2026 NW Cherry and Stone Fruit Research Review

Volunteers, including WTFRC staff member Melissa Garcia, helped plant cherry trees for the LTARE project at Sunrise Orchard in Rock Island as part of the Soil Health Initiative led by Tianna DuPont.

Photo Source: Paige Beuhler

November 4, 2025

Hybrid | Yakima, WA

Northwest Cherry and Stone Fruit Research Review

Yakima Convention Center

November 4, 2025

Hosts: WA Tree Fruit Research Commission (WTFRC) & Oregon Sweet Cherry Commission (OSCC)
Guests: California Cherry Board (CCB), Northwest Nursery Improvement Institute (NNII)
Chairs: Craig Harris (WTFRC), Ian Chandler (OSCC)

Pesticide Credits: Oregon and Washington credits pending

Time	Presenter	Title^	Status*	Year(s)
9:00		Registration		
9:30	Chairs	Welcome		
	Hanrahan	Meeting Etiquette & Housekeeping **(LIVE)**		
Project Reports				
		Little Cherry Disease		
9:45	Northfield	Overview of LCD activities + Taskforce updates **(LIVE)**		
9:55	Galimba	Determining Sweet Cherry Rootstock Sensitivity to X-Disease	NCE/CONT	23-25
10:05	Galimba	Physiology-based identification of X-disease infected trees	NCE/FINAL	22-24
10:15	Northfield	Developing a Leafhopper DD Spray Program for Cherry IPM	NCE/FINAL	22-24
10:25	Kohntopp	Real-Time Detection of LCD using Detector Canines *(out of cycle)*	NCE/CONT	23-24
		Canine Detection Strategies for Managing LCD *(out of cycle, no report)*	CONT	25-26
Break 10:35 – 11:05				
11:05	Whiting	Highlights of the Xth International Cherry Symposium **(LIVE)**		
11:20	Zhao	A Field Deployable Diagnosis of XDP Based on Optimized CAS12A Method	CONT	25-26
11:30	Pitino	Precision Agriculture: Innovating Cherry Disease Management with DPI	CONT	24-26
11:40	Pitino	Sustainable Cherry Protection with Symbiont	CONT	24-26
		Spotted Winged Drosophila		
11:50	Adams	Ganapsis Brasiliensis for Biological Control of SWD	FINAL	23-25
12:05	Adams	SWD in-orchard movement + overwintering dynamics	NCE/FINAL	22-24
Lunch Break 12:15 – 2:00				
2:00	Sallato	The WSU Tree Fruit Extension Team **(LIVE)**		
		Horticulture/Nutrition/Breeding/Misc.		
2:15	Schmidt	Pesticide residues on WA cherries	FINAL	23-25
2:30	Sallato	Precision Nutrient Management for Sweet Cherry Orchards	NCE/CONT	23-25
	Sallato	Cherry Rootstock Research and Demonstration Orchard *(no report)*	DELAYED	25-27
2:40	Thompson	Evaluating Heat Stress Response in Novel Cherry Rootstocks	CONT	25-27
2:50	McCord	A Robust PNW Sweet Cherry Breeding and Genetics Program	CONT	25-27
3:00	Schmidt	Resources for stakeholders **(LIVE)**		
Afternoon Break 3:10 – 3:40				
		Powdery mildew/bacterial diseases/Food Safety		
3:40	Zhao	Investigating Bacterial Canker Disease of Cherry in Young Orchards	CONT	24-26
3:50	Collum	Evaluation of UV-C for Management of Cherry Diseases and Pests	CONT	24-26
4:00	Murphy	Understanding Food Safety Risks During Post Harvest Cherry Production	CONT	24-26
4:10	McCord	Identifying Sources of Resistance to Pseudomonas and Powdery Mildew	CONT	25-26
4:20	Hanrahan	WTFRC-funded Technology projects **(LIVE)**		
4:30		Networking opportunity		
5:00		Adjourn		

^Titles: some titles have been abbreviated to fit into table, see project reports for full titles *CONT = continuing project report (10 min), FINAL = final project report (15 min), **NCE: no-cost-extension (10 min)** All reports are available for download approximately two weeks prior to the research review from www.treefruitresearch.org. Final reports will be permanently added to the searchable database (https://treefruitresearch.org/advanced-search/) within three months of the research review. Additional inquiries may be addressed to Mackenzie Perrault (Mackenziep@treefruitresearch.com).

Proposal Title: Determining Sweet Cherry Rootstock Sensitivity to X-Disease
Report Type: Continuing Project Report (No Cost Extension)

Primary PI: Kelsey Galimba
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Cooperators: Scott Harper (WSU), Melodie Putnum (OSU Plant Clinic)

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $ 37,323
Total Project Request for Year 2 Funding: $ 38,207
Total Project Request for Year 3 Funding: $ 39,119

Other related/associated funding sources: Awarded
Funding Duration: 2020 - 2023
Amount: $8,000
Agency Name: Oregon State University
Notes: Start-up equipment funding provided by OSU will be used to purchase a hoop house to contain/quarantine X-Disease inoculated potted cherry trees.

Budget 1
Primary PI: Kelsey Galimba
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Item	2023	2024	2025
Salaries1	$ 23,111.00	$23,804.00	$24,518.00
Benefits	$5,609.00	$5,777.00	$5,951.00
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies2	$4,000.00	$4,000.00	$4,000.00
Travel			
Plot Fees			
Miscellaneous			
Total	$32,720.00	$33,581.00	$34,469.00

Footnotes: [1] Partial summer salary for Galimba and salary for 0.75 FTE Master's student.
[2] Rootstocks and potting, grafting, and inoculation supplies, qPCR testing.

Budget 2
Co PI 2: Ashley Thompson
Organization Name: Oregon State University
Contract Administrator: Charlene Wilkinson
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Item	2023	2024	2025
Salaries	$3,836.00	$3,836.00	$3,836.00
Benefits	$767.00	$790.00	$814.00
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies			
Travel			
Plot Fees			
Miscellaneous			
Total	$4,603.00	$4,626.00	$4,650.00

Footnotes: [1] Partial summer salary for Thompson.

Objectives

Objective 1: Collect and analyze data regarding scion and rootstock type from known X-disease infections across OR and WA to determine what germplasm has evidence of susceptibility.

Objective 2: Evaluate the susceptibility of rootstocks that are never, or infrequently, represented in the infected dataset.

Significant Findings

1. X-disease infections without hypersensitive response symptoms have been verified from various testing sources in Gisela 6, Gisela 12, Krymsk 5, Krymsk 6, and Mazzard, indicating these rootstocks are susceptible.

2. Rootstocks that were acquired for this project include: Mahaleb, Mazzard, Gisela 5, MaxMa 14, Cass, Clinton, Crawford, Weigi 2, Weigi 3, Colt and Krymsk 7.

3. All rootstocks were grafted with q-PCR-verified infected material in 2025. Mahaleb, Maxma, Clinton, WeiGi 3, Colt and Krymsk 7 all have at least one successful graft. Clare, Mazzard, Gisela 5, Crawford, and WeiGi 2 did not have any successful grafts. Successful grafts indicate an absence of hyper-sensitivity and suggest those varieties are susceptible – however, qPCR validation is necessary to confirm.

Methods

<u>Objective 1: Collect and analyze data regarding scion and rootstock type from known X-Disease infections across OR and WA to determine what germplasm has evidence of susceptibility.</u>

Records related to X-disease testing performed in OR and WA were obtained and used to determine what scion and rootstock varieties have tested positive for X-disease in the past. Sources included the OSU Plant Clinic, WSU Clean Plant Center, and tests run by the Thompson lab. Unfortunately, many tests were performed with only rootstock or scion data recorded, with the majority being scion-only. Data for the following rootstocks and scion cultivars were acquired:

Cherry rootstocks and scions recorded in X-disease testing data from OR and WA.

Rootstock	**Scion**
Clare	Ann
Gisela 3	Attika
Gisela 5	Bing
Gisela 6	Black Pearl
Gisela 12	Burgundy Pearl
Krymsk 5	Chelan
Krymsk 6	Coral Champagne
MaxMa 14	Lapins

Mazzard	Rainier
Myro 29-C	Regina
Piku 4	Skeena
Weiroot	Starletta
	Stella
	Suite Note
	Van

Objective 2: Evaluate the susceptibility of rootstocks that are never, or infrequently, represented in the infected dataset.

Rootstocks were ordered from multiple nurseries/ tissue culture facilities following notification of funding in December, 2022. Rootstocks arrived winter 2023/spring 2024 and were first potted in one-gallon pots and grown in a greenhouse. Later in the season, rootstocks were transferred to 3.5-gallon pots and moved outside, to a weed-cloth covered area. Weigi 2 and Weigi 3 were the only rootstocks available with a grafted scion (Chelan), these were potted in 3.5-gallon pots on arrival and root suckers were used to propagate additional, non-grafted plants. In fall, 2024, rootstocks were transferred to a 20 x 60 high tunnel and were grown there through 2025.

To infect rootstocks with X-disease phytoplasma, we grafted them in July, 2025 with material from three q-PCR-verified infected source trees growing at MCAREC (Fig 1, 3A). Each infected source tree had two-to-three year wood collected, selecting branches that had a similar caliper to the main axis of the rootstock. A combination of one chip-bud and two modified chip-buds consisting of just bark (no bud) was used for each rootstock tree (Fig 1). Three rootstock trees for each of the eleven rootstock types were grafted, with one source tree per tree. Grafts were inspected 45 days later for viability. Grafting was repeated in September for rootstock trees that had no successful grafts – success will be quantified in November.

Figure 1. Grafting examples showing two chip buds (one with bud and one without) in the process of being grafted, two with successful grafts – including one with substantial gummosis, and one with an unsuccessful, rejected graft.

Because the X-disease phytoplasma is not usually present uniformly throughout infected plants, it is possible that that grafting did not transfer the infection. Each rootstock tree will be verified for infection using qPCR next year; if rootstocks are able to successfully house the infection, they will be considered non-resistant.

We also explored additional methods of inoculation. We received dodder (*Cuscuta pentagona*) from the Cooper Lab (USDA-ARS). Dodder is a parasitic plant that can form vascular connections between host plants, and we hoped to use it to transfer the X-disease phytoplasma from infected trees to rootstock trees. Unfortunately, we were only able to successfully germinate a few dodder seeds (Fig. 2) and we were unsuccessful in getting them to parasitize any of the commonly-used host plants. This method may be explored more in the future. We also explored the possibility of using leafhoppers in clip cages to infect rootstocks. Leafhopper colonies for this purpose are currently being propagated by the Harper Lab (WSU).

Figure 2. Dodder tendril growing on *Impatiens* host plant.

Results and Discussion

Objective 1

We identified twelve cherry rootstock varieties in available X-disease testing records. From those, Gisela 6, Gisela 12, Krymsk 5, Krymsk 6, and Mazzard had positive results. These tests came from a number of sources, including tests requested by growers, done on trees with fruit symptoms as well as from research projects looking at X-disease spread. Because none of this testing was originally done to identify susceptibility, it is important not to give too much weight to the numbers, since they are not limited to symptomatic trees and are almost certainly lean more heavily towards the negative for that reason. For the sake of this project, we are considering any positives to be evidence that a particular rootstock is susceptible. Commercially important rootstock varieties are highlighted.

Rootstock	Number Tested	Number Positive	% Positive
Clare	2	0	0%
Gisela 12	102	60	59%
Gisela 3	2	0	0%
Gisela 5	3	0	0%
Gisela 6	44	3	7%
Krymsk 5	44	1	2%
Krymsk 6	62	39	63%
MaxMa 14	2	0	0%
Mazzard	62	13	21%
Myro 29-C	3	0	0%
Piku 4	2	0	0%
Weiroot	2	0	0%

A number of scions were also present in testing records, and the results are listed here. Again, testing purposes varied, so numbers are subjective. If records can be increased, this information may eventually help inform breeding for X-disease resistance.

Scion	Number Tested	Number Positive	% Positive
Ann	4	2	50%
Attika	10	1	10%
Bing	25	6	24%
Black Pearl	71	9	13%
Black Tartarian	6	0	0%
Burgandy Pearl	30	0	0%
Chelan	3	1	33%
Coral Champagne	27	1	4%
Lapins	3	1	33%
Rainier	5	1	20%
Regina	2	1	50%
Royal Ann	4	0	0%
Skeena	12	4	33%
Starletta	2	0	0%
Stella	4	0	0%
Suite Note	10	3	30%
Van	4	2	50%

Objective 2

Eleven rootstocks were obtained for this trial. From the original list, nine were obtained, with an additional two (Colt and K7) added because they were available. Both negative (Mazzard) and positive (Mahaleb) controls were acquired. Rootstocks acquired are highlighted in green below.

Rootstock	Notes
Mahaleb	*P. mahaleb*. Positive Control: exhibits hypersensitive response to X-Disease.
Mazzard	*P. avium*. Negative Control: susceptible to X-Disease.
Gisela 5	*P. cerasus* x *P. canescens*
Maxma 14	*P. avium* x *P. mahaleb.* Higher chance of hypersensitive response to X-Disease based on parentage.
Clinton	*P. cerasus* x *P. canescens*
Lake	*P. avium* x *P. fruticosa*
Crawford	*P. cerasus* x *P. canescens*
Cass	*P. avium* x *P. cerasus* x *P. fruticosa*
Clare	*P. avium* x *P. cerasus* x *P. fruticosa*
Weigi 1	*P. cerasus*
Weigi 2	*P. avium* x *P. canescens*
Weigi 3	*P. avium* x *P. cerasus* x *P. canescens*
Weiroot 720	*P. cerasus*
Colt	*P. avium* x *P. pseudocerasus*

Krymsk 7	*P. lannesiana*

The rootstocks vary greatly in size and health after the first season of growth at MCAREC, and the variability is caused both by the size of the rootstocks when they arrived and the amount of growth each rootstock put on in 2024. In 2025, all eleven rootstock varieties had a high enough caliper to graft. Grafting success was low overall, with 15%, 18%, and 9% of all attempted grafts from source trees 1, 2 and 3, respectively, undergoing successful fusion to rootstock trees (Fig 3 B). Grafting success varied by rootstock type (Fig 3 C). Mahaleb, Maxma 14, Clinton, Weigi 3, Colt and Krymsk 7 all had successful grafts, while Mazzard, Gisela 5, Crawford, and Cass had none. It is surprising that Mahaleb is apparently able to house the phytoplasma without dying, since this was meant to be a hyper-sensitive positive control. It is possible the one successful graft in this case did not have phytoplasma in the phloem. Verification with further grafting and q-PCR testing is needed.

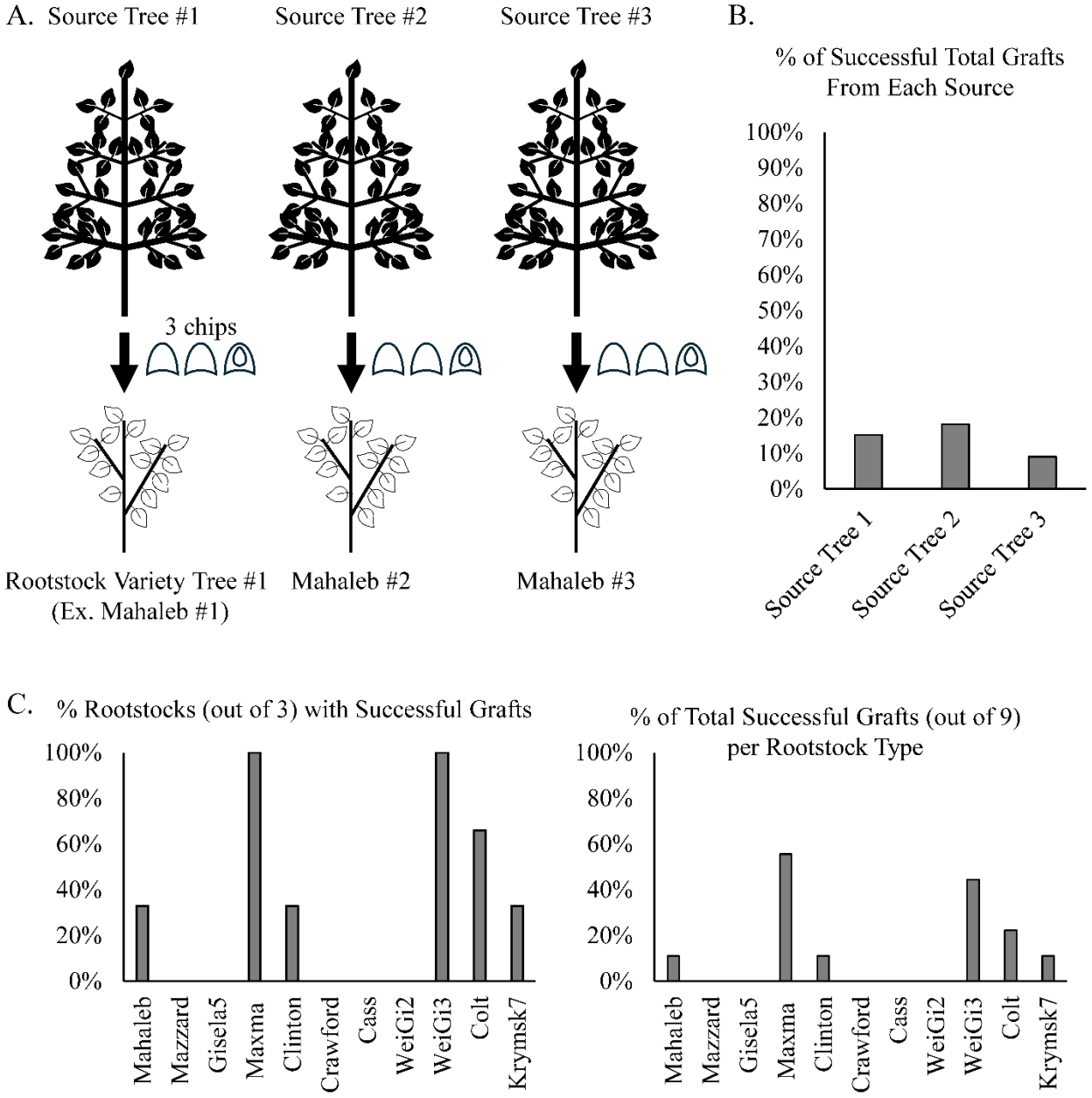

Figure 3. A. Schematic showing grafting from three source trees on to one rootstock type with three individuals. B. Percent of total graft that were successful, from each source tree. C. Percentages of individual rootstock trees and total grafts per rootstock type that were successful in the first round (July).

Justification for NCE

- Trees were ordered in the first year, and many were sourced from tissue-culture and were very small at arrival.
- The proposed project relied solely on grafting to transfer the phytoplasma to rootstocks. This has been performed once, with a small number of successful grafts. An additional year will allow for more grafting to take place, raising the chances of successful inoculation.
- This extra time will also allow for the use of infected leafhoppers to transfer the phytoplasma, an additional method that will make results related to resistance more robust.

Project Title: Physiology-based identification of X-disease infected cherry trees.

Report Type: Final Project Report (No-Cost Extension)

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Cooperators: John Byers (Grower), Tim Hudson (Grower), Ken Newman (Grower), Garret Bishop (G.S. Long), Mike Omeg (Orchard View)

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $30,657
Total Project Request for Year 2 Funding: $42,419
Total Project Request for Year 3 Funding: $33,596

Budget 1
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Item	2022	2023	2024
Salaries[1]	$14,356	$14,787	$15,230
Benefits	$7,928	$8,166	$8,411
Wages			
Benefits			
Equipment[2]		$9,552	
Supplies[3]	$2,000	$2,000	$2,000
Travel	$500	$1,000	$1,000
Miscellaneous			
Plot Fees			
Total	$24,784	$35,505	$26,641

Footnotes:
[1] Estimated salary for one FRA to perform sample collection, testing and data analysis + 2 weeks of PI summer salary.
[2] Field testing equipment for NIR and Ca2+.
[3] Lab supplies and reagents.

Budget 2
Co PI 2: Ashley Thompson
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Item	2022	2023	2024
Salaries	$3,836	$3,836	$3,836
Benefits	$2,037	$2,078	$2,119
Wages			
Benefits			
Equipment			
Supplies[1]			
Travel[2]			
Miscellaneous			
Plot Fees			
Total	$5,873	$5,914	$ 5,955

Budget 3
Co PI 2: Corina Serban
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Item	2022	2023	2024
Salaries			
Benefits			
Wages			

Benefits			
Equipment			
Supplies[1]		$500	$500
Travel[2]		$500	$500
Miscellaneous			
Plot Fees			
Total		$1,000	$1,000

Footnotes: 1&2 Travel and supplies to sample leaves from newly-infected trees and send them to MCAREC for starch testing.

Justification

X-disease is caused by the pathogenic phytoplasma, *Candidatus* Phytoplasma pruni, and in sweet cherry causes small, pale, unpalatable fruit.[1] Currently, potentially infected cherry trees are identified by visually inspecting fruit close to harvest. Vegetative material is then gathered and sent to a lab for PCR analysis in order to confirm presence of the phytoplasma.[2,3] This mode of identification presents a number of challenges for cherry growers. Symptom scouting must take place at the same time as harvest preparations, symptoms may be caused by a number of other diseases or disorders, and lab confirmation is expensive and results may be delayed. These issues highlight the need for more convenient and efficient disease detection. In this project, we aimed to take advantage of the documented physiological changes that have been shown to be caused by related phytoplasmas in related plant hosts, in order to develop a physiology-based assay capable of detecting X-disease infected sweet cherry trees.

Phytoplasmas colonize phloem tissues, where they trigger a Ca^{2+} influx that induces occlusion of the sieve tube elements.[4] This blockage is a defensive mechanism aimed at controlling the spread of the pathogen, but it also drastically alters the plant's ability to transport photoassimilates from the leaves to other areas.[5] This restriction of sugar transport has been shown to occur in phytoplasma infections of a variety of economically-important fruit species including apple, grape, coconut, papaya, jujube and citrus.[6–11] In all of these species, phytoplasma infections cause significant increases in starch content in the leaves, which may be visually undetectable or mimic symptoms of nutrient imbalance. In the most economically-devastating examples of phytoplasma infection, Huanglongbing (HLB) in citrus and Flavescence dorée (FD) in grape, a number of field-based assays have recently been developed to help identify infected trees, either by testing leaf starch content directly[12] or inferring starch content based on specific changes in spectral reflectance.[13–16] Spectral reflectance methods, particularly when combined with new technologies like UAVs, have the potential to be adapted to high-throughput, large scale X-disease identification approaches in the future.

Excess starch accumulation in aerial portions of the tree also has the potential to alter phenology, which has been shown in girdling experiments involving different tree species,[17,18] and to a limited extent in phytoplasma infected fruit trees like apricot and plum.[19,20] X-disease infected cherry trees have been reported to have early bud-break in the spring and late leaf senescence in the fall, indicating that a simple, visual, phenology-based observation may also be useful in identifying potentially infected trees. If this proves to be the case, this is another potential avenue for aerial imaging to be used in order to identify potential areas of outbreak in large orchards.

Objectives

1. Characterize the degree of leaf starch content changes in sweet cherry trees with verified *Candidatus* P. pruni infections (both established and new), using lab-based methods.

2. Identify accurate, efficient procedures to test leaf starch content in a field setting, by comparing methods such as iodine tests and spectroscopy.

3. Explore other potential physiology-based methods for identification to determine if any of them can be developed further as X-disease identification tools.

Significant Findings

- Starch content varies by leaf, and is not statistically different between positive and negative trees. Starch content is not an indicator of infection.

- Iodine tests (which test for starch) do not show differences between positive and negative trees and are not a feasible method for diagnosing X-disease.

- Leaf spectrometry (with our specific method, see below) also does not show a difference between positive and negative tress, and is not a feasible method for diagnosing X-disease.

Methods

Objective 1. Characterize the degree of leaf starch content changes in sweet cherry trees with verified *Candidatus* P. pruni infections (both established and new), using lab-based methods.

Starch assays attempted using a Cell Biolabs kit were attempted in 2023, and were resumed in 2025 using positive trees identified at MCAREC. Unfortunately, the kit gave unreliable results, with standard controls not matching expected values. This led us to explore other methods. Eventually we were successful with a modified protocol originally used for quantifying starch in cherry floral primordia.[21] Sample preparation consisted of flash freezing leaf tissue, grinding it to a fine powder, weighing, and washing with ETOH to remove pigments. Samples were then digested, converting starch to glucose using enzymatic hydrolysis. Glucose was quantified using an enzyme-coupled assay, using a spectrometer to take readings at 340 nm before and after the addition of glucose-6-phosphate. Using the slope and intercept calculated from a standard curve, the volumes of reagents and the original weight of the leaf tissue, we calculated the final values for mg starch/g sample. This assay was performed on three trees in the field, located at MCAREC, that were verified as positive for X-disease in 2023. These trees are part of a breeding trial and are different accessions; for each positive tree a neighboring tree of the same accession was used as a control, resulting in three "sets".

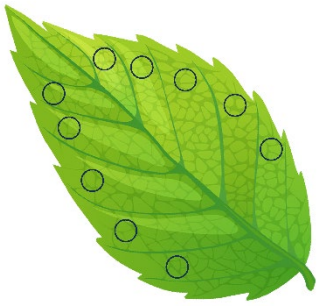


Figure 1. Leaf punches that were collected and pooled for starch assays.

Identification using trained canines (Ruff Country K9) was performed in 2025 – the three positive trees were alerted on, and the control trees were not. Q-PCR validation is pending. We also performed the assay on three infected and three non-infected Mazzard plants received from the Harper Lab (WSU). These small plants are from tissue culture (not seeds) but will be referred to as "seedlings" in the text to represent their small size. For field trees, four leaves were collected for starch analysis, one from the N, S, E and W side of each tree. For the seedlings, one leaf was collected. For each leaf, multiple punches were taken from the perimeter of the leaf blade (avoiding vascular tissue) and pooled (Fig 1).

Objective 2. Identify accurate, efficient procedures to test leaf starch content in a field setting, by comparing methods such as iodine tests and spectroscopy.

Iodine Methods

For the iodine testing, a number of approaches were evaluated during the duration of this project. The first followed the protocol outlined in Takushi *et al.* 2007. The adaxial surface of each leaf was scratched with a one-inch square of fine-grit sandpaper until the surface of the paper was coated. Sandpaper was added to a plastic bag with diluted iodine and color of the solution was observed.

The second method involved clearing leaves before staining with iodine. At first whole leaves were attempted, but the size of the leaves proved difficult to manipulate and limited the number of leaves we could process. To optimize, we switched to using 1x1 inch squares that were cut from the basal portion of each leaf blade, avoiding the midrib if possible (Fig. 2). Leaf squares were boiled for two minutes, then soaked in room temperature (RT) water to cool. They were then boiled in 90% EtOH until bleached, changing out EtOH if needed. Once squares were bleached, they were placed in RT water bath to rehydrate. Pictures were taken of bleached squares and then they were added to a glass dish with iodine solution. They were allowed to remain in contact with iodine for ~1 minute before being rinse in a water bath and photographed.

In 2025, the first protocol (sandpaper/bag) method) was repeated on the three positive field trees and the three positive lab trees (plus negative controls) used for starch analysis. Fresh Lugol's solution was used.

Spectral Methods

In order to determine whether a spectral signature could be used to identify X-disease infected trees, we used a handheld leaf spectrometer (C1-710, CID Biosciences, WA). This tool takes absorbance, transmittance, and reflectance data from a small portion of the scanned leaf, recording values for wavelengths from 360 to 1100 nm.

Figure 2. Handheld leaf spectrometer used for recording spectral data from infected and non-

In 2024, reflectance data taken from orchards in The Dalles, OR and at MCAREC from 2023-2024 was analyzed. In order to visualize the difference between infected and non-infected trees, Princial Component Analysis (PCA) was performed. PCA is often used with large, multi-dimensional datasets to create a 2D visualization of the differences or similarities between groups in the data. PCA decomposes data into axes called principal components (PCs). PCs are ordered such that the first PC explains most of the variation in the data, the second PC less, and so on. We created biplots of the first two PCs for each day and

location. In these biplots, points that are closer together are more similar to each other, and points that are further apart are more different.

In 2025, we took multiple scans of leaves from the three infected field trees located at MCAREC that were used in starch testing and iodine testing. We also scanned lab seedlings. Numbers of leaves scanned matched those tested for starch. Individual values, average values, and PCAs were plotted for visualization.

Objective 3: Explore other potential physiology-based methods for identification to determine if any of them can be developed further as X-disease identification tools.

In 2022, phloem sap was collected from trees used in Objective 1. ¾ inch sections of first- and second-year wood were cut, scored, and centrifuged as in Hijaz & Killiny 2014, then flash frozen for further analysis. Collected liquid should consist of both xylem and phloem contents.

In 2023, visual observations were made of leaf senescence in infected and non-infected limbs, with the goal of determining whether this can be used a field diagnostic marker. No clear pattern related to infection was established, indicating that while X-disease may alter leaf phenology in the fall, it is not consistent enough to be used as a reliable marker and so this objective was not advanced.

Results and Discussion

Objective 1. Characterize the degree of leaf starch content changes in sweet cherry trees with verified *Candidatus* P. pruni infections (both established and new), using lab-based methods.

Three field trees and three lab seedlings with qPCR-verified X-disease infections were tested alongside negative controls, using a starch protocol optimized for cherry material. Starch content for individual leaves did not show an obvious pattern in either field trees (Fig. 3A) or in seedlings (Fig. 3D), with leaves from infected trees exhibiting both higher and lower levels of starch than leaves from uninfected trees. When values were averaged across leaves (Fig. 3B), there were no significant differences between infected and uninfected trees (ANOVA, Tukey HSD). This was true when tree values were averaged as well (Fig. 3C, 3E) with no significant differences between positive and negative categories (Student-t test). The lack of correlation between infection status of trees and starch levels in leaves indicates that this will not be a useful marker for X-disease detection.

Objective 2. Identify accurate, efficient procedures to test leaf starch content in a field setting, by comparing methods such as iodine tests and spectroscopy.

Iodine Results

A number of methods using iodine staining to visualize starch were used throughout the duration of this project. Results are summarized in Table 1.

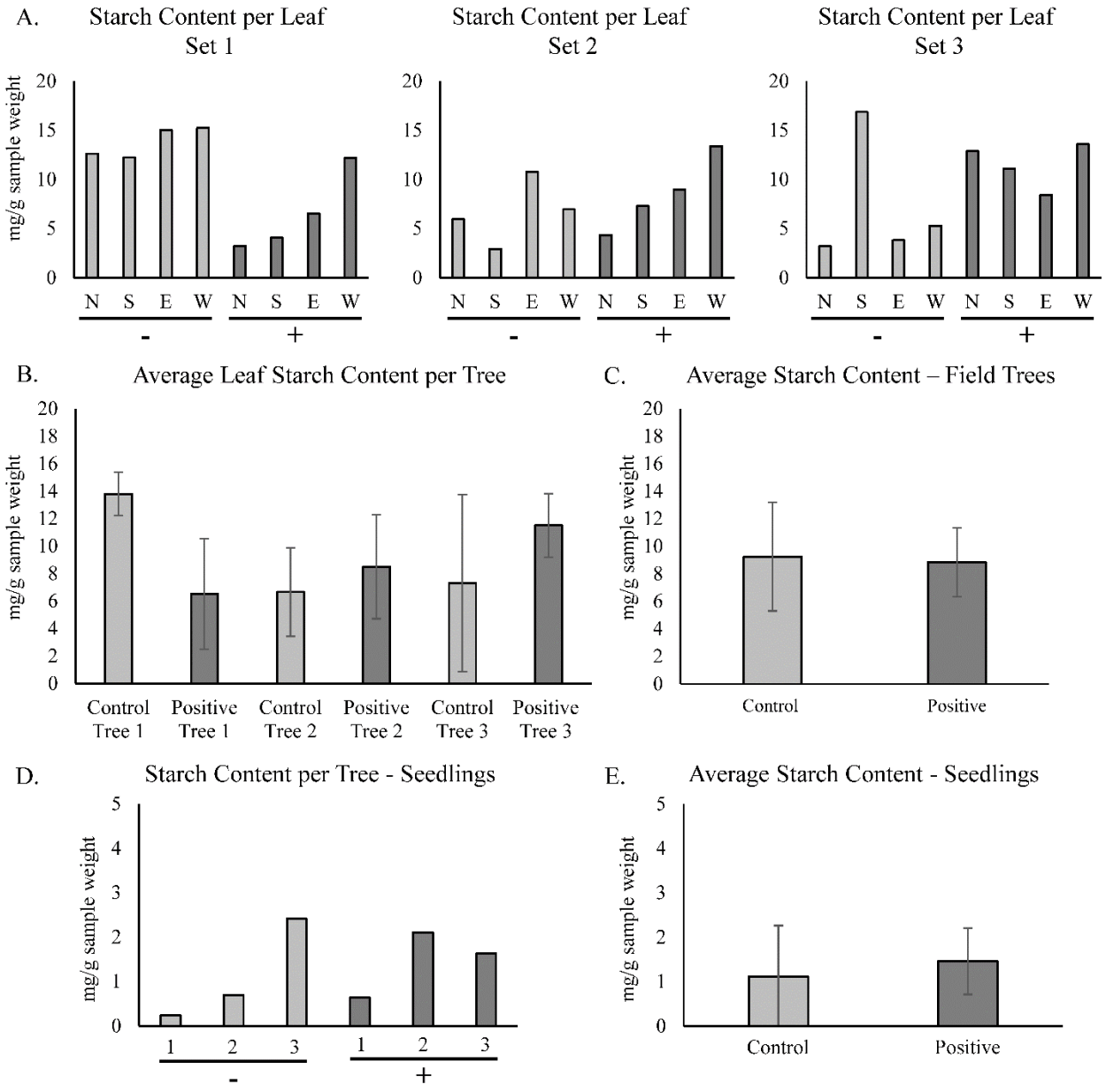


Figure 3. Amounts of starch in X-disease positive and negative trees of different ages. A. Starch content from three infected and three uninfected four-year-old trees in the field, located at MCAREC. For each tree, four leaves were tested. B. Starch content averaged between the four tested leaves for each tree. Values were statistically similar between all trees (ANOVA, Tukey HSD). C. Starch content averaged between positive and negative trees. Values are statistically similar (Student-t). D. Starch content from three infected and three uninfected one-year-old "seedlings". One leaf for each tree was tested. E. Averaged starch content between positive and negative trees. Values were statistically similar (Student-t).

In 2025, we repeated the sandpaper/bag method reported in 2023, on the same trees from the field and the same seedlings that were analyzed for starch content. There were no visual indications of the dark color that occurs when Lugol's reagent comes in contact with starch (Fig. 4). Both positive and negative leaves appeared to be similar in color. In combination with the lack of starch in lab tests, this indicates that iodine testing is not a feasible method for X-disease detection.

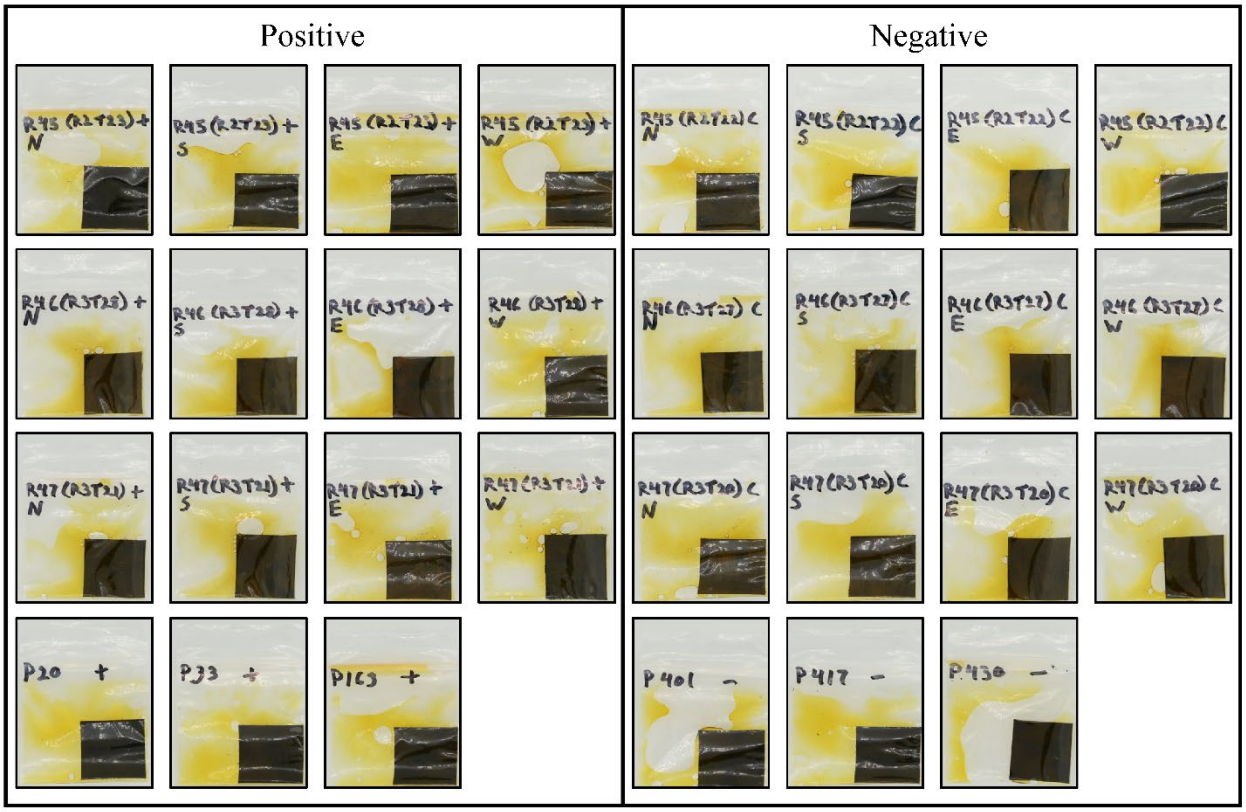


Figure 4. Iodine staining of leaf material to detect starch. One-square-inch pieces of sandpaper were rubbed on four leaves from three infected and three uninfected (top three rows) trees in the field at MCAREC, and on one leaf from three infected and uninfected seedlings. Once enough leaf material was gathered, it was immersed in Lugol's solution in plastic bags to observe darkly colored starch staining. No dark color was present in these tests, independent of X-disease status.

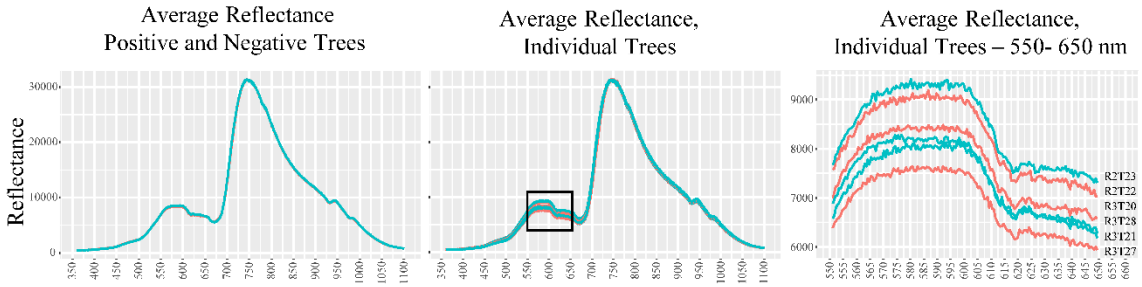
Spectral Results

Spectral analyses in past years did not result in any clear patterns that correlated with X-disease infection (Table 1). In one instance (one site in The Dalles, OR, 2024), a principal component analysis (PCA) indicated a difference between infected and uninfected leaves. In that biplot, we could see clear separation between the control and infected groups. However, on the other days and locations, there was much less dissimilarity between the control and infected trees. Ultimately, it was determined that the differences between infected and uninfected trees in that single analysis was due to differences in reflectance values outside of the wavelengths that

the CI-710s can reliably scan. Files from each scan contain data outside of the 360-1100 nm range, but communication with the company had made it clear this outside data is too "noisy" to be used reliably.

In 2025, we scanned leaves in July, August, and September from the field sets at MCAREC and the seedlings. Scans were done similarly to collections for starch analysis and for iodine testing. Data was trimmed to the appropriate range before analyzing. Reflectance values for each wavelength were averaged and plotted, with average values for infected and uninfected scans appearing very similar in both field and seedling trees (Fig. 5). When values associated with individual trees were plotted, some differences were observed in the 550-650 nm range. However, these differences did not correlate with infection status, making them unlikely to help with disease identification. PCA likewise, did not illustrate any grouping by infection status (Fig. 6). It is possible that scans using our spectrometer are too spatially limited to detect infection, so any further studies with similar objectives should focus on whole-tree scanning and/or wavelengths outside of the 360-1100 nm range.

A. Reflectance plots of qPCR positive and negative (control) field trees, August 2025.



B. Reflectance plots of qPCR positive and negative (control) seedlings, September 2025.

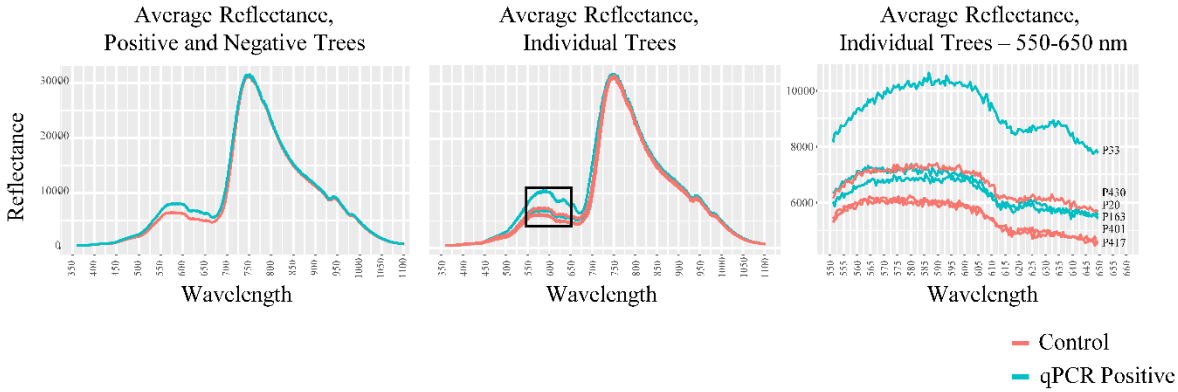


Figure 5. Reflectance values for wavelengths 360-1100 nm. A. Average values for infected and uninfected field trees, individual field trees, and zoomed into the 550-650 nm range for individual field trees. B. Average values for infected and uninfected seedlings, individual seedlings, and zoomed into the 550-650 nm range for individual seedlings. In both cases, infected (blue) and uninfected (red) values are apparently random, with no clear correlation between reflectance values and X-disease infection.

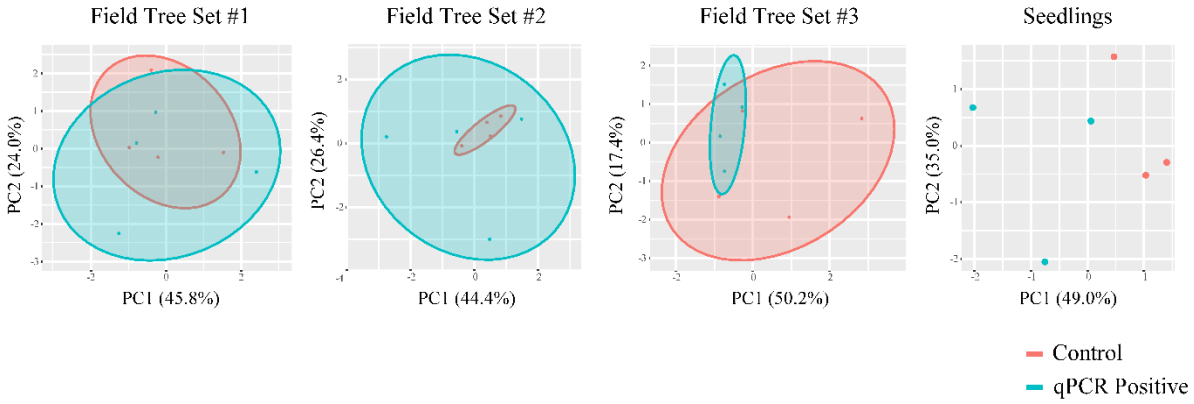


Figure 6. Principal Component Analysis (PCA) for September scans of three field sets (three infected and three uninfected trees, four leaves each) and seedlings (three infected, three uninfected, one leaf each). No clear division of positive/negative is present and PC scores are relatively low for all plots.

Table 1. Compilation of tests and results associated with this project.

Year Reported	Material	Method	Results	Comments
2023	qPCR positive trees control trees from three orchards in The Dalles, OR	Starch testing – Cell Biolab kit	N/A	Method didn't work
2025	qPCR positive and control trees from MCAREC and seedlings	Starch testing – Santolaria *et al.* 2025	No statistical difference in starch levels between +/- trees.	Method worked well.
2023	qPCR positive trees control trees from three orchards in The Dalles, OR	Iodine testing – sandpaper/bag	No difference between +/- trees.	Starch staining not visible from any samples.
2023	qPCR positive trees control trees from three orchards in The Dalles, OR	Iodine testing – clearing whole leaves	Variable starch staining – no difference between +/- trees	-
2023	Uninfected (not qPCR verified) trees from MCAREC – sun vs shade and different times through the day.	Iodine testing – clearing leaf squares	Variable starch staining – no obvious temporal or sun/shade patterns	-
2025	qPCR positive and control trees from MCAREC and seedlings	Iodine testing – sandpaper/bag	No difference between +/- trees.	Starch staining not visible from any samples.
2024	qPCR positive trees control trees from three orchards in The Dalles, OR	Spectrometer	Difference at one site, for one date in The Dalles	Differences only occurred outside of the acceptable wavelength range for this equipment.
2024	qPCR positive and control trees from MCAREC	Spectrometer	No difference between +/- trees.	-
2025	qPCR positive and control trees from MCAREC and seedlings	Spectrometer	No difference between +/- trees.	-

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Executive Summary

Project Title: Physiology-based identification of X-disease infected cherry trees.

Keywords: X-disease, starch, spectral signature, iodine, diagnostic

Abstract:

The cherry industry is in need of more convenient and efficient detection methods for cherry X-disease, a phytoplasma-based disease that causes small, pale, unpalatable fruit. In this project, we aimed to take advantage of the documented physiological changes that have been shown to be caused by related phytoplasmas in related plant hosts, in order to develop a physiology-based assay capable of detecting X-disease infected sweet cherry trees. Phytoplasma infections have been shown to cause a build-up of starch in the leaves of apple, grape, coconut, papaya, jujube and citrus. To determine if this occurs in X-disease infected cherry as well, we tested starch levels in leaves, comparing both young and older infected and uninfected trees. Starch levels varied between leaves, but no correlation was present between infection and starch level. We also explored field-based methods to detect starch in the field. The first, an iodine-based assay, also did not result in a correlation between infected and uninfected leaves, which is expected given the lack of differences in starch levels. The second method, which relies on spectral readings and analysis, could potentially detect starch levels or some other difference independent of starch. However, our analyses did not reveal any signature that correlated with infection status of trees. This project explored methods that could have been valuable for X-disease detection, but it appears that the physiological changes present in infected cherry trees differ from other crop/disease changes and will likely not be useful tools in this situation. Further studies into spectral diagnostics should focus on different scanning methods and/or include wavelengths outside of the range we used.

Project Title: Developing a Leafhopper Degree-day Spray Program for Cherry IPM

Report Type: Final Project Report

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Cooperators: Teah Smith, Garrett Bishop, Jenna Voelker, Dr. Katlyn Catron, Dr. Rodney Cooper, Dr. Scott Harper, Dr. Adrian Marshall, Dr. David Crowder

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $ 77,930
Total Project Request for Year 2 Funding: $ 80,918
Total Project Request for Year 3 Funding: $ 84,025
Total Request: $242,873

Other related/associated funding sources: Awarded
Funding Duration: 2020–2023
Amount: $36,000

Agency Name: Corteva Agriscience, Gowan Co, Brandt Co., Nichino America, Kemin Industries, GroPro
Notes: Funds from chemical industry contribute to salaries and research in Obj. 3.

Other related/associated funding sources: Awarded
Funding Duration: 2023-2025
Amount: $249,813
Agency Name: WSDA Specialty Crop Block Grant
Notes: Project "Leafhopper phenology model development and habitat assessment to improve cherry X-disease management" was awarded to PI Orpet and co-PI Northfield to support leafhopper sampling and expand monitoring to habitat bordering orchards.

Other related/associated funding sources: Awarded
Funding Duration: 2022-2025
Amount: $324,588
Agency Name: USDA NIFA, Crop Protection and Pest Management
Notes: Project "Integrating vector and pathogen phenology to optimize X-disease management" was awarded to PI Northfield and co-PIs Adams, Nottingham, and Galimba to sample leafhoppers and X-disease infected trees for two years.

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Item	2022	2023	2024
Salaries	$47,727.00	$49,636.00	$51,621.00
Benefits	$17,498.00	$18,198.00	$18,926.00
Wages	$5,760.00	$5,990.00	$6,230.00
Benefits	$551.00	$573.00	$596.00
RCA Room Rental	$0.00	$0.00	$0.00
Shipping	$0.00	$0.00	$0.00
Supplies	$0.00	$0.00	$0.00
Travel	$0.00	$0.00	$0.00
Plot Fees	$0.00	$0.00	$0.00
Miscellaneous	$0.00	$0.00	$0.00
Total	$71,536.00	$74,397.00	$77,373.00

Footnotes:
[1]Nottingham Salary: $7,612 x 12 mo x 2% FTE = $1,827 for year 1 x 1.04 each additional year. Benefits at 29.9%
[2]Postdoc Salary: $4,250 x 12 mo x 90% FTE = $45,900 for year 1 x 1.04 each additional year. Benefits at 36.9%.
[3]Non-student temporary employee: $15/hr x 16 hrs/wk x 24 wks = $5,760 for year 1 x 1.04 each additional year. Benefits at 9.6%
[4]Funded with industry gifts and leveraged matching funds.

Budget 2
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Item	2022	2023	2024
Salaries	$0.00	$0.00	$0.00
Benefits	$0.00	$0.00	$0.00
Wages	$5,760.00	$5,875.00	$5,993.00
Benefits	$634.00	$646.00	$659.00
RCA Room Rental	$0.00	$0.00	$0.00
Shipping	$0.00	$0.00	$0.00
Supplies	$0.00	$0.00	$0.00
Travel	$0.00	$0.00	$0.00
Plot Fees	$0.00	$0.00	$0.00
Miscellaneous	$0.00	$0.00	$0.00
Total	$6,394.00	$6,521.00	$6,652.00

Footnotes:
[1]Non-student temporary employee: $15/hr x 16 hrs/wk x 24 wks = $5,760 for year 1 x 1.04 each additional year. Benefits at 11%.
[2]Funded with industry gifts and leveraged matching funds.

Objectives

1. Collect model development and validation data: scout selected WA and OR orchards containing high populations of leafhoppers twice per week while compiling weather data.

Deviations: Scouting occurred weekly in 2022, 2023, and 2024. This project was leveraged to obtain a federal grant to continue collecting through 2025.

2. Modeling phenology curves: Use leafhopper abundance data to parameterize and validate the model.

Deviations: The objective is completed, but we are using one more year, funded by federal funding leveraged by this project to fit the model to improve model coverage across multiple years.

3. Insecticide trials: Screen conventional and organic insecticides for efficacy against leafhoppers and partner with growers to test spray timings.

Deviations: Some new laboratory tests were conducted. In addition, we conducted simulation models to evaluate different timings on orchard longevity. Models suggested orchards survive the longest when insecticides are timed at early adult emergence, rather than during the nymphal stage as previously hypothesized. The degree day model can supplement trapping to inform this timing.

Significant Findings

- Relative abundance of leafhoppers of concern in Washington and Oregon orchards have been defined. From 2022–2023, a total of 28,661adult leafhoppers were counted on sticky cards and 26,188 from vacuum samples. Most of the leafhoppers were species we can assume are innocuous to tree fruit production (64% and 77% of total leafhopper catch from sticky card and vacuum sampling, respectively). Among the three main assumed potential vectors of X-disease, the most common were *Colladonus reductus* (20%, 6%) and *Euscelidius variegatus* (14%, 16%). Oregon sites tended to have relatively more *E. variegatus.* The third species, *Colladonus geminatus,* was least common (1.3%, 0.4%).
- *Colladonus montanus reductus* adults have three seasonal peaks in orchards. Adults first appear as early as May, peaking in June–July. A summer peak usually occurs in August. A final peak occurs in fall, with adults persisting through November. The size of the peaks can vary greatly between peaks, locations, and years.
- Visual inspection, dissection, molecular diagnosis, and literature review conducted among the laboratories of co-PIs and collaborators indicated *E. variegatus* adults vary greatly in pigmentation. This results in a gradient of light and dark forms. Due to earlier confusion, adults of this species were previously undercounted on this project in Washington. They were re-counted for the current report.

Methods

Site selection. In 2022, 10 Washington and three Oregon orchards were selected for vacuum and sticky card sampling (Table 1) in consultation with industry cooperators Bishop, Voelker, and Smith. Orchards were chosen where large numbers of leafhoppers were expected. The orchards were either apricot, cherry, nectarine, apple, or pear. In 2023, the same sites were used, except Richland 1 was replaced with Richland 2 because few leafhoppers were found at Richland 1 in 2022. In 2024, two sites were cut to improve sampling route efficiency and reliability of weekly coverage (Richland 2 and Prosser 1).

Leafhopper monitoring. In 2022–2024, leafhoppers were monitored from May through October using sticky card and vacuum methods. Variable methods were used in 2022 between Washington and Oregon, but methods were standardized among locations starting in 2023.

For sticky cards, in 2022, two yellow cards were collected and replaced from the lowest-height tree limbs for Washington sites, and one sticky card was collected and replaced from a wooden post between two trees for Oregon sites. In 2023, all sites used two sticky cards placed on low limbs. In 2024, three sticky cards were placed per orchard, on wooden posts. The number of *C. reductus* adults, *C. geminatus* adults, *E. variegatus* adults, and other leafhopper adults on each card was recorded.

For vacuum samples, in 2022, Washington site sampling was conducted in a standardized area based on nine 0.81-m (32-inch) diameter circular areas per site whereas Oregon sites standardized sampling based on walking 56–82 m (180–270 ft). In 2023 and 2024, sampling was standardized to nine 0.81 m (32-inch) diameter circular areas per site. A 5-gallon paint strainer bag, held on the vacuum tube with rubber bands, collected the insects and prevented them from being sucked into the motor. After vacuuming, the contents of the paint strainer bag were emptied into a zip-top bag. Zip-top bags were returned to the lab and frozen for later quantification of leafhoppers.

Temperature monitoring. At each site temperature sensors, METER Group ZL6 Advanced Cloud Data Loggers with ECT/RT temperature sensors were installed to record air temperature every 15 minutes. However, temperature data from loggers was found to be unreliable will not be used. Rather, we have used the nearest Agweathernet and AgriMet weather stations, using hourly average temperature.

Table 1. Site names, fruit crops, and years sampled for leafhoppers in 2022–2024.

State	Site	Fruit Crop	Years sampled
Washington	Peshastin	Pear	2022, 2023, 2024
	Wenatchee	Pear	2022, 2023, 2024
	Rock Island 1	Apple	2022, 2023, 2024
	Rock Island 2	Apple	2022, 2023, 2024
	Royal City 1	Cherry	2022, 2023, 2024
	Wapato	Apricot	2022, 2023, 2024
	Zillah	Cherry	2022, 2023, 2024
	Richland 1	Nectarine	2022
	Richland 2	Apple	2023
	Prosser 1	Cherry	2022, 2023
	Prosser 2	Cherry	2022, 2023, 2024
Oregon	Hood River	Cherry	2022, 2023, 2024
	Mosier 1	Cherry	2022, 2023, 2024
	Mosier 2	Cherry	2022, 2023, 2024

Model development. A Johnson SB model (Jones and Wiman 2012) was fit to leafhopper counts on traps in 2022 through 2024. Sites with particularly low counts (never more than one leafhopper per trap per day) were removed before analyzing. The model measures degree day accumulation, measured hourly [(temperature – base)/24], where "base" is the base temperature. Initially we used the base temperature of 58ºF, which is the minimum temperature for development of beet leafhopper. However, after evaluating other base temperatures, a minimum value of 45 ºF was found to fit the data better. We assumed a negative binomial error distribution to account for the nature of the count data.

Simulation model. To better understand ideal spray timings, we developed and evaluated a simulation model inspired by *C. reductus* biology. Given the month-long latency period in *C. reductus*, we assumed that vectors acquire the phytoplasma as nymphs and transmit as adults. Thus, we hypothesized that it would be most effective to target nymphs, so that they were killed before they could transmit. We parameterized the model to match current understanding of *C. reductus*. Currently, the full model description and results are available in Laura Flandermeyer's Master's thesis available at the WSU thesis repository: https://rex.libraries.wsu.edu/esploro/.

Insecticide trials. Bioassay methods for leafhoppers were developed on a related project entitled "Insecticidal control of leafhoppers in cherries" led by co-PI Nottingham funded by the Washington Tree Fruit Research Commission. The same methods were applied in 2023 to test two rates of a new chemical, Wrath (geraniol 30%, peppermint oil 1%, cottonseed oil 0.1%, and rosemary oil 0.01%; manufactured by GroPro) in comparison with PyGanic. Data were reported in last year's continuing report for this project. In 2024, another chemical was tested, Sefina 4.89% afidopyropen and cyclopropanecarboxylate).

Results and Discussion

Model development. Leafhopper counts from sticky cards (Figure 1) and vacuum sampling (Figure 2) show similar patterns during 2022–2024. The species *C. reductus* clearly had three distinct periods of adult peaks. Phenology of *E. variegatus* was more idiosyncratic, and *C. geminatus* were too uncommonly found to visualize clear patterns. For both methods, most of the leafhoppers collected did not belong to any of these species and were probably of no concern as potential vectors of X-disease.

The best fit model for the sticky traps suggests that the *C. reductus* base temperature for development was approximately 45ºF, with adult generations emerging onto traps from approximately 500 degree days (dd) to 1600dd in generation one, 1600dd to 3700dd in generation two, and 3700 to approximately 4500dd in generation 3 (Figure 3). There was significant variation between years, with 2022 typically occurring earlier in the season on a degree day scale than 2023 or 2024. This could be due to a number of reasons. First, it is possible that weather allowed greater movement in 2022 than in other years, allowing earlier movement. In addition, there could have been increased mortality in 2022 that reduced the amount of leafhopper capture later in the generations. Further research on movement and mortality, as well as evaluating counts in the 2025 data currently being collected will help identify variability in year-to-year variation.

There were some differences between the two sampling methods. Vacuum sampling seemed to have more variation in counts between samples than sticky cards, and vacuum samples tended to catch relatively more *E. variegatus* than sticky cards. Similarly, it was more difficult to distinguish between generations with vacuum sampling (Figure 4) than with sticky cards. Because sticky cards are typically used to monitor *C. reductus*, we focused modeling efforts on predicting counts on traps, allowing growers to compare counts with the model. With both methods, the three Oregon orchards tended to have more *E. variegatus.* In Washington orchards, *C. reductus* was clearly the dominant species with sticky card monitoring, whereas in Washington vacuum samples, *E. variegatus* were often found in similar or greater numbers than *C. reductus.*

Previous research suggests *E. variegatus* has a single generation in Italian vineyards (Bosco et al. 1997, Ann. Appl. Biol. 130:1), and this may also be the case in Pacific Northwest orchards.

Simulation model. The simulation model found that contrary to our hypothesis, the optimal timing for insecticide treatments to reduce transmission was when adults numbers are increasing (Figure 5). This may seem counterintuitive, given that adults may be able to transmit prior to treatment. If coverage and mortality was 100%, earlier treatment would indeed be more effective. However, with more

realistic coverage and mortality, we found that earlier sprays killed leafhopper nymphs that may have died naturally before emerging as adults anyway. By moving treatments later in the season, it targets only the leafhoppers with the greatest likelihood of transmission, reducing efforts on killing nymphs that may not have survived anyway. Thus, we recommend timing insecticides to occur when adults first start emerging on traps, which our predictive model can help detect.

The PI from 2023–2024, Dr. Orpet, departed Washington State University but remained as a co-PI on the project, and Dr. Northfield started leading in 2025 to oversee the remaining work in leafhopper quantification and model development. The datasets will be augmented by additional data in 2025 related to a Washington State Department of Agriculture Specialty Crop Block Grant project entitled "Leafhopper phenology model development and habitat assessment to improve cherry X-disease management" awarded to PI Orpet and co-PI Northfield. That grant supports technical assistance and leafhopper sampling in three-point transects within orchards: point 1 is adjacent vegetation, point 2 is the orchard edge, and point 3 is the orchard center. This expanded sampling will help us understand the role of orchard-adjacent habitat as a potential leafhopper source and its role in phenology of the leafhoppers.

Insecticide trials. Wrath, Sefina, and Pyganic, which were tested in associated with the current project in 2023 and 2024, each increased mortality to exposed *C. reductus*. A single publication that has all insecticide tests funded by WTFRC/OSCC and elsewhere has been published (Marshall et al. 2025) and information has been incorporated in the WSU Crop Protection Guide for Tree Fruits in Washington.

Resulting peer reviewed publications from this study (please contact Tobin at tnorthfield@wsu.edu if you have any difficulty accessing them):

Marshall, AT, KA Catron, RJ Orpet, RT Curtiss, TD Northfield, LB Nottingham. 2025. Insecticide and repellent tests on Washington leafhopper vectors of cherry X-disease. *Journal Economic Entomology*, toaf247. https://doi.org/10.1093/jee/toaf247

Flandermeyer, LE, LB Nottingham, SJ Harper, CG Adams, CR André, KA Catron, AE Clarke, ST DuPont, AT Marshall, TD Melton, TD Northfield. 2025. Methods for sampling X-disease *Colladonus* spp. (Hemiptera: Cicadellidae) leafhopper vectors in orchards. Washington State University. 2025. https://doi.org/10.7273/000007491

Clarke, AE, KA Catron, C Reyes Corral, AT Marshall, CG Adams, WR Cooper, SJ Harper, LB Nottingham, TD Northfield. 2024. *Colladonus* spp. (Hemiptera: Cicadellidae) vectors of X-disease: Biology and management in western United States. *Journal of Integrated Pest Management*, 15:13. https://doi.org/10.1093/jipm/pmae005

Student thesis:

Laura Flandermeyer's Master's Thesis:
https://rex.libraries.wsu.edu/esploro/outputs/graduate/Sampling-and-management-of-X-disease-phytoplasma/99901125941101842

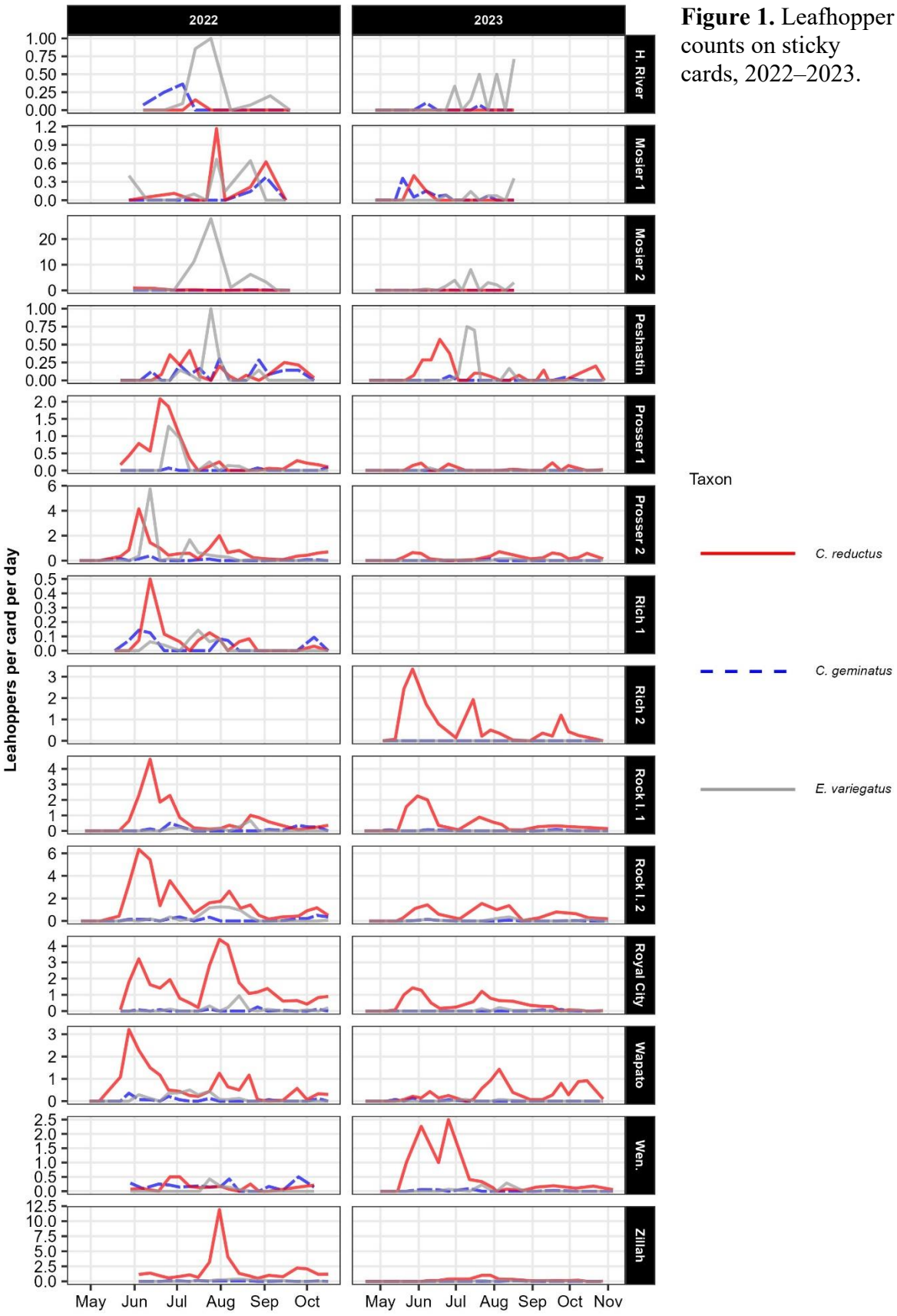

Figure 1. Leafhopper counts on sticky cards, 2022–2023.

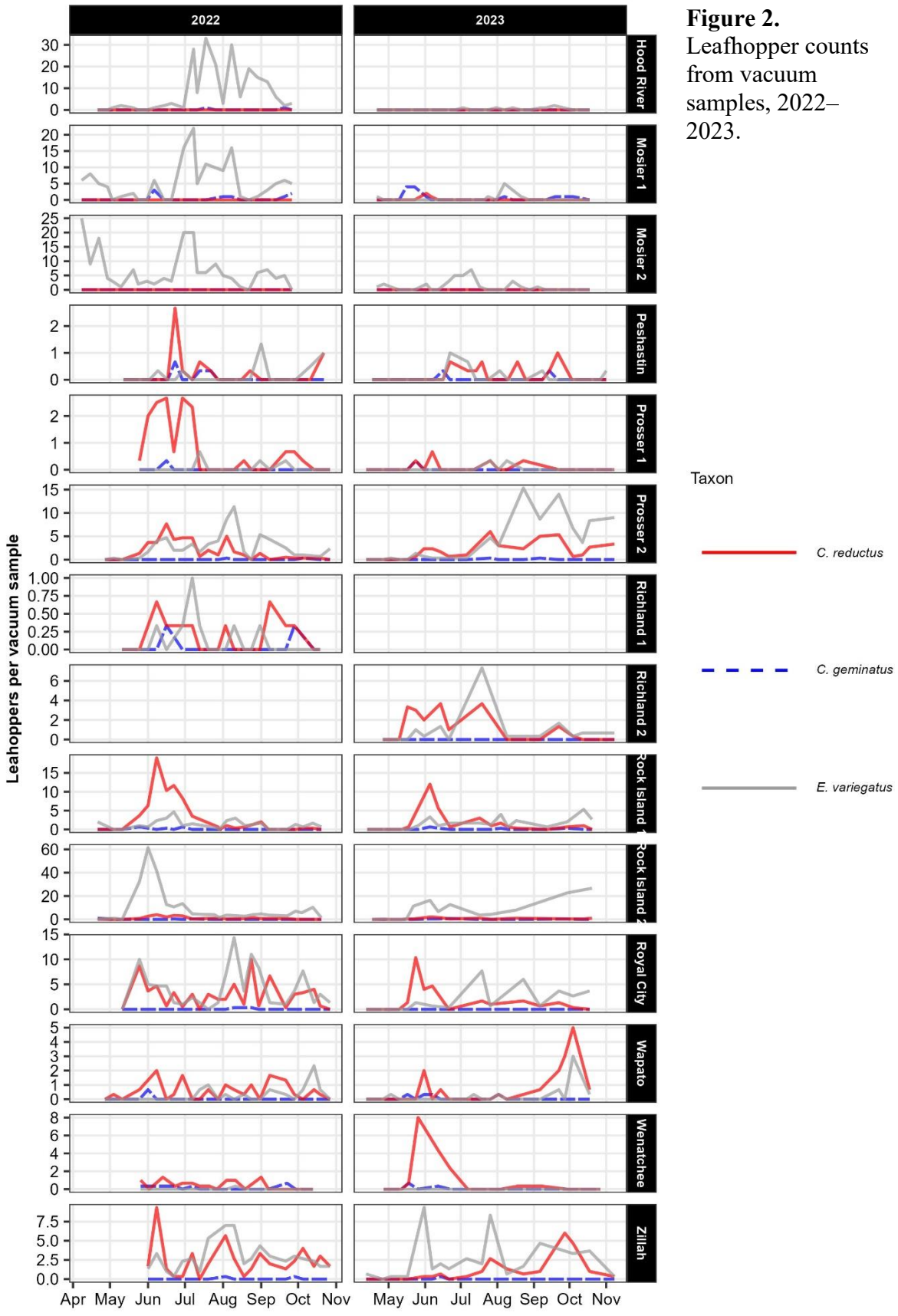

Figure 2.
Leafhopper counts from vacuum samples, 2022–2023.

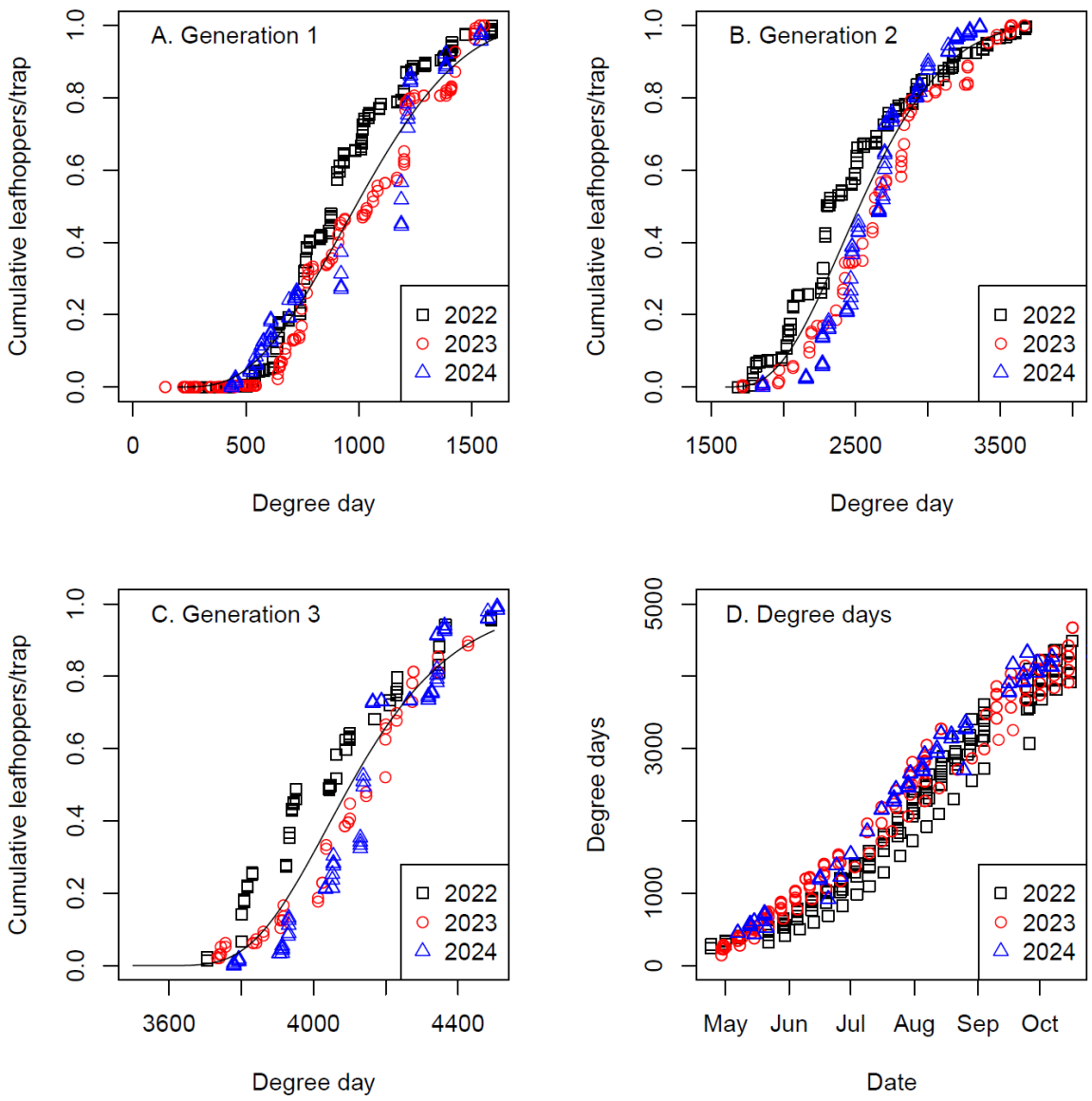

Figure 3. Data collected from sticky traps and best fit models showing three generations, occurring from approximately 500 to 1600 degree days (A), 1600 to 3700 degree days (B), and 3700 and beyond (C), along with site-specific degree day accumulation over the course of the season. The model used a base temperature of 45 degrees Fahrenheit, and are shown for 2022 (black squares), 2023 (red circles), and 2024 (blue triangles).

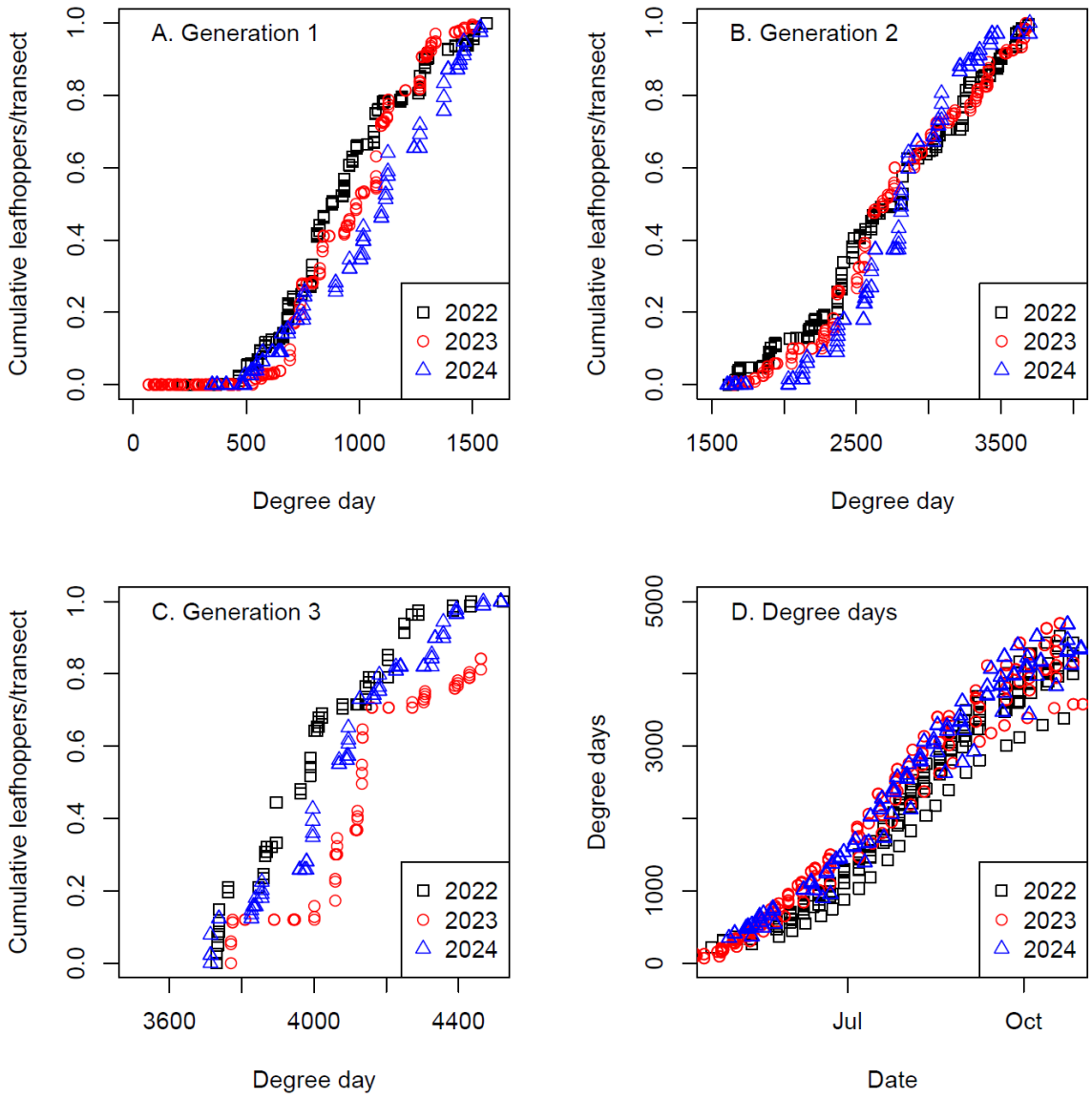

Figure 4. Data collected from vacuum sampling, showing three generations, occurring from approximately 500 to 1600 degree days (A), 1600 to 3700 degree days (B), and 3700 and beyond (C), along with site-specific degree day accumulation over the course of the season. The degree day calculation used a base temperature of 45 degrees Fahrenheit, and are shown for 2022 (black squares), 2023 (red circles), and 2024 (blue triangles).

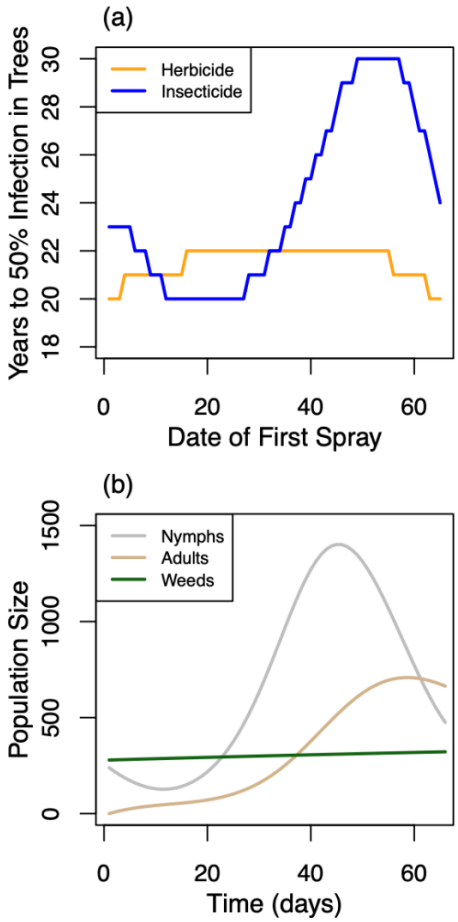

Figure 5. Optimal timing (a) of insecticide (blue) and herbicide (orange) in simulation models that maximizes orchard longevity, relative the relative abundance of nymphs (gray), adults (goldenrod), or weeds (green). Time is presented relative to the start of a given generation, and is most useful in comparison to the leafhopper stages present. Insecticides were assumed to be effective on each nymphs and adults, with 80% coverage.

Project Title: Real-time Detection of Little Cherry Disease using Detector Canines

Report Type: Continuing Report - No Cost Extension

Primary PI: Jessica Kohntopp
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Project Duration: 2-Year - No-cost Extension

Total Project Request for Year 1 Funding: $74,267
Total Project Request for Year 2 Funding: $116,045

Other related/associated funding sources: None

Budget 1
Primary PI: Jessica Kohntopp
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Item	2023	2024
Salaries	$40,000.00	$40,000.00
Benefits	$13,600.00	$13,600.00
Wages		
Benefits		
RCA Room Rental		
Shipping		
Supplies	$2,640.00	$500.00
Travel	$8,338.00	$12,276.00
Plot Fees		
Miscellaneous		
Total	$64,578	$66,376

Footnotes: Year-1: PI Jessica Kohntopp requests salary based on below median detection dog trainer salary survey conducted by Highland Canine Training LLC School for Dog Trainers. Supplies for boxes/tins, harnesses, booties, canine training supplies, camera for videos, tripod, mini freezer etc. Travel to cover 5 trips of 5 days each to bring the canines to WA for blind studies, mock orchards, demonstration and outreach days. **Year-2** travel costs were doubled because canines will be in WA more times for in depth studies.

Budget 2
Co-PI 3: Scott Harper
Organization Name: Washington State University
Contract Administrator: Anastasia (Stacy) Mondy
Telephone: (509)-335-2885
Contract administrator email address: arcgrants@wsu.edu

Item	2023	2024
Salaries		
Benefits		
Wages	$6,233.00	$43,631.00
Benefits		
RCA Room Rental		
Shipping		
Supplies	$955.80	$3,538.20
Travel		
Plot Fees		
Miscellaneous		
Total	$7,189	$47,169

Footnotes: Year-2: Co-PI Harper requests allocation of $30,996 in salary (0.5 FTE) and 11,159 in benefits, with 12,203 in supplies and consumables for the generation of peptides for detector dog training.

Budget 3
Co-PI: Corina F. Serban
Organization Name: Washington State University
Contract Administrator: Anastasia (Stacy) Mondy
Telephone: (509)-335-2885
Contract administrator email address: arcgrants@wsu.edu

Item	2023	2024
Salaries		
Benefits		
Wages		
Benefits		
RCA Room Rental		
Shipping		
Supplies	$500.00	$500.00
Travel	$1,809.00	$1,809.00
Plot Fees		
Miscellaneous	$191.00	$191.00
Total	$2,500	$2,500

Footnotes: Year-1: Co-PI Serban requests $500 towards extension and outreach activities; $1,809 for travel and accommodation; and $191 for miscellaneous items such shipping costs. **Year-2:** request is similar to Year-1.

Objectives:

Year one (May 10, 2023 - May 10, 2024):

(1) Train canines to detect the three pathogens that cause Little Cherry Disease: Little Cherry Virus-1 (LChV-1), Little Cherry Virus-2 (LChV-2), and X-disease phytoplasma (XDP) by using infected plant samples. (Target odors 1-3).

(2) Validate the accuracy, sensitivity, and specificity of the canines to detect Little Cherry Disease (LCD) pathogens in young cherry trees in a Blind Controlled Study.

(3.0) When canines are proficient (95% or higher) in Objective 2, canines will be brought to a mock field setting. (Due to the season, titer levels will not be at their highest. This first step will be to introduce canines to a field setting and for trainer to observe their behaviors).

(4) Extension and Outreach – monthly meetings, demonstration at field day and technical factsheet (Year one-two).

Year two (May 10, 2024 - May 10, 2025):

(3.5) Due to timing and season a majority of mock field training will be done in the beginning of year two when titers are high.

(5) Orchards: When canines are proficient in both parts of Objective 3, Blind studies will be performed in cherry orchards where the observer knows where positive trees are but the handler does not.

(6) Dormant study: Determine if canines can detect LCD in dormant trees in both young nurseries and older orchard blocks.

(7) Temporal Study: Determine how early on in the infection cycle canines are able to detect LCD in comparison to molecular qPCR methods of artificially infected trees.

(8) Nursery study: Determine if canines can find LCD in newly grafted rootstocks, young trees in storage bins, etc.

Objective Deviations:

Given the challenges associated with sampling for this disease, particularly due to its patchy distribution, it is determined that continued qPCR testing of the trees we are monitoring is not an efficient use of resources. A no-cost extension was filed - and accepted - to allow continued monitoring of these trees with canines as well as visual assessment for symptoms over the next couple years.

Significant Findings:

- Five canine-alerted trees (initially tested qPCR negative) were dug up and dissected; all five trees were confirmed qPCR positive, requiring more than 76 samples per tree.
- Canines have alerted on both sap extrudate and ground up tissue samples diluted to low levels.
- Canines are consistently re-alerting on previously identified trees with a few additional alerts.
- Two canine-alerted trees from a newly planted USDA block were dug up and dissected; both trees were positive.

Methods:

Dormancy Study: Canines were re-deployed in the previously used Younger Block B to evaluate their ability to detect LCD pathogens in dormant trees. Assessments were conducted in November 2024, December 2024, and January 2025 under varying seasonal conditions.

- November: Leaves had changed color and were in the process of dropping.
- December: About 99% leaves had dropped, though a few remained. Weather conditions alternated between rain and snow during the canine runs.
- January: All leaves had dropped. While there was no snow cover, the ground was frozen solid and outdoor temperature was 21℉.

Figure 1. Trees pictured from each month respectively for the Dormancy Study.

Bundle Study: We had the opportunity to run canines by partially known bundles before the canines had ever been trained on bundles. The results very much showcased the importance of starting canines on confirmed material. Initially, the canines did not alert, then began attempting to alert indiscriminately, indicating confusion. It was later determined that most, if not all, of the trees were likely positive. This study reinforced the need to begin with known material so the canines can become confident with their responses before going blind and exposing the canines to scenarios where the majority of the trees may be positive.

Dissection Study: Five trees were selected from the Younger Block B to be dug up and dissected in February 2025. These trees had been previously alerted on by the canines and qPCR tested negative (1 sample taken per tree). Each tree was dissected including roots and samples were taken every four inches. The diameter of the wood determined the number of samples taken from specific cross sections (1-4 samples).

Figure 2. Trees being excavated for dissection study

Figure 3. Tape represents where tissue was sampled for qPCR

Younger Block D: Canines were deployed in a younger block of unknown trees, similar to Younger Block B. These trees were not qPCR tested and any canine-alerted trees were removed from the block. Some of those removed trees were transplanted in two different locations: WSU Prosser and USDA Moxee. The long term objective is to determine whether removing canine alerted trees will reduce the number of future new alerts when the block is re-evaluated by canines.

Rerunning Younger Block B: Due to the patchy distribution of the disease and the difficulty of obtaining reliable qPCR samples, it was decided that continued blind sampling would not be an efficient use of resources. Instead, canines will be periodically re-run through the block to compare alerts with previous findings and assess consistency. We will keep visually monitoring the trees to see if symptoms will begin to appear in the next year or two.

Sap extractions: As a first step in developing an understanding of what molecules the dogs imprinted on and determine their levels of sensitivity, we applied two methods to extract phloem contents: 1) For a crude extract, phloem-containing tissues were collected from known X-disease phytoplasma positive and negative plants from our greenhouse-maintained collection, diced into fine pieces and macerated with a mortar & pestle in 1x TBS buffer. The resulting solution was clarified via low-speed centrifugation to remove residual tissues. 2) For greater recovery and downstream analysis, bark sections from the same known positive and negative plants were collected, peeled and sectioned, then washed in ddH20 before being placed in columns for high-speed centrifugation to collect phloem exudate. In both cases, an aliquot of each extract or exudate was used for nucleic acid extraction and qPCR to determine phytoplasma titer.

Once determined, a 10-fold dilution series was prepared for each (to 10^{-9}). 100 µl aliquots of each series were pipetted onto cotton pads, with buffer negatives as additional controls. These were used in a series of blind trials in bird feeder cages to prevent direct contact by the dogs.

Figure 4. Example of a canine reacting to a positive sap exudate sample.

Given that the dogs reacted to positive sap exudates, we then prepared additional aliquots for untargeted LC-MS metabolomic and proteomic analysis to determine differences in the compound(s) are present between infected and uninfected samples. This analysis is still underway and will be reported on in the next performance period.

Results and Discussion:

Dormancy Study: In November and December, canines consistently alerted on the same trees without issue. However, in January, performance differed, with canines struggling more to detect what are presumed to be more weakly infected trees - sometimes only alerting on a second pass, or not at all. When brought to Older Block A the next day, both canines demonstrated fewer difficulties in alerting on positive trees. It should be noted, CT values in Younger Block B ranged from 37 to 39, while those in

Older Block A ranged from 35 to 38. In January, the dogs were only ran past eight of the previously alerted on trees in Younger Block B when the handler saw they were struggling. It was not beneficial to reinforce the canine's second-pass alert behavior. The January results are as follows:

Table 1: Canine Dormancy Study results for January

January Results: Younger Block B				
Canine	**# of Previously alerted trees**	**Alerted first pass**	**Alerted second pass**	**Did not alert at all**
Humma	8	6	1	1
Aika	8	3	5	0
January Results: Older Block A				
Humma	9	7	2	0
Aika	9	8	0	1

We believe the frozen ground and the biology of this disease contributed to the canines' difficulty in detecting certain trees, particularly lower infected trees. This factor will be considered when determining the most effective seasonal timeframe for canine deployment.

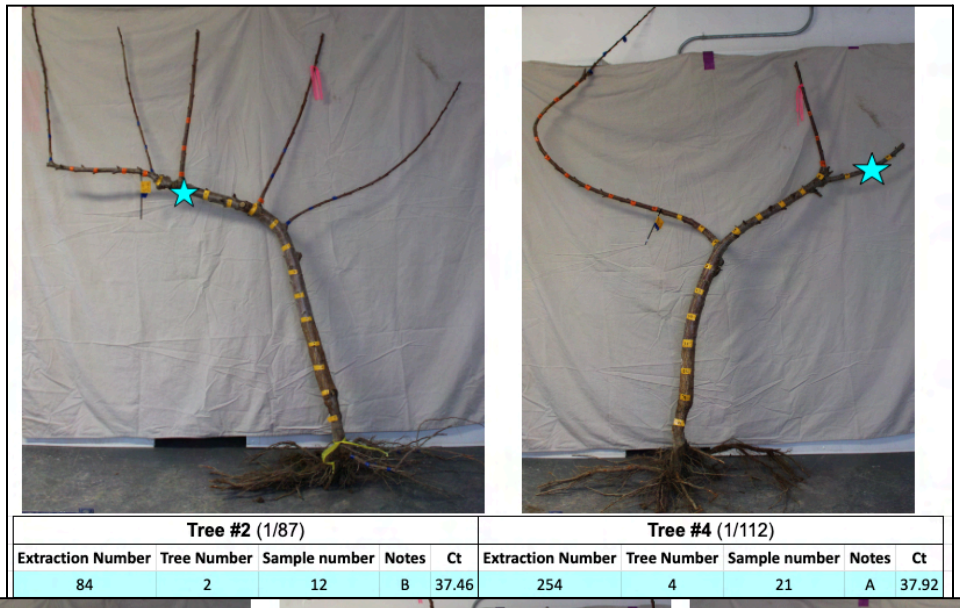
Figure 5. Aika alerting on dormant tree

Figure 6. Humma alerting on dormant tree

Dissection Study: Trees were dissected at four inch intervals, each tree was a different size so the amount of samples per tree varies and can be found on the table below. Although only a few samples tested positive, each tree had at least one positive result, confirming the canines' alerts. Overall, 13 out of 464 samples showed positive results with CT values ranging between 37-38. These results highlight the challenges of selecting the correct tissue for accurate qPCR testing. The images below show each tree, with colored stars indicating the positive qPCR results and corresponding CT values. The colored tape lines that can be seen are where each sample was taken. As seen in the photos, the disease is highly patchy with no distinct pattern, making sampling difficult. This shows the challenges in obtaining accurate qPCR results for young, weakly infected trees and why confirming canine alerts through conventional testing is inherently difficult. These results show how imperative it is to have a diagnostic tool that can sample the whole tree rather than just a portion of it.

Table 2: Dissection Study Results

	Sample Size	PCR Positive Results	% Chance of Infected Tissue Sample Selection	Canine Success
Tree #1	76	5	6.58%	100%
Tree #2	87	1	1.15%	100%
Tree #3	95	3	3.16%	100%
Tree #4	112	1	0.89%	100%
Tree #5	94	3	3.19%	100%
Total	**464**	**13**	**2.80%**	**100%**



Tree #2 (1/87)

Extraction Number	Tree Number	Sample number	Notes	Ct
84	2	12	B	37.46

Tree #4 (1/112)

Extraction Number	Tree Number	Sample number	Notes	Ct
254	4	21	A	37.92

Tree #1 (5/76)

Extraction Number	Tree Number	Sample number	Notes	Ct
10	1	7	A	38.54
19	1	11	B	38.53
36	1	20	B	37.41
48	1	23	D	37.45

Tree #3 (3/95)

Extraction Number	Tree Number	Sample number	Notes	Ct
142	3	5	A	37.69
178	3	3	B	36.91
222	3	26	D	36.895

Tree #5 (3/94)

Extraction Number	Tree Number	Sample number	Notes	Ct
337	5	7	C	37.21
338	5	7	D	37.57
460	5	R3	A	37.77

Figure 5. Dissected trees 1 through 5 with color corresponding stars with CT value table for positive qPCR results.

Younger Block D: Both canines alerted on the same 52 trees. There were eight trees the canines did not agree on - either Aika alerted and Humma did not, or vice versa. These trees were not qPCR tested but were transplanted to alternate locations (WSU Prosser and USDA Moxee) for ongoing monitoring of future potential visual symptoms.

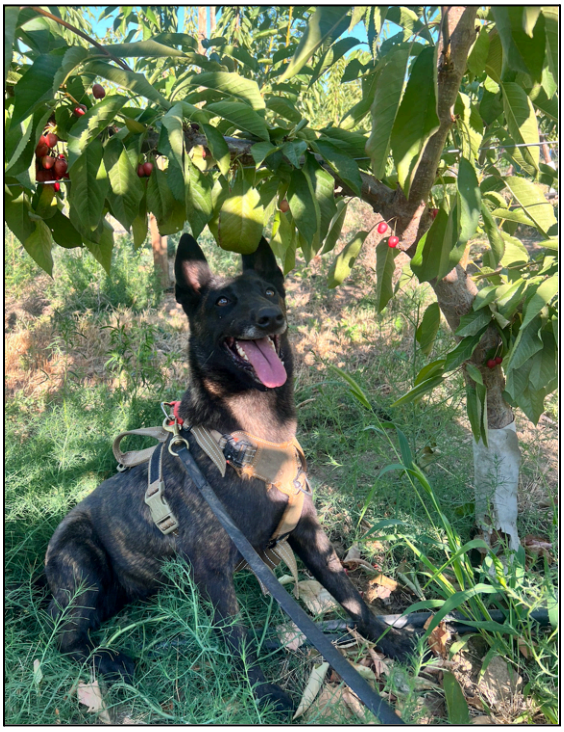


Figure 7. Humma alerting on a cherry tree

Figure 8. Aika alerting on a cherry tree

Re-running Younger Block B: In July 2025, both canines were re-run on the entire Younger Block B to check the consistency of previous alerts to what canines did this time. The results were as follows:

Table 3: Younger Block B Re-run Results

Canine	# of Previously Alerted Trees	Alerted on First pass	Did not alert	New alerts
Humma	43	40	3	12
Aika	47	44	3	12

The 12 new alerts occurred within close proximity to previously alerted trees, suggesting either potential new infections or the canines may have missed detection during the initial run. The trees the canines did not alert on could be due to mapping inconsistencies or just simply the canines missed it. Canines were brought back to these trees for a second pass but showed no interest.

Sap extractions: Canines successfully alerted on the positive samples of both types of sap/phloem tissue exudate, with positive sap exudates being alerted on down to a dilution of 10^{-9}. No reaction to known phytoplasma negative or control panels was observed. As this dilution is considerably lower than can be detected by qPCR, it indicates that the compound(s) that the canines have imported on are expressed, either by the pathogen, or induced by the pathogen and expressed by the plant host, at concentrations

higher than 1:1 with the pathogen itself (Table 4).

Table 4. Dilution series of sap exudates from a known X-disease positive plant, with number of canine alerts and approximate phytoplasma concentration as determined by qPCR.

Dilution Step	Approximate phytoplasma concentration (cells)	Canine Alerts (Humma // Aika)
1	200,000	Alert // Alert
10^{-1}	20,000	Alert // Alert
10^{-2}	2,000	Alert // Alert
10^{-3}	200	Alert // Alert
10^{-4}	20	Alert // Alert
10^{-5}	2	Alert // Alert
10^{-6}	0.2	Alert // Alert
10^{-7}	0.02	Alert // Alert
10^{-8}	0.002	Alert // Alert
10^{-9}	0.0002	Started to struggle // Alert

This, combined with the known scattered in planta distribution of the pathogen early in the infection cycle, underlines the difficulty, if not futility, in attempting validating every positive canine alert using molecular methods: the canines have imprinted on a compound/or series of compounds that is/are present at greater concentration than the pathogen. Therefore, to provide an accurate measure of sensitivity and a tool for validation it is imperative that the compounds in question be identified; to this end, untargeted LC-MS metabolomic and protein analysis is underway and will be reported on during the next performance period.

Future Steps:
Monitoring of Younger Block B will continue using canines, alongside looking for visual symptoms in the next couple years. Canines will also be re-deployed in Younger Block D at the one-year mark. With all previously alerted trees removed, we aim to evaluate whether the incidence of new alerts is lower compared to the rate observed during the re-run of Younger Block B. Finally, efforts will be focused on determining the putative compound(s) the canines have imprinted on.

Project Title: A field-deployable diagnosis of XDP based on optimized Cas12a method

Report Type: Continuing Project Report

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Cooperators: Garrett Bishop (G. S Long); Teah Smith (Zirkle Fruit Co);

Project Duration: 2-Year

Total Project Request for Year 1 Funding: $ 183,029

Total Project Request for Year 1 Funding: $ 93,786
Total Project Request for Year 2 Funding: $ 89,243

Other related/associated funding sources: None

WTFRC Collaborative Costs: None

Budget 1

Primary PI: Dr. Frank Zhao
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Station Manager/Supervisor: Naidu Rayapati
Station manager/supervisor email address: naidu.rayapati@wsu.edu

Item	1/3/2025	1/3/2026
Salaries[1]	$20,925.00	$21,762.00
Benefits[1]	$8,563.00	$8,905.00
Wages		
Benefits		
RCA Room Rental		
Shipping		
Supplies[2]	$14,398.00	$15,080.00
Travel	$2,000.00	$2,000.00
Plot Fees		
Miscellaneous-equipment[3] (ge	$8,000.00	
Total	$53,886.00	$47,747.00

Footnotes: 4% inflation for year 2. [1]Postdoc salary for 4.5 months and postdoc benefit rate at 40.9%. [2]Including budget for co-PI Scott Harper: $6000 for year 1 and $6240 for year 2. [3]Equipment for field test.

Budget 2

Co PI 2: Dr. Yinong Yang
Organization Name: Penn State University
Contract Administrator: Haessly Nachman
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Station Manager/Supervisor: María del Mar Jiménez Gasco
Station manager/supervisor email address: mxj22@psu.edu

Item	1/3/2024	1/3/2025
Salaries	$24,311.00	$25,283.00
Benefits	$8,752.00	$9,102.00
Wages		
Benefits		
RCA Room Rental		
Shipping		
Supplies	$4,837.00	$5,111.00
Travel	$2,000.00	$2,000.00
Plot Fees		
Miscellaneous		
Total	$39,900.00	$41,496.00

Footnotes: 4% inflation for year 2.

Budget 3 & 4

Co-PI 3 & 4: Dr. Scott Harper; Dr. Amit Kesharwani
Organization Name: WSU-IAREC Prosser
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Footnotes: Scott and Amit's budget is included in PI Zhao's budget 1.

Objectives:

1. **To optimize one-pot RPA/Cas12a assay for field-deployable diagnosis using Genie III device or visual detection method.**

2. **To validate, apply and recommend the field-deployable RPA/Cas12a assay for field sample diagnosis.**

Significant Findings:

- Optimized the concentration of each reagent in the reaction and time duration for one-pot DETECTR assay using a Genie III device.
- Optimized a 30-minute one-pot visual detection (VDet) under UV/blue light.
- Compared one-pot, two-pots, and VDet assays with qPCR to detect XDP.
- Designed a pin-prick method without DNA isolation for detection of XDP.
- Tested potted cherry trees for detection of XDP using pin-prick method without DNA isolation.

Methods

Procedures and Methodology. In our previous funded project (2023-2024), we have established a two-pot RPA/Cas12a method for specific, sensitive, and rapid detection of cherry X-disease phytoplasma (XDP). The drawback of this method is twofold, i.e. requiring an expensive fluorescence microplate reader and with potential cross-contamination risk due to the pipetting of amplicons required for the two-step process. In this new project, we will try to overcome these limitations and optimize a one-pot assay using a less expensive portable Genie III platform (OptiGene) and/or by visual detection (VDet).

1. One-pot RPA/Cas12a DETECTR assay

The one-pot assay combined a two-step process into a single step by merging the isothermal RPA and CRISPR-Cas12a reactions in a single tube. Briefly, the concentration of all ingredients, including rehydration buffer, RPA forward/reverse primers, MgOAc, CrRNA, FAM-reporter, NEBuffer2.1, and DNA template, were optimized and added into a PCR tube except LbaCas12a nuclease. LbaCas12a nuclease was placed on the cap of PCR tube. Initial reaction was incubated at 37°C for 15 min. After incubation, vortex and short spin the tube to mix the Cas12a nuclease into the reaction to initiate cleavage of fluorescent oligos at 37°C for 15 min. Fluorescent signals were then recorded at 37°C within 30 minutes by using Genie III device (**Fig. 1**).

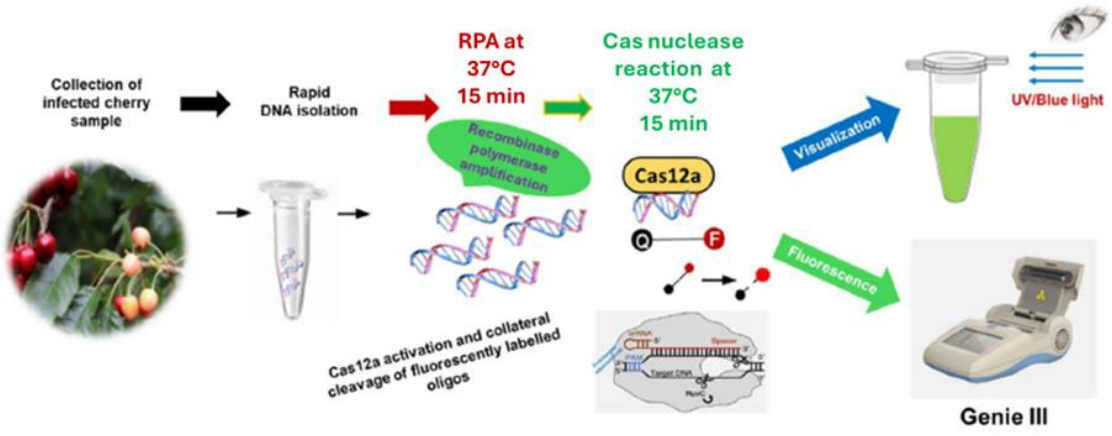

Fig 1. Illustration of one-pot DETECTR and visual detection (VDet) assay. The assay combines rapid DNA extraction, XDP amplification using RPA, followed by Cas12a detection using Genie III device or visual detection with UV/blue light (362-595 nm).

2. One-pot RPA/CRISPR-Cas12a-based visual detection (VDet)

Alternatively, fluorescent signals were checked under UV/blue light (362 to 595 nm) and visualized by naked eye (**Fig. 1**). This can also be completed by using two-pot assay.

3. Designed a Pinprick method for one-pot RPA/Cas12a DETECTR assay without DNA isolation and tested potted cherry trees for XDP

Based on a successful test detection using phytoplasma-positive phloem exudate (extracted by the Harper lab as part of the Detector Dog project), we hypothesized that the RPA/Cas12a method could be used without needing a nucleic acid extraction step. Here we used a pinprick method to obtain cherry sap for the one-pot RPA/Cas12a DETECTR or VDet assay (**Fig. 2**). Briefly, petioles of cherry leaves of known phytoplasma positive or negative plants were punched using a needle. The tip of the needle was then dipped into the one-pot reaction to mix the sap with the RPA reaction. Fluorescence signals were recorded either by Genie III device or visually checked under UV/Blue light (362-595nm) (**Fig.2**).

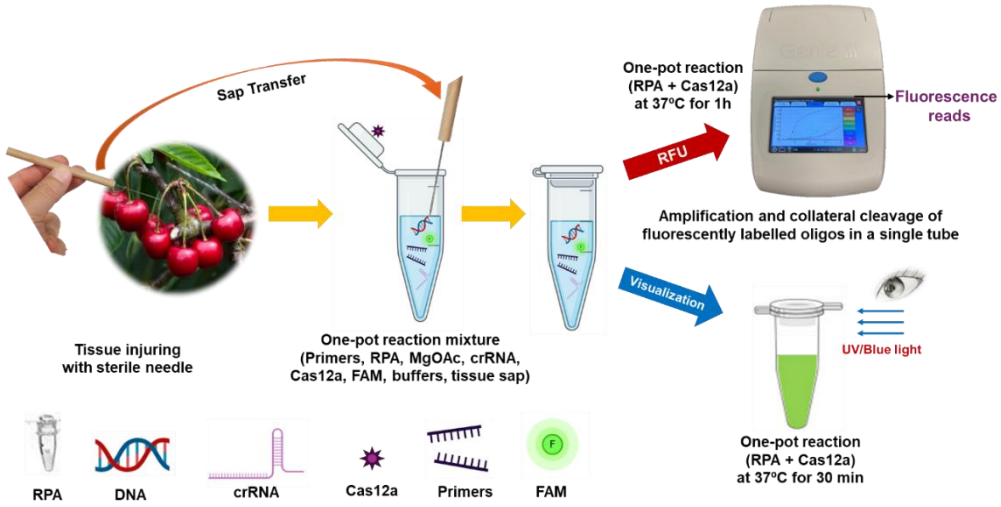

Fig. 2 Illustration of pinprick method for one-pot DETECTR and visual detection (VDet) assay. The assay used plant sap without DNA isolation and Genie III device or VDet using UV/blue light.

Types and timing of anticipated results. In the first year of the project, we optimized the one-pot RPA/Cas12a method with Genie III detection or VDet combined with either rapid DNA extract method or pinprick method without DNA isolation or tissue destruction for detection of XDP in a field-deployable way. We also validated our methods with potted cherry trees in the greenhouse. We will finalize and apply the protocol for the field-deployable one-pot RPA/Cas12a-based assay.

Potential problems or limitations. We have not encountered significant problems so far as the method is technically straightforward. However, when bacterial titer is low in a sample (with CT value >35), especially for young nursery trees, there is a possibility of false negative/positive due to sample volume (1-2 µl) tested for the experiment. On the other hand, by using a one-pot assay, we were able to largely eliminate the risk of cross-contamination by removing the need to handle/manipulate amplified DNA between the first and second stages of the two-step assay; in addition, the one-pot and two-step assays seem to have similar sensitivities. Since qPCR cannot directly use plant sap for detection due to inhibition, one-pot assay with pinprick method will be an advantage for field deployable diagnosis. In the future, we plan to compare multiple platforms, including two-pot, one-pot, qPCR and visual detection to see how to eliminate both false positive and false negative results or establish a confident threshold for detection of XDP in young cherry trees without any symptoms.

Results and Discussion:

1. Sensitivity of one-pot DETECTR assay

The sensitivity of the one-pot DETECTR assay was evaluated with estimated copy number of pure DNA dilutions of XDP. Copy number of the pure DNA sample was estimated through real-time PCR based on the Ct values as previously published. One pM (picomolar) DNA per µl were diluted up to 1aM (attomolar) DNA per µl to obtain one copy DNA per µl. These dilutions were used for one-pot RPA/Cas12a nuclease reactions using Genie III device. Time course of one-pot DETECTR assay indicated that this assay could detect fluorescence signals with ~one copy DNA (1aM) of XDP and provided the minimum fluorescence ≥ 6,500 RFU, while reached ≥ 37000 RFU with 1pM DNA (**Fig. 3A**). However, the RFU value for 1aM DNA was very close to that of negative control (**Fig. 3A**). Therefore, interpretation of the low titer DNA result should be cautious, otherwise it will lead to either false positive or false negative result. When we compared the one-pot assay with two-pot DETECTR assay, similar levels of sensitivity were obtained except the difference of the RFU value due to detection thresholds of the instruments used (**Fig. 3B**). This result suggests that the Genie III device can detect fluorescence signals using the smallest amount of XDP DNA *i.e.,* one attomolar (10^{-18}) or one copy DNA. This is comparable to qPCR which has a similar limit of detection with a high-quality DNA extract, but lower-quality extracts (i.e. those containing inhibitory compounds) can lower qPCR sensitivity. In this respect, RPA/Cas12a is better because its chemistry is not affected by inhibitory compounds present in cherry extracts.

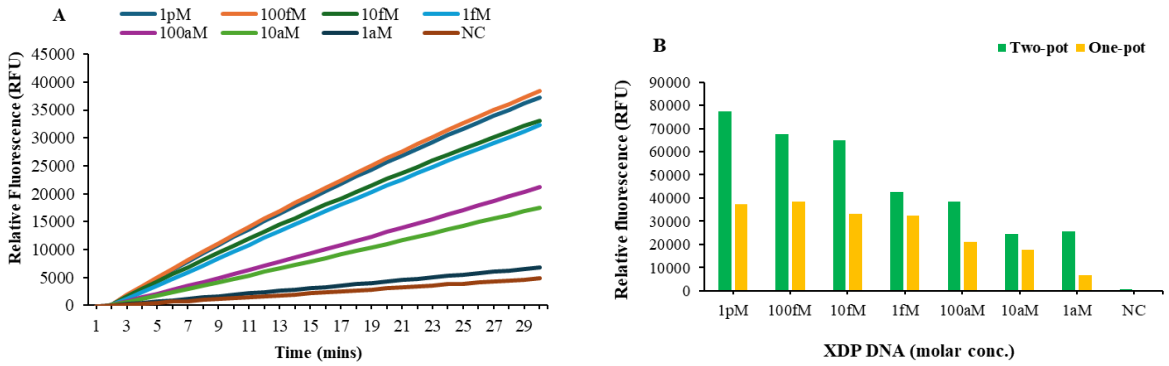

Fig. 3 Sensitivity of one-pot DETECTR assay. Dilutions of quantified DNA of the XDP *secY* gene were used for testing sensitivity of the assay. **A)** Time course of one-pot DETECTR using Genie III device. **B)** Comparing one-pot to two-pot DETECTR assay using same dilutions of XDP DNA. One-pot assay provides similar sensitivity of XDP detection as compared to two-pot DETECTR assay at one attomolar level (10^{-18} or one copy DNA/µl). NC, negative control. pM: picomolar; fM: femtomolar; aM: attomolar. RFU: Relative Fluorescence Unit.

2. Sensitivity of one-pot RPA/CRISPR-Cas12a-based visual detection (VDet) of XDP

The sensitivity of visual detection (VDet) assay was also evaluated using estimated copy number of pure DNA dilutions of XDP *i.e.,* 1pM (picomolar) to 1aM (attomolar). The reactions were incubated at 37°C for 30 minutes to visualize the fluorescence under UV/blue light (362 to 595 nm). Green fluorescence (UV/Blue light) or whitish background (UV) could be seen for dilutions of the XDP up to 1aM level, which is similar to those of the one-pot and two-pot DETECTR assays (**Fig. 4**). In practice, interpretation of the low titer DNA (1 aM) result may be difficult because visual detection is inherently subjective. However, it should be noted that the VDet assay requires only 30 mins to complete, which is about half of the time required for the one-pot or two-pot assays using plate reader or Genie III device, and as a bonus, does not require such specialized equipment.

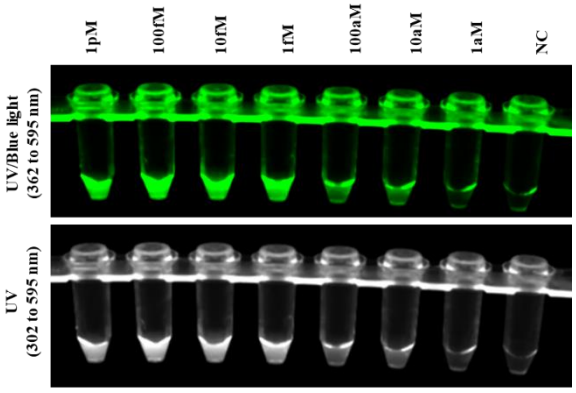

Fig. 4 Sensitivity of visual detection of XDP using one-pot DETECTR assay. Dilutions of quantified DNA of XDP *secY* gene were used for testing sensitivity of the assay. The assay could detect one attomolar level (10^{-18} or one copy DNA) within 30 minutes. The gel-imager instrument was used for visualization of green fluorescence (UV/Blue light) or whitish background (UV) using UV/blue spectrum from 362 to 595 nm. NC, negative control.

3. Comparing one-pot and two-pot, along with visual detection assays with real-time PCR using isolated DNA samples from cherry trees

For comparison, we collected tissues at 7 different spots on each tree (approx. 10 cm gaps between each spot) from six young sweet cherry trees for a total of 42 samples. Nucleic acids were isolated from these 42 samples and used for detection of XDP by one-pot, two-pot, along with visual detection assay and compared with real-time PCR assay in two labs. As shown in **Fig. 5**, 21 out of 42 DNA samples were tested positive with two-pot RPA/Cas12a based detection method and visual detection with the two-pot setup (**Fig. 5A, B**). Similarly, 21 out of 42 samples were tested positive using the one-pot assay as well as visual detection assay with one-pot setup. However, among the 21 positives, only 10 samples were commonly detected positive by both the two-pot and one-pot assays as well as visual detection assay (**Fig. 5AB**, data not shown for the one-pot visual detection).

Of the 42 samples, only 7 and 6 samples out of 42 were tested positive using real-time PCR with Ct values > 35 in two labs, respectively. However, 3 out 7 were commonly tested positive in both labs. Furthermore, only one sample out of 42 was tested positive by all three methods. These results indicate that when the pathogen titer is relatively low (Ct value > 35), a) the one-pot, two pot, and VDet assays are effective than qPCR, because the RPA/Cas12a is not as affected by inhibitory compounds as qPCR, and b) pipetting DNA from a solution, as opposed to water/buffer, becomes an issue when concentration is low and unless large numbers of replicates are run, or unless the reaction volume is increased to use all of the extracted sample, reproducibility is, and will remain, an issue.

4. A pinprick method for one-pot and visual detection (VDet) assay without DNA isolation

Instead of isolating DNA from cherry samples, we tested cherry samples without nucleic acid extraction using a needle-pinprick method (**Fig. 2**). Cherry trees were obtained from an undisclosed commercial source and potted in greenhouse. Samples were also collected from a greenhouse-grown apple tree. The tip of the needle was dipped into the one-pot reaction to mix the sap with the RPA reaction. Fluorescence signals were recorded either by Genie III device or visually checked under UV/Blue light (362-595nm) (**Fig. 6**). Among the five trees tested, two cherry trees and one apple tree showed positive (S2, S5, and S6).

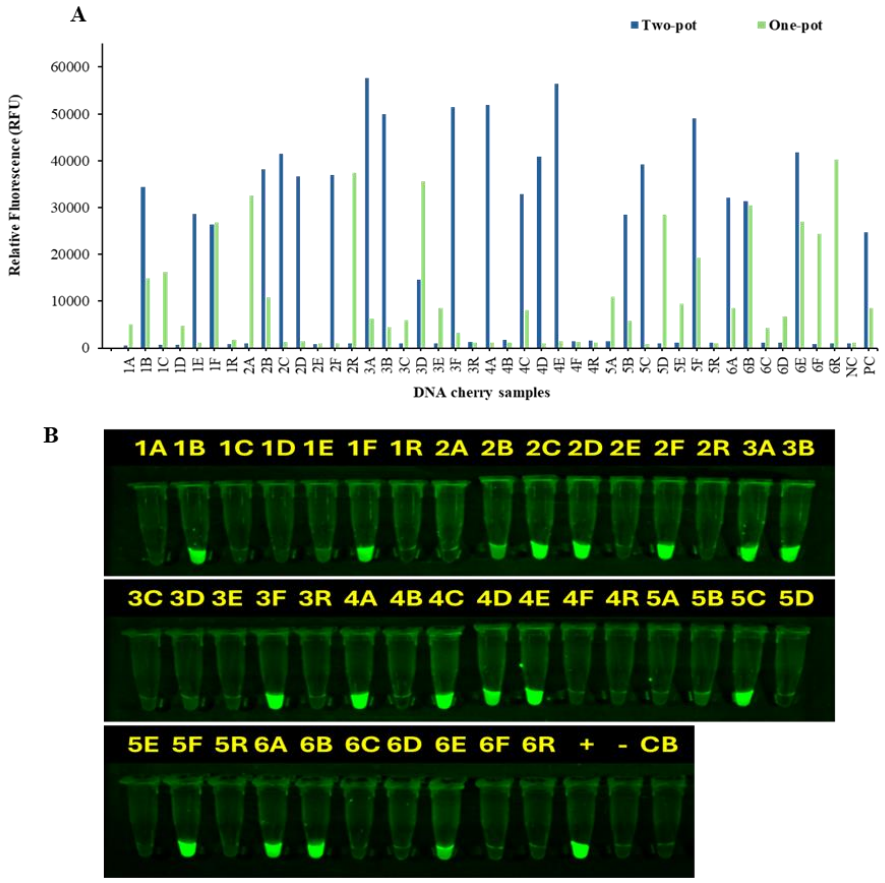

Fig.5 Comparative analysis of one-pot, two-pot, and visual detection of XDP with real-time PCR. A) Fluorescence signals of 42 cherry DNA samples after detected by one-pot and two-pot assays. B) Visual detection set up along with two-pot assay for 42 cherry DNA samples. PC, positive control; NC, negative control.

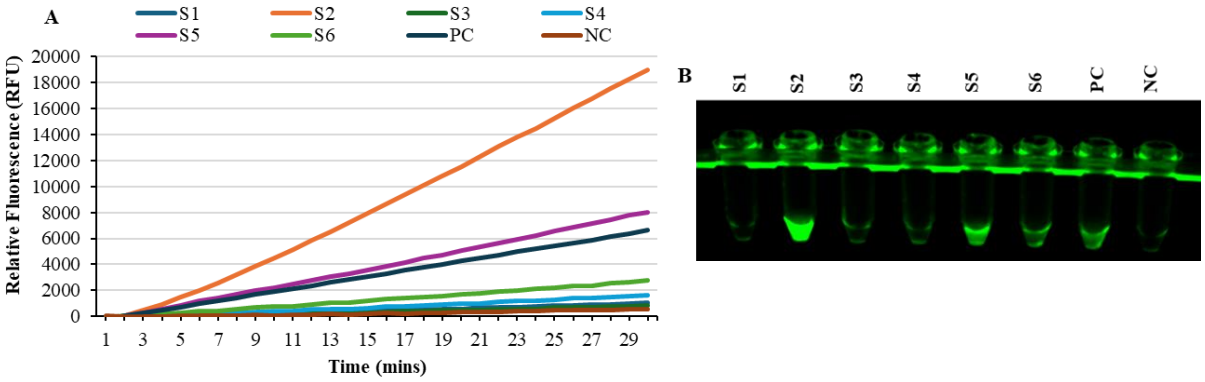

Fig. 6. A pinprick method based one-pot and visual detection (VDet) assay using RPA/Cas12a. A) XDP detection in cherry and apple plant samples using Genie III device. **B)** VDet of cherry and apple plant samples using UV/blue light (362-595 nm). PC: positive control; NC: negative control; S1 to S5 - cherry samples; S6- apple sample. Three out of six samples were detected positive with the pinprick method.

5. Comparing one-pot, two-pot, and VDet assays using the pinprick method

Three cherry trees obtained from an undisclosed commercial source and maintained in pots in greenhouse were used for the comparison. Saps from the petioles of 5 different spots per cherry tree were obtained (bottom to top) using dissection needles and dipped into the one-pot, two-pot, and VDet reactions (**Fig. 7**). The reactions were incubated at 37°C (98.6°F) for 1h using Genie III device for one-pot DETECTR assay to obtain the fluorescence signals and 30 mins for VDet assay to visualize the detection with UV/blue light, respectively. A total of 18 samples (6 x 3), including 3 apple samples were tested and compared. As shown in **Fig. 7**, 11 and 10 samples were tested positives for XDP by one-pot/VDet assay and two-pot assay, respectively (**Fig 7A, B**) and 10 samples were commonly detected positive by all three assays. This pinprick method can not be used for qPCR as it requires nucleic acid extraction to remove the inhibitors present in the sap. Therefore, it was not included as a comparison.

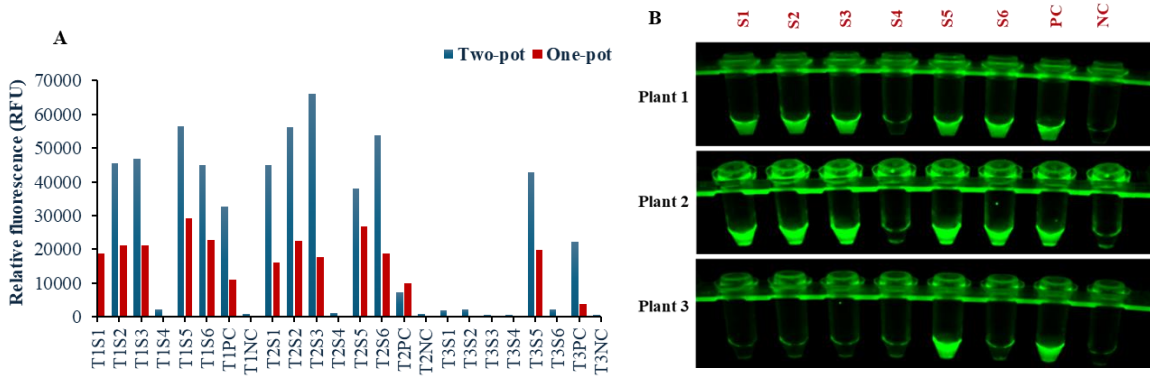

Fig. 7. Comparative analysis of one/two-pot/VDet detection of XDP based on RPA/Cas12a using pinprick method. A) Pinprick method for XDP detection with one-pot and two-pot reactions using Genie III device and fluorescence microplate reader, respectively. Difference was noticed in RFU values that corelates with the instrument's thresholds. **B)** Illustration of pinprick based one-pot VDet of XDP using UV/blue light (362-595 nm). PC: positive control; NC: negative control. T1-T3 – tree numbers; S1-S5 - cherry sample numbers from the same tree (Plant 1 to 3); S6- apple sample.

Significance to the industry and potential economic benefits.

A reliable and inexpensive method for early disease detection in non-fruit-bearing trees and in non-symptomatic trees/rootstocks in both orchards and nurseries is critically needed for the cherry industry, as this would not only save growers and nurseries diagnostic costs, but also reduce economic losses due to tree removal and replacement. However, the major challenge for early detection of XDP in non-fruit-bearing trees and in non-symptomatic trees/rootstocks is due to its low titer and uneven distribution of the pathogen within infected plants. This will potentially lead to false positive and false negative results due to the DNA volume tested in each reaction (1-2 µl) if copy number is below the limits of detection of the currently used qPCR assays, whereas no molecular method can detect the pathogen if it isn't in the small piece of tissue (i.e. 100 mg, or about the size of a penny) collected and in the solution tested.

The RPA/Cas12a-based one-pot and two-pot assays we have developed can detect one copy of DNA in a sample, and unlike qPCR, is not as susceptible to producing false negatives due to inhibition of its chemistry by compounds present in cherry. This also allowed us to develop a pinprick method without DNA isolation, which has its advantage by not destructively sampling trees. The pinprick method also allows users to skip the nucleic acid extraction process, saving time and money and allowing-in field diagnosis of phytoplasma infection. However, more work remains to be done by verifying and standardizing the RPA/Cas12a method based on pinprick method to turn it into an end-user diagnostic tool.

Project Title: Precision Agriculture: Innovating Cherry Disease Management with DPI
Report Type: Continuing Project Report

Primary PI: Marco Pitino

Report is Forthcoming

Project Title: Sustainable Cherry Protection with Symbiont
Report Type: Continuing Project Report

Primary PI: Marco Pitino

Report is Forthcoming

Project Title: *Ganaspis brasiliensis* for Biological Control of SWD
Report Type: Continuing Project Report

Primary PI: Christopher Adams
Organization: OSU
Telephone: 248-850-0648
Email: chris.adams@oregonstate.edu
Address: 3005 Experiment station drive
Address 2:
City/State/Zip: Hood River, OR 97031

Cooperators:
Mike Omeg, Stacey Cooper, Brian Nix

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $10,120
Total Project Request for Year 2 Funding: $10,422
Total Project Request for Year 3 Funding: $10,922
Total Request: $31,464

Other related/associated funding sources: N/A

WTFRC Collaborative Costs:

Budget 1
Primary PI: Christopher Adams
Organization Name: OSU
Contract Administrator: Charlene Wilkinson
Telephone: 541-737-3228
Contract administrator email address: Charlene.wilkinson@oregonstate.edu
Station Manager/Supervisor: Brian Pearson
Station manager/supervisor email address: brian.pearson@oregonstate.edu

Item	2023	2024	2025
Salaries[1]	$5,794.00	$5,967.00	$6,147.00
Benefits	$4,326.00	$4,455.00	$4,775.00
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies[2]			
Travel[3]			
Plot Fees			
Miscellaneous			
		*total	
Total	$10,120.00	$10,422.00	$10,922.00

Footnotes:
[1]FRA salary: $4,166 x 12 mo x 10% FTE = $5,794 for year 1 x 1.03 inflation rate /yr. Benefits at 73%

***Total - I have three copies of this document in my files. I have copies where this middle year is wrong at $4,000. Not sure which version you have on file at WTFRC, but if there is a discrepancy that is why.**

Objectives

1. Establish and increase a colony of *Ganaspis kimorum* (formally *G. brasiliensis*) wasps.

Deviations: As part of this project, we established colonies of three parasitoid wasps; *Ganaspis kimorum* (formally *G. brasiliensis*), *Leptopilina japonica,* and *Pachycrepoideus vindemmiae*. We struggled to maintain our target wasp, *G. kimorum*, and were unable to reestablish this colony after it collapsed.

2. Conduct releases at selected orchards (years 2 & 3)

Deviations: We were unable to release *Ganaspis kimorum*. However, we released both *Leptopilina japonica* and *Pachycrepoideus vindemmiae*.

3. Measure establishment of wasps (year 3)

Significant findings

- In our initial year zero surveys for SWD parasitoids we used infested fruit. Those surveys recovered *Leptopilina japonica,* an introduced SWD parasitoid, and *Pachycrepoideus vindemmiae,* a native generalist pupae parasitoid of several fly species including Drosophila and Tephritidae. Those insects were brought into our insectary and reared as additional biocontrol wasp species.
- We started three parasitoid wasp colonies, *Ganaspis kimorum, Leptopilina japonica,* and *Pachycrepoideus vindemmiae* in an effort to hedge our bets and increase our impact. While we struggled with *Ganaspis kimorum*, we have released 1075 *Leptopilina japonica* at 20 locations across Hood River and The Dalles OR, and 100 *Pachycrepoideus vindemmiae.*
- Establishment of released wasp was measured by placing cup traps baited with apple cider vinegar. A total of 67 SWD parasitoid wasps were recovered in year three of this project (Table 1). Wasp have been collected and identified to genus, but not to species. Some key identifying characteristics are extremely small and require magnification beyond what we have in the lab. Species level identification will be completed in the coming months. Parasitic wasps of SWD were recovered at 95% release locations. Genetic work will need to be done to determine if these wasps were adventive (arrived on their own) or augmentative (released by us) and now established populations.

Methods

1. **Establish a colony of *Ganaspis kimorum* wasps**. Rearing protocols have been published by several authors (Rossi-Stacconi et al. 2022) and I have visited with ODA to see their colony rearing procedures (Figure 1). Fresh organic blueberries are purchased, washed in bleach water, and presented to SWD adults. Female flies are allowed to lay eggs in fruit. Fruit and developing larvae are then moved to a new container (Figure 1) with adult wasps. Female wasps must find developing larvae and inject and egg with their ovipositor (stinger). The SWD larvae continues to eat and grow, as the wasp larvae develops inside the SWD maggot. The wasp can take up to a month to develop.

2. **Conduct releases of *G. kimorum* at selected orchards**. Several orchard locations have been selected, and we are scouting for others. We have been collecting year zero data on the parasitoid wasp populations during the first year as we built the colony. Release sites will be selected based on suitable habitat outside cherry orchards where SWD populations are likely to escape pesticide sprays.

3. **Measure establishment of wasps**. In year three we will measure establishment of introduced wasps by collecting fruit from release locations. Fruit will be brought back to the lab and held to observe the emergence of flies and wasps. Emerged wasps will be sent for positive identification. We will describe the habitats where wasp establishment is most successful and report findings in extension and peer reviewed publications. The effectiveness of outside-orchard habitat supporting beneficial insects will be communicated to stakeholders at grower meetings.

Figure 1. *Ganaspis kimorum* rearing container (left). Blueberries are placed in SWD colony to allow flies to lay eggs. Blueberries with eggs and young SWD larvae are placed in containers with newly emerged wasps, *Ganaspis kimorum* (right) on a penny for scale.

Results and Discussion

Rearing wasps. Because of the conditions in which SWD larvae live (rotting fruit) rearing wasps is difficult and often challenged with mold (Figure 2, right) and other arthropod pests like mites (Figure 3). Researchers around the country have had similar challenges. Both ODA and USDA have experienced similar struggles and loss of their *Ganaspis kimorum* colonies. Typically, we share insects with and between other labs as needed, to help start or augment insect colonies. However, because of these challenges, we have not been able to borrow wasps from others to restart our *Ganaspis kimorum* colony, because everyone is struggling to build and maintain this species.

The established methods for rearing this wasp requires using fresh blueberries. We had successfully started a colony of *Ganaspis kimorum*, however we lost our colony from excessive mold. Using fresh blueberries is a major bottleneck for this project because, even after bleach water washing, mold continually overtakes the berries and destroys fruit before wasps can emerge. Conversely, a slight decrease in humidity causes blueberries to desiccate and dry up (Figure 2, left) before SWD larvae and wasp parasitoids can develop. This balancing act is the biggest hurdle to scaling up this project. A regional working group is working on ways to improve rearing techniques and to develop an artificial diet to replace the need for fresh fruit.

New developments. To address the challenges of mold and desiccation and mites associated with fresh fruit, we developed a new SWD diet. Wasps appear to require the host volatile cues (smell) of infested blueberries during their search for SWD larvae and will not search for larvae in the standard SWD diet (starch and agar-based mix). Our lab has developed a new diet using frozen blueberries incorporated in the standard agar-based fly diet (Figure 4). *Leptopilina japonica* has been successfully reared on this new frozen blueberry and auger-based diet mix. Future research will look at rearing *Ganaspis kimorum* on this new diet.

Establishment. In year three SWD monitoring traps recovered a total of 67 SWD parasitoid wasps (Table 1). While promising these numbers do not represent a sufficient population of SWD parasitoids to significantly impact SWD populations. Riparian areas outside of managed orchards are critical to survival and establishment of parasitoids. While not a pesticide replacement, parasitoids are an important part of an overall IPM strategy against SWD.

<u>Resulting peer reviewed publications from this study.</u>

Gariepy T.D., Abram P. K. Adams C., Beal D., Beers E., Beetle J., Biddinger D., Brind'Amour G., Bruin A., Buffington M., Burrack H., Daane K.M., Demchak K., Fanning P., Gillett A., Hamby K., Hoelmer K., Hogg B., Isaacs R., Johnson B., Lee J.C., Levensen H.K., Loeb G., Lovero A., Milnes J.M., Park K.R., Prade P., Regan K., Renkema J.M., Rodriguez-Saona C., Neupane S., Jones C., Sial A., Smythman P., Stout A., Van Timmeren S., Walton V.M., Wilson J.K., Wang X. Widespread establishment of adventive populations of *Leptopilina japonica* (Hymenoptera, Figitidae) in North America and development of a multiplex PCR assay to identify key parasitoids of *Drosophila suzukii* (Diptera, Drosophilidae)

Figure 2. Blueberries experiencing both desiccation (left) and excessive mold (right). Because these wasps must be reared in fresh fruit, we struggle with both too much and too little humidity. Mold is the biggest challenge to rearing these wasps, followed by mites.

Figure 3. *Ganaspis kimorum* covered in mites. Photo courtesy Max Ragozzino ODA. Mites are a constant struggle in the insect colonies. Even with our bleach water washing protocol, fresh fruit from the grocery store seems to reintroduce mites.

Figure 4. New frozen blueberry and artificial diet recipe.

Table 1. Capture of SWD parasitoids in SWD monitoring traps in year 3 (2025)

Wasp recapture in SWD monitoring traps 2025				
Genus:	Ganaspis sp	Leptopilina sp	Pachycrepoideus vindemmiae	other
Site #				
1	1	5		2
2		4		5
3			7	
4				6
5		2		
6	3		7	
7			2	1
8		3		
9			2	2
10			2	2
11	2			
12			3	
13	1			
14			5	1
15		2	2	
16			4	
17		1	1	2
18			3	
19	1			
20			4	1
total	8	17	42	22

Project Title: SWD in-orchard movement and overwintering population dynamics

Report Type: Final Project Report

Note* - This is a winter project. Everything has been offset by one year.

Primary PI: Christopher Adams
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Address: 3005 Experiment station drive
Address 2:
City/State/Zip: Hood River, OR 97031

Cooperators:
M3 Agriculture Technologies

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $43,952
Total Project Request for Year 2 Funding: $41,137
Total Project Request for Year 3 Funding: $42,254
Total Request: $127,343

Other related/associated funding sources: not funded
Agency name: Helium Foundation, IoT grant
Amount: $100,000
Funding duration: 2021 - 2023
Status: Preproposal approved and highly ranked. Leadership at the foundation changed while in review for the full proposal, and project was not funded.

WTFRC Collaborative Costs:

Budget 1
Primary PI: Christopher Adams
Organization Name: OSU
Contract Administrator: Charlene Wilkinson
Telephone: 541-737-3228
Contract administrator email address: Charlene.wilkinson@oregonstate.edu
Station Manager/Supervisor: Brian Pearson
Station manager/supervisor email address: brian.pearson@oregonstate.edu

Item	2021	2022	2023
Salaries[1]	$21,266.00	$21,904.00	$22,561.00
Benefits	$14,886.00	$15,333.00	$15,793.00
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies[2]	$6,800.00	$2,900.00	$2,900.00
Travel[3]	$1,000.00	$1,000.00	$1,000.00
Plot Fees			
Miscellaneous			
Total	$43,952.00	$41,137.00	$42,254.00

Footnotes:
[1]Adams lab Faculty Research Assistant at 0.40 FTE (2 days /wk), with 3% increase in years 2 and 3; OPE 70%
[2]Dragino LHT65 temp & humid sensor ($50 each) x 80, Long Range Transceiver ($450 each) x2
& Research consumables, SWD bait and traps (40 traps per rep x 3reps = 120 traps) bait replaced weekly for 24 weeks (Jul-Dec) aprox. $100 x 24 wk = 2400,
[3]Travel to field plots

Objectives

1) Collect micro-climate temperature data and trap for overwintering SWD. (Year 1 beginning fall/winter 2022)

   Deviations: Because of an early snow in 2022 we collected very little catch data in the first year.

2) Correlate terrain, vegetation and microclimate temperature data with trap counts to determine if specific in-orchard habitats are more favorable to overwintering SWD. (year 2 & 3)

3) Determine if overwintering SWD can be targeted with off season sprays or attractive baits. (yr 3)

   Deviations: We had planned to target overwintering locations with bait sprays in the 3rd year of the project. However, because the early snow fall cut off trapping data in the first year, we would like to first collect two good seasons of trapping data before we attempt to target these populations with a spray. We will plan to target overwintering populations outside the managed orchard, in year four.
   Deviation: One of the key orchards was removed this past year and so a replacement orchard will need to be identified. One season of data is probably not sufficient to draw reasonable conclusions. I full 3-year proposal has been submitted to properly address this objective.

Significant findings

- We established 4 orchard blocks in The Dalles and installed 20 remote temperature and humidity sensors in each, with wireless a network, paired with 20 insect traps. (Figure 2). We were able to successfully collected a grided network of hourly temperature data across these orchards and transmit that data to the cloud for remote data collection.
- Winter micro-climate temperatures are not correlated with SWD catch data.
- Elevation was not correlated with higher overwinter SWD catch data.
- The specific habitat (oak or conifer) was not correlated with higher SWD catch data.
- Snow cover (not cold weather) significantly affects SWD winter catch data (SWD activity).
- In year two we saw a pattern in all four orchards. Large numbers of SWD are present throughout managed orchards in late fall (October). By early November, the majority of catch data is recorded in traps just outside the border of the managed orchard, in oak or pine habitats. In some cases, “habitat” might just be 2 or 3 pine trees.
- It is not clear if SWD are *surviving* better in off-site habitats (reproducing) or if they are *moving* to these habitats for shelter or food.
- There is a larger SWD population in the off season than during the growing season. This means that sprays applied at this time would produce a much greater return on investment (kills per dollar) than sprays applied during the growing season. Attract and kill strategies could be extremely impactful to SWD populations. A full 3-year proposal has been submitted to address this question.
- Erythritol (non-sugar sweetener) was applied as a non-pesticide winter control tactic in a separate un-funded on-farm field trial in The Dalles. Results were inconclusive. We plan to look at Combi-ProTec in combination with a pyrethroid or OP in subsequent experiments.

Brief Overview

The inspiration for this research came from visiting an orchard in The Dalles (Figure 1) and observing that there were no wild resources outside of the managed cherry orchard. The conventional wisdom is that SWD leave cherry orchards after harvest to find suitable overwintering sites. In addition, this area experiences high winds most of the year, so flies might struggle with directed migration, and might need to seek shelter in place, alternatively we might see catch increase in downwind populations. At the center of the orchard is a valley with some wild blackberry habitat. Our hypothesis was that overwintering SWD would move to the valley at the center of the orchard to seek shelter for the winter, or find microclimate locations with favorable conditions. To capture the microclimate across the orchard we partnered with M3 Agriculture Technologies to equip the orchard with 20 temperature and humidity readers and connected them to a central hub modem with cell phone connectivity for remote data access (Figure 1). Each temperature reader was paired with a baited trap. While catch data from the traps in the valley was the highest (>4000), other traps still caught SWD at very high levels (1000s). This would suggest that flies are not moving out of the orchard, but are seeking shelter within the orchard. How and where SWD overwinter, and what environmental conditions SWD need to survive is still unclear. Many questions remain to be answered about winter movement. This project was designed to expand this initial research project to three other orchards and try to replicate these results, to understand winter survival of this important invasive species.

Methods

1. Collect micro-climate temperature data and trap for overwintering SWD.

Site selection. In fall of 2022 four orchards were equipped with an array of temperature and humidity sensors and a central solar powered modem to collect and transmit data. Each temperature sensor was paired with a baited (apple cider vinegar and wine) cup trap. Traps were deployed in a grided pattern across each block, with the last row of traps on a border (where possible) outside the orchard.

SWD data collection. Traps were checked weekly or bi-weekly (every other week) from October through February in 2022/2023, and 2023/2024. Because of the volume of traps (80 traps spread across The Dalles) SWD numbers were estimated* when catch in the traps was high, e.g. above 100 (*note even numbers in catch data on maps of Fig.2). SWD parasitoids were also collected when they got caught in monitoring traps.

Temperature monitoring. Remote Sensors collect temperature and humidity data hourly and send data to the cloud for storage (Figure 1)

2. Correlate terrain, vegetation and microclimate temperature data with trap counts to determine if specific in-orchard habitats are more favorable to overwintering SWD. (year 2 & 3)

Data analysis. We tried to find correlation of temperature, humidity, elevation, and habitat with SWD trap catch data. Only habitat was correlated with high late season trap catch data.

3. Determine if overwintering SWD can be targeted with off season sprays or attractive baits.

Insecticidal trails. Bait sprays will be applied in year four after (we hope) we see two consecutive years of catch data to document overwintering locations in all four blocks.

Results and Discussion

SWD catch data showed a similar pattern in most orchards (Figure 2). Traps hung at the edge of the blocks in pine, cedar, or along oak fragment habitat caught more flies in late winter. Often the catch in these border traps was in the thousands in November and December. In some cases, the "outside orchard" habitat was just a couple of pine trees within the larger orchard. A severe cold snap in January of 2024 did not completely shut down catch of SWD; catch followed a downward trend, but we still saw catch data in February (Figure 4). Snowfall ***did*** completely shut down all catch in traps early in the fall 2022.

There was some difference in catch data between orchards, with some blocks still catching hundreds of SWD in the interior of the block, while others dropped to zero within the cherry trees. Elevation and micro-climate humidity and temperature were not correlated with high catch data. High catch within the trapping grid did not correlate with prevailing winds at any of the locations.

Orchards 2, 3, and 4* had no other wild berries in the orchard or in the surrounding outside orchard habitat (*orchard 4 has a personal raspberry planting. A trap placed at this location was not the highest catching trap of that block). Orchard 1 had extensive wild Himalayan blackberry throughout the orchard that was *all* removed in the winter/summer of 2022, and currently has no more fruiting (non-cherry) food sources for SWD in or around the orchard. With no outside habitat the possibility for completely eliminating SWD in this isolated orchard seems possible.

We do not have any data to understand if flies are *moving* from the interior of the orchard to the pine or oak habitat, where we get thousands of flies in the monitoring traps in November and December. However, there are no resources within the orchards post-harvest, other than a handful of fallen cherries. SWD has a *winter morph,* which is a slightly different physical form that is more robust looking, and designed to over-winter. This winter-morph is thought to suppress reproductive activity, slow its metabolism and simply *survive* the winter. We do not know how the population goes from near zero (most monitoring traps in Wasco Co. catch zero during June, July, and August, see Fig. 5) to the thousands we find in November. It is likely that flies are reproducing across the landscape in non-cherry fruit such as Himalayan blackberries.

Figure 1. SWD trap, temperature sensor, and solar panel powered modem for data collection.

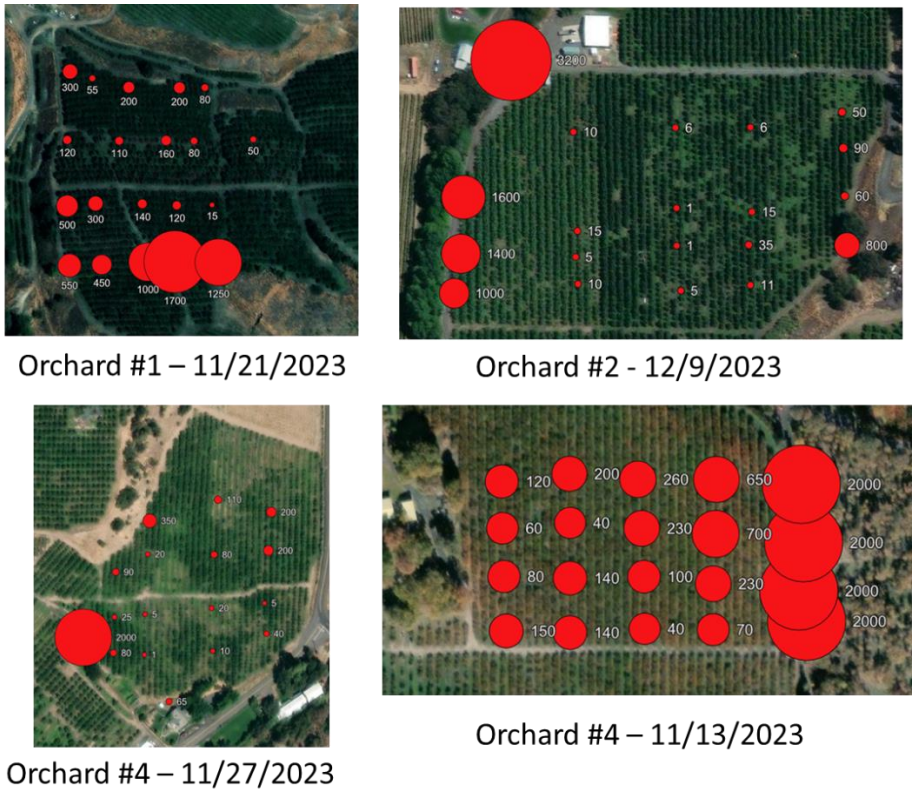

Figure 2. Catch data in the four orchard blocks in late November and early December of 2023. Red dots indicate locations of temperature sensors and SWD traps. Size of the red dots is relative to catch data. Numbers indicate estimated catch for the week. Blocks are between 5 and 10 acres with elevation change of 5 – 20 meters.

Figure 3. Visualization of hourly temperature data across block #4, showing variable temperatures (in °C) across the orchard. Micro climate temperature and humidity did not correlate with increased SWD catch in traps.

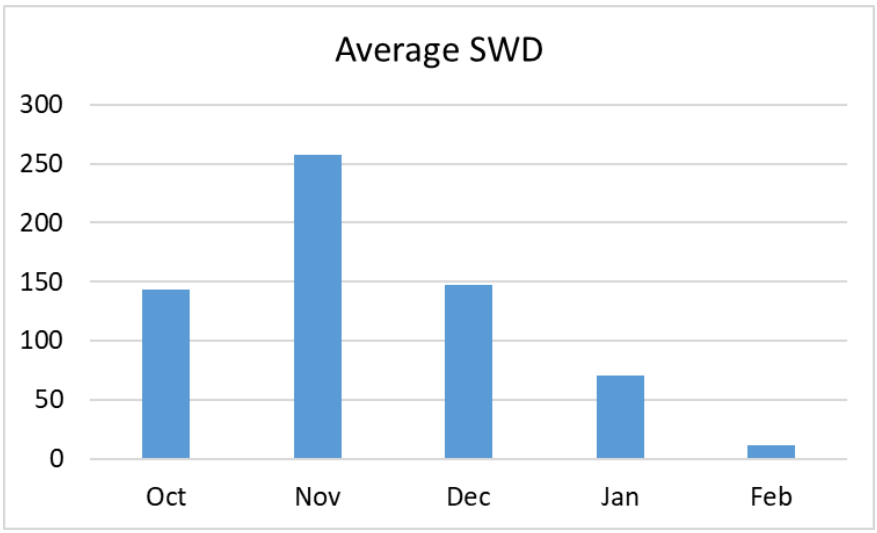

Figure 4. Winter 2023/2024, average catch per trap across all four sites (n = 80 traps) by month. The graph shows peek catch of SWD is in November. This indicates when a pesticide spray or an attract and kill bait would be most effective. This indicates where the control tool would need to be effective, not necessarily when it would need to be applied. For example, an attractive bait applied in September would need to still be viable and effective in November and December to target peek winter populations.

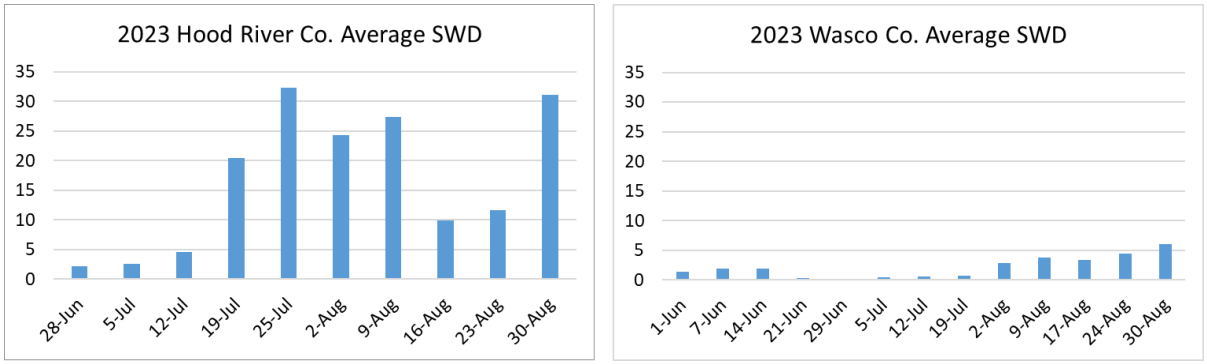

Figure 5. Summer 2023 average catch per trap data across our trapping network in both Hood River and Wasco counties. Data shown for comparison to winter catch (Figure 4). Prophylactic protective sprays are necessary when fruit is present on the trees and *must* be applied regardless of catch in monitoring traps. However, control tactics targeting off-season SWD could be much more impactful and possibly reduce the pressure/population in the following season much more that in-season sprays.

Project Title: Pesticide residues of PNW cherries
Report Type: Final report

Primary PI: Tory Schmidt
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Cooperators: Gerardo Garcia (WTFRC), Northwest Horticultural Council, Pacific Agricultural Labs (Sherwood, OR), OMIC USA Labs (Portland, OR), Orondo Orchards, Gale Fource Orchards, WSU Sunrise Orchard

Project Duration: 3 Years

Total Project Request for Year 1 Funding: $ 6000
Total Project Request for Year 2 Funding: $ 6250
Total Project Request for Year 3 Funding: $ 6500

Other related/associated funding sources: Most chemical products donated by registrants; sample shipping and lab fees for prohexadione residue testing paid by registrants

Primary PI: Tory Schmidt
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Item	2023	2024	2025
Salaries			
Benefits			
Wages1	$1,500.00	$1,600.00	$1,700.00
Benefits1	$800.00	$850.00	$900.00
RCA Room Rental			
Shipping2	$400.00	$425.00	$450.00
Supplies	$300.00	$300.00	$300.00
Travel3	$1,000.00	$1,025.00	$1,050.00
Plot Fees			
Miscellaneous			
Analytical lab fees	$2,000.00	$2,050.00	$2,100.00
Total	$6,000.00	$6,250.00	$6,500.00

Footnotes:
Schmidt estimates 8% of his time is dedicated to this project on an annual basis
Most pesticides tested are donated by their registrants or an ag chemical supply company
1 Wages & benefits primarily for Garcia (spray applications), crew help for Garcia, and Stone (data entry & review)
2 Est. costs to ship cherries overnight to Sherwood, OR
3 Travel costs include hauling equipment to & from plots

2025 WTFRC CHERRY PESTICIDE RESIDUE STUDY

Since 2011, the WA Tree Fruit Research Commission has conducted annual studies of residues of commonly used pesticides on cherry fruit at harvest. Digital versions of this report and similar studies on apple and cherry including comprehensive summaries of multiple years' results are available at www.treefruitresearch.org. For current information on maximum residues levels (MRLs) and other regulatory issues, please consult the Northwest Horticultural Council website at https://nwhort.org/export-manual/.

Spraying trial block at 200 gal/acre

TRIAL DETAILS

- Trial conducted in mature 'Skeena'/K.6 central leader trees on 10' x 16' spacing near East Wenatchee, WA
- 15 insecticides/acaricides, 3 fungicides, and 1 plant growth regulator were applied at or near maximum rates and minimum pre-harvest and re-treatment intervals; products were applied twice as allowed by product labels
- Applications made by Rears PakBlast PTO-driven airblast sprayer with 8 oz non-ionic surfactant (Regulaid)/100 gal water at 200 gal water/acre
- Roughly 0.01" of rain fell on the trial block on June 21; this precipitation likely did not affect residues on the fruit
- Fruit samples shipped overnight to Pacific Agricultural Labs (Sherwood, OR) for standard residue analysis and OMIC USA Laboratory (Portland, OR) for analysis of prohexadione calcium residues

RESULTS & DISCUSSION

Through the years, the primary objective of these studies has been to simulate a *worst case scenario* for residues of legally applied pesticides by using aggressive rates, timings, and spray intervals. As in the past, most materials were applied twice as allowed by product labels, whether or not typical commercial use patterns would do the same. With that approach, **all 2025 residues complied with domestic tolerances but some exceeded foreign MRLs for important export markets: Kudos 27.5WDG, Bexar, Esteem, Asana XL, Nealta, Torino, and Carbaryl 4L.**

While residue levels for prohexadione calcium (Kudos 27.5WDG) in this study are concerning, it should be noted that prohexadione products are rarely, if ever, applied at these late timings; residues from plots sprayed at at more typical industry rates and timings were found to be considerably lower in a separate 2025 WTFRC trial to evaluating various prohexadione calcium programs on Sweetheart cherries. Those results may be found in a supplemental summary focused on prohexadione calcium residues available at www.treefruitresearch.org.

MRLs are known to change frequently and cherry producers should routinely monitor the most current information (https://nwhort.org/export-manual) to facilitate compliance with constantly evolving foreign standards.

Dried residues on cherries at harvest

Measured residue levels vs. MRLs for pesticides applied to cherry fruit at 200 gal water/acre. 'Skeena'/K.6, East Wenatchee, WA. WTFRC 2025.

Common name	Trade name	Application rate[1]	Application timing(s)	Measured residue	US tolerance[2]	Lowest export tolerance[2]
		per acre	*days before harvest*	*ppm*	*ppm*	*ppm*
prohexadione calcium	Kudos 27.5WDG	20 oz	35, 21	**0.40**	0.4	0.01 (THA)
tolfenpyrad	Bexar	27 oz	28, 14	**0.39**	2	0.01 (many)
pyriproxyfen	Esteem	16 oz	28, 14	**0.48**	1	0.01 (THA)
thiamethoxam*	Actara	5.5 oz	21, 14	0.183	0.5	0.5 (many)
esfenvalerate	Asana XL	14.5 oz	21, 14	**0.17**	3	0.01 (THA)
lambda-cyhalothrin	Warrior II	2.56 oz	21, 14	0.17	0.5	0.3 (many)
chlorantraniliprole	Altacor eVo	2.2 oz	21, 11	0.11	2.5	0.5 (KOR)
cyflumetofen	Nealta	13.7 oz	21, 7	**0.15**	1.5	0.02 (AUS)
quinoxyfen	Quintec	8.7 oz	21.7	0.055	0.7	0.4 (many)
cyclaniliprole	Verdepryn 100SL	11 oz	14, 7	0.082	1	0.6 (TWN)
cyflufenamid	Torino	8 oz	14, 7	**0.097**	0.6	0.01 (THA)
flonicamid	Beleaf 50SG	2.8 oz	14, 7	0.28	0.6	0.6 (many)
emamectin benzoate	Proclaim	4.8 oz	14, 7	<0.01	0.09	0.005 (THA)
carbaryl	Carbaryl 4L	96 oz	11, 4	**3.1**	10	0.01 (THA)
zeta-cypermethrin	Mustang Maxx	4 oz	11, 4	0.26	2	1 (KOR)
spinosad	Entrust SC	6.8 oz	11, 4	0.021	0.2	0.2 (many)
pydiflumetofen	Miravis	5.1 oz	11, 1	0.15	2	2 (many)
mefentrifluconazole	Cevya	5 oz	11, 1	0.30	4	1.5 (TWN)
hexythiazox	Onager	24 oz	7	0.17	1	0.2 (KOR)
pyrethrins	Pyganic 5.0EC	15.6 oz	4, 1	<0.05	1	0.01 (THA)

[1] All materials were applied by Rears PakBlast sprayer with 8 oz Regulaid/100 gal water
[2] Major export markets for Pacific Northwest cherries; 29 July 2025. http://mrldb.nwhort.org/#top_markets
* Reported thiomethoxam values reflect sum total of thiomethoxam and clothianidin residue levels

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

WASHINGTON TREE FRUIT RESEARCH COMMISSION

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

Preliminary findings of prohexadione calcium residues on cherries Supplemental report – WFTRC 2025

Due to industry concerns about the lack of posted Maximum Residue Levels (MRLs) for prohexadione calcium in Codex and several key Asian export markets, combined with the relative dearth of information regarding those residues on cherries, the Washington Tree Fruit Research Commission (WTFRC) conducted extended testing of residues from two cherry trials where Kudos 27.5WDG was sprayed in 2025. Applications were made in both trials with a PTO-driven Rears Pak-Blast calibrated to 200 gal/acre with 12 oz of a non-ionic surfactant (Regulaid) and 16 oz of ammonium monosulfate (Bronc) per 100 gallons of water per standard industry practices. All fruit samples were held in cold storage (34 F) and shipped overnight to OMIC USA Laboratory (Portland, OR) for analysis of prohexadione calcium residues. Special thanks to Fine Americas and Kumiai America for helping support these trials.

TRIAL A: ROCK ISLAND SWEETHEART

This trial was established primarily to evaluate the efficacy of new formulations of prohexadione calcium for controlling shoot growth in cherry. These new products were compared to a standard program of Kudos 27.5WDG applied twice at industry standard timings in the spring; fruit was sampled from this standard treatment at typical Sweetheart harvest timing (78 days after full bloom), as well as 12 days earlier as a proxy for typical Bing harvest timing (66 DAFB). While these results only reflect single data points, they do suggest that prohexadione calcium residues may decline significantly as the cherry harvest season progresses.

Measured residues of prohexadione calcium on field run cherry fruit sprayed with Kudos WDG27.5 at 20 oz/acre during bloom and 16 oz/acre at shuck fall. 'Sweetheart'/G.6, Rock Island, WA. WTFRC 2025.

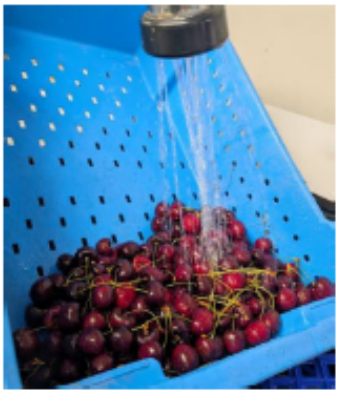
Sample timing	Measured residue	US MRL[1]	Lowest export MRL[1]
	ppm	*ppm*	*ppm*
June 5 (66 DAFB/Bing timing)	0.15	0.4	0.01 (THA)
June 17 (78 DAFB/Sweetheart timing)	0.02		

[1] Major export markets for Pacific Northwest cherries; 29 July 2025. http://mrldb.nwhort.org/#top_markets

TRIAL B: EAST WENATCHEE SKEENA

A prohexadione calcium product was included in WTFRC's annual cherry pesticide residue study for the first time in 2025. To generate the highest possible residues while still following product label guidelines, Kudos 27.5WDG was applied at the maximum rate (20 oz/acre) at the minimum preharvest interval (21 days before harvest) and retreatment interval (14 days). Fruit from this trial was sampled on July 1, roughly 3 days prior to commercial harvest of that block. Some of that fruit was immediately shipped overnight for processing at the analytical lab. After later conferring with industry collaborators, we decided to use some fruit remaining in our cold storage (34 F) from

that harvest sample to try to get a rough sense of how tenacious prohexadione calcium residues may be during the packing process.



Six days after the fruit was sampled from the field, some of the remaining fruit was packaged without any washing (field run) to serve as a control treatment; other fruit was subjected to a rudimentary simulation of a cherry packing process in the WTFRC lab. Fruit in that treatment were rinsed in the sink for 3 minutes to simulate hydrocooling and then floated in a large tub of water with gentle agitation for 10 minutes to simulate time spent in the dump tank and floating down a commercial packing line. Both samples were shipped overnight for processing by the analytical lab.

Residue levels show a modest decrease between the July 2 and July 8 dates of processing samples from the same lot of fruit, suggesting that prohexadione calcium residues may diminish during cold storage after harvest. Our unsophisticated simulation of a cherry cooling and packing process also produced a modest reduction of prohexadione calcium residues, as indicated in the table below.

Measured residues of prohexadione calcium on cherry fruit sprayed with 20 oz/acre Kudos WDG27.5 at 35 and 21 days before harvest. Samples harvested on July 1. 'Skeena'/K.6, East Wenatchee, WA. WTFRC 2025.

Treatment	Sample processing date	Measured residue	US MRL[1]	Lowest export MRL[1]
		ppm	*ppm*	*ppm*
Field run (no washing)	July 2	0.40	0.4	0.01 (THA)
Field run (no washing)	July 8	0.29		
Simulated packing (with washing)	July 8	0.23		

[1] Major export markets for Pacific Northwest cherries; 29 July 2025. http://mrldb.nwhort.org/#top_markets

Results of these trials are shared for informational purposes only and should not be construed as endorsements of any product, reflections of their efficacy in managing vegetative growth in tree fruit, or a guarantee of similar results regarding residues for any user. Cherry growers should consult their extension team members, crop advisors, and warehouses to develop responsible pest control and vegetative growth management programs.

Reports from other pesticide residue studies on apple and cherry which provide a broader context for these results are available on the WTFRC website at www.treefruitresearch.org. We encourage growers and consultants to stay abreast of current information on international MRLs, which often change in response to trade negotiations and/or political developments. For more information, visit the Northwest Horticultural Council website, www.nwhort.org.

WASHINGTON TREE FRUIT RESEARCH COMMISSION

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

EXECUTIVE SUMMARY

Project Title: Pesticide Residues of PNW Cherries

Keywords: MRL, pesticide, residue, cherry

Abstract: The Washington Tree Fruit Research Commission (WTFRC) has conducted annual pesticide residue studies in apple and cherry since 2011 to provide basic data to the tree fruit industry regarding residue levels of commonly used pesticides. This information is used by growers, consultants, and fruit sales desks to help guide management decisions regarding spray programs for fruit being exported to foreign markets which may have more stringent Maximum Residue Level (MRL) tolerances than those allowed for fruit sold domestically.

For these studies, various fungicides, insecticides, acaricides, and plant growth regulators were sprayed at typical rates and timings used by the PNW cherry industry. Applications were made to a commercial 'Skeena' orchard with an airblast sprayer calibrated to 200 gallons water/acre. Fruit were then sampled at standard harvest timing and submitted to commercial labs for residue analysis.

Results have consistently found that residue levels of all pesticides applied were safely below tolerances set for the United States market by the Environmental Protection Agency (EPA), but many residues have exceeded MRLs for some important cherry export markets. Most of these potentially problematic cases have been for countries that have either set their MRLs at the minimum limit of detection for those particular products, or have not posted MRLs for those pesticides; in those cases, imported fruit in those markets cannot have residues which exceed that nation's default level for MRLs, which is frequently a very stringent value such as 0.1 or 0.01 ppm.

Due to keen interest from industry, we did additional testing in 2025 of cherries sprayed with prohexadione calcium at various rates and timings, including some 'Sweetheart' cherries from another WTFRC trial site. All samples tested detected some residues of prohexadione, which would potentially be problematic for fruit destined for export markets with minimal MRLs of 0.01 ppm such as Thailand. Field run fruit sprayed at the maximum rate and minimum pre-harvest interval (PHI) had residue levels close to the US EPA tolerance but was still in compliance. Crude simulations of hydrocooling and packing line processes slightly reduced prohexadione residues on the fruit.

Reports of all WTFRC pesticide studies on apple and cherry since 2011 are available at www.treefruitresearch.org.

Project Title: Precision Nutrient Management for sweet cherry orchards

Report Type: Continuing Project Report NCE

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Cooperators: Douglas Fruit, Cameron Nursery, Dory Linneman, Dana Sirota and Judy Robinson (PNNL), Lav Khot (WSU, AgWeathernet)

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $ 70,004
Total Project Request for Year 2 Funding: $ 20,321
Total Project Request for Year 3 Funding: $ 20,650

Budget 1
Primary PI: Bernardita Sallato
Organization Name: Washington State University
Contract Administrator: Hollie Tuttle
Telephone: (509) 335-2885
Contract administrator email address: arcgrants@wsu.edu

Item	2023	2024	2025
Salaries			
Benefits			
Wages[1]	7,190	7,478	7,777
Benefits	734	763	793
RCA Room Rental			
Shipping			
Services	56,680	6,680	6,680
Supplies	4,400	4,400	4,400
Travel	1,000	1,000	1,000
Total	70,004	20,321	20,650

Footnotes: [1] Salary for 50% FTE for 4 month per year for Juan Munguia, Research Assistant at Sallato's laboratory for data collecting processing fruit in the laboratory. [2] Services include root monitoring equipment and PNNL services. Supplies include laboratory analysis and processing of samples for nutrient test.

OBJECTIVES

Our main objective is to determine nutrient demand in sweet cherry orchards and validate a method for extraction-based estimations for young orchards.

Specific objectives are:

1. Demand: Investigate biomass and nutrient demand in different orchards and rootstocks.

In 2025, samples were collected from the 2018 'Benton' on MxM14, Gisela 12 and Gisela 5 rootstocks and 2023 Black Pearl on G.6, G.12 and Mazzard rootstocks. The data reported here correspond to fruit collected in June 2024, leaf biomass collected in fall 2024, and whole-tree partitioning determined in winter 2025. The analyses of third year data (2025) is underway.

2. Timing: Identify strategies to monitor root growth in sweet cherry.

   2.1. In 2025 root growth was monitored via root windows and CI-600 CID-Bioscience minirhizotron. Data collected via Electrical resistivity tomography (*ERT*) in 2024 were analyzed by PNNL collaborators (Dory Linneman, Dana Sirota, and Judy Robinson). The comparison among methods will be reported in 2026 final report.

   2.2. Nitrogen leaching: We continue evaluating nitrogen leaching (N, NO3 and NH4) at three depths throughout the season. Results from 2025 will be presented in the final report.

SIGNIFICANT FINDINGS

1. Fruit tissue can reliably estimate nutrient demand at lower cost, given that fruit flesh accounts for about 95% of the fruit weight.

2. 'Benton' on MxM14 rootstock had 15–59% higher total biomass and greater accumulation of N, K, and Mg than on Gisela 5 or 12, yet fruit yield remained similar (≈28–33.5 kg per tree).

3. Nitrogen demand for mature orchards was quantified. If the trees have reached full size and production, N demand ranges between 51–58 lb N/acre, regardless of the rootstock. If pruning wood or leaves are removed, additional N must be supplied to replace the loss.

4. In a 2-year-old 'Black Pearl' orchard, N demand ranged from 44–55 lb/acre, P demand was below 15 lbs/acre, K ranged between 14-19 lbs.acre and it was less than 10 lbs for Ca and Mg (at 1,000 trees/acre), with no differences among rootstocks.

5. ERT (Electrical Resistivity Tomography) shows potential for non-invasive root monitoring, showing higher root density in Gi12 compared to Gi5. However, ERT is highly influenced by irrigation and infrastructure, which will require correction for reliable interpretation.

METHODS

1. Demand: Investigate biomass and nutrient partitioning differences in three distinct rootstocks and systems.

Biomass partitioning is being evaluated in the following conditions:

- Benton on MxM14, Gisela 12 and Gisela 5 planted in 2018
- Skeena and Selah on G.12. planted in 2012
- Black Pearl on G.6, G.12 and Mazzard planted in 2023

At harvest, all fruit were collected to determine yield and nutrient concentration and determine nutrient extraction, from four replicated trees per rootstock / cultivar. Before leaf fall a net was placed around the trees to collect dropped leaves and determine total leaf weight and leaf counts. After leaf drop during winter, three trees per site and rootstock were excavated for whole tree biomass and nutrient partitioning by weighing each tree component, drying a subsample to determine dry matter and the dry samples were sent to a laboratory for nutrient analysis (N, P, K, Ca, Mg and micronutrient B) (Figure 1).

Figure 1. Leaf collection during the fall (left) and whole tree excavation (right) of 7th leaf Benton trees.

In all sites, a subsample of leaves and soils were collected each year to monitor nutrient levels following standard methods (Sallato et al., 2019).

2. Timing: Identify strategies to monitor root growth

Root growth was monitored with three techniques: 1. root windows, 2. CI-600 Bioscience minirhizotron and 3. Electrical resistivity tomography (*ERT*) method by Pacific Northwest National Laboratory (PNNL).

Root monitoring started during bloom and continued in a biweekly basis until leaf fall. Images were captured on a 1 ft grid for the root window and evaluated manually, while the minirhizotron (CI-600 In-Situ Root Imager, CID-Bioscience) and evaluated with RootSnap! Software (Figure 2).

The Electrical resistivity tomography (*ERT*) was installed in April 2024 in three replicated trees per rootstock (Figure 2, right). Each study tree was instrumented with 30 electrodes in 5 rows of 6 electrodes centered around the trunk of the tree. The electrodes were spaced 18 inches apart and contained within protective boxes. This procedure is repeated for 230 combinations of source and

receiver electrodes per survey. This survey design was conducted on the six study trees and was repeated 13 times between April and September 2024 (Figure 2, right)

Figure 2. Root growth monitoring via root window (left) and electrical resistivity tomography (ERT) (right).

3. Soil Nutrient Availability and Leaching

Soil total nitrogen (N), nitrate (NO3) and ammonium (NH4) were determined in all the test sites at three depths and six times throughout by collecting samples with soil auger and samples were sent for chemical testing to external laboratory.

RESULTS AND DISCUSSION

1. Demand: Investigate biomass and nutrient partitioning differences in three distinct rootstocks and systems.

Fruit extraction of macronutrients remained within reference ranges with no significant differences among rootstocks ($p<0.05$). We have seen that nutrient values have shown minimal variability across the years of sampling (represented by ranges). More details provided in the 2024 continuing report and explained in this video https://treefruit.wsu.edu/videos/optimizing-tree-fruit-nutrition-for-fruit-quality/

Fruit flesh represents approx. 95% of the total weight and 86% of the flesh dry matter, thus can provide reliable estimation of nutrient demand at a lower economic cost, compared to whole fruit partitioning (pit, peel, stem and flesh).

Benton orchard – 7th leaf

Mean biomass (fresh weight) of whole trees ranged between 99 and 157 kg per tree, being 15% and 59% higher in MxM14, for 2023 and 2024 respectively (data not shown). Fresh weight partitioning among different tree components were not different across rootstocks (Figure 2), except for the proportion of spurs, being half on MxM14 compared to both Gisela rootstocks (Table 1).

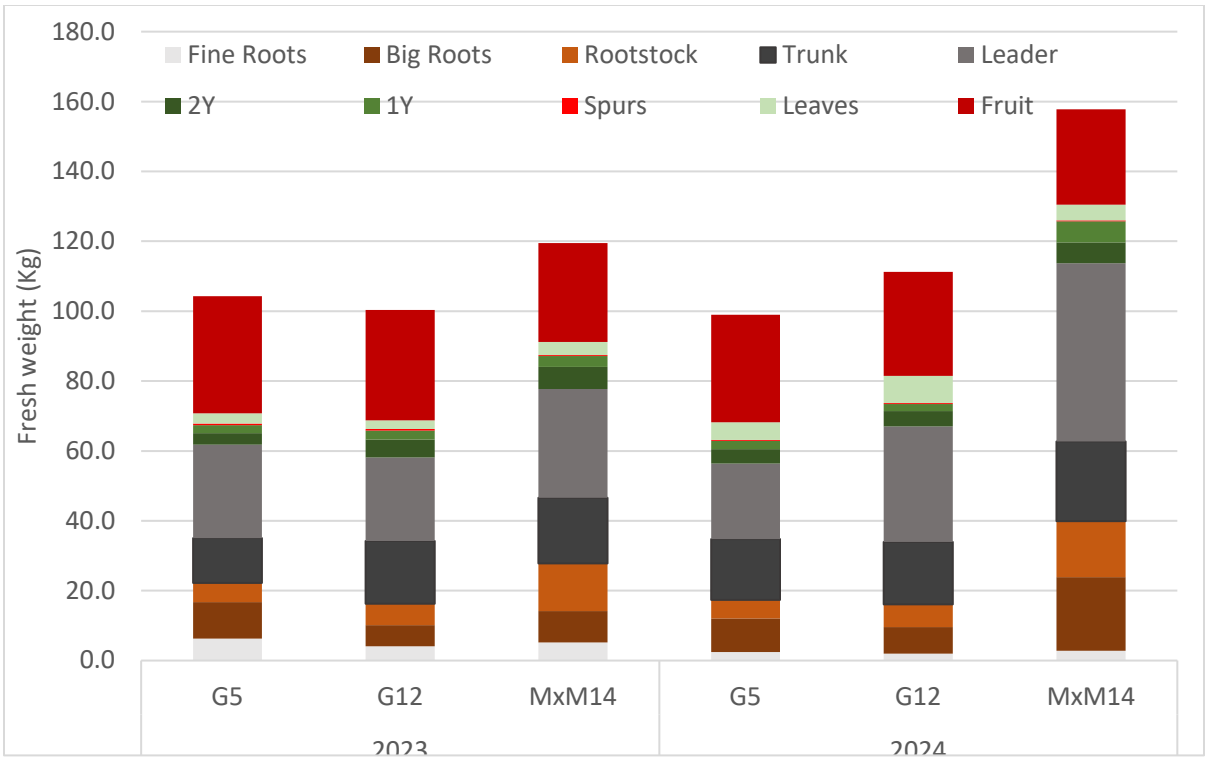

Figure 2. Biomass (fresh weight) partitioning on 'Benton' trees grown on Gisela 5 (G5), Gisela 12 (G12) and Maxma (MxM14) rootstock, for 2023 and 2024. Corresponding to a 6th and 7th leaf orchard. 2Y; 2-year-old shoots, 1Y; 1-year-old shoots.

Table 1. Fresh weight partitioning in 7th leaf 'Benton' trees

Rootstock	Fresh Partitionin (%) 7th leaf							
	FineRoots	BigRoots	Rootstock	Trunk	Leader	2nd.Yr.	1st.Yr	**Spurs**
G5	4%	15%	8%	28%	34%	7%	4%	**0.5% a**
G12	3%	10%	9%	24%	45%	6%	3%	**0.4% a**
MxM14	2%	17%	13%	18%	41%	5%	5%	**0.2% b**
p value	0.324	0.321	0.582	0.060	0.286	0.596	0.269	**0.012**

Rootstock had little effect on fresh weight partitioning in 6th (data not shown) and 7th leaf 'Benton' trees (Table 2). In every case, the majority of fresh weight was found in the leaders (ca. 40%), with the trunk and big roots being the next highest. Partitioning to fine roots was low and varied from 2% to 10%. There were significant differences in partitioning to spurs in both years, with the least in MxM14-rooted trees. Despite this, there were no differences in fruit yield which varied between 28 and 33.5 kg per tree, equivalent to ca. 14.5 and 17.8 tons per acre.

In mature orchards, it is common to estimate nutrient application rates by determining the nutrient extracted by the fruit (fruit demand) minus the supply that is associated with the nutrients available in the soils and water (Sallato et al., 2019). This method assumes that all other components of the tree remain in the orchard and will eventually recycle. In 2024, the average yield was 15.6 tons per acre, with a N demand of 52.4, 51.4 and 58.1 lbs of N/acre (calculated for 479 trees per acre) (Table 2). If growers removed pruning wood, or leaves are blown away, additional N should be considered. In Table 2 we provide an example of the additional N required by new shoots and leaves.

Table 2. Nitrogen demand estimation for adult Benton orchard with 479 trees per acre

Rootstock	Nitrogen Demand				
	New shoots (g/tree)	Fruit (g/tree)	Leaf (g/tree)	Total lb/acre	Fruit only lbs/acre
G5	12.2	49.2	58.7	127.9	52.4
G12	10.8	48.3	54.5	120.9	51.4
MxM14	28.0	54.5	33.5	123.6	58.1
p value	0.043	0.800	0.992	-	-

In the 'Benton' orchard, the accumulated nutrient content in 2023 was not different among rootstocks, while in 2024, MxM14 accumulated higher N, K and Mg, compared to the Gisela rootstocks (Table 3). For both Gisela rootstocks, nutrient content did not change significantly between 2023 and 2024, while MxM14 had significantly higher content accumulated in 2024.

Table 3. Accumulated macronutrient content nutrient content per tree by year and rootstock. Different letters indicate significant differences among rootstocks within column and year.

Rootstock	Rootstock	Tree Weight (Kg)	Nutrient Content (Kg)				
			N	P	K	Ca	Mg
2023	G5	67.9	337.2	64.5	126.3	441.5	52.8
6th leaf	G12	66.2	326.1	69.9	120.6	425.6	54.8
	MxM14	87.4	397.0	73.3	160.2	434.3	70.9
	p value	0.179	0.685	0.847	0.169	0.992	0.350
2024	G5	63b	256.3 b	48.8	136.2 b	661.0	42.2 b
7th leaf	G12	74b	336.9 b	68.7	165.8 b	638.6	52.3 ab
	MxM14	126a	527.0 a	112.5	328.2 a	1063.9	90.4 a
	p value	0.022	0.019	0.065	0.023	0.232	0.047

Black Pearl – 2nd leaf.

In 2023, Mazzard had higher root mass compared to both Gisela rootstocks, while G12 had increased mass partitioning towards the main leader, compared with G6 and Mazzard. However, in 2024 (2nd leaf), Mazzard rootstock had increased partitioning toward 2 year-old shoots and reduced proportion of spurs, compared to both Gisela rootstocks (Figure 3).

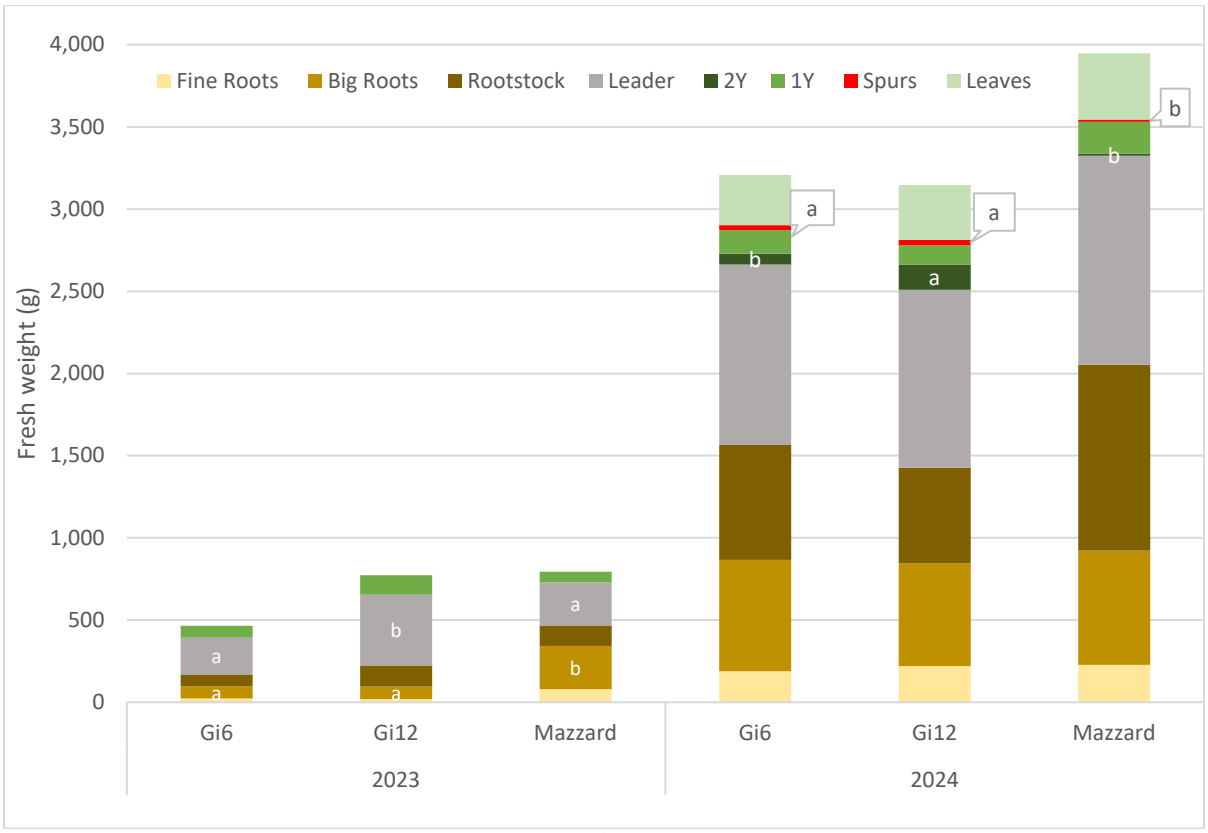

Figure 3. Biomass (fresh weight) partitioning in 2nd leaf Black Pearl grown on Gisela 6 (G6), Gisela 12 (G12) and Mazzard rootstock.

Despite the differences in fresh partitioning, total N accumulation was similar among rootstocks. Only N content in 2-year-old wood was higher in Gi12 compared to Gi6 and Mazzard (Table 4).

Table 4. Nitrogen content accumulation in a 2-year-old Black pearl orchard.

Rootstock	N content (gr/tree)								
	Total	Leaves	Leader	2 Y Old	1 Y Old	Spurs	Rootstock	Big Roots	Fine Roots
Gi6	22.46	1.49	8.14	0.45b	0.95	0.29	4.40	5.20	1.51
Gi12	19.92	1.50	8.08	1.2a	0.81	0.26	2.90	3.60	1.45
Mazzard	24.57	1.98	7.23	0.05b	1.20	0.12	7.00	5.30	1.64
Pr > F(Model)	0.301	0.093	0.802	0.006	0.364	0.109	0.099	0.055	0.913

* Equivalent to Kg per acre for 1000 trees per acre planting

In young orchards, to determine adequate rate one should consider all component of tree demand. In Table 3 we provide an example of the accumulated nutrient demand on a two-year-old orchard, at 1000 trees per acre.

Table 5. Calculated nutrient demand per acre at 1000 trees per acre.

Rootstock	Tree Weight	Nutrient Demand Y2 (lb/acre)				
	(g)	N	P	K	Ca	Mg
Gi6	3208.3	49.9	15.2	16.9	7.8	7.2
Gi12	3145.7	44.3	10.8	14.3	10.4	6.5
Mazzard	3948.7	54.6	12.9	19.3	8.0	8.3
Pr > F(Model)	0.165	0.301	0.119	0.234	0.941	0.280

2. Timing: Identify strategies to monitor root growth (Judy Robinson, Dory Linneman and Dana Sirota (PNNL)

Electrical resistivity tomography (ERT) is a direct-current imaging method that can be used to estimate the distribution of electrical resistivity. The method connects positive and negative electrodes in the ground in a nested array (Figure 4).

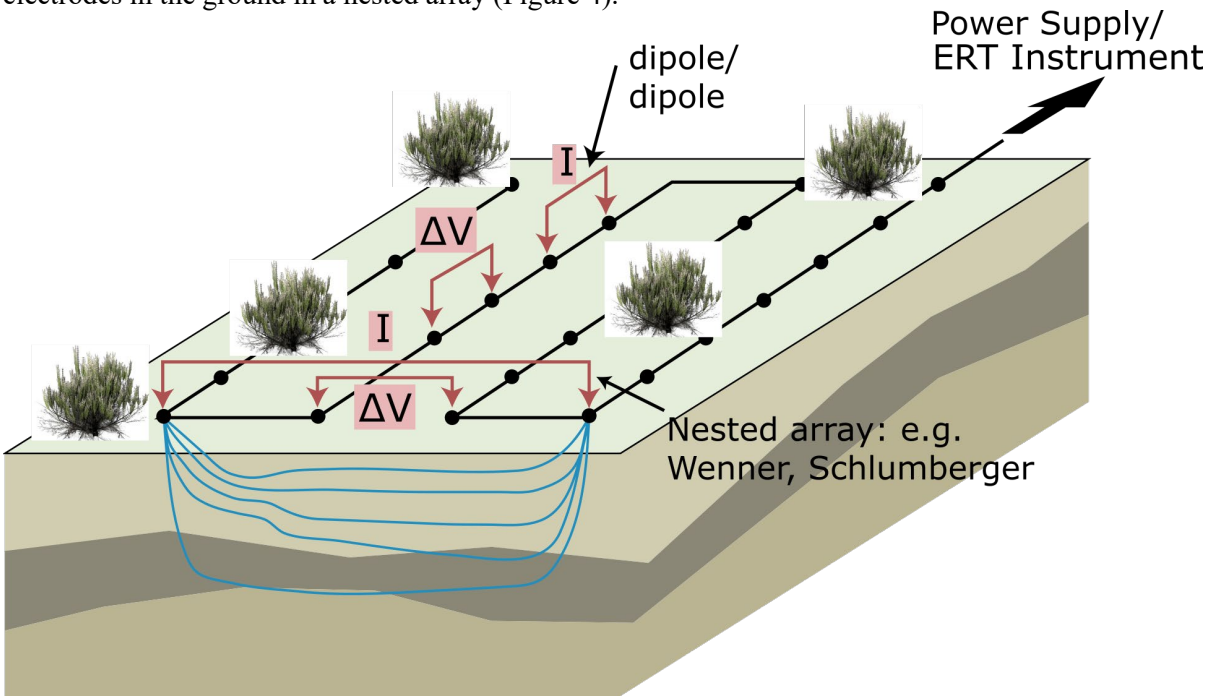

Figure 4. Illustration of a basic Electric Resistivity Tomography layout. I; current from positive to negative (amps). ▼V; measured of positive and negative potential (volts).

The resistance measured by the field instrument is then processed and converted to an "apparent conductivity" between electrode pairs with a geometric factor determined by the source and receiver electrode positioning. The processed data can be visualized as a tomographic image which reflects the soil bulk resistivity (bulk electrical conductivity). The bulk electrical conductivity is related to several properties of the subsurface including rock/soil type, porosity, ionic strength of the pore fluids, and surface conductivity of geologic materials. The advantage of the process is that is non-invasive, is spatially continuing data collection and relatively inexpensive.

In this study, we discarded data with standard deviations exceeding 5%. Of the nearly 18,000 data points collected, approximately 3% were culled using these criteria. The tree with the highest number of 'bad' data points culled less than 5% of the data. This is a strong indication of high data quality throughout the dataset.

The time-lapse ERT images show changes in bulk electrical conductivity (BEC) surrounding the center of Gi5 and Gi12 trees. These images use April 4, 2024 as a reference and were inverted for changes from this date. Greens, yellows and red colors show increases in conductivity and cyan and blue colors show decreases in conductivity from April 4th. The ERT resolution depth is reliable to approximately 1 m.

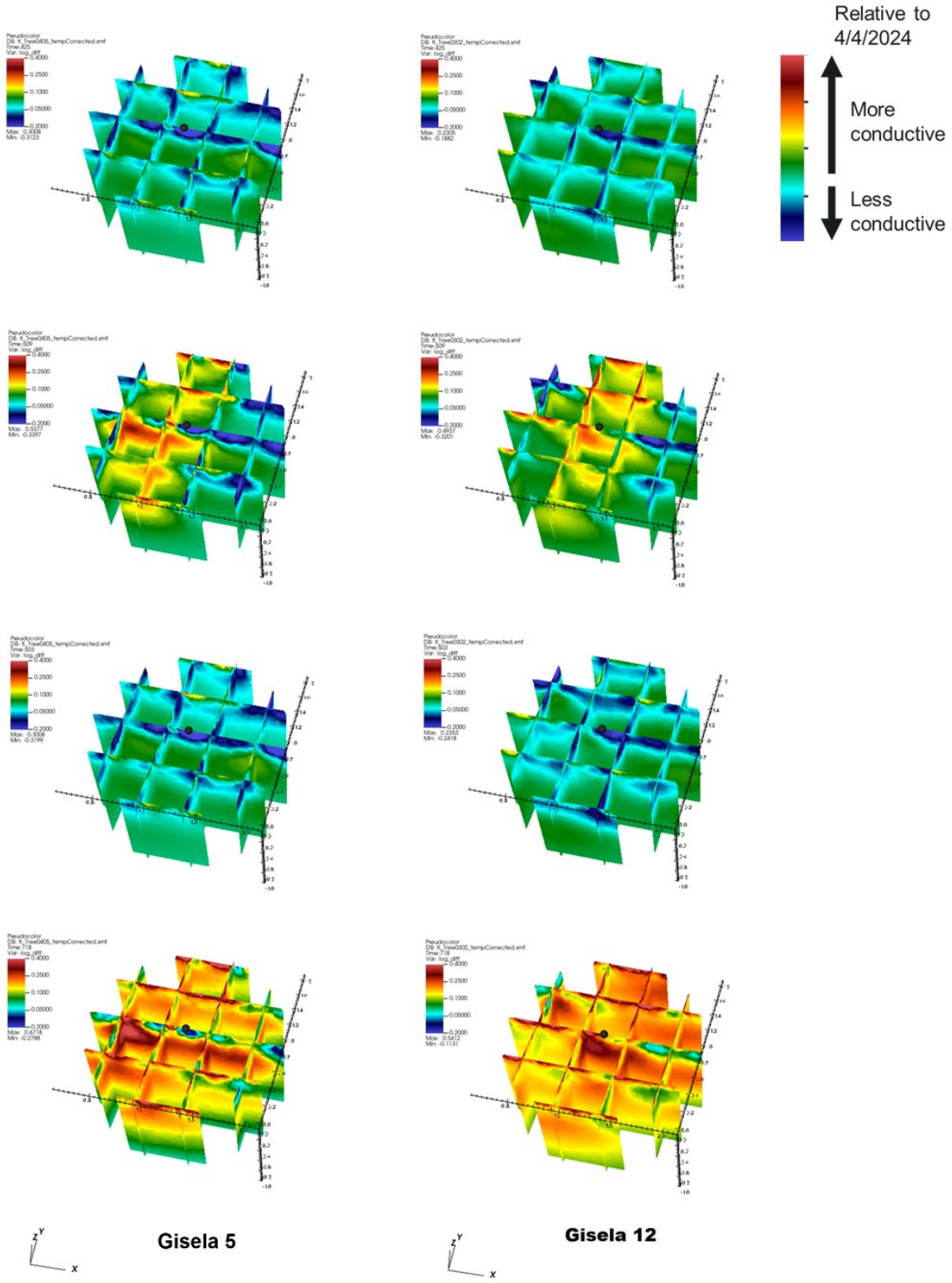

Figure 5. Time-lapse ERT images for Gisela 5 and Gisela 12 during four sampling dates, processed relative to April 4th. Darker red indicates more conductive and blue less conductive.

Time-lapse BEC images are sensitive to changes in temperature, irrigation, water conductivity and saturation. A temperature correction was applied and irrigation water conductivity is not expected to have a large range, thus the logarithmic changes in BEC in the ERT images are likely caused by changes in saturation surrounding the root structure of the cherry rootstocks.

The changes in saturation in the ERT images were dependent on irrigation and rootstock density and structure relative to April 4, 2023. Larger irrigation resulted in increased in BEC. Larger, more dense rootstock presumably will hold more water relative to a less dense, smaller rootstock, and this would appear as a more dense, higher conductivity feature in the ERT images.

The images show that in the very near-surface, the BEC changes of Gi5 trees are lower compared to Gi12 trees. In addition, the Gi12 trees appear to have more concentrated regions of higher BEC, particularly on 7/18 and 7/25, relative to Gi5 trees.

The high correlation with irrigation and temperature, along with field variability, prevents conclusive interpretation of root growth timing. Plot scale dynamics with full soil temperature, soil moisture and saturation instrumentation might help correct for these factors and provide more conclusive results.

Project Title: Evaluating Heat Stress Response in Novel Cherry Rootstocks

Report Type: Continuing Project Report

Primary PI: Ashley Thompson
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Cooperators: Dave Weil (Tree Connections/Varieties International), Dane Klindt (K&K Land Management)

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $ 48,306
Total Project Request for Year 2 Funding: $ 31,193
Total Project Request for Year 3 Funding: $ 31,721

Other related/associated funding sources: Awarded
Amount: $14,500
Agency Name: Oregon State University
Notes: Start-up equipment funding (Pearson) provided by OSU will be used to purchase the LI-COR LI-600 Porometer/Fluorometer.
Amount: 180 cherry trees
Agency: Varieties International
Notes: Trees for testing were donated by Varieties International.

Budget 1
Primary PI: Ashley Thompson
Organization Name: Oregon State University Extension
Contract Administrator: Charlene Wilkinson
Telephone: (541) 737-3228

Contract administrator email address: charlene.wilkinson@oregonstate.edu
Station Manager/Supervisor: Nicole Strong
Station manager/supervisor email address: Nicole.strong@oregonstate.edu

Item	2025	2026	2027
Salaries[1,2]	$9,070.00	$9,341.50	$9,622.28
Benefits	$4,523.00	$4,692.50	$4,869.57
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies	$18,179.00	$500.00	$500.00
Travel			
Plot Fees			
Miscellaneous			
Total	$31,772.00	$14,534.00	$14,991.84

Footnotes:
1. Two weeks summer salary for Thompson.
2. One month salary for one FRA to assist with orchard activities, data collection, and data analysis.

Budget 2
Co PI 2: Kelsey Galimba
Organization Name: Oregon State University MCAREC
Contract Administrator: Charlene Wilkinson
Telephone: (541) 737-3228
Contract administrator email address: charlene.wilkinson@oregonstate.edu
Station Manager/Supervisor: Brian Pearson
Station manager/supervisor email address: brian.pearson@oregonstate.edu

Item	2025	2026	2027
Salaries[1,2]	$7,929.00	$8,011.00	$8,039.00
Benefits	$4,117.00	$4,160.00	$4,202.00
Wages[3]	$4,080.00	$4,080.00	$4,080.00
Benefits	$408.00	$408.00	$408.00
RCA Room Rental			
Shipping			
Supplies			
Travel			
Plot Fees			
Miscellaneous			
Total	$16,534.00	$16,659.00	$16,729.00

Footnotes:

1. Two weeks summer salary for K. Galimba.
2. Estimated salary for one FRA to perform harvest, quality tests, trunk measurements, etc.
3. Wages for hourly student employees to assist with orchard activities, harvest, and quality tests.

Objectives:

1. Quantify stomatal conductance, leaf temperature, and chlorophyll fluorescence heat tolerance of the heat sensitive variety 'Skeena' grafted on to A9 x VSL2, Rulan 8, Krymsk 7, EC 11, EC 12 and RVL 4, Krymsk 5, Mazzard, and Gisela 12 to understand how new rootstocks respond to environmental conditions in a region where heat stress is common. In addition, through the measurement of stomatal conductance in conjunction with quantified evapotranspiration and plant available water of soil within irrigated rows to determine how irrigation management practices influence stomatal conductance, plant growth, and fruit development.

> *Deviations:* None. Trees were bud grafted at Willow Drive Nursery and will arrive for planting in February 2026. We purchased appropriate equipment to begin this study once the trees are planted.

2. Assess horticultural traits, including tree growth (as trunk cross sectional area), yield, flower counts.

> Deviations: None. We will measure horticultural traits at planting in 2026, and in subsequent years.

3. Measure fruit quality (size, firmness, stem retention, sugars, color, and surface pitting) at harvest and following three weeks of cold storage.

> Deviations: None. Fruit quality will be measured in year three.

Our objectives target the WTFRC priority "Fruit quality and Safety" sub-section 1.h "Mitigation of heat stress".

Tangible Progress:

- Grafted trees were ordered in 2024 and will arrive for planting in the winter of 2026 from Willow Drive Nursery.
- LI-COR LI-600 porometer for measuring stomatal conductance was purchased in the summer of 2025. Santosh Kalauni, Faculty Research Assistant in the Galimba Lab, attended a three-day LI-COR training held in Lincoln, NE to learn how to use the LI-COR LI-600 effectively. This summer, Kalauni used the LI-600 to collect stomatal conductance data from another research project. Kalauni will assist in data collection for this project to ensure data accuracy.
- An Onset RX3000 remote weather monitoring station with the ability to measure evapotranspiration rates and 36 HOBOnet soil moisture EC-5 sensors were purchased this fall with plans to install them at planting in the spring.
- UC Davis Analytical Laboratory will create soil moisture release curves this winter.

Methods:

Changes to methods section

- Study location now listed
- Make and model of equipment is now listed

Study location, Experimental Design, and Treatments

This experiment will be conducted at K&K Land Management in The Dalles, OR (45.578146,-121.165315) on silt loam/silt soil using a randomized complete block design (RCBD), with five trees per experimental unit. Trees will be planted on a 10' x 15' spacing. Each rootstock treatment will be replicated four times. The rootstock treatments include A9 x VSL2, Rulan 8, Krymsk 7, EC 11, EC 12, and RVL 4. Mazzard, Krymsk 5 and Gisela 12 will serve as controls. Rootstocks were bud-grafted with the cultivar 'Skeena'. Trees will be managed uniformly for pests and diseases by K&K Land Management. Irrigation will be managed uniformly across all treatments. Trees will be irrigated to maintain a water status above 50% of maximum allowable depletion based on the soil moisture curve described below.

Evapotranspiration (ET), Plant Available Water, and Stomatal Conductance

A monitoring station (RX3000) will be installed at the research site to collect local, on-farm weather conditions (temperature, humidity, wind speed, and solar radiation) and to calculate evapotranspiration (ET). Prior to planting, a soil moisture release curve will be developed for the research site to assess the relationship between irrigation application volume and plant available water (PAW). Soil moisture sensors (EC-5) will be installed in each treatment and replicant. This will allow monitoring of PAW and adjustment of irrigation to avoid drought stress and its ability to decrease transpiration (non-heat related transpirational effect).

A porometer (LI-COR LI-600) will be used to record stomatal conductance (measurement of leaf gas exchange and transpiration), leaf temperature, and chlorophyll fluorescence (an indicator of photosynthetic energy conversion). Measurements will be taken at regular intervals midday when plant water stress is highest and with a device that supports rapid sample collection to ensure environmental conditions are similar and comparable to elucidate differences due to scion-rootstock interactions.

Measuring Tree and Fruit Response

Tree growth of the middle three trees in each experimental unit will be measured as trunk cross sectional area (TCSA) by measuring the trunk diameter 12 inches (30 cm) above the graft union at planting and annually thereafter. Trees will be monitored for mortality monthly. In the second and third years, we will count the number of flowers on the middle three trees in each experimental unit. We anticipate fruiting in year two. Fruit will be harvested and weighed from the middle three trees of each experimental unit and replicant to access total yield. Yield efficiency will be calculated as the ratio of total fruit weight to TCSA. Fruit quality measures, including fruit size, firmness, color, soluble solids (°Brix), stem retention and surface pitting, will be measured. Additionally, we will document heat damage, such as 'Skeena' suture burn, as described by WTFRC (Dinny, 2016). Post-harvest, fruit will be placed in cold storage for three weeks, after which fruit quality will be re-evaluated using the metrics listed above.

Project Title: A Robust PNW Sweet Cherry Breeding and Genetics Program

Report Type: Continuing Project Report

Primary PI: Per McCord
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Cooperators: Allan Bros. Fruit, Cherry River Farms, Custom Orchards, Inc. Orchardview Farms, Stemilt Growers, Breeding Program Advisory Committee (BPAC) members

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $ 238,252
Total Project Request for Year 2 Funding: $ 247,700
Total Project Request for Year 3 Funding: $ 257,493

Other related/associated funding sources:
Awarded
Funding Duration: 2022 - 2026
Amount: $75,000
Agency Name: Sun World International LLC
Notes: 'Agreement for Joint Cherry Germplasm Exchange, Evaluation, Data Sharing and Commercialization'.

Awarded
Funding Duration: 2023 - 2026
Amount: $22,376
Agency Name: WSDA Nursery Research Funding
Notes: 'Evaluating new stone fruit cultivars for western Washington'.

Awarded
Funding Duration: 2024 – 2026 (No cost extension)
Amount: $50,000
Agency Name: USDA NIFA Specialty Crops Research Initiative Planning Grant
Notes: 'Understanding and enhancing climate resilience in *Prunus* crops'. PI: Per McCord. Multiple co-PI's, including Cameron Peace and Matthew Whiting from WSU.

Awarded
Funding Duration: 2025-2026
Amount: $24,531
Agency Name: Washington Tree Fruit Research Commission/Oregon Sweet Cherry Commission/Northwest Nursery Improvement Institute
Notes: 'Identifying sources of resistance to Pseudomonas and powdery mildew'. PI: Per McCord; co-PI's Cameron Peace, Alexandra Johnson.

Requested
Funding Duration: 2026 - 2029
Amount: $645,222
Agency Name: USDA AFRI Foundational and Applied Science Program
Notes: 'Facilitating machine harvestability of sweet cherry through plant growth regulators and genetics'. PI: Per McCord; co-PI's Matthew Whiting and Amit Dhingra.

Requested
Funding Duration: 2026 - 2030
Amount: $5.85 million
Agency Name: USDA NIFA Specialty Crops Research Initiative
Notes: 'Integrated strategies for managing bacterial canker and blossom blast disease of stone fruits'. PI: Frank Zhao. Multiple co-PI's, including Per McCord, Bernardita Sallato, and Matthew Whiting from WSU.

Requested
Funding Duration: 2026 - 2029
Amount: $220,000 (estimate)
Agency Name: WSDA Specialty Crop Block Grant
Notes: 'Establishing rootstock and training system recommendations for new WSU sweet cherry selections'. PI: Per McCord; co-PI's Matthew Whiting and Rob Blakey.

Requested
Funding Duration: 2026 - 2029
Amount: $649,998
Agency Name: USDA AFRI Foundational and Applied Science Program
Notes: 'Accelerating the pace and efficiency of sweet cherry breeding'. PI: Per McCord; co-PI Cameron Peace.

Requested
Funding Duration: 2026 - 2028
Amount: $58,218
Agency Name: Washington Tree Fruit Research Commission/Oregon Sweet Cherry Commission
Notes: 'Screening cherry rootstocks for resistance to X-disease phytoplasma'. PI: Per McCord; co-PIs Scott Harper, Adrian Marshall, and Rodney Cooper.

WTFRC Collaborative Costs: None

Budget 1
Primary PI: Per McCord
Organization Name: Washington State University
Contract Administrator: Kevin Rimes
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Station Manager/Supervisor: Naidu Rayapati
Station manager/supervisor email address: naidu.rayapati@wsu.edu

Item	2025	2026	2027
Salaries[1]	$80,924.00	$84,161.00	$87,527.00
Benefits	$27,715.00	$28,824.00	$29,796.00
Wages[2]	$21,760.00	$22,630.00	$23,535.00
Benefits	$2,191.00	$2,279.00	$2,370.00
RCA Room Rental			
Shipping			
Supplies[3]	$64,000.00	$66,560.00	$69,222.00
Travel[4]	$7,500.00	$7,800.00	$8,112.00
Plot Fees	$12,080.00	$12,563.00	$13,066.00
Miscellaneous			
Total	$216,170.00	$224,817.00	$233,628.00

Footnotes: [1]Includes 1.0 FTE laboratory research technician and 0.5 FTE field technician. [2]Includes temporary labor for crossing, harvesting, seed extraction/transplanting. [3]Supplies for fruit evaluation, DNA extraction/genotyping, embryo rescue, propagation supplies/services, orchard maintenance, and equipment maintenance. [4]Includes fuel, insurance, vehicle maintenance, and lodging/per diem costs (the latter during pollination season).

If project duration is only 1 year, delete Year 2 and Year 3 columns.

(*Complete the following budget tables if funding is split between organizations,* ***otherwise delete*** *extra tables.)*

Budget 2
Co PI 2: Kelsey Galimba
Organization Name: Oregon State University MCAREC
Contract Administrator: Charlene Wilkinson
Telephone: 541-737-3228

Contract administrator email address: charlene.wilkinson@oregonstate.edu
Station Manager/Supervisor: Brian Pearson
Station manager/supervisor email address: brian.pearson@oregonstate.edu

Item	2025	2026	2027
Salaries[1]	$5,700.00	$5,759.00	$5,818.00
Benefits	$4,081.00	$4,123.00	$4,166.00
Wages[2]	$6,256.00	$6,256.00	$6,256.00
Benefits	$616.00	$616.00	$616.00
RCA Room Rental			
Shipping			
Supplies[3]	$2,500.00	$2,500.00	$2,500.00
Travel			
Plot Fees[4]	$2,625.00	$3,325.00	$4,025.00
Miscellaneous[5]	$304.00	$304.00	$304.00
Total	$22,082.00	$22,883.00	$23,685.00

Footnotes: 1. Estimated salary for one FRA to perform planting, pruning, training, data collection, and analysis.
2. Wages for hourly student employees to assist with orchard activities, harvest, and quality tests.
3. Irrigation, trellising, block maintenance, training and quality testing supplies.
4. Research plot fees ($3,500/acre).
5. 2 months cold storage room fee ($1.28/square foot)

If project duration is only 1 year, delete Year 2 and Year 3 columns.

Original Objectives

1. Rigorously evaluate seedlings and selections at all phases of the breeding program, including those now in Phase 3.
2. Increase the quality of seedlings planted in the first phase of the breeding program
    a. Continue to utilize DNA information for superior and complementary parent selection and seedling screening.
    b. Deploy newly developed DNA tests for fruit size.
    c. Increase seed germination rates to allow for more stringent DNA-based selection for priority traits
3. Maintain or improve the overall health of breeding program orchards via continued screening for viruses and phytoplasma.
4. Continue to implement timely and proper practices for orchard management (training/pruning, pest and disease monitoring and control, irrigation and nutrient management)

Significant Findings

- Phase 3 selections R19, R3, and R29 evaluated
    - R19 recommended for release by WSU Cultivar Release Committee
        - First WSU cherry release since 2007
    - R3 being evaluated one extra year before considering for release

    - First year of Phase 3 evaluations for R29. Excellent size, 'Bing' timing
- Data collected from **eight** selections in current Phase 2 trials. R35B, a mid-late blush selection, advanced to Phase 3
- **283** unique seedlings (Phase 1) selected in the field and evaluated in the fruit quality laboratory. **Four** seedlings advanced to Phase 2 trials
- Utilized up to 9 different DNA tests to evaluate potential parents as well as seedlings
- 2025 crosses guided by DNA tests of parents for fruit size, firmness, cracking, self-fertility, and powdery mildew resistance
- A record high germination rate (73%) resulted in more than **4,280 seedlings** from 2024 crosses
- Eliminated **1,119 seedlings** based on DNA test results (highest cull percentage since 2018)
- Eliminated stored pollen samples infected with high levels of prune dwarf virus and prunus necrotic ringspot virus
- Tested 112 trees for X-disease phytoplasma (21 also tested for Little Cherry Virus 2)
    - 3 trees positive for X-disease, 3 positive for Little Cherry Virus 2 (none at Prosser)
    - 2 trees identified at Prosser by canines tested negative by PCR
- Implemented spray recommendations for control of aphids, mites, peach twig borer, Western cherry fruit fly and SWD, leafhoppers, and powdery mildew
- Mixed results from spring budding greenhouse-grown seedlings onto field-grown rootstocks

Methods

Fruit evaluation
In 2025, the Cherry Breeding Program took additional steps to streamline fruit evaluation processes. Fruit from all harvests were run over the sorter for sizing and defect detection, and we eliminated manual defect scoring in the fruit lab, except for postharvest fruit luster and stem browning evaluation. Seedlings (Phase 1) were not screened for postharvest characteristics, and juice analysis for °Brix and titratable acidity was not performed on mahogany seedlings.

As in prior years, breeding program advisory committee (BPAC) members were invited to inspect P1 seedlings during the harvest. Walkthroughs were conducted generally twice per week (once with BPAC members). Selection criteria in the field were based on fruit size, firmness, flavor, and crop load. Fruit from all harvested trees (standard cultivars, selected seedlings and Phase 2/3 selections) were run over a Tomra Invision II sorter for evaluation of fruit size and defects. Standards and Phase 2/3 selections were also sorted for color using the CTIFL scale programmed into the sorter. P1 fruits were classified as either mahogany or blush. In the fruit quality lab, fruit were evaluated for weight and firmness (via Firmtech). Standards, Phase 2/3 selections, and blush P1 selections were also evaluated for °Brix and titratable acidity. An induced cracking test (4-hour soak in deionized water) was performed for Phase 2/3 selections as well as Phase 1 selections with enough fruit. Phase 2/3 selections were also treated with a fungicide soak (Shield-Brite® FDL-230SC) and evaluated postharvest after 4 weeks' refrigerated storage (approximately 35 °F) in modified-atmosphere packaging.

In preparation for filing for Plant Patent/Plant Variety Protection, additional data were collected for the Phase 3 selections, such as bloom timing, growth habit, and detailed measurements of flowers, leaves and fruit.

Crossing and DNA tests
Prior to the 2025 crossing season, more than 100 potential parents (including but not limited to parents used in 2024) were screened using DNA tests for cracking, firmness, and fruit size. The

results of these tests were used to guide crossing combinations. Additional criteria used in crossing decisions included ripening time, powdery mildew resistance, resistance to X-disease, self-fertility, and enhancement of genetic diversity. Crosses were made in cages with mason bees (as well as by hand) in the field and in the breeding program's crossing greenhouse. Embryo rescue was used on seeds from crosses targeting early ripening. All other seed were treated with Captan fungicide and stored in moist vermiculite. Both embryo rescued and conventionally treated seed were kept in a walk-in cooler until germination began.

In the winter of 2024 and spring of 2025, germinated seedlings from the 2024 crosses were screened with the DNA tests for the markers expected to segregate in the individual crosses. Seedlings with inferior genotypes were culled prior to being transplanted into larger pots.

In May 2025, the breeding program employed a professional grafter to spring bud approximately 550 seedlings onto Gisela-12 rootstocks that had been planted the previous August. Prior to budding, the seedlings were given a 6-8 week cold treatment. As seedlings matured throughout the summer and early fall, we budded 481 more seedlings. An additional 656 seedlings were planted in late spring on their own roots.

Disease screening and orchard maintenance
Stored pollen (for use in crosses) was screened via quantitative RT-PCR (qRT-PCR) for the presence of *prune dwarf virus* (PDV) and *Prunus necrotic ringspot virus* (PNRSV). Samples (fruit stems when available) of trees with symptoms typical of Little Cherry Disease were sampled at Prosser and Phase 3 locations. In addition, mother trees at Sagemoor of R35B (which was advanced to Phase 3) and 2 trees detected by canines at Prosser were also sampled for a total of 112 trees. All were tested via PCR for X-disease phytoplasma, and a subset of 21 trees were also screened for *Little cherry virus 2* (LChV2).

Orchards were sprayed beginning with a delayed dormant spray on 24 March targeting aphids and scale insects. Pre-harvest cover sprays (late April-early July) targeted cherry fruit fly, peach twig borer, and spotted-wing *Drosophila*. Powdery mildew was also targeted in the Phase 2 block. Post-harvest (to date, late July and late August), spray applications targeted leafhoppers and spider mites. Orchard irrigation was managed primarily by the IAREC farm crew, who also winter pruned the parental blocks at the Roza and summer pruned (via hedging) the older seedling blocks at the Headquarters farm. The younger seedlings at Headquarters as well as the Phase 2 block were pruned by the breeding program in the spring.

The remaining Phase 1 blocks at the Roza farm were removed (approximately 2.4 acres), except for a few trees being held for budwood and further research. The Phase 1 and Phase 2 blocks at Prosser are now located only at the Headquarters farm.

Phase 2 and 3 locations away from Prosser were managed by the participating growers (including Oregon State University for the Hood River planting).

Results and Discussion

Fruit evaluation
In Phase 1, 283 unique seedlings were selected in the field and further evaluated on the sorter and in the fruit quality laboratory. Four seedlings were advanced to Phase 2 based on at least 2 years' data:

- PC-3 (PSC2020019-001), early mahogany
- PC-4 (PSC2020019-151), early blush
- PC-5 (PSC2018039-036), mid-late mahogany

- PC-6 (PSC2019081-936), late blush

We have implemented a shorthand naming system for seedling advanced to Phase 2. 'PC' stands for 'Prosser Cherry' and is similar to the system used by long-time WSU cherry breeder Dr. Tom Toyama.

We completed 3 years' evaluation of the 2021 cohort of Phase 2 selections (Table 1). R35B, a mid-late ripening blush selection, was advanced to Phase 3. It ripens 7-12 days after 'Rainier', with larger, firmer fruit. R47, an early-ripening selection, has very desirable fruit characteristics but also consistently low fruit set. The program will evaluate R47 again in 2026 before making a decision whether to advance to Phase 3. We will examine the use of different pollinizers and potentially the use of Retain to see if fruit set can be improved. CR01T078 and R45 were acceptable at harvest but had poorer postharvest performance compared with 'Skeena', and were not advanced to Phase 3. Data was also collected for Phase 2 selections in the 2022-2024 cohorts (Table 1). FR09T084 (not shown) was dropped based on repeated poor fruit set across locations. CR11T019 is not as late ripening as hoped, but may be a good replacement for 'Skeena' as it appears to have less cracking. R25 fruited more quickly than expected as it was only planted last year, suggesting it is highly precocious. It is also very early, ripening just before 'Chelan' at Sagemoor.

Table 1. Characteristics of Phase 2 selections and standard cultivars planted in replicated multi-location trials. Data are averaged over all locations where that selection was harvested. [1]PROS, Prosser; SAGE, Sagemoor; HRIV, Hood River (MCAREC); NACH, Naches (Valley View).

ID	Class	Harvest Timing (vs. 'Bing')	Fruit weight	Firmness (g/mm)	°Brix/TA	Locations[1]	Notes
R35B	Blush	+10	14.5	298	24.3/0.88 (MCAREC only)	PROS, SAGE, HRIV	**Advanced to Phase 3**
Rainier	"	+0	9.4	238	19.4/0.61 (MCAREC only)	PROS, SAGE, HRIV	Standard blush
R25	Mahogany	-26	7.9	258	22.8/0.33	PROS, SAGE, HRIV	Data only from Sagemoor
Chelan	"	-18	8.2	215	21.6/0.43	PROS, SAGE	Standard mahogany (early)
FR31T011	"	-14	12.1	225	21.3/0.26 (Naches only)	PROS, HRIV, NACH	Light red skin, white flesh. Unique flavor.
R47	"	-9	10.9	321	24.3/0.75	PROS, SAGE, HRIV	Light crops
Benton	"	-4	10.5	230	23.8/0.81	PROS, HRIV	Standard mahogany (mid)

Bing	“	0	8.9	244	22.9/0.53	PROS, SAGE, HRIV	Standard mahogany (mid)
CR01T078	“	+4	12.4	322	21.8/0.53	PROS, SAGE, HRIV	Weak stems, more pitting postharvest than ‘Skeena’. Not advanced.
Skeena	“	+3	10.6	294	22.4/0.52	PROS, SAGE, HRIV, NACH	Standard mahogany (mid-late)
CR11T019	“	+5	11.4	310	23.7/0.66	PROS, HRIV, NACH	
R45	“	+6	13.3	317	22.7/0.49	PROS, SAGE, HRIV	Postharvest worse than ‘Skeena’. Not advanced
CR21T043	“	+18	10	284	26.9/0.57	PROS, HRIV, NACH	
Sweetheart	“	+19	9.4	288	24.0/0.69	PROS, HRIV	Standard mahogany (late)

We completed three years of Phase 3 evaluations for R19 and R3, and one year for R29 (Table 2). R19 on Krymsk-6 rootstock did well at the Cheri Barn location, and also did well there last year. Based on its consistent early ripening time and superior fruit characteristics, the WSU Cultivar Release Committee has recommended that R19 be released as a new sweet cherry variety. **This will be the first new WSU cherry since 2007**. We are applying for funding from the WSDA Specialty Crop Block Grant program to establish a rootstock and training system trial for R19 and R3.

R3 struggled this year at the Phase 3 sites (Cheri Barn, Buena and Dallesport) but did well at existing older Phase 2 locations in Prosser and Sagemoor (data not shown). We will evaluate it one more year in Phase 3 and release it in Fall 2026 if it performs well in the early-mid category.

R29, a mid-season selection, did well at Dallesport in its first year of full evaluations, with good fruit set, high tree vigor, and very large firm fruit. Smaller test plantings in Desert Aire and Chelan also looked promising (data not shown). This selection will be evaluated in 2026 and 2027 before making a decision whether to release.

Table 2. Characteristics of Phase 3 selections R19, R3 and R29.

ID	Location	Rootstock	Harvest Date	Weight (g)/Row Size (peak)	Firmness	°Brix/TA	Notes
R19	Cheri Barn	Krymsk-6	6/5	8.3/10	331	28.1/0.28	
R19	Cheri Barn	Mazzard	6/2	9.0/10	329	31.3/0.36	Poor fruit set on Mazzard.
Chelan	Cheri Barn	Mazzard	6/6	6.8/Under	306	23.8/0.39	
R3	Cheri Barn	Krymsk-6	6/17	8.6/10.5	252	23/--	Poor color, flavor
Chelan	Buena	Gisela-12	6/10	7.3/11	252	23.3/0.29	Poor set
R3	Buena	Gisela-12	6/16	9.7/10.5	278	--/0.46	Poor set
R19	Dallesport	Gisela-12	6/9	8.6/10	257	27.2/0.24	Light crop
Chelan	Dallesport	Gisela-12	6/9	9.6/10.5	257	23.8/0.36	
R3	Dallesport	Gisela-12	6/16	11.3/9	328	25.1/0.13	Light crop
R29	Dallesport	Gisela-12	6/23	12.5/9.5	387	25.2/0.25	Good vigor in cold site.
Bing	Dallesport	Gisela-12	6/23	7.8/10	340	23.8/0.28	

Crossing and DNA tests

In 2025, the breeding program made 43 crosses, of which 42 were bi-parental. Total seed produced was estimated at 7,007, essentially the same as in 2024. Extensive use was made of DNA test results to guide parental combinations. For seeds from the 2024 crosses, the excellent overall germination rate of 73% resulted in more than 4,280 seedlings. DNA testing was also used to cull inferior seedlings from these crosses. Testing was more extensive this season due to the deployment of three newly developed tests for fruit size. A total of 1,119 seedlings were eliminated based on DNA test results. Additional seedlings were culled for mildew infection (especially in late-ripening families) and stunted growth. Despite the large number of discarded seedlings, the high germination rate allowed for more than 1,600 high quality seedlings from 2024 crosses to be planted in the field.

Bud take of spring-grafted seedlings was mixed, with 33% showing active growth, and approximately the same amount with live buds but no active growth (total take ~66%). This was the first large-scale attempt at spring budding seedlings. Bud take was better in some families, and this could partly be due to the difference in bud size. Scheduling difficulties with the grafter also resulted in some buds beginning to push several weeks after being removed from cold storage. The larger size of the rootstocks relative to the scions also resulted in more suckers, necessitating more pruning. We will continue to explore budding seedlings onto precocious rootstocks.

Disease screening and orchard maintenance

Out of 50 stored pollen samples, 19 were found to have high levels of PDV or PNRSV and were discarded. Out of 112 trees tested for X-disease, 3 tested positive, none from Prosser. A total of 21 trees were also tested for *little cherry virus 2* (LChV-2); 3 tested positive, none from Prosser.

The spray regime was effective at protecting trees from visible insect damage, and from powdery mildew in the case of the Phase 2 block. A spring herbicide application was effective at controlling early season weeds in the tree row, but summer weeds [primarily marestail (*Conyza canadensis* and prickly lettuce (*Lactuca serriola*)] were not well controlled especially in the younger seedlings. We will coordinate with the IAREC farm crew and our crop consultant to ensure an additional herbicide application to control summer weeds.

Proposal Title: Investigating bacterial canker disease of cherry in young orchards

Report Type: Continuing Project Report

Primary PI: **Frank Zhao**
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Cooperators: Garrett Bishop (G. S. Long); Todd Cameron (Cameron Nursery); Bennett Mayo (Mike and Brian Nursery)

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $51,014

Total Project Request for Year 2 Funding: $52,575

Total Project Request for Year 3 Funding: $54,198

Other related/associated funding sources: None

WTFRC Collaborative Costs: None

Budget 1
Primary PI: Dr. Frank Zhao
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Item	1/3/2024	1/3/2025	1/3/2026
Salaries[1]	$28,800.00	$29,952.00	$31,150.00
Benefits[1]	$10,131.00	$10,536.00	$10,957.00
Wages			
Benefits			
RCA Room Rental			
Shipping[2]	$83.00	$87.00	$91.00
Supplies	$9,000.00	$9,000.00	$9,000.00
Travel	$2,000.00	$2,000.00	$2,000.00
Plot Fees	$1,000.00	$1,000.00	$1,000.00
Miscellaneous			
Total	$51,014.00	$52,575.00	$54,198.00

Footnotes: [1]Postdoc salary for 6 months at $4,800/month and postdoc benefit rate at 35.2%. 4% inflation for years 2 and 3.
[2]Shipping materials.

Objectives:

1. **To determine the etiology of bacterial canker disease (BCD) and their copper and kasugamycin resistance status;**
2. **To survey nurseries and young orchards and investigate factors contributing to the outbreak of BCD;**
3. **To develop and recommend best management practice (BMP) for BCD in nurseries and young orchards.**

Significant Findings:

- 5% and 10% lime sulfur rapidly reduced bacterial populations within 6 hours.
- Clove oil exhibited a rapid and concentration-dependent killing of bacteria.
- 0.125% clove oil was sufficient to achieve bactericidal activity within minutes.
- When pruning was done in hot/dry conditions, lime sulfur, tetracycline, Actigard and Kasumin treatment significantly reduced canker length in 2024 trial.
- Tetracycline, Actigard and Kasumin reduced canker length when pruning was conducted under cold/humid conditions in 2024 trial.
- Kasumin treatment (two formulations: K2L or K8L) act similarly and significantly reduced canker length in 2025 trial.
- Vacciplant plus Kasumin did not improve or inhibit canker development as compared to Kasumin alone.
- The overall canker length and percentage of trees developing BCD was much shorter and lower when pruning was done in hot/dry conditions than in cold/humid conditions, respectively.

Methods

Procedures and Methodology:

1. **Antibacterial activity of lime sulfur (LS) and clover oil against *Pseudomonas syringae* pv *syringae* (Pss) and *P. amygdali* pv. *morsprunorum* (Pam)**

Overnight cultures were washed twice with sterile phosphate-buffered saline (PBS) and adjusted to OD_{600} at 1.0. Tenfold serial dilutions (10^{-1} to 10^{-8}) of bacterial suspensions were treated with LS at concentrations of 2%, 5%, and 10% (v/v) for 0 min, 30 min, 6 h, 24 h, and 48 h and aliquots were plated immediately after the exposure. Similarly, based on the minimum inhibitory concentration, tenfold serial dilutions (10^{-1} to 10^{-8}) of bacterial suspensions were treated with clover oil at concentrations of 0.5%, 0.125%, 0.0625%, and 0.0312%, as well as combinations of 0.125% clove oil + 100 ppm kasugamycin and 0.0625% clove oil + 100 ppm kasugamycin for 0 min, 30 seconds, 10 min, and 30 min. Following the incubation of the bacteria with LS or clover oil, each sample was washed twice with PBS to remove residual LS or clover oil and subsequently plated on Luria Bertani agar (LB) plates. Plates were incubated at 28 °C for 24 h, and bacterial survival was determined by colony enumeration and expressed as CFU/mL.

2. **Field trials to evaluate chemicals against bacterial canker disease in 2024 and 2025**

Field trials in 2014 were conducted at the Washington State University (WSU) Roza Experimental Orchard on newly planted sweet cherry trees. 200 cherry young trees, *Prunus avium* L. cv. Coral Champagne, were purchased from nurseries and planted in Roza in April 2024. In 2024, head cutting experiments were conducted during early May, characterized by sunny, dry, and hot weather, and late May, marked by rainy, humid, and wet conditions (**Fig. 1**). Nine treatments included 2% lime sulfur (T1), Actigard at 150 ppm (T2), tetracycline at 0.067% (T3), latex paint (T4), latex paint + badge X2 at 2lb /4gal latex paint (T5), badge X2 at 2lb /4gal water (T6), kasugamycin at 100 ppm (T7), control with head-cut and bacterial inoculation (T8), and control with head cut without bacterial inoculation (T9) (**Fig. 1**). In both early and late May, treatments were applied either 24 hour pre-inoculation or 24 hour post-inoculation (**Fig. 1**). Cut sites were either inoculated with a rifampicin-resistant isolate of *Pss* (S2 Rif^R) adjusted to 10^6 CFU/mL or left uninoculated as a negative control. 100 µL of bacterial suspension was pipetted directly onto each wound site. Canker length and ooze formation on each tree was recorded at 3, 5, and 8 weeks after inoculation and treatment.

In 2025, field trial was performed on a two-year-old sweet cherry block, *Prunus avium* L. cv. 'Coral Champagne', comprising 160 sweet cherry trees. The experiment has a randomized block design (RBD) and treatments included Kasumin 2L (K2L) at 64 fl oz/A, Kasumin 8L at 16 fl oz/A (K8L), Kasumin 2L plus Vacciplant at 14 fl oz/A (K2L + V), Kasumin 8L plus Vacciplant at 14 fl oz/A (K8L + V), clove oil (5% stock solution) at a concentration of 0.125%, and 0.125% clove oil plus 100ppm Kasumin 2L. The treatment design is as follows: chemical treatment followed by bacterial inoculation (CT_I), chemical treatment followed by bacterial inoculation and a second chemical application four days after inoculation (CT_I_CT). Inoculated untreated check (IUC) was used as a negative control and chemical treatment only (CT) and uninoculated untreated check (UUC) were used as positive controls. Each chemical × treatment type combination was replicated on eight cherry trees. The initial chemical treatments were applied on cutting wound on May 13, 2025 using a calibrated handheld sprayer. Bacterial inoculation was done 24 hours after chemical treatment by dispensing 50µL of a standardized bacterial suspension 10^6 CFU/mL of *Pss* strain S2 directly into cutting wound sites, followed by a second application four days later. Canker development was evaluated by measuring canker length at 3, 5, and 8 weeks post-inoculation.

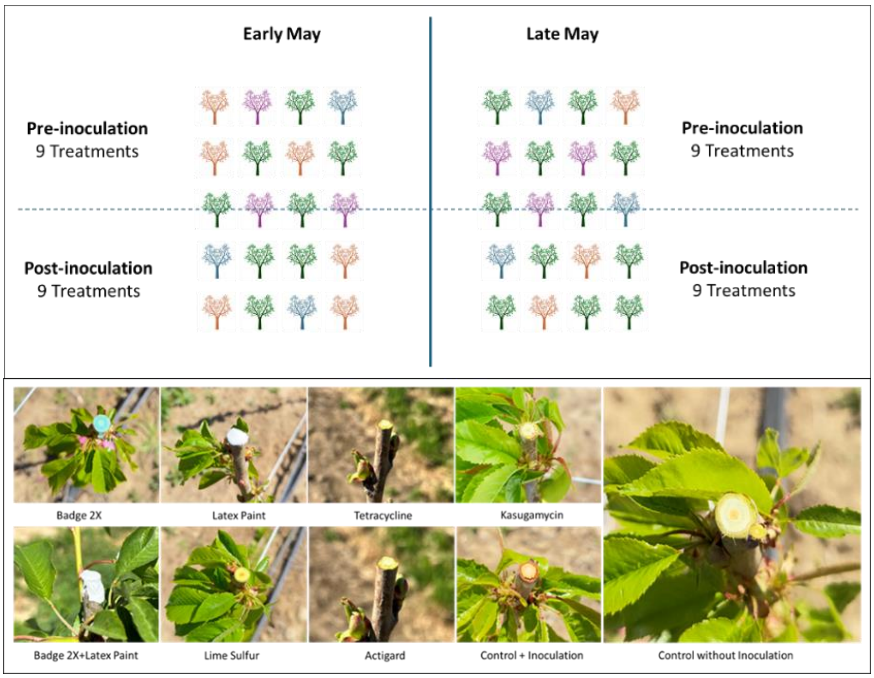

Fig. 1. Experiment design of field trial in early and mid-May 2024 (top)**.** A total of nine treatments were conducted in 2024 (bottom)**.**

3. **Effect of pruning time on bacterial canker incidence**.

After completing the field trials in early July and mid-July in 2024, cankers were removed from trees after 8 weeks. Trees were then left untreated, uninoculated, and monitored for canker development from natural infection once a month until May 2025. In the Early May block, pruning was conducted under hot, dry conditions, which was followed by a week of similarly warm and dry weather. In the Late May block, pruning was performed under relatively cooler, humid conditions and was followed by a week of persistently cool and moist weather.

Types and timing of anticipated results:

In 2024 and 2025, we determined the efficacy of LS and clove oil against bacterial canker pathogens in the lab and in the field. We also found the most effective chemicals to protect cut-wound from pathogen infection in the spring and how weather conditions affected disease incidence and severity. In addition, by monitoring canker development for the entire year, we found the optimal pruning time/condition to reduce infection rate and disease spread.

Potential problems or limitations: We did not encounter any problems and limitations so far as the method is technically straightforward.

Results and Discussion:

1. Antibacterial activities of lime sulfur (LS) and clover oil against canker pathogens in the laboratory

Under continuous contact, 5% and 10% LS rapidly reduced bacterial populations from ~4-6 $\log_{10}$ CFU/mL at 0 h to undetectable levels by 6 hrs as compared untreated control (**Fig. 2acd**). In contrast, 2% LS consistently allowed survival of 5–6 $\log_{10}$ CFU/mL at the same time points but reduced to undetectable levels at 24 to 48 hrs (**Fig. 2b**). When LS was removed after exposure, ~3-6 $\log_{10}$ CFU/mL of bacteria remained alive at 24-48 h across the concentration range of LS used. These results suggest that LS exerts statistically significant and time-dependent bactericidal activity against

Pss and Pam. Maximum efficacy was achieved with 5% and 10% LS under continuous contact, which eliminated recoverable *P. syringae* cells within 6-24 h.

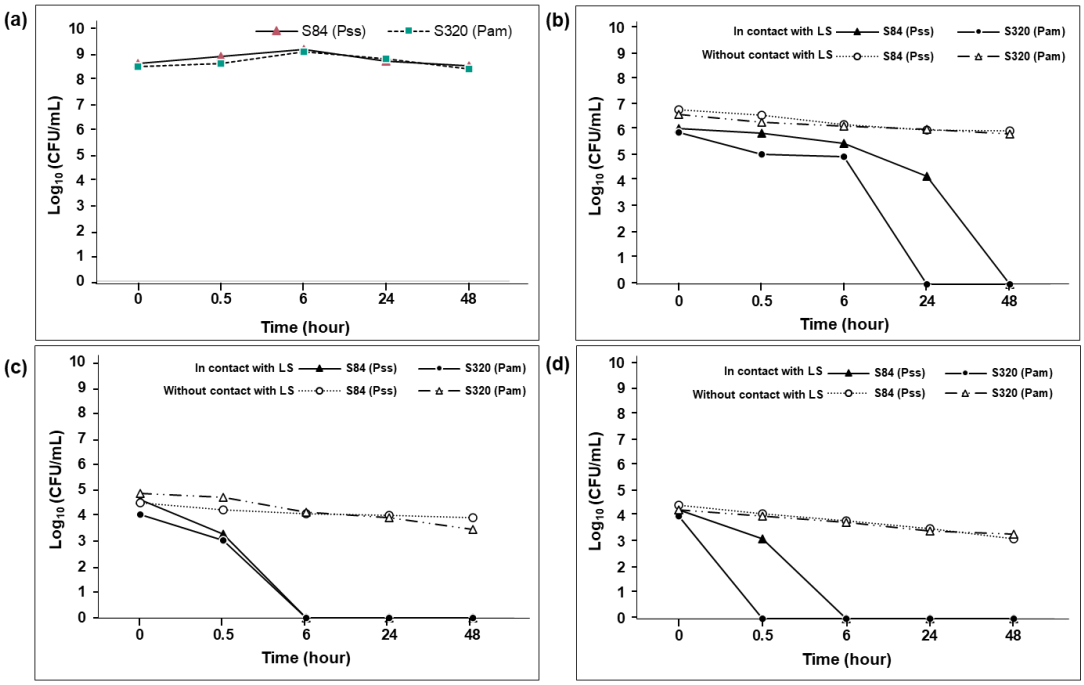

Fig. 2. Antibacterial activity of lime sulfur (LS) against *Pseudomonas syringae* pv. *syringae* (Pss) and *P. amygdali* pv. *morsprunorum* (Pam). Bacterial survival (log_{10} CFU/mL) over time (0-48 h) is shown under continuous contact and post-contact removal for untreated control (A), 2% LS (B), 5% LS (C), and 10% LS (D). LS at 5% and 10% eliminated recoverable cells within 6-24 h under continuous contact.

The minimum inhibitory concentration (MIC), defined as the lowest concentration that inhibits visible bacterial growth after 24 h, was first determined for clove oil against the two bacterial strains. The MIC was 0.125% for Pss strain S84 and 0.0625% for Pam strain S320. Serial concentrations of clove oil were selected for the survival assays using plate counts. Our results showed that clove oil exhibited a rapid and concentration-dependent killing of bacteria (**Fig. 3**). For both pathovars, no significant differences were detected at 0.125% and 0.50% clove oil, indicating that 0.125% was sufficient to achieve bactericidal activity against both pathogens. All clove oil concentrations above 0.0625% (alone or in combination with 100 ppm kasugamycin) significantly reduced bacterial survival relative to the untreated control at every time point. For Pam, survival dropped to undetectable levels at all time points tested. Compared to Pam, Pss was slightly less sensitive to clove oil at 0.0625% concentration and population reduction was about 4 logs at 30 min. However, at 0.03% clove oil level, both pathogens permitted limited survival, with mean log_{10} CFU values declining from 7.98 at 0 min to 6.74 at 30 min (**Fig. 3**). Overall, these results demonstrate that clove oil at concentrations above 0.0625% achieved complete inhibition of both Pam and Pss within 30 s of exposure.

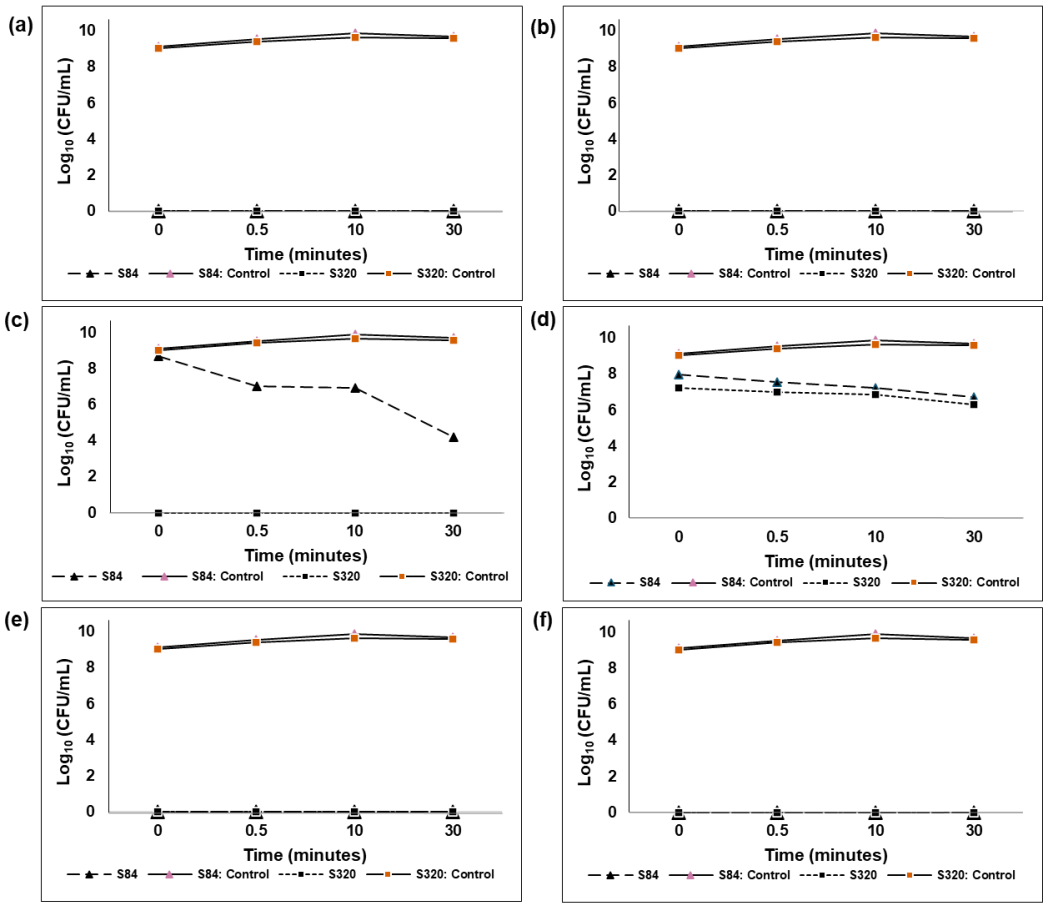

Fig. 3. Survival of *Pseudomonas syringae* pv. *syringae* (S84) and *P. amygdali* pv. *morsprunorum* (S320) following exposure to clove oil and clove oil–kasugamycin combinations. (a) 0.5% clove oil, (b) 0.125% clove oil, (c) 0.0625% clove oil, (d) 0.0312% clove oil, (e) 0.125% clove oil + 100 ppm kasugamycin, (f) 0.0625% clove oil + 100 ppm kasugamycin. Data points represent means of three biological replicates with three technical replicates each.

2. 2024 field trial results. Quantitative evaluation of canker progression from weeks 3 to 8 under Early May conditions showed consistent increased canker lengths across treatments (**Fig. 4ab**). No statistically significant differences were observed between pre-inoculation and post-inoculation treatments across all weeks and treatment groups. For the inoculated untreated checks, mean canker length ranged from 31.8, 45.2, and 55.7 mm at 3, 5, and 8 weeks, respectively. Among the chemical treatments, lime sulfur, Actigard, and tetracycline treatment showed intermediate levels of canker progression, reaching 22.3, 24.7, and 15.3 mm in length, respectively, by week 8. Latex paint, latex paint combined with Badge X2, and Badge X2 alone treatment exhibited comparatively longer canker lengths, averaging 36.7, 40.8, and 46.2 mm by week 8. Kasugamycin treatment consistently maintained lower canker progression across all time points, with mean canker lengths of 4.0, 6.3, and 9.8 mm at 3, 5, and 8 weeks, respectively. For the uninoculated untreated checks, mean canker length ranged from 0.6, 1.8, and 2.2 mm at 3, 5, and 8 weeks, respectively.

Under Late May conditions, canker development advanced progressively between 3 and 8 weeks across all treatments (**Fig. 4cd**). There were no statistically significant differences in treatment efficacy between pre-inoculation and post-inoculation treatments. The inoculated untreated check consistently displayed the longest canker lengths, with mean lengths of 36.0 mm at week 3, 44.3 mm

at week 5, and 58.2 mm at week 8. In contrast, the uninoculated untreated check maintained shortest canker lengths, ranging from 1.0 mm at week 3, 3.4 mm at week 5, and 8.2 mm at week 8. Among the chemical treatments, kasugamycin, Actigard, and tetracycline treatment showed intermediate levels of canker progression, reaching 37.4, 32.8, and 26.2 mm, respectively, by week 8. For lime sulfur treatment, mean canker lengths increased from 23.9 mm at week 3 to 32.3 mm at week 5 and reached 50.4 mm by week 8. The canker lengths for Latex Paint (T4), latex Paint + Badge X2 (T5) and Badge X2 alone reached 49.1, 56.7 and 43.9 mm by week 8, respectively. These results indicate that when pruning was done in hot/dry conditions, lime sulfur, tetracycline, Actigard and kasugamycin significantly reduced canker length (**Fig. 4b**); whereas tetracycline, Actigard and kasugamycin also reduced canker length when pruning under cold/humid conditions (**Fig. 4d**).

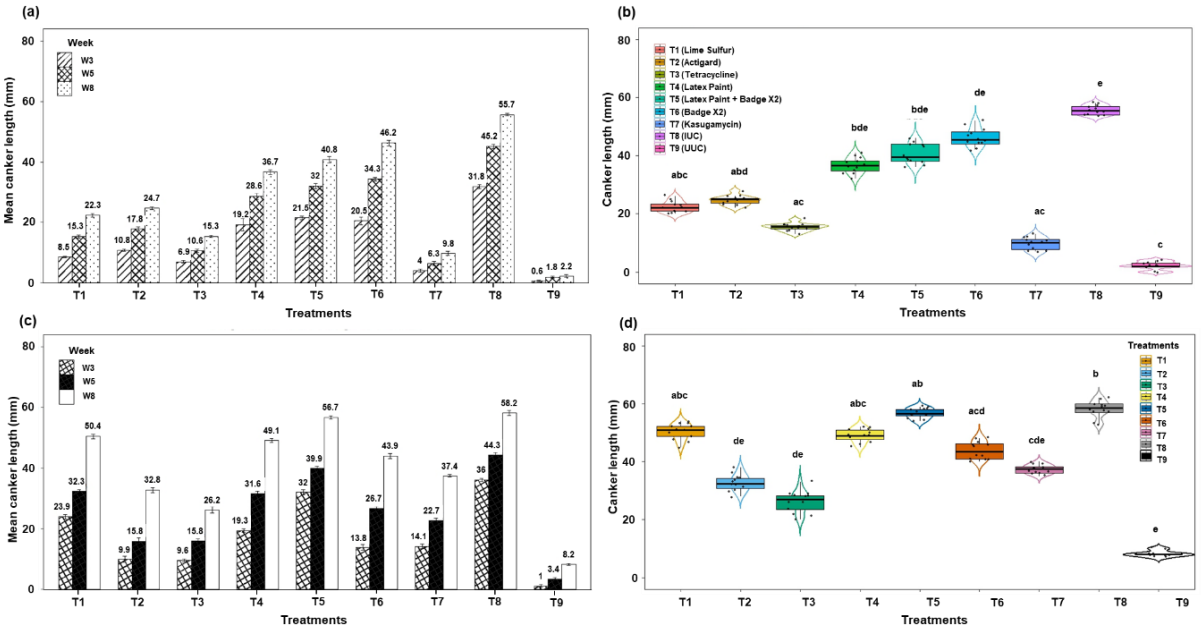

Fig. 4. **Canker length (mm) in sweet cherry trees under different chemical treatments at three assessment times, 3 (W3), 5 (W5), and 8 (W8) weeks post-inoculation**. (**a, b**) Early May block (**a**) bar plot of mean canker lengths (± standard error) over time; **(b)** violin plot of canker length distributions across treatments with statistical groupings indicated by compact letter display (CLD) based on Dunn's test with Holm correction. **(c, d)** Late May block **(c)** bar plot of mean canker lengths over time; **(d)** violin plot with CLD groupings. Treatments include T1, Lime Sulfur; T2, Actigard; T3, Tetracycline; T4, Latex Paint; T5, Latex Paint + Badge X2; T6, Badge X2; T7, Kasugamycin; T8, inoculated untreated control (IUC); and T9, uninoculated untreated control (UUC).

3. 2025 field trial results. Quantitative evaluation of canker progression from weeks 3 to 8 revealed distinct temporal patterns among treatments (**Fig. 5**). The inoculated untreated check (IUC) exhibited the largest increase in canker length, ranging from an average of 9 mm at week 3, 17.8 mm at week 5, and 27.1 mm at week 8. Treatments containing Kasumin showed substantially slower rates of disease progression. Canker length for Kasumin 2L (K2L) treatment was 0.2 mm at week 3, 4.9 mm at week 5 and 11.5 mm by week 8; Similarly, canker length for Kasumin 8L (K8L) treatment also exhibited limited progression from 3.4 mm at week 3, 9.4 mm at week 5, and 15.6 mm by week 8.

Vacciplant plus Kasumin did not improve or inhibit canker development as compared to Kasumin alone (**Fig. 5a**). Canker length for K2L combined with Vacciplant (K2L+V) treatment was 1.1 mm at week 3, 7.7 mm at week 5, and 12.8 mm at week 8. Similarly, canker development for K8L combined with Vacciplant (K8L+V) treatment was slightly worse as compared to K2L+V, ranging from 2.2 mm

at week 3, 8.6 mm at week 5 and 15.0 mm at week 8. In contrast, clove oil and the combined treatment with Kasumin 2L (CO+K2L) did not significantly differ from IUC, indicating limited efficacy. Canker length for clove oil increased from 5.6mm in week 3 to 14.9mm at week 5, and 22mm by week 8. For the clove oil treatment combined with Kasumin 2L, canker length was 4.1mm in week 3, 9.5mm in week 5, and 18.3mm in week 8. The uninoculated untreated check (UUC) maintained minimal canker development, remaining at 0 mm at week 3, increasing to 2.1 mm at week 5 and 4.9 mm at week 8.

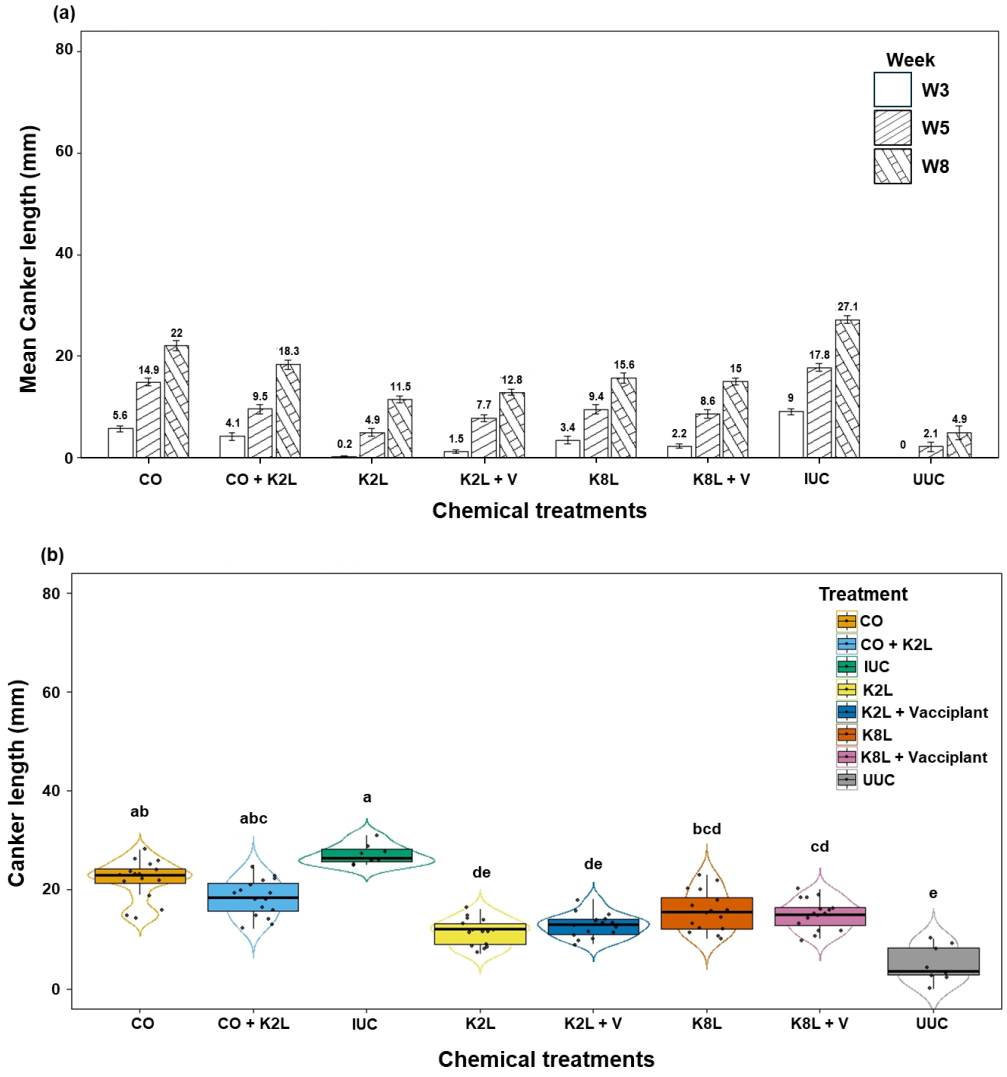

Fig. 5. Canker length (mm) in sweet cherry trees under different chemical treatments at three 3, 5, and 8 weeks post-inoculation. (a) Progression of canker length (mm) over time across treatments at 3 (W3), 5 (W5), and 8 (W8) weeks post-inoculation. Bars represent mean canker length ± standard error for each treatment group. (b) Comparative efficacy of treatments at week 8. Violin plot shows the distribution of canker lengths (mm) across treatment groups at 8 weeks post-inoculation. Median values are indicated by horizontal bars, and individual data points are overlaid. Statistical groupings based on Dunn's post hoc test with Holm correction ($\alpha = 0.05$) are denoted by different letters. Treatments sharing a letter are not significantly different.

Kasumin-based treatments showed substantial reductions in canker length relative to the positive control, inoculated untreated check (IUC, median 27.1 mm) (**Fig. 5b**). Specifically, Kasumin 2L (K2L, median 11.5 mm) and Kasumin 2L combined with Vacciplant (K2L+V, median 12.8 mm,) treatments were statistically indistinguishable from each other and significantly more effective than IUC. Kasumin 8L (K8L, median 15.6 mm) and Kasumin 8L combined with Vacciplant (K8L+V, median 15.0 mm) also exhibited significant canker length reduction relative to IUC. In contrast, clove oil (CO, median 22.0 mm) and the combined CO+K2L treatment (median 18.3 mm) did not significantly differ from IUC, indicating limited efficacy. The uninoculated untreated check (UUC, median 4.9 mm) demonstrated minimal canker progression, significantly different from all inoculated treatments. These results further indicate that two formulations of kasugamycin significantly reduced canker length. Although clover oil could kill *Pseudomonas* in laboratory in less than one minute, field experiment showed that at 0.125% concentration, clove oil did not significantly reduce canker length as compared to untreated control.

4. Pruning time affects infection incidence and disease spread in the field. Infection incidence increased steadily from fall through spring, with marked differences between pruning blocks (**Fig. 6**). By May 2025, 78% of Late May block–pruned trees were infected compared to 41% in Early May block. These results showed that the overall canker length (**Figs. 4, 5**) and percentage (**Fig. 6**) of trees developing BCD was much shorter and lower when pruning was done in hot/dry conditions than in cold/humid conditions, respectively.

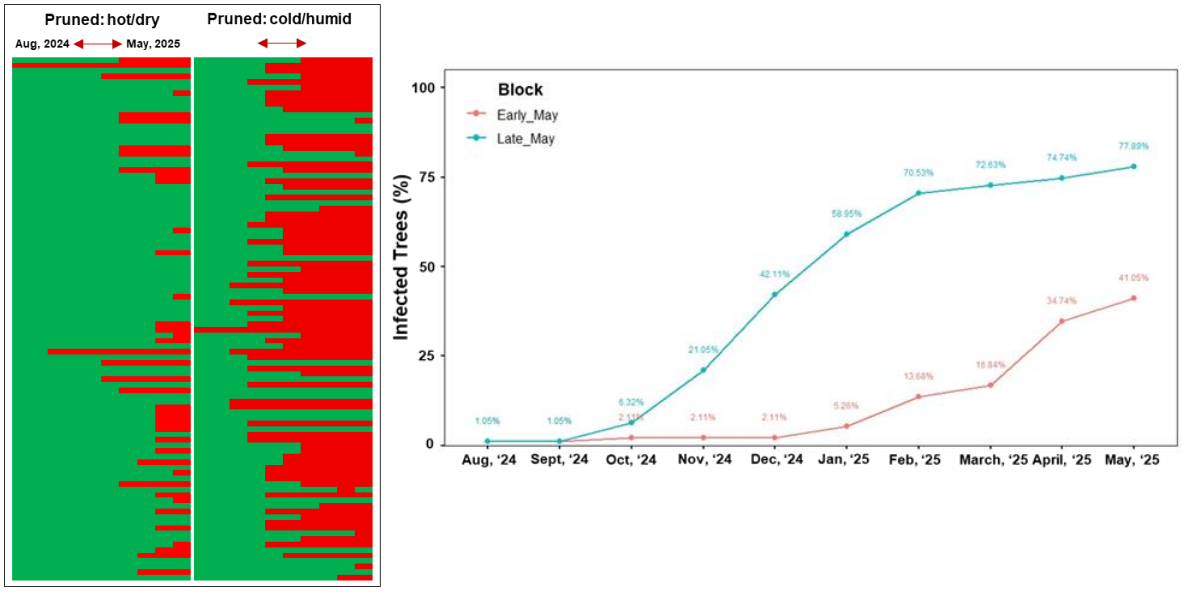

Fig. 6. Monitoring bacterial canker (BCD) development of sweet cherry trees pruned during hot or cold weather conditions from August 2024 to May 2025 (Left). Early May block (hot/dry); Late May block (cold/humid). Red-bar represented individual infected tree throughout the season. A total of 200 trees were monitored. **Percentage of sweet cherry trees developing bacterial canker pruned during hot or cold weather conditions from August 2024 to May 2025 (Right).** Pruning performed in early May block during hot, dry weather resulted in slower disease development, with infection incidence reaching 41%. Late May block pruning under cool, humid conditions resulted in rapid disease progression, with disease incidence reaching 78% at the end of the experiment.

Significance to the industry and potential economic benefits:

Widespread copper resistance in *Pseudomonas* spp. pathogens has left growers with little to no management options, leading to outbreaks of BCD in WA. Finding alternatives to copper is critical for management of bacterial canker disease. We evaluated lime sulfur and clove oil and found both have excellent antibacterial activities in the lab at 5% and 0.125%, respectively. During 2024 and 2025 field trials, we evaluated various chemicals, including copper, lime sulfur, Actigard, Vacciplant, tetracycline, kasugamycin, paint and clover oil, for managing bacterial canker disease in combination of optimal pruning time. When pruning was done in hot/dry conditions, lime sulfur, tetracycline, Actigard and kasugamycin significantly reduced canker length; whereas tetracycline, Actigard and kasugamycin also reduced canker length when pruning under cold/humid conditions. Although clover oil could kill *Pseudomonas* in laboratory in less than one minute, field experiment showed that at 0.125% concentration, clove oil did not significantly reduce canker length as compared to untreated control. When monitoring disease incidence combination with optimal pruning time, we found that the overall canker length and percentage of trees developing BCD was much shorter and lower when pruning was done in hot/dry conditions than in cold/humid conditions, respectively. These findings are significant and could provide direct economic benefits to growers and the cherry industry by limiting copper use, selecting optimal pruning time and alternative treatments, thus reducing costs and increasing profitability. We recommend that due to copper resistance, growers should rely more on cultural control measures for managing bacterial canker disease, including but not limited to timely prune canker limbs under warm and dry conditions.

Project Title: Evaluation of UV-C for Management of Cherry Diseases and Pests
Report Type: Continuing Project Report

Primary PI: Tami Collum

Report is Forthcoming

Project Title: Understanding Food Safety Risks During Post-Harvest Cherry Production

Report Type: Continuing Project Report

Primary PI: Claire Murphy
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City/State/Zip: Wenatchee, WA 98801

Cooperators: Four cherry packinghouses in Washington and four packinghouses in California were cooperators for objective 1. An additional four cherry packinghouses in Washington were cooperators for objective 2. The identity of the operations serving in cooperator roles will be kept confidential.

Project Duration: 3 Years

Total Project Request for Year 1 Funding: $74,255
Total Project Request for Year 2 Funding: $76,231
Total Project Request for Year 3 Funding: $69,785

WTFRC Collaborative Costs:

Item	2024	2025	2026
Salaries	$3,900.00	$4,056.00	$4,218.00
Benefits	$1,218.00	$1,267.00	$1,317.00
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies			
Travel			
Plot Fees			
Miscellaneous			
Total	**$5,118.00**	**$5,323.00**	**$5,535.00**

Footnotes:
Salary/benefits: an estimate of the percent of time spent for Mendoza (4%) at a 31.2% benefit rate and 2% annual increases.

Budget 1
Primary PI: Claire Murphy
Organization: Washington State University Irrigated Agriculture Research and Extension Center
Contract Administrator: Hollie Tuttle
Telephone: 509-786-2226
Contract administrator email address: prosser.grants@wsu.edu
Station Manager/Supervisor: Naidu Rayapati
Station manager/supervisor email address: 509-786-9215

Item	2024	2025	2026
Salaries	$32,981.00	$34,300.00	$35,672.00
Benefits	$2,848.00	$2,848.00	$2,848.00
Wages	$9,955.00	$10,353.00	$10,767.00
Benefits	$1,353.00	$1,407.00	$1,463.00
RCA Room Rental			
Shipping			
Supplies	$20,500.00	$20,500.00	$13,000.00
Travel	$1,500.00	$1,500.00	$500.00
Plot Fees			
Miscellaneous			
Total	**$69,137.00**	**$70,908.00**	**$64,250.00**

Footnotes:
Salaries: $32,981, $34,300, and $36,672 are requested in years 1, 2, and 3, respectively, for a graduate research assistantship for a Ph.D. student to work on all objectives
Benefits: $2,848 is requested in years 1, 2, and 3 for benefits tied to the graduate research assistantship
Wages: $9,955, $10,353, and $10,767 are requested in years 1, 2, and 3, respectively, for summer student hourly employees to assist with sample collection and conducting experiments related to all three objectives
Benefits: $1,353, $1,407, and $1,463 are requested in years 1, 2, and 3, respectively, for benefits tied to the hourly employees
Supplies: $20,500, $20,500, and $13,000 are requested in years 1, 2, and 3, respectively, to purchase disposal supplies such as glassware, inoculation loops, pipette tips, sponge swabs, microbiological media, Petri dishes, PCR reagents, etc.
Travel: $1,500, $1500, and $500 are requested in years 1, 2, and 3, respectively, for mileage and travel-associated cost, adhering to all university policies

Objectives:

1. Evaluate the prevalence of *Listeria* spp., as an indicator for *L. monocytogenes*, on food contact surfaces on the packing line (e.g., conveyor belt surfaces, stainless steel, etc.) within commercial cherry packinghouses at three time points (beginning, middle, and end of the season) over two packing seasons (2024 and 2025).
2. Evaluate the microbiological status of postharvest water and cherries to understand the change in microbial status from receiving to packing over a packing day.
3. Evaluate the risks associated with hydrocooling during laboratory-simulated hydrocooling with water contaminated with generic *E. coli* or *L. monocytogenes* at varying levels under a variety of relevant industry conditions.

Significant Findings:

Objective 1:

- *Listeria* spp. was detected in all packinghouses across both years, with an overall prevalence of 2.0% (38/1,920) in Washington and 2.4% (15/636) in California.
- Bristle flaps, solid conveyor belts, and singulators were the most frequently contaminated food contact surfaces in both years and both states.
- *Listeria* spp. prevalence was slightly higher during packing (2.19%) than pre-packing (1.96%).
- Only 2 sites (3.9%) were positive during the same sampling event, both pre- and during-packing, indicating that most *Listeria* spp. detections were transient rather than persistent**.**

Objective 2:

- Generic *E. coli* was detected on 1.5% of cherries (4/261) and in 2.5% of water samples (5/204), while total coliforms were detected on 18.0% of cherries (47/261) and in 45.1% of water samples (92/204). The low detection rates indicate that *E. coli* and total coliforms are not reliable indicators in cherry packing systems; Aerobic plate count (APC) and *Enterobacteriaceae* (EB) provided more consistent measures of microbial load.
- Over 10 hours of operation, microbial loads in water remained stable, with no significant increase in APC or EB despite changes in physiochemical water quality (e.g., turbidity).
- APC levels on cherries significantly fluctuated between flumes, suggesting localized cross-contamination. However, an increased chlorine ppm in water systems significantly reduced both APC and EB counts. Thus, structural equation modeling (SEM) is being employed to allow for a more comprehensive understanding of the complex interdependencies between microbial load, water quality parameters, and operational practices that may contribute to cross-contamination events within the cherry packing environment.

Methods:

<u>Objective 1 (Years 1 and 2)</u>

<u>Study Design:</u> Four commercial cherry packinghouses in Washington and four in California were recruited into this study and swabs of food contact surfaces were collected from each of the packinghouses during three separate visits (beginning, middle, and end of the season). The food contact surfaces for sampling were selected at each operation before the first sampling event. Sampling sites for each packinghouse were photographed and described in detail to ensure consistency during all sampling events. Samples were collected prior to the start of packing, as well

as 5 h into packing. Sampling was conducted during the 2024 and 2025 seasons in WA, and the 2025 season in CA.

Sample Collection: Swab samples were collected from each site using sponge sticks that were pre-moistened with 10mL of Dey Engley neutralizing broth. Each sponge stick was aseptically removed from the bag with sterile gloves, used according to the manufacturer's specifications: returned to the bag, sealed, and placed in a cooler. All samples were processed within 24h at the Murphy Bio-Safety Level 2 Laboratory at Washington State University's Irrigated Agriculture Research and Extension Center.

Sample Processing: The isolation, detection, and confirmation of *Listeria* spp. was conducted following a modified FDA Bacteriological Analytical Manual method (FDA, 2022). Briefly, 90 mL of buffered *Listeria* enrichment broth was added to each sample bag, stomached, and incubated at 30ºC. Following a 4h incubation period, a 360µL aliquot of *Listeria* selective enrichment supplement was added to each bag and returned to the 30ºC incubator for 20h. At both 24h and 48h, the enriched sample was sub-streaked onto modified oxford agar and incubated for 48h at 30ºC. Presumptive positive colonies were re-streaked onto modified oxford agar and incubated for 48h at 30ºC before being PCR-confirmed.

Objective 2 (Year 2):

Protocol development: A protocol for validating, verifying, and monitoring postharvest water systems and produce through the postharvest water system was developed at WSU. The protocol is set up so that once validated by the team at WSU, it can be created into an extension document that producers can follow to use within their system, including what samples to collect, what to ask a third-party lab for, and how to analyze data. To generate feedback on the protocol, it was sent to extension and industry professionals to provide feedback on the layout, feasibility, etc.

Sample collection and processing: Triplicate water samples (100 mL) and cherries (whole) were collected from each distinct water system in the postharvest process over the course of a packing day. Water and produce samples were tested for aerobic plate counts (APC), *Enterobacteriaceae* (EB), total coliforms (TC), and generic *E. coli*. Water physicochemical properties and antimicrobial levels in water were monitored throughout the sampling day as well. Trends in microbial populations in water and on produce throughout the production run were evaluated to determine the microbial target(s) and physiochemical parameters that best correlate with water quality. For validation, populations of target microorganisms must remain the same or decrease compared to Time 0.

Objective 3 (Years 3)

Study Design: Simulated cherry hydrocooling trials will be performed in duplicate, at minimum, for each combination of the following experimental variables treatments: (i) bacteria (generic *E. coli*, *L. monocytogenes*) (ii) inoculum [low (3 log CFU/mL) and high (6 log CFU/mL)], (iii) water temperature [2°C (35.6°F) and 6°C (42.8°F)], (iv) contact time, and (v) physiological state (cracked and whole). To simulate the maximum field temperatures of cherries exposed to direct sunlight before harvest, fruits will be equilibrated to 40°C (104°F) before use. All parameters are subject to change, based on input from stakeholders, to ensure industry-relevant conditions. For each treatment combination option, cherries will be subjected to a simulated hydrocooler.

Sample processing: For evaluating cross-contamination on the surface of cherries during hydrocooling, cherries will be placed into a stomacher bag with 1:10 mL (weight/volume) of peptone

water and hand-rubbed for 90 min to detach cells from cherry surfaces. The rub solutions will be 10-fold serially diluted and surface on duplicate appropriate agar plates.

Results and Discussion

Objective 1:

The prevalence of *Listeria* spp. on food contact surfaces was assessed in four Washington State cherry packinghouses over two packing seasons. *Listeria* spp. was isolated from all four packinghouses during both the 2024 and 2025 packing seasons. Among all tested samples, 2.0% (38/1920) were confirmed positive for *Listeria* spp. (Table 1). Of the 14 food contact surface types sampled, *Listeria* spp. was most frequently isolated from bristle flaps (6.3%; 59/144), followed by solid conveyor belts (4.2%; 6/144) and singulators (3.8%; 5/132; Table 1). One year of data was collected from four California packinghouses, where the overall prevalence of *Listeria* spp. was 2.4% (15/636). The three most frequently contaminated surfaces were the same as those in WA, though in a different order: solid conveyor belts (6.3%; 3/48), bristle flaps (4.6%; 3/65), and singulators (4.5%; 1/22).

Table 1: Frequency of isolation of *Listeria* spp. by food contact surface in Washington State and California

Food Contact Surface	Washington			California		
	No. of Total Samples	No. of Positive Samples	Frequency	No. of Total Samples	No. of Positive Samples	Frequency
Bristle Flap	144	9	6.3%	65	3	4.6%
Cherry Elevator	168	1	0.6%	50	0	0.0%
Cluster Cutter	84	1	1.2%	24	0	0.0%
Interlocking Conveyor Belt	228	1	0.4%	122	3	2.5%
Packing Guide	60	2	3.3%	43	0	0.0%
Plastic Flap	24	0	0.0%	30	0	0.0%
Singulator	132	5	3.8%	22	1	4.5%
Sizing Rollers	84	1	1.2%	32	1	3.1%
Solid Conveyor Belt	144	6	4.2%	48	3	6.3%
Sorter Cups	96	3	3.1%	32	0	0.0%
Sorter Flap	84	1	1.2%	56	1	1.8%
Stainless Steel Flume	312	4	1.3%	76	3	3.9%
Textured Conveyor Belt	180	0	0.0%	-	-	-
Transfer Point	180	4	2.2%	36	0	0.0%
Total	1920	38	2.0%	636	15	2.4%

Only a handful of previous studies have examined the prevalence of *Listeria* spp. on food contact surfaces (zone 1) of whole fresh market produce packinghouses. On tomato packing lines in Tennessee, 10.9% (62/565) of food contact surfaces sampled from three packinghouses were positive for *Listeria* spp. (Hamilton, 2018). A previous study assessing the prevalence of *Listeria* spp. in apple packing houses in Washington state over two seasons found the prevalence on food contact surfaces to be 4.6% (136/2,988; Ruiz-Llacsahuanga et al., 2021), with the greatest prevalence of *Listeria* spp. on polishing brushes (19.6%), dividers under fans/blowers (17.4%), dryer rollers (10.5%), and brushes under fans/blowers (9.7%). Unlike apples, cherries are not waxed and do not utilize brushes throughout the packing line. However, the bristle materials that make up many of the brushes within

the apple packing line are used in cherry packing lines to help direct the flow of fruit as the cherries move on the line. Food contact surfaces that utilize this bristle material can retain debris and moisture, thus maintaining an environment that can support *Listeria*. Given that bristles are frequently recognized as common harborage sites for *Listeria* spp. in tree fruit operations due to their complex hygienic design, the cherry industry should prioritize mitigation strategies for these materials. This includes replacing them with alternative materials when feasible, regularly cleaning and sanitizing them, and replacing them when they show signs of wear.

When the prevalence of *Listeria* spp. was analyzed by operational step (Table 2), the receiving stage had the highest prevalence of *Listeria* spp. (3.1%; 22/720) both overall and when broken down by pre-packing (2.22%; 8/360) and during-packing (3.89%; 14/360). However, when the operational step frequency was broken down by year and location, differences were observed. For example, in Washington in year 1, the highest prevalence of *Listeria* spp. pre-packing was during the receiving step (2.2%, 1/45), during packing was the packing step (6.7%, 8/120), and overall was the packing step (3.75%; 9/240). On the other hand, in Washington in year 2, the highest prevalence overall was found to be during the sorting operation (2.4%; 15/630), while the highest number of positives in California overall were found during the receiving stage (4.17% 2/48), all of which were collected during packing. Because *Listeria* spp. prevalence varied by step, year, and location, strong good agricultural practices (GAPs) in the field and consistent post-harvest sanitation protocols remain critical, but if time or resources are limited, efforts should focus on likely harborage sites.

Table 2: Frequency of isolation of *Listeria* spp. by operation step and based on sampling time during a sampling event

Step	Percentages of positive samples		
	Pre-packing	**During-packing**	**Total**
Receiving	2.2% (8/360)	3.9% (14/360)	3.1% (22/720)
Sorting	0.9% (1/144)	1.8% (2/114)	1.3% (3/228)
Packing	2.0% (16/802)	1.5% (12/802)	1.7% (18/1604)
Total	2.0% (25/1276)	2.2% (28/1276)	2.1% (53/2552)

In addition to the food contact surface and unit of operation, the timing of sample collection had an impact on *Listeria* spp. prevalence. Of the 1,279 pre-packing samples, 1.96% (25/1276) were positive for *Listeria* spp. compared to the 2.19% (28/1276) of the samples during packing (Table 3). In addition, the timing of *Listeria* spp. isolation was evaluated among all of the positive samples based on three scenarios: (i) the sampling site testing positive pre-packing and negative during-packing, (ii) the sampling site testing negative pre-packing and positive during-packing, or (iii) the sampling site testing positive both pre and during packing (Table 4). For all years and locations combined, the largest number of positive samples fell into scenario 2 (50.98%; 26/51), followed by scenario 1 (45.10%; 23/51) and 3 (3.92%; 2/51). However, similar to the operational step, when the scenarios were broken down by year and location, differences were observed, with the majority of positive samples in WA year 1 and CA falling into scenario 2 (64.7% and 66.7%, respectively), while the majority in WA year 2 were scenario 1 (68.4%). Pre-packing sampling occurred after any cleaning and/or sanitation events but before the product was run for the day, which indicates the effectiveness of the cleaning and sanitation. The higher prevalence of *Listeria* spp. during packing in WA year 1 and CA, compared to pre-packing, indicates that cleaning and sanitation protocols are relatively effective and that incoming crop is cross contaminating the FCS. Previous research in apple packinghouses in Washington (Ruiz-Llacsahuanga et al., 2021), a stone fruit packinghouse in

Virginia (Bardsley et al., 2024), and an avocado processing facility in South Africa (Strydom et al., 2016) also found that *Listeria* spp. prevalence was greater during packing. Additionally, only 2 sampling sites tested positive both pre- packing and during packing, during the same sampling event: one singulator and one transfer point. The low prevalence of paired positive samples during both pre- and during-packing suggests that most *Listeria* spp. findings are transient (sporadic introductions that do not persist) rather than resident (persistent strains that survive and establish in the facility over time).

Table 3: Frequency of *Listeria* spp. isolation for a specific sampling site based on sampling time by location and year

	Scenario	Operational Timepoint		No. of Sites	Frequency (%)[a]
		Pre-packing	During-packing		
Washington Year 1	1[b]	Positive	Negative	5	29.4%
	2[c]	Negative	Positive	11	64.7%
	3[d]	Positive	Positive	1	5.9%
Washington Year 2	1	Positive	Negative	13	68.4%
	2	Negative	Positive	5	26.3%
	3	Positive	Positive	1	5.3%
California	1	Positive	Negative	5	33.3%
	2	Negative	Positive	10	66.7%
	3	Positive	Positive	0	0.0%
Overall	1[b]	Positive	Negative	23	45.10%
	2[c]	Negative	Positive	26	50.98%
	3[d]	Positive	Positive	2	3.92%

[a] Total number of sampling sites with at least one positive detection of *Listeria* spp. in Washington Year 1, Year 2, and California was 17, 19, and 15, respectively.
[b] Sampling sites in which *Listeria* spp. were detected in only the pre-packing sampling.
[c] Sampling sites in which *Listeria* spp. were detected in only during-packing sampling
[d] Sampling sites in which *Listeria* spp. were detected in paired pre- and during-packing samplings.

Objective 2:

The microbial quality of discrete water systems and cherries before and after each system was evaluated in four Washington State cherry packinghouses. Each packinghouse had three or four discrete water systems. In total, 261 cherry samples (90 from packinghouse A, 75 from packinghouse B, 48 from packinghouse C, and 48 from packinghouse D) and 204 water samples (72 from packinghouse A, 60 from packinghouse B, 36 from packinghouse C, and 36 from packinghouse D) were collected as part of this study. Since only 4 cherries (1.5%) and 5 water (2.5%) samples had detectable levels of generic *E. coli* (≥ 1.0 log CFU/cherry and 0.0 log CFU/mL, respectively), and 47 cherries (18.0%) and 92 (45.1%) water samples had detectable levels of generic total coliforms (≥ 1.0 log CFU/cherry and 0.0 log CFU/mL, respectively), *E. coli* total coliform data were not utilized for cross-contamination analysis. In agricultural water quality assessments, total coliforms and generic *E. coli* are commonly employed as indicator organisms. Total coliforms represent a broad group of bacteria present in soil, vegetation, and aquatic environments, and their detection indicates general microbial contamination. In contrast, generic *E. coli* originates from the intestines of warm-blooded animals and is associated with fecal matter; thus, its presence serves as a more specific indicator of fecal contamination. However, the current study found a low prevalence of cherry and water samples that were at or above the limit of detection for generic *E. coli* and total coliforms. Similarly, a study

examining the microbial quality of pre- and post-processed tomatoes in Florida, New Jersey, and Maryland found that 93.8% (1,498/1,597) of samples had generic *E. coli* counts below the detection limit (1.3 log CFU/tomato; Schneider et al., 2017) and unpublished data by Murphy et al. (2025) noted that only 1.6% of pre- and post-flume apples (4/225) and 1.3% of postharvest water samples (2/159) had detectable levels of generic *E. coli*. While generic *E. coli* is an effective fecal indicator, using it alone in a postharvest setting to assess water quality and cross-contamination risk may be insufficient across various commodities. The detection of total coliforms on other produce commodities suggests that their occurrence may be crop-specific; for example, their limited detection in cherries indicates that total coliforms may not serve as a reliable indicator organism for this commodity. Instead, relying on *Enterobacteriaceae* (EB) or aerobic plate count (APC) as indicators can provide a broader and more consistent assessment of microbial load, water quality, and cross-contamination risk across different crops. APC measures the total number of viable bacteria capable of growing in oxygenated conditions, providing an overall estimate of microbial load. EB is a bacterial family that includes many species commonly associated with environmental and fecal contamination, making it a useful indicator of sanitation effectiveness and potential postharvest cross-contamination.

Table 4: Comparison of average and standard deviations (log CFU/mL) for Aerobic Plate Count (APC) and *Enterobacteriaceae* (EB), in water over time in PHA

Water Hold Time (hours)	$Water_1$		$Water_2$		$Water_3$		$Water_4$	
	APC	EB	APC	EB	APC	EB	APC	EB
0	1.57±0.33a[a]	0.88±0.37a	0.78±0.26a	0.05±0.12a	0.66±0.39ab	0.18±0.21a	0.50±0.55a	0.26±0.22a
2	1.22±0.30ab	0.15±0.25b	0.90±0.36a	0.00±0.00a	0.80±0.50ab	0.10±0.16a	0.63±0.45a	0.15±0.25a
4	1.62±0.23a	0.00±0.00b	1.31±0.87a	0.00±0.00a	0.76±0.28ab	0.00±0.00a	0.50±0.55a	0.10±0.16a
6	0.89±0.32b	0.13±0.21b	1.37±0.44a	0.83±0.67a	1.35±0.65a	0.37±0.48a	1.06±0.95a	0.05±0.12a
8	1.26±0.17ab	0.05±0.12b	0.63±0.43a	0.05±0.12a	0.50±0.55b	0.00±0.00a	0.50±0.55a	0.00±0.00a
10	1.29±0.17ab	0.15±0.16b	1.30±0.59a	0.74±1.04a	1.26±0.31ab	0.30±0.27a	0.50±0.55a	0.08±0.19a

[a] Different letters in the same column indicate a significant difference between values over time ($P < 0.05$).

Microbial water quality was monitored over time in four discrete water systems in Packinghouses A and B, and in three systems in Packinghouses C and D. Cherry microbial quality was assessed prior to entry into the first system and again as cherries exited each discrete system (five sampling locations in Packinghouses A and B, and four in C and D). Tables 4 and 5 present results from Packinghouse A; due to space limitations, equivalent tables for Packinghouses B, C, and D are not shown here but are available upon request from the project director. Within each discrete water system at the packinghouses, APC and EB levels (log CFU/mL) fluctuated over time, with some statistically significant differences observed ($P < 0.05$). However, microbial levels at later time points were never significantly higher than those measured at time 0 (the start of water usage). These findings suggest that, over the 10 h holding period evaluated in this study, bacterial loads remained stable despite changes in physicochemical parameters (e.g., increasing turbidity). In other words,

while water quality characteristics shifted during use (due to increased throughput of cherries), microbial counts did not show progressive accumulation. Overall, APC levels showed greater variability compared to EB, suggesting that APC may provide a more sensitive measure of microbial changes in cherry postharvest water quality during extended holding times.

Similarly, log CFU/cherry for APC and EB on cherries varied over time at each sampling location; however, microbial levels were never significantly higher than those measured at time 0. Significant differences were observed between pre-water samples (before entering the postharvest water system) and various post-water sampling points (post-water 1–4). These differences, however, were not consistent across sampling points or packinghouses. For example, in Packinghouse A at 10 hours, APC levels were significantly lower on cherries at all post-water sampling locations compared to pre-water, whereas no significant differences in APC between locations were observed at 2, 4, 6, or 8 hours at the same packinghouse. In contrast, EB levels at the same packinghouse were significantly higher at post-water 1 compared to both pre-water and post-water 2–4 at 0 h.

Table 5: Comparison of average and standard deviations (log CFU/cherry) for Aerobic Plate Count (APC) and *Enterobacteriaceae* (EB) between cherries going into and exiting the water in PHA

Water Hold Time (hours)	APC					EB				
	Pre-water$_1$	**Post-water$_1$**	**Post-water$_2$**	**Post-water$_3$**	**Post-water$_4$**	**Pre-water$_1$**	**Post-water$_1$**	**Post-water$_2$**	**Post-water$_3$**	**Post-water$_4$**
0	2.02±0.27abAB[a]	2.45±0.56aA	1.75±0.31aB	2.20±0.38aAB	1.79±0.45aAB	1.00±0.00aB	1.76±0.61aA	1.53±0.66aAB	1.05±0.12aB	1.05±0.12aB
2	2.23±0.31abA	2.11±0.24aA	2.28±0.34aA	1.80±0.64aA	1.70±0.41aA	1.00±0.00aA	1.00±0.00bA	1.20±0.31abA	1.00±0.00aA	1.05±0.12aA
4	2.24±0.49abA	1.94±0.60aA	1.58±0.49aA	1.73±0.57aA	1.58±0.58aA	1.22±0.34aA	1.00±0.00bA	1.00±0.00bA	1.00±0.00aA	1.81±1.25aA
6	1.65±0.45bA	1.53±0.58aA	1.53±0.52aA	1.50±0.55aA	1.55±0.50aA	1.00±0.00aA	1.10±0.16bA	1.00±0.00bA	1.00±0.00aA	1.00±0.00aA
8	1.67±0.38bA	1.71±0.58aA	1.58±0.58aA	1.55±0.50aA	1.56±0.49aA	1.00±0.00aA	1.00±0.00bA	1.00±0.00bA	1.18±0.32aA	1.00±0.00aA
10	2.58±0.18aA	1.55±0.50aB	1.80±0.43aB	1.68±0.52aB	1.60±0.49aB	1.08±0.19aA	1.00±0.00bA	1.00±0.00bA	1.05±0.12aA	1.00±0.00aA

[a] Different lowercase letters within a column indicate significant differences over time ($P < 0.05$). Different uppercase letters within a row for a given indicator shows significant differences ($P < 0.05$).
[b] * indicates a significant difference between pre- and post-flume apple for a given indicator bacteria.

When a linear model was used to characterize the change in APC and EB levels on cherries based on sampling location and controlling for packinghouse, significant differences by location were observed (Table 6). With the model determining and average starting population of 2.42 log CFU/cherry, APC levels significantly decreased by 0.29 and 0.44 log CFU/cherry, respectively, after exiting flume 1 and flume 4, compared to pre-flume cherries. However, post flume 2, APC levels on cherries statistically increased by 0.35 log CFU/cherry compared to pre-flume cherries. No significant change was observed in EB levels was cherries exited flumes 1, 3, and 4, while a slight significant increase in EB level was observed following flume 2. In cherry packinghouses, dump tanks and flume systems are widely used to facilitate the movement of cherries through postharvest processing. However, these systems are also recognized as critical control points for potential cross-contamination. Results indicated that levels of APC and EB on cherries before entering the postharvest water system were fairly low. Linear model findings did indicate that while microbial reductions occur at some points in the system, the significant increases in APC and EB levels after flume 2 provide evidence that cross-contamination of cherries may be occurring. Linear model findings also demonstrate that ppm of sanitizer in water systems (all discrete water systems in all packinghouses studied used chlorine), had an impact on APC and EB count in both water and on cherries (Table 7). Results indicated that as sanitizer levels increased, APC and EB levels significantly decreased. Thus, moving forward to further elucidate the underlying drivers of these interactions, structural equation modeling (SEM) is being employed. SEM is a multivariate analytical framework that enables simultaneous evaluation of multiple dependent relationships, incorporating both direct and indirect pathways. This approach will allow for a more comprehensive understanding of the complex interdependencies among microbial load dynamics, water quality parameters, equipment characteristics, and operational practices that may contribute to cross-contamination events within the cherry packing environment.

Table 6: Linear model estimates of change in microbial counts compared to pre-flume cherries for all four packinghouses

	Intercept	**Effect Estimate[a]**	**95% CI**	**p-value**
Aerobic Plate Count	2.42			
Post-Water$_1$		-0.29	-0.58, 0.00	0.05
Post-Water$_2$		0.35	0.05, 0.64	0.02
Post-Water$_3$		0.25	-0.05, 0.54	0.10
Post-Water$_4$		-0.44	-0.78, -0.10	0.01
Enterobacteriaceae	1.16			
Post-Water$_1$		-0.08	-0.21, 0.05	0.23
Post-Water$_2$		0.23	0.10, 0.36	<0.001
Post-Water$_3$		-0.01	-0.16, 0.13	0.93
Post-Water$_4$		0.01	-0.14, 0.16	0.89

[a] The effect estimates represent the estimated difference in mean microbial counts (log CFU/cherry) compared to the reference group (pre-water)

Table 7: Linear model estimates of change in microbial counts in water (CFU/mL) and on cherries (CFU/cherry) as a result of increases in parts per million (ppm) sanitizer and turbidity (NTU)

	Intercept	Effect Estimate[a]	95% CI	p-value
Water				
Aerobic Plate Count	2.69			
ppm		-0.02	-0.03, -0.02	<0.001
turbidity		0.00	0.00, 0.00	0.07
Enterobacteriaceae	1.44			
ppm		−0.01	-0.01, 0.00	<0.001
turbidity		0.00	0.00, 0.00	0.79
Cherry				
Aerobic Plate Count	2.69			
ppm		-0.01	-0.02, -0.01	<0.001
turbidity		-0.00	0.00, 0.00	0.50
Enterobacteriaceae	1.29			
ppm		0.00	0.00, 0.00	0.26
turbidity		0.00	0.00, 0.00	0.02

[a] The effect estimates represent the estimated change in average microbial counts for each unit increase in PPM of sanitizer or turbidity.

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Project Title: Identifying Sources of Resistance to Pseudomonas and Powdery Mildew

Report Type: Continuing Project Report

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Cooperators:

Project Duration: 2-Year

Total Project Request for Year 1 Funding: $ 14,971
Total Project Request for Year 2 Funding: $ 11,684

Other related/associated funding sources:

Awarded
Funding Duration: 2025 - 2027
Amount: $752,386
Agency Name: Washington Tree Fruit Research Commission/Oregon Sweet Cherry Commission
Notes: 'A robust PNW sweet cherry breeding and genetics program'. Co-PIs Kelsey Galimba, Cameron Peace.

Requested
Funding Duration: 2026 - 2030
Amount: $5.85 million
Agency Name: USDA NIFA Specialty Crops Research Initiative
Notes: 'Integrated strategies for managing bacterial canker and blossom blast disease of stone fruits'. PI: Frank Zhao. Multiple co-PI's, including Per McCord, Bernardita Sallato, and Matthew Whiting from WSU.

WTFRC Collaborative Costs: None

Budget 1
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Item	2025	2026
Salaries	$7,353.00	$6,361.00
Benefits	$2,996.00	$2,651.00
Wages		
Benefits		
RCA Room Rental		
Shipping		
Supplies	$3,400.00	$1,400.00
Travel	$1,222.00	$1,272.00
Plot Fees		
Miscellaneous		
Total	$14,971.00	$11,684.00

Footnotes:
[1]0.1 FTE Field technician (both years) to run screening assays and collect tissue/perform extractions for DNA work; 9 days (Year 1) and 4 days (Year 2) salary for Dr. Johnson for training technician in screening assays.
[2]Includes petri dishes, growth media, pipets for screening assays, and supplies for DNA extraction, sequencing, and test development.
[3]3 overnight trips per year (Pullman to Prosser) for Dr. Johnson. Includes lodging, meals, and mileage.

Original Objectives
1. Identify genetic factors for bacterial canker resistance (Year 1).

2. Identify critical DNA sequence differences in the 3 confirmed powdery mildew resistance factors (alleles) of Pmr1 (Year 1)
3. Collect robust phenotypic data for bacterial canker and powdery mildew resistance on Breeding Program parents and selections (Years 1 and 2)
    a. Confirm what resistance levels we have in the Breeding Program (*Pseudomonas*)
    b. Generate critical data for non-genotyped parents and selections (*Pseudomonas* and powdery mildew)
4. Develop new or refined DNA tests for screening for PM and Pseudomonas (Year 2)
    a. Genetically characterize Breeding Program parents/selections with new tests

Significant Findings

- Preliminary evidence identified for genetic factors for canker resistance – to be validated next on more individuals
- A total of 27 out of 137 genes in the *Pmr1* region predicted to be associated physiologically with disease resistance – we will focus next on these next to identify critical DNA sequence differences associated genetically with the confirmed mildew resistance alleles
- Initial rounds of phenotypic screening completed for canker and powdery mildew resistance
    - Canker: confirmed partial resistance of 'Regina'
    - Mildew: confirmed resistance of 'Regina'

Methods

Identify genetic factors

Prior research by Drs. Johnson and Peace resulted in a dataset of 69 individuals from the USDA *Prunus* collection that had been screened for *Pseudomonas* resistance and genotyped for over 3,950 DNA markers. These data were used to perform an association mapping study using GAPIT software (Wang and Zhang 2021). Association mapping studies can be influenced by the underlying composition or structure of the populations being analyzed. We explored this population structure using principal component analysis (PCA), also via GAPIT. Based on the PCA, we focused our association analysis on the 30 sweet cherry cultivars in the dataset, plus 1 possible hybrid that grouped closely with the sweet cherries.

Gene targeting for sequencing Pmr1

We utilized the list of markers known to be near the *Pmr1* region to locate the corresponding region of the latest version of the 'Tieton' sweet cherry reference genome. From the list of predicted genes within this region, we identified those with features common to genes involved in disease resistance, similar to the method used by Wöhner and Emeriewen (2024).

Screening assays

We conducted two rounds each of bacterial canker and powdery mildew assays in August and September. Both assays utilized detached 10 mm leaf discs (abaxial side up) supported on water agar. Leaves were sterilized by immersion for 30 seconds in 70% ethanol, followed by two rinses with sterile deionized water. After inoculation, plates were wrapped with Parafilm and incubated at room temperature with a 14-hour photoperiod provided by LED grow lights.

For canker, air-dried leaf discs were inoculated with a suspension of an overnight culture of *Pseudomonas syringae* pv. *syringae*, that had been diluted to an optical density of ~0.2. A 10µL drop of suspension was applied to each leaf disc, and excess solution removed with a sterile cotton swab after 30 minutes. Plates were scored approximately 1 week after inoculation.

For mildew, leaf discs were inoculated with a suspension of mildew spores harvested from symptomatic leaves, diluted to a concentration of 5,000 conidia/mL. A 20 µL drop was applied to

each leaf disc and the excess solution removed with a sterile cotton swab after 15 minutes. Plates were scored approximately 2 weeks after inoculation.

Results and Discussion

Genetic factors for bacterial canker

Association mapping of the 31 sweet cherry accessions identified a region on chromosome 2 that is associated with *Pseudomonas* resistance (Figures 1 and 2). Since this is based on a small number of data points, the effectiveness of the markers will need to be validated in 2026 on more individuals.

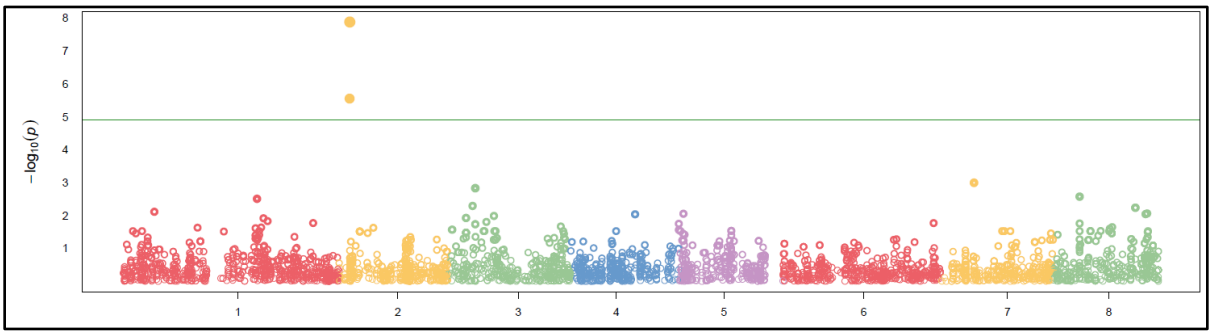

Figure 1. Plot displaying the statistical association of markers with *Pseudomonas* resistance. Two markers on chromosome 2 are significantly associated with the trait.

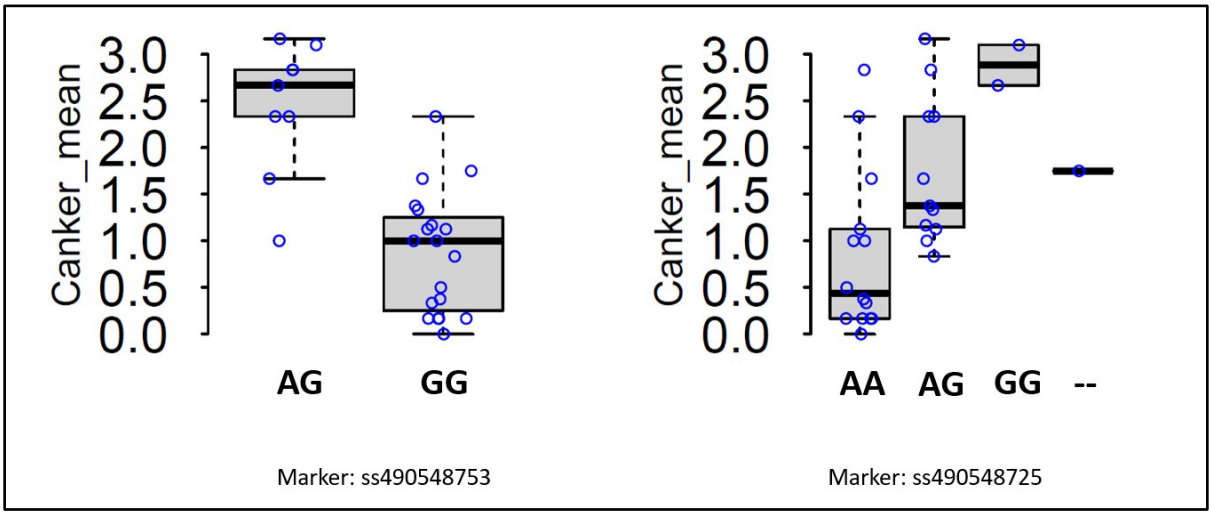

Figure 2. Effects of different alleles for the two markers associated with *Pseudomonas* resistance. Lower mean values are more resistant.

Gene targeting for sequencing Pmr1

A total of 137 predicted genes are found in the *Pmr1* region of chromosome 5. We identified 27 genes with features involved in disease resistance. We will design CRISPR guide RNAs to target genes for sequencing. These RNAs will allow the CRISPR enzyme to cut the DNA at the desired locations, which will enrich the sequencing reaction for these regions. The targeted sequencing will be conducted in the third quarter of 2025.

Screening assays
A total of 24 seedlings and 5 controls were screened in the first mildew assay. Mildew growth occurred on all but 1 of the seedlings, which were from a single family thought to be segregating for powdery mildew resistance. We confirmed the resistance of 'Hedelfingen' (which carries resistance allele *Pmr1b2*). 'Regina', which in prior assays appears partially resistant, was resistant in our screen. 'Regina' is of interest because current DNA testing results suggest it does not carry the *Pmr1a/b1/b2* resistance alleles. Heavy natural disease pressure allowed us to confirm many of our leaf disc assay results, as well as eliminate the need for screening individuals now known to be susceptible.

The second round of mildew assays included 56 seedlings and 3 controls. However, excess humidity inside the assay plates prevented satisfactory mildew growth on nearly all leaf discs. We determined that the likely cause was the presence of a grow light on the bottom side of the shelf which increased the temperature inside the plates. To mitigate this problem we will utilize the bottom-most shelf in the future.

For canker, 21 seedlings and 2 controls were screened in the first assay. Bacterial growth was present but weak, and we determined that the overnight culture had likely grown too long and was no longer highly virulent. The second round of screening included 63 seedlings and breeding program selections and two controls. We grew the bacterial culture for 6-7 fewer hours, which resulted in a more virulent inoculum. We confirmed prior results that 'Regina' is partially resistant. We also confirmed field results that R19 (which will be released for the early mahogany market) is susceptible.

The assays for both diseases will be repeated in 2026, and we will conduct testing in both spring and summer to provide more replication and increase the number of individuals screened.

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