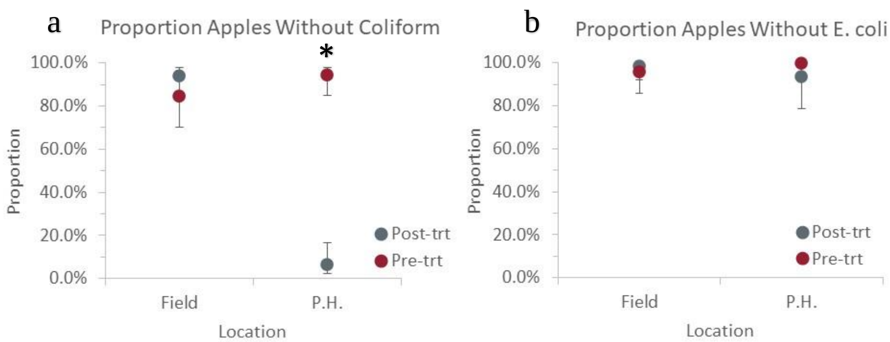

Figure 7. Proportion of apples without coliform (a) and *E. coli* (b) populations for apples pre- and post-drench treatment for Field and Packing House (P.H.) drenchers. There is a significant difference in apples with coliform (*=P-value>0.001) between the pre and post treatments in the packing house. While the field drencher showed no significant differences. And there was not a significant increase in apples with *E. coli* (b) pre or post drench for either treatment

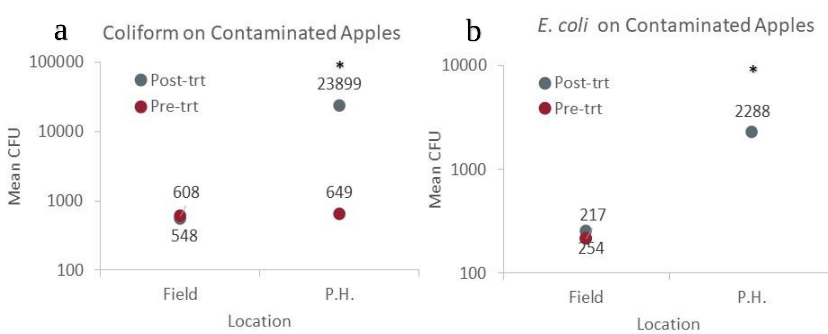

Figure 8. Considering only apples that were contaminated, this is the average colony forming units (CFU) of coliform (a) and *E. coli* (b). There was a significant difference (P> 0.001) between the mean pre and post applications in the packing house for both Coliform and *E. coli* CFUs. While the field drencher showed no change in coliform and *E. coli* CFUs

Water samples*:* There was an estimated mean of 17 (95% CI: 9-33) thousand coliform CFU in the typical field drencher water sample and a mean of 0.6 (95% CI: 0.3-1.3) million coliform CFU in the typical packing house drencher water sample. This is an estimated 35 (95% CI: 13-93) times the number of coliform CFU in the packing house compared to the field.

There was an estimated mean of 111 (95% CI: 24-523) *E. coli* CFU in the typical field drencher water sample and a mean of 2 (0.5-7.1) thousand coliform CFU in the typical packing house drencher water sample. This is an estimated 17 (95% CI: 2-131) times the number of E. coli CFU in the packing house compared to the field.

Future steps:

- 2024 — Conduct outreach activities
- Dec. 2024 — Provide a final report.

Project Title: Understand and mitigate fungicide resistance in *Penicillium* spp.

Report Type: Final Project Report

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Project Duration: 3 Years

Total Project Request for Year 1 Funding: $97,795
Total Project Request for Year 2 Funding: $92,068
Total Project Request for Year 3 Funding: $93,730

Other funding sources: Awarded
Amount: $9,643.20
Agency Name: State Horticultural Association of Pennsylvania
Notes: Awarded to co-PI Jurick II in 2018 entitled "Evaluating the efficacy of a new postharvest fungicide and developing tools to monitor fungicide resistance in blue mold populations."

WTFRC Budget: None

Budget 1

Primary PI:	Achour Amiri
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Item	2020	2021	2022
Salaries[1]	40,925	42,562	44,264
Benefits[2]	13,464	14,003	14,563
Wages			
Benefits			
Equipment			
Supplies[3]	7,000	3,000	2,400
Travel[4]	885	885	885
Miscellaneous			
Plot Fees			
Total	$62,274	$60,450	$62,112

[1 & 2] Salaries for a Postdoc at 4872/month for 12 months at 0.7FTE and benefit rate of 32.9%. A 4% annual inflation is included for Year 2 and 3

[3] Supplies for lab work for fungal growth and maintenance, molecular reagent for detection and sequencing

[4] Travel to packinghouses for sampling and collaborative work for 1,500 miles a year at $0.59/mile

Budget 2:

Co-PI (2):	Wayne M. Jurick II
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Item	2020	2021	2022
Salaries			
Benefits			
Wages[1]	$30,118	$30,118	$30,118
Benefits			
Equipment			
Supplies[2]	$4,900	$1,000	$1,000
Travel[3]	$500	$500	$500
Plot Fees			
Miscellaneous			
Total	$35,518	$31,618	$31,618

[1]Wages will be used to hire a GS-3 level employee to help conduct the research at USDA-ARS.

[2] Supplies for laboratory work including: genomic DNA isolation, library construction and whole genome sequencing, PCR, and media for fungal growth

[3] Travel for sampling packinghouses and collaborative work for 800 miles a year at $0.69/mile

OBJECTIVES

1. **Evaluate the pathogenic fitness of resistant populations having different fungicide resistance phenotypes.** Conidial germination assays indicated a fitness penalty to a lesser extent, but the *in vivo* assay demonstrated that the resistant isolates were more aggressive in disease establishment. The objective is being accomplished and projected to be completed in 2022.
2. **Determine the genetic makeup of *Penicillium* species exhibiting various fungicide-resistant phenotypes to postharvest fungicides.** We identified single, double, and triple fungicide resistant *P. expansum* isolates, and single spore cultures were obtained. Each isolate was confirmed to be *P. expansum* by sequencing of three DNA bar code genes. Elucidation of genome sequences and analysis of resistant phenotypes is underway. One genome has been assembled with a fully sensitive phenotype and 3 other *P. expansum* isolates with single and double resistance to TBZ and FLU that have been sequenced. Assembly and annotation of these genomes is in progress.
3. **Assess the efficiency of various approaches to mitigate resistance in *Penicillium* spp.** Due to limited physical resources and access to commercial facilities due to the ongoing pandemic, we could not accomplish annual and two-year fungicide rotation experiments. However, we screened several chemo-sensitizing agents (CSA) to be used in mitigation strategies and identified four CSAs that are being further evaluated.

Significant findings

- Fifteen *Penicillium expansum* isolates with resistance to single-, double, or three fungicides showed some fitness penalties in vitro for some parameters but not all.
- *In vivo* trials on Fuji apples showed that fungicide- resistant isolates can outcompete sensitive isolates.
- Three major *Penicillium* species, i.e., *P. solitum*, *P. roqueforti* and P. commune, apart from *P. expansum,* are found to be abundant in the PNW packinghouses. These *Penicillium* species have different sensitivities to the current postharvest fungicides.
- Four chemo-sensitizing agents (CSA) were tested alone or mixed with current postharvest fungicides on detached fruit to control decay caused by resistant isolates. Some efficacy was seen but higher doses of CSA need to be tested in the future.
- Of the 18 isolates from the Mid-Atlantic region, 15 were *P. expansum,* two were *P. solitum* and one isolate was *P. paneum*
- Whole genome sequence data has been obtained for a total of 36 isolates encompassing fully sensitive, single, and double, and triple resistant *P. expansum* isolates. A mutation (E198K) was found to correlate with TBZ resistance in *P. solitum* and was not observed in our samples representing *P. expansum*.
- None of the isolates examined at the genome level contained known mutations in CYP51A1 that correlate with difenoconazole resistance.
- Known mutations in the Mrr1 or MDL1 genes, that correlate with multiple drug resistance phenotypes, were not discovered.
- Eighteen isolates from PNW have an intact patulin gene cluster indicating their potential to produce this harmful toxin. Thirteen out of fifteen *P. expansum* isolates and one *Penicillium paneum* from Mid Atlantic area have an intact patulin cluster.

Results and discussion

1. Fitness evaluations

We have selected 15 *Penicillium expansum* isolates isolated from decayed apples collected from packinghouses in the pacific northwest (PNW). These isolates exhibited sensitivity or single, double, or triple resistance to thiabendazole (TBZ), pyrimethanil (PYR), and fludioxonil (FDL). Isolate fitness was evaluated both *in vitro* and *in vivo*. *In vitro* experiments were carried out to compare the ability of the 15isolates to germinate, grow, and sporulate under different conditions at 35°F and 72°F. As shown in Table 1, most resistant isolates had lower germination than the sensitive isolates om different agar media (IM. PDA, and WA) regardless of the temperature. The mycelial growth of the resistant isolates does not seem to be affected at 72°F and is slightly affected for some isolates at 35°F (Table 1). The ability of resistant isolates to survive under dry conditions (water stress) and oxygen imbalance (oxidative stress) were measured on PDA in vitro and showed that dual and triple resistant may incur fitness penalties compared to sensitive and single-resistant isolates (Table 1) and would not survive under such harsh conditions.

Table 1. Change in conidial germination and mycelial growth abilities of single, dual and trip resistant isolates *in vitro* in comparisons with sensitive isolates.

			Change in resistant isolate fitness relative to the sensitive isolates *in vitro*														
			Germination						Mycelial growth								
			72°F			35°F			72°F			35°F			Osmotic stress		Oxidative stress
Sensitivit Group	Sensitivity Phenotype	Isolate	IM	PDA	WA	IM	PDA	WA	PDA	AJA	IM	PDA	AJA	IM	72°F	35°F	72°F
Single-Resistant	TBZ^{R}PYR^{S}FDL^S	*Pe* -1	0%	-4%	-22%	-10%	4%	-15%	0.2	0.4	0.2	-0.5	-0.1	-0.2	40.98%	-6.25%	19.05%
		Pe -2	0%	0%	-16%	-20%	9%	-32%	0.7	0.4	0.0	-0.4	-0.2	-0.2	21.31%	-2.50%	24.34%
		Pe -3	0%	1%	-11%	-20%	-14%	-34%	0.9	0.6	0.0	0.1	0.1	0.0	32.79%	-27.50%	37.04%
Dual-Resistant	TBZ^{R}PYR^{R}FDL^S	*Pe* -4	0%	-1%	-5%	-4%	-13%	-23%	0.1	0.1	0.3	-0.5	0.0	0.0	42.62%	35.00%	-1.59%
		Pe -5	0%	-1%	-25%	-15%	-13%	-26%	0.3	0.6	0.3	-0.2	0.2	-0.1	34.43%	2.50%	10.58%
		Pe -6	0%	0%	-19%	-16%	-16%	-31%	0.3	0.6	0.2	0.0	0.0	-0.1	-77.05%	-10.00%	17.20%
	TBZ^{S}PYR^{R}FDL^R	*Pe* -7	0%	0%	-10%	17%	42%	-26%	0.0	0.0	-0.1	0.2	0.0	0.1	-100.00%	-45.00%	13.23%
		Pe -8	0%	-2%	-9%	7%	7%	-17%	0.2	0.3	0.0	-0.2	0.1	0.1	-65.57%	-57.50%	-40.21%
		Pe -9	0%	0%	-19%	6%	-4%	-36%	0.1	0.3	0.0	0.2	0.2	0.0	24.59%	22.50%	-73.28%
Triple-Resistant	TBZ^{R}PYR^{R}FDL^R	*Pe* -8	0%	0%	-26%	0%	-5%	-25%	0.3	0.4	0.4	-0.7	-0.2	0.1	14.75%	-73.75%	11.64%
		Pe -9	0%	-1%	-19%	-10%	1%	-25%	-0.2	0.0	0.2	0.3	0.6	0.5	8.20%	-80.00%	-56.88%
		Pe -10	0%	1%	-17%	-15%	-8%	-25%	0.4	0.6	0.2	0.6	0.6	0.3	-49.18%	-77.50%	-83.86%

Blue and green colors indicate fitness loss and gain, respectively. Values in each case indicate the change in % (germination) or in cm (for growth) relative to the control.

The virulence of 15 *P. expansum* isolates was assessed *in vivo* on Fuji apples by measuring lesion diameter after 90 days at 35°F. Results show a decreased decay severity in triple and dual-resistant isolates (Figure 1).

Virulence of the same isolates was also tested on Gala, Honeycrisp, Granny Smith, and WA 38. Overall, Gala and Granny Smith were the least susceptible (data not shown).

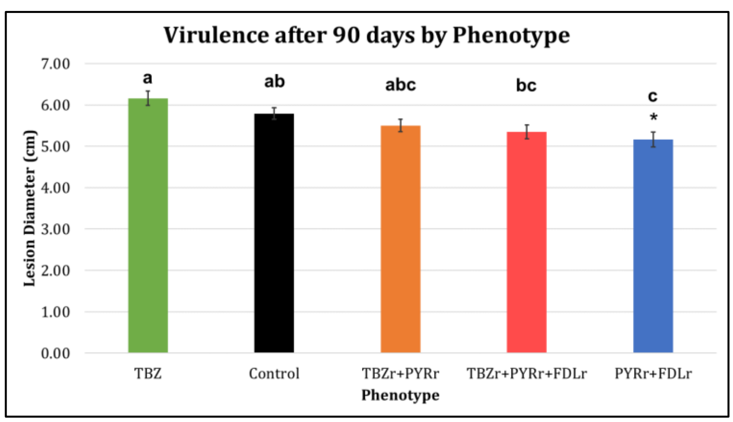

Figure 1. Lesion diamaters (cm) caused on Fuji apples after 90 days of incubation by sensitive and resistant isolates of *P. expansum*.

Activity 2.1. The genetic makeup of *Penicillium* isolates

A total of 644 *Penicillium* isolates that did not exhibit characteristics "*expansum-like"* symptoms when grown on fungal isolation media were characterized using visual distinctions that included the predominant color on PDA, CYA, YES, and MEA after 10 days (colorless, green tint, or orange), the color of the fungal colony (dark green, tan/green, or cream), colony appearance (flat or raised), size of the colony (<2 cm, 2.5-3 cm or >3.5 cm), and the color of the colony on the reverse side of the plate. Based on these criteria, isolates were sequenced using 3 genetic markers for species identification. The DNA sequencing results confirmed that the predominant *Penicillium* species in the PNW was *P. expansum* followed by *P. solitum, P. roqueforti, and P. commune.*

Isolates from these major species were tested for fungicide sensitivity to four postharvest fungicides, i.e., fludioxonil (FDL), pyrimethanil (PYR), thiabendazole (TBZ) and difenoconazole (DIF). The results indicated that a large percentage of major "non-expansum" species developed a high level of tolerance to FDL, TBZ, and PYR but not to DIG.

Activity 2.2. Elucidate whole genome sequences of *Penicillium* isolates with different fungicide resistance phenotypes.

We have identified isolates with varying levels of resistance to postharvest fungicides (Tables 2). These isolates were obtained from commercial packinghouses in WA, OR, PA and MD from infected fruit and cull piles. Single spore isolates were obtained, and glycerol stocks were preserved for each isolate. High quality genomic DNA was isolated for each isolate and quantified using gel and spectrophotometric methods. Intact DNA was then used to make libraries for NGS Illumina HiSeq 150bp paired end reads. Twenty-nine isolates have their genomes sequenced, assembled and annotated. Common mutations in B-tub locus have been identified and correlate 100% with resistance phenotypes. We have observed no mutations in the CY51A1 genomes of these 29 isolates, so they should be controlled by postharvest fungicides containing difenoconazole labeled for pome fruit (e.g. Academy). No mutations in common genes (MDL1, Mrr1) were detected as well. Most of these isolates have intact patulin gene cluster and, therefore, are expected to be active producers of patulin.

Table 2. Isolates *P. expansum* obtained from commercial storage in the Mid-Atlantic (MD, PA, WV) and Pacific Northwest (WA, OR) regions for their fungicide phenotypes and whole genome sequence analysis.

Region	Isolate #	Phenotype	Total Number of Raw Sequences	GC% Total Reads	Mutation in B-tubulin
Mid-Atlantic	ARS1	TBZ^{R}PYR^{S}FDL^{S}	15,864,382	47.4	Yes (E198V, L240F)
	ARS2	TBZ^{R}PYR^{S}FDL^{S}	15,779,790	47.3	Yes (E198V, L240F)
	ARS3	TBZ^{S}PYR^{S}FDL^{R}	16,053,534	46.7	No
	ARS6	TBZ^{S}PYR^{R}FDL^{S}	16,070,506	47.1	No
	ARS11	TBZ^{R}PYR^{s}FDL^{R}	16,187,888	46.8	Yes (E198A)
	ARS15	TBZ^{S}PYR^{S}FDL^{S}	17,755,220	47.2	No
	ARS16	TBZ^{S}PYR^{S}FDL^{S}	16,460,740	46.6	No
PNW	219	**TBZ**^{R}PYR^{S}FDL^{S}	16,053,974	47.0	Yes (E198V, L240F)
	184	**TBZ**^{R}PYR^{S}FDL^{S}	16,178,330	46.9	Yes (E198V, L240F)
	23	**TBZ**^{R}PYR^{S}FDL^{S}	16,280,196	47.1	Yes (E198V, L240F)
	2570	TBZ^{S}PYR^{S}**FDL**^{R}	16,042,060	47.2	No
	2558	TBZ^{S}PYR^{S}**FDL**^{R}	16,062,764	47.7	No
	2555	TBZ^{S}PYR^{S}**FDL**^{R}	16,101,260	47.2	No
	2483	**TBZ**^{R}**PYR**^{R}FDL^{S}	16,052,898	47.1	Yes (E198V, L240F)
	2311	**TBZ**^{R}**PYR**^{R}FDL^{S}	16,301,890	47.5	Yes (E198V, L240F)
	8	**TBZ**^{R}**PYR**^{R}FDL^{S}	16,306,660	47.0	Yes (E198V, L240F)
	2501	TBZ^{s}**PYR**^{R}**FDL**^{R}	16,037,532	47.0	No
	153	TBZ^{s}**PYR**^{R}**FDL**^{R}	15,117,556	47.6	No (G235G, silent)
	2517	TBZ^{s}**PYR**^{R}**FDL**^{R}	16,045,202	47.6	No
	164-5-48	**TBZ**^{R}PYR^{s}**FDL**^{R}	16,029,930	47.4	Yes (E198K)
	164-4-39	**TBZ**^{R}PYR^{s}**FDL**^{R}	16,152,548	47.1	Yes (E198K)
	162-5-42	**TBZ**^{R}PYR^{s}**FDL**^{R}	16,000,486	47.4	Yes (E198K, L240F)
	3045	**TBZ**^{R}**PYR**^{R}**FDL**^{R}	16,184,410	47.2	Yes (F167Y), G235G*
	2754	**TBZ**^{R}**PYR**^{R}**FDL**^{R}	15,118,376	46.8	Yes (F167Y), G235G*
	1020	**TBZ**^{R}**PYR**^{R}**FDL**^{R}	16,135,502	47.1	Yes (E198V, L240F)
	1267	TBZ^{S}PYR^{S}FDL^{S}	16,203,278	47.3	No
	40	TBZ^{S}PYR^{S}FDL^{S}	16,039,432	46.9	No
	3339	TBZ^{S}PYR^{S}FDL^{S}	16,024,584	47.1	No

*: silent mutation, not associated with fungicide resistance

Activity 3.2. Chemo-sensitizing approaches to mitigate fungicide resistance in *Penicillium* spp.

Of the eight chemo-sensitizing agents **(**CSA) tested initially *in vitro*, the four most effective ones, i.e., cinnamaldehyde, carvacrol, octyl gallate, and thymol, were tested solo or in tank-mix with FDL, PYR, or TBZ on detached Fuji apples inoculated with spore suspensions of four P. expansum isolates with different sensitivity phenotypes to FDL, PYR, and TBZ. Cinnamaldehyde, carvacrol, octyl gallate, and thymol applied solo at 100, 500, 100, and 500 ppm, respectively, showed little efficacy against the 3 isolates after 4 months of storage at 35°F except for oxyl-galate which showed some reduction of the triple-resistant isolate Pe1020 (Table 3). Oxyl-galate and cinnamaldehyde significantly reduced blue mold incidence of the TBZ-resistant isolate P23, whereas all CSAs tank-mixed with pyrimethanil reduced blue mold incidence of the triple-resistant Pe1020. Fludioxonil alone or in tank-mixes was fully effective. This trial indicates some potential for the CSAs to reduce incidence of resistant populations but additional tests including different doses and additional *Penicillium* and *Botrytis* isolates will be needed.

Table 3. Blue mold incidence on Fuji apples treated with different fungicides and chemo-sensitizing agents and inoculated with different *Penicillium expansum* isolates after 4 months of storage at 35°F.

	Isolate			
	Pe1267	**Pe23**	**Pe08**	**Pe1020**
Treatment\ Phenotype	**TBZ PYR FDL**	**TBZ* PYR FDL**	**TBZ PYR* FDL**	**TBZ* PYR* FDL***
Control	**100.0 a**	**100 a**	**100 a**	**100 a**
TBZ	16.7 bc	91.7 ab	91.7 a	91.7 a
PYR	0.0 c	0 c	100 a	16.7 cd
FDL	0.0 c	0 c	0 b	0 d
Thymol	100.0 a	91.6 ab	100 a	100 a
Carvacrol	83.3 a	100 a	83.3 a	100 a
Octyl gallate	83.3 a	91.7 ab	100 a	66.7 ab
Cinnamaldehyde	100.0 a	91.7 ab	100 a	100 a
TBZ+ Thymol	33.3 b	100 a	100 a	100 a
TBZ+ Carvacrol	0.0 c	75 ab	91.7 a	83.3 ab
TBZ+ Octyl gallate	0.0 c	16.7 c	83.3 a	66.7 ab
TBZ+ Cinnamaldehyde	0.0 c	66.7 b	100 a	83.3 ab
PYR + Thymol	0.0 c	0 c	100 a	0 d
PYR + Carvacrol	0.0 c	0 c	100 a	0 d
PYR + Octyl gallate	0.0 c	0 c	100 a	0 d
PYR + Cinnamaldehyde	0.0 c	0 c	91.7 a	50 bc
FDL + Thymol	0.0 c	0 c	0 b	0 d
FDL + Carvacrol	0.0 c	0 c	0 b	0 d
FDL + Octyl gallate	0.0 c	0 c	0 b	0 d
FDL + Cinnamaldehyde	0.0 c	0 c	0 b	0 d

Asterisks next to each fungicide indicate that the isolate is resistant to it. Values within the same column followed by different letters are significantly different.

Executive summary

Project Title: Understand and mitigate fungicide resistance in *Penicillium* spp.

Key words: Blue mold, new species, non-expansum, genome, chemo-sensitizers.

Abstract:

Blue mold of apples is a major threat to apples in storage. In this three years project, we conducted a risk assessment study to assess whether populations of the blue mold fungus *Penicillium expansum* that acquired resistance to one, two, or three postharvest fungicides could cause a greater or lower risk to the packers. We have shown that the resistant populations may endure some fitness penalty which does not seem to prevent them from being as virulent as the sensitive populations on detached fruit. This warrants the implementation of adequate resistant mitigation approaches to reduce risks of control failure. We also investigated whether other *Penicillium* species other than P. expansum can cause a greater risk for fruit packers. We have identified 13 different *Penicillium* species, of which three species are widespread, that can cause blue mold on apples. Most isolates from the 13 *Penicillium* species showed high *in vitro* tolerance to the three fungicides most commonly applied to fruit at harvest. Preliminary detached fruit data indicate that these *Penicillium* species are less virulent than *P. expansum* after two months in storage on apples treated with thiabendazole, pyrimethanil or fludioxonil. Whether their virulence increases after 9 to 12 months is being studied. We used whole genome sequencing to obtain full sequences of 36 *P. expansum* isolates from the west and east coasts and their genomes are being annotated. The knowledge will serve to develop molecular tools for detection of resistant populations in the future. In an effort to help mitigate resistant populations of *P. expansum* to thiabendazole, pyrimethanil and fludioxonil, we have tested eight chemo-sensitizing agents (CSAs) *in vitro* of which four were tested on apple fruit. Results indicate that tank-mixing the three fungicides to which resistance is observed with some of the CSAs could potentially enhance their control although additional studies are warranted to optime dosage of the CSAs.

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

Report Type: Final

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Cooperators: Allan Brothers. Inc., Stemilt Growers LLC., Guardian Manufacturing, Inc. AgroFresh Inc.

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $118,779
Total Project Request for Year 2 Funding: $121,797
Total Project Request for Year 3 Funding: $125,404

WTFRC Collaborative Costs:

Item	2018	2019	2020
Salaries	4,141	4,224	4,308
Benefits	1,367	1,394	1,422
Wages	4,500	4,703	5,267
Benefits	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

Budget 1
Primary PI: Meijun Zhu
Organization Name: Washington State University
Contract Administrator: Anastasia Kailyn Mondy
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Item	2018	2019	2020
Salaries	37,124	38,609	40,154
Benefits	12,412	12,909	13,424
Wages	15,340	15,953	16,592
Benefits	1,094	1,137	1,183
Equipment	0	0	0
Supplies	25,500	25,500	25,500
Travel	2,000	2,000	2,000
Miscellaneous	5,000	5,000	5,000
Plot Fees	0	0	0
Total	98,470	101,108	103,853

OBJECTIVES

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

SIGNIFICANT FINDINGS

1. A higher die-off rate of *Listeria innocua* on fresh apples was evident within the initial 6 weeks, irrespective of the apple varieties tested (Granny Smith, Red Delicious, and Cosmic Crisp).

   After 6 weeks of cold storage under either commercial RA or CA conditions, Granny Smith and Red Delicious apples exhibited a 1.5-2.0 log reduction in *L. innocua*, while Cosmic Crisp apples showed a slightly higher reduction of 2.8-2.9 logs.
2. Following the initial 6 weeks of RA or CA storage, the die-off rate of *Listeria* on apples diminished. After 36 weeks of CA storage, there was a 2.2-3.0 log reduction on Granny Smith and Red delicious apples and 3.4 log reduction on Cosmic Crisp apples.
3. The die-off rate of *Listeria* on apples of the selected varieties under CA storage with ozone gas was comparable to that observed during RA/CA storage over the initial 6 weeks of storage. During this period, the concentration of ozone gas steadily increased, eventually reaching the targeted concentration.
4. The introduction of ozone gas promotes the die-off of *Listeria* during the 6-24 weeks of storage, irrespective of 1-MCP pretreatment and the apple varieties tested (Granny Smith, Red Delicious, and Cosmic Crisp).
5. The application of ozone gas during a 36-week storage period resulted in an additional reduction of 2.5~3.0 log CFU/apple on Granny Smith and Red Delicious apples compared to CA storage alone.
6. In Cosmic Crisp apple storage study, gaseous ozone was only applied during the initial 24 weeks, followed by standard CA conditions for an additional 12 weeks. This approach achieved a comparable anti-*Listeria* efficacy to Granny Smith and Red Delicious apples treated with 36 weeks of ozone gas application. These findings suggest that the duration of ozone gas application can be shortened to 24 weeks in future industry application.
7. Ozone gas at 50-87 ppb exhibited comparable antimicrobial efficacy across all tested apple varieties. This suggests that a lower concentration within this range could be considered for practical applications in apple industry.
8. The initial indigenous yeast/mold counts of uninoculated apples across the tested apple varieties ranged from 4.5-5.0 log_{10} CFU/apple. These counts remained stable during the initial 12 weeks of RA regardless of apple varieties. By 24 weeks of storage and beyond, the yeast/mold counts on apples under RA were higher for Granny Smith and Cosmic Crisp apples or similar for Red Delicious apples compared to those during CA storage. The application of low doses of ozone gas decreased yeast/mold counts on apples.
9. Continuous low-dose ozone gas application for 9 months or 6 months at 50-87 ppb did not cause adverse effects on fruit quality or the occurrence of internal and external disorders for all tested apple varieties. However, Granny Smith apples under CA storage could develop ozone burn-like symptoms.
10. *E. faecium* NRRL B-2354 proves a suitable surrogate of *L. monocytogenes* for apple cold storage study.

E. faecium NRRL B-2354 displays survival profiles comparable to *L. innocua* during 36 weeks of RA and CA storage of Cosmic Crisp apples. However, it demonstrates greater resistance to gaseous ozone treatments, regardless of ozone concentration and MCP treatment, in comparison to *L. innocua* on Cosmic Crisp apples.

11. Detailed findings related to Granny Smith apples and Red Delicious apples are available in the published papers by Shen et al (2021) and Sheng et al (2022).

METHODS

<u>Objective 1. Assess the fate of *Listeria* on apple surfaces stored under RA and CA with continuous low doses of ozone.</u>

1. Strain, inoculum preparation, inoculation, and establishment on the apple surface

E. faecium NRRL B-2354 was acquired from the USDA-ARS culture collection located in Peoria, Illinois. For *L. innocua* storage study, a 3-strain *L. innocua* cocktail was utilized, including *L. innocua* NRRL B-33197 (USDA-ARS culture collection), *L. innocua* isolates from the Avocado facility and the Apple facility-Bidart (acquired from Dr. Trevor Suslow, University of California). This cocktail was prepared by mixing equal numbers of each respective strain into a suspension. Unwaxed and unbruised apples of the selected varieties at commercial maturity were individually and separately inoculated to establish 1×10^6 CFU/apple of 3-strain *Listeria* cocktail or *E. faecium* NRRL B-2354 inoculum through dipping inoculation. The inoculated apples were then held at room temperature for 24 h prior to various storage storages.

2. Cold storage treatments in a commercial packing facility

Apples of selected varieties, inoculated with $\sim1\times10^6$ CFU/apple of *L. innocua* or *E. faecium* NRRL B-2354, were randomly separated and assigned into six groups and subjected to three different storages: refrigerated air (RA, 1 °C/ 33 °F), controlled atmosphere (1 °C/ 33 °F, 2 % O_2, 1 % CO_2) treated with (CAMCP) or without 1-methycyclopropene (CA), CA with a low dose gaseous ozone and MCP-1 treatment (CAMCPLowPO_3), CA with high dose gaseous ozone with (CAMCPHigh O_3) or without MCP-1 treatment (CAHighO_3) for up to 36 weeks.

In Cosmic Crisp apple storage study, gaseous ozone application was limited to the initial 24 weeks of storage, followed by standard CA for an additional 12 weeks. Apples under different storage conditions were sampled at 0, 3-, 6-, 12-, 18-, 24-, 30-week, and 36-week of storage, when the counts of *L. innocua* or *E. faecium* NRRL B-2354 survived on apples were enumerated.

3. Microbial analysis

On each sampling day, apples under the respective storage condition were sampled and transferred to sterile Whirl-Pak bags containing 10 ml of 0.1% buffered peptone water. The apples were then gently rubbed to release attached microorganisms, then the resulting microbial suspension was subjected to serial diluted. Appropriate dilutions were plated on agar plates. Plates were incubated at 35˚C (95˚F) for 48h and enumerated manually. Enrichments were done when bacterial levels were under the detection limit of 10 CFU/apple following our previous publication (Sheng et al., 2018).

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

1. Cold storage treatments in a commercial packing facility

Non-waxed, uninoculated apples of the selected cultivars were subjected to different storage conditions (RA, CA, CAMCP, CAMCPLowO_3, CAMCPHingO_3, CAHingO_3) as described previously. Apples were sampled at 0-, 6-, 12-, 24, and 36 weeks of storage for total plate count and yeast and mold enumeration.

2. Survival microorganism analysis

On 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, followed by serial dilution. The appropriate dilution was plated onto TSAYE plates for total plate count (TPC) and potato dextrose agar (PDA) plates for yeasts and molds, respectively per our established methods (Shen et al., 2019; Sheng et al., 2018; Sheng et al., 2020). 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 (TSS), and titratable acidity (TA) were performed at harvest, after 6-month and 9-month storage per our established methods (Sheng et al., 2018). 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 the sun and shade side of the apples. Total soluble solids were evaluated using Atago PR-32 digital Brix refractometer. The titratable acidity 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 for external disorders and 7 days at room temperature 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.

RESULTS AND DISCUSSION

1. Survival of *L. innocua* on apples of selected varieties under storage conditions with or without low-dose ozone gas.

The survival of *L. innocua*, initially inoculated at levels of 6.0-6.5 Log10 CFU/apple, on Granny Smith, Red Delicious, and Cosmic Crisp apples were investigated under refrigerated air (RA), controlled atmosphere (CA), and CA conditions with varying doses of O3 gas (51-87 ppb).

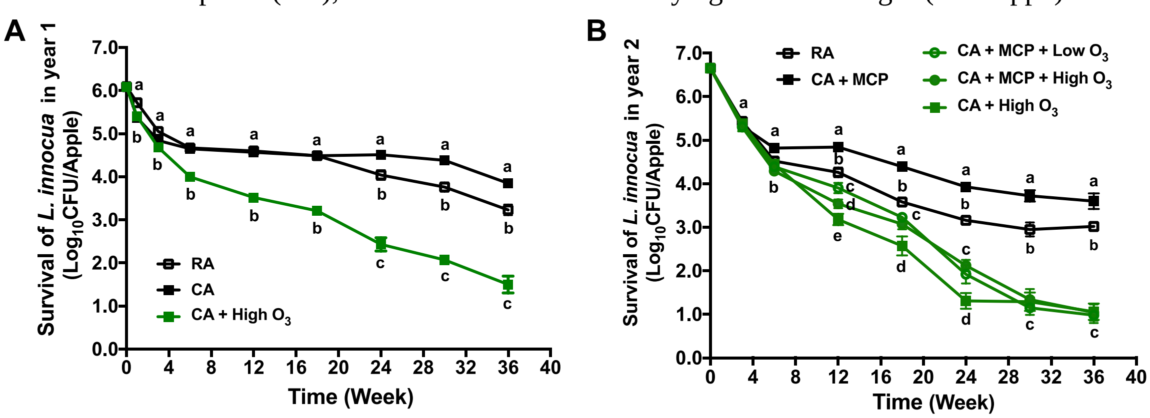


Figure 1. Survival of *Listeria* on Granny Smith apple under commercial cold storages. RA: refrigerated atmosphere (33°F); CA: controlled atmosphere (33°F, 2% O2, 1% CO2). Year1, CA + High O_3: CA with 87 ± 38.8 ppb ozone gas; Year 2, CA + Low O_3; Ozone gas concentration at 51 ± 5 ppb; CA + High O_3: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

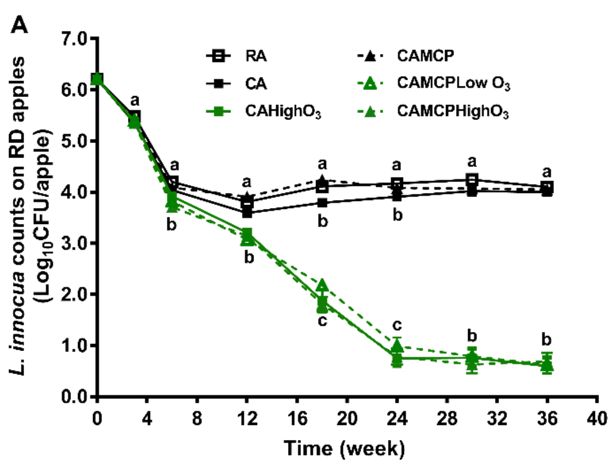
1.1 Survival of *L. innocua* on Granny Smith apples

Fates of *L. innocua* inoculated on Granny Smith apples at a level of 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 during two cropping seasons. Over the 3-week cold storage, *L. innocua* was reduced by 1.0-1.5 $\log_{10}$ CFU/apple on Granny Smith apples 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 a 2.9-3.5 log reduction of *L. innocua* on Granny Smith apples over 36 weeks of cold storage under a commercial RA environment, and a 2.2-2.7 log reduction under CA storage conditions (Figure 1) (Sheng et al., 2022). Continuous low dose ozone gas application in CA storage generated additional ~2-log reduction of *L. innocua* on Granny Smith apples. The test range of ozone gas doses (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 that in the CA+ High O3 group (Figure 1B) (Sheng et al., 2022).

1.2 Survival of *L. innocua* on Red Delicious apples

Studies on Granny Smith apples revealed that continuous low dose ozone gas application in CA cold storage effectively eliminates or controls *L. innocua*, resulting ~ 5 Log_{10} CFU/apple reduction. We further evaluated efficacy of low dose ozone gas against *L. innocua*, on Red Delicious apples. Results revealed a reduction of 0.7-0.9 $\log_{10}$ CFU/apple on Red delicious apples stored in RA, CA, and CA plus different doses of O_3 with a die-off rate of 0.24-0.29 $\log_{10}$ CFU/apple/week over 3 weeks of cold storage (Figure 2) (Shen et al., 2021), which is smaller than that observed on Granny Smith apples (Sheng et al., 2022). There was ~2.2 Log_{10} CFU/apple reduction of *L. innocua* on Red Delicious apples over 36 weeks of cold storage under a commercial RA and CA storage environment. This reduction was smaller, especially in RA storage, compared to that in Granny Smith apples (Sheng et al., 2022). In comparison to Granny Smith apples, low dose ozone gas at similar dose (60-80 ppb for Red Delicious apples vs 51-87 ppb for GSA) was more effective against *L. innocua* on Red Delicious apples (Figure 1-2) (Shen et al., 2021; Sheng et al., 2022). An additional 3.3-3.4 Log_{10} CFU/apple reduction was observed compared to RA or CA storage. MCP-1 treatment prior to storage had no effects on *L. innocua* survival on Red Delicious apples ($P > 0.05$) (Figure 2), while MCP-1 application in CA room slightly decreased anti-*Listeria* efficacy of ozone gas on Granny Smith apples (Sheng et al., 2022).



B

Reduction of *L. innocua* during storage ($\log_{10}$CFU/apples)

		CA		CA+1-MCP		
Week	RA	0	High O_3	0	Low O_3	High O_3
0	0.00	0.00	0.00	0.00	0.00	0.00
3	0.73	0.86	0.83	0.80	0.81	0.86
6	2.01	2.17	2.29	2.12	2.39	2.50
12	2.40	2.62	3.00	2.30	3.14	3.07
18	2.10	2.42	4.33	1.97	4.03	4.40
24	2.04	2.30	5.46	2.13	5.22	5.43
30	1.97	2.19	5.45	2.14	5.42	5.58
36	2.15	2.21	5.61	2.18	5.59	5.52

Figure 2. **Survival of *L. innocua* on Red Delicious apples during 36-week of commercial cold storage.** A. *L. innocua* count on apples over cold storage period. Mean ± SEM, n = 32-40. Different letters (a-c) at each sampling point indicated significant differences ($P < 0.05$); B. Reduction of *L. innocua* on apples under different storages. Mean averaged from 32-40 apples. RA: refrigerated atmosphere; CA: controlled atmosphere; MCP: apples were treated with 1-methycyclopropene before subjecting to cold storage; CAHighO3: CA storage with continuous gaseous O_3 application at 78.7 ± 13.2 ppb; CAMCPLowO3: CA storage with continuous gaseous O_3 application at 60.2 ± 5.7 ppb, where apples were treated with 1-methycyclopropene treatment before subjecting to storage.

1.3 Survival of *L. innocua* on Cosmic Crisp apples

Data collected from Fuji (Sheng et al., 2018), Red Delicious (Sheng et al., 2022), and Granny Smith (Sheng et al., 2022) apples revealed that the most substantial reduction in *L. innocua* counts occurred within a 24-week storage period. Therefore, in this study, a low-dose continuous gaseous ozone was applied only during the initial 24 weeks of CA storage, followed by an additional 12 weeks of CA storage. The survival *L. innocua* on Cosmic Crisp apples were evaluated throughout the 36-week storage under different conditions.

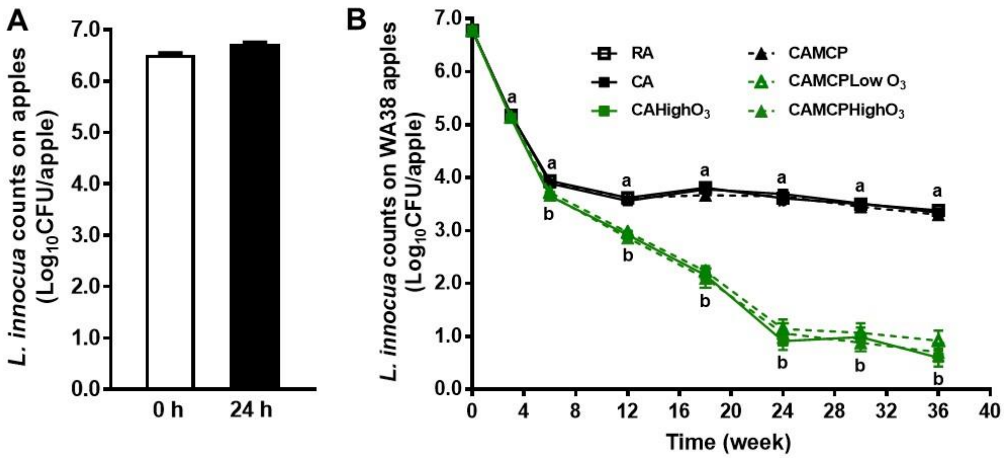


Figure 3. Fates of *L. innocua* on Cosmic Crisp apples during 36 weeks of cold storage under different storage regimes. A. The initial bacterial population on apples; B. Survival of *L. innocua*; RA: refrigerated atmosphere; CA: controlled atmosphere; MCP: apples were treated with 1-methycyclopropene prior to cold storage; CAHighO$_3$: CA storage with continuous gaseous O_3 application at 78.2 ± 12.2 ppb; CAMCPHighO$_3$: CA storage with continuous gaseous O_3 application at 78.2 ± 12.2 ppb, where apples were treated with 1-methycyclopropene prior to cold storage; CAMCPLowO$_3$: CA storage with continuous gaseous O_3 application at 55.5 ± 8.8 ppb, where apples were treated with 1-methycyclopropene prior to cold storage. Different letters (a-b) at each sampling point indicated significant differences ($P < 0.05$).

As results observed in Granny Smith and Red Delicious apples, the greater die-off rate of *L. innocua* on Cosmic Crisp apples was observed during the initial 6 weeks of storage (Figure 3). During the initial 3 weeks, before reaching the target ozone concentration, the populations of *L. innocua* on Cosmic Crisp apples exhibited a reduction of 1.6-1.7 log10 CFU/apple across all storage conditions (Figure 3). This reduction surpassed that observed on Red Delicious apples (Shen et al., 2021). Over the entire 36-week storage period, regardless of atmospheric conditions or 1-MCP treatment, there was a substantial 3.4-3.5 log10 CFU/apple reduction in *L. innocua* on Cosmic Crisp apples (Figure 3), surpassing reductions seen in Granny Smith and Red Delicious apples (Shen et al., 2021; Sheng et al., 2022).

The continuous application of low-dose ozone gas during the initial 24 weeks of CA cold storage caused an additional 2.4-2.8 $\log_{10}$ CFU/apple reduction when the ozone gas was discontinued. The anti-*Listeria* efficacy of 24 weeks of gaseous ozone application was similar to that seen in Granny Smith and Red Delicious apples treated with ozone gas for 36 weeks (Shen et al., 2021; Sheng et al., 2022). In alignment with Red Delicious apples (Shen et al., 2021), pre-storage1-methylcyclopropene (1-MCP) treatment had a minor effect on *L. innocua* survival on Cosmic Crisp apples ($P > 0.05$) (Figure 3).

2. Fates of resident microbiota on apples of the selected varieties under RA, CA, and CA with low-dose ozone gas.

Resident bacteria, mold and yeast cause postharvest decay of apples (Janisiewicz & Korsten, 2002). Therefore, the impacts of ozone gas application on the counts of resident bacteria, mold and yeast were examined on Granny Smith, Red Delicious, and Cosmic Crisp apples using uninoculated apples under the above stated various storage conditions, mirroring those applied to the inoculated apples.

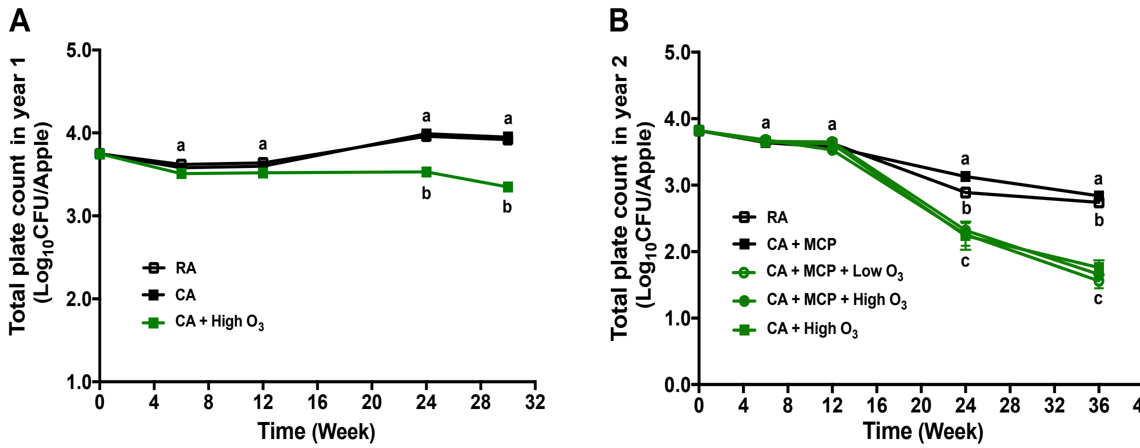


Figure 4. **Survival of resident bacteria on Granny Smith apple under commercial cold storages.** RA: refrigerated atmosphere (33°F); CA: controlled atmosphere (33°F, 2% O2, 1% CO2). A: Year1; B. Year 2; In year 1, CA + High O_3: CA with 87 ± 38.8 ppb ozone gas; In year 2, CA + Low O_3; Ozone gas concentration at 51 ± 5 ppb; CA + High O_3: Ozone gas concentration at 68 ± 7 ppb. Mean ± SEM; n = 40. Different letters (a-c) at each sampling point indicated significant differences ($P < 0.05$).

The initial total plate count for background bacteria in Granny Smith apples was 3.5-4.0 $\log_{10}$ CFU/apple, maintaining relative stable over 36-week storage at RA or CA (Figure 4) (Sheng et al., 2022). Continuous low dose ozone gas application in CA room significantly decreased resident bacteria in Granny Smith apples after 24 weeks of storage, with a more pronounced reduction observed in the year 2 study (Figure 4B) (Sheng et al., 2022).

Regarding indigenous yeast and mold (Y/M) counts, the initial level in un-inoculated Granny Smith apples were 4.5-5.0 $\log_{10}$ CFU/apple, remaining relatively stable during the first 12 weeks of storage in RA and CA conditions before gradually increased (Figure 5) (Sheng et al., 2022). By the 24th week of storage and beyond, the Y/M count in Granny Smith apples stored under RA was significantly higher than that in the CA room (Figure 5) (Sheng et al., 2022). The Y/M count in Granny Smith apples under CA storage with different doses of ozone gas decreased during the initial 24 weeks of storage, followed by a gradual increase (Figure 5) (Sheng et al., 2022).

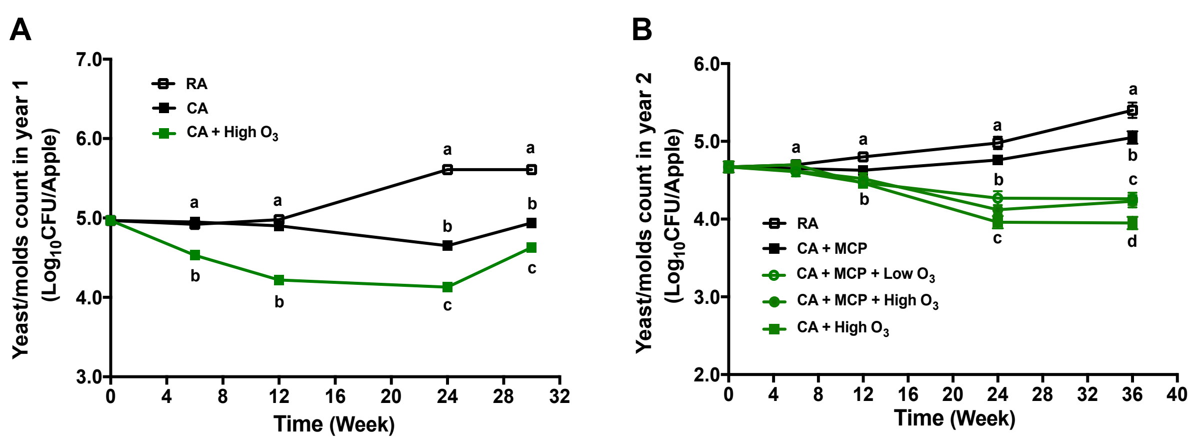


Figure 5. The yeast/mold counts of Granny Smith apple under commercial cold storages. RA: refrigerated atmosphere (33°F); CA: controlled atmosphere (33°F, 2% O2, 1% CO2). A. Year1; B. Year2. In year 1, CA + High O_3: CA with 87 ± 38.8 ppb ozone gas; In year 2, CA + Low O_3; Ozone gas concentration at 51 ± 5 ppb; CA + High O_3: Ozone gas concentration at 68 ± 7 ppb. Mean ± SEM; n = 40. Different letters (a-d) at each sampling point indicated significant differences ($P < 0.05$).

Red Delicious apples had similar initial bacterial count (~ 3.8 Log_{10} CFU/apple). It increased by one log_{10} CFU/apples after 12-week storage at RA or CA at 1°C/33°F and maintained this high level throughout 36-week storage (Figure 6A) (Shen et al., 2021). Ozone gas application at different doses in CA storage decreased resident bacteria in Red Delicious apples by 1.2-1.3 Log_{10} CFU/apple after 36 weeks of storage (Figure 6A) (Shen et al., 2021).

The initial level of indigenous Y/M counts of uninoculated Red Delicious apples was 4.7 log_{10} CFU/apple, which was similar to that of uninoculated Granny Smith apples. The Y/M count gradually increased in apples under RA and CA storages (Figure 6B). By 36-week of storage, the Y/M counts of Red Delicious apples stored under RA or CA room were increased by 1.1-1.3 Log_{10} CFU/apple (Figure 6B) (Shen et al., 2021). On the other hand, the Y/M count on Red Delicious apples gradually decreased under CA with 60-80ppb ozone gas; there was ~ 0 .7 Log_{10} CFU/apple reduction of Y/M at the end of cold storage (Figure 6B) (Shen et al., 2021).

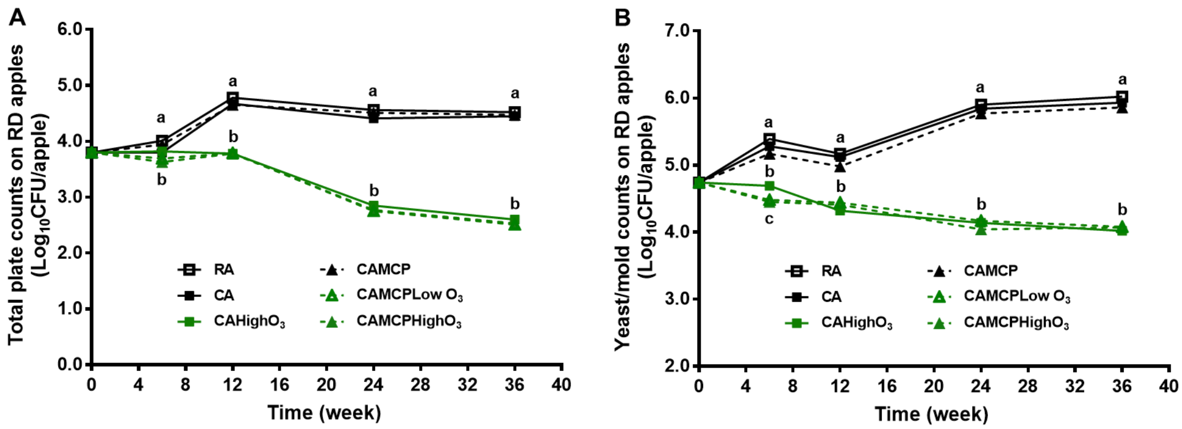


Figure 6. Resident bacteria and natural decay microorganisms on Red Delicious apples during 36 weeks of commercial cold storage. A. Total plate counts on apple over storage; B. Yeast and mold counts on apples during storage; Mean ± SEM, n = 40. Different letters (a-c) at each sampling point indicated significant differences ($P < 0.05$). RA: refrigerated atmosphere; CA: controlled atmosphere; MCP: apples treated with 1-methycyclopropene prior to cold storage; CAHighO_3: CA storage with continuous gaseous O_3 application at 78.7 ± 13.2 ppb; CAMCPHighO_3: CA storage with continuous gaseous O_3 application at 78.7 ± 13.2 ppb, where apples were treated with MCP-1 prior to different storages; CAMCPLowO_3: CA storage with continuous gaseous O_3 application at 60.2 ± 5.7 ppb, where apples were treated with 1-MCP prior to cold storage.

For Cosmic Crisp apples, the initial resident microflora and indigenous yeast/mold count in the receiving apples were 3.75 log_{10} CFU/apple and 4.87 log_{10} CFU/apple, respectively (Figure 7). These levels fall within the range observed for Granny Smith and Red Delicious apple (Shen et al., 2021; Sheng et al., 2022). The resident bacteria on Cosmic Crisp apples showed an increase of 0.6-0.7 log_{10} CFU/apple under both RA and CA storage within the first 12 weeks of storage (Figure 7A). Total plate counts on Cosmic Crisp with low dose ozone treatment were initially reduced by 1.2-1.3 log_{10} CFU/apple at 24 weeks of storage, followed by subsequent increase, although still lower than that at harvest (Figure 7A).

The Y/M counts of Cosmic Crisp apples exhibited an increase of 0.5-0.6 log_{10} CFU/apple after 36 weeks of both RA and CA storages (Figure 7B). However, Yeast/mold counts decreased by 0.2-0.4 log_{10} CFU/apple on Cosmic Crisp apples treated gaseous ozone for 24 weeks (Figure 7B).

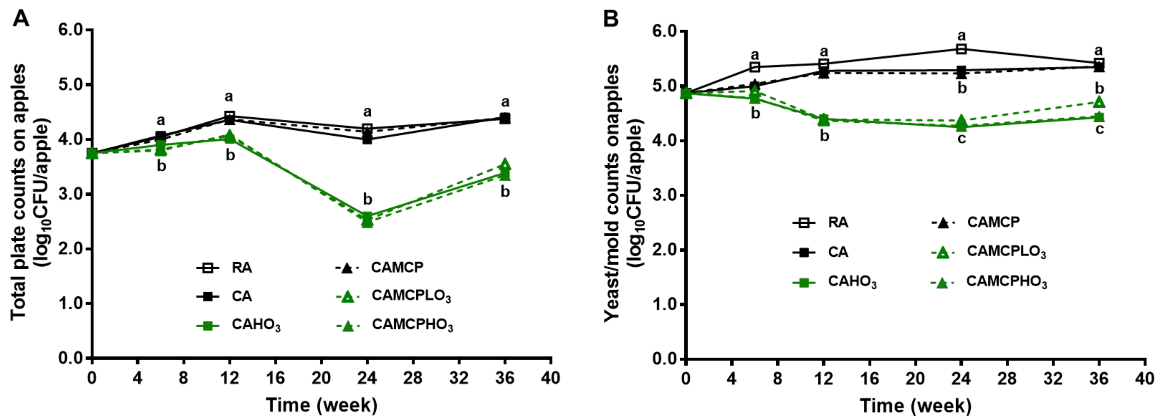


Figure 7. Resident bacteria and natural decay microorganisms on Cosmic Crisp apples during 36 weeks of commercial cold storage. A. Total plate counts on apple over storage; B. Yeast and mold counts on apples during storage; Mean ± SEM, n = 32. [a-b] Different letters (a-c) at each sampling point indicated significant differences ($P < 0.05$). RA: refrigerated atmosphere; CA: controlled atmosphere; MCP: apples were treated with 1-methycyclopropene before cold storage; CAHighO_3: CA storage with continuous gaseous O_3 application at 78.2 ± 12.2 ppb during the initial 24 weeks of storage; CAMCPHighO_3: CA storage with continuous gaseous O_3 application at 78.2 ± 12.2 ppb during the initial 24 weeks of storage, where apples were treated with MCP-1 before different storages; CAMCPLowO_3: CA storage with continuous gaseous O_3 application at 55.5 ± 8.8 ppb during the initial 24 weeks of storage, where apples were treated with 1-MCP before cold storage.

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

Fruit quality is a crucial factor influencing consumer preference. CA storage is known to help maintain fruit quality for extended periods (Tasdelen & Bayindirli, 1998) and retard apple decay during prolonged storage (Xuan & Streif, 2005). Improper cold storage can resulted in a significant decline in fruit quality (Davis & Blair, 1936). The aforementioned data suggest the potential efficacy of low-dose ozone gas in *Listeria* control; however, uncontrolled or excessive ozone gas administration may lead to ozone burn and quality deterioration, resulting in substantial financial losses. Therefore, apple fruit quality parameters were evaluated both at harvest and after storage.

For Granny Smith apples, both years of study revealed that 6 months of RA storage resulted in a significant reduction of firmness and TA (Sheng et al., 2022). CA storage alone significantly mitigated the loss of firmness and TA. The incorporation of ozone gas in CA storage had a minor impact on Granny Smith apple quality, while the 1-MCP treatment further reduced the loss of firmness and TA in CA storages, independent of ozone gas (Sheng et al., 2022). In terms of physiological disorders, CA storage with or without ozone reduced the incidence of superficial scald and lenticel decay in year 1(Sheng et al., 2022). In year 2, although CA+HO_3 storage alone failed to inhibit superficial scald, 1-MCP pre-storage treatment significantly reduced the incidence of superficial scald on GSA in CA storage with or without ozone (Sheng et al., 2022). However, in the year 2 studies, apples under CA storage showed ozone burn-like symptoms, and apples treated with 1-MCP, and ozone gas showed a higher ozone burn-like symptom (Sheng et al., 2022). To confirm the observed symptoms, we conducted another quality evaluation in year 3, and no ozone burn-like symptoms were observed on any apples regardless of treatment. (Sheng et al., 2022). 1-MCP treatment of Granny Smith apples in CA with ozone resulted in ozone burn-like symptoms in year 2 but not in year 3, indicating the potential role of preharvest properties of apples on their reactions to ozone treatment. CA storage, regardless of ozone gas and 1-MCP treatment, significantly reduced the incidence of internal browning compared to RA storage in all three years (Sheng et al., 2022).

Red Delicious apples are particularly prone to developing watercore compared to other commercially grown apple varieties, posing an increased risk of developing internal disorders such as internal browning during long-term CA storage (Mattheis, 2008). Quality attributes of Red Delicious apple fruits under different storage conditions were further assessed both at harvest and after 6-month or 9-month storage. TSS of Red Delicious apples did not differ among storage treatments and over 9 months storage (Shen et al., 2021). Red Delicious apples subjected to RA storage exhibited significantly lower firmness and TA compared to those with CA with MCP-1 pretreatment at 6-month and 9-month storages (Shen et al., 2021). Ozone gas application significantly improved the firmness and increased TA of apples comparted to RA and CA storage (Shen et al., 2021). Both ozone gas application at 60-80 ppb did not induce ozone burn in Red Delicious apples at both 6-month and 9-month storage (Shen et al., 2021). Neither of ozone application had effects on superficial scale, lenticel decay, Russet, CO_2 damage compared to CA, all of which were significantly better than those observed in RA storage (Shen et al., 2021). Ozone applications further enhanced the visual appearance of apples (Shen et al., 2021).

Cosmic Crisp apples have garnered consumer popularity due to their appealing crisp texture, juiciness, and other desirable traits (Evans et al., 2012). These apples possess remarkable storage capabilities (Evans et al., 2012). The weight and TSS of apple fruits at 9 months of storage were not different from that at harvest, regardless of storage conditions (Table 1). The firmness of Cosmic Crisp apples, after 9 months of CA storage with or without 1-MCP and gaseous ozone treatments, was the same as that measured at harvest. However, the firmness of Cosmic Crisp apples under RA storage was significantly reduced (Table 1). TA of Cosmic Crisp apples after 9-month storage was significantly lower than that of apples at harvest, regardless of storage treatments. Gaseous ozone application had no impact on TA (Table 1). No external disorder or internal disorder was observed in any of Cosmic Crisp apples after 9 months of storage (data not shown).

Table 1. Fruit quality attributes of Cosmic Crisp apples at harvest and after storage

Treatment	**Weight (kg)**		**Firmness (kg)**		**TSS (% Brix)**		**TA (% malic acid)**	
	0-m	9-m	0-m	9-m	0-m	9-m	0-m	9-m
RA	0.26 ± 0.05^{A}	0.25 ± 0.04^{aA}	8.33 ± 0.16^{A}	6.22 ± 0.13^{aB}	NA	14.25 ± 0.25^{a}	0.77 ± 0.03^{A}	0.26 ± 0.02^{aB}
CA		0.26 ± 0.06^{aA}		8.00 ± 0.11^{bA}		15.05 ± 0.15^{a}		0.34 ± 0.02^{bB}
CAMCP		0.24 ± 0.03^{aA}		8.22 ± 0.08^{bA}		14.43 ± 0.17^{a}		0.41 ± 0.00^{bB}
CAMCPLowO_3		0.26 ± 0.04^{aA}		8.24 ± 0.09^{bA}		14.18 ± 0.15^{a}		0.37 ± 0.04^{bB}
CAMCPHighO_3		0.25 ± 0.05^{aA}		8.29 ± 0.10^{bA}		14.38 ± 0.27^{a}		0.41 ± 0.03^{bB}
CAHighO_3		0.26 ± 0.05^{aA}		8.12 ± 0.07^{bA}		14.55 ± 0.19^{a}		0.37 ± 0.09^{abB}

TSS: Total soluble solids; TA: titratable acidity. RA: refrigerated atmosphere; CA: controlled atmosphere; MCP: apples were treated with 1-methycyclopropene before cold storage; CAHighO_3: CA storage with continuous gaseous O_3 application at 78.2 ± 12.2 ppb during the initial 24 weeks of storage; CAMCPHighO_3: CA storage with continuous gaseous O_3 application at 78.2 ± 12.2 ppb during the initial 24 weeks of storage, where apples were treated with MCP-1 before different storages; CAMCPLowO_3: CA storage with continuous gaseous O_3 application at 55.5 ± 8.8 ppb during the initial 24 weeks of storage, where apples were treated with 1-MCP before cold storage. $^{a\text{-}d}$ Mean within a column of the selected quality attribute without a common letter differ significantly ($P < 0.05$). $^{A\text{-}B}$ Mean the comparison of an individual quality parameter at 0-month (at-harvest) and 9-month storage without common letter differ significantly ($P < 0.05$). Mean ± SEM, n=40.

4. *E. faecium* NRRL B-2354 is a suitable surrogate of *L. monocytogenes* for Cosmic Crisp apple during cold storage study with or without ozone gas treatment

For apples stored under regular atmosphere (RA) and controlled atmosphere (CA) conditions, the survival pattern of *E. faecium* NRRL B-2354 on Cosmic Crisp apples closely mirrored that of *L. innocua* over a 24-week storage period (Figures 3B & 8), resulting in a reduction of 3.0-3.1 log10 CFU/apple. However, when Cosmic Crisp apples underwent the gaseous ozone treatment, *E. faecium* NRRL B-2354 exhibited higher resistance to ozone treatment compared to *L. innocua* throughout 36-week storage (Figures 3B & 8). At the conclusion of the 24-week storage, upon discontinuation of the ozone gas treatment, the implementation of ozone gas led to an additional reduction of 1.1-1.2 $\log_{10}$

CFU/apple for *E. faecium* NRRL B-2354, whereas *L. innocua* exhibited a more substantial reduction of 2.4-2.7 log10 CFU/apple (Figures 3B & 8). Data indicate *E. faecium* NRRL B-2354 serves as a suitable non-*Listeria* surrogate for assessing *Listeria* behaviors during commercial cold storage.

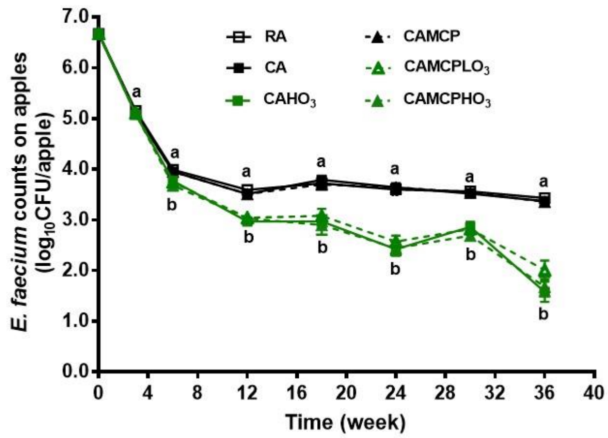


Figure 8. Fates of *E. faecium* NRRL B-2354 on Cosmic Crisp apples during 36 weeks of cold storage under different storage regimes. RA: refrigerated atmosphere; CA: controlled atmosphere; MCP: apples were treated with 1-methycyclopropene prior to cold storage; CAHighO3: CA storage with continuous gaseous O_3 application at 78.2 ± 12.2 ppb; CAMCPHighO3: CA storage with continuous gaseous O_3 application at 78.2 ± 12.2 ppb, where apples were treated with 1-methycyclopropene prior to cold storage; CAMCPLowO3: CA storage with continuous gaseous O_3 application at 55.5 ± 8.8 ppb, where apples were treated with 1-methycyclopropene prior to cold storage. Different letters (a-b) at each sampling point indicated significant differences ($P < 0.05$).

EXECUTIVE SUMMARY

This comprehensive study explores the potential of gaseous ozone in mitigating *L. innocua* contamination on Granny Smith, Red Delicious, and Cosmic Crisp apples during extended commercial cold storage. The study revealed a higher die-off rate within the initial 6 weeks, showcasing reductions of 1.5-2.0 log for Granny Smith and Red Delicious, and a slightly elevated 2.8-2.9 log for Cosmic Crisp apples. Following the initial 6 weeks, the die-off rate diminished, resulting in a 2.2-3.0 log reduction for Granny Smith and Red Delicious, and a substantial 3.4 log reduction for Cosmic Crisp apples after 36 weeks of RA and CA storage. Additionally, the study demonstrated that gaseous ozone application at 50-87 ppb is a viable technology for controlling *Listeria*, leading to an additional 2.5~3.0 log CFU/apple reduction on apples compared to CA storage alone. Given the comparable antimicrobial efficacy of ozone gas at 50-87 ppb across all apple varieties, it suggests that a lower concentration within this range could be considered for practical applications in the apple industry. Data obtained from Cosmic Crisp apple storage revealed that gaseous ozone applied for the initial 24 weeks achieved a comparable anti-*Listeria* efficacy to Granny Smith and Red Delicious apples treated with 36 weeks of ozone gas, indicating potential for shorter duration in future industry applications. Furthermore, continuous low-dose ozone gas application at 50-87 ppb decreased yeast/mold counts on apples and did not adversely affect fruit quality or lead to internal and external disorders across all tested apple varieties. Our data further indicate that. *E. faecium* NRRL B-2354 can serve as a suitable non-Listeria surrogate for *L. monocytogenes* in apple cold storage studies.

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Project/Proposal Title: Interaction of microbiome and *Listeria* on apples during cold storage

Report Type: Continuing Project Report

WTFRC Project Number: AP-20-100A

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Cooperators: Stemilt Growers LLC.; Allan Brothers Inc., Hansen Fruit

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $84,704
Total Project Request for Year 2 Funding: $86,853
Total Project Request for Year 3 Funding: $88,076
Total Project Request for 2024-2025: $ 18,000

WTFRC Collaborative Costs:

Item	2020-2021	2021-2022	2022-2023
Salaries			
Benefits			
Wages	2,509	2,548	2,588
Benefits	1,280	1,272	1,284
RCA Room Rental	6,750	6,952	7,160
Shipping			
Supplies	500	525	550
Travel	650	700	725
Plot Fees			
Miscellaneous			
Total	11,689	11,997	12,307

Footnotes:

Budget 1
Primary PI: Meijun Zhu
Organization Name: Washington State University
Contract Administrator: Anastasia Kailyn Mondy
Telephone:
Contract administrator email address: arcgrants@wsu.edu

Item	2020-2021	2021-2022	2022-2023	2024-2025
Salaries	31,500	32,760	34,070	
Benefits	11,010	11,451	11,909	
Wages	3,200	3,328	3,461	
Benefits	305	317	329	
Equipment				
Supplies	16,000	16,000	15,000	
Travel	2,000	2,000	2,000	
Miscellaneous	9,000	9,000	9,000	18,000*
Plot Fees				
Total	73,015	74,856	75,769	

Footnotes:

*: Funds are requested to partially support the procurement of autoclave and associated maintenance fees. The current autoclave has exceeded its service life and has been experiencing frequent breakdowns. This not only hampers our research progress but also incurs substantial expenses.

OBJECTIVES

1. Examine survival of resident microbiota on apple surfaces stored under RA and CA.
2. Characterize the dynamic change of dominant and differential bacterial and fungal populations in the microbiome of fresh apples in the co-occurrence of *Listeria* under RA or CA storage.

SIGNIFICANT FINDINGS

1. The *L. innocua* count was decreased by 1.5 log_{10} CFU/apple on apples under RA or CA storage.
2. *Enterobacteriaceae* had higher counts on apples contaminated with *L. innocua* than uninoculated control apples at RA or CA storage.

   The *Enterobacteriaceae* count was lower on apples under CA storage than those under RA storage.
3. The count of *Pseudomonas* decreased during 36 weeks of cold storage. Introducing *L. innocua* on apples increased the reduction of *Pseudomonas* on apples, especially under CA storage.
4. Lactic acid bacteria count on apples slightly increased after 36 weeks of cold storage regardless of storage condition.
5. Populations of native bacteria and yeast and molds, particularly *Penicillium*, were increased on apples with or without *L. innocua* inoculation after 36 weeks of RA or CA storage.
6. A 9-month CA or RA storage had a great influence on fungal community structure; these significant differences were found on the phylum level, family level, genus level, and species level.
7. Inoculation with *L. innocua* significantly impacts the fungal community on apples at the selected sampling time (before commercial storage or after 9 months of CA or RA storage).
8. *Basidiomycota* followed by *Ascomycota* are dominant fungal phyla of Fuji apples, regardless of *L. innocua* inoculation and storage condition.
9. The relative abundance of *Basidiomycota* of the non-inoculated apples decreased after 9 months of CA or RA storage while *Ascomycota* increased.
10. The relative abundance of *Basidiomycota* and *Ascomycota* in the inoculated apples remained stable after 9 months of RA storage; the relative abundance of *Basidiomycota* of inoculated apples decreased and *Ascomycota* increased after 9 months of CA storage.
11. *Bulleribasidiaceae* is the dominant family in non-inoculated apples followed by *Filobasidiaceae*. *Filobasidiaceae* is the dominant family in inoculated apples followed by *Bulleribasidiaceae* and *Pleosporaceae*. The abundances of these families changed after 9 months of CA or RA storage.
12. *Vishniacozyma* and *Filobasidium* are dominant genera in non-inoculate apples accounting for 52.7% and 25.6% of total fungal genera. *Filobasidium*, *Vishniacozyma*, and *Alternaria* are dominant genera in inoculated apples with 27.8%, 22.6%, and 21.6% relative abundance, respectively. These genera were changed after 9 months of CA or RA storage.
13. *Vishniacozyma victoriae and Filobasidium magnum* were the main species detected on non-inoculated apples and inoculated apples. The relative level of *Filobasidium magnum* content decreased after 9 months of CA and RA storage regardless of inoculation.
14. *Tausonia pullulans* level in apples was low regardless of the inoculation but was extremely elevated in the inoculated apples after 9 months of RA storage (Fig. 5), increasing from 0.1% to 26.2%.
15. The introduction *of L. innocua* led to a significant decrease in bacterial diversities and richness on apples.
16. Over the 36 weeks of CA storage, alpha diversities of bacterial communities were gradually increased in *L. innocua*-contaminated apples while decreasing on uninoculated apples.
17. Dynamic alterations were evident in bacterial communities at the phylum, family, genus, and species levels.

18. *Deinococcus*, *Hymenobacter*, *Rhodococcus* and *Pantoea* were positively correlated with *L. innocua*, while *Massilia* and *Pseudomonas* were negatively correlated with *L. innocua* on apples.

METHODS

1. Apple cultivar selection

We acknowledge that the different varieties may behave differently in terms of bacterial adhesion and dynamic change of the microbiome on their surface during cold storage. Thus, four popular varieties, Fuji, Granny Smith, Cosmic Crisp, and Pink Lady apples were used in this study.

2. Strain selection and inoculum preparation

L. innocua is a widely used nonpathogenic surrogate for *L. monocytegenes* (Sheng, Shen, & Zhu, 2020). To elucidate the impact of strain variability on their survival under cold storage, *L. innocua* isolates from Bidart apple facility and other processing plants were used to prepare a 3-strain cocktail of *L. innocua* inoculum per our well-established method (Sheng et al., 2018).

3. Inoculation

Washed and unwaxed apples of selected varieties were individually and separately inoculated to establish 1×10^6 CFU/apple using a 3-strain cocktail of *L. innocua* per our well-established method (Sheng, Edwards, Tsai, Hanrahan, & Zhu, 2017; Sheng et al., 2018; Sheng et al., 2020).

4. Cold storage treatments

Unwaxed and uninoculated or inoculated apples of selected varieties were randomly divided and subjected to well-controlled RA or CA storage for 9 months. 1% CO_2 and 1.2% O_2 were used in CA storage following the practices of commercial packing facilities for the selected varieties. A storage temperature of 33 ºF (1°C) was chosen for the selected apples. All fruits were subjected to 1-methyl cyclopropane (1-MCP, a maturation inhibitor) treatment once before they are put in their respective storage rooms.

5. Sampling during cold storage

Fruits were sampled right before storage, at 3, 6, 12, 18, 24, 30, and 36 weeks of storage. Four replicates of 10 fruits each will be used on each sampling day at each storage condition.

6. Surviving Listeria analysis

On each sampling day, four sets of 10 apple fruits under the respective storage conditions were sampled and transported to the Food Microbiology Lab on the Pullman campus of Washington State University for microbial analyses. Upon arrival, *Listeria* survival of apple surfaces was analyzed immediately or within 24h per our well-established method (Sheng et al., 2017; Sheng et al., 2018). If survival of *Listeria* on apple fruits was below the detection limit, the suspension was enumerated for Presence/Absence after 48h enrichment in Buffered *Listeria* Enrichment Broth (BLEB) and streaking onto a selective *Listeria* agar plate. Presumptive positive colonies were further confirmed by PCR (FDA, 2015).

7. Resident microbiota enumeration

To enumerate *Enterobacteriaceae*, the detached microbiota suspension was plated on TSAYE overlaid with Violet Red Bile Glucose agar and incubated at 35°C for an additional 24 h.

To enumerate *Pseudomonas*, the detached microbiota suspension was plated on TSAYE plates overlaid with *Pseudomonas* selective agar supplemented with 10 µg/ml of cetrimide, 10 µg/ml of Fucidin, and 50 µg/ml of cephalosporin, and then incubated at 28°C for 5 days.

Total native bacteria were enumerated on TSAYE plates and incubated at 30°C for 3 days. Lactic acid bacteria were enumerated by pour plate method using de Man, Rogosa and Sharpe (MRS) agar and incubated at 35°C for 48 h.

Yeast and mold were plated on potato dextrose agar (PDA) supplemented with 100 μg/ml chloramphenicol and incubated at room temperature (~22°C) for 5 days. Colonies were classified into yeasts, molds, and *Penicillium* regarding morphological characteristics.

8. Next-generation sequencing analysis of microbiome on apple surfaces

1) Microbial detachment from apple surface

At each sampling day, 4 composite replications containing 16 uninoculated and/or inoculated apple fruits were collected. Microbial suspension detached from 16 apples was pooled together and used for DNA extraction as described in the following.

2) DNA extraction and purification

Genomic DNA was extracted from microbial samples collected above using commercial DNA extraction and purification kit from Qiagen (Valencia, CA) per our established method (Kang, Yang, Zhang, Ross, & Zhu, 2018). The concentration and quality of DNA will be measured using Nanodrop spectrometry (Thermo Scientific), while the quality of DNA will be monitored by DNA agarose gel.

3) Next-generation DNA sequencing

Next-generation sequencing of the microbiome was performed by the Initiative for Bioinformatics and Evolutionary Studies (IBEST) Genomics Resources Core at the University of Idaho using Illumina MiSeq dual-barcoded two-step PCR amplicon sequencing. To produce amplicons for sequencing, the V4 region of the bacterial 16S rRNA gene was amplified using universal primers (515F: GTGCCAGCMGCCGCGGTAA, 806R: GGACTACHVG GGTWTCTAAT) with flanking regions ACACTGACGACATGGTTCTACA or TACGGTAGCA GAGACTTGGTCT at F515 or R806, respectively, for the first PCR reaction. The PCR products obtained from the first PCR were diluted and used as the template for the second PCR to add barcodes and sequencing adapters. Equal amounts of amplicons were pooled to create a composite sample, which was then normalized, and denatured prior to sequencing per the Illumina protocol for a 2×301 MiSeq run (Illumina, Inc., San Diego, CA).

For fungal community, the internal transcribed spacer region (ITS1) of the fungal ribosomal RNA gene will be amplified using the prepared microbial DNA and universal primers of ITS1F: 5'-CTTGGTCATTTAGAGGAAGTAA-3' and ITS2: 5'- GCTGCGTTCTTCATCGATGC-3' with flank regions ACACTGACGACATGG TTCTACA and TACGGTAGCAGAGACTTGGTCT at ITS1F and TIS2, respectively, for the first PCR. The second round of PCR (PCR2) will be performed to add sample-specific barcodes and Illumina adapters by priming the common tag sequences (Schlatter, Yin, Hulbert, & Paulitz, 2020; Schoch et al., 2012) and using the first PCR product as a template. Barcoded amplicons of PCR2 were quantified andcombined at equal amounts to construct the fungal ITS library (Schlatter et al., 2020; Schoch et al., 2012).

4) Bioinformatics analysis of apple microbiome under storage

Raw DNA sequence reads from the Illumina MiSeq will be demultiplexed and classified using the established method by bioinformaticist at IBEST (Kang et al., 2018).

9. Fruit quality analysis

At harvest or 36-week storage, fruit quality such as firmness, total soluble solids, and titratable acidity, as well as external and internal disorders, including superficial scald and lenticel decay, were assessed at the end of cold storage by the WTFRC quality lab using established methods (Sheng et al., 2018). A sample size of 10 apples per replicate with 4 independent replicates per wax type was used for internal and external disorder assessment.

10. Statistical analysis.

Data were analyzed with IBM SPSS 19.0 (Chicago, IL). Mean differences were compared by the one-way analysis of variance (ANOVA) followed by a Tukey multiple comparison test. *P* values less than 0.05 were considered significant differences.

RESULTS AND DISCUSSION

In preceding years, we conducted an analysis that revealed significant differences in the fungal community structure at various taxonomic levels (phylum, family, genus, and species) among apple samples obtained during different sampling times as well as between apples inoculated with or without *L. innocua* at the selected sampling time. Our ongoing investigation now extends to the analysis of bacterial community structure, the results of which are detailed below.

1. Overall comparison of bacterial composition of apples right after inoculation

Figure 1 depicts the microbiome distribution and diversity on Fuji apples before and immediately after inoculation with *L. innocua*. Prior to the introduction of *L. innocua*, 15 bacteria phyla were identified, with *Proteobacteria*, *Deinococcus-Thermus*, *Actinobacteria*, *Cyanobacteria*, *Bacteroidetes,* and *Firmicutes* being the most prevalent phyla (Fig. 1A). However, following *L. innocua* inoculation, there is a notable reduction in bacterial phyla diversity (Fig. 1B-D), with only five phyla, including *Proteobacteria*, *Firmicutes, Bacteroidetes, Cyanobacteria* and *Actinobacteria* being identified (Fig. 1A).

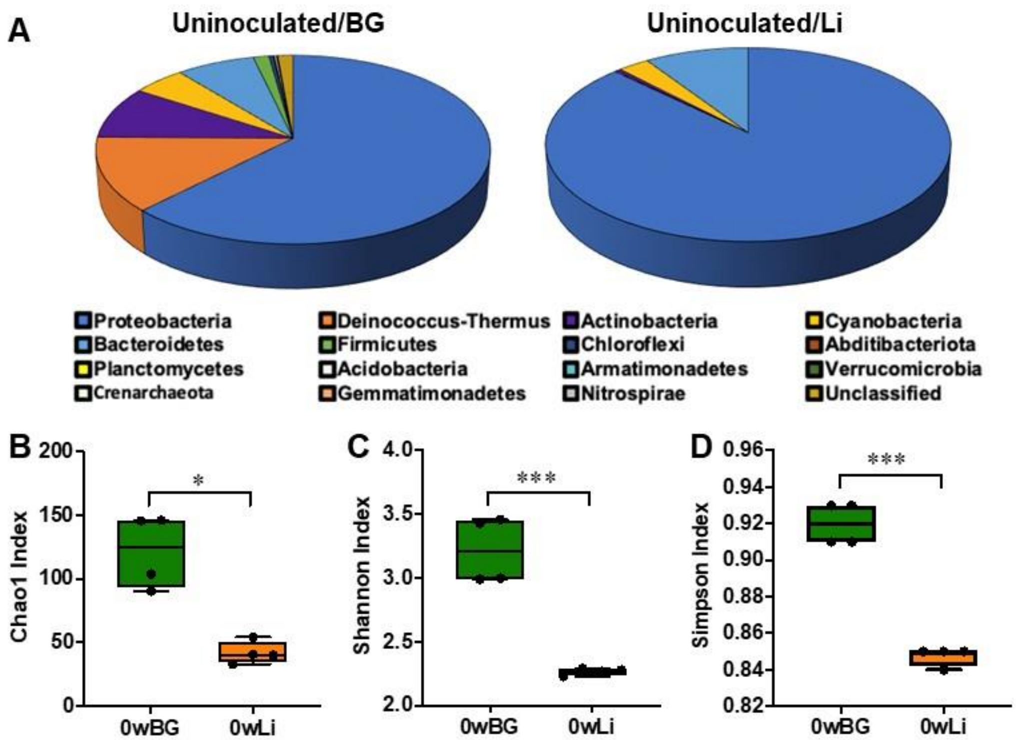

Figure 1. The microbiome distribution and diversity on apples contaminated with or without *Listeria innocua.* A. The bacterial phyla on apples. B. Alpha diversity of Chao 1 index. C. Alpha diversity of Shannon index. D. Alpha diversity of Simpson index. Inoculate/0wLi: apples inoculated with *L. innocua*; Uninoculate/0wBG: apples without *L. innocua* inoculation. Mean ± SEM, n = 4, each replicate has 16 apples.

2. Overall comparison of bacterial composition of apples during 36 weeks of CA storage

Figure 2 illustrates the microbiota diversity of apples with and without *L. innocua* contamination over a 36-week period of CA storage. Over the course of 36 weeks of CA storage, alpha diversities, reflecting the variety of species within individual samples, showed a gradual increase in *L. innocua*-inoculated apples, while a decline was observed in uninoculated apples (Fig. 2A-C).

Meanwhile, beta diversities, highlighting differences in bacterial community compositions, demonstrated distinct bacterial community composition profiles between *L. innocua*-inoculated and uninoculated apples throughout the 36 weeks of CA storage (Fig. 2D-E).

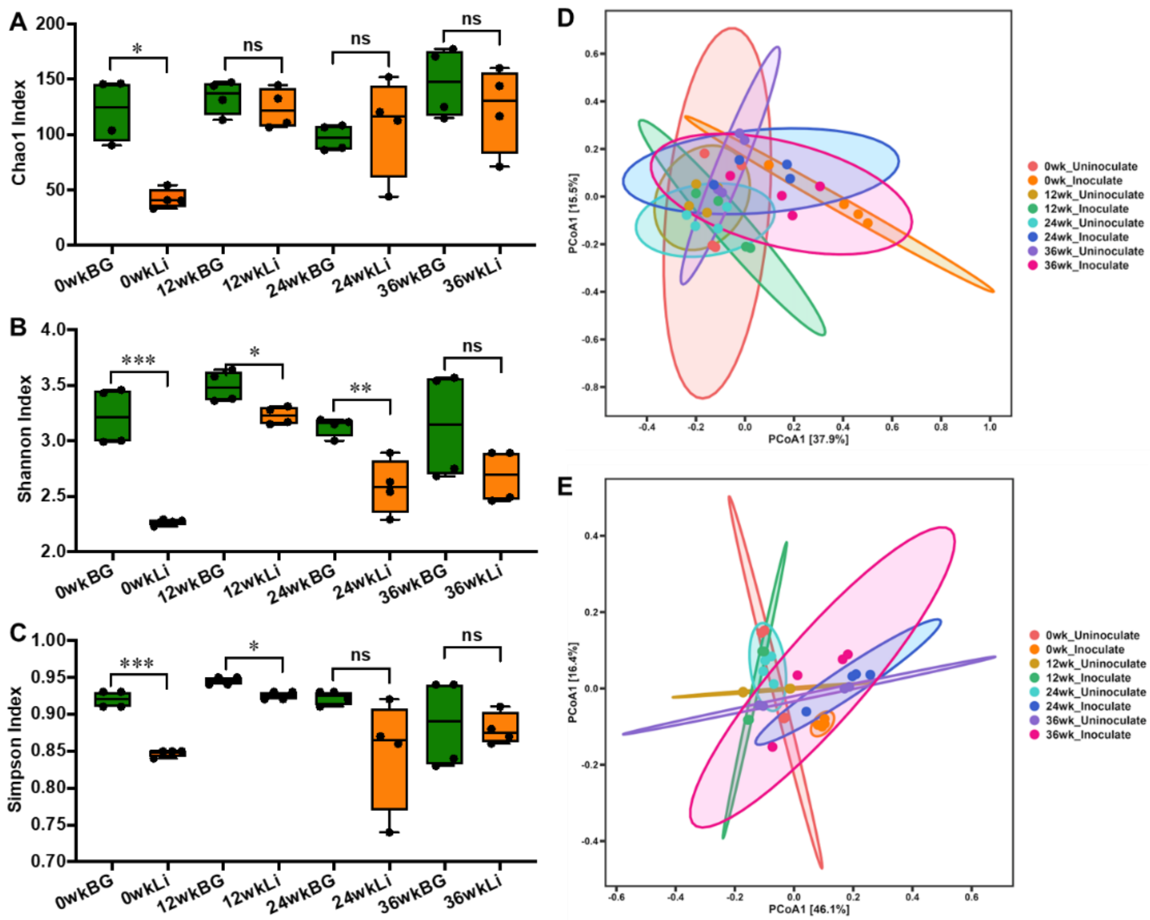

Figure 2. Microbiota diversity of apples with or without *L. innocua* contamination during 36 weeks of controlled atmosphere (CA) storage. A. Alpha diversity of Chao 1 index. B Alpha diversity of Shannon index. C. Alpha diversity of Simpson index. D. Principal coordinate analysis (PCoA) based on the Bray-Curtis distance. E. PCoA based on the weighted UniFrac distance. Inoculate/Li: apples contaminated with *L. innocua*; Uninoculate/BG: apples without *L. innocua* contamination; 0 wk: apples before storage; 12 wk: 12-week storage; 24 wk: 24-week storage; 36 wk: 36-week storage. Mean ± SEM, averaged from 4 replicate, each with 16 apples.

3. Bacterial composition of apples inoculated with or without Listeria innocua at the phylum level during 36 weeks of CA storage

Figure 3 presents a Chord diagram illustrating the relative abundances of bacterial phyla on apples with and without *L. innocua* contamination throughout a 36-week CA storage period. During this duration of CA storage, there were dynamic changes in bacterial phyla on both L. innocua-inoculated and uninoculated apples (Figure 3). Specifically, over the 36 weeks of CA storage, *Proteobacteria* decreased by 24.48% and 45.48% on uninoculated apples and *Listeria* inoculated apples, respectively (Figure 3). *Deinococcus-Thermus*, initially absent on *Listeria*-inoculated apples, increased to ~11% by the end of the storage period (Figure 3). In contract, *Deinococcus-Thermus* remained relatively stable ($P > 0.05$) on uninoculated apples during 36 weeks of CA storage (Figure 3). *Actinobacteria* significantly increased by 24.38% on *Listeria*-inoculated apples and 26.38% on uninoculated apples after 36 weeks of CA storage (Figure 3). *Cyanobacteria* significantly increased on *Listeria*-inoculated apples while remaining unchanged on uninoculated apples over the entire CA storage (Figure 3). *Bacteroidetes* decreased by ~5% on uninoculated apples but showed no change on *Listeria*-inoculated apples after 36 weeks of CA storage (Figure 3).

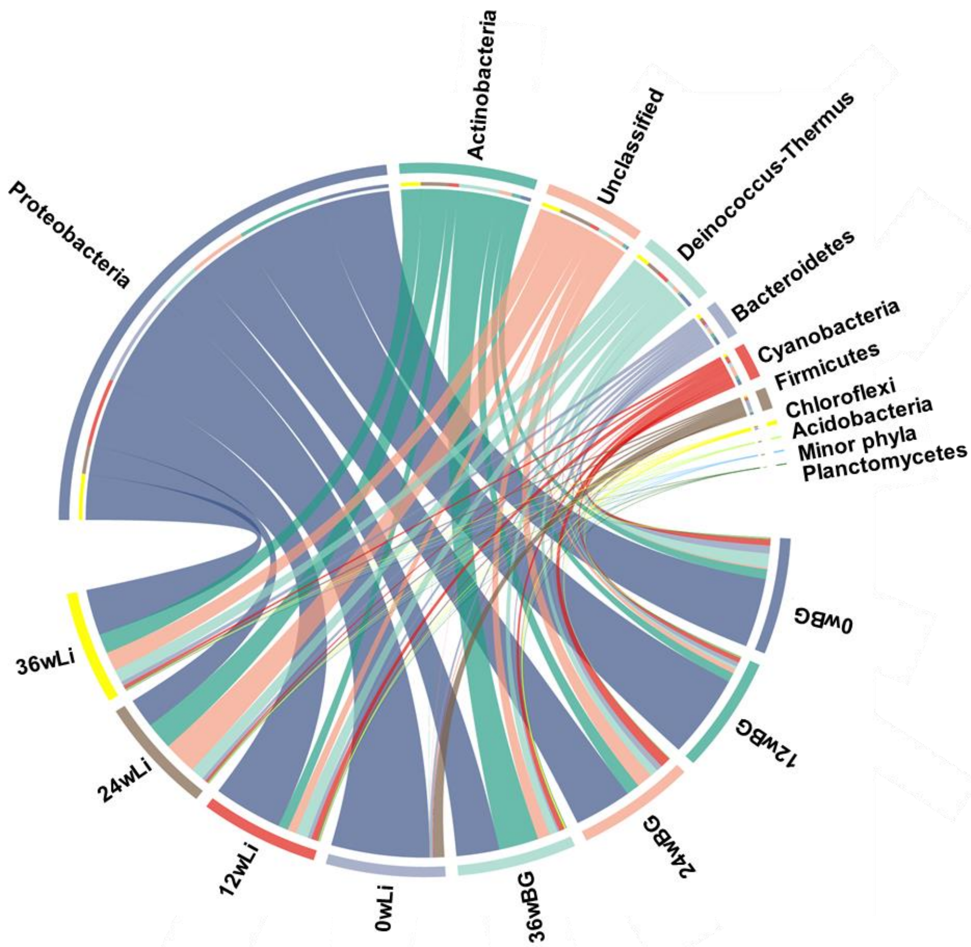

Figure 3. Chord diagram showing the relative abundances of bacterial phyla on apples with or without *L. innocua* contamination during 36 weeks of controlled atmosphere (CA) storage. Minor phyla: average relative abundance less than 0.1%. 0 w: apples before storage; 12 w: 12-week storage; 24 w: 24-week storage; 36 w: 36-week storage. Li: apples contaminated with *L. innocua*. BG: apples without *L. innocua* contamination. The relative abundance in each treatment was averaged from four replicates, each with 16 apples.

4. Bacterial families of Fuji apples inoculated with or without Listeria innocua during 36 weeks of CA storage.

Figure 4 employs Venn diagrams to illustrate shared and unique bacterial families and a heatmap to showcase abundance and clustering patterns of these families on apples with and without *L. innocua* contamination during a 36-week CA storage period. Throughout this storage period, uninoculated apples had higher number of overlapping bacterial families compared to *Listeria*-inoculated apples (Figure 4A-B). Specifically, *Pseudomonadaceae* and *Exiguobacteraceae* were significantly more abundance on uninoculated apples than on *L. innocua*-inoculated apples under CA storage (Figure 4C). The introduction of *L. innocua* on apples significantly increased the relative abundance of *Pseudomonadaceae* but decreased the relative abundance of other bacterial families under 36 weeks of CA storage (Figure 4C). *Cytophagaceae* was significantly higher on *L. innocua*-inoculated apples compared to uninoculated apples after 36 weeks of CA storage (Figure 4C). As anticipated, *Listeriaceae* was exclusively found on *L. innocua*-inoculated apples and gradually decreased during the 36 weeks of CA storage (Figure 4C).

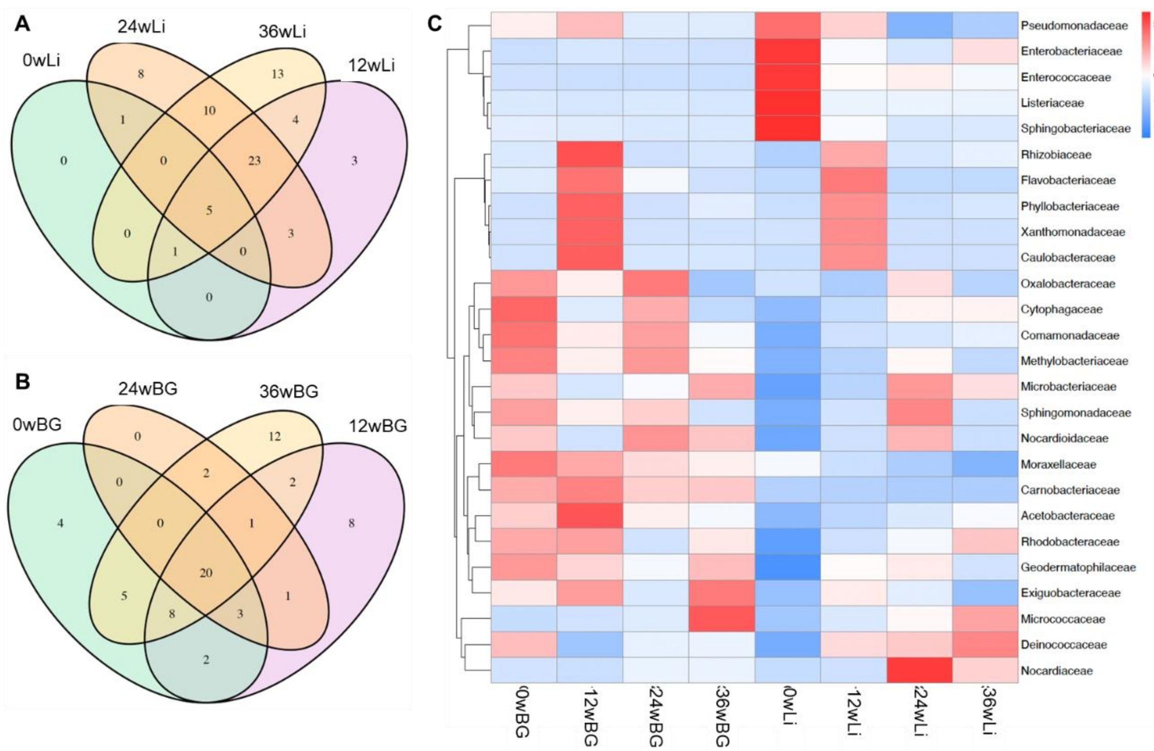

Figure 4. Venn diagrams and heatmap of bacterial families on apples with or without *L. innocua* contamination during 36 weeks of controlled atmosphere (CA) storage. A. Venn diagram of bacterial families on *L. innocua* contaminated apples. B. Venn diagram of bacterial families on uncontaminated apples. C. Heatmap of bacterial families on apples during storage. Li: apples contaminated with *L. innocua;* BG: apples without *L. innocua* contamination; 0 w: apples before storage; 12 w: 12-week storage; 24 w: 24-week storage; 36 w: 36-week storage. The relative abundance in each treatment was averaged from four replicates, each with 16 apples.

5. *Bacterial genera of Fuji apples inoculated with or without Listeria innocua during 36 weeks of CA storage.*

Figure 5 presents a star diagram to depict the relative abundances of bacterial genera on apples with and without *L. innocua* contamination throughout a 36-week CA storage period, offering a comprehensive visual overview of the distribution and dynamics of these genera over time. Notably, *Pseudomonas* was significantly higher on *L. innocua*-inoculated apples compared to uninoculated apples before storage (Figure 5), decreasing on *L. innocua*-inoculated apples while increasing on uninoculated apples during the 36 weeks of CA storage (Figure 5). By the end of CA storage, *Deinococcus* and *Hymenobacter* on *L. innocua*-inoculated apples were significantly higher than that on uninoculated apples (Figure 5). *Rhodococcus* and *Pantoea* were significantly higher on *L. innocua*-inoculated apples compared to uninoculated apples throughout the 36 weeks CA storage (Figure 5).

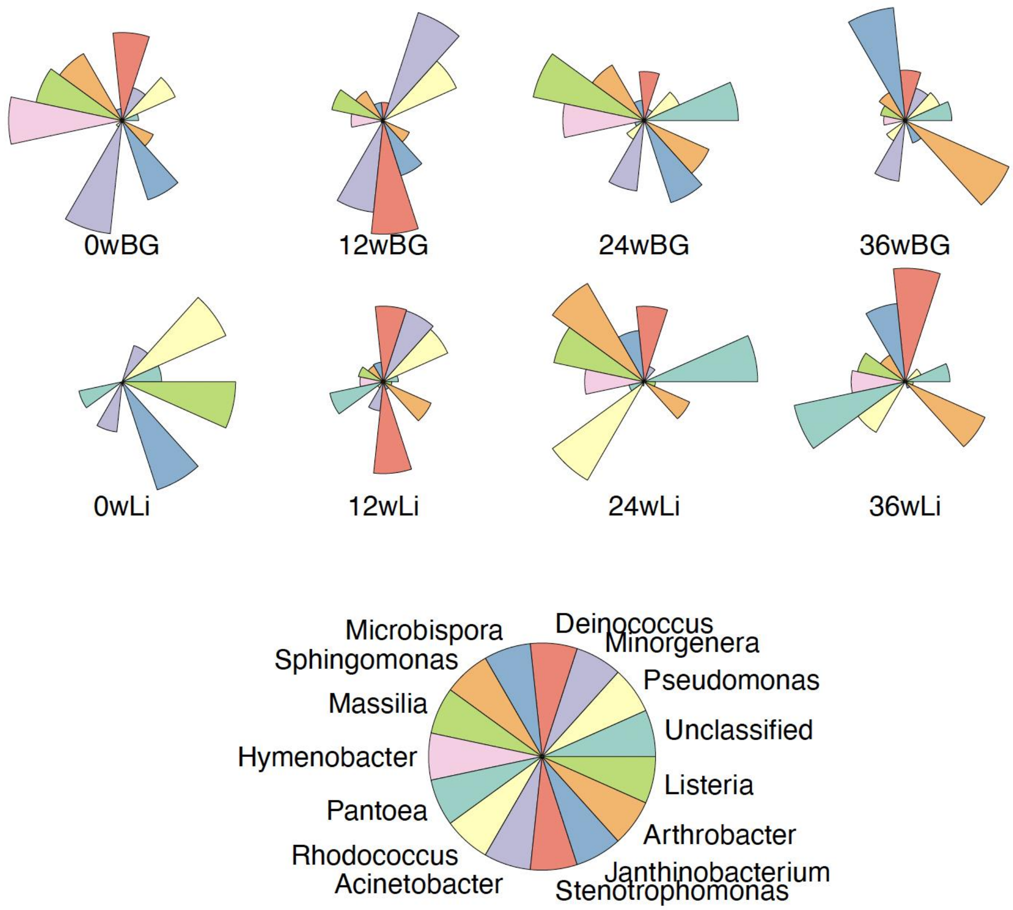

Figure 5. Star diagram showing the relative abundances of bacterial genera on apples with or without *L. innocua* contamination during 36 weeks of controlled atmosphere (CA) storage. Minor genera: average relative abundance less than 2.0%. Each sector represented the relative abundance of respective genus. 0 w: apples before storage; 12 w: 12-week storage; 24 w: 24-week storage; 36 w: 36-week storage. Li: apples contaminated with *L. innocua*. BG: apples without *L. innocua* contamination. The relative abundance in each treatment was averaged from four replicates, each with 16 apples.

6. Bacterial species on Fuji apples inoculated with or without Listeria innocua during 36 weeks of CA storage.

Relative abundances of bacterial communities were further analyzed at species level. After 36 weeks of CA storage, *Pseudomonas umsongensis*, *Pseudomonas veronii*, *Comamonas terrigena*, and *Acinetobacter lwoffii* exhibited higher abundance on uncontaminated apples compared to *L. innocua*-inoculated apples (Figure 6). In contrast, *Rhodococcus fascians* were significantly higher on *L. innocua*-inoculated apples than on uncontaminated apples at the end of 36 weeks of CA storage (Figure 6). *Candidatus nitrososphaera* and *Aeromonas sharmana* showed significantly higher abundances on uncontaminated apples compared to *L. innocua*-contaminated apples within 36 weeks of CA storage (Figure 6).

In summary, the introduction *of L. innocua* led to a significant decrease in bacterial diversities and richness on apples. Over the 36 weeks of CA storage, alpha diversities of bacterial communities were gradually increased in *L. innocua*-contaminated apples while decreasing on uninoculated apples. Dynamic alterations were evident in bacterial communities at the phylum, family, genus, and species levels.

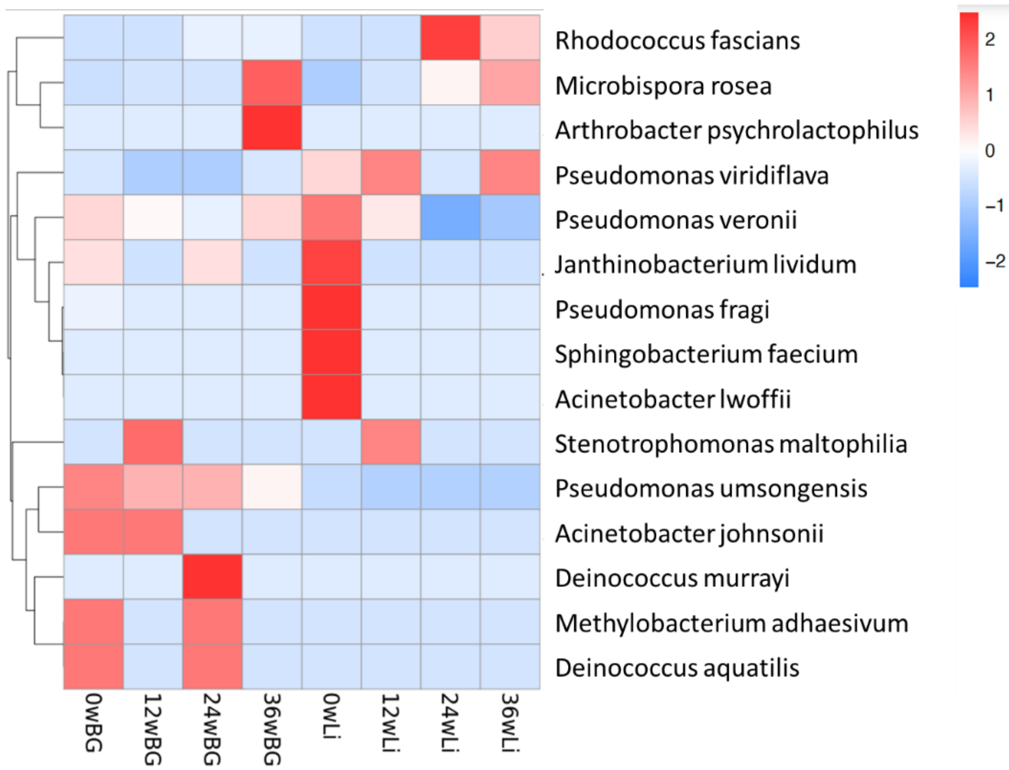

Figure 6. Heatmap of relative abundance of bacterial species on apples with or without *L. innocua* contamination during 36 weeks of controlled atmosphere (CA) storage. Li: apples contaminated with *L. innocua;* BG: apples without *L. innocua* contamination; 0 w: apples before storage; 12 w: 12-week storage; 24 w: 24-week storage; 36 w: 36-week storage. The relative abundance in each treatment was averaged from four replicates, each with 16 apples.

Project Title: Reducing CO_2-related disorders during Honeycrisp rapid CA treatment

Continuing Project Report Year 2 of 3

Primary PI: David Rudell
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City/State/Zip: Wenatchee, WA 98801

Cooperators: Emmi Klarer, Stemilt Growers LLC.

Report Type: Continuing Project Report

Project Duration: 3 Years

Total Project Request for Year 1 Funding: $22,000
Total Project Request for Year 2 Funding: $85,000
Total Project Request for Year 3 Funding: $85,000

Other related/associated funding sources: Awarded
Funding Duration: 2022 - 2025
Amount: $115,317/3 yrs.
Agency Name: USDA-ARS, In-house project
Notes: In-house project with complimentary objectives. Funds for storage maintenance and costs ($8000/yr), supplies and materials ($3000/yr), travel ($1000/yr), and 0.1 FTE (PI) and 0.1 FTE (technical).

Other related/associated funding sources: Awarded
Funding Duration: 2023 - 2027
Amount: $555,828/4 yrs.
Agency Name: USDA-NIFA
Notes: Project Title: "Benefits of and barriers to dynamic controlled atmosphere (DCA) storage: Analyses needed for technology uptake by the U.S. apple industry". Lead institution: Cornell University (PD: Chris Watkins). Project was funded through the SCRI program beginning in FY24. Project activities commenced this fall.

Budget
Primary PI: David Rudell
Organization Name: USDA-ARS
Contract Administrator: Sharon Blanchard
Telephone: 509-664-2280 (SB)
Contract administrator email address: Sharon.Blanchard@usda.gov

Item	2022	2023	2024
Salaries (GS-7)*		43,311	45,764
Benefits (40%)		18,124	18,305
Wages	5,000	5,000	5,000
Benefits			
Equipment			
Supplies	5,000	4,565	3,931
Travel			
Miscellaneous**	12,000	12,000	12,000
Plot Fees			
Total	22,000	85,000	85,000

Footnotes: *Estimated 3% salary increase; **22% of instrument service contract

Objectives:
1. Determine influence of CO_2 levels on disorder development during rapid CA treatment.
2. Determine impact of initial fruit temperature and delay of CA establishment during conditioning on disorder development.
3. Monitor flesh chemistry to indicate which treatment conditions may elevate risk of developing soft scald/soggy breakdown or CO_2-related/other disorders.

SIGNIFICANT FINDINGS

1. Rapid CA does not compromise quality (6 months) regardless of how long it was delayed.
2. Significant CO_2-related symptoms did not develop as a result of rapid CA under CO_2 levels up to 5% in the first season.
3. Leather blotch developed on apple from one orchard but was not impacted by delaying rapid CA establishment.

METHODS

Objective 1: Determine influence of CO_2 levels on disorder development during rapid CA treatment.

In year 1, Honeycrisp apples were harvested approximately 1 week prior to commercial harvest, at commercial harvest, and 1 week after commercial harvest from the same block in Quincy, WA. Harvest maturity (starch index, internal ethylene concentration, firmness, titratable acidity, and soluble solids) and external/internal appearance were evaluated, and fruit were imaged using a digital camera. Apples were treated with 1-MCP (1 ppm), then stored in 2.5% O_2 and (0.5, 1, 2, 3, or 5%) CO_2 for 7 days at 50 °F. To distinguish soft scald from CO_2-related internal browning, 2 trays of DPA (2000 ppm drench) treated apples and 2 trays of untreated (no 1-MCP or DPA) apples from the last harvest were stored in 2.5% O_2 and 5% CO_2 for 7 days at 50 °F in separate CA chambers as controls. Following conditioning, apples were stored for 6 months in 2.5% O_2 and 0.5% CO_2 at 37 °F upon which external and internal disorders, firmness, titratable acidity, soluble solids, and defect incidence were evaluated.

Objective 2: Determine impact of initial fruit temperature and delay of CA establishment during conditioning.

To determine the impacts of delayed CA establishment during conditioning, Honeycrisp apples were harvested at commercial harvest from 3 different orchards: 2 near Quincy, WA and 1 near Mattawa, WA. Harvest maturity (starch index, internal ethylene concentration, firmness, titratable acidity, and soluble solids) and external/internal defects were evaluated, and fruit were imaged using a digital camera. Apples were treated with 1-MCP (1 ppm) then conditioned at 50 °F in CA (atmosphere established at 0, 4, or 8 days) in 2.5% O_2 and (2.5 or 5%) CO_2. Once in CA, apples were conditioned at 50 °F until day 10 after harvest. Following conditioning, apples were stored in 2.5% O_2 and 0.5% CO_2 at 37 °F for 6 months upon which external and internal disorders, firmness, titratable acidity, soluble solids, and defect incidence were evaluated.

To determine the impacts of conditioning temperature during rapid CA, Honeycrisp apples were harvested approximately one week after commercial harvest from an orchard in Quincy, WA. Harvest maturity (starch index, internal ethylene concentration, firmness, titratable acidity, and soluble solids) and external/internal defects were evaluated, and fruit were imaged using a digital camera. Apples were treated with 1-MCP (1 ppm) and immediately placed in CA in 0.5% O_2 and 2.5% CO_2 at (37, 46, or 50 °F) for 7 days. Following conditioning, apples were stored in 2.5% O_2 and 0.5% CO_2 at 37 °F for 6 months upon which external and internal disorders, firmness, titratable acidity, and soluble solids were evaluated.

Objective 3: Monitor flesh chemistry to indicate which treatment conditions may elevate risk of developing soft scald/soggy breakdown or CO_2-related browning.

Honeycrisp apples were picked at three timepoints (from Objective 1; 1 week prior to commercial harvest, at commercial harvest, and 1 week after commercial harvest), treated with 1-MCP, and immediately pulled down to 2.5% O_2 and 0.5, 1, 2, 3, or 5% CO_2 and conditioned for 1 week at 50 °F (as described in objective 1). One tray of each CO_2 treatment was sampled at 0-, 2-, 4- and 7-days during conditioning to determine if markers of CO_2 sensitivity increase with disorder risk and/or symptom development. The cortex samples were flash frozen in liquid nitrogen and cryo-preserved for chemical analyses. Data processing targeted chemistries associated with CO_2-sensitivity and internal browning discovered in our previous project (Rudell and Mattheis, 2023).

Current (Year 2) season:

The season in year 1 had an unusually light crop with weather events during pollination and significant hail damage in one of the blocks we used. Disorder (soft scald/soggy breakdown, bitter pit, CO_2-related internal browning) incidence was insignificant requiring repetition of all year 1 activities in year 2 to meet project objectives. We added additional air stored treatments merely to establish weather disorders would develop under those conditions if not the CA/conditioning treatments already employed.

RESULTS AND DISCUSSION

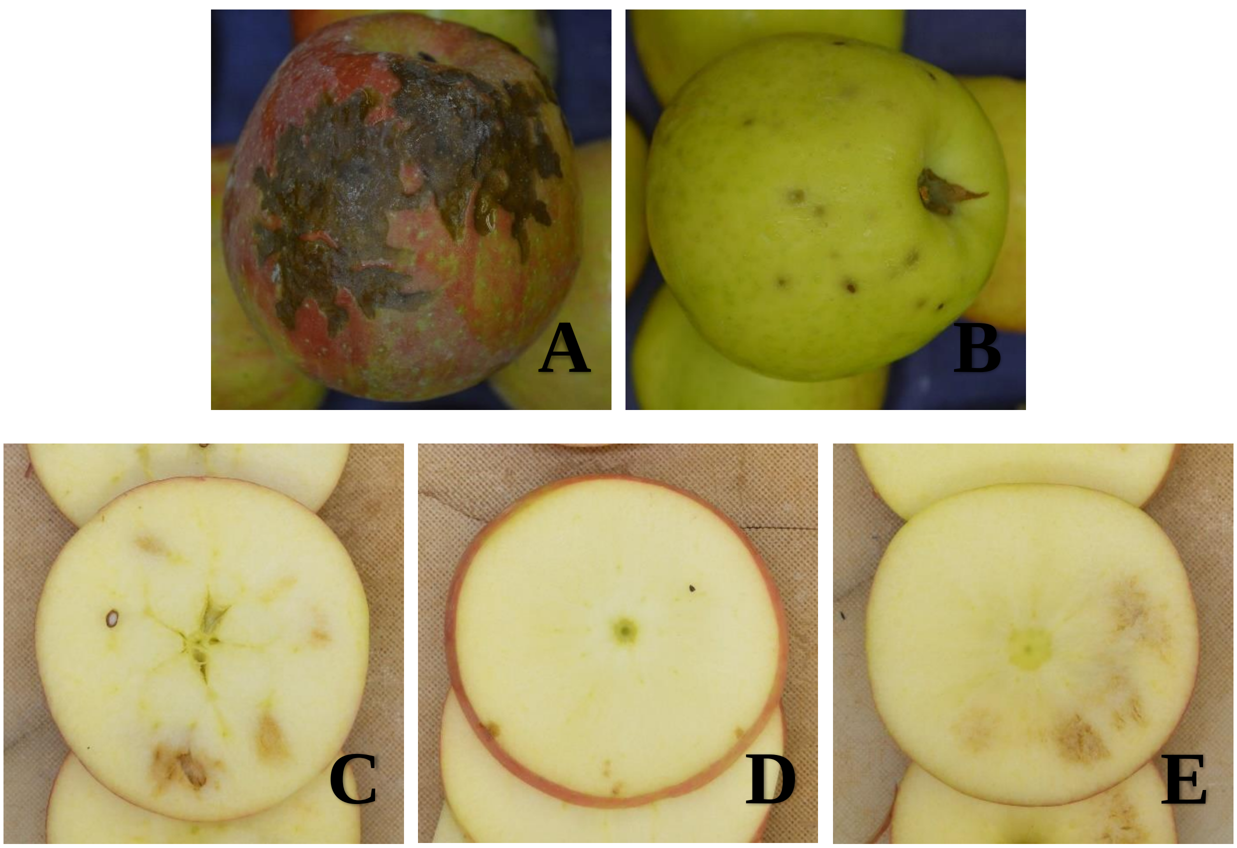

Figure 1. Disorders of Honeycrisp observed in the current study. (A) Leather blotch, (B) bitter pit, (C) CO_2-related lens-shaped cavities, (D) cork spots, and (E) CO_2-related internal browning. Apart from apples developing leather blotch from one of the orchards, disorder incidence was insignificant in Year 1.

Table 1. Disorder incidence after 6 months of storage of Honeycrisp harvested at different maturities and conditioned at 50 °F alongside rapid CA with 2.5 % O_2 and varying CO_2 levels to test impacts of harvest maturity on CO_2 sensitivity. Data were analyzed comparing CO_2 conditioning treatments within a single harvest using a pooled z-test (n=36, $p<0.05$). No significant differences of disorder incidence were observed within a single harvest.

Harvest	**CO_2 Treatment**	**Bitter Pit**	**Leather Blotch**	**Cork Spot**	**Cavities**
Early	0.5%	2.77 a	2.77 a	2.77 a	0 a
	1%	5.55 a	2.77 a	2.77 a	0 a
	2%	2.77 a	5.55 a	2.77 a	0 a
	3%	8.33 a	5.55 a	2.77 a	0 a
	5%	5.55 a	5.55 a	0 a	0 a
Commercial	0.5%	0 a	0 a	0 a	0 a
	1%	0 a	0 a	0 a	0 a
	2%	0 a	0 a	0 a	0 a
	3%	5.55 a	0 a	0 a	2.77 a
	5%	0 a	0 a	0 a	0 a
Late	0.5%	0 a	0 a	0 a	0 a
	1%	0 a	2.77 a	0 a	0 a
	2%	0 a	2.77 a	0 a	0 a
	3%	0 a	0 a	0 a	0 a
	5%	0 a	2.77 a	0 a	0 a
	No 1-MCP (5%)	0 a	0 a	0 a	2.77 a
	DPA (5%)	0 a	0 a	0 a	0 a

Objective 1: Determining influence of CO_2 levels on disorder development during rapid CA treatment

To account for any differences of susceptibility to bitter pit, softscald/soggy breakdown, and CO_2-sensitivity related to harvest maturity, Honeycrisp was harvested from a single orchard 3 times at 1 week intervals around commercial harvest. Starch index values were 3, 4, and 5 and internal ethylene was 2.4, 2.1, and 9 ppm for harvests 1, 2, and 3, respectively. Less than 10% developed bitter pit and leather blotch on fruit from the early harvest stored in all levels of CO_2 by 6 months of storage (Table 1). There were a few apples from the other harvests that developed these disorders. Cork spot also developed at an insignificant level in early harvest apples stored at all CO_2 levels. No cork spots developed in fruit from any other harvest. No softscald/soggy breakdown or CO_2-related disorders developed. Fruit quality was not impacted by storage atmospheric composition, even when apples were not treated with 1-MCP (Table 2). Apples harvested at the commercial and late pick dates were less acidic than the early harvest.

Leather blotch is typically associated with bitter pit and, potentially, cork spot as a more severe form of these disorders that develops during storage. It is typically linked with 1-MCP treatment and, like bitter pit, more likely to develop on more immature apples of susceptible cultivars such as Honeycrisp. High CO_2 did not, and was not expected to, have any impact on incidence of these disorders. It is important to note that incidence of bitter pit and bitter pit-related disorders were insignificant (too low to statistically separate from no incidence), and this may be expected as all of the apples were conditioned with rapid CA, a strategy expected to reduce bitter pit.

Likewise, softscald/soggy breakdown would be expected to be, at least, reduced by conditioning at 50 °F and storage at 37 °F. As neither of these disorders developed in any instance, it is difficult to conclude the impact of rapid CA, in this case. As no symptom of CO_2 sensitivity developed, even in apples stored under the highest CO_2 levels, we cannot conclude that CO_2 levels with relation to maturity during rapid CA had no impact on CO_2 sensitivity. While published evidence is limited with regard to rapid CA, previous reports support that CO_2 accumulation during 1-MCP treatment in air may have little impact on development of CO_2-related disorders in this cultivar (Mattheis et al., 2015). However, softscald/soggy breakdown incidence and CO_2 sensitivity is variable by season for Honeycrisp, and, for this orchard, this season was an outlier with regard to cold weather during pollination, hail events, and a relatively light crop. All of these factors may have contributed to the lack of disorder development and, consequently, prompt a repeat of this experiment in subsequent year to help confirm any lack of CO_2 sensitivity with regard to harvest maturity during rapid CA conditioning.

Table 2. Fruit quality after 6 months of storage of Honeycrisp harvested at different maturities and conditioned at 50 °F alongside rapid CA with 2.5 % O_2 and varying CO_2 levels to test impacts of harvest maturity on CO_2 sensitivity. Data were analyzed comparing CO_2 conditioning treatments within a single harvest using SAS Proc ANOVA (Tukey's, $p<0.05$). Letters indicate significant groups within a harvest timepoint.

Harvest	**Conditioning CO_2**	**Firmness (lb)**	**TA (g/L)**	**°Brix**
Early	0.50%	14.31 a	5.08 a	12.37 a
	1%	14.45 a	5.45 ab	12.51 ab
	2%	14.49 a	5.71 b	12.66 ab
	3%	14.64 a	5.21 a	12.61 ab
	5%	14.69 a	5.17 a	12.91 b
Commercial	0.50%	13.79 a	4.58 a	12.17 ab
	1%	13.67 a	4.34 a	11.90 a
	2%	13.96 a	4.59 a	12.22 ab
	3%	13.69 a	4.55 a	12.27 ab
	5%	13.97 a	4.63 a	12.32 b
Late	0.50%	13.10 a	4.31 ab	12.40 a
	1%	13.15 a	4.33 ab	12.48 a
	2%	13.38 a	4.60 ac	12.33 a
	3%	13.12 a	4.88 c	12.40 a
	5%	13.25 a	4.80 c	12.49 a
	No 1-MCP	12.90 a	4.06 b	12.05 a
	DPA	13.01 a	4.40 ac	12.33 a

Objective 2: Determining impact of initial fruit temperature and delay of CA establishment during conditioning

Honeycrisp was harvested from 3 different orchards (Orchard 2 was also used for Objective 1) and rapid CA was initiated at different times after establishing conditioning to determine whether a delay would reduce the incidence of CO_2-related internal browning while still reducing bitter pit and softscald/soggy breakdown incidence. Two CO_2 levels were also used as a means to establish the relative level of CO_2 that led to any related internal browning.

Table 3. Quality of Honeycrisp harvested from different orchards following 6 months CA storage. Apples were conditioned to reduce softscald/soggy breakdown with CA (with 5 % or 2.5 % CO2) established following delays of 0, 4, or 8 days. Data were analyzed comparing conditioning treatments (CO_2 and CA) within an orchard using SAS Proc ANOVA (two-way, Tukey's, p<0.05). Different lower-case letters indicate significant differences within the same orchard.

Orchard	**CA Start Day**	**Conditioning CO_2**	**Firmness (lb)**	**TA (g/L)**	**°Brix**
Orchard 1	0	2.50%	15.37 a	5.24 a	12.48 a
		5%	15.16 a	5.25 a	12.45 a
	4	2.50%	15.18 a	5.10 ab	12.50 a
		5%	14.93 a	5.30 a	12.52 a
	8	2.50%	15.29 a	4.88 b	12.29 a
		5%	15.14 a	5.19 ab	12.46 a
Orchard 2	0	2.50%	13.97 a	4.52 a	12.19 a
		5%	13.57 a	4.74 a	12.01 ab
	4	2.50%	13.57 a	4.47 a	12.34 a
		5%	13.67 a	4.46 a	11.78 b
	8	2.50%	13.83 a	4.47 a	12.08 ab
		5%	13.62 a	4.48 a	12.08 ab
Orchard 3	0	2.50%	14.42 a	5.06 a	13.18 a
		5%	13.94 a	4.83 ab	13.42 a
	4	2.50%	13.59 a	4.77 ab	13.32 a
		5%	14.09 a	4.79 ab	13.02 a
	8	2.50%	13.63 a	4.88 ab	13.24 a
		5%	14.27 a	4.53 b	13.61 a

Fruit quality was not consistently impacted by treatment (Table 3). As with the early harvest of Orchard 2 (objective 1), apples from Orchard 1 developed leather blotch during storage, albeit at much higher frequency of 30 %, in one instance (Table 4). Very little bitter pit was found but cork spot also developed immediately under the peel of apples from orchard 1 regardless of treatment. Cork spot incidence was statistically insignificant at less than 17%. Neither storage atmosphere nor delay of CA establishment during conditioning had any impact on incidence of these disorders. Incidence of these disorders in apples from Orchards 2 and 3 was nearly absent. As with the results from objective 1 activities, the least mature fruit harvested from Orchard 1 (starch index = 2) developed the highest levels of bitter pit-related disorders compared with Orchards 2 and 3 (starch index = 4).

Softscald/soggy breakdown did not develop during this activity. However, a few instances of internal browning and even more internal cavities were found in apples from Orchard 1 stored under 5% CO_2 beginning at 0 or 4 days during temperature conditioning. These levels were not statistically significant. Cavities are a more common internal symptom of CO_2 sensitivity in relatively immature apples in many cultivars rather than internal radial browning more typical of more mature fruit. While this fits a pattern where apples are more sensitive to CO_2 earlier during conditioning or the longer the apples are exposed to high CO_2 during conditioning, significant levels of symptoms are required for any conclusions. It also emphasizes the importance of orchard factors and seasonal factors when considering CO_2 sensitivity.

Table 4. Percent incidence of observed external and internal disorders in Honeycrisp fruit harvested from different orchards and varied conditioning treatments after 6 months of storage. Data were analyzed comparing conditioning treatments (CO_2 and CA) within an orchard using a pooled z-test (n=36, $p<0.05$). Different lower-case letters indicate significant groups within an orchard.

Orchard	**CA Start Day**	**Conditioning CO_2**	**Bitter Pit**	**Leather Blotch**	**Cork Spot**	**Cavities**	**Internal Browning**
Orchard 1	0	2.50%	0 a	0 a	0 a	0 a	0 a
		5%	0 a	30.55 b	5.55 a	16.67 a	2.77 a
	4	2.50%	2.77 a	27.78 b	13.89 a	0 a	2.77 a
		5%	0 a	8.33 a	5.55 a	2.77 a	0 a
	8	2.50%	0 a	19.44 ab	11.11 a	0 a	2.77 a
		5%	2.77 a	13.89 ab	16.67 a	0 a	0 a
Orchard 2	0	2.50%	0 a	0 a	0 a	0 a	0 a
		5%	0 a	0 a	0 a	0 a	0 a
	4	2.50%	0 a	0 a	0 a	0 a	0 a
		5%	0 a	0 a	0 a	0 a	0 a
	8	2.50%	0 a	2.77 a	2.77 a	0 a	0 a
		5%	0 a	2.77 a	0 a	0 a	0 a
Orchard 3	0	2.50%	0 a	0 a	0 a	2.77 a	0 a
		5%	0 a	0 a	0 a	0 a	0 a
	4	2.50%	0 a	0 a	0 a	2.77 a	2.77 a
		5%	0 a	0 a	0 a	5.55 a	2.77 a
	8	2.50%	0 a	2.77 a	0 a	0 a	0 a
		5%	0 a	0 a	0 a	5.55 a	5.55 a

Temperatures of 37, 46, and 50 °F were also tested during rapid CA to account for any impacts of conditioning temperature. CA was established immediately for this test using apples picked one week after commercial harvest from orchard 2. Conditioning temperature had no influence on apple quality (not shown). Furthermore, statistically insignificant levels of CO_2-related symptoms developed only in apples conditioned at 37 °F (not shown).

Objective 3: Monitoring flesh chemistry during conditioning period

Flesh tissue of Honeycrisp of all 3 harvests and storage environments from the Objective 1 activity were collected multiple times during the conditioning period to monitor levels of natural chemicals found to be associated with risk of CO_2-related internal browning in our previous project (Rudell and Mattheis, 2023). We will briefly summarize those findings with an example of those compounds. These examples are also useful for monitoring superficial scald risk (Rudell et al., 2010) and, potentially, softscald and soggy breakdown (Leisso et al., 2016; Leisso et al, 2015). These compounds indicated disorder risk during CA storage (0.5 % O_2) of Fuji associated with various levels of CO_2 (0, 1, 2.5, and 5 %). Fuji stored under 5 % CO_2 developed significant levels of internal browning while those stored under 2.5 % developed less and 1 % still less (Figure 2). The ratio of 2 of these chemicals, one that increases in concentration with risk (ASG) and one that decreases with risk (SE), increased between CA establishment and the first evaluation at 2 weeks. This ratio is many folds higher in already browned tissue (not shown).

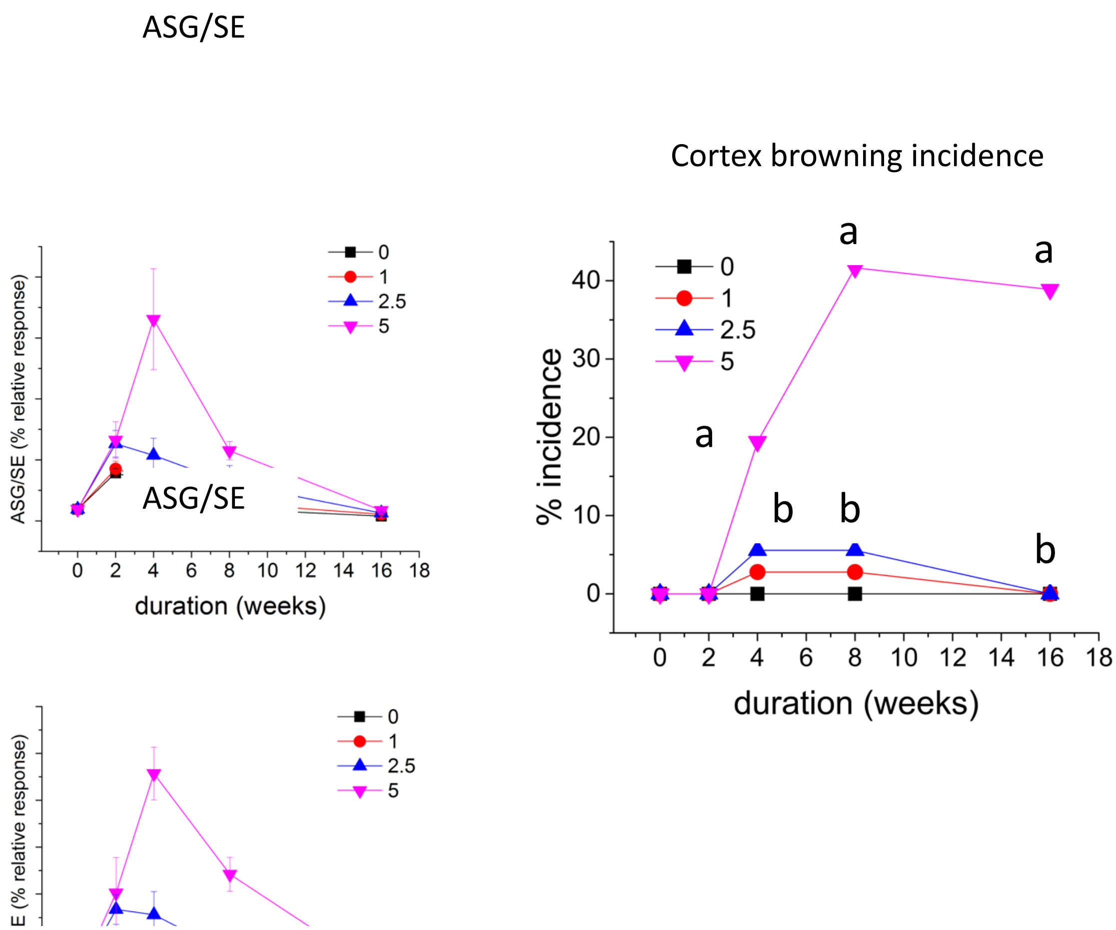

Figure 2. Two ratios of natural chemicals found in apple flesh associated with risk of developing internal CO_2-related browning (left) and internal browning development in Fuji stored at 2.5 % O_2 and 0.5, 1, 2.5, and 5 % CO_2 (right). This experiment was the outcome of an objective of our previous project (Rudell and Mattheis, 2023) where natural peel chemicals associated with risk of CO_2-related disorders were found. Apples stored under the highest levels of CO_2 developed browning and this was reflected by the ratio of ASG/SE before symptoms developed.

Unlike the former project where risk was not assessed before 2 weeks, we are testing whether we can detect risk within the first week of storage during the conditioning period. Unfortunately, disorder incidence was very low during this activity (Table 1). Risk assessment marker values seemed to reflect this relative lack of risk (Figure 3). However, no conclusions could be drawn. This experiment is being repeated.

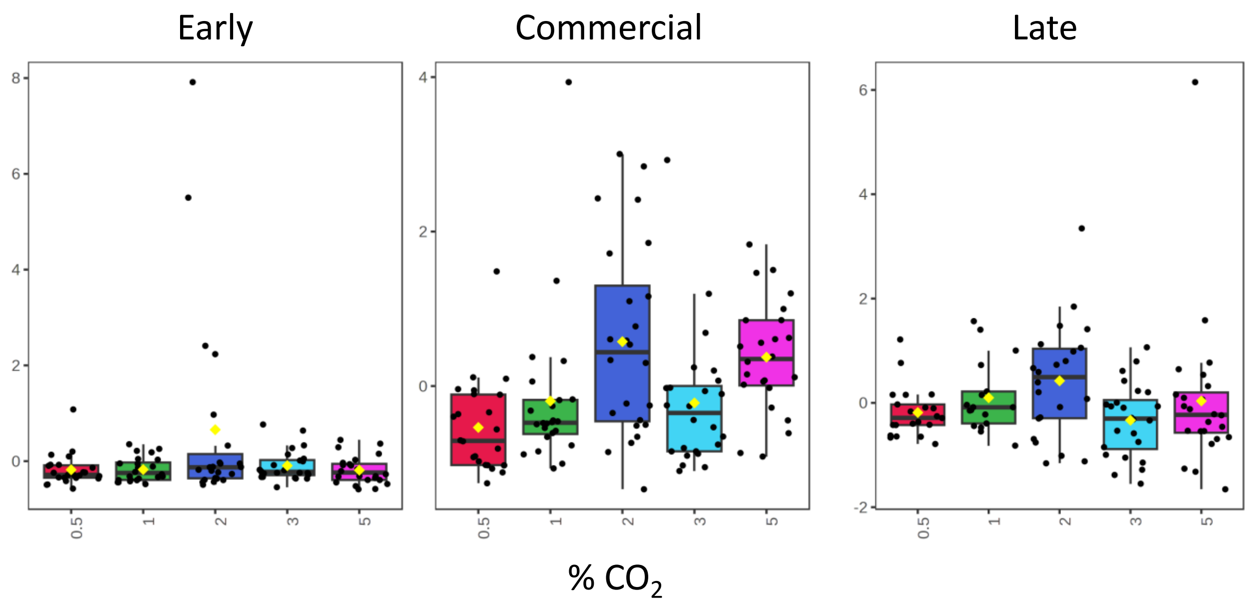

Figure 3. Ratio of ASG/SE, natural chemicals associated with risk of developing CO_2-related internal browning, in Honeycrisp during rapid CA conditioning at 50 °F and 2.5 % O_2 and 0.5, 1, 2.5, or 5 % CO_2. Apples were harvested at 1 week before commercial harvest, commercial harvest, and 1 week following commercial harvest. Each bar represents a compilation of results from samples taken at 0, 2, 4, and 8 days during conditioning. Apples did not develop CO_2-related internal browning.

References:

Leisso, R.S., Buchanan, D.A., Lee, J., Mattheis, J.P., Sater, C., Hanrahan, I., Watkins, C.B., Gapper, N., Johnston, J.W., Schaffer, R.J., Hertog, M.L.A.T.M., Nicolaï, B.M., Rudell, D.R. 2015. Chilling-related cell damage of apple (Malus x domestica Borkh.) fruit cortical tissue impacts antioxidant, lipid and phenolic metabolism. Physiol. Plant. 153:204-220.

Leisso, R.S., Gapper, N.E., Mattheis, J.P., Sullivan, N.L., Watkins, C.B., Giovannoni, J.J., Schaffer, R.J., Johnston, J.W., Hanrahan, I., Hertog, M.L., Nicolaï, B.M., and Rudell, D.R. Gene expression and metabolism preceding soft scald, a chilling injury of 'Honeycrisp' apple fruit. BMC Genomics 2016;17:798.

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Rudell, D., Mattheis, J., Zhu, Y. 2010. Finding scald control tools using apple peel chemistry. WTFRC Final Report. https://treefruitresearch.org/report/finding-scald-control-tools-using-apple-peel-chemistry/

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Project/Proposal Title: Fate of *Listeria* on fresh apples as affected by commercial apple waxes

WTFRC Project Number: AP-20-104A

Report Type: Continuing Project Report

Primary PI: Meijun Zhu
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Cooperators: Stemilt Growers LLC.; Hansen Fruit; Allan Brothers; Pace International LLC.; Jones-Hamilton Co.

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $ 83,842
Total Project Request for Year 2 Funding: $ 85,841
Total Project Request for Year 3 Funding: $ 86,419
Total Project Request for 2024-2025: $ 18,000

WTFRC Collaborative Costs:

Item	**2020-2021**	**2021-2022**	**2022-2023**	
Salaries				
Benefits				
Wages	2,104	2,135	2,168	
Benefits	1,089	1,093	1,110	
RCA Room Rental	2,250	2,316	2,385	
Shipping		275	275	
Supplies	250	275	275	
Travel	350	375	400	
Plot Fees				
Miscellaneous				
Total	6,043	6,469	6,613	

Footnotes:

Budget 1
Primary PI: Meijun Zhu
Organization Name: Washington State University
Contract Administrator: Anastasia Kailyn Mondy
Telephone:
Contract administrator email address: arcgrants@wsu.edu

Item	**2020-2021**	**2021-2022**	**2022-2023**	**2024-2025**
Salaries	32,667	33,974	35,333	
Benefits	6,632	6,898	7,173	
Wages				
Benefits				
Equipment				
Supplies	28,500	28,500	28,500	
Travel	2,000	2,000	2,000	
Miscellaneous	8,000	8,000	6,800	18,000*
Plot Fees				
Total	77,799	79,372	79,806	

Footnotes:

*: Funds are requested to partially support the procurement of autoclave and associated maintenance fees. The current autoclave has exceeded its service life and has been experiencing frequent breakdowns. This not only hampers our research progress but also incurs substantial expenses.

OBJECTIVES

1. Examine the fates of *Listeria*, resident bacteria, and yeast/mold on apples applied with commercial apple wax under subsequent cold storage.
2. Evaluate the fates of *Listeria* on waxed apples contaminated during wax application under subsequent cold storage.
3. Investigate the killing effects of residual sanitizers on the fates of *Listeria* and resident microbes on waxed apples under subsequent cold storage.

SIGNIFICANT FINDINGS

1. The dry temperature, whether at ~22 °C/72 °F, 45 °C/113 °F, or 60 °C/140°F, showed no discernible impact on the survival of *L. innocua* on wax-coated apples.
2. The population of *L. innocua* on unwaxed apples decreased by 1.9 log_{10} CFU/apple over the course of 18 weeks of refrigerated air storage.
3. *L. monocytogenes* reduced by 1.8-2.0 log_{10} CFU/apple on waxed apple during 12-week cold storage, regardless of the type of wax coating.
4. The fate of *Listeria* on wax-coat apples was similar to that on unwaxed apples.
5. The die-off rate of *L. monocytogenes* on wax-coated apples contaminated during wax coating was not significantly different from those contaminated-on apples before wax coating.
6. We identified a significant risk of cross-contamination of *L. monocytogenes* from inoculated apples to waxing brushes and from contaminated brushes to uninoculated apples during wax coating application process.
7. The fate of *L. innocua* on Granny Smith apples exhibited comparable trends to those observed on Fuji apples, irrespective of the specific type of wax coating.
8. Different *L. monocytogenes* serotypes, including 1/2a, 1/2b, and 4b, exhibited distinctive survival profiles on Granny Smith apples.
9. Serotype 1/2a displayed the highest resilience, sustaining a high population on Granny Smith apples throughout storage, whereas serotype 4b, linked to the caramel apple outbreak, exhibited the lowest survivability on apples, with a rapid decline observed within 48 h of attachment at 22 °C/72 °F.
10. The inclusion of fungicides in wax coating effectively reduced yeasts and molds on wax-coated apples; however, it did not have an impact on *L. monocytogenes*.
11. Wax coating had no impact on the survival of yeasts and molds on apples, irrespective of the apple cultivars; an increase of 0.4-0.5 log_{10} CFU/apple was observed after 18 weeks of cold storage, regardless of the type of wax treatment applied.
12. Wax coating increased the glossiness of apples regardless of wax treatment.
13. The application of wax, regardless of the wax coating type, maintained total soluble solids (TSS) in apples after 18 weeks of cold storage, whereas TSS significantly increased in unwaxed apples.
14. The titratable acidity (TA) decreased in both unwaxed and waxed apples after 18 weeks of cold storage. The application of wax coating, irrespective of its type, had no impact on interior and exterior disorders on Fuji apples, while it significantly reduced internal browning in Granny Smith apples.

METHODS

1. Strain selection

<u>*L. monocytogenes* strains for BSL2 lab storage:</u> To elucidate the impact of strain variability, a panel of *L. monocytogenes* serotypes consisting of serotypes 1/2a, 1/2b, and 4b was selected and used in this study. *L. monocytogenes* strains for serotype specific survival profiles included serotypes 1/2a (celery isolate with erythromycin resistance), 1/2b (cantaloupe isolate with erythromycin and rifampicin resistance), and 4b (Bidart apple outbreak strain with streptomycin resistance).

<u>*L. innocua* strains employed for commercial cold storage:</u> *L. innocua*, a widely used surrogate for *L. monocytegenes*, was used to investigate the fates of *Listeria* during commercial cold storage. A 3-strain cocktail of *L. innocua* isolates, sourced from an apple packing facility and other fresh produce processing plants, was prepared using our established methodology.

2. Apple inoculation

<u>Apples were contaminated with *Listeria* prior to the waxing application:</u> Washed and unwaxed apples of the selected varieties without cuts or bruises were individually and separately inoculated to establish 1×10^6 CFU/apple of 3-strain cocktail of *L. monocytogenes* or *L. innocua* per our well-established method. The inoculated apples were held at 22 °C for 24h before the wax coating was applied.

<u>Apples were contaminated during waxing application:</u> To test the potential of *L. monocytogenes* cross-contamination from apple-to-brush and brush-to-apple, one waxing brush was used to coat one *L. monocytogenes* inoculated apple; then, this contaminated brush was used to wax five uninoculated apples in a sequence (Fig. 1).

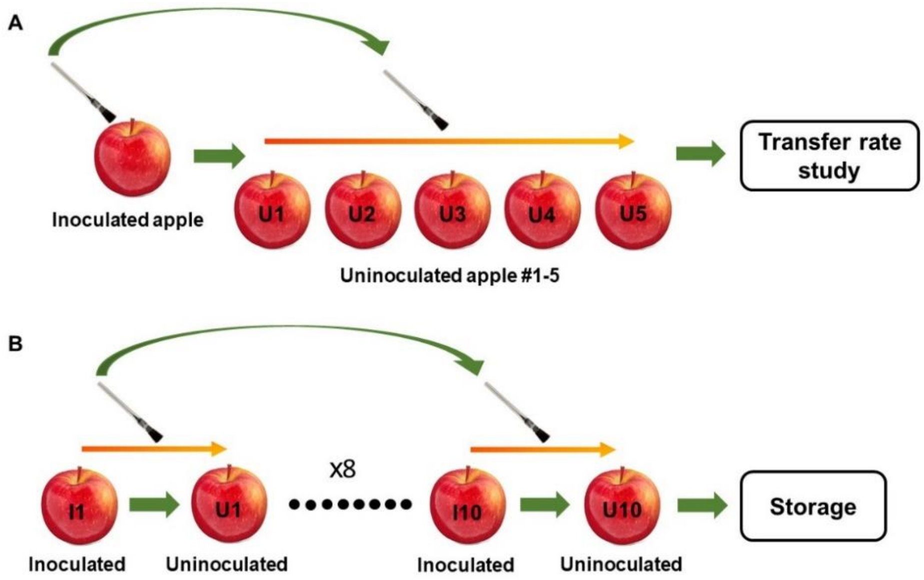

Fig. 1. Illustration for the preparation of waxed apples contaminated with *Listeria monocytogenes* during wax coating. A. Wax-coated apples for the apple-to-brush and brush-to-apple transfer rate study. B. Wax-coated apples for the storage study. I: inoculated apple; U: uninoculated apple.

3. Waxing application

<u>Wax selection:</u> Three commercial apple fruit waxes, namely Prima Fresh 360 HS (PF360), Prima Fresh 606 EU (PF606) or Shield Brite AP-450 (AP-450) were used in the proposed studies.

Waxing application: Each wax solution was manually applied evenly to both inoculated and uninoculated apple surfaces of the selected cultivars unless specified. To assess the fate of *Listeria* on waxed apples subjected to cross-contaminated during the waxing process, brushes contaminated with *L. monocytogenes* were employed for the manual application of wax to apples, while cross-contamination of *L. monocytogenes* to uninoculated apples (Fig. 1).

4. Wax coating drying

To evaluate the impacts of wax coating drying conditions/temperatures on the survival of *Listeria* on waxed apples, apples immediately following wax coating were subjected to different drying temperatures (~22 °C/72 °F, 45 °C/113 °F, or 60 °C/140°F) for 2 min, followed by an additional 5-h drying at room temperature (~22 °C/72 °F) before being subjected to cold storage.

5. Cold storage treatments and sampling

BSL2 lab cold storage: Uninoculated or inoculated apples of the selected cultivars were subjected to 1°C/33°F storage for 16 weeks and sampled weekly/biweekly for enumeration of *L. monocytogenes* or resident microbiota (background bacteria or yeast/mold), respectively. Two independent and sequential trials were conducted using different lots of fruits. In each independent trial, twenty apples per treatment were sampled on each designated sampling day.

Commercial facility storage: Uninoculated apples and apples inoculated with a 3-strain *L. innocua* cocktail, coated with different wax coating, were subjected to storage at 1°C/33°F for 12-18 weeks in refrigerated air (RA) room of the commercial packing facility. Apples of each treatment combination were sampled after 2, 4, 6, 9, 12, and 18 weeks to enumerate the survival of *L. innocua* and yeast/mold. Studies were conducted over two consecutive years. Four sets of 10 fruits were used for each wax treatment on each sampling day in each independent study.

6. Survival microorganism analysis

Listeria enumeration: At each sampling day, *Listeria* survival on waxed apples under the respective storage (BSL2 or commercial facility) were detached and serially diluted. Appropriate dilutions were plated on trypticase soy agar supplemented with 0.6% yeast extract (TSAYE) plates overlaid with modified Oxford agar per our established method. For the serotype-specific survival profile analysis, the detached microbial suspensions were plated onto TSAYE plates with erythromycin, erythromycin and rifampicin, and streptomycin for the enumeration of serotype 1/2a serotype 1/2b, and serotype 4b, respectively.

All plates were incubated at 35˚C/95 °F for 48 h and subsequently enumerated. If the survival of *Listeria* on apple fruit fell below the enumerative detection limit, the suspension was assessed for presence/absence after 48h of enrichment in Buffered *Listeria* Enrichment Broth (BLEB) and streaked onto a selective *Listeria* agar plate. Presumptive positive colonies were further confirmed by PCR (FDA, 2015).

Resident microbiota: Microbial suspension at appropriate dilutions were also plated on duplicate Potato Dextrose Agar plates supplemented with 0.1 g/l chloramphenicol for yeast and mold counts. The PDA plates were incubated at room temperature (~22°C/72 °F) for 5 days.

7. Fruit quality analysis

At harvest or 18-week storage, fruit quality parameters such as firmness, total soluble solids, and titratable acidity, as well as external and internal disorders, including superficial scald and lenticel decay, were assessed at the end of cold storage by the WTFRC quality lab using established methods (Sheng et al., 2018). A sample size of 10 apples per replicate with 4 independent replicates per wax type was used for internal and external disorder assessment.

8. Glossiness measurement

The gloss index of apples was determined at 60° with a glossmeter (Novo-Curve, Rhopoint Instrumentation, East Sussex, UK). The gloss units (GU) were directly measured on the fruit surface with 10 randomly selected spots per fruit. A total of 10 apple fruits per treatment condition was used for gloss analysis.

9. Statistical analysis.

Data were analyzed with IBM SPSS 19.0 (Chicago, IL). Mean differences were assessed through one-way analysis of variance (ANOVA), followed by a Tukey multiple comparison test. *P* values less than 0.05 were considered significant differences.

RESULTS AND DISCUSSION

1. Serotype-specific survival of L. monocytogenes on Granny Smith apples during 48 h of attachment

Different *L. monocytogenes* serotypes exhibited distinctive survival behavior on Granny Smith apples, with 4b exhibiting a sharp population decrease of 1.36 log CFU/apple in the initial 48 hours at 22°C/72 °F. In contrast, populations of serotypes 1/2a and 1/2b increased by 0.44 and 0.50 log CFU/apple during the 48-h attachment period (Figure 2). The trend of the 3-strain cocktail is mirroring the behaviors of ½ a and ½ b serotypes with high resistance.

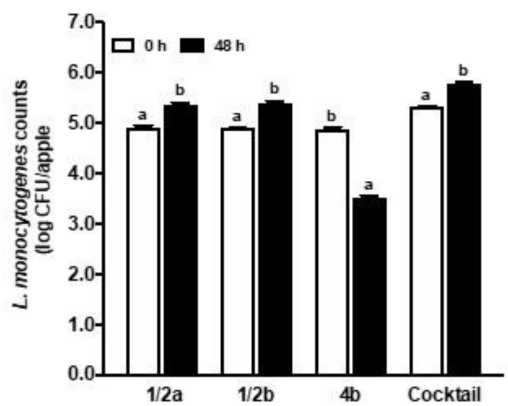

Figure 2. Recovery of *L. monocytogenes* serotypes on Granny Smith apple right after inoculation (0 h) and 48 h post-inoculation (48 h). Data were presented with mean ± SEM, n = 30. [a-d] Means at each sampling point without a common letter differ significantly ($P < 0.05$).

2. Survival of L. monocytogenes on waxed apples contaminated during different waxing schemes

Over the 12 weeks of 1°C/33°F storage, distinct survival profiles were observed for different *L. monocytogenes* serotypes. After a sharp decrease during the initial 48 hours of attachment, serotype 4b maintained a consistently low but stable population on apples across all treatments throughout the 12 weeks of storage (Figure 3A-C). Serotype 1/2a displayed remarkable resilience, sustaining a high population throughout the entire storage period. Serotype 1/2b exhibited stability during the 48h of attachment and initial 9 weeks of storage but underwent a drastic reduction from 9 to 12 weeks of storage. The counts of the 3-strain *L. monocytogenes* cocktail on Granny Smith apples remained relatively stable, mirroring serotype 1/2a behavior (Figure 3A-C). The fate of each serotype strain exhibited comparable outcomes on both unwaxed and waxed fruits, as well as on PrimaFresh 360 HS (PF 360) and Shield-Brite AP 450 (AP 450) coated fruits (Figure 3). The behavior of these serotypes on Granny Smith apples stored at 22°C/72°F displayed parallel trends to those observed under cold storage conditions (Figure 4).

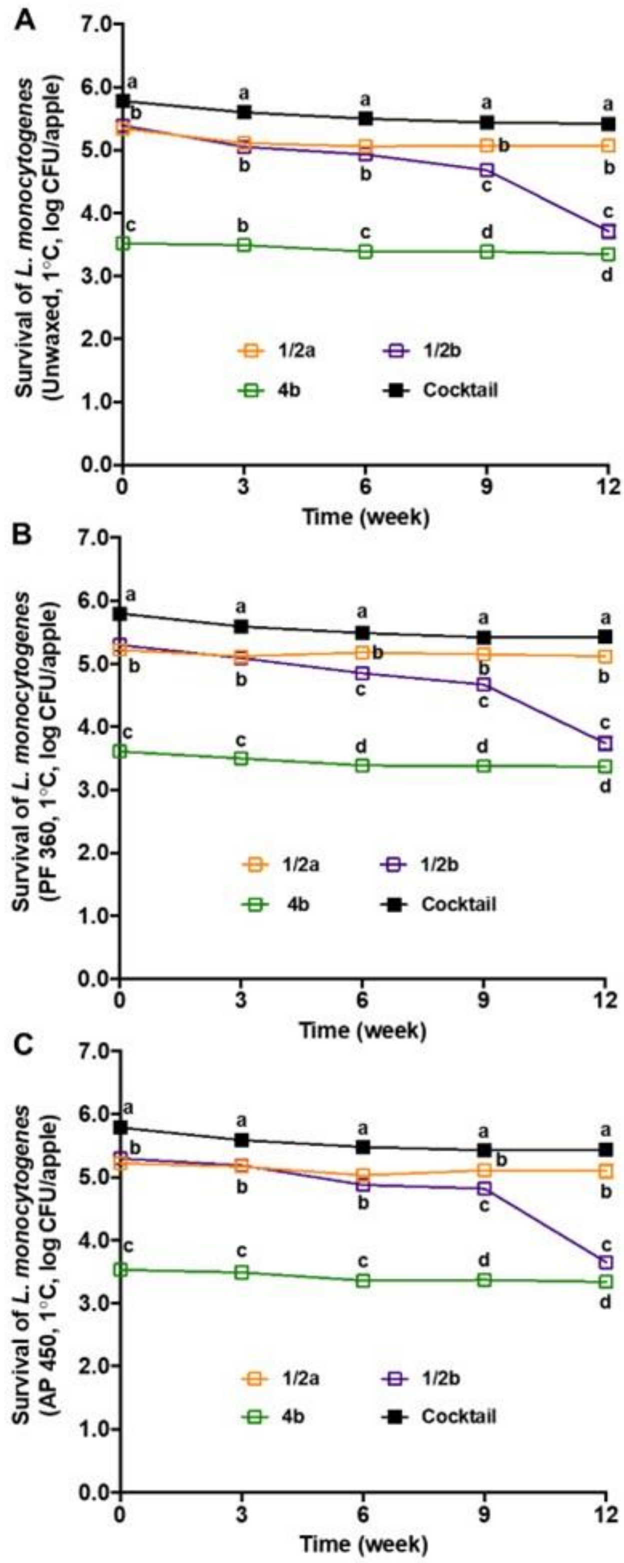

Figure 3. Survival of different *L. monocytogenes* serotypes on unwaxed and waxed Granny Smith apples during 12 weeks of 1 °C/33 °F storage. A. Unwaxed apples. B. PrimaFresh 360 HS (PF 360) coated apples; C. Shield-Brite AP 450 (AP 450) coated GSA. Mean ± SEM, n = 40. Different letters (a-d) indicate significant differences at each samping point ($P < 0.05$).

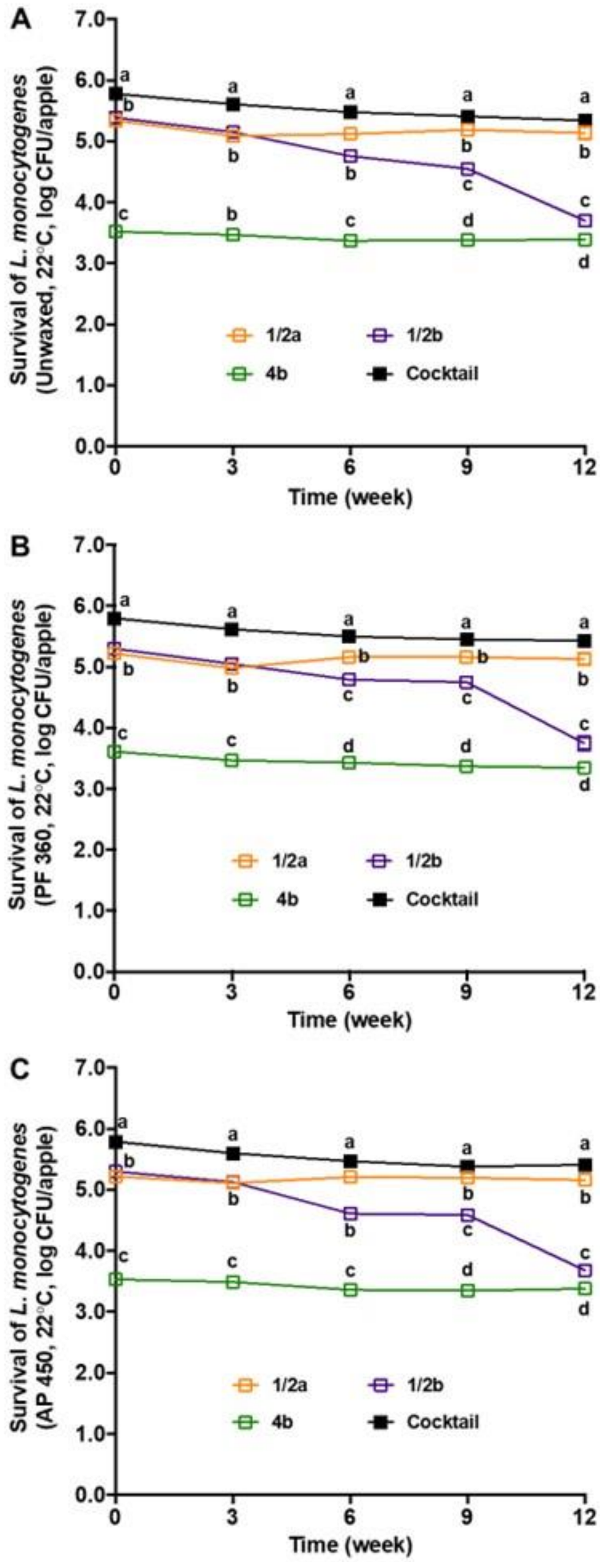

Figure 4. Survival of different *L. monocytogenes* serotypes on unwaxed and waxed Granny Smith apples during 12 weeks of 22 °C/72 °F storage. A. Unwaxed apples. B. PrimaFresh 360 HS (PF 360) coated apples; C. Shield-Brite AP 450 (AP 450) coated GSA. Mean ± SEM, n = 40. Different letters (a-d) indicate significant differences at each samping point ($P < 0.05$).

3.2 Fate of Listeria and residential yeasts and molds on wax-coated Granny Smith apples during commercial cold storage

By the end of 18 weeks of commercial RA storage, there was a reduction of 1.64 log CFU/apple on unwaxed Granny Smith apples, and a reduction of 1.76 and 1.96 log CFU/apple on PF 360 and AP 450 coated Granny Smith apples, respectively (Figure 4A). These reductions are comparable to those observed on Fuji apples (Su et al., 2023), although slightly greater reduction was noted on waxed Granny Smith apples compared to their unwaxed counterparts (Figure 4A).

Populations of yeasts and molds were increased by 0.63 log CFU/apple after 18 weeks of commercial RA storage, irrespective of wax coating or the type of wax coating. This observation aligns with our previous findings on unwaxed Granny Smith apples (Sheng et al., 2022) and waxed Fuji apples during 18 weeks of commercial RA storage (Su et al., 2023).

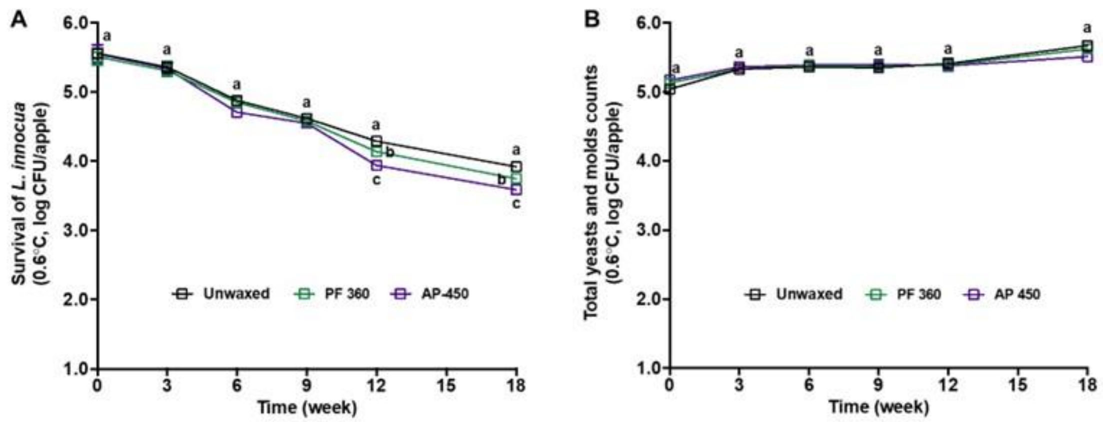

Figure 4. Fates of *L. innocua* and yeasts and molds on Granny Smith apples coated with or without wax during 18 weeks of commercial refrigerated air storage. A. *L. innocua*; B. yeasts and molds counts. PF 360: PrimaFresh 360 HS; AP 450: Shield-Brite AP 450. Mean ± SEM, n = 40. Different letters (a-c) indicate significant differences at each sampling point ($P<0.05$).

4.3 Quality attributes of wax-coated GSA during commercial cold storage

Granny Smith apples coated with AP 450 exhibited protection against apple weight loss compared to unwaxed apples after 18 weeks of cold storage. This protective effect of AP 450 application on weight loss was also observed in Fuji apples during 18 weeks of cold storage (Su et al., 2023). TA decreased in both unwaxed and wax-coated GSA after 18 weeks of cold storage, consistent with our earlier finding on unwaxed Granny Smith apples during 24 weeks cold storage (Sheng et al., 2022). The application of wax coating, regardless of wax type, increased TSS in apples, improved firmness loss, and reduced internal browning compared to unwaxed apples after storage (Data not shown).

References:

FDA. (2015). BAM Protocol: Simultaneous Confirmation of *Listeria* species and *L. monocytogenes* isolates by real-time PCR. Retrieved from https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm279532.htm

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 Microbiol, 76*, 21-28. doi:10.1016/j.fm.2018.04.006

Sheng, L., Shen, X., Su, Y., Xue, Y., Gao, H., Mendoza, M., Green, T., Hanrahan, I., & Zhu, M. J. (2022). Effects of 1-methylcyclopropene and gaseous ozone on *Listeria innocua* survival and fruit quality of Granny Smith apples during long-term commercial cold storage. *Food Microbiol*, *102*, 103922. doi:10.1016/j.fm.2021.103922

Su, Y., Shen, X., Hua, Z., Zhu, H., Chiu, T., Wang, Y., Mendoza, M., Hanrahan, I., & Zhu, M. J. (2023). Fate of *Listeria innocua* on wax-coated Fuji apple surfaces under commercial refrigerated air storage. *Postharvest Biol Technol*, *198*, 112236.

Project Title: WA38 applied research and demonstration block

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Cooperators: Keith Oliver, Garret Henry, Derek Hill, David Gleason, Valent BioScience, Bleyhls Co-op, Burrows tractor Inc, Drape Net, Corsi Consulting.

Report Type: Final report

Project Duration: 3 -Years

Total Project Request for Year 1 Funding: 5,733
Total Project Request for Year 2 Funding: 9,605
Total Project Request for Year 3 Funding: 9,907

Other funding sources: **Awarded**
Amount: $152,938
Agency Name: Root Growth Management to Reduce Ca Deficiency Disorders in Apples and Cherries. Washington State USDA- Specialty Crop Block Grant. $152,938. P.I. B. Sallato. Co-P.I.s; L. Kalcsits, M. Whiting.
Notes: Costs associated with objective 1 and wages for hourly support during sample collection will be covered by this proposal.

Other funding sources: **Awarded**
Amount: $50,000
Agency Name: IAREC – WSU
Notes: Funding support for five years (2020 – 2025) provided by Naidu Rayapati, IAREC Director for tree fruit orchards maintenance and plot fees.

Other funding sources: **Awarded**
Amount: $15,000
Agency Name: Valent BioScience
Notes: Costs associated with ReTain and Pollen spray for fruit set.

Organization Name: Washington State University
Contract Administrator: Anastasia Mondy
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Station Manager: Naidu Rayapati
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Item	**2021**	**2022**	**2023**
Salaries			
Benefits			
Wages	3,000	6,520	6,781
Benefits	673	1,025	1,066
Equipment			
Supplies[1]	2,060	2,060	2,060
Travel			
Miscellaneous			
Plot Fees			
Total	5,733	9,605	9,907

Footnotes: [1] Wages and benefits to support data collection. Supplies include tissue samples and chemical analyses if peel and fruit for nutrient differences in apples with and without physiological disorders (activity a and b), and fruit quality supplies: sampling bags, iodine, flagging tape, etc.

OBJECTIVES

1. Evaluate horticultural practices on WA 38 grown on G41 and M9-Nic 29 for better production and fruit quality.

We managed, monitored and conducted experiments in the WSU WA 38 Roza orchard. Parallel projects include a heat stress monitoring and mitigation project, led by PIs Khot, and "Ca-related disorders management for vigorous conditions", led by PI Sallato. Information regarding these projects will be reported separately.

2. Utilize the WA 38 Roza farm as a demonstration block for community engagement and outreach.

The WA 38 Roza farm provided a venue for community engagement and outreach. The Roza Farm hosted over 20 field days, workshops and visitors, reaching over 150 people. The workshops were delivered in English and Spanish.

SIGNIFICANT FINDINGS

- At the WA 38 Roza farm, the optimum load per tree, based on fruit load and fruit quality, ranged between 90 and 110 fruits per tree on bi-axis and spindle (1210 trees per acre), and between 65 and 80 fruits per tree on V-trellis (2420 trees per acre)
- WA 38 can overcrop (as seen in 2021) and induce alternate bearing. Thus, there is a need to identify the maximum production potential under each growing condition.
- At the Roza site, WA 38 requires management to prevent excessive vigor, given that the soil is deep, with high water retention and no evidence of limiting conditions. We were able to reduce vigor and increase crop load by implementing summer pruning (between June and August), reducing N and K levels, and controlling water
- G41 exhibited more vigor than M9-nic 29, which was related to higher root growth, root growth rate, and a longer root growth period. Higher root growth translates into higher leaf nutrient uptake, vigor, and green spot incidence
- There is a strong relation between green spot (GS) and nutrient imbalance, specifically between calcium (Ca) and nitrogen (N), an imbalance caused by excessive vigor (Sallato et al., 2021). Green spot severity positively correlated with N and B concentration. Ca was significantly lower in severe GS ++ compared to the control with no symptoms.
- Supplemental pollen at a rate of 30 g per acre via electrostatic application applied at bloom increased fruit set by 36% and final fruit load by 48% compared to the untreated control.
- AVG (ReTain®) applied alone, in combination with pollen or after petal fall, increased fruit per tree between 45% to 85% when compared with the control. However, these treatments did not always lead to increased fruit set or yield.
- While supplemental pollen spray aims to increase the availability and transfer of pollen, ReTain® aims to increase ovule longevity. Thus, when pollen availability, bee activity, or a short pollination period is expected, these tools could improve production, while there is no benefit if these factors are not limiting.
- Summer pruning increased fruiting wood and fruit yield in the second season after the summer prune. There were no differences among the different timings of summer pruning; however, the earliest pruning (May) could induce bud break in the fall (fall blooms). Spring pruning can affect fruit size if done aggressively, as it removes carbohydrate sources; however, it did not induce fruit drop.

METHODS

1. ***Evaluate horticultural practices on WA 38 grown on G41 and M9-Nic 29 for better production and fruit quality.***

The WSU WA 38 block was planted in 2013 in a 0.8-acre block, to evaluate rootstock and training systems. The orchard is divided in three training systems: Spindle 3 x 10 ft (rows 1 to 4), V trellis with spindle training at 1.5 x 12 ft (rows 5 to 8) and bi-axis at 3 x 10 ft spacing (row 9 to 11), on two rootstocks, Geneva 41 (G.41) and M9-NIC29 (Figure 1). Rootstocks are randomly distributed within each training system in blocks of 10 or 22 trees.

Figure 1. WA 38 at WSU Roza Farm with three training systems; spindle (3 x 10 ft), V-trellis (1.5 x 12 ft.) and bi-axis (3 x 10 ft).

Initially the pollinizers were Granny Smith and Chehalis at density approximately of 14% (9% in V-Trellis and 18% in Bi-axis) on M-26 rootstock. In 2017, the Roza Farm was affected by a hail event during bloom accompanied by favorable conditions for fire blight development. Consequently in 2018, 24% of the WA 38 on M9-nic 29 and 11% of the pollinizers died due to fire blight infection and trees were removed. In 2020, we replaced the removed trees with WA 38 on Geneva 11 (G.11) and added missing pollinizers Snowdrift and Evereste crab apple.

Soil conditions: The block is located on a silt loam soil, corresponding to the Warden series over basalt rock. The depth varies slightly between 2.5 feet of effective soil depth to more than 4 ft. Above the basalt rock, some areas have $CaCO_3$ (Caliche), with pH ranging between 7.0 and 7.8. Soil P, S and B levels are usually low.

Training Systems

Spindle; row 1 to 4, with 28 blocks of 10 trees. Initially trained by bending branches, which led to blind wood and low productivity. Since 2018, we been slowly transitioning to traditional spindle. This section is notoriously more vigorous than V-Trellis and bi-axis, providing us the opportunity to learn about green spot and vigor management. Since 2021 we been using these blocks for the PGR trial to evaluate Ca related disorders (Sallato's final report on PGR's for vigor control)

V-Trellis; row 5 to 8, with 28 blocks of 22 trees. This block continues to be managed with winter pruning, summer pruning and hedging. Six trees in this section have a root window (rhizotron) to monitor root growth differences between rootstocks (Obj 1).

Bi-axis; row 9 to 11, with 20 blocks of 10 trees. This section was planted a year later (2014). Since 2018 trees have been pruned lightly during the winter to remove undesired branches; redundant, hanging, and renew wood, followed by summer pruning and hedging. Since 2021, these blocks have been used to evaluated heat monitoring and mitigation practices (Khot's heat stress final report).

General management

Disease and pest management is under advice from Jeff Sample (Blehyl Co-op). Mayor challenges have been fire blight (2018-2019), thrips (2021), and mildew (2019-2022). In 2020, the irrigation system was upgraded and divided for each training system, utilizing Wiseconn Engineering monitoring and controls platform. A set of moisture and temperature sensors were installed on each section, and one weather monitoring system for the entire block. A Venturi system was installed for fertigation in 2021. Additional monitoring systems have been installed in the bi-axis section, associated to the heat stress project (for more details review Khot et al, 2021 report)

Soil and nutrient management

Initial soil analysis (2019) indicated mineral deficiencies of phosphorous (10 mg/kg), sulphur (8 mg/kg), zinc (0.50 mg/kg) and boron (0.12 mg/kg) according to recommended levels (http://treefruit.wsu.edu/orchard-management/soils-nutrition/fruit-tree-nutrition/). In 2019, we applied 100 lbs. per acre of mono ammonium phosphate (MAP), 25 lbs of $ZnSO_4$/acre and 2 lbs of B/acre. Since 2019, we have continued with spring ground application of P (MAP) at 150 lbs/acre and foliar B and Zn (fall and spring). In 2022, we added 23 g of Urea per tree (individually) to all young, replanted trees and new pollinizers.

Research project

1.a. Differences in root growth and nutrient uptake between M9-Nic29 and G41. (Funding source Washington State USDA- Specialty Crop Block Grant. $152,938. Ending 2021). (Sallato)

Root windows (3 x 3 x 3-foot cubes with Plexiglas on one and plywood for other sides) were installed on three random trees per rootstock since 2019. Evaluation of root growth starts prior to bloom and continues every week during spring period when roots are actively growing, and every other week during the summer and fall. Each root window is treated as a replicate unit. Monitoring of root growth is done manually by drawing a quadrant (1.5 x 1.5 ft.) in the middle of the plexiglass and monitoring white roots during the growing season. New growth is recorded and measured on site, then marked with different colors to identify period of growth. At the end of the season, each tree is strip harvested to determine yield, crop load and fruit quality. 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/)

1.b. Green spot nutrient composition differences, rootstock, and vigor. (Partially funded by Washington State USDA- Specialty Crop Block Grant. $152,938) (Sallato).

From 2018 to 2021, fruit with and without green spot (GS) have been collected from trees on G41 and M9-Nic 29 rootstocks. At harvest, fruit from different rootstocks and training systems were collected to determine fruit per tree, crop load and GS incidence. From each experimental unit and rootstock, fruit from six representative trees with (GS+) and without green spot (GS-) symptoms were collected for quality analysis. Then, each individual fruit were separated into peel, flesh, core and seeds to determine fresh and dry matter proportions. Subsequently, each tissue sample was dried, homogenized and sent to a commercial laboratory for nutrient analysis; nitrogen (N), phosphorous (P), potassium (K), calcium (Ca) magnesium (Mg), iron (Fe), zinc (Zn), cupper (Cu), manganese (Mn) and boron (B) analyses following the method recommended for total tissue analyses (Gavlak et al., 2005). In 2020 and 2021 we added an additional level of GS severity associated to milder symptom (greening), to determine relation with nutrient concentrations.

1.c. Use of AVG (ReTain ®) and artificial pollination to improve fruit set and production (Sallato).

In 2019 to 2021, we studied the effect of an ethylene inhibitor (AVG; ([S]-trans-2-amino-4-(2-aminoethoxy)-3-butenoic acid hydrochloride) (ReTain ®, Valent) and supplemental pollen application on WA 38 fruit set. The trials were conducted in the WA 38 Roza farm and in three commercial orchards; **Buena** 4th and 5th leaf WA 38 trial consisted of five treatments. 1. Pollen, 2. Pollen + ReTain ® at 80% bloom, 3. Pollen + ReTain ® at petal fall, 4. ReTain ® alone at petal fall and 5. Untreated control. All pollen treatments consisted of two applications (approximately at 30 and 80% open flowers) with 15 g of pollen/acre (70% Red Delicious and 30% Granny smith) each provided as in-kind by Firman Pollen. Treatments were applied with electrostatic sprayer provided as in-kind by OnTarget, USA. **Roza WA 38**, 9th leaf consisted of four treatments: 1. Pollen, 2. Pollen + ReTain®, 4. ReTain ® alone and 4. Untreated control. All treatments consisted of one application at 80% bloom of 30 g/acre equivalent. The application was conducted with battery powered backpack sprayer. ReTain® application were all at 333g/acre rate (1 pouch), provided as in-kind by, Valent Bioscience, USA. In all trials we determined the percent of open flowers prior to the application, fruit set (July) and percent of single, double or triple at harvest. Results from this and the other commercial sites have been shared in the pre-harvest field day (2021) and 2022 WSTFA annual meeting.

1.d. Pruning strategies to promote fruiting wood (Sallato).

During the summer, random sections of sets of tree trees throughout the block were selected and pruned at different timings in 2021: June 26th, July 26th and August 25th. In 2022, the same set of trees were left unpruned during the winter, and again pruned during the summer on May 30th, June 16th or September 8th. Fruit yield was monitored during harvest.

In addition, in 2022 and 2023 forty random trees with equivalent bloom density were selected. All trees received a light winter pruning to remove excessively vigorous shoots (thinning cuts) and reduce long hanging shoots. During the growing season a set of 10 trees each were pruned at 0 leaf (0L), five leaf (5L), ten leaf (10L) or left unpruned (control). Total fruit weight, fruit per tree and defects were evaluated at harvest.

1.e. Fruit ripening variability between systems and rootstocks (Sallato, Bolivar).
In 2020 and 2021, three trees per training system and rootstocks were selected during harvest, and each fruit was evaluated for starch content utilizing the WA 38 starch index chart (Hanrahan et al, 2019) http://treefruit.wsu.edu/wa38-starch-scale/.

RESULTS

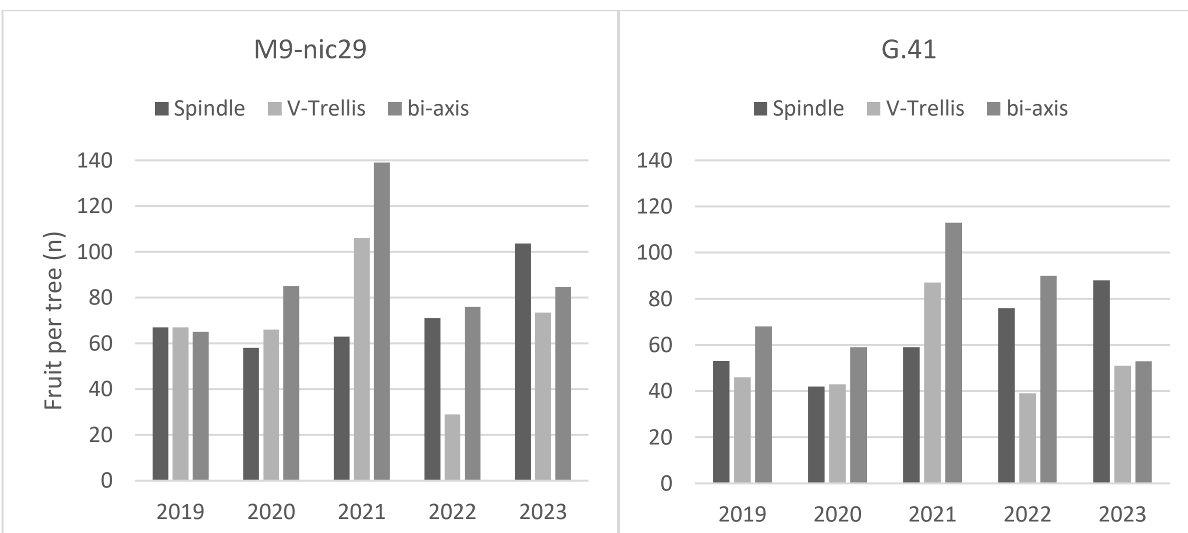
In this report, we provide a summary of key results and focus only on results not reported elsewhere, with focus on final recommendations for WA 38.

Evaluate horticultural practices on WA 38 grown on G41 and M9-Nic 29 for better production and fruit quality.

Since 2018, we have focused our management to reduce excessive vigor, controlling irrigation, reducing fertilizer application based on demand, soil and tissue test, and throughout minimal winter pruning and more intensive summer pruning. Table 1 shows the changes in yield per tree, fruit size and green spot incidence for the last five years, and an estimation of the most frequent box size (based on fruit weight) and bins per acre (based on average fruit weight and a 980 lb, discarding the percent of fruit with green spot). Note that in this estimation, we are considering between 10 to 50 trees per system and rootstock, we are not accounting for other defects such as cracks, thrips or bird picks that were significant in 2021 and 2022.

Overall M9-Nic29 had higher productivity (fruit per tree) compared to G.41 (Figure 1). As shown in Figure 1, in the spindle system, production has been increasing steadily, which has also increased fruit quality and reduced green spot incidence that was very high during 2018 – 2019 (Table 1). In 2023, fruit size averaged 289 g and 272 g for M9-Nic29 and G.41 respectively, and both picked at 81 mm and estimated 64 – 72 box size, the best quality obtained in 2023 across all years.
On the V-trellis, for both rootstocks, the productivity increased dramatically in 2021, to more than 100 fruit per tree in M9nic29 and more than 80 fruit per tree in G.41, leading to smaller fruit in both rootstocks and alternate bearing. In both rootstocks the production dropped to more than half in 2022, and while we didn't see an increase in green spot, there was high level of cracking (apro. 30%). In 2023 fruit yield increased as expected, however to a moderate level of 70 fruit per tree in M9-nic 29 and only about 50 fruit per tree in G.41.

Similarly in 2021, bi-axis trees were also overcropped on M9-nic 29 with almost 140 fruit per tree, leading to small fruit size approx. 237 g average and between 69 and 85 mm diameter, while reduced green spot incidence (below 4%). Consequently, in 2022 we had reduced fruit load, although slightly better fruit size (241 g). In 2023 crop load was slightly increased in M9-nic 29 with no differences in fruit size. In contrast, on G.41 a high crop load in 2021 (average 113 fruit per tree), led to a slight reduction in 2022 (90 fruit per tree). Surprisingly, we observed a greater reduction in 2023 with 53 fruit per tree.



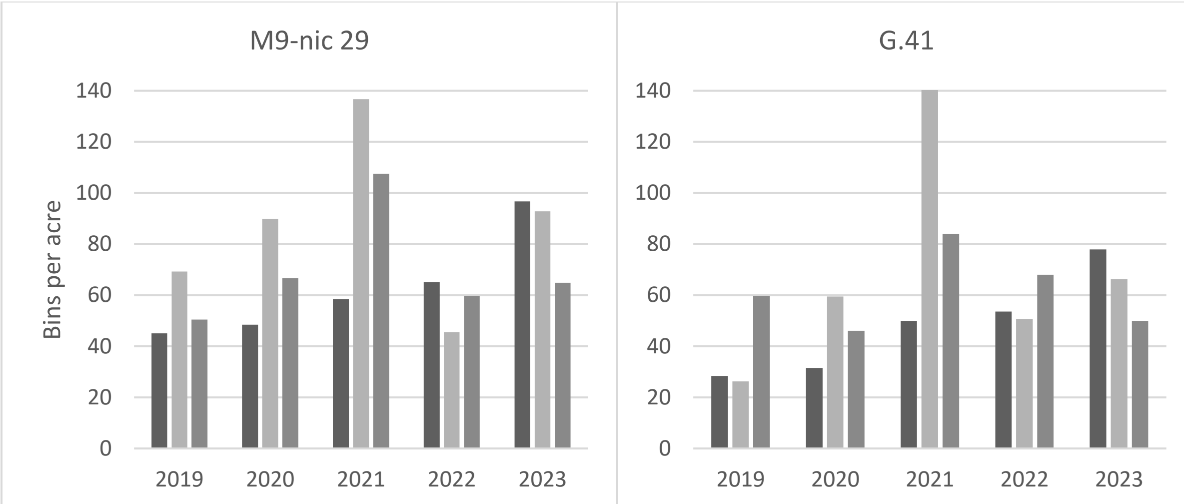


Figure 1. Top; fruit per tree (n) in WA 38 on M9-Nic 29 (a) and G.41 (b) trained as vertical spindle at 3 x 12 ft, on V-trellis at 1.5 x 12 ft and vertical bi-axis at 3 x 12 ft. Bottom; bins per acre in WA 38 on M9-Nic 29 (c) and G.41 (d) estimated based on average fruit weight and a 980 lb bin, accounting for fruit losses associated to green spot only.

Table 1.

Rootstock	System	2019	2020	2021	2022	2023
				Fruit weight (g)		
M9 -Nic29	**Spindle**	290	276	290	287	289
	V-Trellis	221	259	237	289	237
	bi-axis	240	241	237	241	235
G41	**Spindle**	298	316	298	230	272
	V-Trellis	239	284	298	244	241
	bi-axis	328	257	237	241	289
				Green spot (%)		
M9 -Nic29	**Spindle**	29	7.3	2	2	1
	V-Trellis	14	3.4	0	0	2
	bi-axis	1	0.3	0	0	0
G41	**Spindle**	45	27.1	13	6	0.3
	V-Trellis	56	10.4	0	2	1
	bi-axis	18	7	4	4	0
				Box size (n), 40 lb box		
M9 -Nic29	**Spindle**	63	66	63	63	63
	V-Trellis	82	70	77	63	77
	bi-axis	76	75	77	75	77
G41	**Spindle**	61	57	61	79	67
	V-Trellis	76	64	61	74	75
	bi-axis	55	71	77	75	63

Based on our results and growing conditions, we estimate that the optimum load on a bi-axis and spindle systems ranged between 90 to 100 fruit per tree, for both rootstocks. For V-trellis the optimum load ranged between 65 and 80 fruit per tree.

Soil nutrient levels were initially high in potassium (K) while low in phosphorous (P-Olsen), sulfate (SO4), and boron (B) (Table 2). We have been able to reduce the levels of K by not adding any K since 2018. Levels of have increased slowly as a consequence of our annual application of mono ammonium phosphate to the ground (100 – 150 lbs/acre). Levels of S and B remain low.

Table 2. Soil chemical levels from 2020 to 2023.

Parameter	Unit	Optimum	4/4/2020	5/4/2021	3/21/2022	4/17/2023
pH	-	5.0 – 7.0	7	7	7.1	7.3
K	mg/kg	150 - 250	224	271	237	171
	meq/100g	0.5 - 0.65	1.9	2.3	2.0	1.4
Ammonium_N	mg/kg	-	1.8	2.8	9.2	2.1
Nitrate-N	mg/kg	-	0.9	0.6	1.8	1.2
O.M	%	> 1	1.5	1	1	1.9
Ca	meq/100g	4.0 - 20	8.3	8.4	8.7	8.6
Mg	meq/100g	0.5 – 2.5	3.6	2.9	2.9	3.1
CEC	meq/100g	11 - 40	13.9	13.7	13.7	13.2
P Olsen	mg/kg	15 - 40	7.0	7.0	8.0	16.0
Sol.Salts	mmhos/cm		0.1	0.3	0.6	0.1
Sulfate-S	mg/kg	9 - 20	0.9	6.0	6.0	2.3
Na	meq/100g	< 0.5	0.2	0.1	0.1	0.1
Zn	mg/kg	0.6 – 1.0	4.3	1.2	0.9	11.4
Fe	mg/kg	-	14.0	20.0	12.0	103.0
Mn	mg/kg	1 - 4	6.7	1.6	2.2	5.1
Cu	mg/kg	0.6 – 1.0	2.3	2.2	2.2	2.3
B	mg/kg	1.0 – 1.5	0.07	0.13	0.10	0.21

Leaf nutrient levels have remained within normal ranges. Leaf N levels were adequate despite the low N fertilization rate applied since 2019, consisting of one application of 33 lbs/acre during spring. Likewise, all other nutrient levels are within adequate ranges, including Ca, considering the orchard does not receive Ca ground or foliar application.

Table 3. Fruit nutrient concentration from 2021 to 2023. Different letters indicate statistical differences at p value < 0.001 (Tukey test, XSLTAT, Andisoft)

	N	P	K	Ca	Mg	S	Zn	Fe	Mn	Cu	B
Year	%						ppm				
2021	2.0	0.58	2.20	2.36	0.27	0.18	22.4	742	33.7	9.2	52
2022	2.2	0.39	2.36	1.46	0.29	0.17	12.5	301	28.9	20.3	56
2023	2.2	0.56	1.95	1.66	0.29	0.14	13.8	499	24.7	8.1	54
*	1.7–2.5	0.2-0.3	1.2-1.9	1.5-2.0	0.2-0.3	0-10	15-200	-	25-150	5-12	20-60

* Recommended values for apples leaf samples.

Research projects;

1.a Differences in root growth and nutrient uptake between M9-Nic29 and G41. (Munguia-Sallato)

In all three years of evaluation (2019 – 2022) root growth started when temperatures were above 59 °F in the soil. Consistently G41 has shown higher total root length, root growth rate and longer growth period, compared to M9-nic29. In 2022, after the roots started to growth, soil temperatures got below 44.6 F at 8 inches of soil, delaying root growth on both rootstocks (data not shown, reported in 2022). Increased root growth rate and volume in G.41 contributed to higher vigor and green spot incidence observed in this rootstock between 2018 and 2021, when compared with M9-nic 29. Results from this research objective can be seen in our web page http://treefruit.wsu.edu/videos/rootstock-differences-in-wa-38/.

1.b. Green spot nutrient composition differences, rootstock, and vigor. (Sallato-Munguia-Whiting)

Our previous work suggested that there is a strong relation between GS and nutrient imbalance, specifically between calcium (Ca) and nitrogen (N), an imbalance caused by excessive vigor (Sallato et al., 2021). In 2021 we evaluated different levels of GS to incorporate an intermediate level of severity. We observed a positive correlation between N and B concentration and GS severity (Table 4). Ca was significantly lower in severe GS ++ compared to the control with no symptoms. Nutrient concentration of P and K were higher in GS fruit, irrespective of the severity.

Note that Ca related disorders and the nutrient imbalances extensively reported for bitter pit, green spot and other physiological disorders do not imply a deficiency of Ca, the need of additional Ca application nor the causal factor. Nutrient imbalances have been useful as indicators, however the causes have been associated more strongly to excessive vigor.

Table 4. Nutrient concentration in the peel of WA 38 fruit on G41 without green spot (GS -) and two levels of GS: flecking (GS +) and spots (GS ++).

Nutrient	GS -	GS +	GS ++	Pr > F(Model)
N %	0.40 c	0.48 b	0.54 a	<0.0001
P %	0.08 b	0.09 a	0.09 a	0.001
K %	0.83 b	0.95 a	0.94 a	0.007
Ca %	0.09 b	0.10 a	0.08 c	<0.0001
Mg %	0.11 b	0.12 a	0.13 a	<0.0001
B mg/kg	32.5 b	38.1 ab	43.5 a	0.038

1.c. Use of AVG (ReTain ®) and artificial pollination to improve fruit set and production. (Sallato-Whiting)

Between 2020 and 2021, we evaluated the impact of supplemental pollen spray and AVG ([S]-trans-2-amino-4-(2-aminoethoxy)-3-butenoic acid hydrochloride; ReTain®, Valent Bioscience Inc) on WA 38 fruit set and yield. The treatments included supplemental pollen application with or without ReTain® in six different trials, two at the WSU Roza WA 38 orchard. The supplemental pollen was always applied at a rate of 30 g per acre (70% 'Red Delicious' and 30% 'Granny Smith', Firman Pollen Inc., Yakima, USA) suspended in a proprietary suspension media. In all trials except at the WSU Roza farm (Prosser, WA), the treatments were applied with a commercial electrostatic sprayer, while at the Roza farm we used an electrostatic backpack sprayer (OnTarget Spray Systems, Mt. Angel, OR). The AVG treatment consisted of one pouch (333g) of ReTain® formula.

In 2020, in a commercial 'WA 38' orchard near Buena, WA, fruit set varied between 60.0 ± 9.3 and 82.5 ± 9.3 fruitlets, being 36% higher with supplemental pollen spray treatment compared to the untreated control and ReTain®. At harvest total fruit per trees were 85%, 80% and 48% higher in the ReTain®, ReTain®+ pollen, and pollen treatments respectively, when compared with the control

(Figure 2). The following year, we added two treatments: ReTain® at petal fall (PF) and pollen at bloom +ReTain® at PF. Again, supplemental pollen, pollen + ReTain® at bloom and ReTain® alone at petal fall increased the number of fruit per tree by 66.5%, 58.5% and 45.4% respectively, when compared with the control (Figure 1).

Similar results were observed at the WSU Roza experimental station on 'WA 38' on M9-Nic 29 rootstock, where fruit set was 74% higher with supplemental pollen application, followed by ReTain® and ReTain® + pollen spray. At harvest, yield per tree was 29% higher with ReTain® + pollen, when compared with the control, while the other treatments were not different (data not shown). The same treatments were imposed on 'WA 38' on G.41 rootstock, with no differences among treatments, however, crop load (fruit number per trunk cross sectional area) was the lowest in the control (4.7 ± 0.7 fruit/TCSA cm2) and 25.5% higher in the supplemental pollen spray treatment (5.9 ± 0.7). In two commercial trials conducted on a 4th leaf 'WA 38' in 2021, the application of ReTain®, supplemental pollen or the combination of both led to no differences in fruit set or yield. However, the grower reported 37% more bins per acre in the pollen and the ReTain® treated blocks, when compared with the untreated control.

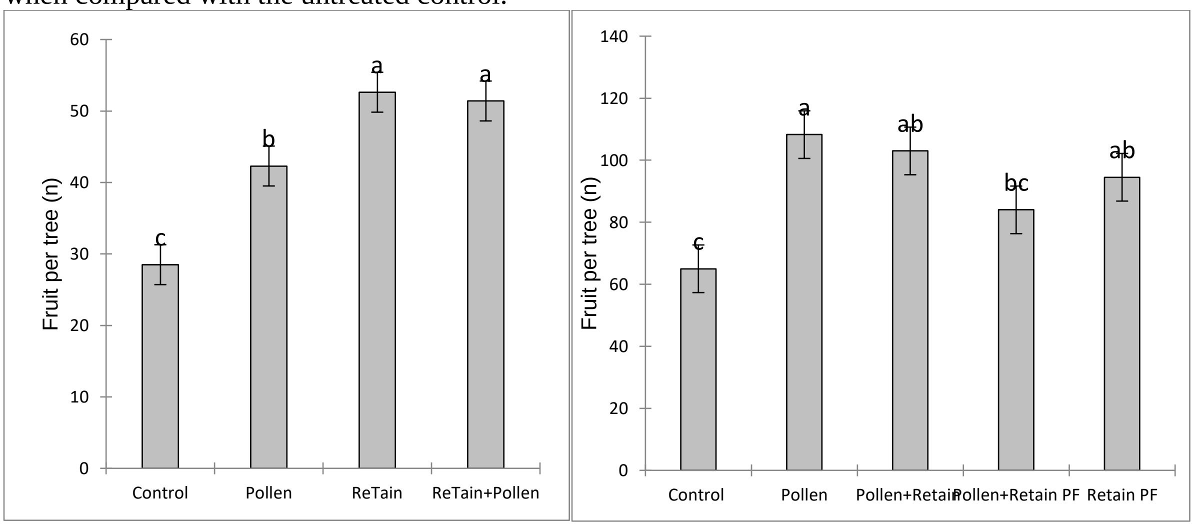


Figure 2. Total fruit (n) per tree at harvest in 2020 (left) and 2021 (right) commercial 'WA 38' orchard near Buena, WA. Treatments included an untreated control, pollen (at bloom), ReTain® at bloom, ReTain®+Pollen, Pollen + ReTain PF (at petal fall) and ReTain® PF (at petal fall). Bars indicate mean values and error bars correspond to the standard error (n=10). Different letters indicate significant differences between treatments ($p < 0.001$).

The outcomes of supplemental pollen application and ReTain® varied across the orchards, possibly due to different limiting factors. While supplemental pollen spray aims to increase the availability and transfer of pollen, ReTain® aims to increase ovule longevity. Thus, when pollen availability, bee activity or short pollination period is expected, these tools could improve production, while there is no benefit if these factors are not limiting. Results for this objective were reported in detail in 2021 and shared with WA industry at the WSU WA 38 pre harvest field days (2021, 2022 and 2023), pollination field days (April 19th and April 21, 2022) in Spanish with infographics "*Mejora de la cuaja en 'WA 38' (*Improving fruit set in 'WA 38'). And at the WSTFA annual meeting Dec 6, 2022 newsflash.

1.d. Pruning strategies to promote fruiting wood (Sallato)

Summer pruning response varied depending on the year. In 2021, early summer pruning (between May and June 16th) led to current season regrowth and in a few cases, induced flower buds to break during the fall. These late blooms can increase the risk of fire blight infection while losing the bud for the following season. Later summer pruning (after June 16th) did not induce fall bloom and the regrowth varied depending on the vigor and angle of the shoot being pruned. The later the pruning, the lesser the regrowth. However, in 2022 we did not induce flowering in the fall, regardless of the timing of pruning, and regrowth shoots were shorter than those in 2021.

In all regrowth cases, the new shoots were weaker, and, in most cases, we observed symptoms of iron or zinc deficiency (chlorosis), likely due to fast regrowth rate and reduced mobility of these elements in the plant. Regardless, the following year, there were no signs of deficiency.

By the end of the second-year, summer pruned trees had higher crop load compared to those that were only pruned in the winter. There were no differences among the different timings of summer pruning. This higher crop load in 2022 led to a dramatic reduction in fruit set in 2023, thus we couldn't isolate the impact of consecutive summer pruning.

Regarding the summer pruning trial, based on pruning levels, the early and more aggressive summer pruning led to higher fruit per tree compared with the pruning trial at 5 leaf or 10 leaf stage, however it was not different from the unpruned control. These intensive pruning treatments led to small fruit at harvest. In 2023, there were no differences among treatments in regards of fruit yield, size or defects (data not shown).

Table X. Total fruit yield, fruit per tree and average fruit weight by pruning intensity.

	Yield (lbs/tree)	Fruit per tree	Fruit weight (g)
Control	19.6 a	39.0 ab	243.9 ab
10 L	11.4 ab	20.4 b	237.3 ab
5 L	9.2 b	14.6 b	293.7 a
0 L	20.2 a	48.2 a	198.8 b
Pr > F(Model)	0.030	0.011	0.021

Summer pruning helped reduce excessive shoot growth and helped increase tree productivity by two main effects; 1. Reducing vigorous upright shoots that can become blind wood or further increase vegetative growth with winter pruning. 2. Increased fruiting wood for the following and subsequent season. A later summer pruning (August) seemed more appropriate and with lesser risk in inducing fall blooms, however, summer pruning did expose fruit that were previously shaded, leading to increased sunburn risk. To prevent this higher risk, we applied sunburn protectants to the individual trees, however growers can utilize other mitigation practices, or avoid summer pruning when temperatures and sun exposure is expected to be high.

1.c. Fruit ripening variability between systems and rootstocks (Bolivar – Sallato)

Detailed information was shared in previous report and at the Jan 2022, Pom Club meeting (Sallato), and in the Spanish field days led by CoPI Bolivar, "Perfil de maduración de WA38 en dos portainjertos y tres sistemas de producción- Año 2020. Jenny Bolivar-Medina, Bernardita Sallato. (WA38 maturation profile on three production systems and 2 rootstock types- 2020). An infographic of the results can be found http://treefruit.wsu.edu/perfil-de-maduracion-de-wa-38-en-dos-portainjertos-y-tres-sistemas-de-produccion-2020/

Utilize the WA 38 Roza farm as a demonstration block for community engagement and outreach.

The WA 38 Roza farm provided a venue for community engagement and outreach in multiple field days, workshops, group visits, etc. In 2022, Sallato led a full day workshop for the WSTFA – WSDA and WSU collaborative “Agricultural Leadership Program”, where we covered the areas of “plant physiology” (M. Whiting), Crop load management (D. Gleason), Irrigation (A. Moreno), IPM (T. DuPont) and Soil and Nutrient management (B.Sallato), in English and Spanish, for 33 students. Co PI Bolivar hosted 3 field days in Spanish in 2022 and Sallato has hosted a pre-harvest WA 38 every year.

In 2021 we reached over 60 people during the field days, in 2022 we reached over 80 people and in 2023 we reached over 100 people including a Spanish and English speaking growers, three international visits (Italy, Chile, Brasil), K-12, Yakima Community College students among others.

A survey conducted after the field days reported 80% increase of knowledge and 50% of the participants, indicated intention to change their management practice for pollination and nutrient management. At the pre harvest WA 38 field day (Spanish), we had 33 attendees and 24 responded to our survey. A 100% of the respondent indicated they see value in the WA 38 demonstration site and field days, which had led to changes in practices including training systems, rootstock selection, fruit set management strategy, pruning, among others.

EXECUTIVE SUMMARY

Project Title: WA38 applied research and demonstration block.

Keywords: Cosmic crisp, vigor, green spot, supplemental pollen

With the development of new varieties and rootstocks, numerous challenges emerge before achieving enhanced growing practices. With over 20 million WA 38 plants, growers face various unanswered questions. The Roza WA38 block, established in 2013 at the Washington State University Irrigated Agriculture Research and Extension Center in Prosser, serves as a unique research and extension hub in the heart of the Yakima Valley. The planting design has three training systems; vertical spindle at 1210 trees per acre, V trellis at 2420 trees per acre and a vertical wall bi-axis at 1210 trees per acre, and two rootstocks; G 41 and M-9 Nic 29, arranged in a randomized design, creating a distinctive experimental setting for targeted inquiries and validation. The project objectives were to 1. Evaluate horticultural practices on WA 38 grown on G41 and M9-Nic 29 for better production and fruit quality and 2. Utilize the WA 38 Roza farm as a demonstration block for community engagement and outreach.

Over the three-year period, insights revealed that at the WA 38 Roza farm, the optimal fruit load per tree, based on fruit load and quality, ranged between 90 and 110 fruit per tree on bi-axis and spindle (1210 trees per acre) and between 65 and 80 fruit per tree on V-trellis (2420 trees per acre). These findings underscored that WA 38 can experience overcropping (as seen in 2021), leading to alternate bearing, necessitating the identification of maximum production potential under each growing condition. Managing excessive vigor at the Roza farm, achieved through summer pruning (June to August), reduced N and K levels, and water control, resulted in increased fruiting wood and fruit yield in the second season post-summer prune. Although no differences were observed among various summer pruning timings, the earliest pruning in May could induce bud break in the fall (fall blooms). Spring pruning, if done aggressively, could impact fruit size by removing carbohydrate sources, but it did not induce fruit drop. Despite this, G41 exhibited more vigor than M9-nic 29, correlated with higher root growth, root growth rate, and a longer root growth period. Higher root growth translated into increased leaf nutrient uptake, vigor, and higher green spot incidence during the initial two study years. In 2022 and 2023, green spot levels significantly decreased in both rootstocks, strongly linked to nutrient imbalance, particularly between calcium (Ca) and nitrogen (N), a result of excessive vigor.

Another significant limitation of WA 38, as reported by surveyed growers, is low productivity (low fruit set). The evaluation of supplemental pollen application via electrostatic, with and without AVG (ReTain®, Valent), yielded varied results across orchards and years. However, it seems that when weather conditions limit natural pollen availability or transfer, supplemental pollen can boost yield by 48%. Similarly, AVG (333 g, ReTain®) applied alone, in combination with pollen, or after petal fall also increased fruit per tree compared to an untreated control.

In summary, the Roza WA38 project provided valuable insights into tailoring horticultural practices, managing vigor, and addressing productivity challenges, contributing to the ongoing enhancement of WA 38 cultivation.

FINAL PROJECT REPORT **PERIOD:** 3 year of 3 years

Project Title: **'WA 38': SOP from planting to cropping**

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Cooperators: Several companies growing 'WA 38' in combination with different rootstocks have been contacted.

Project Request: **Year 1:** $90,860 **Year 2:** $88,523 **Year 3:** $87,292 **(Total $266,675)**

Other funding sources:
Project #AP14-103A: "WA 38 rootstocks and training systems" (2014-2016+1yr NCE) total funds $ 242,519 provided the support to maintain the orchard for this project.

BUDGET
Primary PI: Stefano Musacchi
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Budget

Musacchi-Serra-Lewis-Sallato			
Costs	Year 1 (2021)	Year 2 (2022)	Year 3 (2023)
Salaries[1]	$ 37,800	$ 39,312	$ 40,884
Benefit[2]	$ 16,760	$ 17,431	$ 18,128
Wages[3]	$ 12,000	$ 12,480	$ 12,980
Supplies[4]	$ 16,800	$ 11,800	$ 7,800
Travel[5]	$ 7,500	$ 7,500	$ 7,500
total	*$ 90,860*	*$ 88,523*	*$ 87,292*

Footnotes:

[1] Salary for a 75% Research assistant ($4,200/month) (Musacchi)
[2] Benefit on salary at 38.98%. Benefits on temporary at 22.4%
[3] Non-student temporary for 20 wks: 40hrs/wk at $15/hr (Musacchi/Lewis/Sallato).
[4] Labware/consumable, tree cost (Musacchi). Supplies include video recording and editing, printing material for outreach. Supply includes software fees for outreach material and translation. Supplies include video recording and editing, printing material for outreach.
[5] 13,043 miles/year for domestic travel ($0.575/mile) to go to the orchard. Travel to visit 10 blocks x 4 visits/year in Columbia Basin and North Central WA (Lewis). Travel to visit 10 blocks x 4 visits/year in south-central WA from Prosser (Sallato)

RECAP-OBJECTIVES:

1. Determine the rootstock effects on flower bud formation, fruit set, and spur extinction from planting to cropping (3rd year).
2. Investigate the 'WA 38' fruit set in the different types of bearing wood and assess the return bloom the following year.
3. Investigate the cultural management practices developed in 'WA 38' private orchards and summarize them in a list of recommended guidelines for growers.

SIGNIFICANT FINDINGS:

1. ***Determine the rootstock effects on flower bud formation, fruit set, and spur extinction from planting to cropping (3rd year) (Musacchi-Serra)***
   - In "No Stub 2022" (unpruned) trees, the smallest proportion of blind wood was reported in 'WA 38'/'G.890' and 'G.11' approximately 11 months after planting, while the largest proportion was in 'M.9-T337', followed by 'Bud10'.
   - 'WA 38'/'Bud9' trees in the "No Stub 2022" treatments presented the highest number of lateral shoots per branch.
   - 'WA 38'/'Bud9' trees for both 2022 pruning scenarios reported the highest average number of Flower Buds/branch (or stubs with shoots).
   - "No Stub'22+Stub3X'23" trees had left the 52% of flower clusters in comparison to "No Stub'22+unpruned'23", whereas "Stub'22+Click(STD)'23" trees only the 26%.
   - No significant difference in fruit set between the three treatments in trial in 2023.
   - Early bearing tendency was observed for 'WA 38'/ 'Bud9', 'M.9-T337', and 'G.935' (regardless of the pruning treatment), while 'G.890' did not produce fruit at harvest in 2023.
   - 'WA 38' grafted on 'Bud9', 'Bud10', 'M9-T337', and 'G.11' reported the lowest TCSA when managed with stub after planting and click pruning in the following year, while 'WA 38'/ 'G.890' showed the highest TCSA at the end of the second year after planting.

2. ***Investigate the 'WA 38' fruit set in the different types of wood and assess the return bloom on the different bearing woods (Musacchi-Serra)***
   - Largest part of 'WA 38' productions in 2021 and 2022, as avg. of 3 sites, was held as single apple/cluster (65% to 73%), followed by double apples/cluster (23% to 32%), and the residual as triple apples/cluster.
   - As average across the 2021 and 2022 scenarios (5 and 3 sites, respectively), 61 to 64% of the 'WA 38' apples were borne on spurs, followed by 12 to 27% on 'Brindilla' and 12% to 24% on 1-year-old shoot lateral buds ('Ramo misto').
   - Apples on 1-year-old shoot lateral buds ('Ramo misto') were confirmed to belong mainly to the small size class; while spurs produced apples with more variable sizes, from small to extra-large.
   - Quality analysis comparing apples from 3 bearing woods did not find consistent trends in 2 years.

3. ***Investigate the cultural management practices developed in 'WA 38' private orchards and summarize them in a list of recommended guidelines for growers (Lewis-Sallato)***
   - Fruit load varied between 63 and 143 fruit per tree, averaging a 70% increase as the trees mature and fill the space, with one site having reduced crop compared to 2021.
   - Crop load was highly variable among sites, ranging from 7.8 to 20 fruit per trunk cross section area (cm^2), influenced more by the year and site (management) than by rootstock.
   - Fruit size vary between 183 g and 304 g, 15% smaller than in 2021, except for one orchard (in both rootstocks), where fruit size was 22% higher.

RESULTS AND DISCUSSION

Objective 1) Determine the rootstock effects on flower bud formation, fruit set, and spur extinction from planting to cropping (3rd year) *(Musacchi-Serra)*

At eight months (8M) after planting (February 2023), the combinations in trial reported significant differences in terms of trunk cross sectional area and growth in 8M within both 2022 treatments (Table 1). For "No Stub 2022", the least vigorous combinations were 'WA 38'/'G.969', 'Bud9', and 'Bud10' and the most vigorous ones were 'WA 38'/'G.890', followed by 'WA 38'/'G.935' (Table 1). The "No Stub 2022" combinations that reported the highest growth in TCSA were grafted on 'G.935', 'G.213', 'G.41' and 'G.890'. For "Stub 2022" trees, a similar trend of vigor was confirmed, with 'WA 38'/'G.890' and 'G.935' being the most vigorous, in contrast to 'WA 38'/''Bud10'' and 'Bud9', the least vigorous combinations (Table 1). 'G.11', 'M.9-T337', 'Bud10', and 'Bud9' showed a low and comparable TCSA growth in 8M, while the most considerable growth was recorded in the graft combination with 'G.41' and 'G.935' (Table 1). In February 2023, only 4 trees in the whole orchard (543 trees total) were deceased by unknown cause: 3 'WA 38'/'G.969' and 1 'WA 38'/'M.9-T337'.

Table 1. *'WA 38' trunk cross sectional area (TCSA, cm^2) on 2/6/23 and trunk growth in 8 months (8M) for trees grafted on 9 different popular rootstocks for WA sorted by 2022 imposed treatment: No Stub vs Stub. Significance root: *** = p≤ 0.001). Trees were planted in 2022 at 11 ft x 3ft (1320 trees/A).*

TRT 2022	Rootstock for WA38	N trees '22	TCSA (cm^2) on 02/06/2023		TCSA Growth (cm^2) from June '22 to Feb '23	
No Stub	Bud9	33	**3.37**	D	**1.14**	E
	Bud10	34	**3.55**	D	**1.40**	D
	G969	28	**3.62**	D	**1.57**	CD
	G11	35	**4.22**	C	**1.81**	BC
	G213	35	**4.25**	C	**2.20**	A
	G41	34	**4.28**	C	**2.12**	A
	M9T337	33	**4.42**	BC	**1.73**	BC
	G935	34	**4.68**	B	**2.21**	A
	G890	33	**5.79**	A	**1.99**	AB
Significance rootstock			***		***	
Stub	Bud10	35	**3.37**	F	**1.29**	D
	Bud9	34	**3.50**	EF	**1.24**	D
	G11	34	**3.75**	DE	**1.39**	D
	G969	32	**3.78**	DE	**1.73**	C
	G213	33	**3.95**	CD	**1.94**	ABC
	M9T337	35	**3.98**	CD	**1.36**	D
	G41	33	**4.29**	BC	**2.09**	A
	G935	34	**4.44**	B	**2.02**	AB
	G890	34	**5.41**	A	**1.80**	BC
Significance rootstock			***		***	

Before imposing new treatments by pruning, at the end of winter 2022-2023 (March 2023), the experimental trees were assessed for branch measurements, blind wood incidence, branching ability and flower buds count. Looking at the proportion of blind wood (as a portion of the branch with no vegetative/flower bud break over the total length of the branch then averaged between the 4 selected branches/tree) in the "No Stub 2022" trees (N=81), some significant differences emerged: the largest proportion of blind wood was found in combination with 'M.9-T337' followed by 'Bud10' (and similar to other 5 combinations, Table 2), while the smallest proportion of blind wood was reported in 'WA 38'/'G.890' and 'G.11' (Table 2) partially confirming some of the results of July 2022 (data not shown). No significant differences in the average internode length in the blind wood portion of the branches (data not shown), but distinctions between original branch lengths in the unpruned trees emerged; this parameter ranged from 77.2 cm (30") in 'WA 38'/'Bud9' to 112.8 cm (44", on average) in 'WA 38'/'G.890' (Table 2). With different levels of significance across the 4 branches, a consistent tendency of 'WA 38'/M9-T337 presenting the highest number of "blind nodes" and 'WA 38'/'G.11' and 'G.41' the lowest number of "blind nodes" emerged, confirming some results already observed in 2022 (data not shown). At the time of branch measurements and blind wood assessment, we also counted the number of 1-year-old shoots longer than 5 cm (~2") inserted in the 4 basal branches. "No Stub 2022" 'WA 38'/Bud9 trees reported the highest number of lateral shoots per branch (2.9), an average significantly superior to the other 8 combinations in trial. Moreover, those lateral shoots (> 5 cm) were

longer on average in ‘WA 38’/’G.890’ (43 cm, 17”) and ‘WA 38’/’G.213’ (37.3 cm, 15”) while shorter in ‘WA 38’/’Bud10’ (12.3 cm, 5”, Table 2).
The scion-rootstock combination impacted the average number of flower buds (FB) per branch in the “No Stub 2022” trees in March 2023. ‘G.11’, ‘Bud9’, and ‘Bud10’ reported the highest average number of FB/branch (16.9-17.7 FB/branch), while ‘G.213’ was the lowest (9.6 FB/branch).

Table 2. *‘WA 38’ combinations with 9 different rootstocks before pruning 2023: No Stub (2022) unpruned trees (N=9/combinations with 4 branches/tree) measurements on blind wood carried out in March 2023. Parameters reported are: the proportion of the branch affected by blind wood expressed as % and average of 4 basal branches/tree, the average length unpruned branches (cm), the average length of blind wood per branch (cm), the average number of lateral shoots (1-year-old and >5cm long) emerging from the branches, the average length of lateral shoots (cm), and the average number of flower buds (FB) for branch. Significance: NS= not significant, ** = p≤ 0.01, *** = p≤ 0.001 and letters of separation within each treatment were provided by SNK test. Trees were planted in 2022 at 11 ft x 3ft (1320 trees/A).*

Trt 2022	Rootstock comb. with WA 38 (2023)	N trees (N branches/ tree)	% Blind Wood		Avg. length of unpruned branch (cm)		Avg. length of blind wood in unpruned branch (cm)	Avg. Num 1 yr-old lateral shoots (>5 cm)/branch		Avg. length of 1 yr-old lateral shoots (>5 cm)/branch (cm)		Avg. Num Flower Buds (FB)/branch	
			(average of 4 branches/tree)										
No Stub (= unpruned)	**Bud10**	9 (4)	**17.1**	AB	78.9	B	**12.8**	**2.9**	A	**12.3**	C	**17**	A
	Bud9	9 (4)	**13.4**	ABC	77.2	B	**10.0**	**0.8**	B	**15.9**	BC	**18**	A
	G11	9 (4)	**10.9**	BC	88.9	B	**9.5**	**1.3**	B	**24.7**	B	**18**	A
	G213	9 (4)	**14.3**	ABC	82.7	B	**11.3**	**1.1**	B	**37.3**	A	**10**	C
	G41	9 (4)	**14.0**	ABC	83.6	B	**10.1**	**1.2**	B	**26.4**	B	**14**	ABC
	G890	9 (4)	**10.7**	C	112.8	A	**11.8**	**1.8**	B	**43.0**	A	**13**	ABC
	G935	9 (4)	**14.3**	ABC	86.9	B	**11.7**	**0.8**	B	**27.6**	B	**15**	AB
	G969	9 (4)	**16.4**	ABC	81.7	B	**12.3**	**0.8**	B	**15.5**	BC	**13**	ABC
	M9-T337	9 (4)	**18.1**	A	88.2	B	**14.2**	**1.3**	B	**18.9**	BC	**11**	BC
	Significance		**		***		*NS (0.0504)*	***		***		***	

Similar measurements were carried out in the “Stub 2022” trees (N=81) before pruning them at the end of winter 2022-2023 (Table 3). The 9 combinations that got stubbed after planting in 2022 did not show significant differences in the number of 1-year-old shoots produced from each stubbed branch (=stub), ranging on average between 1.5 (‘G.890’) and 2.0 (‘G.11’) per stub, proving that with stub pruning we zeroed the growth and allowed all combinations to start over. On the other hand, significant differences emerged for the average length of 1-year-old shoots, with ‘WA 38’/’G.890’ presenting the longest shoots (81 cm, 32”), while ‘WA 38’/’Bud9’, ‘M.9-T337’, ‘Bud10’ the shortest ones (37.5 cm, 15”, to 41.4 cm, 16”, Table 3). Also, the total length of 1-year-old shoots emerging from the stubs showed significant differences, with tendencies reflecting the relationship between rootstock vigor and vegetative growth. ‘G.41’, ‘G.890’, and ‘G.935’ reported the longest total growth of shoots/stub (111.4 cm, 44”, to 116.6 cm, 46”, on avg.), while ‘M.9-T337’, ‘Bud9’, ‘Bud10’, and ‘G.213’ had the shortest total lengths (Table 3). Regarding the average number of flower buds per stub, the order of magnitude in the “Stub 2022” trees is lower than in “No Stub 2022”; in fact, the (click)-stubbing approach after planting led to a reduction of flower buds on a tree for the following year. ‘WA 38’/’Bud9’ presented an average of 2.3 FB/stub, while ‘WA 38’/’G.890’ presented only 1.3 FB/stub, representing the two average extreme values among the 9 combinations (Table 3).

In general, the average longest internode in the 1-year-shoots emerging from stubs (from stubs 1 to 4 in “Stub 2022” trees) was found in ‘G.890’, ‘G.41’, ‘G.969’, or ‘G.11’, while the shortest internode was found in ‘M.9-T337’ (data not shown). This might confirm that ‘M.9-T337’ responded well to click pruning after planting to minimize the blind wood with respect to unpruned trees.

***Table 3**. 'WA 38' combinations with 9 different rootstocks before pruning 2023: Stub (2022) or click-pruned trees (N=9/combinations with 4 branches/tree) measurements carried out in March 2023. Parameters reported are: the average number of 1-year-old shoots emerged from the 2022 stubs, the average length of 1-year-old shoots emerged from the 2022 stubs, the total length of 1-year-old shoots emerged from the 2022 stubs, and the average number of flower buds (FB) for branch. Significance: NS= not significant, * = p≤ 0.05, ** = p≤ 0.01, *** = p≤ 0.001 and letters of separation within each treatment were provided by SNK test. Trees were planted in 2022 at 11 ft x 3ft (1320 trees/A).*

Trt 2022	Rootstock comb. with WA 38 (2023)	N trees (N branches/ tree)	Avg. Num 1 yr-old shoots/stub	Avg. length 1-yr-shoots/stub (cm)		Total length 1-yr-shoots/stub (cm)		Avg. Num Flower Buds (FB)/stub (1yr-old shoots included)	
			(average of 4 branches/tree)						
Stub	**Bud10**	9 (4)	**1.8**	**41.4**	D	**72.7**	B	**1.7**	AB
	Bud9	9 (4)	**1.9**	**37.5**	D	**67.6**	B	**2.3**	A
	G11	9 (4)	**2.0**	**45.7**	CD	**88.3**	AB	**1.9**	AB
	G213	9 (4)	**1.6**	**51.2**	BCD	**78.9**	B	**1.8**	AB
	G41	9 (4)	**1.8**	**65.5**	B	**116.6**	A	**1.7**	AB
	G890	9 (4)	**1.5**	**81.0**	A	**115.0**	A	**1.3**	B
	G935	9 (4)	**1.8**	**64.3**	B	**111.4**	A	**1.9**	AB
	G969	9 (4)	**1.6**	**58.9**	BC	**88.2**	AB	**1.4**	AB
	M9T337	9 (4)	**1.8**	**39.7**	D	**67.2**	B	**1.9**	AB
	Significance		*NS*	***		***		*	

In fact, in July 2022 measurements, 'WA 38'/M9-T337 unpruned trees showed the highest number of "blind nodes" (9), but when that combination was click-pruned (stubbed) after planting, the number of blind nodes decreased to one-third (3, -66.7%, data presented in the previous report).

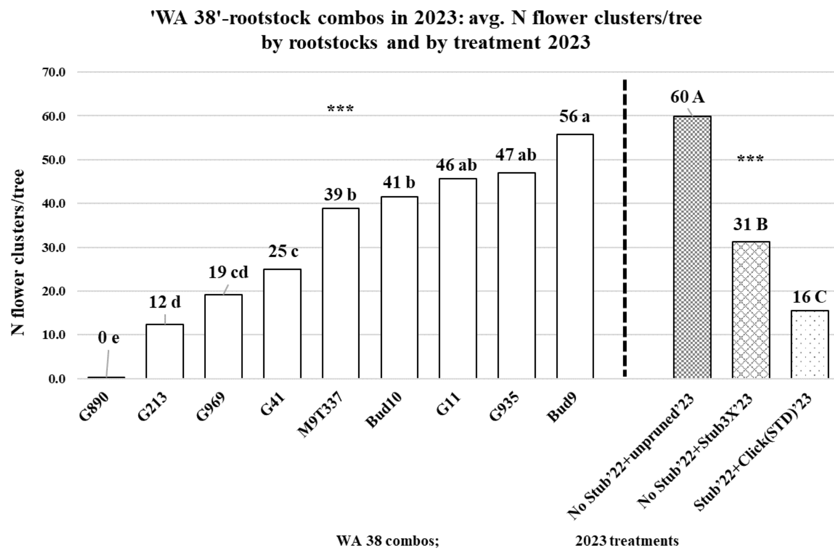
On 03/28/23, we imposed new pruning treatments to the existing trial, dividing the 81 "No Stub 2022" trees into two scenarios: "No Stub'22+unpruned'23" (45 trees=5 trees* 9 rootstocks) and "No Stub'22+Stub3X'23" (36 trees= 4 trees*9 rootstocks). In the first scenario, we only did light branch simplification cuts on top where needed or when too strong branches were competing with the leader. Whereas, in the second case, we pruned the long mid-basal branches (ground to 2nd wire = 4') at a length equal to 3 times the length of the 2022 stub (~11 cm = 4.3") in the "Stub 2022" trees (approx. 33 cm = 1') and the tree-top was handled with regular click pruning, making sure to select the most vertical shoot as leader and remove competitors. On the other hand, the "Stub 2022" trees (81 trees= 9 trees* 9 rootstocks) were all pruned again as: "Stub'22+Click(STD)'23" following the criteria of clicking the 1-year-old shoots emerged from the stubs at a 2X-stub length, so, approx. 22 cm = 8.7", while when branch (shoot or stub) was strong or vertical the click was done at 1X.

On 4/21/23 (12 days before full bloom), flower clusters (FC) number per selected branches (or stubs) was counted in all the experimental trees, and on 6/30/23 (58 DAFB), after the end of the natural fruit shedding, the count of apples per branch and tree was carried out to assess the fruit set by the different 2023 treatments. In early June 2023, each row in the orchard was implemented with peat moss (5 m^3/row=177 ft^3/row=0.05m^3/tree=1.6 ft^3/tree) to conserve water in the hot summer, and 6 applications of 16-16-16 fertilizer (112g/tree/application) were distributed from 5/10 to 7/24/2023. Regardless of the pruning treatments, a highly significant difference emerged when comparing the number of flower clusters/tree among the different rootstock combinations (Figure 1).

The highest averages of flower clusters/tree were reported in combination with 'Bud9' (56 FC/tree), 'G.935' (47 FC/tree), and 'G.11' (46 FC/tree), while 'G.213' (12 FC/tree) and 'G.890' (0 FC/tree) presented the lowest numbers (Figure 1). When comparing the 3 treatments (Figure 1), the effect of the pruning (2022+2023) was very clear on the residual numbers of FC/tree: the "Stub'22+Click(STD)'23" trees presented only 26% of FC in comparison to "No

Stub’22+unpruned’23”, while “No Stub’22+Stub3X’23” had a 52% of FC left with respect to “No Stub’22+unpruned’23”. The interaction rootstock * treatment resulted significant and revealed some different trends between treatments within some rootstock; one example is ‘WA 38’/’G.213’, where the highest N FC/tree was recorded in “No Stub’22+unpruned’23”, followed by “Stub’22+Click(STD)’23” (statistically similar). At the same time, the lowest number was found in “No Stub’22+Stub3X’23”, a different trend than what was reported in Figure 1 (data not shown). At the end of the natural fruitlet shedding, the highest calculated fruit set (%) was reported for ‘WA 38’/’Bud9’ (2.4%), followed by ‘M.9-T337’ (1.9%), and ‘G.935’ (1.3%), while the other combinations were lower and all comparable (data not shown).

Figure 1. *‘WA 38’ scion-rootstock combination in trial in 2023 in Rock Island (WA). On the left of the bar chart, the avg. N of flower clusters/tree is reported by rootstocks regardless of the trt (N=18/combo), and, on the right, the same parameter displayed by pruning treatments in 2023 (N=45, 36, 79 respectively). Significance: ***=p<0.001 and letter of separations discriminate means for p=0.05; in lower case letters for the 9 combos and capital letters for the three treatments 2023. Trees were planted in 2022 at 11 ft x 3ft (1320 trees/A).*



On 9/28/23, the first crop was harvested, but not all 162 experimental trees were bearing fruit. Therefore, the proportion of bearing trees versus not-bearing trees was surveyed by rootstock*treatment combination, and the combinations with less than 3 trees were excluded by statistical analysis (Figure 2). In general, ‘Bud9’, ‘M.9-T337’, and ‘G.935’ showed an early bearing tendency, with 78 to 72% of trees bearing fruit, while, on the opposite side, ‘G.890’ did not bear fruit until harvest (Figure 2).
Comparing the three treatments in 2023, yield per tree was higher in “No Stub’22+unpruned’23” (2.0 kg/tree) and “No Stub’22+Stub3X’23” (1.6 kg/tree) in comparison to “Stub’22+Click(STD)’23” trees (0.5 kg/tree, data not shown). The different 2023 treatments showed to have impacted the average fruit mass, with the lowest apple weight found in “Stub’22+Click(STD)’23” (224 g, 80 apples/box size) and the highest in “No Stub’22+Stub3X’23” (289 g, 72-64 apples/box size, data not shown).
Only 8 combinations of scion-rootstock-treatment were statistically analyzed (Figure 3), and the most productive one, in the first cropping season, was ‘WA 38’/’Bud9’_No Stub’22+unpruned’23 (avg. 3.8 kg/tree =8.4 lb/tree), followed by ‘WA 38’/’Bud9’_No Stub’22+Stub3X’23 (2.3 kg/tree =5.1 lb/tree), and ‘M.9-T337’_No Stub’22+unpruned’23 (2.1 kg/tree=4.6 lb/tree); all the other combinations produced less than 2 kg/tree (4.4 lb/tree). On average, the apple weight fluctuated from 203 g to 288 g without significant differences between the combinations (Figure 3).

In fall 2023, tree dimensions (height, widths, trunk circumference) were measured, and trunk cross sectional area, trunk growth, and canopy volume, were calculated for all experimental trees.
‘WA 38’/’G.890’ was the combination that, in general, regardless of the pruning treatment imposed, showed the highest tree height, widths (S-N and E-W), canopy volume and TCSA and growth, while, on the opposite side of the range, we had ‘WA 38’/’Bud9’ and ‘WA 38’/’Bud10’ (similar) with the shortest tree height, the narrowest canopies (smaller widths), the smallest TCSA and canopy volume,

and the lowest trunk growth in 2023 season (Table 4). These results confirmed the expected effect of different rootstocks in controlling tree size.

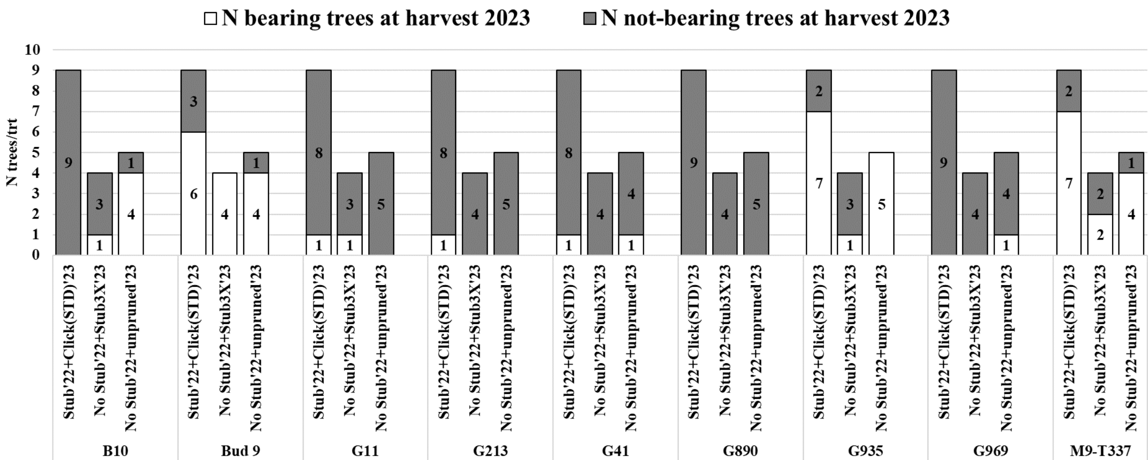


Figure 2. *'WA 38' scion-rootstock-pruning treatment combinations in trial in 2023 in Rock Island (WA). The combinations with less than 3 trees/combo were excluded from statistical analysis by combinations on yield 2023 data set.*

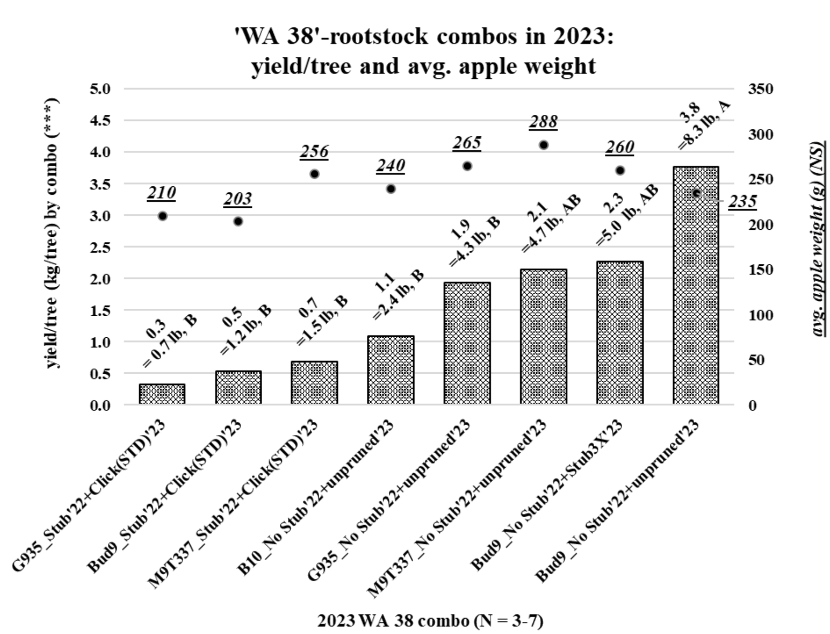


Figure 3. *Productive data for 'WA 38' scion-rootstock-pruning treatment by combinations in trial in 2023 in Rock Island (WA). Parameters reported are: on the primary Y-axis, the yield as kg/tree (on top of each bar kg are also converted in lb/tree), and on the secondary Y-axis, the average apple weight (g). Significance: NS= not significant, ***=p<0.001. Trees were planted in 2022 at 11 ft x 3ft (1320 trees/A).*

In the comparison of the 3 pruning treatments in 2023 for tree dimensions and volume, regardless of the rootstock, it became evident how the "No Stub'22+unpruned'23" trees are significantly wider (data not shown) and with a larger canopy volume than "No Stub'22+Stub3X'23" and "Stub'22+Click(STD)'23" (Table 4). These last two treatments showed a 20% canopy volume reduction compared to unpruned trees (Table 4). When looking at TCSA by treatments, no significant differences emerged, but the interaction rootstock * treatment showed a trend for 'WA 38'/'G.213' and 'WA 38'/'G.890', opposite to the majority of the other combinations. In fact, for those combinations, the trees subjected to No Stub'22+Stub3X'23 reported the highest TCSA than the other two treatments (data not shown).

Table 4. *'WA 38' combinations with 9 different rootstocks in October 2023: tree dimensions (height, widths, trunk circumference) were measured, and canopy volume, trunk cross sectional area, and trunk growth were calculated for all experimental trees. Significance: NS= not significant, * = p≤ 0.05, *** = p≤ 0.001 and letters of separation within each treatment were provided by SNK test. Trees were planted in 2022 at 11 ft x 3ft (1320 trees/A). TCSA = trunk cross sectional area, Growth 8 M= TCSA growth in 8 months, Canopy volume was calculated as a cone. Canopy widths were not reported.*

October 2023 measurements									
WA 38 rootstocks	**N=**	**TCSA (cm^2)**		**Growth 8M (cm^2)**		**Tree height (cm)**		**Canopy volume (m^3)**	
Bud9	18	7.0	D	3.4	D	278	F	1.1	F
B10	18	7.8	CD	4.3	C	290	EF	1.0	F
G11	18	8.7	C	4.7	C	303	DE	1.7	CDE
M9T337	18	8.7	C	4.5	C	311	CDE	1.4	E
G969	18	9.9	B	5.9	B	326	BCD	1.6	DE
G41	18	10.3	B	5.9	B	345	B	2.1	B
G935	18	10.3	B	5.7	B	332	BC	1.9	BC
G213	18	10.5	B	6.4	B	323	BCD	1.9	BCD
G890	18	12.8	A	7.3	A	375	A	3.0	A
Significance by root		***		***		***		***	
trt 2023									
No Stub'22+unpruned'23	45	9.6		5.3		327.6		2.0	A
No Stub'22+Stub3X'23	36	9.6		5.3		323.1		1.6	B
Stub'22+Click(STD)'23	81	9.5		5.3		315.1		1.6	B
Significance by trt23		*NS*		*NS*		*NS*		***	
*Significance rootstock*trt23*		*		* (p=0.0408)		*NS*		*NS*	

Objective 2) Investigate the 'WA 38' fruit set in the different types of wood and assess the return bloom on the different bearing woods *(Musacchi-Serra)*

From harvest 2022, samples for quality analysis from the two sites where 'WA 38' was trained at spindle were stored for 3.5 months in RA storage. The different quality parameters across the locations were I_{AD}, firmness, and titratable acidity (Table 5). The first two parameters confirmed the same behavior as the previous year. Apples harvested from 'WA 38'/M9337-GS_Spindle_SRO site – regardless of the bearing wood – showed to be riper with lower firmness, lower $I_{AD,}$ and TA than apples from 'WA 38'/NIC29_Spindle _Quincy orchard (Table 5). Despite the lack of significance for yield and average apple mass in 2022 between the two sites (N apples/tree and kg/tree), SRO site showed a slightly lighter crop than Quincy (data not shown).

When comparing the 3 bearing woods – major interest for this analysis – firmness, SSC, and DM% did not differ in 2022, while they did in 2021. On the other hand, apples harvested from spur had a larger diameter and mass, higher N of healthy and mature seeds, and higher TA compared to apples from 'Ramo misto' and/or 'Brindilla' (Table 5). While in 2021 crop, apples borne on 'Brindilla' were smaller and had lower SSC and DM% than the other bearing woods, in 2022, this wood formation showed a lower apple mass and higher I_{AD} (less ripe). Regarding mature-healthy seeds/fruit, apples harvested from spurs showed an average of 8.7 good seeds/fruit, significantly higher than 7.8 good seeds in apples borne on 'Ramo misto' (Table 5). The latter bearing wood showed a delay in bloom compared to inflorescences on spurs and 'Brindilla' that can cause a slightly lower number of healthy and enduring seeds. In the two years of quality analysis comparing bearing woods, consistent trends for the internal parameters were not found. In the last year of data, DM% did not show differences among the 3 bearing woods, suggesting apples with a similar consumer eating quality. Further investigation would be needed in the future to test this hypothesis.

Table 5. *'WA 38' quality analysis after 3.5 months (M) of regular air 34°F storage from harvest 2022 for the locations 'WA 38'/NIC29_Spindle_QUINCY and 'WA 38'/M9337-GS_Spindle_SRO investigated in the 2021-2022 survey for objective 2. Sorting criteria for those apples were: only single (S) apples in the cluster, all best color, absence of defects and size range 216-339 g (80-64 apples/box). Significance: *= p≤ 0.05, ** = p≤ 0.01, *** = p≤ 0.001, NS= not significant and letters of separation within each treatment were provided by SNK test. DM% and TA have a different number of replications not corresponding to N apples reported.*

WA 38 harvest 2022 (obj. 2 survey)	*N apples*	Apple maximum diameter (mm)		Apple mass (g)		I_{AD} at 3.5M		Firmness (lb)		N mature-healthy seeds/tree		N underdeveloped seeds/tree		SSC (°Brix)	DM%	TA (% malic ac.)	
QUINCY	*90*	75.9		209		0.75	A	15.47	A	8.3		1.6		12.9	13.49	0.41	A
SRO	*78*	76.3		218		0.36	B	14.58	B	8.1		1.7		12.9	13.39	0.35	B
Significance location		NS		NS		***		***		NS		NS		NS	NS	***	
Brindilla	*55*	73.8	C	194	C	0.67	A	15.11		8.1	AB	1.6		12.8	13.24	0.36	B
Ramo Misto	*55*	75.9	B	212	B	0.54	B	15.05		7.8	B	1.9		13.1	13.45	0.36	B
Spur	*58*	78.4	A	233	A	0.50	B	15.01		8.7	A	1.4		12.8	13.62	0.40	A
Significance wood		***		***		***		NS		*		NS		NS	NS	**	
Significance location*wood		NS		NS		NS		NS		**		**		NS	NS	***	
QUINCY_B_S	*30*	74.2	c	191	c	0.85	a	15.34	a	8.67	a	1.27	b	12.8	13.28	0.37	b
QUINCY_RM_S	*30*	76.0	bc	212	bc	0.73	b	15.43	a	7.57	b	2.30	a	13.2	13.52	0.38	b
QUINCY_S_S	*30*	77.6	ab	224	ab	0.67	b	15.64	a	8.67	a	1.23	b	12.7	13.66	0.46	a
SRO_B_S	*25*	73.3	c	197	c	0.46	c	14.83	b	7.52	b	2.08	ab	12.9	13.20	0.36	b
SRO_RM_S	*25*	75.8	bc	212	bc	0.32	d	14.59	b	8.16	ab	1.36	b	13.0	13.38	0.34	b
SRO_S_S	*28*	79.3	a	242	a	0.31	d	14.34	b	8.68	a	1.61	ab	12.9	13.58	0.34	b
Significance combo		***		***		***		***		**		**		NS	NS	NS	

Objective 3) Investigate the cultural management practices developed in 'WA 38' private orchards and summarize them in a list of recommended guidelines for growers *(Lewis-Sallato)*

A total of nine commercial 'WA 38' blocks were selected in 2021 (Table 6). The selection considered the industry standard rootstocks; 'G.41', 'M.9, 'G.890', 'G.11' and 'Bud10' and prioritized locations with more than one rootstock for comparative analysis. Sites were monitored during bloom to rate bloom density and date, by the end of the growing season to measure max shoot growth, and at harvest to determine fruit yield, estimate crop load, and trunk cross sectional area. We collected photos and videos on each site/stage to develop a comprehensive database to share with WA growers and extension and outreach products. In addition, we interviewed 31 growers to understand the most common practices utilized in 'WA 38' orchards and continue assessing challenges and knowledge gaps to guide future efforts.

Table 6. *Sites of 'WA 38' monitored for Objective 3 in 2022-2023. Single Drip + Sprinklers =SD+S, Single Drip =SD, double drip=DD, overhead cooling =OC. All sites were planted at 12 ft row spacing and two leaders (Bi-Axis) training system.*

Site	Rootstock	Planting year	Year of first crop	Soil History	Training	Leader spacing (inches)	Irrigation	Heat mitigation
1	G41	2018	3rd	Replanted	Vertical – Bi-axis	30	SD+S	OC
2	M9-NIC29	2018	3rd	Replanted	Vertical – Bi-axis	30	SD+S	OC
3	G890	2018	3rd	Replanted	Vertical – Bi-axis	30	SD	Netting
4	M9-NIC29	2018	3rd	Replanted	Vertical – Bi-axis	30	SD	Netting
5	Bud10	2018	3rd	Replanted	Y Angle – Bi-axis	24	DD	Sprays
6	G.11	2017	3rd	New	Vertical-Bi-axis	30	SD+S	OC
7	M9-NIC29	2018	3rd	New	Vertical-Bi-axis	30	SD+S	OC
8	G41	2019	2nd	Replanted	Vertical- Bi-axis	30	SD+S	Sprays
9	M9-NIC29	2019	2nd	Replanted	Vertical- Bi-axis	30	SD+S	Sprays

Overall bloom density increased over the tree years, being generally high in 2022 and 2023, ranging between 68 and 244, higher than in 2022. However, fruit yield (number per tree) was more than 30% lower in three blocks (1, 2 'G.41' and 2 'M.9'), equivalent to the previous year, and only orchard 5 ('M.9-NIC29') was 30% higher, overall, it ranged between 37 and 141 fruit per tree (Figure 4). Note that growers were expecting higher crops as the trees filled the space. The changes in relation to the previous year appear to be more associated with the orchard than the rootstock, thus a response to environmental conditions or management. For example, in orchard 1, both 'G.41' and 'M.9' had lower fruit per tree, while in orchard 5, both 'G.41' and 'M.9-NIC29' slightly increased fruit yield.
In contrast, as a measure of fruit per trunk cross sectional area, crop load ranged between 3.8 and 6.9 was reduced in all combinations but orchard 5 (Figure 5).

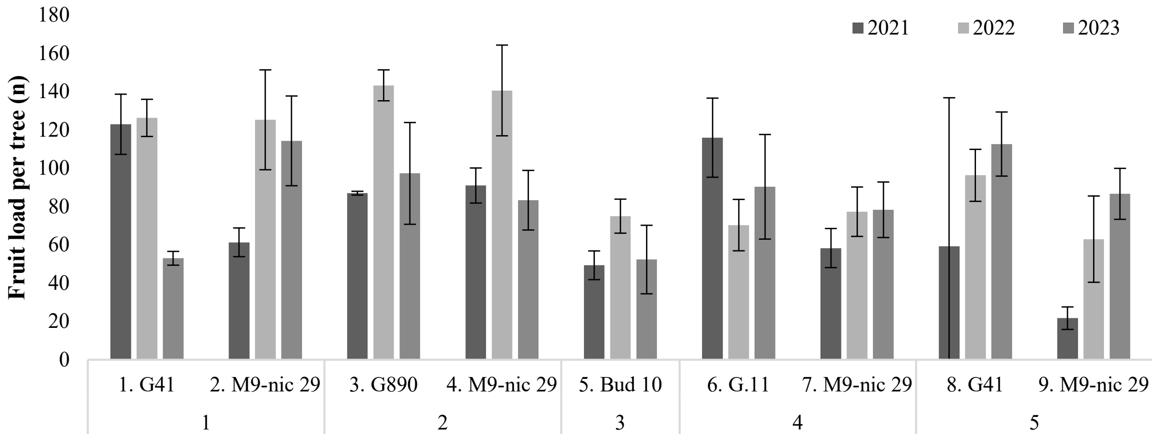


Figure 4. *Fruit load per tree for 2021, 2022, and 2023 by orchard (number) and rootstock. Bars indicate the mean value of 6 representative trees; error bars indicate standard deviation.*

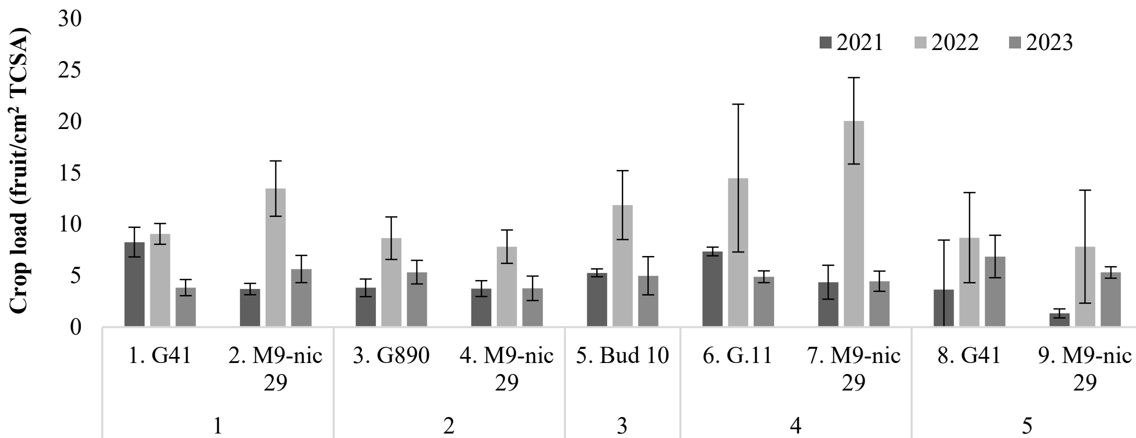


Figure 5. *Crop load (N fruit per trunk cross sectional area in cm²) for 2021, 2022, and 2023 by orchard (number) and rootstock. Bars indicate the mean value of 6 representative trees, error bars indicate standard deviation.*

Survey results

With more than 20 million trees ordered, 'WA 38' on 'G.41' remains the most planted **scion-rootstock** combination (23%), followed by M9-337 (20%) and M9-Nic 29 (12%). In the last two years, 'G.969' and G11 were the second and third most ordered trees. Over half of the surveyed growers planted their 'WA 38' orchards at 3 x 12 ft (20%). Regarding **training system**, trees with two leaders (bi-axis) are the most popular (50%), trained vertically. Spindle trees were the second most popular choice, with half of the trees trained on a V trellis. Only a quarter of the sites were three or more leader trees. Regarding the **pollinizers**, the density varied between 5 to 11%. The most popular are crabapple pollinizers: Everette, Snowdrift, and Indian Summer. Due to low crop load, several growers added more pollinizers after the third or fourth year. Most growers use double drip **irrigation** plus sprinklers (38%). Only 9 out of 31 blocks had drip only (29%). Four indicated the intention to add sprinklers (S) for better water coverage or sunburn or frost protection. Many growers started with no **sunburn mitigation strategies**; however, after 2021 heat events, 67% started using overhead cooling (OC). Two of the surveyed sites installed netting at planting. Most growers complemented these strategies with sunburn protectants (sprays) starting in June when temperatures are expected to be over 90 °F. **Summer pruning** is used by 34% of the surveyed sites. The timing varies among growers, but preferred time is during active shoot growth (end of May to end of July). Apogee® was used by 10% of the respondents. Among those adopting summer pruning, spraying sunburn protectants (i.e., Eclipse, Surround) was indicated as a practice after summer pruning. Except for two sites, all orchards have **cover crops** in the inter- row. One of 29 respondents thinned their 'WA 38' during bloom, but only for one year. Regarding challenges currently relevant for 'WA 38', the most mentioned is the low productivity (low fruit set, high drop). Among the practices adopted to increase set, 54% used supplemental pollen, either dusted, sprayed in solution, or placed in the beehives. Ten of them also sprayed ReTain® during bloom or after ten days. And 20% of them are using both Pollen + ReTain® as a standard practice.

New challenges in 2023 field days are bird damage control and identifying the best rootstock combinations.

Outreach/extension activities

A) Nine field days were organized: five in Spanish and four in English.

- October 13th, 2023: Covering vigor management, heat mitigation and harvest maturity (Sallato, Khot, Torres) (35 attendees)
- September 13th, 2023: 'WA 38' Pre-Harvest Field Day hosted by Musacchi, Serra, Lewis, Sallato (estimated 29 attendees). Looking at rootstock differences and pruning response, fruit set and bee exclusion by netting.
- July 20th, 2023: Dia de Campo en la Roza (Spanish). Field day in the WSU Experimental orchard Roza farm in Prosser in Spanish (16 attendees).
- October 19th, 2022: Actualización de conocimientos en 'WA 38' (Spanish), hosted by Sallato. We had 33 attendees and 23 responses to the survey. 81.6% indicated increased knowledge, and 83.3% intention to apply some of the learnings during the session. E.g., means for vigor control, summer pruning and irrigation, when to apply calcium. The highest-rated topics are soils-root-tree and general characteristics of 'WA 38'.
- September 15th, 2022: 'WA 38' Pre-harvest Field Day hosted by Musacchi, Serra, Lewis, Sallato. Invited presenter: Kalcsits. At the preharvest field day, we had 89 attendees. Of 22 respondents, over 93.4% indicated they were satisfied with the content. Learnings included: pruning, harvest timing, and maintaining vigor.
- July 5th, 2022: Día de campo en 'WA 38': Nutrición, vigor y estrés por calor. Sallato presenter (Spanish). WSU- Huerta la Roza, IAREC (10 attendees). Topics: Nutrition, vigor and heat stress).
- April 21st, 2022: Día de Campo 'WA 38'- Polinización y cuaja de fruta Sallato presenter (Spanish). Quincy and Royal City, (14 attendees). Topics: Pollination and fruit set.
- April 19th, 2022: Día de Campo 'WA 38'- Polinización y cuaja de fruta. Sallato presenter (Spanish). WSU Experimental orchard Roza farm in Prosser (16 attendees). Topics: Pollination and fruit set.
- Sept 17th, 2021: 'WA 38' research and harvest management update (attendees 115 in Sunrise and 95 in Quincy) hosted by Musacchi, Serra, Lewis, Sallato. A survey was conducted, and we obtained 30 responses (26%). The most interesting topics were associated with improving fruit set and the farm manager interview. Forty percent of the respondents indicated gained knowledge, and 27% indicated an intention to implement changes in their operation based on the information reported during the field day.

B) A "*'WA 38' SOP manual*" made of 8 independent chapters is currently in preparation, and it is forecasted to become available as a WSU Extension publication (each chapter independent from others) in Summer 2024.

GLOSSARY (Figure 6)**:**

A. 1X stub = residual portion of a pruning cut made on lateral branches, 1X stands for length of the remaining wood = 11 cm/4.3”. Approach utilized after planting the first year in the “Stub” trt.
B. 3X stub = residual portion of a pruning cut made on lateral branches during the second year after planting, specifically stubbed at 3X length (33 cm/~1ft) as a “recovery approach.”
C. Spur = 2+ year-old short and compact fruiting formation.
D. Brindilla = tip bearing 1-year-old shoot with vegetative lateral buds.
E. Ramo Misto = 1-year-old shoot bearing on lateral buds and on tip bud.

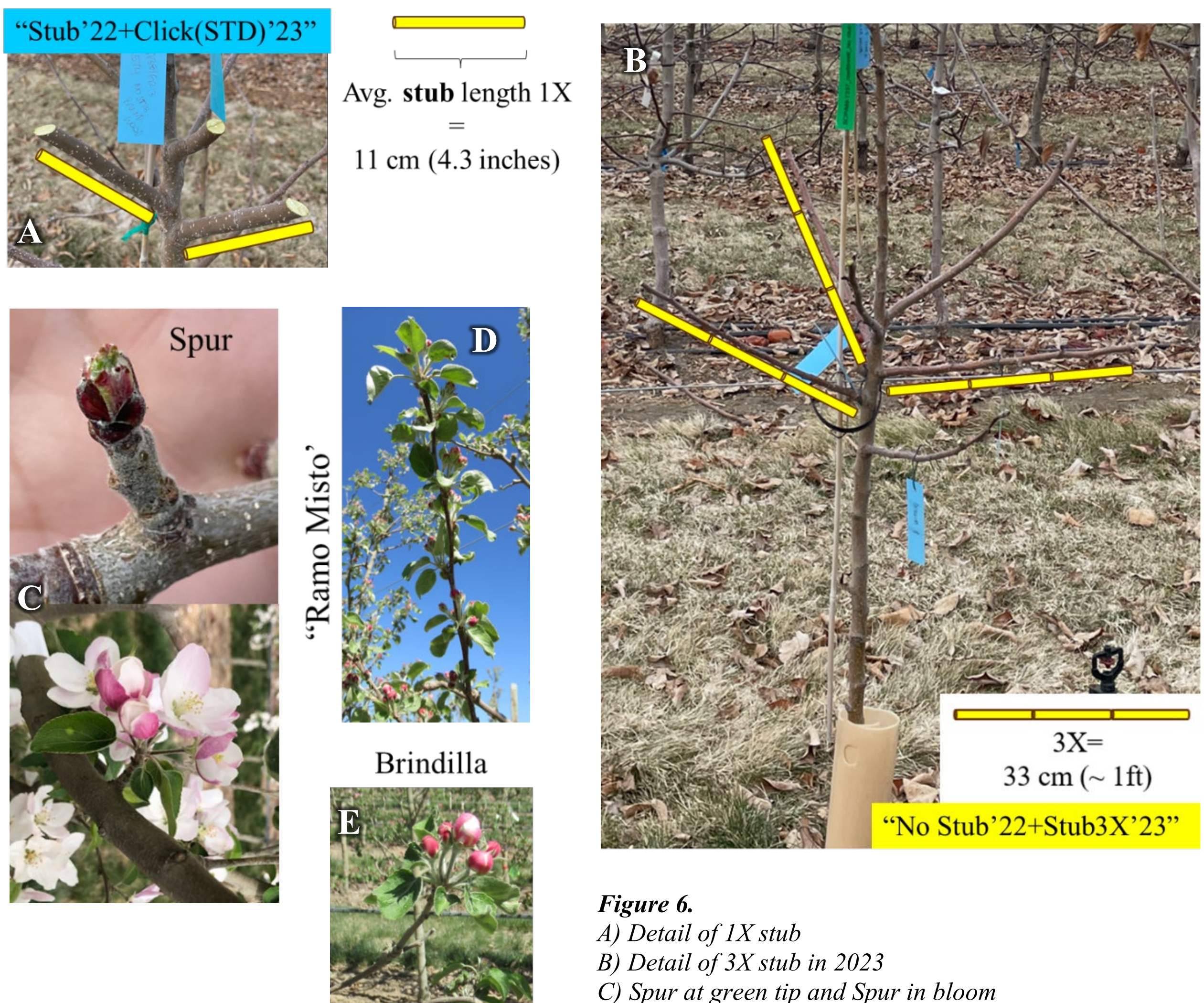


Figure 6.
A) Detail of 1X stub
B) Detail of 3X stub in 2023
C) Spur at green tip and Spur in bloom
D) Ramo Misto at loose pink balloons
E) Brindilla tip with inflorescence at pink balloon

Executive Summary

Project Title: **"WA 38: SOP from planting to cropping"**

Keywords: *blind wood, rootstocks, click pruning, bearing woods*

The overall goal of the project was to develop a protocol from orchard establishment to cropping for 'WA 38' in combination with 9 of the most popular and adopted rootstocks in WA: 'Bud9', 'Bud10', 'G.11', 'G.213', 'G.41', 'G.935', 'G.969', 'G.890', 'M.9-T337'. Due to poor quality trees originally planted and the severe heat waves that hit the PNW in June 2021, the orchard was replanted with well-feathered trees in April 2022, allowing us to plant 2 additional combinations.

With the first objective, we aimed to characterize the rootstock effect on blind wood, vegetative growth, flower buds formation, fruit set, and yield by comparing two contrasting scenarios: A) "No Stub" at planting and, B) "Stub" at planting; being the latter the recommended approach to minimize the blind wood for this variety. During the first season, the incidence of the blind wood (% blind portion of a branch over the total length of it) on unpruned basal branches of "No Stub" trees was significantly higher in combination with 'G.213', 'M.9-T337' and 'G.969', while smaller with 'G.11'. The effect of clicking or stubbing immediately after harvest allowed an overall reduction in the length of blind wood from 15.3 cm in the unpruned trees to 5.6 cm in the stubbed branches. Moreover, 64 to 85% of the nodes in the stubbed branch vegetated after the cut. Before the imposition of pruning at the end of March 2023, vegetative measures revealed that the smallest proportion of blind wood in the unpruned trees was found in combination with 'G.890' and 'G.11', while the largest incidence in M9-T337 and 'Bud10'. Regarding the flower bud (FB) formation, 'WA 38'/'Bud9' trees registered the highest average number of FB/branch (or stub with shoots) in both 2022 pruning scenarios, suggesting a specific trait of conferring early bearing for this rootstock. During pruning, a third treatment was added to the comparison between "No Stub 2022+2023" and "Stub2022+click2023"; this additional pruning approach had the purpose of following the evolution of not stubbed trees at planting but stubbed at 3X length during the second season as a sort of "recovery approach". Both pruning approaches in 2023 showed an impact on the number of flower clusters/trees in comparison to unpruned trees, but, at the end of the natural abscission window, the fruit set was not different between them. At harvest 2023, not all the combinations had bearing trees. In fact, 'WA 38'/'G.890' did not bear fruit at harvest, confirming the effect of a vigorous rootstock in delaying cropping. At the end of the 2023 season, trunk cross sectional areas across combinations allowed us to build our own WA tree vigor/size scale for 'WA 38' on the 9 rootstocks in trial, mimicking Cornell's one widely adopted for the Geneva series rootstocks. 'WA 38' vigor scale based on "Stub'22+Click'23" approach lists in ascending order of vigor the following rootstocks: 'Bud9'='Bud10'='G.11'='M.9-T337' < 'G.213'='G.935'='G.969'='G.41' < 'G.890'.

With the second objective, we investigated the different types of fruiting wood, and we surveyed that the 61-64% of the crop of 2 years was held on spurs (2+ year-old short fruiting formations), followed by 'Brindilla' (tip bearing 1-year-old shoots), and 'Ramo misto' (1-year-old shoots bearing on lateral buds). 'WA 38' as a natural fruiting habit is a type 4 variety characterized by an inclination to set fruit on 'Brindilla' in addition to spurs. Training systems and pruning styles are key factors in impacting the proportion of the different types of wood in the tree.

With the third objective, we explored the cultural management practices adopted and developed in 'WA 38' private orchards and summarized them in this report. Moreover, nine 'WA 38' field days were organized in 3 years. "'WA 38': SOP from planting to cropping" will become part of a WSU extension article collection made of 8 independent chapters with a forecasted publication in Summer 2024.

Future prospective:

The three treatments imposed in March 2023 ("No Stub'22+unpruned'23", "No Stub'22+Stub3X'23", "Stub'22+Click(STD)'23") will be further investigated with the goal of collecting information about the effectiveness of the "recovery approach" as a potential tool to rescue a 'WA 38' block and convert it to click pruning.

Project Title: Mitigating WA 38 greasiness and related quality defects

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

Primary PI: Carolina Torres
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City/State/Zip: Wenatchee/WA/98801

Cooperators: Anne Plotto, USDA-ARS, USHRL
Project Duration: 3 Years
Total Project Request for Year 1 Funding: $ 84,050.00
Total Project Request for Year 2 Funding: $ 84,012.00
Total Project Request for Year 3 Funding: $ 86,092.00

Other related/associated funding sources: None

WTFRC Collaborative Costs: None

Budget 1
Primary PI: Carolina Torres
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Station Manager/Supervisor: Chad Kruger
Station manager/supervisor email address: ckruger@wsu.edu

Item	Type year of project start date here	(Type year start date of year 2 here if relevant)	(Type year start date of year 3 here if relevant)
Salaries	54,000	56,160	58,406
Benefits	20,050	20,852	21,686
Wages			
Benefits			
Equipment			
Supplies	4,000	1,000	
Travel			
Miscellaneous			
Plot Fees			
Total	78,050	78,012	80,092

Footnotes:

Salaries: Research personnel to carry out field and laboratory work, fruit evaluations and data analyses in years 1, 2, and 3.
Benefits: $20,050, $20,852, and $21,686 are requested for benefits tied to the research personnel.
Supplies: Supply costs of $4,000 in year 1 and $1,000 in year 2 are requested to pay for supplies for fruit quality evaluation.

Budget 2
Co-PI: David Rudell
Organization Name: USDA-ARS
Contract Administrator: Chuck Myers and Sharon Blanchard
Telephone: (510) 559-5769 (CM, 509-664-2280 (SB)
Contract administrator email address: Chuck.Myers@usda.gov, Sharon.Blanchard@usda.gov

Item	Type year of project start date here	(Type year start date of year 2 here if relevant)	(Type year start date of year 3 here if relevant)
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies	3,000	3,000	3,000
Travel	3,000	3,000	3,000
Miscellaneous			
Plot Fees			
Total	6,000	6,000	6,000

Footnotes:

Supplies: Supply costs of $4,000 in year 1 and $1,000 in year 2 are requested to pay for supplies for fruit quality evaluation.
Travel: $3,000 is requested in years 1, 2, and 3, respectively, for associated travel for Dr. Anne Plotto.

Objectives:
1. Further, define harvest maturity guidelines limiting greasiness in the cold chain.
2. Establish ethylene mitigation protocols that reduce greasiness for both conventional and organic production.
3. Determine the limitations of wax/detergent for mitigating greasiness in the post-storage cold chain.
4. Identify and determine protocols for mitigating off-flavors associated with greasiness.

Significant Findings:
1. Maturity progression varied between growing sites. Fruit from Quincy was greasier than that from Mattawa, and it was most severe in air storage. Greasiness severity did not increase over time during storage, but it did following storage after 7 days at 68°F (shelf-life). Differences in maturity progression have been seen in 2022 and 2023. Further analysis will include seasonal weather.
2. 1-MCP and AVG treatment altered maturity and ripening, but not greasiness incidence. Additional years are needed to study this further.
3. All detergent and coating treatments removed and continued to control greasiness during the cold chain.
4. Off-flavor was linked with a higher ratio of aroma compounds typical of unripe apples compared with ripe apples.

Objective 1: Further define harvest maturity guidelines limiting greasiness in the cold chain.

Year 1: During 2022, fruit was harvested twice (a week apart) from 2 commercial orchards: Mattawa (4th-leaf trees) and Quincy (3rd-leaf trees). Maturity progression was evaluated weekly starting at four weeks before commercial harvest. Following each harvest, fruit was stored 33°F in air or in controlled atmosphere (2.5% O_2, 1.5% CO_2). Fruit quality (ripeness, skin greasiness, physiological disorders) was evaluated monthly until 6 months. Skin greasiness was rated using a 4-point subjective scale, rubbing the fruit against the hand, and rated as (0) no greasiness to (3) severe greasiness.

Maturity was different in fruit from both locations with ethylene production, starch index, and chlorophyll degradation (DA meter) reflecting these differences. Table 1 shows the maturity indices of fruit at harvest from both locations. Figure 1 shows the frequency of SI values in fruit harvested commercially and one week later in 2022.

Table 1. Apple maturity and ripeness at commercial harvest and one week afterward (WAH) from Mattawa and Quincy sites during seasons 2022 and 2023.

	Location	Harvest	Weight (g)	Red skin coverage (%)	Backg. Color (1-4)	Soluble Solids (°Brix)	SI (1-6)	I_{AD} (0-2.2)	Ethylene (ppm)	Firmness (lb)
2022	Mattawa	Harvest	283.8±38.6	91.9±5.1	3.6±0.5	13.3±1.2	3.0±0.9	0.7±0.3	0.5±0.3	15.4±0.9
		1 WAH	268.9±40.1	90.5±5.7	3.8±0.4	13.3±1.2	3.3±0.8	0.6±0.2	0.8±0.4	17.5±1.4
	Quincy	Harvest	313.1±62.3	87.7±6.4	3.5±0.5	13.8±0.8	2.1±0.7	1.2±0.3	0.0±0	19.5±1.1
		1 WAH	317.5±41.3	90.3±4.7	3.6±0.5	13.9±0.9	1.8±0.6	0.7±0.3	0.3±0.5	15.8±0.8
2023	Mattawa	Harvest	268.8±43.9	85.7±5.7	3.7±0.5	12.8±0.9	2.1±0.5	0.7±0.3	0.4±0.3	21.9±0.4

	1 WAH	296.4±34.1	94.7±1.2	3.9±0.2	16.2±1.2	2.9±0.5	0.4±0.2	5.9±1.5	23.7±0.4
Quincy	Harvest	312.9±27.3	82.7±6.4	3.7±0.5	14.6±1.6	2.0±1.0	1.0±0.3	0.2±0.3	22.9±0.3
	1 WAH	249.4±27.3	93.6±2.9	3.9±0.2	14.5±0.5	3.1±0.7	0.4±0.1	11.8±1.9	20.4±0.2

[Y] Average ± Standard Error

During storage, CA-stored impeded softening better than air storage (Table 2). Ethylene was lower in fruit sorted in CA compared with those in air, with few exceptions (Table 2). Greasiness did not increase with storage duration when evaluated after 7 days at 68°F following removal (Table 2). Fruit from Quincy was greasier than that of Mattawa, and it was more severe in air storage (Table 2).

Year 2: Table 1 shows maturity and ripeness of WA 38 harvested in 2023. The frequency of the starch index values at both, harvest 1 (commercial) and 2 (1 week after; wah) are shown in Fig. 2. Fruit was placed in RA and CA storage, and fruit quality assessments are ongoing.

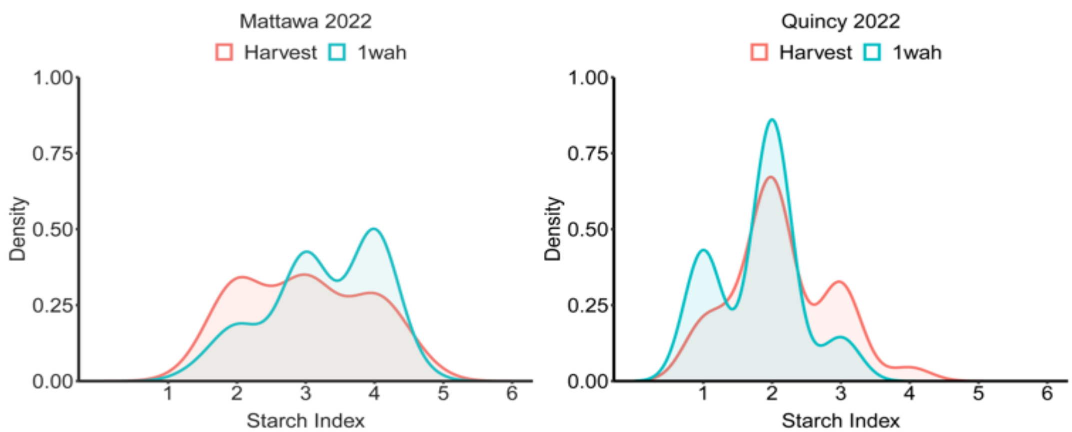


Figure 1. Density plots from the starch index of fruit from Mattawa and Quincy in 2022 at commercial harvest and one week later (wah). They show no differences in the starch degradation population in fruit sampled at each time-point.

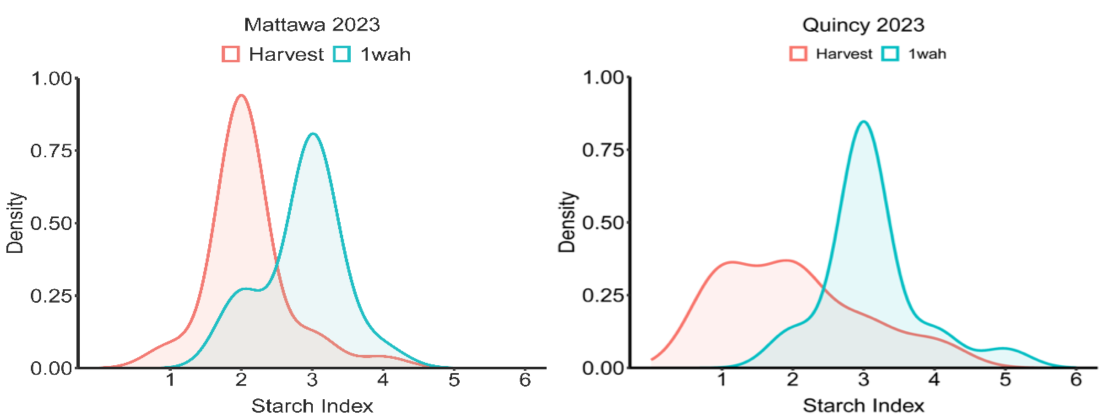


Figure 2. Density plots from the starch index of fruit from Mattawa and Quincy in 2023 at commercial harvest and one week later (wah). They show different starch degradation populations in fruit sampled at each time-point than that from 2022.

Table 2. Relative ripeness during air and CA storage at 33ºF.

	Harvest	Storage Eval.	Firmness (lb)		Backg. Color (1-4)		I_{AD} (0-2.2)		SI (1-6)		Ethylene (ppm)		Grease Severity (0-3)	
			RA	CA	RA	CA	RA	CA	RA	CA	RA	CA	RA	CA
Mattawa	H1	1mo+1d	16.0	15.7	3.5	3.4	0.6	0.6	4.1	4.1	54.5	182.8*	0.1	0.0
		2mo+1d	17.5	19.1	3.3	2.9	0.4	0.7*	4.9	4.8	69.3	14.4*	0.0	0.0
		3mo+1d	17.4*	18.3	3.9	3.9	0.4	0.6*	5.9	5.5*	86.2	13.6*	0.1	0.0
		4mo+1d	18.1*	20.2	3.9	3.8	0.3	0.5*	6.0	6.0	80.5	20.4*	0.2	0.0*
		5mo+1d	17.4*	19.3	3.7	3.0*	0.4	0.6*	6.0	6.0	78.6	7.0*	0.0	0.0
		6mo+1d	17.0*	19.5	3.9	3.4*	0.3	0.5*	6.0	6.0	36.9	13.5*	0.0	0.0
	H2	1mo+1d	15.4	15.8	3.3	3.7	0.5	0.6	4.6	3.8	66.4	13.7*	0.5	0.1
		2mo+1d	18.3	17.8	3.8	3.9	0.6	0.6	5.6	5.2*	57.0	16.7*	0.2	0.0*
		3mo+1d	18.8	18.9	4.0	4.0	0.3	0.5*	5.9	5.8	148.2	16.0*	0.0	0.0
		4mo+1d	21.5	21.4	3.9	3.8	0.3	0.5*	6.0	6.0	102.0	9.8*	0.0	0.0
		5mo+1d	18.3*	20.0	3.7	3.7	0.2	0.4*	6.0	6.0	38.1	0.4*	0.0	0.0
		6mo+1d	20.8	21.4	3.8	3.8	0.4	0.6*	6.0	6.0	1.4	10.0	0.2	0.0*
Quincy	H1	1mo+1d	15.6	16.1	3.7	3.5	0.5	0.8*	3.3	2.6*	11.1	5.71*	0.9	0.4*
		2mo+1d	20.0	20.1	3.2	3.2	0.7	0.8	4.3	4.2	15.6	39.0	1.2	0.4*
		3mo+1d	21.2	21.2	3.7	3.6	0.4	0.5	5.8	5.5*	5.4	4.1	0.6	0.1*
		4mo+1d	20.3	20.5	3.6	2.6*	0.3	0.6*	6.0	6.0	99.1	9.7*	1.5	0.3*
		5mo+1d	20.8	20.8	3.6	3.7	0.4	0.5	6.0	6.0	22.9	6.5*	1.5	0.2*
		6mo+1d	19.4	20.9	3.4	2.9*	0.4	0.7*	6.0	6.0	40.0	2.1*	1.4	0.3*
	H2	1mo+1d	15.9	15.3	2.7	2.7	0.6	0.5	3.3	3.1	46.6	3.2*	1.3	0.9*
		2mo+1d	18.8	18.1	4.0	4.0	0.6	0.5	4.5	4.3	79.1	46.5*	0.6	0.5
		3mo+1d	21.4	18.6*	3.1	3.8*	0.6	0.6	5.9	5.8	126.3	25.7*	0.7	0.1*
		4mo+1d	20.2	19.4*	3.8	3.7	0.3	0.6*	6.0	6.0	11.0	82.0*	1.0	0.2*
		5mo+1d	18.1*	20.2	3.8	3.6	0.2	0.5*	6.0	6.0	54.6	4.1*	1.3	0.3*
		6mo+1d	18.0*	20.5	4.0	3.0*	0.2	0.5*	6.0	6.0	53.7	2.3*	0.5	0.5*

* Indicates significant differences between storage regimes (RA, CA) (ANOVA, $P \leq 0.05$)

Objective 2: Establish ethylene mitigation protocols that reduce greasiness for both conventional and organic production.

Year 1: AVG (Retain®) and 1-MCP (Harvista™ and SmartFresh™) were applied in different rates and timing (Table 3). Both products were applied in different commercial orchards. Retain trials were conducted in Zillah, WA (2nd-leaf trees); Harvista™/Smartfresh™ (SF) trial was conducted in Royal City (4th-leaf trees).

Preharvest application of Retain®, either at half or full rate, suppressed ethylene production in a dose-dependent manner. Retain® treatments also delayed starch degradation pattern. Fruit greasiness was present at the time of the first application (2 weeks before harvest). Postharvest treatments did not consistently affect ethylene production (Table 4). Starch was fully cleared by 4 months with no differences among treatments.
Harvista™, either applied at a single (1x) or double (2x) rate, was not able to reduce ethylene production nor affect the starch degradation pattern on the fruit compared to the UTC. Apples were not greasy at harvest. Ethylene production was suppressed using Harvista™ 1x plus SF (T5) and UTC plus SF (T4) for up to 8 months plus 7 days at 68ºF. Firmness was higher for most of the storage period when apples were treated with Harvista™. The postharvest SI was not affected by the treatments. No fruit were bitter in this test.

Year 2: Table 3 shows treatments applied in 2023. Maturity and ripening progression were determined are ongoing after fruit for up to 12 months.

Table 3. PGR treatments.

Year	Treat.	Material/a.i.	Rate	Timing	Others
2022	1	Untreated control (UTC)	NA	NA	• Orchard location: Zillah, WA • Sylcoat (0.1% OSi) added to Retain® applications. • Two harvests: At the time of UTC (commercial), and 1 week later.
	2	AVG (ReTain®)	Full rate (24 oz/acre)	7 DBH	
	3	ReTain®	Half-rate (12 oz/acre)	7 DBH	
2023	1	Untreated control (UTC)	NA	NA	
	2	ReTain®	Full rate (24 oz/acre)	21 DBH	
	3	ReTain®	Full rate (24 oz/acre)	14 DBH	
	4	ReTain®	Full rate (24 oz/acre)	7 DBH	
2022 & 2023	1	UTC			• Orchard location: Royal City, WA • Two harvests: At the time of UTC (commercial), and 1 week later. • Only harvest 1 for SF treats.
	2	1-MCP (Harvista™ 1.3 SC) (1x)	Full dose	14 DBH	
	3	Harvista™ (2x)	Full dose	14 & 7 DBH	
	4	UTC plus 1-MCP (SmartFresh™; SF)	100 ppm	At harvest	
	5	Harvista™ 1x plus SF	T2 plus SF (100 ppm)	At harvest	

AVG: Aminoethoxyvinylglicine; 1-MCP: 1-methylcyclopropene; DBH: Days before harvest

Table 4. Fruit maturity/ripeness and quality during storage at 33ºF of WA 38 treated with AVG (Retain®) applied in Zillah, WA.

	Harvest	Treatment	SI (1-6)	Firmness (lb)		Ethylene (ppm)		TA (% malic acid)		Grease Severity (0-3)	
				+1d	+7d	+1d	+7d	+1d	+7d	+1d	+7d
Preharvest	2wbh	Control	2.0	16.8	-	1.1	-	0.5	-	-	-
	1wbh	Control	2.3	15.4	-	6.0 a	-	0.5	-	-	-
		Half Rate	2.4	16.1	-	4.3 ab	-	0.5	-	-	-
		Full Rate	1.5	16.1	-	0.7 b	-	0.4	-	-	-

H1	Harvest	Control	3.9 a[Z]	17.5	-	4.9 a	-	0.4	-	1.0	-
		Half Rate	4.2 a	17.8	-	0.6 b	-	0.5	-	0.8	-
		Full Rate	2.4 b	18.1	-	0.0 b	-	0.4	-	1.1	-
	4 m	Control	6.0	15.8 b	16.1	100.5 a	273.2 a	0.5	0.5	1.2 ab	1.6 b
		Half Rate	6.0	16.2 b	16.7	92.7 b	125.5 b	0.6	0.4	1.6 a	1.7 ab
		Full Rate	6.0	17.6 a	17.0	44.3 b	151.2 b	0.6	0.4	0.9 b	1.9 a
	8 m	Control	6.0	15.3	14.3	59.2	429.0	0.4	0.4	1.4	2.4
		Half Rate	6.0	15.1	13.9	50.1	413.7	0.3	0.3	1.6	2.6
		Full Rate	6.0	14.7	14.5	49.1	399.8	0.3	0.3	1.5	2.6
H2	Harvest	Control	3.4 a	16.8 b	-	4.6 a	-	0.4	-	2.1 a	-
		Half Rate	2.6 b	18.1 ab	-	1.1 b	-	0.6	-	1.9 b	-
		Full Rate	2.1 b	18.6 a	-	0.0 b	-	0.7	-	1.3 b	-
	4 m	Control	6.0	18.0 a	15.4	27.0	220.5	0.4	0.4	0.6	1.1
		Half Rate	6.0	18.9 ab	16.3	28.9	248.1	0.4	0.4	0.6	1.5
		Full Rate	6.0	17.6 b	15.8	35.6	185.4	0.4	0.4	1.1	1.3
	8 m	Control	6.0	14.6	14.2	27.3	-	0.4	0.2	1.0	2.0
		Half Rate	6.0	15.5	15.1	18.8	-	0.4	0.3	1.3	1.8
		Full Rate	6.0	15.2	14.1	20.7	-	0.4	0.3	1.1	2.0

[Z] ANOVA (P≤0.05). Different letters indicate significant differences between treatments (Tukey, P≤0.05).

Table 5. Greasiness of WA 38 orchard in Zillah, WA treated with Retain®.

	Harvest	Treatment	Grease 0		Grease 1		Grease 2+3	
			+1d	+7d	+1d	+7d	+1d	+7d
H1	4 m	Control	46.0 ab	35.1	34.0 ab	23.4	20.0	41.4 ab
		Full Rate	32.0 b	23.4	44.7 b	25.2	23.3	51.4 a
		Half Rate	68.6 a	53.5	26.1 a	21.9	5.2	24.6 b
	8 m	Control	52.8	67.1	28.4	5.1	18.9	27.9
		Full Rate	39.4	56.7	35.2	9.6	25.4	33.7
		Half Rate	37.0	56.2	30.3	4.5	32.7	39.3
H2	4 m	Control	77.1	45.8	22.9	44.3	0.0 b	9.9
		Full Rate	63.7	22.3	27.5	31.9	8.8 a	45.8
		Half Rate	63.5	7.8	32.6	38.2	3.9 ab	54.0
	8 m	Control	100.0	87.8	0.0	8.4	0.0	3.8
		Full Rate	100.0	65.9	0.0	7.6	0.0	26.5
		Half Rate	100.0	66.2	0.0	17.8	0.0	16.0

Table 6. WA 38 ripeness/quality during storage at 33ºF after 1-MCP (Harvista™) application in Royal City, WA.

Evaluation	Treatment	Starch	Firmness (lb)		Ethylene (ppm)		TA (% malic acid)		Grease Severity (0-3)	
			+1d	+7d	+1d	+7d	+1d	+7d	+1d	+7d
3wbh	UTC	1.0	25.9	-	0.4	-	0.8	-	-	-
2wbh	UTC	1.0	17.8	-	0.1	-	0.9	-	-	-
	Harvista™ 1x	1.0	17.5	-	0.4	-	0.6	-	-	-
1wbh	UTC	1.3	16.2	-	0.6	-	0.4	-	-	-
	Harvista™ 1x	1.3	16.2	-	0.5	-	0.5	-	-	-
Harvest	UTC	2.0 a[Z]	19.7 b	-	0.8	-	0.5	-	0.1	-
	Harvista™ 1x	1.8 b	20.1 b	-	1.4	-	0.4	-	0.1	-
	Harvista™ 2x	2.6 a	21.1 a	-	0.6	-	0.5	-	0.1	-

4 m	UTC	6.0	20.8	19.1 b	74.3 ab	22.1 a	0.6	0.4	0. 6 a	0.6 a
	UTC+SF	6.0	20.4	20.2 ab	5.2 c	0.2 b	0.7	0.4	0.0 b	0.0 b
	Harvista™ 1x	6.0	20.9	19.8 b	81.2 a	23.1 a	0.7	0.4	0.6 a	0.3 b
	Harvista ™ 2x	6.0	20.8	20.3 a	57.3 a	31.7 a	0.6	0.4	0.2 b	0.3 b
	Harvista ™ 1x+SF	6.0	20.4	21.3 a	6.8 c	0.3 b	0.7	0.5	0.3 ab	0.3 b
8 m	UTC	6.0	21.5 c	18.0 c	38.8 a	165.9 a	0.3	0.2	0.2 ab	1.4 ab
	UTC+SF	6.0	24.0 ab	21.2 a	5.8 b	27.1 b	0.3	0.3	0.4 a	1.6 a
	Harvista ™ 1x	6.0	21.4 c	18.2 bc	29.6 a	161.2 a	0.3	0.2	0.0 b	1.3 ab
	Harvista ™ 2x	6.0	23.6 b	19.2 b	35.1 a	132.9 a	0.9	0.3	0.2 ab	1.0 b
	Harvista ™ 1x+SF	6.0	25.2 a	21.2 a	13.9 b	17.7 b	0.3	0.3	0.0 b	1.0 b
12 m	UTC	6.0	16.9 b	15.5 b	5.2	46.7 b	0.2	0.2	0.1 a	0.1 b
	UTC+SF	6.0	19.8 a	19.7 a	10.2	107.5 a	0.3	0.3	0.2 ab	0.2 b
	Harvista ™ 1x	6.0	17.3 b	15.3 b	4.6	107.5 a	0.2	0.2	0.0 b	0.3 b
	Harvista ™ 1x+SF	6.0	20.1 a	19.6 a	8.2	65.8 b	0.3	0.3	0.0 b	0.7 a

[Z] ANOVA (P≤0.05). Different letters indicate significant differences between treatments (Tukey, P≤0.05)

Table 7. Greasiness severity (0-3) (n=30) during storage at 33°F following 1-MCP (Harvista™) treatment in Royal City, WA.

Evaluation (months)	Treatment	Grease 0		Grease 1		Grease 2+3	
		+1d	+7d	+1d	+7d	+1d	+7d
4 m	Control	100.0 a	31.9 b	0.0 b	23.6	0.0	44.4 a
	Control-1MCP	100.0 a	72.2 a	0.0 b	22.2	0.0	5.6 b
	Harvista 1x	100.0 a	70.1 a	0.0 b	17.9	0.0	12.0 b
	Harvista 2x	86.7 b	65.3 a	13.3 a	16.7	0.0	18.1 b
	Harvista1x-1MCP	93.3 a	65.3 a	6.7 a	25.0	0.0	9.7 b
8 m	Control	80.2 a	48.1	16.0	38.3 a	3.7 ab	13.6 b
	Control-1MCP	57.9 b	35.0	32.5	33.7 ab	9.7 a	31.3 ab
	Harvista 1x	79.0 a	44.4	18.5	25.9 ab	2.5 b	29.6 ab
	Harvista 2x	63.0 ab	40.7	30.9	21.0 b	6.2 ab	38.3 a
	Harvista1x-1MCP	73.5 a	-	22.2	-	4.3 ab	-
12 m	Control	94.0	89.2	6.0	10.8	0.0	0.0
	Control-1MCP	100.0	85.9	0.0	14.1	0.0	0.0
	Harvista 1x	97.5	90.1	2.5	9.9	0.0	0.0
	Harvista1x-1MCP	95.1	92.6	4.9	4.9	2.5	0.0

Objective 3: Determine the limitations of wax/detergent for mitigating greasiness in the post-storage cold chain.

Year 1: Detergents and coatings treatments are shown in Table 6. Greasy fruit from two different commercial lots were used for both sets of experiments (detergents, coatings). Fruit quality was assessed for up to 7 days (detergents) and 21 days (coatings) at room temperature (68°F) after treatment and after 30 days in cold storage (33°F), in the case of coatings. For the coating experiment, fruit was cleaned with Epi-Clean detergent (60 s), dried in air, and coated with the different formulations (Table 6).

Year 2: Another set of trials will be conducted in Jan 2024 using greasy fruit and another set of commercially available coatings will be included.

Table 6. Detergent and coating treatments.

Product	Treat.	Material	Rate	Application Time
Detergents	1	UTC (Water)	NA	30 s
	2	Acidex Duo	25 ml / L	30 & 60 s
	3	Epi-Clean	25 ml / L	30 & 60 s
Coatings	1	UTC	NA	-
	2	PrimaFresh 360 HS	0.4 g /fruit	30 s
	3	Shield-Brite AP-450	0.4 g /fruit	30 s
	4	Xedasol	0.4 g /fruit	30 s

For the detergent trial, in general, all treatments were able to remove the natural greasiness of the fruit, and it re-appeared after 4 days at 68ºF in both lots of fruit (without greasiness (A) and with low greasiness (B)) (Fig. 3). All coatings tested were able to effectively reduce greasiness on the fruit during the shelf-life period with and without 30 days in cold storage post-application (Fig. 4).

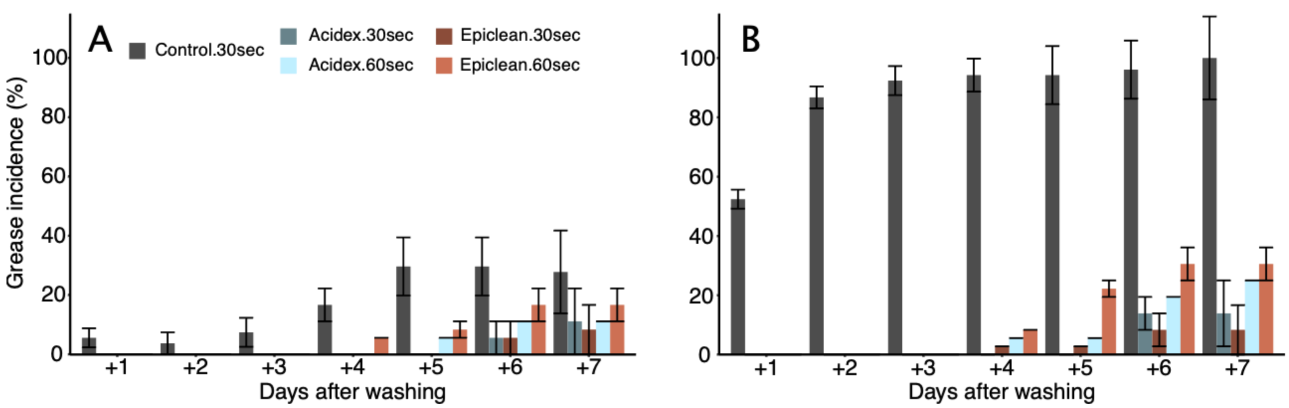


Fig. 3. Greasiness incidence (% fruit) in two lots: without greasiness (A) and with low greasiness (B) (Average ± Standard Error) for up to 7 days at 68ºF after washing with different detergent treatments.

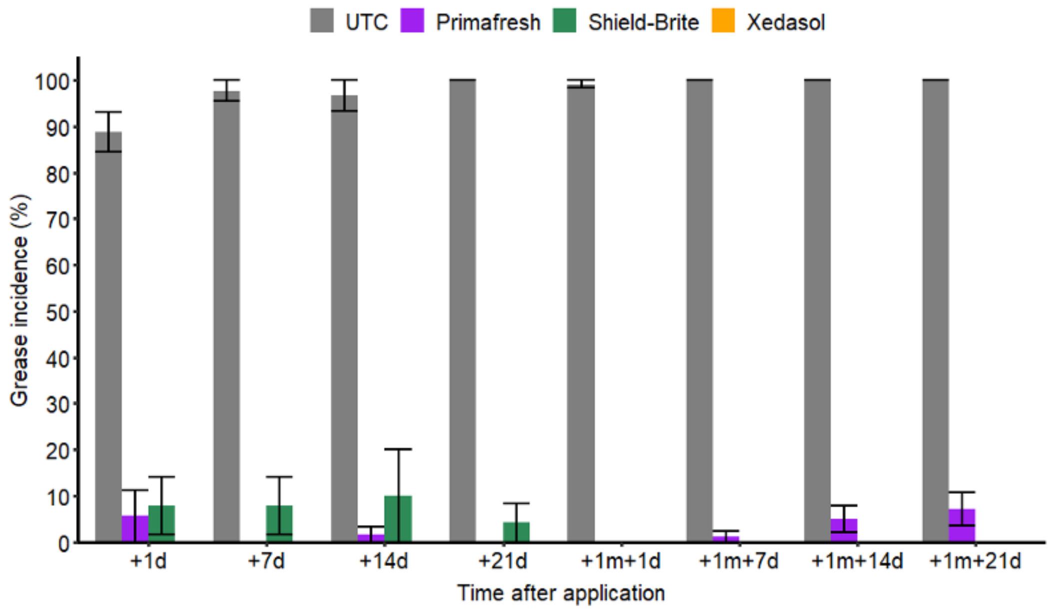


Fig 4. Greasiness incidence (% fruit) in fruit with low greasiness (average ± standard Error) for up to 21 days at 68°F after coating treatments, and after 1 month in cold storage (33°F) and up to 21 days at 68°F.

Objective 4: Identify and determine protocols for mitigating off-flavors associated with greasiness.

Year 1: Fruit were sampled from long-term CA lots where the off-flavor was identified. An *ad hoc* taste panel of individuals accustomed to apples was assembled and trained to identify the "bitter" off-flavor as well as hypothetical off-flavor components. Apples were sampled in sections representing quarters of the fruit (stem/calyx end; sun-facing/shaded). Apple samples were rated for eating quality by the sensory panel, and the adjacent peel/cortex flash was frozen in LN_2 and stored at -80 °C until processing and instrumental analysis. Peel and cortex were considered separately for the taste trial and instrumental aroma analysis. Samples were, first, simply rated as "good", "bad", or "passable" and, then, panelists were asked to identify samples with "bitter", "musty", "chemical", "medicinal", "metallic", "tingly", "astringent", or an "after taste", all descriptors of the off-flavor determined by panelists during training. The aroma profile was analyzed using gas chromatography/mass spectrometry to allow for a snapshot of the aroma, approaching a direct comparison of the profile and the panelists' experience. The sugar and acid profile analyses are underway (Year 2). Data were analyzed using statistical approaches that identified the presence, nature, and associated aroma components linked with those samples identified as imparting an off-flavor.

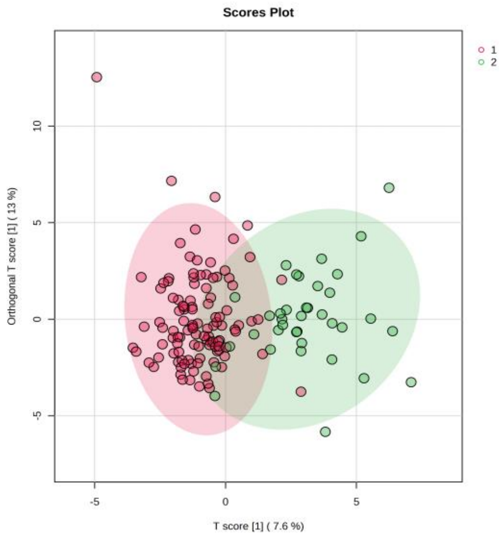
Year 2: Fruit was treated with 3 rates of natural chemicals associated with the off-flavors identified in year 1. This included 3 rates of natural substrates of those aroma chemicals that are components of apple wax, especially greasy apples. Treatments included ECs made with trans-2-hexenal and cis-3-hexenal mixed with Triton X-100 as well as the substrates oleic acid (hexanal), linoleic (hexanal), and linolenic acid (trans-2-hexenal; cis-3-hexanal). Treatment active ingredient concentrations were 0, 0.25, 0.50, and 1.00 mL L-1. Peel will be sampled at 0, 1, 2, and 4 weeks, flash frozen, and stored at -80°C until instrumental aroma analysis. The analysis will indicate if the elevated presence of these natural chemicals leads to enhanced levels of chemicals associated with the off-flavor.

Off-flavor was identified in at-least one sample given to each panelist. Bitter samples were often detected in the same fruit as samples that were not considered bitter. While peel typically produces the highest levels of aroma, bitterness was more commonly associated with cortex tissue. The off-flavor was detected fairly randomly over individual apples. There was no consistent sensory difference between samples taken from the sun or shade side and neither the stem nor calyx end of the apple.

The aroma profile was compared with off-flavor detected by the sensory panel. Off-flavor was determined as a positive response to any of the following descriptors as they could all be considered descriptors of the off-flavor according to the sensory panel survey: “Bitter”, “chemical”, “medicinal”, “metallic”, “tingly”, “astringent”, or “after taste”. The aroma profile (35 natural aroma chemicals) was consistent among samples from the same apple, but links with off-flavor were not obvious until whole apples were categorized according to presence of off-flavor. An apple was considered if off-flavor was detected in 50% or more of the comprising cortex or peel samples. This more clearly revealed associations with aroma chemicals linked with unripe apples, especially more “cut grass” and less ripe apple aroma chemicals (Fig. 5). This classification was even more apparent when projected using the ratio of 2-methylpropyl acetate, an aroma note associated with apples that were considered “good” overall, to one that was associated with “bad” flavored fruit, 2-hexenyl acetate. Consequently, once these associations were confirmed using this binary categorization, we could follow levels of these and related aroma compounds back to compare them with sensory classifications of individual samples finding that links between the limited set of aroma compounds and sensory classification still held true.

Given these results, additional chemical analysis will focus on natural chemicals that impact flavor such as phenolics, sugars, and acids to confirm whether the content of these, in ratio with the aroma profile, also impacts the perception of off-flavors.

Subsequent work will focus on determining any associations among phenotype, unripe aroma notes, and harvest maturity to resolve whether harvesting maturity impacts consumers' experience off-flavors. Rather than any single chemical causing the off-flavor, it is possible that another significant factor, such as maturity, is impacting flavor consumer perception of bitterness such as a pre-association between that attribute and green, grassy aroma notes. Consequently, in year 2, we are treating WA 38 with aroma compounds and the natural chemicals they are produced from, some of which are prominent in apple wax and greasy apples, to determine if other more bitter chemicals are produced from those associated with the off-flavor.



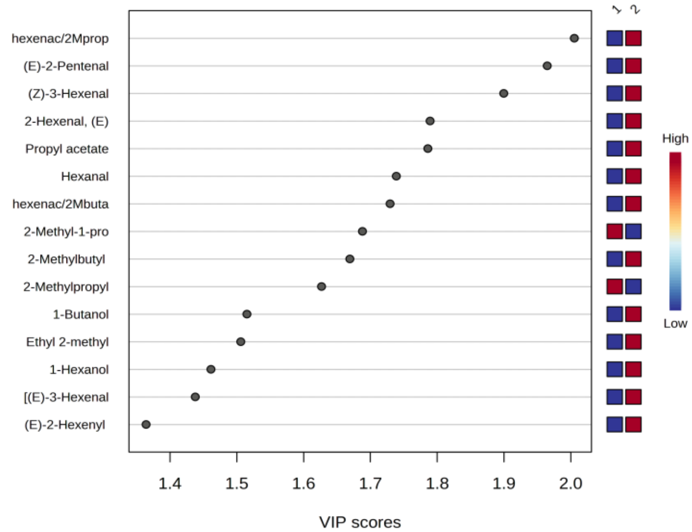


Fig. 5. Flesh tissue samples (left) from WA 38 apples were identified by a sensory panel as having off-flavor (green) or not (red). This indicates that the aroma profile, as a whole, is different between apples considered to have an off-flavor and those with no issue. It also reveals that there are one or more aroma components related to this difference. Natural aroma chemicals (right) that are most associated with off-flavored samples include those typically most abundant in unripe or immature apples, while those prominent in samples considered good are typically more prominent in ripe apple aroma profile. The ratio of unripe to ripe aroma components are also high in this list, further highlighting the relationship between unripe aroma and perceived off-flavor.

Project Title: Managing Ca-related disorders in high vigor conditions

Report Type: Final Project Report

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Project Duration: 3 Year

Total Project Request for Year 1 Funding: $13,728
Total Project Request for Year 2 Funding: $14,041
Total Project Request for Year 3 Funding: $14,366

Other related/associated funding sources: **Awarded**
Amount: $152,938
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Other funding sources: **Awarded**
Amount: $15,000
Agency Name: Valent BioScience
Notes: Costs associated with product supply.

Budget 1
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Item	2021	2022	2023
Salaries	5,800	6,032	6,273
Benefits	2,028	2,109	2,193
Wages[1]			
Benefits			
Equipment			
Supplies[2]	5,900	5,900	5,900
Travel			
Miscellaneous			
Plot Fees			
Total	**13,728**	**14,041**	**14,366**

Footnotes: [1] Salary for 50% FTE for 2 month per year for Juan Munguia, Research Assistant at Sallato's laboratory for trial establishment, collecting data, processing fruit in the laboratory and organizing extension activities. [2] Supplies include laboratory analysis and processing of samples for nutrient test (total of 4 soil initial test at 35 USD and 320 leaves and apple nutrient test at 18 USD/sample).

OBJECTIVES

Given the high and unpredictable levels of Ca related disorders; bitter pit, blotch pit, brown pit, green spot in WA apple orchards, we evaluated the effect of pre-harvest application of ABA, Prohexadione Ca and gibberellic acid (GA) on Ca related disorders in Honeycrisp and WA 38 orchards.

1. *Evaluate and manage Ca related disorders utilizing PGRs.*

2. *Analyze the effect of these products on reducing plant and fruit stresses*.

3. *Develop and distribute new strategies to manage Ca related disorder for excessively vigorous conditions.*

SIGNIFICANT FINDINGS

- The PGR treatments had inconsistent effect on shoot growth and fruit disorders across site, cultivars, and years.
- The pre harvest application of ABA reduced shoot growth in half of the sites and years when compared with the control, however with no significant impact on fruit quality.
- In relation to the untreated control, the application of ABA pre harvest reduced bitter pit incidence by 35% only in Honeycrisp Prosser in 2021. When BP incidence was significantly higher compared to 2022 and 2023.
- In 2022, where fruit cracking in WA 38 was a much greater issue compared to green spot incidence. Only in the Roza site, all treatment had lower cracking compared to the control, while in Prosser site, the control and GA had the lowest cracking incidence.
- In Honeycrisp the application of Prohexadione Ca (Apogee®) pre harvest did not affect bitter incidence.
- In WA 38, the application of Prohexadione Ca (Apogee®) pre harvest increased green spot incidence in the Roza site and only in 2021, while reducing cracking in 2021 and 2022. However in Prosser site, ProCa had 73% higher cracking incidence compared to the control.
- In Honeycrisp Grandview, BP incidence was very high in all three years of study. We conclude that BP incidence was mostly associated to high vigor and consequently low crop load, and while PGRs were able to reduce shoot growth, this reduction was insufficient to make an impact on fruit Ca disorders.
- In Honeycrisp Prosser, BP incidence was also associated to high vigor during the stage where growers push the trees to fill the space, where the ABA treatment was beneficial in reducing shoot growth and BP (2021). Once the orchard filled the space and vigor was controlled with alternative horticulture methods, the PGRs had no significant impact.
- There was a significant and strong correlation between bitter pit incidence and shoot growth ($p<0.001$; $r = 0.722$, $R2 = 0.521$)
- Green spot incidence was relatively low in most sites and years, except for WA 38 Roza in 2021 (> 13%), when ProCa did not reduce shoot length while increased green spot incidence.
- Green spot incidence in both sites seemed directly related to low crop load, increased over the years throughout horticultural practices, where PGRs had no significant impact.
- Fruit nutrient levels appear to reflect vigor differences across orchard, yet unrelated to fruit Ca related disorders. In several cases, fruit Ca concentration, and other nutrients were actually higher in the treatments, years or site that also had higher BP or green spot incidence.
- Leaf nutrient levels appear to reflect excessive vigor when above the recommended range (example Grandview site), while there was no direct relation with either fruit nutrient concentration or fruit quality.

METHODS

The project was conducted in two vigorous Honeycrisp orchards and two vigorous WA 38 orchards, selected due to high incidence of Ca-related disorders during 2020. The ‘Honeycrisp’ trials were a ‘Honeycrisp’ grafted over Fuji/M26 orchard located near Grandview (Honeycrisp Grandview), trained with four leaders per graft on a V trellis. And a ‘Honeycrisp’ on M9-337 located near Prosser (Honeycrisp Prosser), planted in a 5 x 10 ft. spacing, with three vertical leaders spaced 20 inches apart on a vertical wall. The WA 38 trials (WA 38 Prosser) were conducted in a commercial WA 38 on G.41 planted in 2019 located near Prosser, on a V trellis at 2.5 x 10 ft, trained with three vertical leaders on each side of the trellis. Additionally, we used the WSU research orchard Roza in a WA 38 on G41 (WA 38 Roza), planted in 2013 at 3 x 12 ft on a vertical spindle system.

1. *Evaluate and manage Ca related disorders utilizing PGRs*

Plant regulators were applied to 10 randomly selected replicate trees per site and cultivar according to Table 1. The treatments were applied to each canopy, utilizing a battery-powered backpack sprayer (controlled flow) to full coverage. Control trees were sprayed with the same water utilized in the solution.

Table 1. Plant regulator treatments.

Treatments	Concentration	Timing	Reference
ABA *(Protone ®, 20% Valent BioScience) Organic*	400 mg ai/L 20 g/10L	Honeycrisp: Every week, starting five weeks prior to harvest. (approx. 125 DAFB)	Falchi, et.al, 2017.
		WA 38: Every week, starting ten weeks prior to harvest. (approx. 160 DAFB)	
ProCa *(Apogee ®, 27.5%) Not Reg for 48 days before harvest*	300 mg ai/L 10.9 g/10L	Honeycrisp: Every week, starting five weeks prior to harvest. (approx. 125 DAFB)	Amarante et.al, 2020
		WA 38: Every week, starting ten weeks prior to harvest. (approx. 160 DAFB)	
GA_3* (*ProGibb®, Valent Bioscience) Not Reg*	300 mg a.i. /L 7.50 g/10L	Honeycrisp: Every week, starting five weeks prior to harvest. (approx. 125 DAFB)	Amarante et.al, 2020
		WA 38 : Every week, starting ten weeks prior to harvest. (approx. 160 DAFB)	
Control	Water	Same dates as above	-

*GA_3 was included years 2 and 3.

Evaluation

At harvest, each replicated unit (n=10) was individually assessed for total fruit per tree, total fruit weight, trunk diameter to calculate crop load as the total number of fruit per trunk cross sectional area (TCSA), and at harvest for calcium related disorders, which included bitter pit, blotch pit or green spot. In the laboratory, individual fruit were evaluated for weight, diameter and other defects; splits, cracks, sunburn, etc. For Honeycrisp, only healthy fruit were stored at 39 F, for six weeks. After storage, fruit were evaluated for Ca related disorders and total percent disorders was calculated. From each sample unit, a subsample were selected for dry matter and nutrient analysis: N, P, K, Ca, Mg, Cu, Mn, Fe, Zn, B.

1. *Analyze the effect of these products on reducing plant and fruit stresses.*

Treated trees were monitored throughout the growing season for fruit and shoot growth, leaf nutrient levels, stem water potential, stomatal conductance, and leaf temperature. Stem water potential was measured in five sun-exposed leaves per tree. Leaves were collected and stem water potential was determined immediately with a pressure chamber. Measurements were collected the day before the treatment application, then two and three days after the treatment application.

The experimental data were analyzed using analysis of variance (ANOVA) followed by a Tukey's multiple range test, for mean separation of significant treatment effects. All the analyses were done using R Studio and XLSTAT Inc software.

2. *Develop and distribute new strategies to manage Ca related disorder for excessively vigorous conditions.*

The project progress and results have been shared in growers' meetings, field days (English and Spanish), at the Heritage University Graduate Students, in the last three year's newsflash session at the WSTFA annual meeting, and at the newsflash session at the Columbia Basin Tree Fruit Club by Juan Munguia de la Cruz, WSU graduate student and research assistant.

RESULTS

1.1 Evaluate and manage Ca related disorders utilizing vigor controlling products.

Ca related disorder incidence was affected strongly by location/cultivar and year, thus the analysis to determine the influence of the PGR treatments were conducted independently for each location and year.

Honeycrisp Grandview

In the Honeycrisp Grandview site, Ca related disorders were high across all years ranging between 23 to 66% and associated mainly to bitter pit (BP) and to a lesser proportion to botch pit, both disorders being highest in 2022 ($p=0.010$, data not shown). Only in 2022 was BP affected by the treatments, when the ABA treatment had 39% higher BP incidence when compared with the lowest incidence in the ProCa treatment. However, there were no differences between any treatment and the control ($p=0.102$). In 2021, BP mean values in the ABA treatment were three times higher than the control but due to very high variability among trees (standard error = 16.5%), there were no statistical differences among treatments. To better understand this variability, we examined BP incidence within each main leader within a tree, and there were cases where BP incidence ranged from 0 to 75% among leaders. Crop load was similarly variable, averaging 4.6 fruit per cm^2 of trunk cross sectional area (TCSA), but varying between 1.1 and 14.8 fruit per cm^2 TCSA. The correlations between crop load and BP incidence were significant but weak ($p=0.007$; $r=-0.39$) (data not shown).

Among other fruit quality parameters, in 2021, 4.4% of the ABA treated fruit had cracks or splits, compared with no cracks in the control and ProCa (Table 2). In 2022, fruit cracks were higher overall ($p<0.01$), but with no treatment differences, and the same was observed in 2023 (Table 2). In 2022, firmness was 10% lower than in 2021 ($p<0.001$), and no treatment differences.

In 2021 and 2022, shoot growth was reduced by all treatments while there was no effect in 2023. In 2021, ABA and ProCa reduced shoot growth by 21% and 11%, respectively when compared with the control (Table 2). In 2022, with overall higher shoot growth, all treatments (including GA) reduced final shoot length by 17 – 21%. Despite these differences in shoot growth, there were no differences in fruit count, weight, and diameter among treatments for the three years of study, and there was no relationship between BP incidence and shoot growth. Note that the Grandview site has consistently higher vigor overall when compared with 13 'Honeycrisp' orchards we monitored in South Central WA. For 2021 and 2022, shoot lengths were 39% and 21% larger than the regional average (10 cm), respectively. And while the PGRs reduced shoot growth in 2021 and 2022, none of these reductions reduced BP incidence.

Regardless of the treatments, at Grandview, BP incidence was very high all three years of study. The estimated yields were 60, 35 and 50 bins per acre for 2021, 2022 and 2023 respectively, however with average 6 packs per bin. With overall cull associated to Ca disorders and rots. The low crop load (below 2 fruit per TCSA) and large fruit (> 80 mm) lead us to conclude that while PGRs were able to reduce vigor, this reduction was insufficient to make an impact on fruit Ca disorders.

Table 2. Plant regulator effect on shoot growth and fruit quality indicators between 2021 and 2023 at a Honeycrisp orchard near Grandview. Different letters with column and year indicate statistical differences at p value < 0.10 (Tukey text, XSLTAT, Andisoft)

Year	Treatment	Shoot growth (cm)	Fruit (n)	Fruit Firmness (Lb)	Fruit Weight (g)	Fruit Diameter (mm)	Ca Disorders (%)	Cracks (%)
2021	Control	18.1 a	48.5	21.9	231	82.2	22.5	0 b
	ProCa	16.4 b	51.0	22.0	229	82.1	40.3	0 b
	ABA	14.2 c	50.5	21.6	225	81.7	66.1	4.4 a
	p value	<0.0001	0.647	0.765	0.905	0.964	0.225	0.018
2022	Control	23 a	120.5	19.2	216	73.6	59 ab	6.9
	ProCa	19 b	99.7	19.8	217	73.1	49 b	12.3
	ABA	19 b	106.7	19.2	217	75.3	69 a	10.2
	GA+	18 b	110.6	20.0	209	73.6	54 ab	0.1
	p value	0.025	0.11	0.075	0.843	0.300	0.102	0.310
2023	Control	19.2	68.6		242	81.5	40.0	5.9
	ProCa	17.1	67.4		233	80.5	41.9	3.6
	ABA	16.4	75.8		221	79.1	51.2	1.3
	GA+	17.1	79.6		232	80.4	39.0	0.9
	p value	0.22	0.82		0.41	0.41	0.57	0.06

Honeycrisp Prosser

Ca related disorders in the Honeycrisp orchard near Prosser were associated mainly to BP, being highest in 2021 and progressively lower over the years (p<0.001). In 2021, BP ranged between 57% and 77%, being 35% higher in the control when compared with ABA, while ProCa had intermediate BP incidence (Table 3). In 2021, the control also had 10% greater shoot growth compared with ABA and softer fruit (approx. 1 lbs.) (Table 3). In contrast, in 2022 BP was much lower, ranging between 2.8% and 14%, and the ABA had five times more BP incidence when compared with GA, but with no differences with the control and the ProCa treatments (Table 3). Fruit firmness in 2022 was 16% lower than in 2021 (p < 0.001), and the ABA treatment had the firmest fruit (approx 1 lbs) when compared with all other treatments (Table 3). Regardless of the treatments, cracks or splits were 28-times higher in 2022 compared with 2021 and 2023 ($p < 0.001$).

In 2023, BP and cracks were insignificant (below 1%) with no differences among treatments. However, in 2023 crop load was very high ranging between 5.7 and 8.7 fruit per TCSA and between 197 and 288 fruit per tree, 27% higher than in 2022 ($p < 0.001$). Among the treatments, the control had 46% more fruit, leading also to the smallest fruit (111 g and 67 mm diameter) when compared with the GA, but not different from ProCa and ABA.

Considering all years, when correlating shoot length, an indicator of vigor, and BP incidence, there was a significant and strong correlation ($p<0.001$; $r = 0.722$, $R2 = 0.521$) (Figure 1). Regardless, shoot length in 2021 and 2022, respectively, were 25% and 54% larger than the regional average, which corroborates the highly vigorous conditions of the block. However, only the ABA treatment in 2021 reduced shoot length. This may explain the reduction in Ca related disorders; however this same effect was not seen in 2022 or 2023. Here, the decline of BP incidence over the years related directly with orchard maturity, increase fruit yield, higher crop load and reduced fruit size ($p<0.001$). Note that in 2023, BP was reduced to less than 1%, however fruit size and color were also reduced. Here, the orchardist incorporated summer pruning and vigor control measures since the beginning of the project. Thus, we hypothesize that the limiting factor in Honeycrisp Prosser, leading to BP incidence, was related to high vigor during the stage where growers push the trees to fill the space, where the ABA treatment was beneficial in reducing vigor and BP (2021). Once the orchard filled the space and vigor was controlled with alternative horticulture methods, the PGRs had no significant impact on BP. In addition, in 2023, when crop load was high, the GA application appears to have induced fruit drop, leading to larger fruit compared to the control and ProCa treatments.

Table 3. Plant regulator effect on shoot growth and fruit quality indicators between 2021 and 2023 at a Honeycrisp orchard near Prosser. Different letters with column and year indicate statistical differences at p value < 0.10 (Tukey text, XSLTAT, Andisoft)

Year	Treatment	Shoot growth (cm)	Fruit (n)	Fruit Firmness (Lb)	Fruit Weight (g)	Fruit Diameter (mm)	Ca Disorders (%)	Cracks (%)
2021	Control	30.3 a	49.5	19.1 b	316	89.0	77 a	0.0
	ProCa	27.9 ab	52.8	20.3 a	310	86.9	68 ab	0.0
	ABA	27.5 b	51.5	20.5 a	324	89.0	57 b	1.4
	p value	0.044	0.467	0.006	0.587	0.189	0.045	0.133
2022	Control	27.2	181	16.5 b	204	77.8	6.3 ab	8.7
	ProCa	24.4	173	16.6 b	203	77.4	9.5 ab	12.7
	ABA	24.9	181	17.6 a	201	76.9	14.0 a	18.1
	GA+	24.2	206	16.7 b	192	75.3	2.8 b	17.1
	p value	0.208	0.466	0.001	0.648	0.478	0.047	0.149
2023	Control	21.0	288 a		111 b	67 b	0.1	0.04
	ProCa	22.0	235 ab		120 b	68 b	0.3	0.16
	ABA	22.6	220 ab		142 ab	70 ab	0.3	0.07
	GA+	22.1	197 b		167 a	73 a	0.3	0.05
	p value	0.198	0.033		0.031	0.031	0.670	0.465

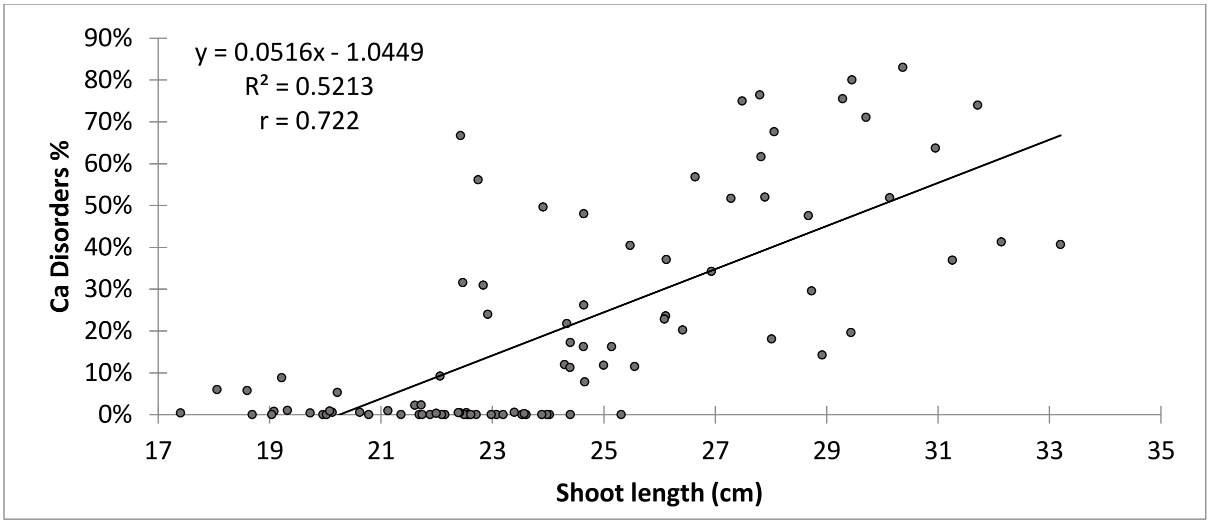

Figure 1. Correlation between incidence of Ca related disorders and shoot length at the Honeycrisp Prosser site, considering data from 2021 to 2023.

WA 38 Prosser

At the Prosser WA 38 orchard, Ca related disorders were associated with green spot and varied between 1.3 and 6.4%, being higher in 2021 and 2022 compared to 2023 ($p < 0.001$). Fruit number per tree increased over the years as the trees filled the space, reaching 66.8 fruit per tree in 2023, 47% higher than in 2021.

The PGR treatments had no effect on fruit number, firmness, weight, diameter, Ca disorders or cracking incidence in 2021 and 2023 (Table 4).

Table 4. Plant regulator effect on shoot growth and fruit quality indicators between 2021 and 2023 at a WA 38 orchard near Prosser. Different letters with column and year indicate statistical differences at p value < 0.10 (Tukey text, XSLTAT, Andisoft)

Year	Treatment	Shoot growth (cm)	Fruit (n)	Fruit Firmness (Lb)	Fruit Weight (g)	Fruit Diameter (mm)	Ca Disorders (%)	Cracks (%)
2021	Control		45	20.5	275	84.5	5.95	0.39
	ProCa		44	20.5	279	85.4	5.45	0.12
	ABA		48	20.6	280	85.1	7.71	0.68
	p value	-	0.818	0.935	0.391	0.135	0.455	0.616
2022	Control	37.4 a	48	20.9 b	292	79.7	4.7 ab	19.2 b
	ProCa	30.1 b	59	21.1 b	274	79.1	2.9 b	33.3 a
	ABA	31.7 b	56	22.4 a	274	79.0	7.1 ab	23.7 ab
	GA+	38.8 a	54	21.2 b	273	78.5	9.9 a	19.0 b
	p value	<0.0001	0.335	0.003	0.383	0.808	0.088	0.018
2023	Control		67	20.5	263	81.2	2.0	0.0
	ProCa		78	19.9	265	81.5	0.7	0.0
	ABA		57	20.1	253	80.2	1.5	0.0
	GA+		66	19.9	261	80.6	1.2	0.0
	p value	-	0.201	0.238	0.849	0.853	0.326	-

In 2022, green spot incidence varied between 2.9% and 9.9%, while cracking varied between 19% to 33%, being a greater issue for WA 38 growers across the state. In 2022, ProCa and ABA reduced shoot growth by 19.5% and 15.2% respectively when compared with the control and GA, which were similar (Table 4). In the GA treatment, green spot incidence was 3.4 times higher compared with the ProCa that had the lowest green spot incidence (2.9%). While it seems to be a relation between shoot growth and green spot, given the differences in GA and ProCa treatments ($r = 0.53$, $p = 0.015$, data not shown), the ABA had equivalent green spot incidence of that obtained in the control and GA, regardless of having lower shoot growth. Unfortunately, in 2021 and 2023 season, shoots were summer pruned before our measurements were collected thus, we were not able to determine impact on shoot growth for both years. Vegetative growth suppression by PGRs typically lasts or 2 to 5 weeks per application during the current growing season (P. Francescatto, Valent). Similar to Honeycrisp, in 2022, the ABA application seemed to have promoted Ca related disorders.

In 2022, when fruit cracks were significantly higher compared to 2021 and 2023, ProCa had 73% more cracking when compared with the control and GA treatments. ProCa has been previously associated with an increase in fruit cracking on apple varieties known to be prone to cracking. Fruit firmness, was higher in 2022, opposite to the Honeycrisp sites where 2022 led to softer fruit, being approximately 1 lb higher in the ABA treatment, compared to all other treatments (Table 4).

WA 38 Roza

The WA 38 Roza orchard had no crop load or fruit count differences across the three years of study, which reflect its mature nature. In 2021, green spot incidence ranged between 13.4% and 32%, 3.8 and 5.5 times higher than in 2022 and 2023, respectively. In 2021, ProCa had the highest green spot incidence compared to both the control and ABA (Table 5). Only the ABA treatment in 2021 induced reduced shoot growth compared with the control and other treatments, but this shoot growth reduction did not affect any other fruit quality indicators (Table 5).

Table 5. Plant regulator effect on shoot growth and fruit quality indicators between 2021 and 2023 at a WA 38 orchard at WSU Roza farm. Different letters with column and year indicate statistical differences at p value < 0.10 (Tukey text, XSLTAT, Andisoft)

Year	Treatment	Shoot growth (cm)	Fruit (n)	Fruit Firmness (Lb)	Fruit Weight (g)	Fruit Diameter (mm)	Ca Disorders (%)	Cracks (%)
2021	Control	26.9 a	81	19.4	281	85.0	13.4 b	5.9 a
	ProCa	25.2 a	51	19.2	274	84.4	32.0 a	2.6 b
	ABA	23.1 b	60	19.2	278	84.2	20.1 b	5.6 ab
	p value	0.000	0.218	0.639	0.329	0.323	0.004	0.062
2022	Control	33.2	76	19.5	230	77.6	6.1	37 a
	ProCa	32.6	62	19.8	243	76.8	9.0	27 b
	ABA	31.1	81	19.4	218	75.0	4.8	26 b
	GA+	34.2	85	19.2	231	77.8	3.2	20 b
	p value	0.570	0.110	0.200	0.345	0.349	0.185	0.000
2023	Control	21.7	88	20.1	272	80.9	2.2	1.7
	ProCa	19.8	80	20.7	255	80.9	3.3	1.0
	ABA	20.6	71	20.4	267	81.8	5.3	1.3
	GA+	19.3	62	20.4	271	83.1	5.7	3.0
	p value	0.202	0.279	0.270	0.539	0.305	0.254	0.530

Here the PGRs had no impact on fruit number, firmness, weight, or diameter (Table 5). Note that fruit weight ranged between 230 and 277 g, with smaller fruit (weight and diameter) in 2022. Fruit firmness was higher in 2023 (20.4 lbs) with no differences between 2021 and 2022.

Similar to the other sites, overall fruit cracks were 6 and 16 times higher in 2022 when compared with 2021 and 2023, respectively. Regardless, in 2021 and 2022 the highest crack incidence was found in the control treatment, compared with ProCa in 2021, and with all PGR treatments in 2022 (Table 5).

Shoot growth in 'WA 38' sites in 2021 (higher green spot incidence) were 58 and 70% higher in the Prosser and Roza sites, respectively, when compared with fifteen other 'WA 38' orchards being monitored in South-Central WA. In both sites, shoot growth length has progressively been reduced while fruit load increased, while we observe a dramatic reduction in green spot incidence. The higher green spot incidence in WA 38 Roza with the ProCa treatment is still unknown.

Nutrient levels
While there were fruit and leaf nutrient concentration differences among treatments, sites and years, we focused our discussion on differences that translated in fruit quality, and in relation to the PGR treatments.

Grandview Honeycrisp orchard had the highest BP incidence in 2022, and also higher BP in the ABA compared to the ProCa. In 2022, N, P, K, Ca, Fe, Cu and B levels varied among treatments, however these differences were not associated to fruit quality indicators (Table 6). For example, ABA had lower N concentration compared with GA and the control, while no difference with ProCa, however fruit quality differences were only found between ABA and ProCa. ABA also had lower P and K levels compared to all other treatments, and lower fruit Ca compared with the control, however all levels were above the recommended values (4.0% – 5.5%) (Table 6). In the Honeycrisp Prosser site, when in 2022 the ABA-treated fruit also had higher BP, however compared to the GA. Yet, analysis of fruit nutrient content showed that Ca concentration was actually twice as high in the ABA treatment compared to GA-treated fruit (opposite to what might be expected).

The GA treatment increased the K:Ca and Mg+K:Ca ratios significantly (> 24), compared to all other treatments (< 16.7), in both Grandview and Prosser site (Table 6), however this difference had no consistent influence in fruit quality. When comparing fruit nutrient levels between the two Honeycrisp sites, all fruit nutrients, except Zn, were significantly higher in the Grandview orchard ($p < 0.05$).

Leaf nutrient levels in Honeycrisp Grandview were not different in 2021 and 2023, despite the differences in 2021 shoot growth and cracking incidence. While in 2022, the ProCa application had 13% more leaf Ca compared with ABA, both having the lowest and highest BP incidence. ProCa also had 16% more Zn compared with all other treatments. In Grandview, leaf N and Mg levels were above the recommended range in all three years of the study (data not shown).

In the Prosser site, leaf Ca levels in the ABA treatments were 14% higher than in the control and ProCa treatments in 2021, and 14% higher than the GA in 2022. Note that in 2021 BP incidence was much higher than in 2022, being lowest in the ABA, and the opposite in 2022 when compared with GA. Thus, leaf Ca differences don't have a consistent relation with the treatments nor the BP incidence. In 2022, B levels were also significantly higher in the control and ABA, compared with the GA treatment. In 2023 (lowest BP incidence), nutrient levels were unrelated to fruit quality differences. When comparing across years, given the differences in BP incidence (highest in 2021), only Ca, Mn and B levels were significantly higher in 2021, compared to 2022 and 2023. More so, Ca levels in 2021 were 2.7% much higher than the recommended level of 2.0%.

Table 6. Fruit nutrient analysis in 2022 Honeycrisp sites. Different letters with a column indicate statistical differences within site.

Site	Treatment	% Dry	N	P	K	Ca	Mg	S	Zn	Fe	Mn	Cu	B	N/Ca	K/Ca	Mg+K / Ca
			mg*100 g^{-1}						mg/kg = ppm							
Grandview	Control	13%	52.5 a	9.9 a	115.0 a	9.3 a	4.13	3.88	0.49	10.3 a	0.30	4.9 a	2.0 a	5.8 b	13 b	13 b
	ProCa	13%	49.5 ab	10.1 a	112.6 a	9.2 ab	4.15	3.65	0.49	9.0 ab	0.27	4.4 a	2.1 a	5.5 b	13 b	13b
	ABA	13%	41.9 b	8.3 b	97.1 b	7.0 bc	4.29	3.65	0.44	6.5 ab	0.25	4.0 ab	1.2 b	7.2 ab	16 b	17 b
	GA	13%	50.0 a	10.7 a	121.4 a	5.6 c	4.09	3.70	0.55	5.2 b	0.27	3.2 b	1.2 b	9.9 a	24 a	25 a
p value		ns	0.020	0.001	0.000	0.001	ns	ns	ns	0.023	ns	0.010	0.002	0.010	0.001	0.001
Prosser	Control	12%	42.9 a	7.86	79.8	7.7 a	4.0 a	3.39	0.48 b	5.68	0.22	2.6 b	1.5 a	6.0 b	11 b	12 b
	ProCa	12%	32.8 b	7.03	79.9	5.9 a	3.6 ab	2.86	0.55 ab	4.68	0.18	3.3 a	1.0 b	6.6 b	16 b	17 b
	ABA	12%	33.7 b	7.42	77.2	6.5 a	3.5 ab	3.15	0.53 ab	5.03	0.22	2.6 b	1.2 ab	6.2 b	14 b	14 b
	GA	12%	37.1 ab	7.90	74.6	3.2 b	3.0 b	2.88	0.69 a	5.66	0.21	2.8 ab	0.5 c	12.5 a	25 a	26 a
p value		ns	0.001	ns	ns	<0.001	0.020	ns	0.031	ns	ns	0.063	<0.001	0.00	0.00	<0.001
Grandview		13%a	48.5 a	9.8 a	111.5 a	7.8 a	4.2 a	3.7 a	0.49 b	7.76 a	0.27 a	4.15 a	1.61 a	7.1	16	17
Prosser		12%b	36.6 b	7.6 b	77.9 b	5.8 b	3.5 b	3.1 b	0.56 a	5.26 b	0.21 b	2.81 b	1.03 b	7.8	16	17
p value		0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.024	0.030	0.001	0.001	<0.001	<0.001	ns	ns	ns

ns; no significant difference at p > 0.05

Table 7. Fruit nutrient analysis in 2022 WA 38 Prosser. Different letters with a column indicate statistical difference

Treatment	% Dry	N	P	K	Ca	Mg	S	Zn	Fe	Mn	Cu	B	N/Ca	K/Ca	Mg+K/Ca
		mg*100 g^{-1}						mg/kg = ppm							
Control	0.14 b	34.1 c	10.8 c	123.0 b	7.6 b	5.8 b	3.5	0.26	5.8 b	0.09 b	2.54 c	1.65 b	4.5 b	16.3 a	17.0 a
ProCa	0.14 b	41.4 b	12.2 b	142.2 a	11.5 a	7.0 a	4.0	0.30	66.8 a	0.42 a	3.68 a	2.39 a	3.7 c	12.7 b	13.4 c
ABA	0.16 a	50.7 a	13.3 a	151.7 a	10.6 a	6.3 b	4.2	0.33	9.6 b	0.13 b	3.29 ab	1.68 b	4.8 ab	14.4 ab	15.0 bc
GA	0.15 b	43.6 b	12.3 ab	122.7 b	8.1 b	5.9 b	4.0	0.26	16.8 b	0.23 b	2.84 bc	1.87 b	5.4 a	15.3 a	16.0 ab
p value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.001	ns	ns	0.001	0.001	<0.0001	0.004	<0.0001	0.001	0.001

ns; no significant difference at p > 0.05

In 2021 fruit nutrient levels of WA 38 from the Prosser block, were not statistically different among treatments. In contrast, in 2022, the GA treatment had lower fruit K, Ca, Mg, Fe, Mn, Cu and B, and higher N:Ca, K:Ca and Mg+K:Ca ratios (data not shown). Similar to fruit nutrient levels, leaf nutrient in WA 38 Prosser, were not different among treatments in 2021. In 2022, when green spot incidence was higher in the ProCa compared with GA, leaf Zn concentration was higher in the GA treatment, while B was lowest. In 2023, Zn and Cu were also higher in the GA, but only compared with the ABA. However, overall levels were within adequate range.

In WA 38 Roza, fruit N, Ca, Mg, Zn, Fe, Mn, B were more than doubled in 2021 (with higher green spot incidence) compared to 2022 and 2023 (Table 7). In 2022 the GA treatment had the highest N, Ca and Mg concentration. In contrast, in 2023 GA had the lowest N, Ca and Mg concentration, and none of these differences related to fruit quality. Regarding leaf nutrient differences across years, 2021 had lower N, but higher P, Ca, S, Zn, Fe and Mn when compared with 2022 and 2023.

When comparing across sites, there was a weak correlation between fruit and leaf nutrient concentration. The strongest correlation was leaf and fruit Ca, with significant ($p<0.001$) positive relation ($r = 0.679$), however the predictability was low $R2 = 0.461$.

2. *Analyze the effect of these products on reducing plant and fruit stresses.*

In 2022, we measured stem water potential in WA 38 Prosser prior to several ABA treatments and again, 48 and 72 hours after those treatments. The ABA treatment apparently reduced stress in the plant because on several sample dates stem water potential was significantly lower in untreated trees (Figure 2). This effect was particularly evident following treatments on 8 August and 23 August where stem water potential was low in untreated trees (ca. 20 bars, equivalent to -2.0 MPa) but unaltered (or slightly improved) in trees treated with ABA. While this didn’t impact green spot or cracking incidence (not different from the control), the ABA had firmer fruit compared to all other treatments, which could be associated to higher dry matter content.

This effect may be due to the role ABA plays in stomatal closure. Overall, it is apparent that weekly treatment with ABA had a stabilizing effect on tree water status though the benefits of this were not manifest as any reduction in green spot incidence.

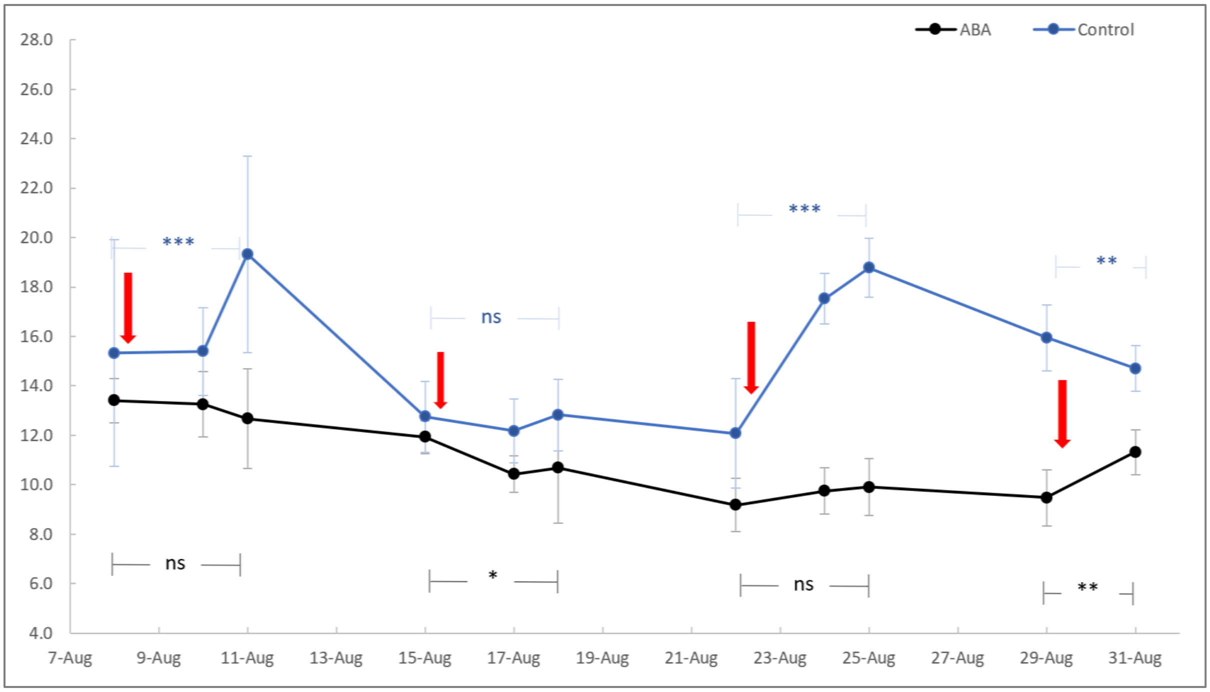

Figure 2. Trends in stem water potential for WA38 trees treated with ABA and untreated. Each arrow indicates the timing of ABA applications. Data are presented in bars (higher readings indicative of greater stress).

In 2021, we observed overall higher sunburn incidence, given the cool spring and hot summer. In WA 38 Roza, where there are no heat mitigation strategies, the ABA led to 48% more sunburn incidence compared to ProCa and the control (data not shown). The higher sunburn incidence could be associated to two independent factors, 1. reduced shoot growth in the ABA treatment reduced the natural protection of the exposed fruit, and or 2. ABA prevented the natural temperature regulation by reducing stomatal conductance during the hot days in the summer, leading to higher fruit temperature.

3. *Develop and distribute new strategies to manage Ca related disorder for excessively vigorous conditions.*

The project progress and results have been shared in 6 growers' meetings by Sallato, and in 6 field days (English and Spanish), at the Heritage University Graduate Students (awarded first place), in the last three year's newsflash session at the WSTFA annual meeting, and at the newsflash session at the Columbia Basin Tree Fruit Club by Juan Munguia de la Cruz, WSU graduate student and research assistant. We will continue sharing the results of this project throughout 2024.

Executive Summary

Project Title: "Managing Ca-related disorders in high vigor conditions"

Keywords: *Bitter pit, green spot, PGRs, crop load*

Calcium-related disorders, such as bitter pit, blotch pit, lenticel breakdown, and others, remain some of the most challenging issues to combat. More recent findings demonstrated that limited xylem functionality in certain cultivars is one of the main causes for cellular Ca deficiency, reducing the translocation of Ca for cell wall. Phloem-mobile nutrients (e.g., nitrogen (N) and potassium (K)) can continue to flow to the fruit, leading to nutrient imbalance in the fruit, which is more pronounced in vigorous conditions. Plant growth regulators have been effective for reducing bitter pit in apples. DeFreitas, (2011) and Falchi et al. (2017) showed reduced Ca related disorders in apples treated pre-harvest with the plant growth regulator abscisic acid (ABA) and increased fruit Ca levels. In 2020, Amarante et al. reported reduced bitter pit incidence in Braeburn apples treated pre-harvest with Prohexadione Ca (Apogee). We evaluated the effect of pre-harvest application of ABA, Prohexadione Ca and gibberellic acid (GA) on Ca related disorders in Honeycrisp and WA 38 orchards between 2021 and 2023 growing season.

The PGR treatments had inconsistent effect on shoot growth and fruit disorders across site, cultivars, and years. The pre harvest application of ABA reduced shoot growth in half of the sites and years when compared with the control, however with no significant impact on fruit quality. In relation to the untreated control, the application of ABA pre harvest reduced bitter pit incidence by 35% only in one site in 2021. In 2022, where fruit cracking in WA 38 was a much greater issue compared to green spot incidence, in the Roza site, all treatment had lower cracking compared to the control, while in Prosser site, the control and GA had the lowest cracking incidence. In Honeycrisp the application of Prohexadione Ca (Apogee®) pre harvest did not affect bitter incidence. In WA 38, the application of Prohexadione Ca (Apogee®) pre harvest increased green spot incidence in the Roza site and only in 2021, while reducing cracking in 2021 and 2022. However, in Prosser site, ProCa had 73% higher cracking incidence compared to the control. The cause leading to higher Ca related disorders remained associated to high vigor, low crop load and big fruit size. While PGRs were able to reduce shoot growth, this reduction was insufficient to make an impact on fruit Ca disorders when vigor is too high and crop load are the most limiting factors. In one case of high vigor, ABA treatment was beneficial in reducing shoot growth and BP (2021). However, once the orchard filled the space, horticultural practices had a greater impact in reducing Ca related disorders, where PGRs become of no benefit. Fruit nutrient levels appear to reflect vigor differences across orchard and years, yet unrelated to fruit Ca related disorders. In several cases, fruit Ca concentration, and other nutrients were actually higher in the treatments, years or site that also had higher BP or green spot incidence. Leaf nutrient levels appear to reflect excessive vigor when above the recommended range (example Grandview site), while there was no direct relation with either fruit nutrient concentration or fruit quality.

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Project/Proposal Title: N, Mg, and K guidelines to control disorders for Honeycrisp and WA 38

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Cooperators: Many Washington State Honeycrisp and WA 38 producers

Report Type: Final Report

Project Duration: 3-Year

Total Project Request for Year 1 Funding: $ 81,270
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WTFRC Collaborative Costs: none

Budget 1
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Item	2020	2021	2022
Salaries	43,200[1]	44,928	46,726
Benefits	15,895[2]	16,530	17,192
Wages	7,800[3]	8,112	8,436
Benefits	1,735[4]	1,805	1,877
Equipment			
Supplies	6,900[5]	6,900	6,900
Travel	3,240	3,240	3,240
Miscellaneous			
Plot Fees	2,500	2,500	2,500
Total	$81,270	$84,015	$86,871

Footnotes:

OBJECTIVES

This project had three objectives aimed at improving fertilizer management and establishing thresholds on fertilizer applications for Honeycrisp and WA 38.

1. Test how varying rates of N, K, and Mg affects fruit quality traits, disorder incidence, return bloom and tree vigor in Honeycrisp and WA 38 orchards.
2. Identify the relation between shoot growth, crop load, and nutrient concentration with disorder incidence for commercial orchards in WA State.
3. Develop clear thresholds for N, K, and Mg fertilization based on fruit and leaf elemental concentrations for Honeycrisp and WA 38 orchards in WA State.

SIGNIFICANT FINDINGS

1. Crop load continues to be one of the main contributing factors for the development of both bitter pit and green spot. Nutrient analysis on fruitlets and fruit need crop load data to accompany them or they are impossible to estimate risk.
2. For commercial sampling, green spot in WA 38 demonstrated the same risk indicators (high vigor, low crop load, and high K: Ca ratios) as bitter pit in Honeycrisp.
3. Green spot decreased in incidence from 2020 to 2021 going from almost 12% in 2020 to 3.7% in 2021 and then 4.2% in 2022. Further evaluations in 2023 indicated minimal green spot again across most orchards.
4. N applications increased tree vigor and green spot incidence in ‘WA 38’ apple and increase bitter pit in Honeycrisp apple.
5. Rootstock can also contribute to green spot and bitter pit incidence through its effect on vigor and fruit set each year.

METHODS

1. **Test how varying rates of N, K, and Mg affects fruit quality traits, disorder incidence, return bloom and tree vigor in Honeycrisp and WA 38 orchards.**

The first objective is being conducted at Sunrise Research Orchard. In response to reviewer comments, in 2020, treatments were applied every two weeks over three applications in liquid form in May and June. For both cultivars, a second experiment was used to measure seasonal response of N, Mg, and K rates on growth, physiology, and fruit quality of both Honeycrisp and WA 38 trees. These experiments were conducted on untreated trees each year to determine seasonal responses of post-bloom applications of each of N, Mg, and K to WA 38 and Honeycrisp. For Honeycrisp, crop load was carefully regulated using the combination of bloom and fruitlet thinning strategies and hand clean-up to target crop loads by June 1. WA 38 was not thinned. Shoot growth was measured at harvest.

Table 1. Rates for nitrogen, potassium, and magnesium at low, medium, and high applications rates for controlled experiments.

lbs/acre applied	Nitrogen (N)	Potassium (K)	Magnesium (Mg)
Low	12	50	25
Medium	25	100	50
High	50	200	100

Fruit quality

At harvest (early September for Honeycrisp and early October for WA 38), all fruit was completely removed from each sample tree (two trees per replicate) and weighed to provide total yield. Then, 48 fruit was randomly selected from each tree. 16 fruit were used for fruit quality at harvest and the other fruit was stored in regular atmosphere for three months at 1° C and used for disorder evaluation after storage. Elemental analysis was performed using a pooled sample consisting of a peel sample collected from the calyx end of eight fruit from each replicate. Samples were dried, ground, and acid digested then analyzed using an Agilent 4200 MP-AES elemental analyzer. N was analyzed separately with a elemental analyzer. Then, after 3 months of storage, bitter pit and green spot incidence and severity along with fruit firmness will be assessed again for fruit from each replicate.

RESULTS AND DISCUSSION

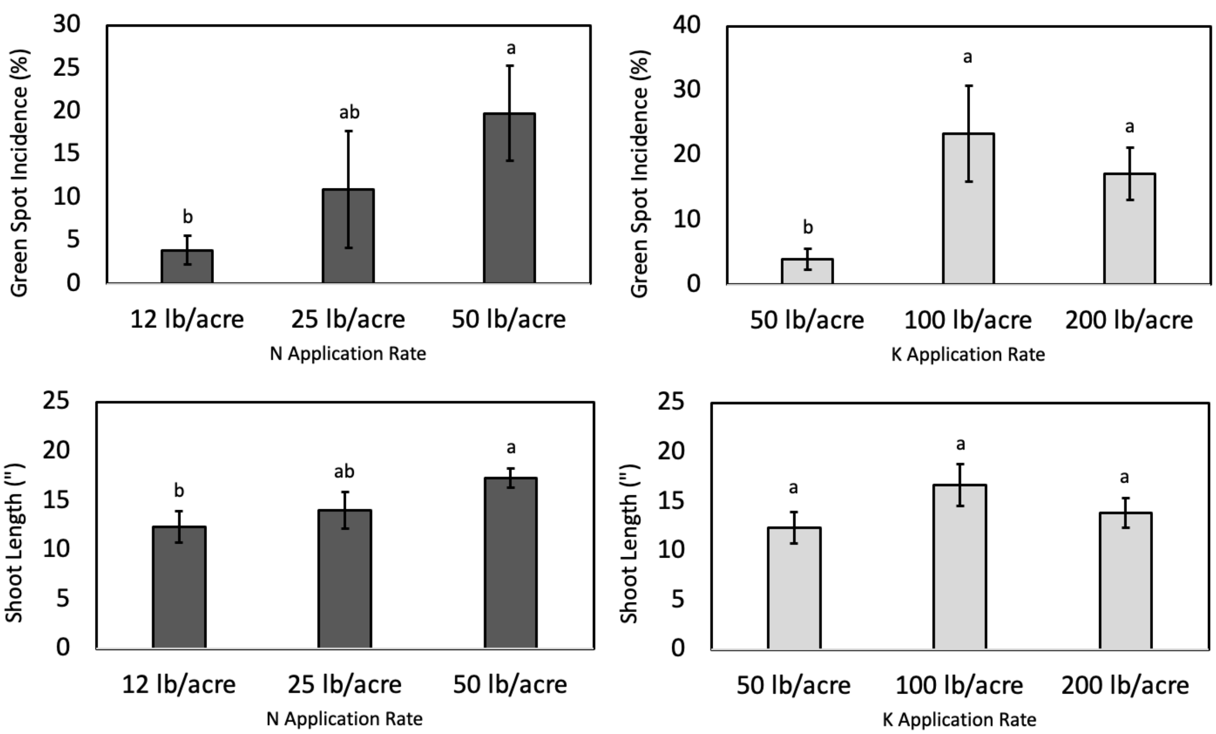

Figure 1. Green spot incidence (top) and mean shoot length (bottom) for WA 38 apple after three months of storage in regular atmosphere when treated with different soil-based application rates of N (left) and K (right). Error bars indicate standard error (N=3) and letters denote differences among treatment means.

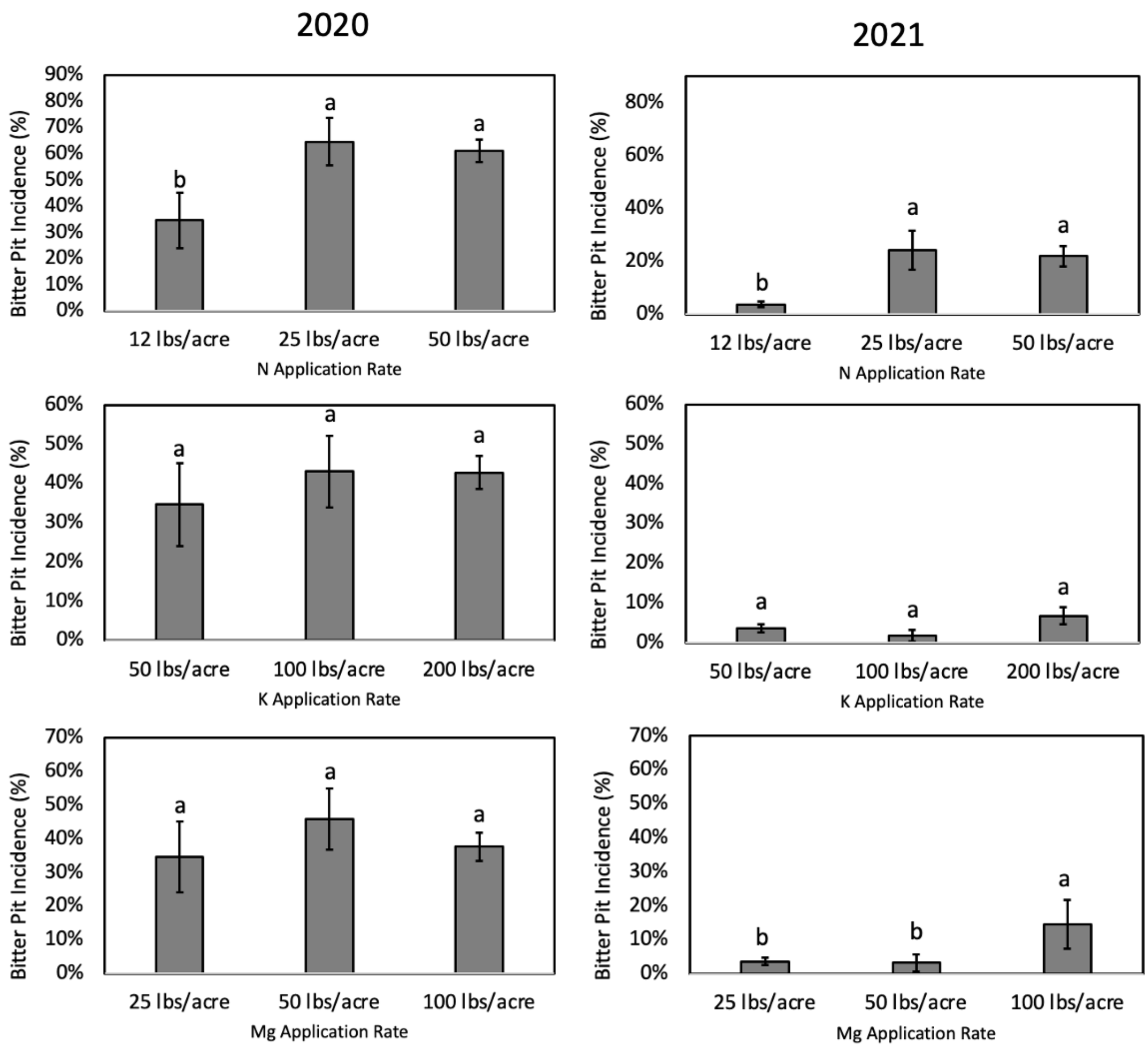

Figure 2. Bitter pit incidence in Honeycrisp apple in 2020 (left) and 2021 (right) after three months of storage in regular atmosphere when treated with different soil-based application rates of N (top), K (middle) and Mg (bottom). Error bars indicate standard error (N=3) and letters denote differences among treatment means.

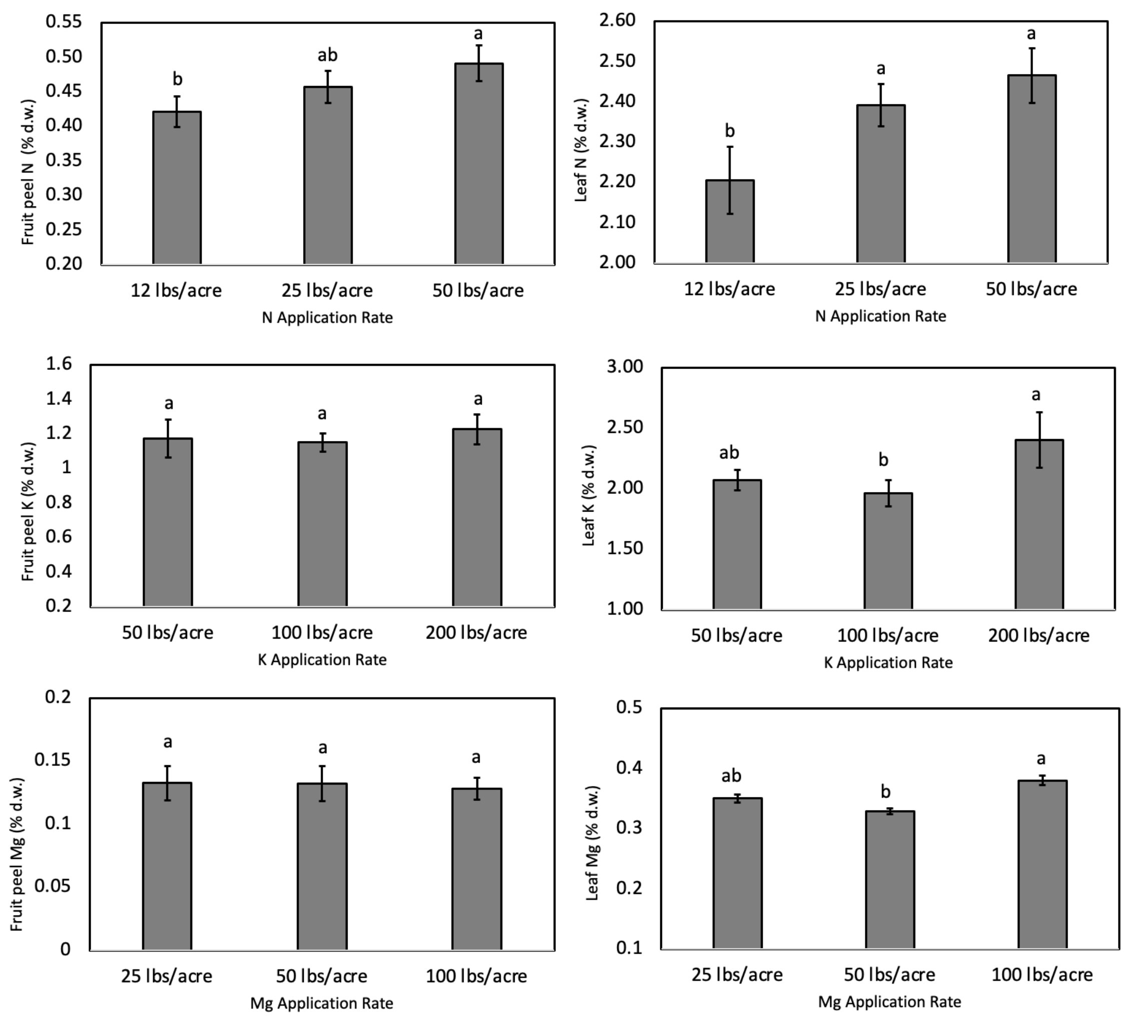

Figure 3. Fruit peel (right) and leaf (left) N (top), K (middle), and Mg (bottom) concentrations for Honeycrisp in 2020 after being treated with different rates of early-season N, K, and Mg soil applications. Error bars indicate standard error (N=3). Different letters denote significant differences among means.

2. Identify the relationship between shoot growth, crop load, and nutrient concentration with disorder incidence at harvest and after storage for commercial orchards.

Experiments conducted in objective 1 were valuable for determining thresholds and impacts of fertilization on fruit and tree physiology along with disorder incidence. However, commercial orchards span a larger range of environments, soil types, ages, training system, management strategies, and rootstocks that underscore the importance of including a thorough sampling approach to capture the range in factors that affect disorder incidence for both Honeycrisp and WA 38.

In 2020, there were a total of 42 orchards sampled for Honeycrisp and WA 38 in total. In 2021, there were 56 orchards sampled. Management information will also be collected that will include soil type, physical and chemical conditions, location and management practices to better help understand key factors on the disorder development.

In all sampled commercial orchards, three representative trees were chosen and diameter measured. Fruitlet and leaf samples were collected at this time for nutrient analysis. We added a component using fruitlet sap analysis in collaboration with Dr. Lailiang Cheng from Cornell University and are working to compile the results from that study. Fruit was harvested within three days of commercial harvest for all sites. At harvest, fruit counts were determined for selected trees and a subsample of 32-48 fruit per tree was collected. Half were placed in cold storage for three months and fruit quality will be measured using the parameters described in objective 1. Shoot growth will also be measured on 20 terminal shoots per tree. Fruit peel elemental analysis was performed as described in objective 1 including N, Ca, Mg, and K concentrations along with $\delta^{13}C$ analysis as an indicator of irrigation management relative to soil type. Elemental analysis was completed for 2020 and is in the process of being completed for 2021. Data for elemental composition will be presented for 2020 and disorder incidence, yield, etc. will be presented for both year.

3. **Develop clear thresholds for N, K, and Mg fertilization based on fruit and leaf elemental concentrations for Honeycrisp and WA 38 orchards in WA State.**

This work started in 2021 and will continue after the end of the project as regular Extension material. This will include Extension deliverables prepared by both Lee Kalcsits and Bernardita Sallato. Peer-reviewed publications are being written in 2024 that will be communicated and summarized for the industry like this existing report.

RESULTS AND DISCUSSION

For all WA 38 orchards that were sampled in all three years, 88%, 82%, and 82% of target crop load was achieved in 2020, 2021, and 2022, respectively. These targets were based on 5 fruit cm^{-2} TCSA in 2020 and 6 fruit cm^{-2} TCSA in 2021 and 2022. For Honeycrisp, crop load targets were 5 fruit cm^{-2} TCSA for all three years. Honeycrisp orchards achieved a higher % target in 2020 at 95% then fell lower again at 82% and 81% of target crop load for 2021 and 2022, respectively. Lower harvested yields compared to optimum is indicative of the hot period and wind events that led to fruit loss in 2021 and then the cold spring in 2022 that may have caused freezing damage and/or poor pollination. For Honeycrisp, orchards that had higher than optimum crop loads in 2020 averaged 7.6 fruit cm^{-2} TCSA and as a result, yields averaged 40% lower the following year in 2021. These same orchards had only 13.5% bitter pit in 2020 compared to an increase to 33.3% in 2021. Off years drive bitter pit risk. Orchards that were able to maintain crop load within 1 fruit cm^{-2} TCSA target in 2020, were able to maintain consistent crop in 2021 averaging 5.24 fruit cm^{-2} TCSA and then 4.56 fruit cm^{-2} TCSA in 2022. Bitter pit in those orchards with an optimum crop load averaged 5.4% and 17.8% in 2020 and 2021, respectively.

Bitter pit incidence was not different between the two years reported here (2020 and 2021) and averaged approximately 15% across both years. Fruit weight was significantly higher for WA 38 than Honeycrisp most likely because they were younger trees than Honeycrisp. There were significant differences in fruit peel nutrient concentrations for WA 38 and Honeycrisp. Fruit potassium concentrations were higher for Honeycrisp (Tables 4). Magnesium concentrations were not different between the two cultivars. Calcium and nitrogen concentrations were higher for WA 38 than

Honeycrisp. When a statistical clustering approach was used to cluster outcomes for groups of orchards into five different categories, there were significant differences in bitter pit and green spot among the clusters (Tables 6 and 7). Orchard years that clustered low for bitter pit in Honeycrisp had low vigor and optimum crop load. Although vigor was higher for WA 38 in general, vigor didn't cluster with green spot for commercial orchards. However, crop load clustered closely to green spot where orchard years with low crop load had clear elevated incidence of green spot. Rapid fruit growth associated with high carbohydrate loading during fruit expansion may be responsible for cracks and green spot developing on the peel of WA 38.

There have been significant discussions about the use of fruitlet and leaf testing for predicting bitter pit at harvest. Our results show that there are significant relationships between fruitlet and leaf concentrations in June compared to fruit peel concentrations at harvest. However, fruitlet (K+Mg)/Ca ratios were related to bitter pit incidence in Honeycrisp. The variability around these ratios limits the predictive power. There are many factors that happen following June sampling that can affect final bitter pit risk. Examples of this include rapid fruit growth, post sampling thinning, summer pruning, irrigation management. All of these can change the nutrient ratios and limit the usefulness of those June fruitlet samples. These samples might indicate if there are some potential problems emerging, but crop load and vigor assessments will probably catch the same issues without needing the nutrient analysis unless the grower is trying a new fertility program or product. Fruitlet and leaf N and K concentrations clustered with bitter pit and green spot risk in Honeycrisp and WA 38 (Tables 8 and 9). Both nutrients are associated with rapid fruit growth and larger fruit sizes. These appear to be targets for early season monitoring and have the potential to be remobilized and accumulate later in the season in developing fruit. N and K also were the most closely correlated with final fruit nutrient concentrations (Figures 4 and 5). We also had the opportunity to test the peel sap method with traditional fruitlet sampling. Ratios in sap were significantly positively related to bulk nutrient ratios in the fruitlets. (K+Mg)/Ca ratios for both methods were significantly correlated with bitter pit risk for Honeycrisp for commercial orchards. However the predictive power of these ratios in fruitlets was relatively low compared to near harvest. Fruit peel N/Ca ratios remain a good indicator of green spot and bitter pit. These results were supported by findings from our controlled experiments presented in 2021 where elevated N and K applications contributed to elevated green spot risk in WA 38.

Table 2. Commercial orchard sampling for WA 38 and Honeycrisp used for objective 2

	'WA 38'	**'Honeycrisp'**
2020	23	28
2021	19	22
2022	17	22
Total 'Orchard Years'	**59**	**72**

Table 3. Descriptive statistics and range in agronomic variables among commercial orchards for 'Honeycrisp'.

	Bitter pit (%)	Shoot length (inches)	Crop load (fruit cm^{-2} TCSA)	Fruit weight (g)
		2020		
Average	16.6	6.5	5.4	231
Minimum	0	1.0	1.1	156
Maximum	94.6	13.3	14.2	325
		2021		
Average	14.4	4.5	4.0	214
Minimum	0	1.8	0.95	111
Maximum	71.9	7.6	9.5	317
		2022		
Average	*	6.6	4.2	253
Minimum	*	3.8	0.85	205
Maximum	*	18.1	11.9	275

Table 4. Descriptive statistics and range in agronomic variables among commercial orchards for 'WA 38'.

	Green spot (%)	Shoot length (inches)	Crop load (fruit cm^{-2} TCSA)	Fruit weight (g)
		2020		
Average	13.47	7.9	4.4	286
Minimum	0	3.0	0.8	186
Maximum	72.2	14.4	11.4	385
		2021		
Average	3.91	8.5	5.3	272
Minimum	0	2.7	1.1	184
Maximum	18.75	13.6	10.9	327
		2022		
Average	4.1	8.0	5.5	277
Minimum	0	5.0	1.8	225
Maximum	19.1	12.6	12.1	306

Table 5. Descriptive statistics and ranges in fruit peel nutrient concentrations among commercial orchards for ‘WA 38’ and ‘Honeycrisp’.

	Calcium (mg g^{-1} dw)	Potassium (mg g^{-1} dw)	Magnesium (mg g^{-1} dw)	Nitrogen (mg g^{-1} dw)
		WA 38		
Average	0.9	7.2	1.0	4.5
Minimum	0.2	5.5	0.7	2.7
Maximum	1.9	13.8	1.9	5.8
		Honeycrisp		
Average	0.7	9.6	1.1	3.9
Minimum	0.1	6.2	0.7	2.5
Maximum	2.7	15.6	1.6	5.9

Table 6. Clustering of variability in bitter pit among 72 commercial orchard years for ‘Honeycrisp’. These are statistically clustered orchards with centered values for each variable.

Risk	Bitter pit (%)	Shoot length (inches)	Crop load (fruit cm^{-2} TCSA)	Fruit weight (g)	Fruit Ca (mg g^{-1} dw)	Fruit K (mg g^{-1} dw)	Fruit Mg (mg g^{-1} dw)	Fruit N (mg g^{-1} dw
Low	8.2	6.5	4.9	226	0.72	9.8	1.02	3.8
Low	11.6	4.9	4.9	179	0.74	9.1	0.98	3.8
Moderate	20.0	8.8	4.6	284	0.64	9.2	1.03	3.8
High	69.2	11.2	3.7	247	0.52	9.2	1.07	3.7
Very High	83.5	17.1	2.8	339	0.27	7.5	1.03	4.0

Table 7. Clustering of variability in green spot among 59 commercial orchard years for ‘WA 38’. These are statistically clustered orchards with centered values for each variable.

Risk	Bitter pit (%)	Shoot length (inches)	Crop load (fruit cm^{-2} TCSA)	Fruit weight (g)	Fruit Ca (mg g^{-1} dw)	Fruit K (mg g^{-1} dw)	Fruit Mg (mg g^{-1} dw)	Fruit N (mg g^{-1} dw
Low	3.1	8.1	6.0	258	0.97	7.1	0.97	4.4
Low	4.1	9.6	7.5	210	1.09	6.5	0.94	4.6
Moderate	8.5	8.3	4.7	301	0.81	7.4	1.00	4.4
Mod-High	17.7	7.3	3.0	356	0.65	8.5	1.11	4.6
Very High	51.9	8.1	1.7	314	0.32	8.0	0.99	4.9

Table 8. Clustering of variability in bitter pit associated with fruitlet and leaf nutrient concentrations that were sampled in late June. These are statistically clustered orchards with centered values for each variable.

Risk	Bitter pit (%)	Fruitlet Ca (mg g^{-1} dw)	Fruitlet K (mg g^{-1} dw)	Fruitlet Mg (mg g^{-1} dw)	Fruitlet N (mg g^{-1} dw	Leaf Ca (mg g^{-1} dw)	Leaf K (mg g^{-1} dw)	Leaf Mg (mg g^{-1} dw)	Leaf N (mg g^{-1} dw
Low	0.8	0.87	11.9	0.78	6.3	21.6	15.5	4.6	26.1
Moderate	10.6	0.81	13.8	0.88	10.7	21.6	16.5	4.1	27.8
Mod-High	23.0	0.83	13.1	0.73	7.1	25.7	16.8	5.2	28.8
High	57.1	0.95	13.7	0.81	10.1	29.5	15.5	5.9	27.5
Very High	77.3	1.05	15.5	1.04	11.5	21.9	17.5	5.4	30.1

Table 9. Clustering of variability in green spot associate with fruitlet and leaf nutrient concentrations that were sampled in late June. These are statistically clustered orchards with centered values for each variable.

Risk	Green spot (%)	Fruitlet Ca (mg g^{-1} dw)	Fruitlet K (mg g^{-1} dw)	Fruitlet Mg (mg g^{-1} dw)	Fruitlet N (mg g^{-1} dw	Leaf Ca (mg g^{-1} dw)	Leaf K (mg g^{-1} dw)	Leaf Mg (mg g^{-1} dw)	Leaf N (mg g^{-1} dw
Low	3.6	1.86	17.4	1.31	10.2	20.4	22.3	4.3	25.8
Moderate	2.5	1.83	17.9	1.36	11.2	21.2	22.9	3.9	28.0
Mod-High	30.5	1.47	19.0	1.26	11.5	15.3	19.7	3.4	24.9
High	38.9	1.92	19.3	1.60	17.6	23.6	21.8	4.6	29.4
Very High	59.3	1.89	20.5	1.64	15.4	24.4	28.9	4.2	29.8

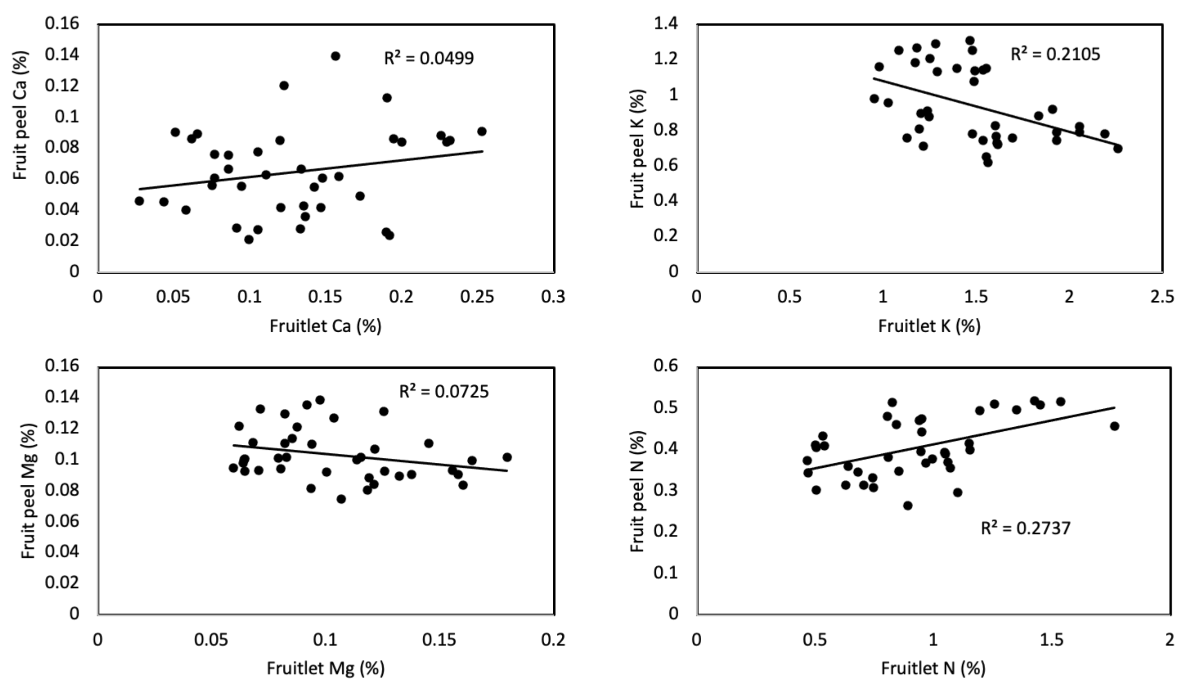

Figure 4. Relationships between fruitlet and leaf and fruit nutrient concentrations for WA 38 and Honeycrisp.

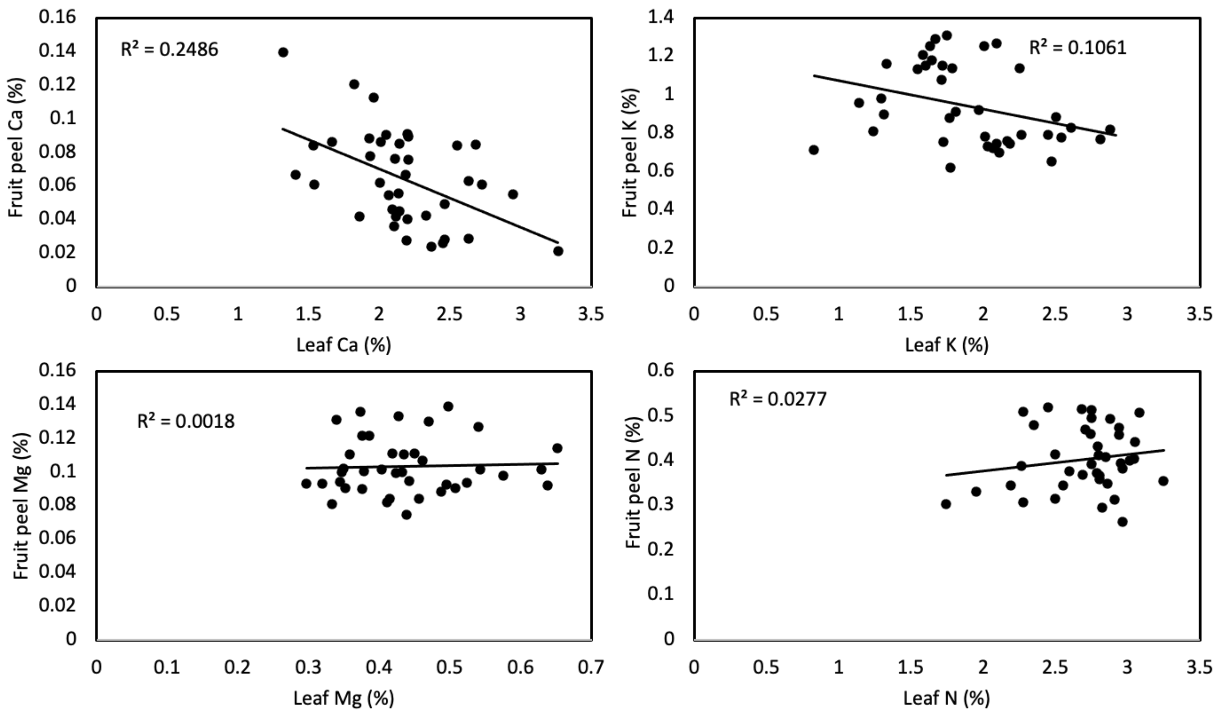

Figure 5. Relationships between fruitlet and leaf and fruit nutrient concentrations for WA 38 and Honeycrisp.

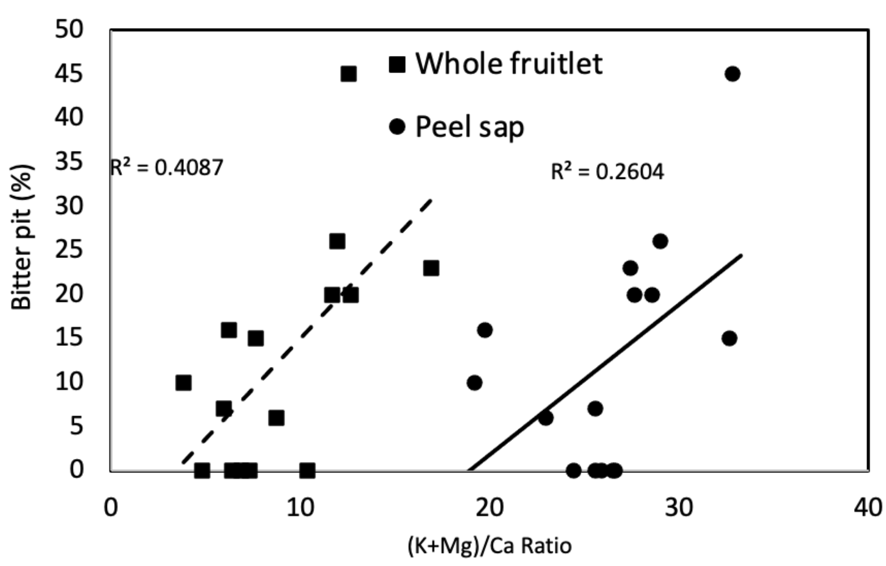

Figure 6. Relationship between peel sap and traditional whole fruitlet analysis and bitter pit in Honeycrisp

EXECUTIVE SUMMARY

Project title: N, Mg, and K guidelines to control disorders for Honeycrisp and WA 38

Keywords: Fertilizer management, bitter pit, green spot, physiological disorders

Abstract: For Honeycrisp, nutrient management is critical for limiting bitter pit. In this project, we used experimental and commercial orchard sampling approaches to better understand the roles of antagonistic nutrient contents in leaves, fruitlets, and fruit peel at harvest on bitter pit and green spot development for Honeycrisp and WA 38, respectively. For experimental approaches, we altered the application of potassium, magnesium, and nitrogen in a non-limiting orchard environment that span the normal range of application rates advised for WA state apple producers. N applications increased tree vigor and green spot incidence in ‘WA 38’ apple and increase bitter pit in Honeycrisp apple. For commercial orchards, bitter pit ranged from near 0 in some orchards to nearly 100% in other orchards. Green spot decreased in incidence from 2020 to 2021 going from almost 12% in 2020 to 3.7% in 2021 and then 4.2% in 2022. Further evaluations in 2023 indicated minimal green spot again across most orchards. For commercial sampling, green spot in WA 38 demonstrated the same risk indicators (high vigor, low crop load, and high K: Ca ratios) as bitter pit in Honeycrisp. Crop load was one of the main contributing factors for the development of both bitter pit and green spot. Rootstock can also contribute to green spot and bitter pit incidence through their effects on vigor, nutrient uptake, and fruit set each year. However, even rootstocks like G.41 can continue to produce high yields of green spot free fruit if crop loads are sufficient every year. Nutrient analysis on fruitlets and fruit need crop load data to accompany them or they are impossible to estimate risk. Leaf analysis was not a useful indicator of final fruit nutrient status. Fruitlet analysis gives early indications of problems, but other factors can lead to divergence from fruitlet values and final fruit nutrient content. Fruitlet sap analysis developed at Cornell corresponded to fruit nutrient concentrations at harvest and may also be an option for early risk identification. However, risk assessment can be as simple as evaluating vigor and crop load including identifying areas of the orchard which may be light in crop load.