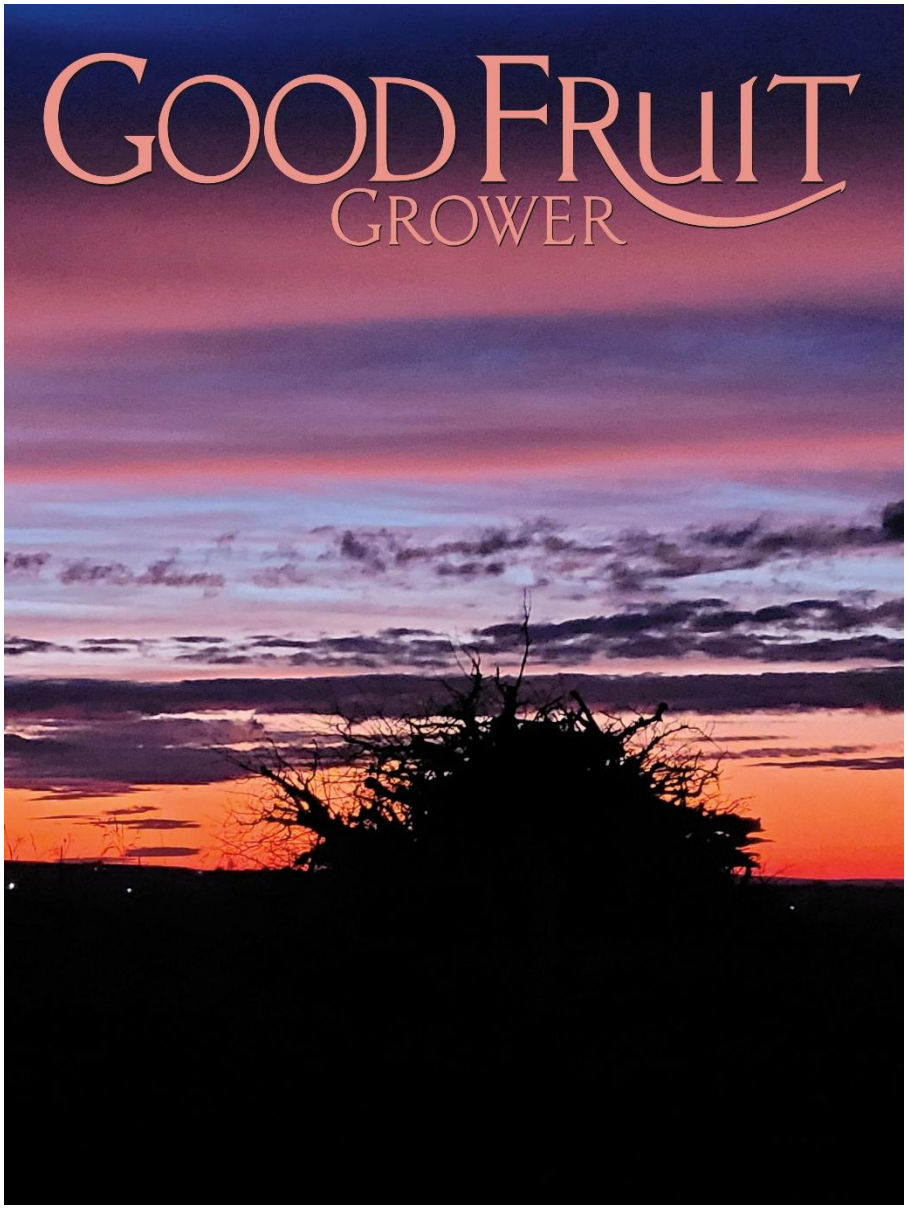
2027 NW Cherry and Stone Fruit Research Review



A cherry orchard removed due to little cherry disease is set against the backdrop of a new sunrise in 2023 near Zillah, WA.

Photo by Ines Hanrahan was selected as the Good Fruit Grower Cover for the issue of March 16, 2026.

November 3, 2026

Hybrid | Yakima, WA

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

Primary PI: Kelsey Galimba
Organization: Oregon State University - MCAREC
Telephone: (541) 386-2030 ext.38218
Email: kelsey.galimba@oregonstate.edu
Address: 3005 Experiment Station Dr.
City/State/Zip: Hood River, OR 97031

Co-PI 2: Ashley Thompson
Organization: Oregon State University
Telephone: (541) 296-5494
Email: ashley.thompson@oregonstate.edu
Address: 400 E. Scenic Dr.
City/State/Zip: The Dalles, OR 97058

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
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	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
Telephone: 541-737-3228
Contract administrator email address: charlene.wilkinson@oregonstate.edu

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. Rootstocks with records of positive infections include Mazzard, Gisela 6, Gisela 12, Krymsk 5 and Krymsk 6, indicating these rootstocks are susceptible (not resistant) to X disease.

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

3. For each rootstock type, three individuals were successfully grafted with q-PCR-verified infected material in 2025/2026.

4. Every grafted rootstock was negative when tested with qPCR in 2026. Testing will need to be repeated in 2027.

Methods

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.

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. These trees were also indicated as positive by Ruff Country K9. 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 1A). 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 (Fig 1B). Grafting was repeated in September, 2025 and again at the beginning of June, 2026 on

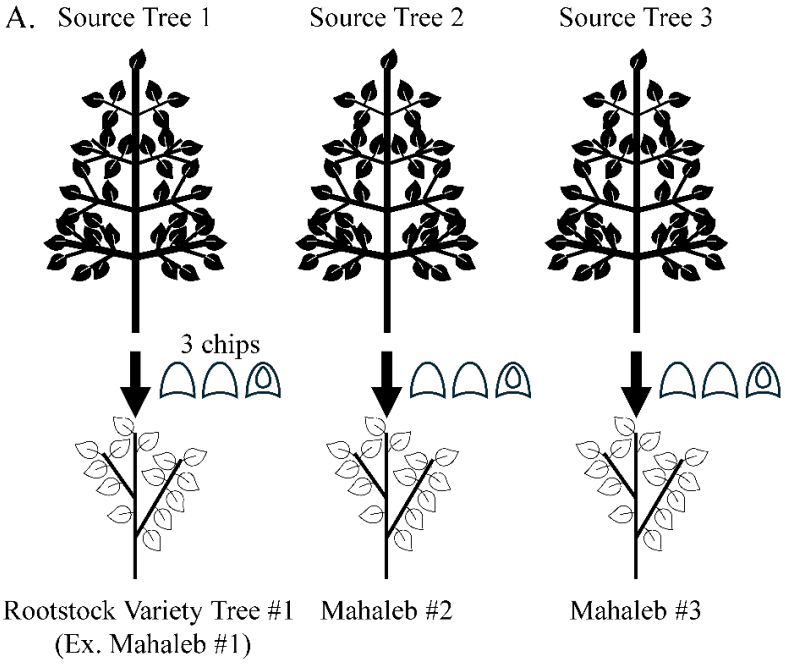




Figure 1. A. Grafting schematic showing method of transferring X disease from infected source trees in the field to rootstocks. Each of three rootstock individuals received chip/blind grafts from one source tree. B. Examples of grafting showing two chip buds (one with bud and one without) in the process of being grafted (top) and two with successful grafts (bottom).

rootstock trees that had no successful grafts in previous rounds.

If rootstocks are able to successfully house the infection, they will be considered non-resistant. Material from all grafted individuals was sent to Qualterra (https://www.qualterraag.com/) for qPCR testing in August 2026. For each individual, two samples consisting of a two to three cuttings, six to eight inches long, with leaves attached and from different sections of the tree were tested.

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 varied greatly in size and health after the first season of growth at MCAREC, and the variability was 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 in the first round in July 2025, with 15%, 18%, and 9% of all attempted grafts from source trees 1, 2 and 3, respectively, undergoing successful fusion to rootstock trees. For this reason, we repeated grafting again in fall (September 2025) and spring (June 2026) for rootstocks where source material did not take. After three rounds of grafting, all individuals had at least one successful graft (Fig. 2).

	Summer Grafting 2025			Fall Grafting 2025			Spring Grafting 2026		
	Source Tree 1	Source Tree 2	Source Tree 3	Source Tree 1	Source Tree 2	Source Tree 3	Source Tree 1	Source Tree 2	Source Tree 3
Mahaleb	X							X	X
Mazzard				X	X	X			
Gisela 5				X	X	X			
Maxma 14	X	X	X						
Clinton		X					X		X
Crawford				X		X		X	
Cass				X				X	X
Weigi 2				X				X	X
Weigi 3	X	X	X						
Colt	X	X				X			
Krymsk 7	X				X				X

Figure 2. Rootstock types and when material from each source tree was successfully grafted.

All grafted individuals were tested for X disease and found negative. This is an unexpected result, as the source trees were verified positive and the grafting methods have been used successfully by others (Personal Communications). With our experimental design, rootstock types exhibiting X disease resistance should reject grafts with infected material, or they should die shortly after infection. Susceptible rootstocks without resistance should accept grafts and should be able to survive while infected. Because all rootstock types (including the susceptible control, Mazzard, and the rootstocks that have been shown to house infections in objective 2 (G6, G12, K5, K6)) tested negative, it is probable that any successful infections are not advanced enough to detect with qPCR.

Mahaleb, the resistant control that should be exhibiting a hypersensitive response when exposed to X disease was also successfully grafted and is still alive. It is possible that the current local strains of the X disease phytoplasma do not induce hypersensitivity in Mahaleb like the strains in CA did in the previous decades' outbreaks, and that likewise it will not induce hypersensitivity in any of our tested rootstocks. While unanticipated, this will still be valuable information for the cherry industry.

Justification for NCE

- All rootstocks have successful grafts from verified source trees but all tested negative for X disease. The oldest grafts were ~one-year-old at testing. It is likely the infections are too young and titer levels are too low to detect with qPCR.
- Repeated qPCR testing after an additional year would potentially be more accurate.
- Cooperating with dog team (Ruff Country K9) to identify infections would potentially be more accurate.
- A NCE will allow for these testing approaches to be used in 2027.

Project Title: Canine Detection Strategies for Managing Little Cherry Disease in Nurseries

Report Type: Continuing Project Report

Primary PI: Jessica Kohntopp
Organization: Ruff Country K9 LLC
Telephone: (208) 602-1293
Email: jesskohntopp@gmail.com
Address: 949 E 4100 N
City/State/Zip: Buhl, ID 83316

Co-PI: Scott Harper
Organization: Washington State University
Telephone: (509) 786-9230
Email: scott.harper@wsu.edu
Address: 24106 N Bunn Road
City/State/Zip: Prosser, WA 99350

Co-PI: Corina F. Serban
Organization: Washington State University
Telephone: (509) 574-1595
Email: corina.serban@wsu.edu
Address: 2403 S 18th Street Suite 100
City/State/Zip: Union Gap, WA, 98903

Co-PI: Rodney Cooper
Organization: USDA-ARS-Temperate Tree Fruit and Vegetable Research Unit
Telephone: (509) 454-6570
Email: rodney.cooper@usda.gov
Address: 5230 Konnowac Pass Road
City/State/Zip: Wapato, WA 98951

Cooperators: Todd Cameron (Cameron Nursery), Bennett Mayo (Mike and Brian's Nursery), Segun Akinbade (WSDA), Allison Schrader (Cameron Nursery), Bill Howell (NNII), Garrett Bishop (G.S. Long Company), Teah Smith (Zirkle Fruit Company), Craig Harris (Harris Farms), Garrett Henry (Douglas Fruit), Tobin Northfield (WSU), Ashley Thompson (OSU)

Project Duration: 2-Year

Total Project Request for Year 1 Funding: $135, 105
Total Project Request for Year 2 Funding: $135,555

Other related/associated funding sources: None

Budget 1
Primary PI: Jessica Kohntopp
Organization Name: Ruff Country K9
Contract Administrator: Jessica Kohntopp
Telephone: (208) 602-1293
Contract administrator email address: jesskohntopp@gmail.com

Item	2025	2026
Salaries	$40,000.00	$40,000.00
Benefits (34%)	$13,600.00	$13,600.00
Wages		
Benefits		
RCA Room Rental		
Shipping		
Supplies		
Travel	$23,940.00	$23,940.00
Plot Fees		
Miscellaneous	$500.00	$500.00
Total	$78,040.00	$78,040.00

Footnotes: Year-1: PI Kohntopp requests Year-1 Salary based on below median detection dog trainer salary survey conducted by Highland Canine Training LLC School for Dog Trainers. Travel to cover 18 trips of 5 days each to bring the canines to WA for training, blind studies, Mother blocks, etc. Year-2: request is similar to Year-1.

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	2025	2026
Salaries	$32,496.00	$33,796.00
Benefits	$11,432.00	$11,704.00
Wages		
Benefits		
RCA Room Rental		
Shipping		
Supplies	$6,163.00	$5,880.00
Travel		
Plot Fees		
Miscellaneous		
Total	$50,091.00	$51,380.00

Footnotes: Year-2: Salaries & benefits for 0.5 FTE Research Technician per year, and laboratory supplies for diagnostics.

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	2025	2026
Salaries		
Benefits		
Wages		
Benefits		
RCA Room Rental		
Shipping		
Supplies	$1,340.00	$500.00
Travel	$1,809.00	$1,809.00
Plot Fees	$635.00	$635.00
Miscellaneous	$3,191.00	$3,191.00
Total	$6,975.00	$6,135.00

Footnotes: Year-1: Co-PI Serban requests $500 towards extension and outreach activities, $840 for netting; $1,809 for travel and accommodation; $635 for plot fees for the use of IAREC; and $3000 in miscellaneous for farm crew Fees, $191 for miscellaneous items such shipping costs Year-2: request is similar to Year-1.

Objectives:

(1) Using WSDA LCD diagnostic results from previous and current years as a guide, canines will screen trees with emphasis on ones that may have previously shown positive but could not be reconfirmed. Any canine alerts will be shared with WSDA for further testing if necessary.

(2) Validate the canines' accuracy, sensitivity, and specificity in a bundle blind study - this includes three different tests: rootstock tissue culture trays, budwood bundles, and 2-year tree bundles.

(3) Assess canines' ability in a mock second year rootstock scenario.

(4) Determine the canines' ability to detect XDP in young trees when a) an infected budwood source is used, and b) when clean sources are used but with the risk of leafhopper incursion during the growth period. Canines will be used to screen these trees throughout the process at four different stages: Mother tree, Budwood, Rootstock, and trees at the end of the two-year period.

(5) Extension and Outreach – monthly meetings, demonstration at field day and technical factsheet

Objective Deviations:

Objective 3 was unable to be performed in the controlled way we were expecting/planning due to irrigation issues at Moxee. However, we had the opportunity to use a mock nursery block that was going to be removed since its previous project was over. Although we were going in blind, we were able to use these trees for several tests with the canines.

Objective 4 has been replaced with *(4) Imprint two canines on the structural proteins of Little Cherry Virus-1 (LChV-1), Little Cherry Virus-2 (LChV-2), and X-disease phytoplasma (XDP) then introduce canines to infected plants.*

(5) Validate canines accuracy, sensitivity, and specificity in a blind controlled study and compare results to previous canines trained the conventional manner.

The original objective 4 addresses inoculation and pathogen accumulation biology, and while interesting from a general scientific perspective, is unnecessary for the purpose of training canines for detection. This spring we demonstrated, with the collaboration of the Northfield and Cooper Labs at WSU and USDA-ARS respectively, that the current dogs were unable to navigate and indicate on closely spaced (6" apart) trees. When the trees were dug up and spaced further apart the dogs operated more successfully, and we propose for the purposes of training and commercialization that this is a better approach than attempting to train in an approximation of the nursery production environment. The plot for this objective was originally intended to be managed by the Co-PI Corina Serban and with her departure our focus has shifted toward an objective that serves as the final crucial piece of the canine project prior to commercialization. Furthermore, in the past report, and subsequently we have demonstrated a better approach to a) some of the molecules that the current dogs have imprinted on, and b) built on this to conduct a trial to see whether this is viable for canine training.

Significant Findings:

- Canines identified WSDA PCR positive mother trees plus additional trees in 2025 and 2026.
- WSDA is proceeding with a dissection study of three canine alerted on mother trees.
- Moxee old nursery block was able to be used across multiple canine studies.
- Canines successfully screened bundles prior to planting.
- Canines alerted on all sections of 10 positive trees
- Multiple mock bundle studies were successfully completed.
- Bundle blind studies showed an average of
    - **99.49%** accuracy, **96.83%** sensitivity, and **100%** specificity for **Humma**
    - **99.10%** accuracy, **94.44%** sensitivity, and **100%** specificity for **Aika**

Methods:

Mother Trees

Working with WSDA and using their PCR results from each year, canines were deployed to search mother trees identified as PCR-positive. Canines searched a randomized number of trees prior to encountering the PCR positive tree. This randomization was critical for reducing the potential for pattern-based responses, such as the canine learning to alert on every third tree, and helped ensure that alerts were based on the target odor rather than the position or sequence of the trees.



Figure 1: *Aika Alerting on Mother Tree*



Figure 2: *Humma Alerting on Mother Tree*

2025: WSDA testing identified 35 PCR positive mother trees across four locations. Canines were deployed to three of the four sites. Humma ran all 34 positive trees across the three locations. Aika ran past 23 of the positive trees but became overwhelmed in a section with a solid line of highly infected trees, ultimately shutting down. This outcome highlights the

importance of lower incidence rates for effective canine performance, as well as the critical role of the handler in recognizing when a canine is not working effectively and redirecting accordingly.

2026: WSDA testing identified 5 PCR-positive mother trees at one location. This time both canines searched all 5 positive trees. As well as 350 additional trees that were PCR negative. It should be noted, that mother trees at this location were spaced in close proximity (trunks ~ 1 foot apart) and have extensively intertwined branches, this may have a large impact on potential root grafting or odor transfer/drift from neighboring PCR positive trees.



Figure 3: *Moxee Nursery Block*

Moxee Mock Nursery Block

Trees in the Ground: This block was previously used to evaluate netting, kaolin clay, and untreated controls in a nursery setting. The treatment groups had been PCR-tested collectively rather than at the individual-tree level, so while the number of positive trees within each group was known, their specific identities were not. Before the trees were removed, the canines searched the block and marked their alerts. The trees were then removed and individually spaced, allowing us to determine whether the canines would re-alert on the same trees or identify additional positive trees.



Figure 4: *Moxee Trees Stored*

Trees Dug/Removed and Stored: All trees were individually marked, before being dug on March 13th, and were placed under traditional nursery storage conditions. This allowed these trees to be used for several tests for the canines over the following months.

Individual Trees: After being removed from the field, the trees were individually spaced out in a manner the canines are used to searching. Trees were then re-evaluated by the canines to determine whether additional alerts occurred and if previously alerted on tree results stayed consistent.



Figure 5: *Moxee Trees Spaced Out*

Mock Second-year Tree

Bundles – Outside: Following the individual tree searches, trees were separated into groups based on canine negative and positive results. These trees were then used to create mock bundles designed to simulate second-year or finished nursery stock bundles. Trees were bundled in groups of five and then stacked to create bundles containing 10, 15, 20, and 25 trees. To simulate a positive bundle, one known positive tree was placed underneath a bundle of negative trees. Individual bundles were positioned approximately 3 feet apart, with slightly greater spacing between larger stacks as bundle surface area increased.



Figure 6: *Humma Running Bundles*

Mock Second-year Tree Bundles – Inside: We wanted to determine whether the canines could identify positive trees within bundled material under colder and less familiar environmental conditions. The indoor environment introduced several potential distractions or challenges, including slick and



Figure 7: *Bundles Indoors*

wet floors, loud noises, confined working areas, and air movement from fans. The canines searched the indoor bundles demonstrating no hesitation or apparent difficulty working in the environment. However, if prolonged indoor searching is conducted under similar conditions, protective booties would be recommended to protect the canines' feet from prolonged contact with cold, wet flooring.

Dissection Study: Ten trees were selected for dissection and evaluated using our established sampling protocol, with PCR samples taken every 4”. Of the ten trees, five were canine alerted, three were canine negative, and two were canine hesitations. A canine hesitation was defined as a tree in which the canine did not alert on but had substantially more interest than a normal negative tree.

Cut Trees Study: Ten positive trees were selected and cut into approximately 16-inch sections. The number of sections varied depending on the size and structure of each tree. Roots were retained primarily as the root ball at the base of the tree, with additional individual root clippings collected where possible. The canines then searched each section individually to determine whether they alerted on all portions of the tree or only specific sections. Negative trees were processed in the same manner and incorporated throughout the searches to provide comparison material and prevent pattern alerting.



Figure 8: *Cut up Positive Tree*

Mock rootstock and scionwood bundles: Trees used in the mock bundles were cut into material representative of commercial rootstock and scionwood bundles. Rootstock bundles contained 50 roots. Scionwood bundles contained 100 sticks per bundle. To simulate positive bundles, one root or stick from a positive tree was placed under the bundle.



Figure 9: *Roots for Bundles*



Figure 10: *Stick for Bundles*

Blind Studies: Blind studies were conducted to evaluate canine accuracy, sensitivity, and specificity using rootstock, budwood, and second-year nursery stock. Each run was completely randomized using a computer-generated sequence, and the trainer was unaware of the true-positive locations. When a canine alerted, the trainer called the alert and an observer verbally confirmed or denied the response before the canine was rewarded or removed. Canines alternated runs, with plants rearranged between searches. Bundle sizes were 50 rootstock, 100 budwood, and 10 second-year nursery trees. Canine performance was evaluated using true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN) to calculate sensitivity, specificity, and overall accuracy as described by Gottwald et al. (2020). The study goal was to achieve >95% accuracy, >90% sensitivity, and >90% specificity.



Figure 11: *Aika Searching Planting Bundles*

USDA Netting Trial Block

Bundles at Planting: Canines were deployed during the planting of a USDA block to evaluate the use of canines during a commercial planting operation. The primary objectives were to determine whether the canines could maintain the pace required during planting without slowing or disrupting the operation, and whether they could continue to perform reliably while working around personnel, planting machinery, and associated noise and activity.

Trees in Planted Block: Following planting, canines were brought back to the block to further evaluate their accuracy in identifying positive bundles. The canines searched each tree individually within the entire block. This follow-up search provided an opportunity to determine whether the canines succeeded in finding all positive bundles or if any were missed.



Figure 12: Aika Alerting

Results and Discussion:

Mother Trees 2025: The canine results on the PCR positive mother trees are as follows in the table below. One PCR positive tree was not alerted on by either canine; however, both canines strongly alerted on the adjacent tree, which had a low CT value of 39.06. This makes it unlikely that the canines were detecting the target odor early or late on the neighboring tree, suggesting instead a possible tissue sampling mix up. All trees alerted on by the canines including those previously confirmed PCR-positive, were subsequently retested, with all samples returning negative results. WSDA does not plan to conduct further PCR testing and has removed all canine-alerted trees from the nurseries.

***Table 1**: Mother Tree Results 2025*

	WSDA Lab	Humma		Aika		Extra Dog Detection	
LCD results	Total Positive PCR	PCR Positive Trees Ran	Trees Alerted	PCR Positive Trees Ran	Trees Alerted	Humma	Aika
Nursery 1	15	15	13	15	12	4	4
Nursery 2	2	2	2	2	1	1	1
Nursery 3	17	17	17	6	5	6	5
Nursery 4	1	0	N/A	N/A	N/A	N/A	N/A
Total	**35**	**34**	**32**	**23**	**18**	**11**	**10**

Mother Trees 2026: The canine results on the PCR positive mother trees are as follows in the table below. WSDA's five positive trees had the following CT values: 14.81, 25.39, 35.61, 36.04, and 36.25. Canines alerted on all PCR-positive trees, as well as 17 additional adjacent trees (Both dogs alerted on 12 and 5 that were one dog alert). Since the mother trees are spaced in close proximity and have extensively intertwined branches, the adjacent canine alerts may reflect potential root grafting or odor transfer/drift from neighboring PCR positive trees. Canines searched an additional 350 mother trees, resulting in 57 canine alerts seen on the table below. Both canines alerted on 19 trees, while 38 trees were alerted on by only one canine. An additional 7 trees were marked as questionable but were not classified as true alerts. Similar to the PCR-positive locations, multiple canine alerts were frequently observed in clusters of closely spaced, adjacent trees. As illustrated in the example to the right, some of the single-canine alerts occurred within these clusters, where the canines did not consistently agree on the specific tree associated with the odor. This pattern highlights the difficulty of pinpointing the precise source of an odor when trees are positioned in close proximity and have extensively intertwined branches, where odor may potentially move between or around neighboring trees. All PCR positive trees alerted on by the canines were subsequently retested, with all samples returning negative results. WSDA plans to do a dissection study on three trees the canines alerted on but resulted in PCR non-detect. However, WSDA does not plan to conduct further PCR testing on any other trees the canine's have alerted on and plans to remove all canine-alerted trees from the nurseries.

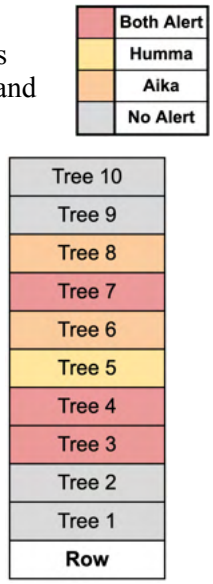


Figure 13: Tree Alert Example

***Table 2**: Mother Tree Results 2026*

	WSDA Lab	PCR Positive Trees			Additional Trees	
Canine	Total Positive	PCR Trees Ran	PCR Trees Alerted	Additional Alerts	Trees Ran	Trees Alerted
Humma	5	5	5	15	350	33
Aika	5	5	5	15	350	35
Totals	**5**	**5**	**5**	**17**	**350**	**57**

Moxee Mock Nursery Block

Trees in the Ground: Canines alerted on 15 of the 180 trees searched. The handler identified an additional 8 trees as potential alerts due to the close spacing of the trees. In several instances, the canine would sit on the first tree and her nose would shift between the tree she alerted on and the next one. With the trees spaced so close together it is difficult to determine which individual tree is the source of the response. In these situations, the handler recorded the adjacent tree as a potential alert even though the canine did not distinctly sit on that specific tree. This resulted in 23 trees of interest moving forward.

Individual Trees: Once we were able to spread the trees out, the canines alerted on all 15 previously alerted on trees, all 8 handler called trees, plus an additional 7 trees. These 7 trees were all in close proximity to previously alerted on or handler called trees.

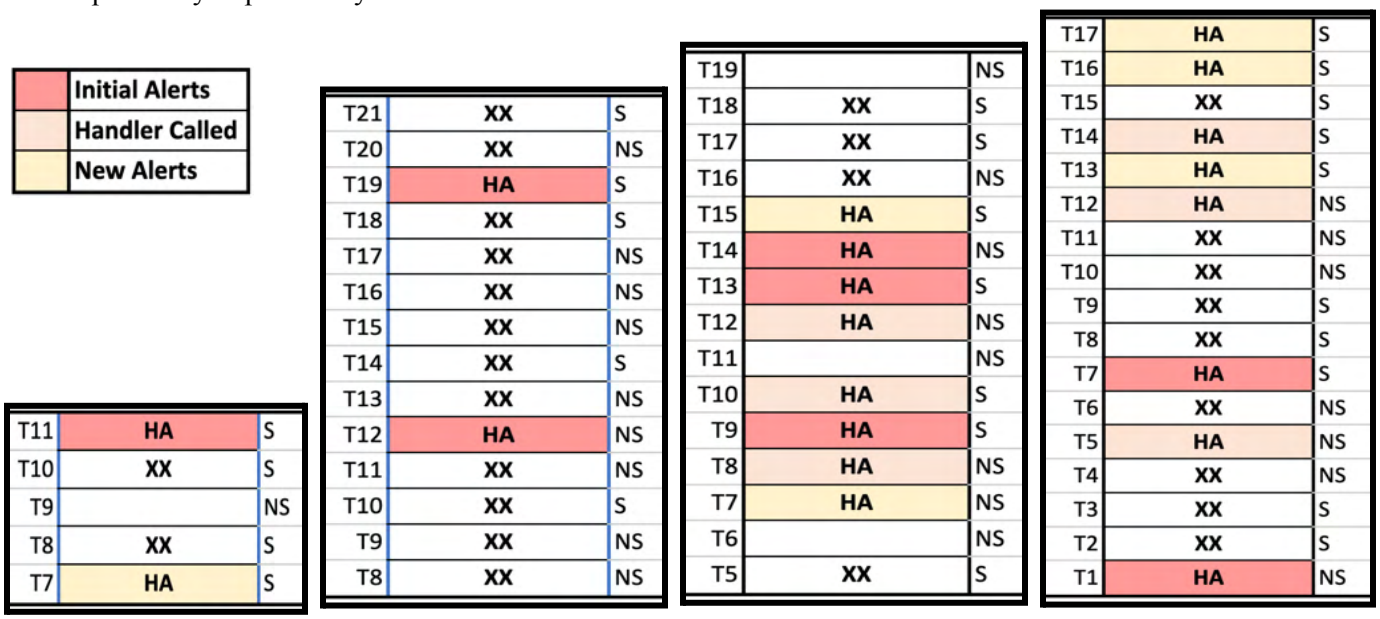


Figure 14: Moxee Nursery Block Canine Alerts

Dissection Study: Samples were collected at 4-inch intervals along each tree, with 1, 2, or 4 samples collected from each cross-section depending on the diameter of the wood. Sample sizes and positive results per tree are found on the table below. All five trees the canines alerted on contained at least one PCR-positive sample, confirming the canines were correct. Of the two trees classified as hesitations, one contained a PCR-positive sample. Additionally, two of the three trees classified as canine-negative were found to contain PCR-positive samples. Although the canines missing is always an option, the canines had multiple opportunities to search these trees during other studies and did not alert on them. Given the repeated exposure to these trees, it is less likely that the lack of alerts can be attributed solely to the canines failing to detect the pathogen. These findings raise the question of the possibility that biology and tree-to-tree variation during dormancy may influence the availability of the target odor. The results suggest that some positive dormant trees may not emit sufficient odor for the canines to identify. To further investigate this possibility, an additional dissection study focused specifically on canine-negative trees during dormancy will be conducted. This study will help determine the frequency at which PCR-positive trees are not identified by the canines and provide further insight into the relationship between disease biology, dormancy, and odor availability.

***Table 3**: Mother Tree Results 2026*

Tree Number	No. Samples	No. Samples Positive	% Chance of Sampling	Canines Response	Average CT Values
1A-T12	101	3	2.97%	Alert	38.5
2A-T1	155	3	1.94%	Alert	37.99
4A-T3	63	3	4.76%	Alert	38.68
1B-T7	123	1	0.81%	Alert	36.22
1B-T11	122	1	0.82%	Alert	39.28
2A-T2	146	1	0.68%	Hesitation	32.21
1A-T11	36	0	-	Hesitation	-
A1-T10	101	0	-	Non-alert	-
2A-T3	113	1	0.88%	Non-alert	38.13
1B-T10	67	3	4.48%	Non-alert	38.61

Cut Tree Study: Canines alerted on all sections of the 10 trees, including the roots, without any apparent difficulty. CT values for the individual trees were unknown, which was a primary reason for including 10 trees in the study. Evaluating multiple trees allowed for a broader assessment of canine responses across different trees and sections of the tree rather than relying on a single tree or known CT value. Tree sections ranged from 39 to 751.

Blind Studies: The three main things that are being looked at in the blind study is the accuracy, sensitivity, and specificity of the canines. Accuracy: The total amount of correct alerts on positive trees and non-alerts on negative trees. Sensitivity: The total amount of correct alerts on positive trees. Specificity: The total amount of correct non-alerts on negative trees. Second-year trees, scionwood, and rootstocks were all ran in separate studies due to the number of material within bundles. Results from the blind studies can be seen on the tables below.

Second-Year Bundles: Bundles containing five trees were created for the blind study. Each canine completed 29 runs; 13 runs consisted of 18 positions containing five-tree bundles, 10 runs consisted of 9 positions containing 10-tree bundles, and 6 runs consisted of 4 positions containing 15-tree bundles. In total, the study included 413 bundles containing 2,550 individual trees.

Rootstock Bundles: Bundles containing 50 roots each were created for the blind study. Each canine completed 13 runs, with 13 bundles included in each run. Five runs contained three positive bundles, four runs contained two positive bundles, and four runs contained one positive bundle. In total, the study included 196 bundles containing 9,800 individual roots.

Scionwood Bundles: Bundles containing 100 sticks each were created for the blind study. Each canine completed 17 runs, with 8 bundles included in each run. Five runs contained three positive bundles, seven runs contained two positive bundles, and five runs contained one positive bundle. In total, the study included 170 bundles containing 17,000 individual scionwood sticks.

***Table 4**: Blind Study Results*

	Second-Year Trees Bundles		Rootstock Bundles		Scionwood Bundles		Bundle Totals	
	Humma	Aika	Humma	Aika	Humma	Aika	Humma	Aika
Sensitivity	**96.92%** (63/65)	**93.85%** (61/65)	**97.30%** (26/27)	**97.30%** (26/27)	**97.06%** (33/34)	**94.12%** (32/34)	**96.83%** (122/126)	**94.44%** (119/126)
Total Bundles Missed	2	4	1	1	1	2	4	7
Specificity	**100%** (348/348)	**100%** (348/348)	**100%** (169/169)	100% (169/169)	**100%** (136/136)	**100%** (136/136)	**100%** (653/653)	**100%** (653/653)
Total False Alerts	0	0	0	0	0	0	0	0
Accuracy	**99.52%** (411/413)	**99.03%** (409/413)	**99.49%** (195/196)	**99.49%** (195/196)	**99.41%** (169/170)	**98.82%** (168/170)	**99.49%** (775/779)	**99.10%** (772/779)
Total Material Screened	**2,550** total Trees		**8,450** total Roots		**13,600** total Branches		**24,600** Total Material	

USDA Larger Netting Trial Block

Bundles at Planting: A total of 154 bundles were searched during the planting operation, with the canines alerting on 13 bundles. These bundles were set to the side and later broken down and researched as individual trees. When bundles were broken down, both canines alerted on 12 trees, Aika alerted on 3 others, Humma alerted on 4 others, and 7 were marked as questionable. All 26 trees were not planted. Individual trees canines alerted on were not planted. The remaining trees from the alerted on bundles were planted in the last rows of the block.

Follow Up Search of Planted Block: Canines were brought back to search the newly planted block after the trees began leafing out. A total of 13 trees were alerted on by both canines. Three were located within a row classified as negative during bundle screening, potentially indicating that a positive bundle was missed or that the trees were sufficiently dormant during the original search that the target odor was not present. The remaining 10 alerts occurred in rows containing previously alerted bundles. These results raise questions about the effects of dormancy, biological activity, and breaking positive bundles into individual trees on odor availability and the canines' ability to detect and pinpoint the target odor. Further testing will be needed to distinguish between these factors.

Future Steps:

Future research will evaluate canine screening during commercial nursery digging and bundling operations at two nurseries to determine whether canines can maintain the pace of normal workflow while detecting positive bundles amid personnel, machinery, and associated activity. Additional studies will investigate whether phytoplasma or virus can be detected in sap from extrafloral nectaries or wounds to better understand how pathogen-associated compounds become available for canine detection. Two new canines will also be trained using the developed training aid and compared with the previously trained canines to determine whether providing a defined target odor improves training efficiency and effectiveness. Finally, further investigation of dormant trees will evaluate whether dormancy affects the biological response of LCD and, consequently, target odor emission. A planned dissection study of trees receiving canine-negative responses will help determine whether LCD is present despite the lack of a canine alert and provide additional insight into factors affecting detection.

Literature Review

Detection dogs have demonstrated remarkable ability to identify odors across law enforcement, medicine, conservation, and agriculture, including plant diseases with reported accuracies up to 99% (Gottwald et al., 2020)[1]. In previous LCD research, these canines achieved 99.72% accuracy, 96% sensitivity, and 100% specificity in blind testing and successfully identified positive trees in unknown-status areas. However, confirming canine alerts through PCR proved challenging due to the pathogen's sporadic distribution within trees. In a study of five canine-alerted trees that initially tested PCR-nondetect, 464 tissue samples were tested, with only 13 testing positive (CT 37.21–38.54); importantly, every tree contained at least one positive sample, confirming all five canine alerts. These findings highlight the value of a nondestructive, whole-tree detection method, particularly for an economically significant disease that has resulted in the removal of more than 238,856 sweet cherry trees across Washington.

1: Gottwald, T., Poole, G., McCollum, T., Hall, D., Hartung, J., Bai, J., Luo, W., Posny, D., Duan, Y.-P., Taylor, E., da Graça, J., Polek, M., Louws, F., and Schneider, W. (2020). Canine olfactory detection of a vectored phytobacterial pathogen, Liberibacter asiaticus, and integration with disease control. Proceedings of the National Academy of Sciences, 117(7):3492-3501. https://doi.org/10.1073/pnas.1914296117

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

Report Type: Final Project Report

Primary PI: Jessica Kohntopp
Organization: Ruff Country K9 LLC
Telephone: (208) 602-1293
Email: jesskohntopp@gmail.com
Address: 949 E 4100 N
City/State/Zip: Buhl, ID 83316

Co-PI: Scott Harper
Organization: Washington State University
Telephone: (509) 786-9230
Email: scott.harper@wsu.edu
Address: 24106 N Bunn Road
City/State/Zip: Prosser, WA 99350

Co-PI: Corina F. Serban
Organization: Washington State University
Telephone: (509) 574-1595
Email: corina.serban@wsu.edu
Address: 2403 S 18th Street Suite 100
City/State/Zip: Union Gap, WA, 98903

Cooperators: Daisy Arias (Stemilt Growers LLC), Teah Smith (Zirkle Fruit Company), Craig Harris (Harris Farms), Bill Howell (NNII) Todd Cameron (Cameron Nursery), Garrett Bishop (G.S. Long Co.), Rodney Cooper (USDA-ARS).

Project Duration: 2 years + 1 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
Organization Name: Ruff Country K9
Contract Administrator: Jessica Kohntopp
Telephone: (208) 602-1293
Contract administrator email address: jesskohntopp@gmail.com

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: 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:
1. Train canines to detect little cherry virus-2 (LChV-2), and the X-disease phytoplasma (XDP) by using infected plant samples (Year 1).
2. Validate the accuracy, sensitivity, and specificity of the canines to detect LCD pathogens in young cherry trees in a single-blind greenhouse/potted plant studies (Year 1) and single-blind studies in commercial orchards (Year 2).
3. Examine the effects of dormancy and temporal infection cycles on canine detection (Year 2)
4. Extension and Outreach – monthly meetings, demonstration at field day and technical factsheet (Years 1 & 2)

Given the challenges associated with sampling for this disease, particularly due to its uneven distribution *in planta*, it was determined that continued qPCR testing of the trees in the absence of complete dissection was 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:
- Canines are capable of detecting LChV-2 and XDP infection in trees in advance of detection by qPCR.
- Canines have shown 99.72% accuracy, 96% sensitivity, and 100% specificity for the detection of LChV2 and XDP in a blind controlled study and successfully displayed odor discrimination from

other diseases and stressors.
- Canines alerted on sap/phloem extracts, and then on pathogen specific proteins, suggesting at least one of the targets imprinted on were components of the pathogens themselves
- Low temperatures inhibited detection of low concentration positives, while close tree spacing limited pinpointing of trees, suggesting environmental and spatial considerations for field deployment.
- Overall, canine detection shows potential as a rapid, non-destructive, whole-tree screening tool for little cherry pathogens.

Methods

Initial training

Two canines were selected for this project and trained to detect the three pathogens associated with Little Cherry Disease (LCD). Known LCD positive and negative potted trees were used as training aids for the canines. Canines underwent a 1-week acclimation period followed by 8–10 weeks of odor and obedience training. Training consisted of 7–10 sessions per day, 4–5 days per week, in a controlled environment. Canines were positively reinforced with verbal praise and brief toy play when they correctly identified the target odor through self-discovery; incorrect alerts were not reinforced. Training continued until canines reliably alert by sitting and remaining at the target odor until released. Once the canines were confirmed to be responding to the target odors, they were evaluated through a series of single-blind greenhouse/potted plant and field-based trials.

Single-blind studies

Two types of single-blind studies were conducted to assess a) Accuracy: The total amount of correct alerts on positive trees and non-alerts on negative trees, b) Sensitivity: The total amount of correct alerts on positive trees, and c) Specificity: The total amount of correct non-alerts on negative trees. The first trial ran the dogs through a greenhouse containing approximately 200 potted negative plants in which seven potted positive plants were randomly placed. The second trial involved placement of 60 positive and negative plants in rows in an open, outdoor location, with randomized replicates in which the observers changed the placement of the positives while the handler was unaware of the locations. Replicates were performed 23 times, giving a total number of 1380 plants scouted.

This process was repeated in a commercial orchard where the observers knew the location of trees that had previously tested positive by standard sampling collection and qPCR testing protocols. However, in practice it was found that the canines alerted on additional trees that had tested qPCR negative. To confirm the canine detection trees were dug out of the ground and dissected, with tissue samples collected vertically down the stem every 4” (~10 cm), with larger diameter pieces divided into two or four samples to capture spatial distribution. These were extracted and tested by qPCR.

Training aid development

To investigate the ability of the canines to detect the LCD-associated pathogens, we first macerated XDP infected leaf and stem tissue in phosphate-buffered saline (PBS) pH 7.4 at a 1:10 (w/v) ratio in a mortar & pestle and removed the sediment by centrifugation. From this we prepared a serial 1:10 dilution series down 10^{-9} and placed these on cotton pads in small cages for scouting by the canines, along with pathogen negative and buffer controls. Following successful indication on these extracts we then performed phloem sap extraction from XDP infected plants by high-speed centrifugation of stem sections and again created a serial dilution panel down to 10^{-9}, using pathogen negative sap as the diluent. Finally, to better identify the target(s) that the canines had imprinted on, we used the strategy of a) performing LC-MS on sap extracts from XDP, LChV2 and PDV positive plants to see if there were significant differences that could be attributed to pathogen activity, and b) expressed the structural coat proteins of LChV-1, LChV-2 and immunodominant membrane proteins of XDP, with PDV and PNRSV coat proteins, and a highly expressed *Pseudomonas syringae* pv. *syringae* protein

as nontarget controls. These proteins were used for the preparation of dilution series as above, pipetted on cotton pads in small cages for scouting by the canines, along with nontarget proteins and buffer controls.

Environmental, temporal and spatial effects on detection
Additional field studies were used to evaluate potential factors that could affect detection, including environmental conditions, tree dormancy, tree spacing, and the presence of nearby infected trees. Previously identified positive trees were evaluated alongside their corresponding qPCR results to examine the effects of tree age and infection level on canine detection. Tree spacing and proximity to neighboring infected trees were also assessed to determine whether adjacent trees or potential odor transfer could influence canine responses. Seasonal and environmental conditions, including dormant trees and frozen ground, were evaluated during searches conducted at different times of the year. Previously searched blocks were re-evaluated using a modified search approach, with each canine sit alert recorded during the search. .These trials were designed to evaluate both the capabilities and practical limitations of using canines as a tool for LCD detection.

Results and Discussion
Initial training
Initial training began with two XDP-positive and 10 negative trees. However, substantial differences in pot size, plant age, foliage, soil, fertilizer, and tagging made the plants unsuitable for reliable disease-specific training. Canines were initially trained using potted plants enclosed in boxes to minimize visual cues and continued training on this material for approximately six weeks while awaiting more suitable training material. The second set consisted of eight positive and 22 negative plants with standardized potting media, fertilizer, tagging, and other characteristics. The canines were re-initialized on the new material before transitioning to an outdoor 24-plant training grid, where they progressed well despite environmental distractions. Distractor training incorporated plants affected by other diseases and environmental stressors to assess the canines' ability to distinguish the target odor from non-target odors. Distractor training was successful, with both canines demonstrating reliable odor discrimination and no apparent difficulty distinguishing the target odor from distractors. Once proficiency was established, a blind study was conducted.

Single-blind studies
In the greenhouse trial, both canines correctly identified all seven positive plants, as well as an additional plant that was later sampled and confirmed positive for XDP. It should be noted that this was the first time the canines had searched inside a greenhouse, the handler was unaware of the positive plant locations, and the canines had never encountered positive plants other of the eight plants used in their training material.

In the outdoor study both canines were ill (due to unfortunate uncontrollable circumstances the day before) although Aika was affected more. Humma remained focused and continued to perform, producing results closer to those observed during the mini study. Aika, however, was frequently distracted by eating grass, resulting in 12 misses. After the canines recovered, the mini study was conducted, and Aika was substantially more focused and her performance reflected that. During the mini blind study, both canines missed two positive plants. It should be noted, in both blind studies, the canines never missed the same positive plant. Missed plants occurred during different runs or at different positive plant locations. Neither canine has ever produced a false alert; although they occasionally missed positive plants, they did not alert on any negative plants, reflected by the 100% specificity. Overall, the number of positive plants missed, excluding Aika's initial run while ill, was relatively low. Under controlled conditions, the mini blind study demonstrated that the canines achieved **99.72% accuracy, 96% sensitivity, and 100% specificity**, demonstrating their ability to reliably distinguish LCD-positive plants from negative plants.

***Table 1**: Blind Study*

	Initial Blind Study		Mini Study
Canine	**Humma**	**Aika**	**Both Canines**
Sensitivity	**95.05%** (96/101)	**88.12%** (89/101)	**96%** (48/50)
Total Positives Missed	**5**	**12**	**2**
Specificity	**100%** (1279/1279)	**100%** (1279/1279)	**100%** (670/670)
Total False Alerts	**0**	**0**	**0**
Positive Predictive Value	**100%**	**100%**	**100%**
Negative Predictive Value	**99.61%**	**99.07%**	**99.70%**
False Positive Rate	**0.00%**	**0.00%**	**0.00%**
False Negative Rate	**4.95%**	**11.88%**	**4.00%**
False Discovery Rate	**0.00%**	**0.00%**	**0.00%**
Accuracy	**99.64%** (1375/1380)	**99.13%** (1368/1380)	**99.72%** (718/720)

Footnotes: *Accuracy: The total amount of correct alerts on positive trees and non-alerts on negative trees. Sensitivity: The total amount of correct alerts on positive trees. Specificity: The total amount of correct non-alerts on negative trees*

During in-orchard testing, the canines alerted on several trees had tested negative by qPCR. These results raised the possibility that standard sample collection methods (i.e. a pooled sample based on a minimum of tissue from collected from four compass points around the tree) were failing to capture infected tissue. Five of the canine-alerted trees that had previously tested PCR-negative were selected for removal and sequential dissection. Between 76 to 112 samples were collected from each tree, and qPCR testing indicated that each of the five trees had at least one positive result, confirming the canines' alerts. The Ct values for these positives ranged between 36 – 38 cycles, or approximately <5 phytoplasma cells per section. The positive tissue samples were unevenly distributed around the trees, with no consistency in location between the five trees dissected.

***Table 2:** 5 Tree Study Results*

	CT Value	Sample Size	PCR Positive Results	% Chance of Selecting Correct Tissue	Canine Success
Tree #1	38.54 38.53 37.45 37.41	**76**	**5**	**6.58%**	**100%**
Tree #2	37.46	**87**	**1**	**1.15%**	**100%**
Tree #3	37.69 36.91 36.89	**95**	**3**	**3.16**	**100%**
Tree #4	37.92	**112**	**1**	**0.89%**	**100%**
Tree #5	37.57 37.21 37.77	**94**	**3**	**3.19%**	**100%**
Total	**---**	**464**	**13**	**2.80%**	**100%**

These results highlight the difficulty of selecting the tissue for PCR testing at early stages of infection, a limitation shared by all methodologies that rely on destructive sample collection, and why repeated collection of individual samples from the same tree to confirm a previous positive is not a viable practice. It also underlines the superiority of the canine detection method, as the canines are not limited by in-planta distribution of the target, as well as being significantly faster and less expensive than full-tree dissection.

Training aid development

Canines successfully alerted on the positive samples of both sap from macerated tissues, and phloem tissue exudates at dilutions as low as 10^{-9}, while no indications were observed with known phytoplasma-negative samples or controls. As this dilution is considerably lower than had be detected by qPCR for either LChV2 or XDP, it may be suggested that the compound(s) the canines were imprinted on may be 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). 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 appear have imprinted on a compound/or series of compounds that are present at greater concentration than the pathogen itself.

***Table 3**. 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

Based on these results we next performed untargeted LC-MS from phloem sap exudates to determine if there was a compound or series of compounds that could be associated with X-disease or LChV2 infection, using PDV infected samples as a control. Unfortunately, this screening was inconclusive due to the small sample size, with no compounds found to be pathogen-associated (data not shown) and needs to be repeated in greater depth.

In parallel, the LChV-1 and LChV-2 coat proteins, as well as the XDP immunodominant membrane proteins were expressed *in vitro*, along with PDV, PNRSV coat proteins and cherry canker effector as negative controls. The canines alerted on dilutions of the LChV-2 and XDP proteins, down to femtomolar (-10^{12}) concentrations, while not reacting to the non-target proteins and controls (PBS buffer and bovine serum albumin). Interestingly, the while not specifically trained on LChV-1 due to the lack of singly infected positive control plants in their initial training, one of the canines (Humma) alerted on the LChV-1 coat protein whereas the other (Aika) passed it up as a negative. This behavior is consistent with the in-field screening of nursery plants performed in conjunction with WSDA in 2025, where Humma had alerted on LChV-1 positive trees whereas Aika did not. As the LChV-1 and LChV-2 proteins are structurally very different (data not shown) it suggests, that the Humma imprinted on LChV-1 in the mixed-infection training materials earlier whereas the Aika did not.

The expressed proteins provide us with a highly adaptable and reproducible training aid. While not representing the entire range of compounds that could be present in or emitted by an infected plant, they are exclusively specific to the individual pathogens unlike, for example, a host-produced volatile such as terpenoid and allows the canines to be trained down to specific targets first, then introducing host background. It also allows the stepping-down of concentrations to determine the limits of detection by the canines and will form the basis of training efforts for the the next generation of canines.

Effects of environmental, spatial and temporal variables on detection

Older Cherry Block C: qPCR Results Samples were taken on Sept 3, 2024 from the nine trees in the table below with their corresponding dog responses and qPCR test results. Both canines alerted hard on Tree D running from both directions and did not alert on nor showed interest in the symptomatic Tree F that came back positive with a CT Value of 20.13 (~480k phytoplasma cells) for XDP. This could be due to the canines' odor threshold at the time. The canines have seen qPCR positive potted plants in those high ranges before, but the trees were much younger and smaller than in this block. It is believed the canines were within their 'normal reward threshold' when they were smelling the adjacent tree shown in the Figure 3 on the right. The handler was concerned the same sort of scenario could have happened with Tree G. Samples were retaken from that tree on Sept 24, 2024, and both trees on either side of it. When results came back, both the adjacent trees came back non-detect and this time Tree G did come back qPCR positive (XDP 33.9, ~54 cells). Tree J could have been missed by both canines as there are times they struggle finding back-to-back trees. Another explanation could be due to the canines alerting on a 33.58 (~66 cells) right before the 39.66 (<5 cells). Looking back to the diagram above, odor is continuously dispersing from the tree and the canines are constantly having to work through the residual rings. With such a weak infection beside a higher infected tree, the canines could have marked it as residual odor and kept searching.

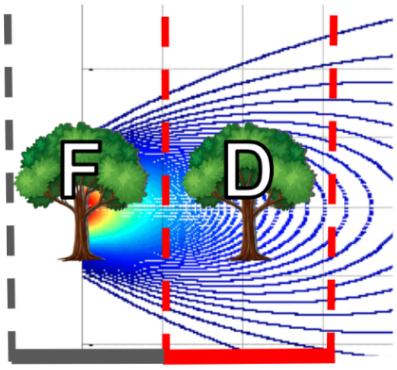


Figure 1: *Odor Threshold*

Table 4: *Block C qPCR Results*

Tree Sample	Humma Alert	Aika Alert	Humma Hesitate	Aika Hesitate	qPCR Result Sept. 3	Retested Sept. 24
Unknown Tree A	X	X			LChV-2 (37.89)	
Unknown Tree B	X	X			XDP (33.58)	
Unknown Tree J (Adjacent to Tree B)					XDP (39.66)	
Unknown Tree C		X	X		-	
Unknown Tree D (Adjacent to Tree F)	2x	2x			-	
Symptomatic Tree F					XDP (20.13)	
Symptomatic Tree E					-	
Unknown Tree G	X	X			-	XDP (33.9)
Unknown Tree H			X	X	-	

Dormancy Study:



Figure 2. *Trees pictured from each month respectively for the Dormancy Study*

In November and December, the canines consistently alerted on the same trees without difficulty. However, in January, their performance differed, with both canines experiencing difficulty detecting some trees presumed to have lower-level infections. In some cases, they alerted only on a second pass, while in others, they did not alert at all. When brought to Older Block A the following day, both canines had fewer difficulties detecting the positive trees. It should be noted that CT values in Younger Block B ranged from 37–39, while those in Older Block A ranged from 35–38.
In January, the canines were only run past eight previously alerted trees in Younger Block B after the handler observed that they were struggling to detect the trees. Continuing to work the dogs until they alerted on a second pass was not considered beneficial, as this could reinforce the behavior of requiring a second pass to make an alert. The January results are as follows:

Table 5: *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.

Monitoring Previously Ran Blocks: The canines have previously searched these blocks twice, with relatively consistent results between searches. and no more than 12 additional canine alerts identified over a one-year period. However, the search methodology used during these previous evaluations may have greatly influenced the results. These two blocks were the first "unknown" areas in which the

canines were deployed, and the handler was initially informed that it was expected to be a low-pressure block. As the canines began alerting on more trees than anticipated, the handler began challenging the alerts. When a canine alerted, the handler would pull the canine off the tree, return several trees back, and rerun the canine through the area. If the canine alerted again on the same tree, the response was recorded; if the canine did not re-alert, the search continued. This process may have resulted in some alerts not being recorded. This same search approach/pattern was used on both blocks during every reevaluation until 2026. This year, a greater number of trees than usual were exhibiting visible LCD symptoms, potentially due to environmental factors. The canines were brought back to the blocks and allowed to search without challenging their responses. Any tree on which the canine produced a sit alert was recorded as an alert. This change in search methodology resulted in a substantially greater number of recorded canine alerts compared with previous searches. Initial alerts on the two blocks were as follows: Block B Humma: 55 trees and Aika: 59 trees, these trees were left within the block. Block D 52 initial alerts by both canines were recorded and trees were removed from the block. After the new way of running, number of alerts jumped to 168 in block B (across 17 rows, omitting 16 rows) and 318 in Block D.

Overall, this project demonstrates the strong potential of canine detection as a whole-tree, nondestructive approach for identifying Little Cherry Disease. Under controlled conditions, the canines demonstrated high accuracy, sensitivity, and specificity, successfully discriminated LCD-associated odors from common disease distractors, and transferred their detection ability from potted plants to greenhouse, field, and commercial orchard settings. Their ability to detect positive sap and phloem exudates at extremely low dilutions further suggests that the canines may be responding to compounds associated with infection that occur at concentrations greater than the pathogen itself. The PCR and tree dissection results also highlighted an important limitation of using conventional molecular testing as the sole method for validating canine alerts. The highly patchy distribution of the pathogen within weakly infected trees means that even extensive tissue sampling may fail to capture infected material. The fact that all five dissected, canine-alerted trees contained PCR-positive tissue, while only 13 of 464 samples testing positive, demonstrates the challenge of confirming whole-tree detection through limited tissue subsampling and the need for a holistic approach. Field studies demonstrated that canine detection can be influenced by infection level, tree spacing, adjacent infections, season, ground conditions, and potentially the biology of the disease. While these factors may affect detection under certain conditions, the canines consistently demonstrated the ability to locate infected trees across a variety of environments. Continued research will focus on training new canines with the newly developed training aid, comparing their abilities to the two canines trained the conventional way, and evaluating the integration of canine screening into commercial nursery and orchard operations.

Literature Review

It has been proven repeatedly that the canine olfactory system is truly remarkable, and the depth of its full potential is not yet fully understood. The use of canines for odor detection is not a new phenomenon. Using canines for hunting dates back >12,000 years and the canine nose has since been used for a range of odor detection and tracking (Gottwald et al., 2020). The ‘traditionally’ accepted and more well-known application is police/military canines that have been trained for explosive and drug detection as well as human tracking. However, canines have also been used in medicine to find cancer, epileptic warnings, diabetic alert, and most recently COVID-19. In outdoor applications, conservation canines have become more prevalent, locating invasive species, endangered species, and wildlife scat. Other canines have been used to find bed bugs, cell phones in prisons, and cadavers (both human and animal). In the agricultural community, canines have been successfully trained to find a variety of plant diseases including citrus greening, citrus canker, and laurel wilt of avocado with accuracies as high as 99% (Gottwald et al., 2020). Canines have been

shown to be a practical tool for pathogen detection, in a quick, accurate, and non destructive manner.

F1K9 had HLB detection canines in orchards and residential properties with canines detection time being ~2s per tree, providing quicker results than any current diagnostic tool. SEIR grafts are models commonly used and accepted by the USDA APHIS to predict infectious disease. Whereas there is a susceptible group (S), exposed group (E), infected group (I), and resistant group (R). A variation of this model, SECIR, was used in the HLB paper adding in asymptomatic trees referred to as cryptic (C). This model accounted for 100+ different variables and the data gathered from the canines in the Temporal Study. This model demonstrates that pathogen control was possible when canine detection was followed by tree removal whereas current methods of PCR and visual detection failed to suppress the exponential trajectory which is shown in Figure 3.

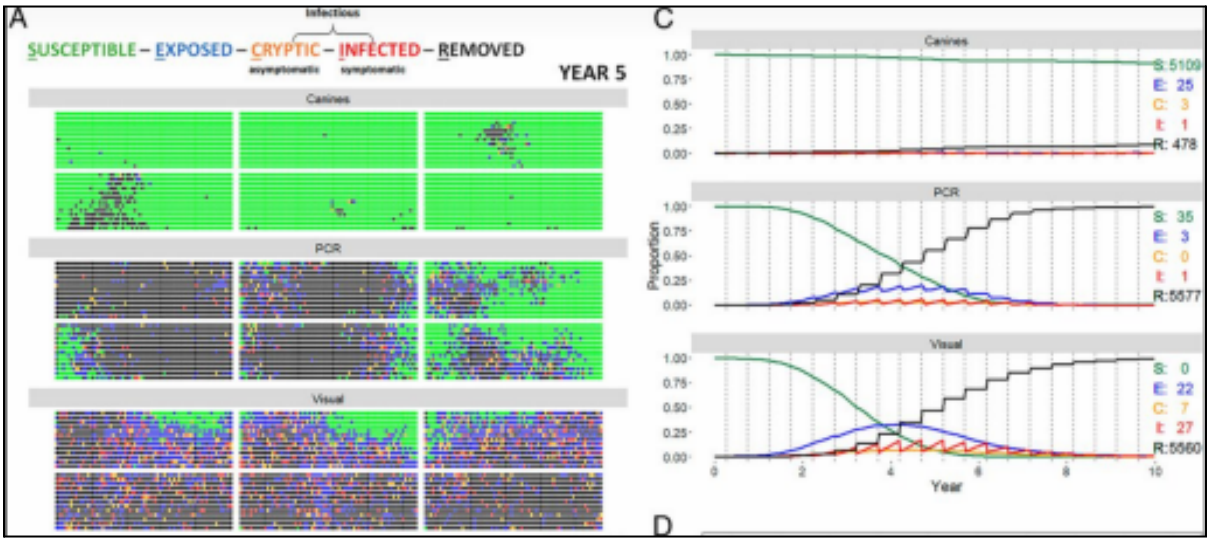
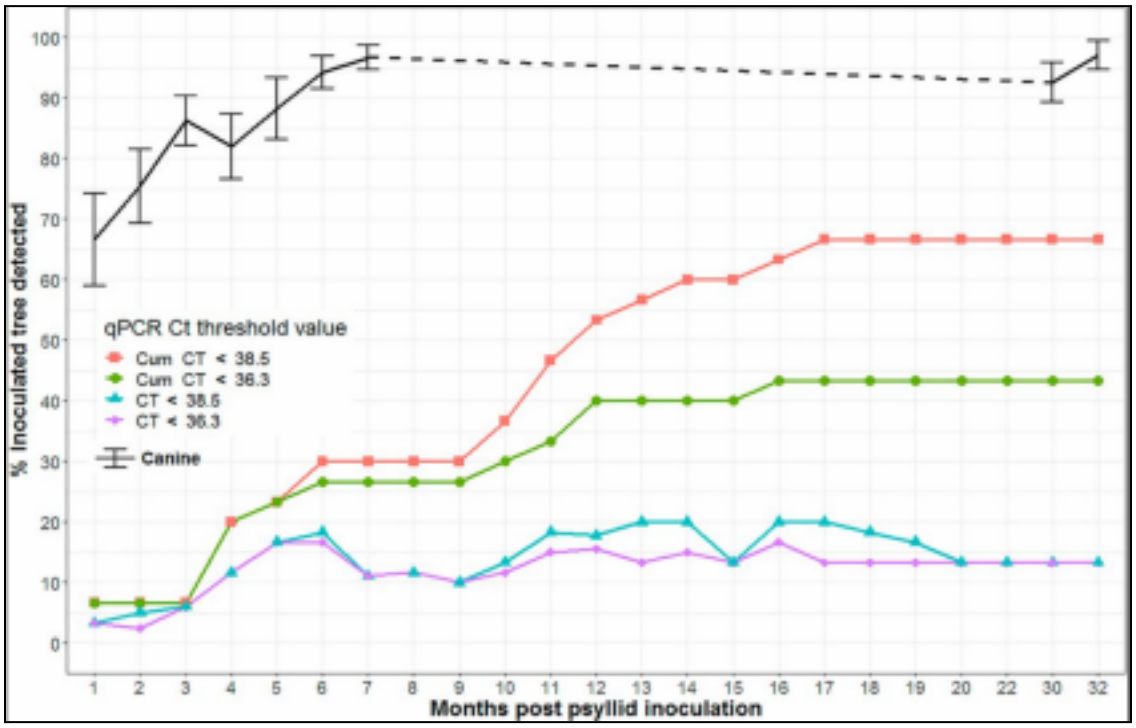


Figure 3. *SECIR Model based simulation of CLas epidemic over 10-year period.*

A Temporal Study conducted within the HLB paper proved canines were able to find infection months to years prior to molecular methods of PCR shown in the table below. Plants in this study were artificially infected, kept track of via numbering system, and kept in a greenhouse to ensure no natural psyllid transmission took place. Every two weeks trees would be put in a grid in a computer-generated randomized order. The canines would run past the trees and data was collected on which trees were alerted on. Samples were taken for PCR at the same time. This test proved canines could be used as an early detection tool (Figure 4).

Figure 4. *Temporal assessment of canine vs qPCR detection of CLas infection.*



LCD is a widespread problem throughout Washington which has resulted in the removal of about 974 acres of sweet cherry trees which is more than 238,856 trees between 2015 and 2020 (Molnar et al., 2022). This has caused a large economic impact on growers of over $115 million (Molnar et al., 2022). Control is very difficult due to the short range of time that visual symptoms are present and the unfeasible cost of testing many trees through PCR. Canines could be the year-round, real-time, non-destructive diagnostic tool that is needed to help mitigate the spread of LCD.

References

- Gottwald, T., Poole, G., McCollum, T., Hall, D., Hartung, J., Bai, J., Luo, W., Posny, D., Duan, Y.-P., Taylor, E., da Graça, J., Polek, M., Louws, F., and Schneider, W. (2020). Canine olfactory detection of a vectored phytobacterial pathogen, Liberibacter asiaticus, and integration with disease control. Proceedings of the National Academy of Sciences, 117(7):3492-3501. https://doi.org/10.1073/pnas.1914296117

- Mendel, J., Burns, C., Kallifatidis, B., Evans, E., Crane, J., Furton, K. G., & Mills, D. (2018). Agri-dogs: Using Canines for Earlier Detection of Laurel Wilt Disease Affecting Avocado Trees in South Florida. HortTechnology, 28(2), 109–116.https://doi.org/10.21273/HORTTECH03791- 17

- Molnar, C., DuPont, T., & Thompson, A. (2022). Estimated Impact of X-disease and Little Cherry Disease in Washington and Oregon from 2015 to 2020. *The Journal of Extension, 60*(4), Article 17. https://doi.org/10.34068/joe.60.04.17

Executive Summary

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

Key words: Little Cherry Disease, Canine, Early Detection, Rapid Detection, Non-destructive Detection

Abstract
Little Cherry Disease (LCD) is a significant threat to the cherry industry, yet early detection remains difficult due to the short-lived and inconsistent nature of visible symptoms. While conventional PCR testing provides a diagnostic option, the patchy and uneven distribution of the pathogen throughout a tree makes it difficult to collect representative tissue and confirm low-level or early infections from limited subsamples. This project evaluated the use of canines as a potential year-round, real-time, nondestructive detection tool that provides a holistic, whole-tree sampling approach rather than relying on individual tissue subsamples.
Two canines were trained to detect the three pathogens that cause LCD and subsequently demonstrated discrimination between LCD and other common diseases and stressors. In blind, controlled testing, the canines achieved 99.72% overall accuracy, 96% sensitivity, and 100% specificity. They were successfully transitioned from potted trees to field and commercial orchard settings. Importantly, several trees alerted on by the canines initially tested non-detect by qPCR. Five of these trees were later removed and extensively dissected, and all five were confirmed positive, with only 13 of 464 tissue samples testing positive. This highlights the highly uneven distribution of pathogen within these trees and demonstrates why confirming early or weakly infected trees through qPCR is so difficult. Further expanding on the need for a method that can sample the whole tree.

Canines also successfully detected positive sap and phloem extracts at dilutions as low as 10^{-9}, suggesting they may be responding to one or more compounds associated with infection and may have learned multiple odor signatures associated with LCD. Field studies identified additional factors affecting detection, including tree spacing, adjacent infections, seasonal conditions, and low pathogen levels. Overall, these results demonstrate strong potential for canine detection as a tool for identifying LCD-infected trees and improving disease management. Continued research will focus on training new canines with the newly developed training aid, comparing their abilities to the two trained the conventional method, and evaluating the integration of canine screening into commercial nursery and orchard operations.

Continued research will focus on training new canines with the newly developed training aid, comparing their abilities to the two canines trained the conventional way, and evaluating the integration of canine screening into commercial nursery and orchard operations.

Project Title: X-disease Transmission Threshold – Measuring the Moment of Spread

Report Type: Continuing Project Report

Primary PI: W. Rodney Cooper
Organization: USDA-ARS
Telephone: 509-454-6570
Email: rodney.cooper@usda.gov
Address: 5230 Konnowac Pass Rd
Address 2:
City/State/Zip: Wapato, WA, 98951

Co-PI 2: Adrian Marshall
Organization: USDA-ARS
Telephone: 509-454-4476
Email: adrian.marshall@usda.gov
Address: 5230 Konnowac Pass Rd
Address 2:
City/State/Zip: Wapato, WA, 98951

CO-PI 3: Tobin Northfield
Organization: Washington State University
Telephone: 509-293-8789
Email: tnorthfield@wsu.edu
Address:
Address 2: 1100 N Western Ave
City/State/Zip: Wenatchee, WA, 98801

Co-PI 4: W. Jacob Pitt
Organization: Washington State University
Telephone: 509-293-8800
Email: William.pitt@wsu.edu
Address:
Address 2: 1100 N Western Ave
City/State/Zip: Wenatchee, WA, 98801

Cooperators: Scott Harper, Marco Pitino, Garrett Bishop

Project Duration: 3 Years

Total Project Request for Year 1 Funding: $ 50,000
Total Project Request for Year 2 Funding: $ 50,000
Total Project Request for Year 3 Funding: $ 50,000

Other related/associated funding sources:
Funding Duration: 2025 - 2027
Amount: $337,500
Agency Name: Washington Research Foundation Postdoctoral Fellowship
Notes: Postdoctoral fellowship supporting co-PI Jacob Pitt

WTFRC Collaborative Costs: None

Budget 1
Primary PI: W. Rodney Cooper
Organization Name: USDA
Contract Administrator: Mara Guttman
Telephone: (510) 559-5619
Contract administrator email address: Mara.Guttman@usda.gov
Station Manager/Supervisor: Rodney Cooper
Station manager/supervisor email address: Rodney.Cooper@usda.gov

Item	2026	2027	2028
Salaries			
Benefits			
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies	$9,394.00	$7,774.00	$6,089.00
Travel			
Plot Fees			
Miscellaneous			
Total	$9,394.00	$7,774.00	$6,089.00

Footnotes:
Supplies – Insect colony supplies: celery seeds, pots, soil, lights, cages. EPG supplies – gold wire, silver glue, and X-disease diagnostics – DNA extraction kits and qPCR reagents.

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

Budget 2
Co PI 3: Tobin Northfield
Organization Name: Washington State University
Contract Administrator: Kevin Rimes
Telephone: 509-293-8803
Contract administrator email address: kevin.rimes@wsu.edu
Station Manager/Supervisor: Lee Kalcsits
Station manager/supervisor email address: lee.kalcsits@wsu.edu

Item	**2026**	**2027**	**2028**
Salaries	$36,800.00	$38,272.00	$39,803.00
Benefits	$3,706.00	$3,854.00	$4,008.00
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies	$100.00	$100.00	$100.00
Travel			
Plot Fees			
Miscellaneous			
Total	$40,606.00	$42,226.00	$43,911.00

Footnotes:
Salaries – two temporary hourly employees (20 weeks) to help conduct transmission trials and EPG assays. One based at the WSU TFREC in Wenatchee and the other at the USDA-ARS in Wapato, WA**.**

Objectives

1. **Determine the infection threshold (Ct 40-35, 34-30, 29-25, <25) at which *Colladonus reductus* can acquire X-disease phytoplasma.**
   Determining at what infection level a leafhopper can acquire the phytoplasma will directly impact the management decision of when to remove an infected tree and guide the sensitivity needed for early detection. For example, if a leafhopper cannot acquire the phytoplasma until infection titer has reached thousands of cells, then the sensitivity and urgency needed for detecting trees for removal is much less than if they can acquire it at tens of phytoplasma. This will directly influence ongoing early detection projects including dogs, qPCR, antibody, and CRISPR studies.

2. **Assess the latent period and the proportion of *Colladonus reductus* that can transmit after acquiring from different titer levels of infected plants.**
   Research conducted in the 1950's - 1970's demonstrated *Colladonus reductus* could transmit the phytoplasma in 30 days, but it was unknown how infected the trees were at acquisition. Pathogen infection levels can now be measured using qPCR and are reported as Ct values. Ct values are inversely related to infection level such that higher Ct values (35-40) indicate low levels of infection whereas lower Ct values (<25) indicate high pathogen concentration. Testing and scouting over the last three years in Washington have shown that the level of phytoplasma in trees and weeds can vary widely from recently infected with a few phytoplasma cells (Ct 35-40) to an established infection of millions of phytoplasma (Ct <25). At lower infection levels (Ct > 30) trees may only present slight symptoms or be completely asymptomatic and can be missed during scouting and removal, allowing them to be sources of phytoplasma for leafhoppers. The only existing data linking the success of transmission to infection levels are from studies conducted last year showing that when *C. reductus* feed on highly infected plants (Ct < 20) nearly 50% of them were able to acquire and transmit X-disease phytoplasma in just two weeks, which is half of the time previously reported. It is currently unknown if leafhoppers can acquire the phytoplasma in the early stages of plant infection, and whether titer levels affect the proportion of infected leafhoppers or the amount of time until they can transmit.

3. **Evaluate the feeding time needed for acquisition and transmission of X-disease by *Colladonus reductus* using EPG**
   C reductus develops on broadleaf weeds that harbor relatively low levels of phytoplasma compared to cherry trees. In fact, it is not clear whether phytoplasma levels are high enough in weeds for *C. reductus* to acquire the pathogen, or if the leafhopper must acquire the pathogen from infected cherry trees. The methods developed last year for acquisition and transmission assays can be used to determine the effect of phytoplasma titers on acquisition rates and the time it takes for leafhoppers to become infective. Through the use of electropenetrography (EPG) it is possible to precisely estimate the feeding time needed for leafhoppers to acquire and transmit X-disease phytoplasma. Understanding the role of infection level and feeding time on transmission success is essential for guiding management decisions. Specifically, we will use results of these trials to determine how quickly pesticides or repellents need to work, how effective antimicrobial molecules need to be, and at what Ct value a tree must be removed to stop leafhopper transmission.

Significant Findings

- Although we originally considered the acquisition threshold of phytoplasma titer in the plant and phloem feeding time two separate objectives, the two factors seem to be interrelated. Initial findings suggest that combining the two factors in the same model provides the best estimate of phytoplasma acquisition ability of *Colladonus reductus*. For instance, a leafhopper that fed for a very long time from the phloem on a tree that had a low phytoplasma titer may acquire a similar level of phytoplasma to a leafhopper that only fed from the phloem for a very short amount of time but on a tree that had a high phytoplasma titer.
- Preliminary results application: removing trees before they are highly infected increases the impacts of insecticides, because it provides a better chance of killing the insect before it feeds enough to acquire the phytoplasma. In contrast, leaving infected trees in the orchard necessitates very intense vector management, because it reduces the amount of feeding time needed to acquire the phytoplasma.

Methods

For all transmission trials, we used *C. reductus* collected from a Phytoplasma non-infected laboratory-reared colony. Colonies are located at the USDA-ARS in Wapato, WA, WSU-TFREC in Wenatchee, WA, and at the WSU-IAREC in Prosser, WA. The leafhoppers were reared on celery (*Apium* graveolens var. *dulce*) at 22°C±2°C (72°F±3°F), 45% relative humidity, and a 16:8 L:D photoperiod. Wild *C. reductus* were routinely added to the colonies to maintain genetic diversity and prevent colony adaptation to laboratory conditions and food plants. Subsequent X-disease free offspring were used for trials since X-disease is not vertically transmitted. Colony testing was conducted to ensure that leafhoppers were X-disease free.

1) Determine the infection threshold (Ct 40-35, 34-30, 29-25, <25) at which Colladonus reductus can acquire X-disease phytoplasma.

Using infected cherry tree branches from established trees has been the most successful method for acquisition success and low leafhopper mortality. Branches from trees with high levels of X-disease (Ct <25) were collected June – August 2026, when leaves and fruit had their highest amount of X-disease. Clean *C. reductus* were added to cages with the clipped branches and the leafhoppers were given three days to feed and acquire X-disease before transferring them to a new cage with a celery plant. DNA was purified from the cherry branches andqPCR used to determine the Phytoplasma infection titer. Leafhoppers were maintained on the celery plants for one week after initial access to the cherries before testing them for Phytoplasma using qPCR. This work will continue to be conducted in 2027 and 2028 until we have at least 200 *C. reductus* per the four infection titer categories.

2) Assess the latent period and the proportion of Colladonus reductus that can transmit after acquiring from different titer levels of infected plants.

Leafhoppers do not transmit X-disease phytoplasma immediately after feeding on an infected plant. The time between pathogen acquisition and transmission (known as the latent period) can vary. The length of the latent period or ability for phytoplasma to absorb into the insect's hemolymph (the insect's "blood"), travel to the salivary glands and grow within the salivary glands may depend upon pathogen levels in the source plant, especially if the immune system is fighting the phytoplasma "infection." In preliminary trials we found that *C. reductus* that were given access to highly infected cherry branches (Ct <25) for three days were able to transmit X-disease phytoplasma in less than two

weeks. However, we did not determine the absolute timing of how quickly they could transmit within the fourteen-day period. This summer, we repeated these trials, putting non-infected leafhoppers on highly infected branches (Ct < 25) for up to three days before transferring them to a mature celery plant for the remainder of the experiment. Each day from two to six days after initial access to the infected branch, 20 adult *C. reductus* were transferred to 20 individual clean celery seedlings contained in small vials and given 24 hours to transmit. After 24 hours the leafhoppers were collected, and the celery were collected the following day. All samples were stored at -20°C until DNA extraction and qPCR testing. This trial was conducted twice in August.

3) Evaluate the feeding time needed for acquisition and transmission of X-disease by Colladonus reductus using EPG.

Branches for Objective 3 trials were obtained from highly infected trees (Ct 16.08 – 21.94) at the USDA Moxee farm. We used these highly infected trees for the first trials (conducted June – September 2026) in order to establish a baseline for future trials to be conducted with less infected trees. Late-instar nymphs (3^{rd} – 5^{th} instar) were used for these trials, as nymphs may have a higher propensity to feed from the phloem (W.J. Pitt, personal observation), and at this life stage they may have the maximum time to feed and transmit as an adult.

Feeding behaviors of *C. reductus* were monitored using a GIGA 8d DC EPG monitor (EPG Systems, Wageningen, Netherlands). Insect electrodes were attached to leafhoppers and feeding behaviors were recorded for 5 hours. Leafhoppers were immobilized using a vacuum suction device, and 18 µm diameter gold wires were attached to the dorsal side of leafhoppers' thorax using a small amount of silver glue. Leafhoppers were connected to the EPG system and placed on the petiole of infected branches housed inside of a faraday cage at a temperature of 22 ± 2°C (72°F ± 4°F). Cut branches were connected to the plant electrode of the EPG system by placing cut branches and plant electrodes in cups of water. After recordings, leafhoppers were removed from branches and stored individually in a freezer at -20°C (-4°F). The petiole that a leafhopper was feeding on was also removed and stored at -20°C. Because leafhoppers were tethered to 2 cm long wires (i.e., were able to move within a 2 cm radius), leafhoppers occasionally moved to the leaf blade during recordings, in which case tissue from the leaf blade was also sampled. EPG recordings were annotated using Stylet+ software (EPG Systems), analyzed using R software v.4.5.1 (R Core Team 2022), and the duration of phloem feeding was calculated for each leafhopper. Approximately 1 – 2-year-old cherry rootstock (Mazzard), which were housed in cages inside a greenhouse and were not infected with X-disease, were also used in recordings to provide clean controls to ensure that leafhoppers were acquiring the phytoplasma from infected branches. DNA was extracted from leafhoppers and cherry petioles and then tested for the phytoplasma using qPCR protocols of Wright et al. (2022). Ct values were converted to estimated number of phytoplasma cells per reaction using the standard curve from Wright et al. (2022), which we refer to as the 'phytoplasma titer'.

During 5-hour recordings, leafhoppers were allowed to freely feed from the phloem and were not terminated after pre-determined lengths of phloem feeding. This allowed us to obtain a natural variation in phloem feeding durations for assessing what duration is necessary for acquisition, without needing to manually observe and artificially terminate EPG recordings in real time, greatly reducing the workload of this objective while also increasing the number of replicates. The phytoplasma titer of leafhoppers was modelled using 1) the phytoplasma titer of the source plant petiole and 2) total duration of phloem feeding as predictor variables. Additionally, a third predictor variable was created by multiplying the total phloem feeding duration by the phytoplasma titer of the petiole; this variable is referred to as 'phloem duration*source plant phytoplasma titer' (**Fig. 2**). Models were created using the packages 'lme4' and 'lmerTest' in R (Bates et al. 2015; Kuznetsova et al. 2017).

Results and Discussion

1) *Determine the infection threshold (Ct 40-35, 34-30, 29-25, <25) at which Colladonus reductus can acquire X-disease phytoplasma.*

Our preliminary studies showed that *C. reductus* can acquire X-disease from highly infected cherry branches (Ct < 25). Consistent with this, results of our initial EPG experiments demonstrate that *C. reductus* acquires X-disease from branches at Ct 16.08 – 21.94 infection levels, but very few replicates run to-date included petioles with lower infection levels (**Table 1**). An additional 21 samples are currently being processed, and these trials will be repeated in 2027 to obtain a greater number of replicates at lower infection levels.

Table 1: Number of replicates for each infection level from initial trials.

Ct value of petiole	Number of replicates
< 25	35
29 - 25	1
34 - 30	1
40 - 35	4

2) *Assess the latent period and the proportion of* Colladonus reductus *that can transmit after acquiring from different titer levels of infected plants.*

We conducted the transmission trials for leafhoppers acquiring from highly infected branches (Ct < 25), those samples are collected and stored in the freezer for DNA extraction and qPCR testing this winter. The higher Ct ranges will be tested in the following years.

3) *Evaluate the feeding time needed for acquisition and transmission of X-disease by* Colladonus reductus *using EPG.*

A total of 16 out of the 41 individual leafhoppers that we evaluated tested positive for the phytoplasma. An additional 21 samples, results from which are not included here, were obtained and are currently being processed. Not all individuals that were recorded fed from the phloem, helping explain the lack of acquisition by some leafhoppers feeding on highly infected branches. EPG waveforms were analyzed for leafhoppers which tested positive for the phytoplasma. Of the leafhoppers that fed from the phloem, the total duration of phloem feeding ranged from 28 seconds to 2.7 hours. The variable 'phloem duration*source plant phytoplasma titer' had a significant positive effect on phytoplasma titer of leafhoppers (*df = 13.6; t = 2.7; P = 0.017; AICc = 52.0*; **Fig. 2**). This model performed better than the model that estimated phytoplasma titer in the leafhopper as a function of phloem feeding (*df = 13.0; t = 2.5; P = 0.025*) and source plant phytoplasma titer (df = 13; *t* = 1.4; P = 0.17) separately (AICc = 56.7).

Results from initial trials for Objective 3 confirm that source plant infection level and duration of leafhopper vector feeding in the phloem are critical components of phytoplasma acquisition. Although phytoplasma acquisition in some cases can occur very quickly, our findings suggest that feeding on enough phytoplasma for the phytoplasma to survive through the latency period within the insect fed for longer time periods (e.g., 56 mins) on highly infected leaves (Figure 2). Further research linking these acquisition rates with transmission will better link these feeding times with subsequent infectivity of leafhoppers following the latent period. Ongoing work for Objective 2 will help better understand this relationship.

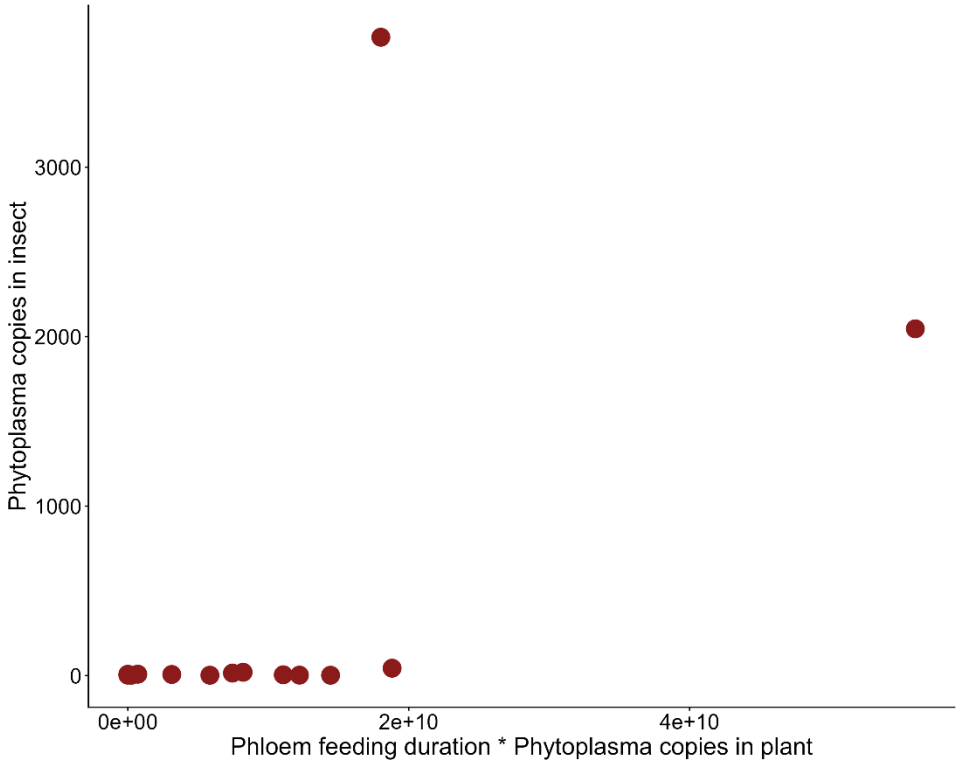


Figure 2: Phytoplasma acquisition by *Colladonus reductus* nymphs from Objective 3 trials. The Y-axis represents the phytoplasma titer of a leafhopper, and the x-axis represents the total phloem feeding duration of a leafhopper multiplied by the phytoplasma titer in the plant it was feeding on. Each point represents an observation of an individual leafhopper.

Literature Review

Candidatus Phytoplasma pruni, is an intracellular, phloem-limited phytoplasma that is the causative agent of X-disease, a debilitating disorder causing significant crop loss in the Pacific Northwest. X disease phytoplasma reduces fruit size and quality in cherries, has no cure, and once infected trees transmit the phytoplasma for the life of the tree (Lenz and Dai 2017). In peaches and nectarines, X disease symptoms include leaf shot-hole appearance, branch dieback and tree death (Davis et al. 2013). The phytoplasma also infects several other Prunus species, such as plums and almonds but does not induce symptoms (Davis et al. 2013). The phytoplasma can be transmitted between trees through grafting, including root grafting, and by several leafhoppers (Rawlins and Home 1931, Rawlins and Thomas 1941, Wolfe et al. 1951, Jensen 1959, 1971, Purcell et al. 1987). The disease has been attributed with the elimination of cherry production in Napa, Sonoma, and Solano counties, in California (Van Steenwyk et al. 1995).

There are no known effective treatments for the disease, so management focuses on keeping the disease out of orchards by removing diseased trees and weedy hosts, and vector management (Lenz and Dai 2017). Leafhoppers can spread the phytoplasma between orchard trees and may acquire the phytoplasma from infected weedy hosts or other *Prunus* sp. crops that can host the pathogen without expressing symptoms (Wolfe et al. 1951, Jensen 1959). A California abatement program in the 1980's focused on infected tree removal and pesticide use and found that the most effective treatment was tree removal, with limited effect of the insecticides available at the time (Van

Steenwyk et al. 1995). Disease detection primarily focuses on fruit size and quality, which is only apparent near harvest and is confounded by horticultural factors such as irrigation and fertilization that also influence fruit traits. These symptoms are often apparent on one section of the tree and are latent for one or two years, allowing the disease to spread within the orchard before detection. Indeed, the California abatement program generally observed symptomatic trees adjacent to areas where trees were removed the year prior (Van Steenwyk et al. 1995). This is due to phytoplasma infection through root grafting and/or movement by leafhoppers. Further complicating matters, Little cherry virus 2, which is transmitted by mealybugs, is present in northern cherry growing regions (e.g., northern Washington) and the virus commonly induces symptoms indistinguishable from those caused by the phytoplasma, making diagnosis difficult without molecular methods (Eastwell and Bernardy 2001).

Given the limited options for pathogen treatment, improving vector management is important for limiting spread. Preliminary insect surveys in southern Washington identified 6 leafhopper species (*Colladonus reductus*, *C. geminatus*, *Fieberiella florii*, *Euscelidius variegatus*, *Scaphytopius* sp., and *Osbornellus* sp.) that tested positive for the *Candidatus* Phytoplasma pruni pathogen (Harper, Ferguson, unpublished data). Recent studies have found *C. reductus* and *C. geminatus* are the most commonly collected leafhopper species and are the most positive testing for the pathogen species in Washington orchards (Marshall et al., 2023, Harper unpublished data). Research conducted in California in the 1980's suggested *Colladonus montanus* is most likely to develop on uncultivated hosts and transmit the phytoplasma to the orchard (Purcell et al. 1987). Recent feeding trials, gut content analysis, and plant infection surveys have continued to confirm this through finding many common weeds and native plants to be both hosts for phytoplasma and *Colladonus* leafhoppers (Cooper et al., 2022, Northfield unpublished, Harper unpublished data).

In 1952 Mervin Nielsen found that in the Dalles region in Oregon the first leafhopper generation of adults occurs in May and June, with a second generation of adults occurring (Nielson 1968). However, recent research by Northfield's group suggests that there may now be an extra generation of leafhoppers, at least for *C. reductus* (not studied by Nielsen) (Marshall et al., 2023). Preliminary analysis of phytoplasma titers within trees suggest that trees have higher titers at the distal parts of branches later in the season, suggesting that fall may be particularly important for disease transmission. Therefore, leafhoppers are most likely to acquire X-disease from cherry trees and transmit to other trees in late summer and early fall which was previously demonstrated in California (Suslow and Purcell 1982).

Due to the periodic nature of X-disease outbreaks occurring on average every 30 years, research on all aspects of the plant pathogen, plant hosts, and the insect vectors has been sporadic. A recently published recovery plan for X-disease has identified five core areas of research, education, and extension necessary for developing an effective area-wide management plan (Harper et al., 2023). Therefore, research efforts towards building a sustainable X-disease management program need to focus on pathogen-host-vector interactions, identifying factors of biological significance in the pathosystem, and developing methods for suppressing pathogen and vector spread. Current studies aim to accomplish this through assessing cultural controls for leafhoppers through physical barriers to alternative hosts (Extenday) or repellency from trees (kaolin clay) (Marshall et al., 2023), new groundcover management to reduce alternative hosts (Northfield unpublished data), and understanding the pathogen disease cycle, strain variety, and host range in the Pacific Northwest (Harper, unpublished data).

References

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of statistical software*, *67*, 1-48.

Cooper, W. R., A. T. Marshall, J. Foutz, M. R. Wildung, T. D. Northfield, and D. W. Crowder. 2022. Directed sequencing of plant specific DNA identifies the dietary history of four species of Auchenorrhyncha (Hemiptera). Annals of the Entomological Society of America 115: 275-284.

Davis, R. E., Y. Zhao, E. L. Dally, I. M. Lee, R. Jomantiene, and S. M. Douglas. 2013. 'Candidatus Phytoplasma pruni', a novel taxon associated with X-disease of stone fruits, Prunus spp.: multilocus characterization based on 16S rRNA, secY, and ribosomal protein genes. International Journal of Systematic and Evolutionary Microbiology 63:766-776.

Eastwell, K. C., and M. G. Bernardy. 2001. Partial characterization of a closterovirus associated with apple mealybug-transmitted little cherry disease in North America. Phytopathology 91:268-273.

Harper, S. J., Northfield, T. D., Nottingham, L. B., DuPont, S. T., Thompson, A. A., Sallato, B. V., ... & Cooper, W. R. (2023). Recovery plan for X-disease in stone fruit caused by 'Candidatus Phytoplasma pruni'. *Plant Health Progress*, *24*(2), 258-295.

Hartig, F. (2016). DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. *CRAN: contributed packages*.

Jensen, D. D. 1959. A plant virus lethal to its insect vector. Virology 8:164-175.

Jensen, D. D. 1971. Vector fecundity reduced by western X disease. Journal of Invertebrate Pathology 17:389-394.

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. (2017). lmerTest package: tests in linear mixed effects models. *Journal of statistical software*, *82*, 1-26.

Lenz, R. R., and W. H. Dai. 2017. Mapping X-disease phytoplasma resistance in Prunus virginiana. Frontiers in Plant Science 8.

Marshall, A. T., T. D. Melton, G. Bishop, A. E. Clarke, C. A. Reyes-Corral, K. A. Catron, L. B. Nottingham, and T. D. Northfield. 2023. Cultural control methods improve management of leafhopper vector of X-disease. Crop Protection. 106445. Cultural control methods improve management of leafhopper vector of X-disease - ScienceDirect

Nielson, M. W. 1968. Biology of the geminate leafopper, Colladonas geminatus, in Oregon. Annals of the Entomologial Society of America 61:598-610.

Purcell, A. H., J. K. Uyemoto, R. A. Van Steenwyk, W. R. Scheader, K. G. Suslow, and B. C. Kirkpatrick. 1987. Buckskin disease of cherry. California Agriculture 41:26-27.

R Core Team. (2022). R: the R project for statistical computing. (v. 4.2.2). https://www.r-project.org/

Rawlins, T. E., and W. T. Home. 1931. 'Buckskin', a destructive graft-infectious disease of cherry. Phytopathology 21:331-335.

Rawlins, T. E., and H. E. Thomas. 1941. The buckskin disease of cherry and other stone fruits. Phytopathology 31:916-925.

Suslow, K. G., and A. H. Purcell. 1982. Seasonal transmission of X-disease agent from cherry by leafhopper *Colladonus* montanus. Plant Disease 66:28-30.

Van Steenwyk, R. A., B. C. Kirkpatrick, A. H. Purcell, C. E. Fouche, J. A. Grant, and J. K. Uyemoto. 1995. Evaluation of an Abatement Program for Western X-Disease in Sweet Cherry. Plant Disease 79:1025-1028.

Wolfe, H. R., E. W. Anthon, G. H. Kaloostian, and L. S. Jones. 1951. Leafhopper transmission of western X-disease. Journal of Economic Entomology 44:616-619.

Wright, A. A., Shires, M., Molnar, C., Bishop, G., Johnson, A., Frias, C., & Harper, S. J. (2022). Titer and distribution of 'Candidatus Phytoplasma pruni'in Prunus avium. Phytopathology®, 112(7), 1406-1412.

Proposal Title: Early Detection Field Kit for X-disease in Cherries

Report Type: New Proposal

Primary PI: Marco Pitino

Organization: USDA-ARS TTFVRU
Telephone: 509-454-4463
Email: marco.pitino@usda.gov
Address: 5230 Konnowac Pass Rd
City/State/Zip: Wapato, WA, 98951

Co-PI 2: Adrian Marshall
Organization: USDA-ARS TTFVRU
Telephone: 509-454-4476
Email: adrian.marshall@usda.gov
Address: 5230 Konnowac Pass Rd
City/State/Zip: Wapato, WA, 98951

CO-PI 3: Scott Harper
Organization: WSU IAREC
Telephone: 509-786-9230
Email: scott.harper@wsu.edu
Address: 24106 N Bunn Rd
City/State/Zip: Prosser, WA 99350

Cooperators: Rodney Cooper, Garrett Bishop, Laine Hackenberg

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $ 40,000
Total Project Request for Year 2 Funding: $ 40,000
Total Project Request for Year 3 Funding: $ 20,000

Other related/associated funding sources: (2025) Aphis awarded - $30,000, but unable to purchase reagent for purchase limitation issues. USDA ARS core funds were used to buy a new fluorescent microscope for effector localization - $30,000, expected delivery November 2026.

Organization Name: USDA ARS
Contract Administrator: Mara Guttman
Telephone: 509 454 6573
Contract administrator email address: Mara.Guttman@usda.gov
Station Manager/Supervisor: W. Rodney Cooper
Station manager/supervisor email address: Rodney.Cooper@ars.usda.gov

Item	(Type year of project start date here)	(Type year start date of year 2 here if relevant)	(Type year start date of year 3 here if relevant)
Salaries	$20,000.00	$20,000.00	$40,000.00
Benefits			
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies	$10,000.00	$10,000.00	$10,000.00
Travel			
Plot Fees			
Miscellaneous			
Total	$30,000.00	$30,000.00	$50,000.00

Objectives

The goal is a sensitive, field-deployable test for early detection of 'Candidatus Phytoplasma pruni', the cause of X-disease in sweet cherry. The original objectives are unchanged:

- Characterize four highly expressed phytoplasma effectors from infected cherry for antibody generation.
- Generate and optimize polyclonal antibodies against those effectors.
- Develop a lateral flow assay (LFA) for effector detection in insect vectors and plant tissue, using Western blot to validate antibodies first.
- Validate the assay on Washington orchard samples for sensitivity, specificity, and practicality against qPCR.
- After the antibody assay is working, explore nanobodies (VHH Variable Domain of Heavy-Chain-Only Antibody) against the same targets to improve stability and, later, phloem delivery.

Deviation from the work plan, not from the objectives: Objective 1 is complete through candidate selection and preliminary localization. Objective 2 has started: polyclonal antibodies are now in hand for all four shortlisted effectors (MCQ9618454, MCQ9618702, MCQ9618819, and MCQ9618875). Western blot optimization, the lateral-flow build, and orchard validation (Objectives 3 and 4) have not started. Objective 5 was pulled forward. Nanobody production has been initiated for three effectors rather than held until the polyclonal assay is finished: MCQ9618454 because it is the most abundant transcript, MCQ9618819 because it localizes to the nucleus, and MCQ9618702 because it is also highly abundant. MCQ9618875 remains on the antibody path only. No objective was dropped.

Significant Findings

- **Genome screen complete.** Twenty-two secreted effector candidates were retained from ‘Ca. Phytoplasma pruni’ after non-secreted membrane proteins were removed.
- **Four targets shortlisted by transcript abundance.** RT-qPCR of 17 candidates in infected cherry fruit stems advanced MCQ9618454, MCQ9618702, MCQ9618819, and MCQ9618875.
- **Lead candidate is far above the genomic marker.** MCQ9618454 mean Ct was 19.30, versus 24.89 for the phytoplasma genomic marker (about 48-fold at 100% efficiency) and more than 100-fold above the plant control GTB1.
- **Expression constructs are in plants.** GFP fusions of the shortlisted effectors were cloned into a binary plasmid and transiently expressed in *Nicotiana benthamiana*.
- **Preliminary localization.** MCQ9618819 is nuclear (DAPI overlap). MCQ9618454 and MCQ9618702 accumulate in cytosolic compartments and vesicles. MCQ9618875 imaging is not finished.
- **Polyclonal antibodies are in hand for all four candidates.** Sera were raised against MCQ9618454, MCQ9618702, MCQ9618819, and MCQ9618875. Western blot confirmation and lateral-flow formatting have not been run yet.
- **Nanobody production has started for three effectors.** MCQ9618454 (highest abundance), MCQ9618819 (nuclear localization), and MCQ9618702 (also abundant). MCQ9618875 was not entered into the nanobody workflow.

Methods

Effector selection

Predicted proteins from the Phytoplasma pruni whole-genome assembly were filtered for secretion. Proteins scored as “non-secreted membrane proteins” were removed, leaving 22 candidates. Seventeen were then quantified by RT-qPCR in infected sweet cherry fruit stems (three stems), with a phytoplasma genomic marker and the host gene GTB1 as references. Fruit stems were used because that tissue is the recommended harvest-timed sample for X-disease testing and is where titer, when present, is most informative. Candidates were ranked by mean Ct. Lower Ct was treated as higher transcript abundance. Fold difference versus the genomic marker was estimated as 2 raised to the Ct difference, assuming 100% amplification efficiency. No standard curve was run, so this ratio is a ranking tool, not an absolute copy-number assay.

Transient expression and localization

Coding sequences were fused to GFP and placed in a binary plasmid. Agrobacterium-mediated transient expression in *N. benthamiana* was used both as a protein biofactory and as a localization screen. Infiltrated tissue was examined on the unit’s existing fluorescence microscope. Nuclear localization was checked by overlap with DAPI. A dedicated fluorescence microscope purchased with ARS core funds ($30,000) is expected in November 2026; localization will be repeated on that instrument for better imaging.

Antibody generation and nanobody start

Recombinant protein from the shortlisted effectors was used to raise polyclonal antibodies. Antibodies are now available for all four candidates: MCQ9618454, MCQ9618702, MCQ9618819, and MCQ9618875. The validation of those antibodies will be done with Western blot on *Nicotiana benthamiana* tissue expressing the candidate effectors and infected cherry tissue. Field samples from Washington orchards will be scored against qPCR on the same extracts.

Nanobody production was started in parallel for three candidates, MCQ9618454 was included because it is the most abundant transcript in infected fruit stems. MCQ9618819 was included because GFP-DAPI imaging places it in the nucleus, so a single-domain reagent against it is useful both as a diagnostic and as a later blocker of a possible host-transcription target. MCQ9618702 was included because it is also highly abundant.

Results and Discussion

X-disease makes sweet cherry fruit small, pale, and unmarketable, and the Pacific Northwest strain often does this without clear leaf symptoms, so visual scouting misses early infections. qPCR is the current standard, but the pathogen is restricted to sieve tubes and is patchy in the canopy. Wright et al. (2022) showed that titer tracks symptomatic limbs and that woody tissue, sampled lower in the tree, is more reliable than leaves before harvest. A negative canopy qPCR can therefore be a sampling miss. Secreted effectors are being tested as an alternative analyte because a signal peptide can put them into the phloem stream, where they may accumulate above the local genome copy number (Figure 1). Once Western blot optimization successfully confirms these target protein signals across complex tissue matrices, our priority for the subsequent grant cycle will transition toward converting these validated markers into field-deployable lateral flow assay (LFA) strips for rapid, point-of-need diagnostics.

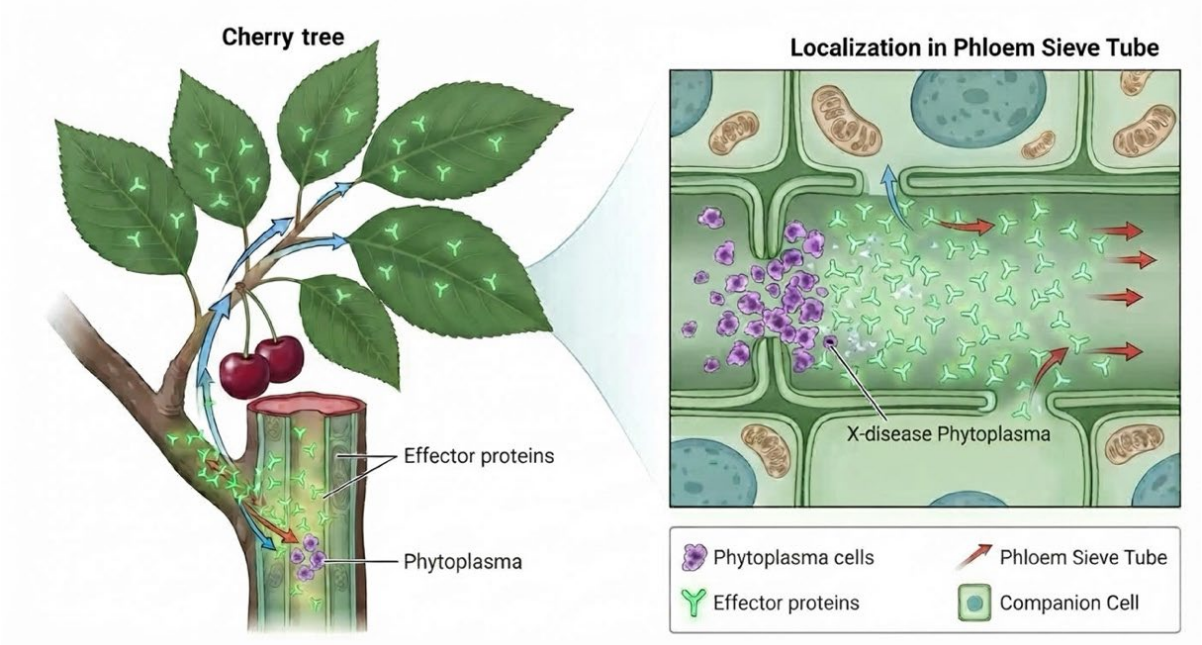


Figure 1. Working model. Phytoplasma cells stay in the sieve tubes. Secreted effectors move in the phloem and are the intended canopy target.

Screening four candidates. MCQ9618454 is the priority: mean Ct 19.30, against 24.89 for the genomic marker. The 5.59-cycle gap is about 48-fold to the bacteria. Figure 2 and table 1 show the full Ct heatmap.

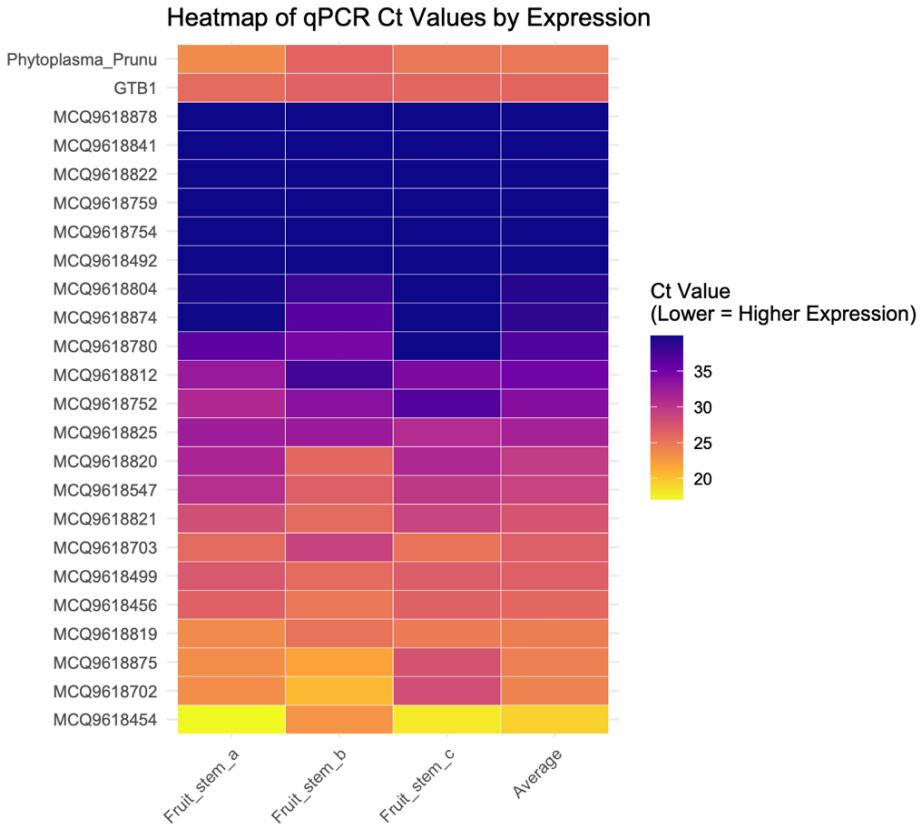


Figure 2. RT-qPCR Ct heatmap, infected fruit stems. Lower Ct is higher transcript abundance (yellow on the color scale). MCQ9618454 is the bottom row. Several accessions at the top were near the useful detection floor and were dropped.

Candidate	Expression result	Localization status
MCQ9618454	Highest; mean Ct 19.30 (~48× genomic marker)	Expressed. Cytosolic compartments and vesicles
MCQ9618702	High; second tier	Expressed. Restricted cytosolic compartments and vesicles.
MCQ9618875	High; second tier	Construct made. Imaging not finished.
MCQ9618819	High	Expressed. Nucleus, confirmed by DAPI overlap.
Genomic marker	Mean Ct 24.89	Cherry DNA Reference

Table 1. Shortlisted effectors and the genomic reference. Fold change is a Ct conversion, not a standard-curve copy number.

GFP fusions confirm that three of the four proteins can be produced in planta (Fig. 3). Nuclear localization of MCQ9618819 matches the pattern of phytoplasma effectors such as SAP11 that move to the host nucleus and alter development and defense signaling. That makes 8819 interesting for later work on how infection suppresses fruit development, but it does not change its status as a diagnostic antigen. Compartment and vesicle patterns for 8454 and 8702 still need a second imaging pass.

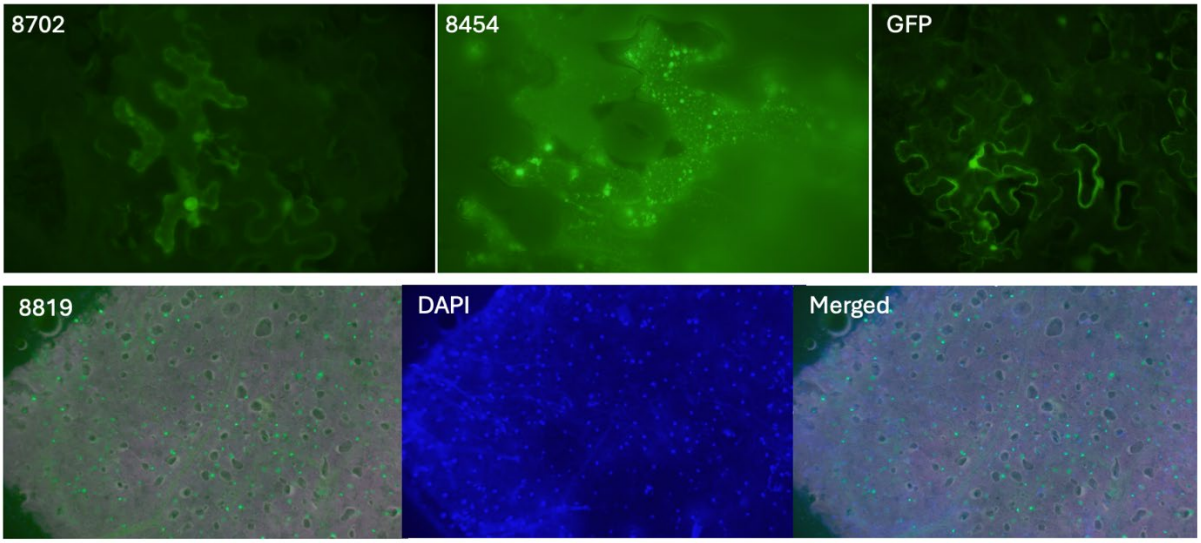


Figure 3. Transient localization of effector-GFP fusions in Nicotiana benthamiana. Images are from the current fluorescence microscope and are preliminary.**Panel legend.**

8702. MCQ9618702-GFP. Signal is restricted to cell-associated compartments and is not uniform across the cytoplasm.

8454. MCQ9618454-GFP. Bright puncta consistent with vesicles, over a weaker cytosolic background. No single organelle has been assigned.

GFP. Free GFP control. Signal follows the cell periphery of pavement cells, without the puncta seen for 8454.

8819. MCQ9618819-GFP. Discrete green foci in the size and position of nuclei.

DAPI. DNA stain of the same 8819 field, marking nuclei.

Merged. 8819-GFP over DAPI. Green foci sit on the blue nuclei, which is the basis for calling MCQ9618819 nuclear

Literature Review

Titer work on sweet cherry showed that the pathogen is not uniform. Severely symptomatic limbs carry more phytoplasma than limbs with mild or no fruit symptoms, and sampling lower in the tree improves detection before harvest. That distribution is the operational reason a DNA test still returns false negatives, and it is the gap an effector protein assay is meant to narrow.

Phytoplasma genomes encode small secreted proteins, several of which are delivered into host cells and act as effectors (Bai et al. 2009). SAP11 from aster yellows phytoplasma enters the plant nucleus and changes development and jasmonate signaling in a way that also favors the insect vector (Sugio et al. 2011). A related precedent comes from another phloem-limited bacterium. Sec-delivered effector 1 (SDE1) of '*Candidatus* Liberibacter asiaticus', the huanglongbing pathogen, was followed into the host, shown to bind the RNA helicase DDX3, and linked to leaf chlorosis (Zhou et al. 2020). They use a secreted effector as a readable sign of a pathogen that is scarce in the sieve tubes. Similarly we are using of the Phytoplasma pruni candidates, as abundant diagnostic antigens and, for a nuclear protein such as MCQ9618819, as a later target for blocking host manipulation. Conventional antibodies remain the first reagent for a lateral-flow test, and those sera now exist for all four candidates. Camelid single-domain nanobodies (VHH) are smaller and more stable, but discovery costs more. Production was still opened for three candidates where the case is strongest: MCQ9618454 and MCQ9618702 on transcript abundance, and MCQ9618819 on

nuclear localization. Whether any nanobody discriminates infected from healthy cherry is still an open result.

Literature Cited

Bai, X., Correa, V.R., Toruño, T.Y., Ammar, E.D., Kamoun, S., and Hogenhout, S.A. 2009. AY-WB phytoplasma secretes a protein that targets plant cell nuclei. Molecular Plant-Microbe Interactions 22:18–30.

Sugio, A., Kingdom, H.N., MacLean, A.M., Grieve, V.M., and Hogenhout, S.A. 2011. Phytoplasma protein effector SAP11 enhances insect vector reproduction by manipulating plant development and hormone signaling. Proceedings of the National Academy of Sciences 108:E1254–E1263.

Wright, A.A., Shires, M.K., Molnar, C., Bishop, G., Johnson, A., Frias, C., and Harper, S.J. 2022. Titer and distribution of ‘Candidatus Phytoplasma pruni’ in Prunus avium. Phytopathology 112:1406–1412.

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

Report Type: Final Project Report

Primary PI: **Dr. Youfu "Frank" Zhao**
Organization: WSU-IAREC Prosser
Telephone: 509-786-9284
Email: youfu.zhao@wsu.edu
Address: 24106 N. Bunn Rd.
Address 2:
City/State/Zip: Prosser, WA 99350

Co-PI 2: **Dr. Yinong Yang**
Organization: Penn State University
Telephone: 814-867-0324
Email: yuy3@psu.edu
Address: 405C Life Science Bldg
Address 2:
City/State/Zip: University Park, PA 16802

CO-PI 3: **Dr. Scott Harper**
Organization: WSU-IAREC Prosser
Telephone: 509-786-9230
Email: scott.harper@wsu.edu
Address: 24106 N. Bunn Rd.
Address 2:
City/State/Zip: Prosser, WA 99350

CO-PI 4: **Dr. Amit Kumar Kesharwani**
Organization: WSU-IAREC Prosser
Telephone: 509-786-9284
Email: a.kesharwani@wsu.edu
Address: 24106 N. Bunn Rd.
Address 2:
City/State/Zip: Prosser, WA 99350

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
Organization Name: WSU-IAREC Prosser
Contract Administrator: Jamie Meek
Telephone: (509)786-9231
Contract administrator email address: jamie.meek@wsu.edu; or prosser.grants@wsu.edu
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 cost.

Budget 2

Co PI 2: Dr. Yinong Yang
Organization Name: Penn State University
Contract Administrator: Haessly Nachman
Telephone: 814-865-5864
Contract administrator email address: him103@psu.edu
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
Contract Administrator: Jamie Meek
Telephone: (509)786-9231
Contract administrator email address: jamie.meek@wsu.edu; or prosser.grants@wsu.edu
Station Manager/Supervisor: Naidu Rayapati
Station manager/supervisor email address: naidu.rayapati@wsu.edu

Footnotes: Scott and Amit's budget is included in PI Zhao's budget 1. Amit resigned in May.

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:

- A one-pot RPA/Cas12a assay was developed for specific detection of XDP.
- Optimized a one-pot DETECTR assay using a Genie III device.
- Optimized a 30-minute one-pot visual detection (VDet) under UV/blue light.
- Designed a method using plant sap without DNA isolation for detection of XDP.
- Detection of various strains of XDP using one-pot and VDet assays.
- Finalized a protocol for a field-deployable one-pot RPA/Cas12a assay for XDP diagnosis.
- Demonstration of the one-pot and VDet assays to growers and consultants.

Methods

Procedures and Methodology. Previously, a two-pot RPA/Cas12a method was developed for specific, sensitive, and rapid detection of cherry X-disease phytoplasma (XDP) (**Tables 1-3**). The first step of the method is RPA reaction (**Table 1**); followed by the second step: cas12a detection reaction (**Tables 2-3**) and fluorescens signal reading using fluorescence microplate reader. In this project, we developed and optimized a one-pot assay using a less expensive portable Genie III platform (OptiGene) and/or visual detection (VDet) (**Table 4**).

Table 1 RPA reaction

Pre-Master Mix:	1x rxn	5x rxn
2x reaction buffer: dNTPs (10 mM): Primer A (10 uM) Primer B (10 uM): 10x Basic E-mix:	10 ul (1x) 3 ul (1.5 mM) 1 ul (0.5 uM) 1 ul (0.5 uM) 2 ul (1x)	50 ul 15 ul 5 ul 5 ul 10 ul
Total	17 ul	85 ul

Table 2 Pre-detection reaction

Pre-Det Mix	10x rxn	Final conc
ddH2O: NEBuffer r2.1 (10x) Lba Cas12a (10 uM) crRNA (10 uM) FAM-reporter (10 uM)	14.75 ul 2 ul 1 ul 1.25 ul 1 ul	N/A 1x 500 nM 625 nM 500 nM
Total	20 ul	

Table 3 Cas12a Detection reaction

Detection Rxn Mix	10x rxn	Final Conc
NEBuffer r2.1 (10x) Pre-Det Mix (Cas12a; crRNA; FAM reporter) ddH2O	20 ul 20 ul 140 ul	1x 50/62.5/50 nM N/A
Total	180 ul	

1. One-pot RPA/Cas12a DETECTR assay using Genie III device

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 (**Table 4**), 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**).

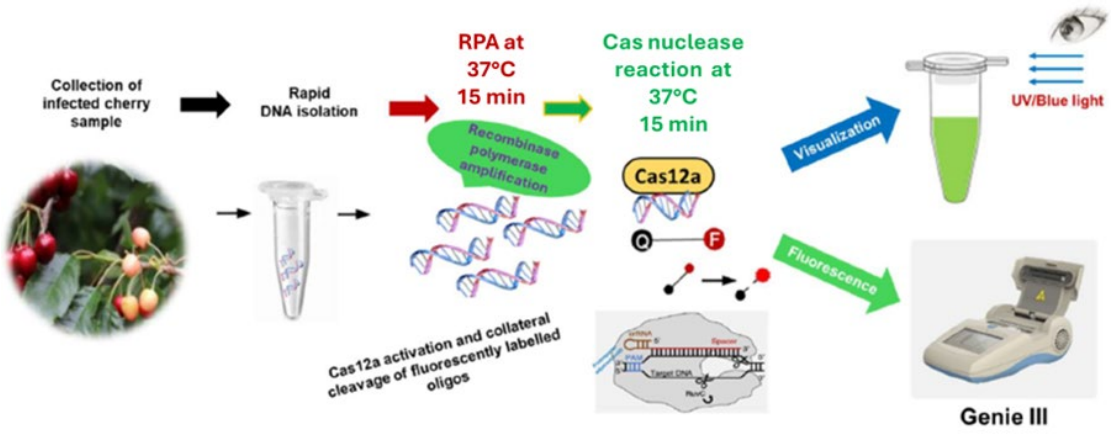


Fig 1. Illustration of one-pot DETECTR and visual detection (VDet) assay. The assay combines rapid DNA extraction, XDP amplification using RPA, followed by Cas12a detection using Genie III device or visual detection with UV/blue light (362-595 nm).

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**).

Table 4. One-pot DETECTR/visual detection reaction

Ingredients	Reaction (1x)	Reaction (10x)
Rehydration buffer	11.80 µl	118.0 µl
Primer A (360 nM)	0.72 µl	7.2 µl
Primer B (360 nM)	0.72 µl	7.2 µl
CrRNA (250 nM)	0.50 µl	5.00 µl
5-mer FAM reporter (200 nM)	0.40 µl	4.0 µl
NEBuffer 2.1	1.60 µl	16.0 µl
MgOAc (4.725 mM)	0.42 µl	4.2 µl
LbCas12a (250 nM)	0.50 µl	5.0 µl
Nuclease free water	1.34 µl	13.4 µl
Template	2.00 µl	20 µl
Total	20.00 µl	200.00 µl

Stock: Primers:10 µM; FAM reporter:10 µM; Magnesium Acetate (MgOAc):280 mM; LbCas12a:10 µM.

3. Using plant sap for one-pot RPA/Cas12a DETECTR assay without DNA isolation

A method was used to obtain cherry sap directly 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**).

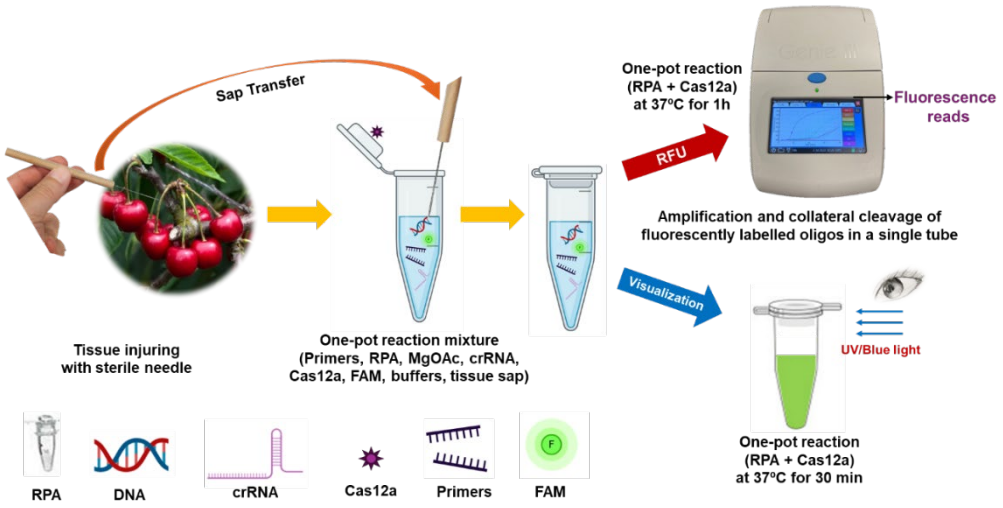


Fig. 2 Illustration of using sap for one-pot DETECTR and visual detection (VDet) assays. The assay used plant sap without DNA isolation and Genie III device or VDet using UV/blue light.

Types and timing of anticipated results. We optimized the one-pot RPA/Cas12a method with Genie III detection or VDet combined with either rapid DNA extract method or sap without DNA isolation or tissue destruction for detection of XDP in a field-deployable way. We finalized our protocols for the field-deployable one-pot RPA/Cas12a-based assay.

Potential problems or limitations. The potential problem for the two-pot RPAS/Cas12a method is false negative when bacterial titer is low in a sample (with CT value >35), especially for young nursery trees and the risk of cross contamination, which may lead to false positive results. By using a one-pot assay, we largely eliminate the risk of cross-contamination but still have similar sensitivities. However, the sensitivity for visual detection might not be as high as using one-pot DETECTR by Genie III device. Another advantage for using sap in one-pot assay is to eliminate DNA isolation, thus it is suitable for field deployable diagnosis. However, the method still requires professional handling of all the reagents (**Table 4**). Therefore, in the future, it will be ideal to develop an end-user diagnostic tool/kit that contains most of the reagents except the enzyme and sample DNA or sap, which should be easy enough for everyone to perform the assay.

Results and Discussion:

1. **Sensitivity of one-pot/two-pot DETECTR assays**

The sensitivity of the one-pot DETECTR assay was evaluated with estimated copy number of pure DNA dilutions of XDP. 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**).

When we compared the one-pot assay with two-pot DETECTR assay, similar levels of sensitivity were obtained at one pM (picomolar) DNA, except the RFU value at this level of DNA was higher for two-pot assay than that of one-pot assay due to detection thresholds of the instruments used (fluorescence microplate reader for two-pot assay versus Genie III device for one-pot assay)(**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, but fluorescence signal is

relatively low. Therefore, interpretation of the low titer DNA result should be cautious, otherwise it will lead to either false positive or false negative results.

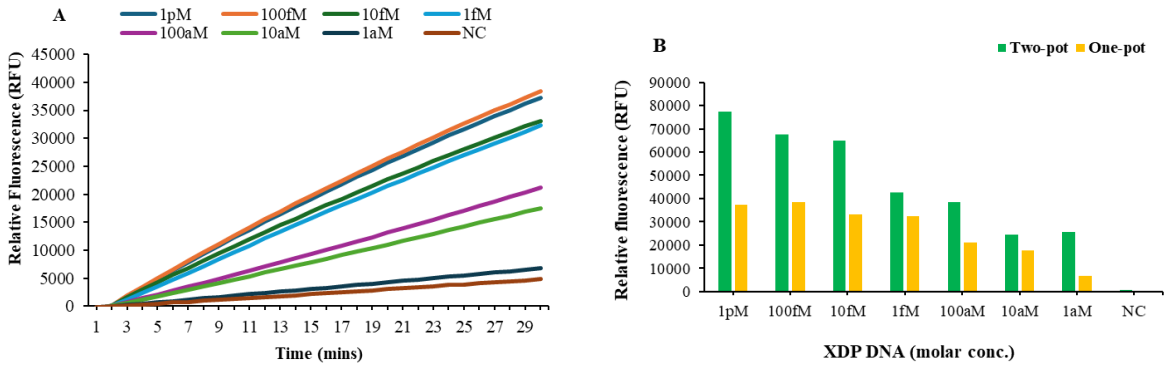


Fig. 3 Sensitivity of one-pot DETECTR assay. Dilutions of quantified DNA of the XDP *secY* gene were used for testing sensitivity of the assay. **A)** Time course of one-pot DETECTR using Genie III device. **B)** Comparing one-pot to two-pot DETECTR assay using same dilutions of XDP DNA. One-pot assay 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)

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**). However, interpretation of the low titer DNA (1aM) result may be difficult in practice due to that visual detection is inherently subjective. Experience may help in interpretation of the results when you compare signals at 1aM versus control (**Fig. 4**). It should also 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 fluorescence microplate reader or Genie III device. Therefore, the sensitivity of VDet might be slightly lower than that of one-pot using Genie III device.

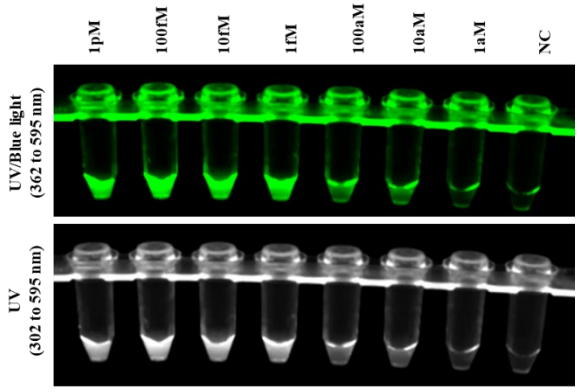


Fig. 4 Sensitivity of visual detection of XDP using one-pot DETECTR assay. Dilutions of quantified DNA of XDP *secY* gene were used for testing sensitivity of the assay. The assay could detect one attomolar level (10^{-18} or one copy DNA) within 30 minutes. The gel-imager instrument was used for visualization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

3. One-pot, two-pot and visual detection (VDet) assays using DNA samples

To determine the effectiveness of the detection assays, we isolated DNA samples from six one-year-old nursery sweet cherry trees grown in the greenhouse and a total of seven samples per tree with each sample about 10 cm apart were collected. These DNA samples were subject to detection of XDP by one-pot, two-pot, and visual detection assays. As shown in **Table 5** and **Fig. 5**, all six trees were tested positive for XDP. However, there are discrepancies among the different assays and not all samples were tested positive with one, two-pot RPA/Cas12a based detection method and visual detection. These results suggested that due to uneven distribution of XDP and low-titer samples of young trees, multiple samples from each tree are needed for accurate diagnosis of XDP infection.

Table 5. Detection of XDP by one-pot and two-pot assays using DNA

Assay	Tree 1		Tree 2		Tree 3		Tree 4		Tree 5		Tree 6	
Sample	**Two**	**One**	**Two**	**One**	**Two**	**One**	**Two**	**One**	**Two**	**One**	**Two**	**One**
A	-	-	-	-	+	+	+	+	-	-	+	+
B	+	+	+	+	+	+	-	-	+	+	+	+
C	-	-	+	+	-	-	+	+	+	+	-	-
D	-	-	+	+	+	+	+	+	-	-	-	-
E	+	-	-	-	-	-	+	+	-	-	+	+
F	+	+	+	-	+	+	-	-	+	+	-	+
R	-	-	-	+	-	-	-	-	-	-	-	+

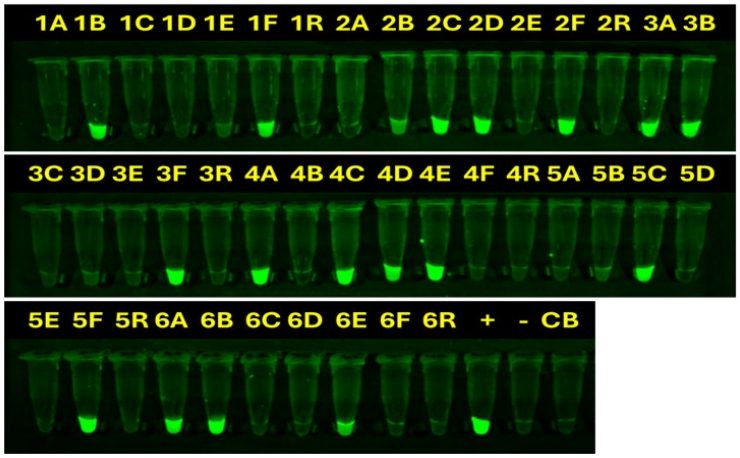


Fig. 5 Visual detection of XDP of tree samples using isolated DNA from six trees, each with seven samples (A to R). +, positive control; -, negative control.

4. Detection of various X-disease phytoplasma strains using one-pot/VDet.

Instead of isolating DNAs from cherry samples, we detected XDP directly using cherry plant saps without DNA isolation. One-pot DETECTR and visual detection assays were used to detect the various strains of XDP (X1-3; EX, WX, CC2) as well as other subgroups (CFB and POIBI) using collected sap from infected plants. Results showed that one-pot VDet assay detected all tested XDP strains and closely related phytoplasmas, demonstrating broad applicability across different 16SrIII subgroups (**Fig. 6**).

5. One-pot, two-pot, and VDet assays using plant saps

Three cherry trees (two-years-old) and one apple tree from an undisclosed commercial source were tested for XDP infection. Saps from petioles of five different spots per cherry tree were obtained 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. Our results showed all four trees were tested positive for XDP and the titers of XDP were relatively high in these trees as shown in the visual detection assay (**Table 6, Fig. 7**). Therefore, the results were much more uniform among the three assays. These results suggested that, when the titer is relatively high, one pot and two-pot assay as well as VDet assay could achieve similar accurate diagnosis of XDP infection. Our findings also indicate that using sap directly for XDP diagnosis without DNA isolation is feasible, which can reduce the risk of cross-contamination and save-time. This also makes the assay field-deployable one step-further.

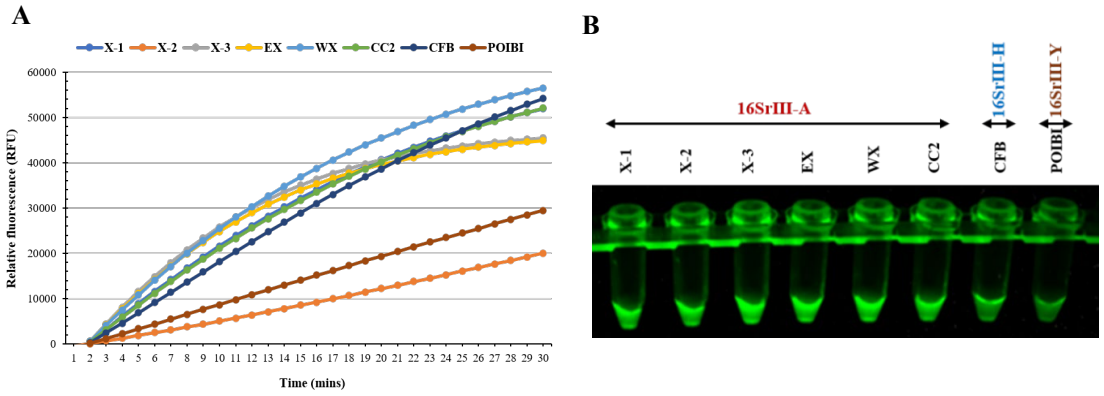


Fig. 6 One-pot and visual detection (VDet) of XDP strains from 16SrIII subgroup A, Y and H infected plants. A) Sap samples were used to detect various strains of XDP and close related subgroups using Genie III device. X-disease phytoplasma (XDP): 16SrIII subgroup A – X-1, X-2, X-3, Eastern X, and Western X:, CC2: *Ca.* phytoplasma pruni; subgroup Y – CFB: *Ca.* phytoplasma pruni; subgroup H – POIBI (Poinsettia branch-inducing phytoplasma JR1); B) Visual detection of XDP strains using UV/blue light (362-595 nm).

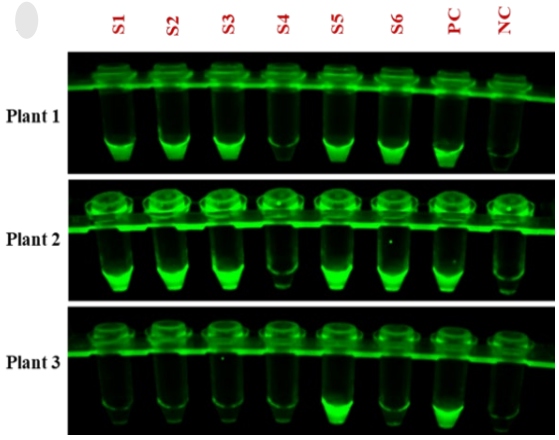


Fig. 7. One pot VDet detection of XDP using plant saps. Signal visualized using UV/blue light (362-595 nm). PC: positive control; NC: negative control. S1-S5 - sap samples from the same tree (Plants 1 to 3); S6- apple samples.

Table 6. Detection of XDP using one-pot and two-pot assays using tree sap

Assay	Plant 1		Plant 2		Plant 3	
Sample	**Two**	**One**	**Two**	**One**	**Two**	**One**
S1	-	+	+	+	-	-
S2	+	+	+	+	-	-
S3	+	+	+	+	-	-
S4	-	-	-	-	-	-
S5	+	+	+	+	+	+
S6 (Apple)	+	+	+	+	-	-

6. Protocol for one-pot and VDet assays

Based on our results, we finalized our protocol for the assays and summarized steps below.

1. Prepared the master mix of eight reactions by adding all the ingredients (**Table 4**) in a 1.5ml sterile Eppendorf tube except template and LbCas12a.
2. Add Twist Amp RPA reaction powder (8x) and mix the suspension with vortex.
3. Aliquot 17.50 µl of reaction mix into PCR tubes and add template DNA (2 ul).
4. Add LbCas12a (0.5 ul) on the cap of the PCR tube.
5. Incubate at 108°F (37°C) for 15 minutes in Genie III device.
6. Vortex and incubate for 15 minutes and then read the relative fluorescence for 30 minutes.

Genie III protocol settings:

1. Turn on the machine, select Lamp and Anneal option
2. Set preheat at 108°F (37°C) for 30 minutes.
3. Set amplification at 108°F (37°C) for 30 minutes.
4. Check the Relative fluorescence graph.
5. Export the results by selecting the export file.

UV/Blue Light: Visualize the one-pot reaction using UV torch by naked eyes or with Gel Doc machine at 362 to 595 nM.

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. The RPA/Cas12a-based one-pot and two-pot assays as well as VDet methods we have developed can detect small amount of DNA in a sample and is not as susceptible to producing false negatives due to inhibition of its chemistry by compounds present in cherry. This also allowed us to use sap directly without DNA isolation in the assay, which has its advantage by not destructively sampling trees and can be performed in orchard environment. In addition, this method also allows users to skip DNA isolation process, saving time and money and allowing-in field diagnosis of XDP infection. However, the method in its current form still requires professional handling of all the reagents. Therefore, in the future, it will be ideal to develop an end-user diagnostic tool/kit that contains most of the reagents except the enzyme and sample DNA or sap, which then should be easy enough for everyone to perform the assay.

Literature Review:

Since 2015, the X-disease phytoplasma (*Candidatus* Phytoplasma pruni) (XDP) became the predominant pathogen in Washington, increased from less than 10% in 2017 to 36% in 2020 (Clarke et al. 2024; Davis et al. 2013; Harper et al. 2020; Wright et al. 2021, 2022). The outbreak has caused severe damage to the cherry and stone fruit industry in Washington and in northern Oregon. Between 2015 and 2020, more than 238,856 cherry tress (974 acres) and 33082 stone fruits trees (>81 acres) were removed due to X-phytoplasma disease, a total of $95 million lost revenue for the industry (Harper et al. 2020). Estimated lost revenue and re-establishment costs were about $115 million over the seven-year period (Molnar et al. 2022). The emergence of new phytoplasma genotypes, increased host range, and abundant vector species makes management of XDP even challenging (Clark et al. 2024; Molnar et al. 2024; Wright et al. 2022). Besides cherry or stone fruits, surveys found that 52 out of 77 plant species were tested positive for XDP (https://treefruit.wsu.edu/ article/alternative-weedy-hosts-for-x-disease-phytoplasma/). In addition, young active growing trees may easily get reinfected if the pathogen or vector presents nearby. These findings suggest that early detection of XDP infections using fast and easy methods is critical to the cherry industry as well as prevention of the disease, thus reducing the economic losses to growers and the cherry industry.

The clustered regularly interspaced short palindromic repeats (CRISPR)-Cas12a system is an emerging biosensor technology where CRISPR-associated proteins (Cas protein) utilize the distinct guide RNA (gRNA) to target a specific site of the nucleic acid (DNA). Upon initial cleavage of specific target DNA, activated Cas12a gains the ability to trans-cleave the fluorescence oligo reporter, which is detectable by a fluorescence microplate reader (Chen et al., 2018; Kaminski et al. 2021; Mao et al. 2022). One of the great advantages of the Cas12a-based detection is its high specificity at the single nucleotide level and super-sensitivity at the attomolar level or even lower concentration (Kaminski et al. 2021). Typically, Cas12a-based detection is coupled with target DNA amplification by using isothermal methods such as recombinase polymerase amplification (RPA) or loop-mediated isothermal amplification (LAMP) for improved specificity and sensitivity. A number of CRISPR/Cas-based detection strategies such as DETECTR (DNA endonuclease-targeted CRISPR trans reporter), have been demonstrated as point of care diagnostic tools for ultrasensitive and specific detection of pathogen nucleic acids in biomedical samples (Chen et al. 2018; Gootenberg et al. 2017; Jiao et al. 2021; Li et al. 2019). The use of RPA/Cas12a DETECTR assay for supersensitive and specific detection of low titer bacterial pathogens such as *Candidatus* Liberibacter asiaticus (Wheatley et al. 2021) and phytoplasmas (Wheatley et al. 2022) has also been previously reported. We also successfully developed a two-pot RPA/Cas12a assay, which has been used in the laboratory setting for detection of XDP in cherry samples, weeds, and insect vectors with a 384-well fluorescence microplate reader. However, our two-pot RPA/Cas12a assay system for XDP detection requires an expensive instrument (microplate reader, > $25,000) and is not field-deployable.

The isothermal RPA/Cas12a-based one-pot assay has been used for the detection of SARS-Cov-2 and plant RNA viruses (Aman et al. 2020; Ding et al. 2020; Malci et al. 2022; Sun et al. 2021). Cas12a has also been combined with loop-mediated isothermal amplification (LAMP) for DNA detection (Wang et al. 2019). However, this LAMP/Cas12a method requires at least 2 hours to complete, and its sensitivity needed to be optimized. More recently, advances have been made in developing a CRISPR/Cas12a-based platform to visually detect nucleic acids and pathogens (Kanitchinda et al. 2022; Qin et al. 2022; Wang et al. 2019). As compared to LAMP/Cas12a, RPA/Cas12a visual detection method requires only 30 min to complete. In our current study, our goal is to make the one-pot RPA/Cas12a assay field-deployable and practically useful to diagnosticians, crop consultants and even growers. Therefore, we optimized a one-pot RPA/Cas12a method with Genie III detection or VDet combined with either rapid DNA extract method or sap without DNA isolation for detection of XDP in a field-deployable way. We also finalized our protocol for the field-deployable one-pot RPA/Cas12a-based assay.

References:

1. Aman, R., Mahas, A., Marsic, T., Hassan, N., Mahfouz, M.M. 2020. Efficient, rapid, and sensitive detection of plant RNA viruses with one-pot RT-RPA-CRISPR/Cas12a assay. Front. Microbiol.11:610872.
2. Chen, J.S., Ma, E., Harrington, L.B., Da Costa, M., Tian, X., Palefsky, J.M. et al. 2018. CRISPR Cas12a target binding unleashes indiscriminate single-stranded DNase activity. Science, 360:436-4359.
3. Clarke, A. E., Catron, K. A., Corral, C. R., Marshall, A. T., Adams, C. G., Cooper, W. R., Harper, S. J., Nottingham, L. B., Northfield, T. D. 2024. *Colladonus* spp. (Hemiptera: Cicadellidae) vectors of X-disease: biology and management in Western United States. J Integ Pest Manag. 15:1–10.
4. Davis, R. E, Zhao, Y., Dally, E. L., Lee, I. M., Jomantiene, R., Douglas, S. M. 2013. *Candidatus* Phytoplasma pruni', a novel taxon associated with X-disease of stone fruits, Prunus spp.: multilocus characterization based on 16S rRNA, secY, and ribosomal protein genes. Int J Sys.Evol.Microbiol., 63:766–776.
5. Ding, X., Yin, K., Li, Z., et al. 2020. Ultrasensitive and visual detection of SARS-CoV-2 using all-in-one dual CRISPR-Cas1 2a assay. Nat. Comm. 11:4711.
6. Gootenberg, J. S., Abudayyeh, O. O., Lee, J. W., Essletzbichler, P., Dy, A. J., Joung, J., et al. 2017. Nucleic acid detection with CRISPR-Cas13a/C2c2. Science 356:438-442.
7. Harper, S., Northfield, T., Nottingham, L., DuPont, T. 2020. X-disease phytoplasma (Western X). http://treefruit.wsu.edu/crop-protection/disease-management/western-x/.
8. Jiao, J., Kong, K., Han, J., Song, S., Bai, T., Song, C., Wang, M., Yan, Z., Zhang, H., Zhang, R., Feng, J., Zheng, X. 2021. Field detection of multiple RNA viruses/viroids in apple using a CRISPR/Cas12a-based visual assay. Plant Biotechnol J. 19:394-405.
9. Kaminski, M. M., Abudayyeh, O. O., Gootenberg, J. S., Zhang, F., Collins, J. J. 2021. CRISPR-based diagnostics. Nat Biomed. Eng. 5: 643-656.
10. Kanitchinda, S., Srisala, J., Suebsing, R., Prachumwat, A., Chaijarasphong, T. 2020. CRISPR-Cas fluorescent cleavage assay coupled with recombinase polymerase amplification for sensitive and specific detection of *Enterocytozoon hepatopenaei*. Biotech. Rep. 27: e00485.
11. Li, L., Li, S., Wu, N., Wu, J., Wang, G., Zhao, G., and Wang, J. 2019. HOLMESv2: A CRISPR-Cas12b-assisted platform for nucleic acid detection and DNA methylation quantitation. ACS Synth. Biol. 8:2228-2237.
12. Malci, K., Walls, L. E., Rios-Solis, L. 2022. Rational design of CRISPR/Cas12a-RPA based one-pot COVID-19 detection with design of experiments. ACS Syn. Biol. 11:1555-1567.
13. Mao, Z., Chen, R., Wang, X., Zhou, Z., Peng, Y., Li., Han, D., Li, S., Wang, Y., Han, T., Liang, T., Ren, S., Gao, Z. 2022. CRISPR/Cas12a-based technology: A powerful tool for biosensing in food safety. Trends Food Sci. Tech. 122:211-222.
14. Molnar, C., DuPont, S.T., Thompson, A. 2022. Estimated impact of X-disease and little cherry disease in Washington and Oregon from 2015 to 2020. J. Ext. 60:17.
15. Molnar C, Shires MK, Wright AA, Hoskins MC, Cowell SJ, Nikolaeva EV, Knier R, Harper SJ. 2024. Putting 'X' into context: the diversity of '*Candidatus* Phytoplasma pruni' strains associated with the induction of X-disease. Plant Dis. 108: 2677-2687.
16. Qin, C., Liu, J., Zhu, W., Zeng, M., Xu, K., Ding, J., Zhou, H., Zhu, J., Ke, Y., Li, L.Y., Sheng, G., Li, Z., Luo, H., Jiang, S., Chen, K., Ding, X., Meng, H. 2022. One-pot visual detection of African swine fever virus using CRISPR-Cas12a. Front. Vet. Sci., 9:962438
17. Sun, Y., Yu, L., Liu, C. et al. 2021. One-tube SARS-CoV-2 detection platform based on RT-RPA and CRISPR/Cas12a. J. Transl. Med. 19: 74.
18. Wang, B., Wang, R., Wang, D., Wu, J., Li, J., Wang, J., Liu, H., Wang, Y. 2019. Cas12aVDet: A CRISPR/Cas12a-Based Platform for Rapid and Visual Nucleic Acid Detection. *Anal. Chem.* 91:12156-12161.

19. Wheatley, M. S., Duan, Y.-P., Yang, Y. 2021. Highly sensitive and rapid detection of citrus Huanglongbing pathogen (*Candidatus* Liberibacter asiaticus) using Cas12a-based methods. Phytopathology 111:2375–2382.
20. Wheatley, M. S., Qin Wang, Q., Wei, W., Bottner-Parker, K., Zhao, Y., Yang, Y. 2022. Cas12a-based diagnostics for potato purple top disease complex associated with infection by '*Candidatus* Phytoplasma trifolii'-related strains. Plant Disease 106: 2039-2045.
21. Wheatley, M. S., and Yang, Y. 2021 Versatile applications of the CRISPR/Cas toolkit in plant pathology and disease management. Phytopathology 111:1080-1090.
22. Wright, A. A., Shires, M., Beaver, C., Bishop, G. M., DuPont, S. T., Naranjo, R., Harper, S. J. 2021. The effect of *Candidatus* Phytoplasma pruni infection on sweet cherry fruit. Phytopathology 111:2195-2202.
23. Wright AA, Shires M, Beaver C, Bishop GM, DuPont ST, Naranjo R, Harper SJ 2022. Titer and distribution of 'Candidatus Phytoplasma pruni' in Prunus avium. Phytopathology 112: 1406-1412.

Executive Summary

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

Key words: X-disease Phytoplasma, Early Detection, Field Diagnosis, Management

Abstract: The cherry X-disease is at epidemic levels in the Pacific Northwest and has caused significant economic losses. 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. A two-pot recombinase polymerase amplification (RPA)/Cas12a diagnostic assay for detection of XDP has been previously established, which is capable of specific, sensitive and rapid detection of XDP-infected samples from the field. However, the assay requires an expensive fluorescence microplate reader and has a high risk of cross-contamination. In this study, we developed and optimized a one-pot RPA/Cas12a assay using a less-expensive portable Genie III platform and a 30-minute one-pot visual detection (VDet) assay under UV/blue light. This method also allows us to apply plant sap directly without DNA isolation for detection of XDP, thus permitting in field diagnosis of XDP infection. Finally, we finalized a protocol for a field-deployable one-pot RPA/Cas12a assay for XDP diagnosis. In summary, the RPA/Cas12a assays for XDP detection not only provide the industry with a much-needed platform for fast, supersensitive, and accurate diagnosis, but also save time and money for both growers and nurseries. However, the method in its current form still requires professional handling of all the reagents. Future studies should focus on developing an end-user diagnostic tool/kit that contains most of the reagents except the enzyme and sample DNA or sap, which should be easy for everyone to perform.

Project Title: Precision Agriculture: Innovating Cherry Disease Management with DPI

Report Type: Continuing Project Report - No-cost extension

PI: Marco Pitino
Organization: USDA-ARS, Wapato, WA
Telephone: 509/454-4463
Email: marco.pitino@usda.gov

PI: W. Rodney Cooper
Organization: USDA-ARS, Wapato, WA
Telephone: 509/454-4463
Email: Rodney.Cooper@ars.usda.gov

PI: Adrian Marshall
Organization: USDA-ARS, Wapato, WA
Telephone: 509-454-6570
Email: adrian.marshall@usda.gov

Cooperators: Michelle Heck USDA-ARS, Ithaca, NY; Scott Harper; Tobin Northfield

Additioanl funds: PPA 772, 1 year 2026 (*in vitro* screening), budget requested for personel and molecules $77,000

Project Duration: 3 Year

Total Project Request: **Year 1**: $35,000 **Year 2**: $35,000 **Year 3**: $35,000 Year 4: 0

2024	2025	2026	2027
$35,000.00	$35,000.00	$35,000.00	
$35,000.00	$35,000.00	$35,000.00	$0.00

Objectives

1. Investigate the mobility of therapeutic molecules and marker signals delivered through direct plant infusion.
2. Evaluate the effectiveness of therapeutic molecules in reducing phytoplasma titer and improving plant health.
3. Assess the impact of therapeutic molecules on the fitness of *Colladonus reductus* (the insect vector).
4. Initiate field evaluations using commercially available trunk injection systems.

Significant Findings

During the 2026 season, this project advanced rapidly from preliminary laboratory and greenhouse screening into full-scale field deployments within commercial sweet cherry and peach orchards. Rather than spending years reinventing injection hardware and delivery chemistry from scratch, we leveraged operational principles, formulation strategies, and injection tooling originally refined in citrus greening programs. This cross-crop knowledge transfer allowed us to fast-track the delivery pipeline directly into Pacific Northwest stone fruit blocks.

Our primary focus quickly shifted toward high-throughput screening and commercial trunk injection deployment, initial trials incorporated edible red dye markers alongside direct plant infusion assays to track vascular mobility. Observing systemic dye uptake confirmed rapid translocation throughout the canopy and into vector feeding sites; subsequent gut analyses in *Colladonus reductus* verified direct ingestion from the vascular system, supporting the hypothesis that systemic therapeutics can simultaneously target the phytoplasma and suppress vector fitness.

To ensure long-term trial continuity and complete data collection, this work expanded beyond its initial scope. With support from complementary grant resources, we secured a dedicated full-time postdoctoral researcher focused on field injection logistics and physiological assessments, alongside a full-time technician supporting molecular screening and high-throughput qPCR titer tracking. Furthermore, because available grant funds consistently carry over toward the end of each annual cycle, we plan to leverage these residual resources to maintain full operational momentum for one additional final year (no extension cost) ensuring all core objectives (vascular mobility, therapeutic efficacy on phytoplasma titer, vector impact assessment, and commercial trunk injection evaluations) are thoroughly completed and validated.

In Vitro, lab condition screening

To rapidly evaluate candidate antimicrobial molecules against Candidatus Phytoplasma pruni, we developed a robust *in vitro* screening platform utilizing infected cherry plants maintained on agar-solidified media supplemented with test compounds across a dilution series. Efficacy is quantified by measuring DNA titer via qPCR at baseline (Time 0) and following a five-week incubation period. Control treatments demonstrate exceptional stability in baseline Ct values over the duration of the assay, confirming the reproducibility of the system. Preliminary screening results illustrate clear differential efficacy: candidate molecule M1 successfully decreased bacterial titer in a dose-dependent manner, with higher concentrations yielding greater reductions, whereas molecule M2 showed low impact on bacterial accumulation compared to untreated controls (Figure 1). This standardized *in vitro* platform provides a high-throughput, controlled environment for prioritizing candidate molecules prior to greenhouse and field validation.

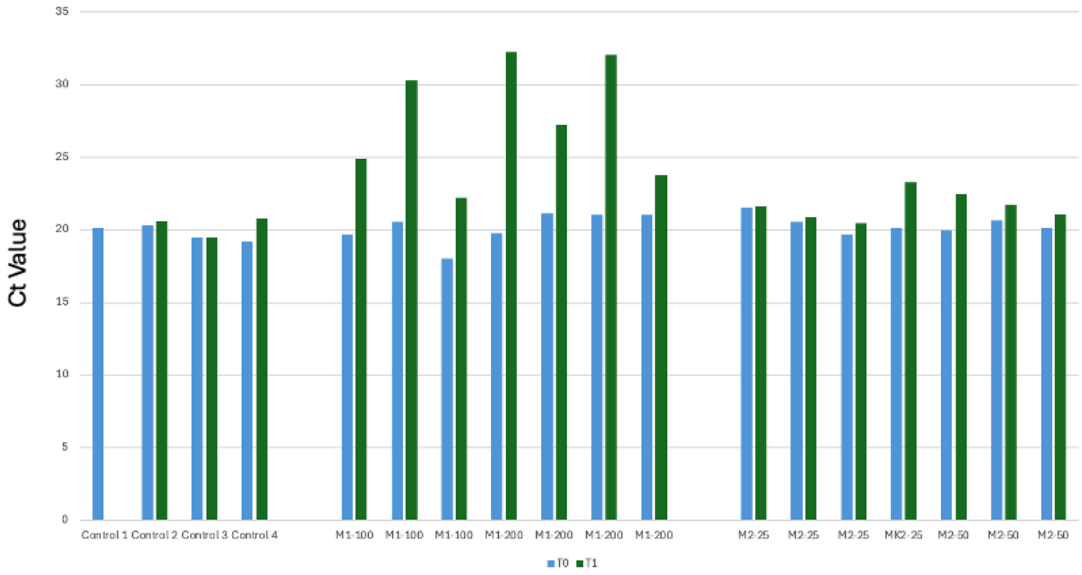


Figure 1: *In vitro* screening of infected cherry plant tissues. Blue bars represent T_0 and green bars represent T_1 qPCR $C_T$differences, demonstrating a drop in bacterial titer for candidate molecule M1 across increasing concentrations.

Early Pilot Trials (USDA Research Farm, 2025)

Before expanding into commercial orchard blocks, we ran initial proof-of-concept pilot evaluations during the 2025 season at our USDA-ARS research orchard. Using targeted injections on infected peach trees and testing multiple formulation chemistries in sweet cherry, our primary objective was to confirm that stone fruit vascular architecture could reliably take up therapeutic formulations. To trace and verify vascular transport, we delivered a red floral dye marker, which confirmed uniform, systemic distribution through the xylem network and out to the distal canopy (Figure 2).



Figure 2: Red dye trunk injection in cherry. We evaluated a new trunk injection system to directly deliver molecules into the vascular system of cherry trees, utilizing red floral dye to measure uptake volume, concentration, and timing.

This foundational farm trial delivered clear visual evidence that vascular delivery could improve symptom expression in peach, where foliar symptoms, such as severe leaf curling, chlorosis, and shot-hole necrosis, are remarkably conspicuous compared to sweet cherry (which primarily expresses symptoms through stunted, unmarketable fruit development). Although this initial evaluation focused on a proof-of-concept injection, the phenotypic contrast between June 2025 and September 2027 was highly encouraging:

- **Non-Injected Control Trees:** Maintained persistent, escalating X-disease decline characterized by severe upward leaf roll, pronounced chlorosis, foliar bronzing, and progressive scaffold dieback (Figure 3, Panels A to C).
- **Injected Trees:** Overcame initial disease symptoms, pushing clean terminal shoot growth and establishing a visually healthy green canopy by the following season (Figure 2, Panels B to D). Farm crews annually prune and trim back dead or dying branches every year.

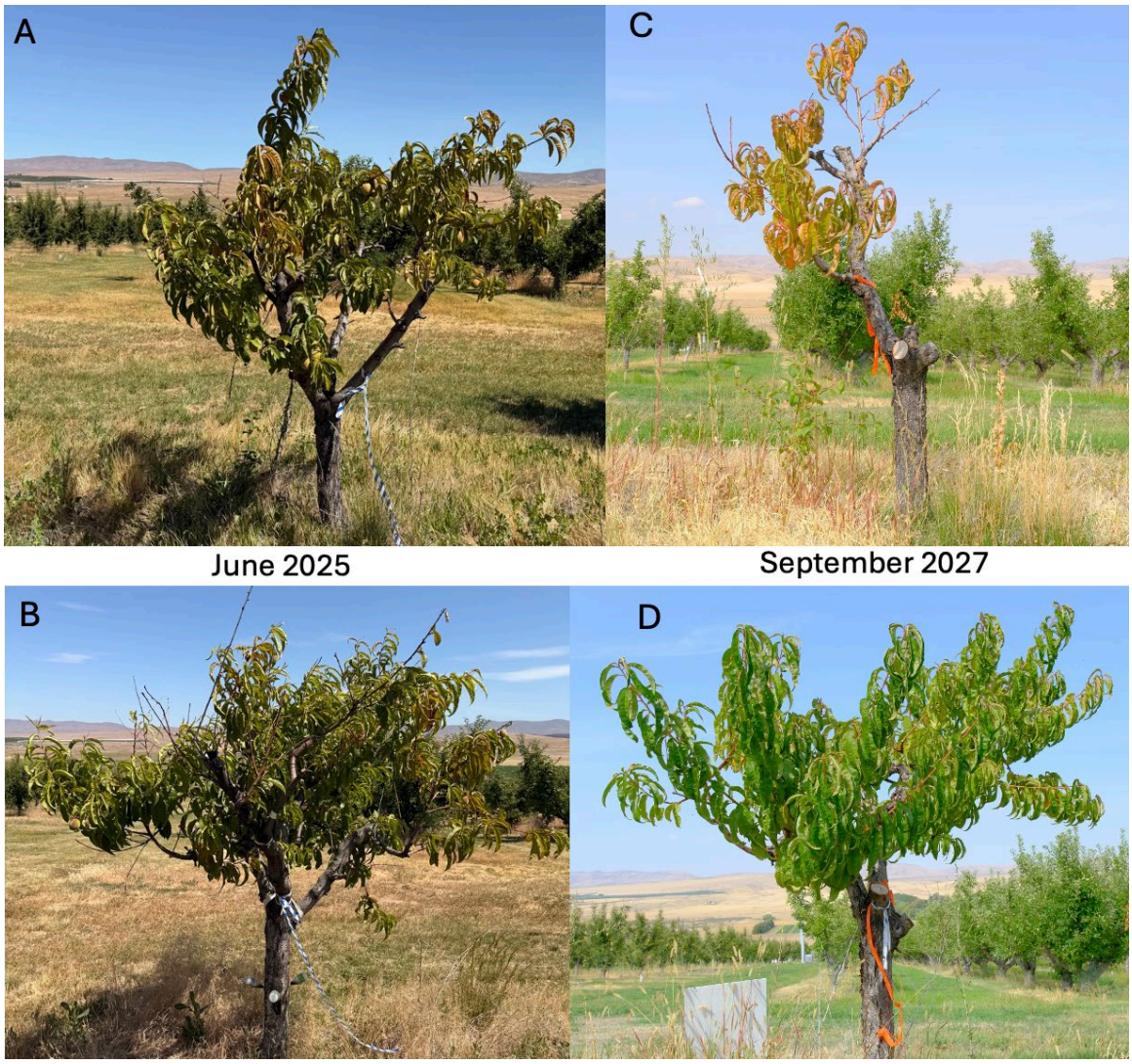


Figure 3: Peach treatment comparisons (A–D). Non-injected controls exhibit escalating X-disease decline, whereas treated trees display robust recovery and clean terminal shoot growth.

While reversing foliar decline in a severely symptomatic tree went beyond our initial baseline expectations, these encouraging phenotypic results directly justified scaling trunk injection from a single experimental research block into multi-acre commercial field trials in 2026. Concurrent injections in sweet cherry confirmed rapid uptake and established baseline tissue tolerance across both stone fruit species, solidifying the operational framework for our regional field expansion.

Delivery Platform and Field Injection Mechanics

For the commercial deployment phase, we selected the **FLexInject™** platform from TJ BioTech (Figure 4), a system proven in commercial citrus applications. This platform operates on low-pressure delivery, enabling the tree to take up therapeutic formulations at its natural transpiration rate rather than forcing high-pressure liquid that risks tearing the bark cambium or causing internal vascular splitting. Injections are performed by drilling a small-diameter

pilot hole of approximately 0.44 cm (11/64-inch) into functional sapwood above the rootstock union, minimizing mechanical trunk injury and promoting rapid seasonal wound compartmentalization. The UV- and heat-insulated design of the injector protects sensitive active chemistries from thermal degradation during hot orchard applications. Across our trials, properly calibrated trees took up standard therapeutic doses within 15 to 45 minutes under active daytime transpiration, demonstrating that the system can be operated by commercial orchard crews without heavy tractor-mounted equipment.



Figure 4 : Trunk injector hardware. Application of trunk injection in commercial orchards utilizing the low-pressure FLexInject™ system.

Commercial Field Trial Scale and Block Architecture

Our 2026 field work covers three separate orchard sites across Washington stone fruit acreage: two commercial sweet cherry blocks and one dedicated peach block, totaling over 350 individually monitored trees.

- **Sweet Cherry Blocks:** Approximately 220 trees were injected across the candidate therapeutic classes.
- **Peach Block:** Includes approximately 60 injected trees, serving as a responsive model system to evaluate rapid foliar recovery alongside fruit symptom tracking.

Individual treatment plots were assigned across multiple rows to capture natural soil moisture, irrigation, and vigor gradients. Every eligible tree was pre-screened by quantitative PCR to establish baseline pathogen levels, ensuring treatments were balanced across heavily infected trees ($C_T \approx 17$–22) and early-stage or borderline infections ($C_T \approx 36$–40). A two-tree buffer gap, when trees were too close to each other, was maintained between injected trees to

eliminate cross-talk from root grafting or overlapping canopies. Structurally undersized replants, missing trees, and trees with non-target trunk damage (such as severe rodent girdling) were excluded. Pre-treatment foliar bronzing was mapped for each tree to provide a benchmark for tracking post-dormancy canopy response.

Physiological Monitoring and Fall Pre-Dormancy Deployment

Because trunk injection relies on the tree's transpiration pull, identifying the operational uptake window in sweet cherry and peach is essential. In the field, uptake speed is directly tied to ambient weather and tree water status. We actively track stomatal conductance, transpiration rates, and photosynthetic parameters using a LI-COR portable gas analyzer to identify peak transpiration windows and ensure trees are physiologically active prior to injection.

Our initial commercial evaluations were deployed during the late-summer to early-fall pre-dormancy window. This post-harvest timing leverages late-season transpiration to distribute therapeutic compounds throughout the vascular framework before winter. Foliar phytotoxicity is negligible during fall applications because the canopy naturally senesces and drops leaves ahead of winter dormancy.

Future Directions: Narrowing Candidates and Field Optimization

As we conclude this grant with the first season of field trunk injection, the immediate priority shifts toward a new scope of work (new proposal submitted to WTFRC) for evaluating how fall treatments translate into spring performance. Over the upcoming winter and early spring, we will track phytoplasma titer fluctuations via qPCR, monitor bud break uniformity, and assess return bloom and canopy vigor in treated sweet cherry and peach blocks.

Building on the foundational data and commercial momentum established over the past three years, we are actively preparing a new grant proposal for the Washington Tree Fruit Research Commission (WTFRC) to fund a subsequent cycle. While this current project established the baseline delivery mechanics, *in vitro* screening platforms, and initial commercial feasibility using the FLexInject™ system, the upcoming grant cycle will focus intensively on commercial orchard-scale tree applications. Specifically, future research will target refining and optimizing precise therapeutic concentrations and delivery formulations for full-scale commercial orchards, standardizing tree-diameter-based dosing protocols across diverse stone fruit varieties, defining optimal seasonal injection windows to maximize therapeutic persistence and minimize labor overhead, and translating these advanced trunk injection technologies into practical, cost-effective management programs that regional growers can readily adopt.

Project Title: Sustainable Cherry Protection with SYMBIONT

Report Type: Continuing Project Report

PI: Marco Pitino
Organization: USDA-ARS, Wapato, WA
Telephone: 509/454-4463
Email: marco.pitino@usda.gov

PI: W. Rodney Cooper
Organization: USDA-ARS, Wapato, WA
Telephone: 509/454-4463
Email: Rodney.Cooper@ars.usda.gov

PI: Adrian Marshall
Organization: USDA-ARS, Wapato, WA
Telephone: 509-454-6570
Email: adrian.marshall@usda.gov

Cooperators: Michelle Heck USDA-ARS, Ithaca, NY; Scott Harper; Tobin Northfield

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $ 48,000
Total Project Request for Year 2 Funding: $ 48,000
Total Project Request for Year 3 Funding: $ 48,000

Organization Name: USDA ARS
Contract Administrator: Mara Guttman
Telephone: 509 454 6573
Contract administrator email address: Mara.Guttman@usda.gov
Station Manager/Supervisor: W. Rodney Cooper
Station manager/supervisor email address: Rodney.Cooper@ars.usda.gov

Other related or associated funding sources: A USDA ARS Headquarters postdoctoral position was awarded for 2 years. Symbiont research is one of USDA ARS's priorities, and several laboratories are involved in this project, including USDA ARS Fort Pierce, Florida the University of Florida, BTI in Ithaca, New York, USDA ARS in Albany, California, and USDA ARS in Wapato, Washington.

2024	2025	2026	2027
$48,000.00	$48,000.00	$48,000.00	
$48,000.00	$48,000.00	$48,000.00	$0.00

While the 2025 project year presented distinct challenges, this past year has marked significant advancements across multiple facets of our engineered symbiont platform. The recruitment of a dedicated USDA-ARS postdoctoral researcher in September, focused full-time on symbiont technology, is already accelerating experimental throughput and enabling more rapid iteration across our molecular and applied pipelines. To support field-level translation, we established a new block of sweet cherry trees at the USDA-ARS farm in Moxee, creating a dedicated site for evaluating the system under natural ambient conditions for future symbiont inoculations.

Molecular design and construct optimization have progressed considerably, supported by active collaboration with laboratories in Ithaca, Fort Pierce, and Albany. These efforts have improved construct stability and overall functional performance. In parallel, we initiated a comprehensive screening program for antimicrobial peptides (AMPs) with diverse modes of action, applying them directly to cherry tissues. Importantly, innovations achieved in the cherry pipeline have also provided substantial bilateral improvements to our citrus platform, demonstrating how iterative development in one system benefits the broader cross-crop symbiont framework.

Original Objectives

1. Symbiont Development in Cherry: Assess the development of engineered symbionts in cherry, comparing with citrus findings.

2. AMPs Screening: Inoculate infected cherry trees with various symbiont plasmids, screen for disease reduction, and develop improved plasmids.

3. Combined AMPs Efficacy: Evaluate new plasmids in greenhouse trials, focusing on titer reduction and plant health improvements.

4. Field Evaluation: Plan and initiate field trials for top-performing symbionts, leveraging citrus trial experience.

Significant Findings and Progress

Symbiont Development and Inoculation Refinement

We completed and significantly expanded our primary symbiont development goals, improving upon the initial inoculation system originally developed for citrus. Because environmental conditions vary substantially between regions, particularly the lower humidity typical of Washington relative to Florida, engineered symbionts require careful micro-environmental control during the establishment phase.

• **Wrapping System Innovation:** We optimized a wrapping system capable of maintaining required humidity levels for symbiont proliferation across woody plant species (fig. 1).



Figure 1: symbiont on cherry plants wrapped with the new optimized material.

• **Refined Wounding Geometry:** Traditional inoculation methods remove a small tissue disk, but this can disrupt essential vascular architecture. We discovered that minimizing wound size preserves vascular integrity, enabling host vascularization to grow directly through and into the symbiont gall. This dramatically improves vascular integration and systemic connectivity (Fig. 2).
• **Cross-Crop Application:** The refinements developed for cherry have been incorporated back into citrus inoculation protocols, improving reproducibility across both systems.

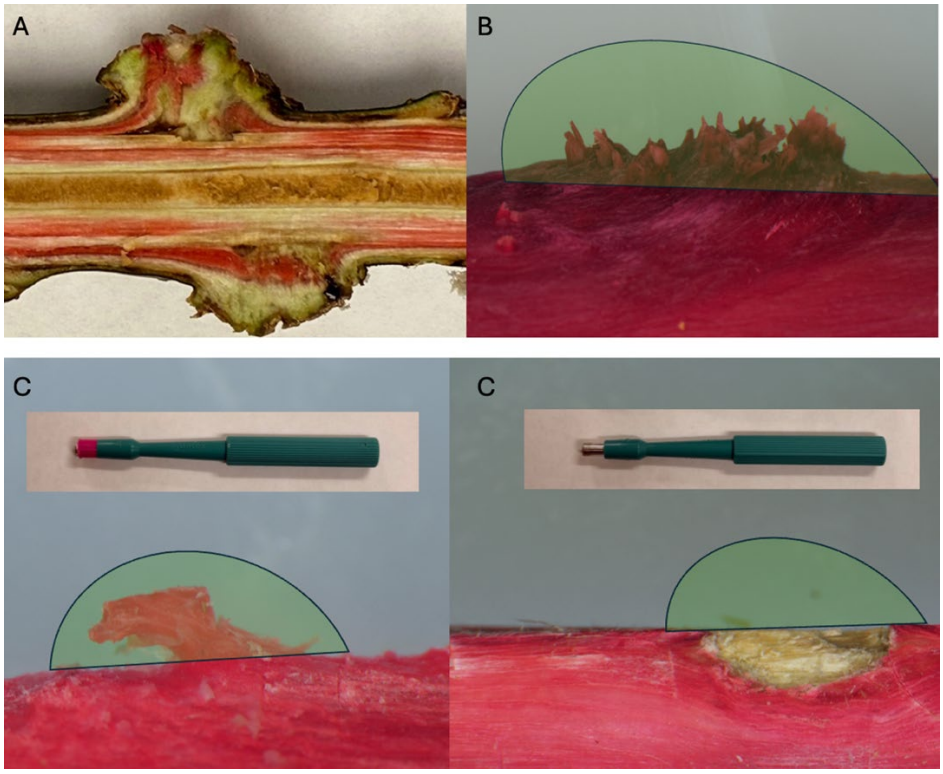


Figure 2. Inoculation results. A: Transverse section of a cherry stem showing the symbiont, with red dye highlighting vascularization inside the symbiont. B: Light green indicates where the symbiont was located before removal, with vascularization continuing to grow actively in the former symbiont area. C: Another example of host vascular tissue forming inside the symbiont (light green). D: Old system inoculation shows an absence of vascularization and potentially limited movement of molecules from the symbiont to the host vascular system.

Establishment of a Reliable Tree Infection Pipeline

Establishing phytoplasma infections in woody perennials is a major biological bottleneck because symptom development in mature field trees can require 3–5 years. To avoid dependency on opportunistic field infection, we built a robust, controlled multi-stage infection pipeline:

• **Annual Nursery Intake:** Each year, 300–500 clean nursery trees enter our regulated exposure pipeline.

• **Vector-Assisted Challenge:** Trees undergo inoculation via successive generations of leafhopper vectors within infected orchard blocks.
• **Staggered Cohort Management:** Plants are advanced through multi-year progression phases, ensuring a continuous, predictable supply of uniformly infected material for AMP screening.

This pipeline represents a major advancement toward predictable, high-throughput disease evaluation in cherry.

Molecular Design and AMP Construct Library

Construct generation and genetic optimization have expanded considerably through collaborations across partner USDA-ARS laboratories.
• Large-Scale Library Expansion: We have developed, cataloged, and are iterating on more than 150 genetic constructs for the symbiont platform.
• Functional Diversity: The library includes single-agent AMPs, multi-effector designs, and synergistic combination constructs.
• Mechanistic Breadth: By integrating peptides with varied mechanisms, from membrane disruption to targeted metabolic interference, we are increasing our chances of reducing pathogen titers effectively while mitigating the risk of resistance.

Field Evaluation and Regulatory Frameworks

We have established a new, dedicated experimental sweet cherry (Prunus avium) orchard block at the USDA-ARS research facility (fig. 3). This block comprises approximately 300 trees representing a diverse array of commercial scions and rootstock combinations, including 'Chelan' on Krimsk-6, 'Samba' on Krimsk-6, and various 'Rainier' selections. Testing confirms that our engineered symbiont platform maintains robust, predictable behavior across these different sweet cherry cultivars and rootstock genotypes, demonstrating that the host's genetic background does not alter core symbiont colonization patterns or performance metrics.
A critical milestone was achieved regarding regulatory clearance for outdoor field applications. Because the engineered symbiont platform relies on a localized, transformed gall rather than a whole transgenic plant, it presents a distinct and highly favorable biosafety profile, specifically mitigating risks related to systemic transgene dissemination or lateral gene flow into the environment. Successfully navigating and finalizing the USDA-APHIS permitting procedure for outdoor deployment clears a path for field-level evaluations under natural environmental conditions.
Currently, authorizations are granted via a year-by-year permitting process. We are actively applying for an outdoor permit to utilize the symbiont at our outdoor USDA laboratory space, allowing us to perform initial inoculations in a controlled environment and hold the plants until the Agrobacterium vector naturally fades away. Once plants achieve readiness for open field deployment, we will submit a secondary permit application to clear the use of Agrobacterium directly in the field. To streamline this regulatory path, we are modeling our approval process after the exact framework utilized for ongoing Florida symbiont field trials targeting citrus greening, significantly accelerating our local approval timeline



Figure 3: New cherry block at Moxee USDA ARS farm.

Results and Discussion: Beyond AMPs and Plasmids

The project has optimized its tree infection cycle by establishing a standardized protocol and utilizing a table-mapped layout inside a regulated shade house. Compliance efforts are underway with USDA-APHIS to permit containment space for engineered symbiont maintenance and subsequent antimicrobial peptide screening. Additionally, tomato plants (*Solanum lycopersicum*) are being evaluated as a rapid model system to accelerate the validation of protein export vectors before testing in slower-growing woody fruit trees.

While we generate plasmids for phytoplasma, we are also collaborating with several external laboratories to improve our inoculation protocols, cellular export efficiency, and systemic delivery systems. Because AMPs targeting phytoplasma can only be successfully screened using woody plants actively infected with the bacteria (such as cherry trees), exploring alternative fast-growing plant models like tomatoes allows protein export and vascular movement to be evaluated rapidly in an easier laboratory setting.

Furthermore, our functional marker analyses demonstrate that a symbiont is more than just a protein factory; it can manufacture and coordinate the complex molecules needed to unlock a plant's entire defensive arsenal and secondary metabolic potential (fig 4):

- Green Fluorescent Protein (GFP): Used as a visual marker to track protein tracking, secretion, and systemic movement throughout the host plant’s vascular system.
- Betanin: A tyrosine-derived pigment used to visually monitor the expression and localization of short-pathway secondary metabolites.
- Anthocyanin: A complex, multi-step flavonoid pigment used to validate the symbiont's ability to trigger or complement intricate, multi-enzyme biochemical pathways in the host

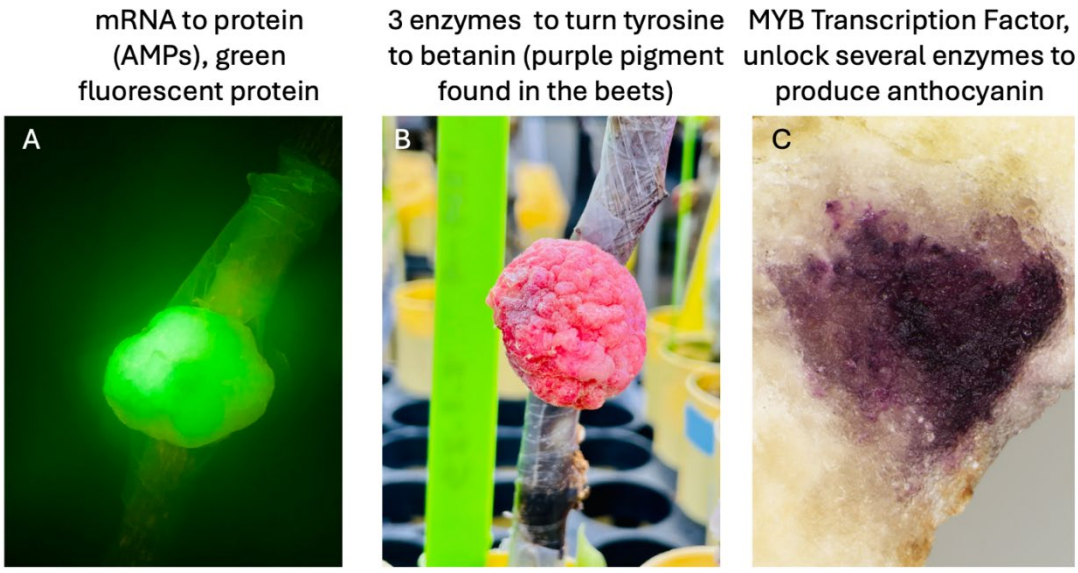


Figure 4: A) Green Fluorescent Protein (GFP): Used as a visual marker to track protein tracking, secretion, and systemic movement throughout the host plant's vascular system. B) Betanin: A tyrosine-derived pigment used to visually monitor the expression and localization of short-pathway secondary metabolites. C) Anthocyanin: A complex, multi-step flavonoid pigment used to validate the symbiont's ability to trigger or complement intricate, multi-enzyme biochemical pathways in the host.

Conclusions and Future Directions

As we conclude the final year of this project, we have successfully met our core objectives while securing a cost-zero extension to finalize remaining milestones. Over the course of the project, we optimized the engineered symbiont platform specifically for cherry, established a robust library of over 150 distinct genetic constructs containing diverse antimicrobial peptides (AMPs), alternative secretory strategies, and varied mechanisms of action. Significant progress was also made regarding regulatory compliance and field preparation. We established a new experimental sweet cherry block at the Moxee farm and initiated formal regulatory engagement with USDA-APHIS. A major milestone achieved through these discussions is the recognition by regulators that the engineered symbiont relies on a localized gall rather than creating a whole transgenic plant, a distinction with a highly favorable biosafety profile that prevents systemic transgene dissemination. While the formal deregulation process for the symbiont as a new genetic tool remains ongoing, this foundational recognition is a major step forward. In the interim, authorizations permit us to utilize and maintain the symbiont cherry plants in our secured outdoor research spaces. Looking toward the immediate future, our final objective for this concluding period is to execute the first complete screening of our AMP library directly against phytoplasma. With our reliable tree infection pipeline now fully established, providing a steady supply of infected plant material, we are well-positioned to complete these evaluations and pave the way for future translational applications.

Project Title: Precision Nutrient Management for sweet cherry orchards

Report Type: Final Project Report

PI: Bernardita Sallato
Organization: WSU- ANR
Telephone: 509-786-9205
Email: b.sallato@wsu.edu
Address: 24106 N Bunn Rd
City/State/Zip: Prosser, WA, 99350

PI: Matthew Whiting
Organization: WSU- Horticulture
Telephone: 509-786-9
Email: mdwhiting@wsu.edu
Address: 24106 N Bunn Rd
City/State/Zip: Prosser, WA, 99350

Cooperators: Douglas Fruit, Cameron Nursery, Dory Linneman, Dana Sirota and Judy Robinson (PNNL), Lav Khot (WSU, AgWeathernet), Juan Munguia de la Cruz, Diana Velazco, Alondra Mendez Guerrero, Dayanara Mendoza.

Project Duration: 3 Year + **NCE**
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 was to determine nutrient demand in sweet cherry orchards and to validate a method for extraction-based estimations for young orchards. Specific objectives were:

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

Completed. We determined fruit nutrient extraction for 'Benton' and completed three consecutive seasons (2023–2025) of whole-tree destructive partitioning in two blocks: 'Benton' on Gisela 5 (Gi5), Gisela 12 (Gi12) and MxM14 (6th to 8th leaf), and 'Black Pearl' on Gisela 6 (Gi6), Gi12 and Mazzard at WSU IAREC (1st to 3rd leaf). In 2025, we also excavated two commercial blocks planted the same year as the WSU block: 'Chelan'/Mazzard and 'Black Pearl'/Mazzard. This report emphasizes the final-year (2025) data.

2. **Timing:** Identify strategies to monitor root growth and nutrient availability in sweet cherry.

2.1 Root growth was monitored with root windows, a minirhizotron (CI-600, CID Bio-Science) and electrical resistivity tomography (ERT), the latter conducted by PNNL collaborators. These findings were presented in the 2024 and 2025 reports.

2.2 Nitrogen leaching: In 2024, we evaluated nitrogen leaching in a silt loam soil (Roza farm), as presented in the 2024 report. In 2025–2026, a new trial evaluated the leaching of post-harvest urea in a sandy and a silt loam soil.

3. **Outreach:** Findings have been incorporated into the WSU Tree Fruit soil and nutrition web page (Nutrient Management in Sweet Cherries | WSU Tree Fruit | Washington State University) and the WSU Tree Fruit video (https://treefruit.wsu.edu/videos/optimizing-tree-fruit-nutrition-for-fruit-quality/). Findings will be presented at the 2026 Northwest Cherry Institute meeting, and a summary will be shared in Fruit Matters. Results were also published in two Good Fruit Grower articles (https://www.goodfruit.com/a-window-to-the-roots/ ; https://www.goodfruit.com/research-to-help-growers-schedule-the-sustenance/).

SIGNIFICANT FINDINGS

1. Fruit nutrient removal by 'Benton' was 3.1–4.0 lb N, 0.8–1.0 lb P and 4.0–5.8 lb K per ton of fruit, within the ranges reported for other Washington cultivars. Fruit flesh is about 94% of fruit weight, so analyzing flesh alone is enough to estimate removal.
2. In the mature 'Benton' orchard, whole-tree N did not increase from the 6th to the 8th leaf. Tissues renewed each year (1-year shoots, spurs and fine roots) held only 9% of tree N (24–38 g/tree, 25–40 lb N/acre), and most of this N is recycled in the orchard through fine-root turnover, leaf fall and pruning residues. In mature orchards, annual N demand is therefore driven mainly by fruit yield: about 31–40 lb N/acre at 10 tons/acre.
3. Nutrient demand of new orchards should be estimated from biomass growth. Third-leaf 'Black Pearl' at WSU IAREC accumulated 27–37 g N per tree, or 64–88 lb N/acre at 1,089 trees/acre. Of this, 13–17 lb N/acre was in renewable tissues (leaves, 1-year shoots and fine roots). Total N did not differ among Gi6, Gi12 and Mazzard, although Mazzard allocated half of its biomass to the trunk and rootstock.
4. The N accumulated by the WSU 'Black Pearl' trees in three years (64–88 lb N/acre) was about twice the N applied (about 34 lb N/acre). Nursery reserves and soil and water N supplied a large share of young-tree demand.
5. Trees of the same age in two commercial blocks were 2–3 times larger than those in the WSU experimental orchard. The extra biomass was mostly in the leader, 2- and 1-year wood and leaves, while trunk + rootstock weight was similar. 'Black Pearl'/Mazzard weighed 23.1 kg at the commercial site and 7.9 kg at WSU IAREC.

6. However, N accumulated in the trees per acre did not differ among the five blocks (49–88 lb N/acre; p = 0.33), even though cumulative N inputs over three years ranged from about 34 lb/acre (WSU) to 75 lb/acre ('Black Pearl', commercial) and 300 lb/acre ('Chelan', commercial).
7. Root windows were the most practical method to monitor root growth. New roots formed 3–4 weeks after full bloom, once the minimum soil temperature at 8 inches exceeded 49 °F, and growth was greatest from early June to the end of July.
8. Most of the post-harvest (September) ground-applied urea was not recovered in the sandy soil (Mattawa): ammonium increased only in the top 2 inches and declined by 60% by April, and nitrate did not increase below 6 inches. In the silt loam (Roza), no change was detected in November, but by January the urea had been converted to nitrate, 72–138 ppm above the control in the top 6 inches and reaching 12–18 inches in one replicate. The urea nitrified while trees were dormant, leaving nitrate exposed to leaching with spring irrigation.

METHODS

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

Biomass and nutrient partitioning were evaluated in the following blocks:

- 'Benton' on MxM14, Gi12 and Gi5 planted in 2018 at the WSU Roza farm (479 trees/acre). Trees were excavated after the 2023, 2024 and 2025 seasons (6^{th} to 8^{th} leaf).
- 'Skeena' and 'Selah' on Gi12 planted in 2012, used only for root monitoring and fruit extraction.
- 'Black Pearl' on Gi6, Gi12 and Mazzard planted in 2023 at the WSU IAREC farm (1,089 trees/acre). Trees were excavated after each season (1^{st} to 3^{rd} leaf). Each tree received 10 g of monoammonium phosphate (MAP) at planting and 10 g of urea in 2024, and the block received 20 lb N/acre in 2025 (about 34 lb N/acre in total).
- Two commercial blocks planted in 2023 (290 trees/acre): 'Chelan'/Mazzard, which received 100 lb N/acre per year since planting, and 'Black Pearl'/Mazzard, which received 25 lb N/acre per year. Trees were excavated after the 2025 season (3^{rd} leaf).

At harvest, all fruit from four replicate trees per rootstock were collected to determine yield and fruit nutrient removal, with fruit separated into flesh, pit and stem. Before leaf fall, nets were placed around the trees to collect all dropped leaves. After leaf drop, three to four trees per rootstock were excavated (Figure 1). Each tree was separated into components (leader, 2-year-old wood, 1-year-old wood, spurs, trunk, rootstock (stump), big roots and fine roots), and each component was weighed fresh. A subsample of each component was weighed fresh and after drying to determine dry matter, and analyzed for total N, P, K, Ca, Mg, S, B, Zn, Fe, Mn and Cu. Whole-tree nutrient content was calculated as the sum, over all components, of fresh weight × dry matter (%) × nutrient concentration (%).



Figure 1. Leaf collection during the fall (left), whole-tree excavation (middle) of 'Benton' trees and dry matter partitioning (right).

Rootstocks within each block were compared with one-way ANOVA, followed by Tukey's HSD test ($p < 0.05$). The three WSU 'Black Pearl' rootstocks and the two commercial blocks, all in the third leaf, were compared together as five groups. For this comparison, the trunk and upper rootstock were combined because they were weighed together at WSU IAREC. The mature 'Benton' block was analyzed separately and was not compared with the young blocks. Per-acre values were calculated with the actual planting density of each block (g/tree × trees per acre ÷ 453.6).

2. Timing: Identify strategies to monitor root growth and nutrient availability.

2.1 Root growth monitoring. The methods and results for root windows, the minirhizotron and ERT were described in the 2024 and 2025 reports and are not repeated here.

2.2 Nitrogen leaching after post-harvest urea application.

During 2023 and 2024, soil samples were collected through the season at the WSU 'Benton', 'Skeena' and 'Black Pearl' sites, but the results were inconclusive. Therefore, in fall 2025, two independent trials were established in soils with contrasting texture. The Mattawa site has a very sandy soil (organic matter 0.7% at 2 inches, decreasing to 0.2% at 24 inches; bulk density 1.5 g/cm^3). The Roza site has a silt loam (organic matter 2.2% to 1.0%; bulk density 1.4 g/cm^3). At each location, four replicate sites received urea at 10 g/tree (Nitrogen) and four sites remained untreated (Control). Urea was applied as granules about 6 inches from the trunk on September 19, 2025, one day after the baseline (pre-treatment) sampling, and was irrigated immediately for 2 hours at Mattawa and 6 hours at Roza. Both sites are drip and sprinkler irrigated. Mattawa then received 2-hour sets daily until mid-October and one 6-hour set at the end of October, while Roza received no further water until the following spring.

Soil samples were collected at 2, 6, 12, 18 and 24 inches one day before application (September 18, 2025) and after application on November 14 and December 13, 2025, and April 8, 2026 (Mattawa), and on November 14, 2025, and January 12, 2026 (Roza). Samples were analyzed for organic matter (OM), total N, nitrate (NO_3-N) and ammonium (NH_4-N). The treatment effect was calculated as the difference between Nitrogen and Control means at each depth, with the standard error of the difference. One core (Roza, January, replicate 4, 6 inches) with 384 ppm NO_3-N and 401 ppm NH_4-N, consistent with a dissolving granule, was excluded from the means.

RESULTS AND DISCUSSION

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

Fruit nutrient removal

Fruit removal of N, P and Ca did not differ among rootstocks. Fruit K, Mg, S, B and Cu removal was highest on MxM14 (Table 1). The largest difference was 1.8 lb K per ton of fruit between MxM14 and Gi12; all other differences were below 0.1 lb per ton for macronutrients and below 3 g per ton for micronutrients. Removal values were within the ranges reported for 'Chelan', 'Coral Champagne' and 'Skeena' (Sallato et al., 2024). Fruit flesh made up 94% of fruit fresh weight, so analyzing flesh alone provides a reliable estimate of removal.

Table 1. Fruit nutrient removal by 'Benton' on three rootstocks, per US ton of fresh fruit. Different letters within a column indicate significant differences (Tukey's HSD, $p < 0.05$).

Rootstock	N	P	K	Ca	Mg	S	B	Zn	Mn	Cu
			lb/ton					g/ton		
Gi5	3.20	0.94	5.09 ab	0.51	0.31 ab	0.20 b	4.3 b	0.82 a	1.22 a	0.99 ab
Gi12	3.11	0.83	4.00 b	0.57	0.27 b	0.17 b	4.3 b	0.52 b	0.99 b	0.86 b
MxM14	4.00	0.97	5.76 a	0.45	0.33 a	0.26 a	6.7 a	0.57 b	0.84 b	1.21 a
p value	0.081	0.053	**0.014**	0.683	**0.010**	**0.003**	**0.024**	**0.001**	**0.001**	**0.009**

Mature 'Benton' orchard – 6th to 8th leaf

Over three seasons, MxM14 trees were consistently the largest and held the most nutrients, but the differences were significant only in 2024 (Table 2). In 2025 (8th leaf), tree weight did not differ among rootstocks (Table 2). The leader held 36–39% of tree biomass, followed by the trunk (21–29%) and big roots (11–16%), while spurs were below 0.5%. The only difference in partitioning was the rootstock section, which was 14.4% of tree weight on MxM14 compared with 7.4% on Gi5 (Gi12 9.6%, intermediate; $p = 0.017$). Whole-tree nutrient content did not differ among rootstocks in 2025, except Mn (higher on MxM14). Nutrient content per kg of tree was the same for all three rootstocks (3.95–4.06 g N/kg).

Table 2. Tree fresh weight and macronutrient content (woody tissues) of 'Benton' trees by season and rootstock. Different letters within a column and season indicate significant differences (Tukey's HSD, $p < 0.05$).

Season	Rootstock	Tree weight (kg)	N	P	K	Ca	Mg
					(g/tree)		
2023 (6th leaf)	Gi5	67.9	337.2	64.5	126.3	441.5	52.8
	Gi12	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 (7th leaf)	Gi5	63 b	256.3 b	48.8	136.2 b	661.0	42.2 b
	Gi12	74 b	336.9 b	68.7	165.8 b	638.6	52.3 ab
	MxM14	126 a	527.0 a	112.5	328.2 a	1,063.9	90.4 a
	p value	0.022	0.019	0.065	0.023	0.232	0.047
2025 (8th leaf)	Gi5	68.5	279.1	51.9	159.9	694.7	50.7
	Gi12	67.9	265.0	46.0	140.2	522.2	42.9
	MxM14	101.6	408.6	77.0	239.1	733.2	70.1
	p value	0.242	0.214	0.187	0.254	0.438	0.282

In the Gisela rootstocks, whole-tree N did not increase between the 6th and 8th leaf (Gi5: 337, 256 and 279 g; Gi12: 326, 337 and 265 g). The mature trees had reached a steady size maintained by pruning,

so little new N was being stored in permanent wood. The N in new tissues (1-year shoots, spurs and fine roots) was estimated at 24–38 g per tree, only 9% of whole-tree N for all three rootstocks, or 25–40 lb N/acre at 479 trees/acre (Table 3). Because total N did not change, most of this N is likely recycled in a mature orchard: fine roots turn over in the soil, leaves resorb part of their N before falling and the rest returns to the orchard floor, and pruned shoots remain in the alley when mulched.

Fruit, in contrast, is exported every year, so annual nutrient demand is driven mainly by fruit yield (Table 3). Additional N is only needed when a deficiency has been diagnosed.

Table 3. Nitrogen in tissues renewed annually by 8th leaf 'Benton' trees, compared with N removed by fruit. Per-acre values at 479 trees/acre.

Rootstock	1-yr shoots g N/tree	Spurs	Fine roots	Renewable	% of tree N	Renewable lb N/acre	Fruit removal[a]
Gi5	5.2	1.3	17.5	23.9	9.2	25.2	32
Gi12	5.4	1.8	16.8	24.0	9.0	25.4	31
MxM14	16.1	1.1	20.6	37.8	9.0	39.9	40
p value	0.312	0.071	0.763	0.329	0.994		

[a]*Fruit N removal (Table 1) × 10 tons/acre.*

Non-bearing sites:
'Black Pearl' (WSU IAREC) – 3rd leaf
In the third leaf, trees weighed 7.7 kg (Gi6), 9.6 kg (Gi12) and 7.9 kg (Mazzard), including leaves, with no differences among rootstocks. Mazzard allocated 50% of its fresh weight to the trunk and rootstock, compared with 34% (Gi6) and 37% (Gi12) ($p < 0.001$; data not shown). As in the first two years, Mazzard invested more of its biomass in the trunk and rootstock, while the Gisela rootstocks invested more in the canopy.

Total N ranged from 26.6 to 36.6 g per tree and did not differ among rootstocks (Table 4). At the actual density of 1,089 trees/acre, this is 64–88 lb N/acre accumulated by the third leaf, compared with 48–59 lb N/acre after the second leaf (2025 report, adjusted to 1,089 trees/acre). Of the total, 13–17 lb N/acre was in tissues that are partially renewed every year (leaves, 1-year shoots and fine roots) and 51–72 lb N/acre was in the permanent structure.



Figure 2. WSU IAREC 'Black Pearl' block planted in 2023 (left) and remaining 3rd leaf trees (right).

Over the three years, this block received about 34 lb N/acre in total: 2.6 lb from MAP at planting, 11 lb from urea in the second year and 20 lb in the third year. The trees, however, held about twice that amount, so N reserves from the nursery, together with soil and water N, supplied a large share of the demand of these young trees.

Table 4. Nitrogen content (g N/tree) by tree component in 3rd leaf 'Black Pearl' (WSU IAREC), and equivalent N per acre at 1,089 trees/acre. Renewable = leaves + 1-year shoots + fine roots.

Rootstock	Leaves	Leader	2-yr	1-yr	Spurs	Trunk + rootst.	Big roots	Fine roots	Total	Total (lb/ac)	Renew. (lb/ac)
Gi6	4.13	8.76	2.33	1.37	0.49	8.23	5.31	1.39	32.0	76.8	16.5
Gi12	4.02	14.24	2.10	1.32	0.61	7.60	5.52	1.18	36.6	87.8	15.7
Mazzard	3.75	6.47	1.16	0.42	0.27	10.85	2.52	1.21	26.6	64.0	12.9
p value	0.926	0.059	0.578	0.061	0.110	0.621	0.082	0.804	0.537		

Commercial blocks compared with WSU IAREC – 3rd leaf

Trees of the same age grown under different conditions differed greatly in total size (Figure 3, Table 5). The commercial 'Black Pearl'/Mazzard (23.1 kg) and 'Chelan'/Mazzard (17.6 kg) trees were 2–3 times heavier than the WSU 'Black Pearl' trees (7.7–9.6 kg). Comparing the same cultivar and rootstock, 'Black Pearl'/Mazzard weighed 23.1 kg at the commercial site and 7.9 kg at WSU IAREC, so site conditions, spacing and nutrient management had a larger effect than rootstock.

The extra biomass of the commercial trees was mostly in the canopy: leader (5.6–7.3 kg vs 1.7–2.9 kg), 2-year wood (1.8–2.1 kg vs 0.2–0.4 kg), 1-year wood (2.5 kg for 'Black Pearl'/Mazzard, the highest of all blocks) and leaves (2.5–4.2 kg vs 0.8–0.9 kg). Trunk + rootstock weight (p = 0.25) and fine-root weight (p = 0.29) did not differ among blocks. 'Chelan'/Mazzard was the only block that produced a measurable crop in the third leaf (4.7 kg of fruit per tree); the 'Black Pearl' blocks were not cropped.

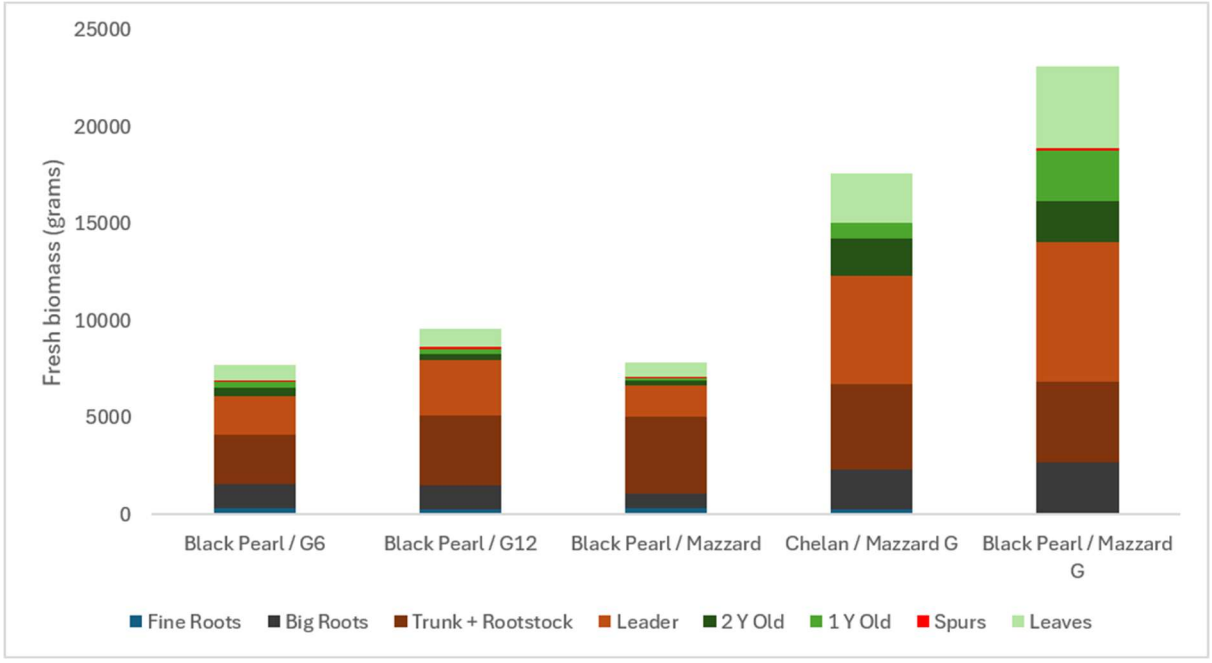


Figure 3. Fresh biomass (g/tree) by tree component in 3rd leaf 'Black Pearl' on Gi6, Gi12 and Mazzard (WSU IAREC, 1,089 trees/acre) and in commercial 'Chelan'/Mazzard and 'Black Pearl'/Mazzard blocks near Pasco (labeled "G"; 290 trees/acre).

Per tree, the commercial trees held two to four times more N, P, K, Ca and Mg than the WSU trees (Table 5). However, the concentration of N in the biomass was similar in all blocks (3.5–4.7 g N per kg of fresh weight; p = 0.06), so the per-tree differences in N were driven by tree size. A similar value (3.95–4.06 g N/kg) was found in the mature 'Benton' trees.

Table 5. Whole-tree fresh weight and macronutrient content of 3rd leaf trees in 'Black Pearl' at WSU IAREC and in two commercial blocks near Pasco. Different letters within a column indicate significant differences (Tukey's HSD, p < 0.05).

Block	Tree weight (kg)	N	P	K	Ca	Mg	N per kg
		(g/tree)					(g/kg)
WSU Black Pearl/Gi6	7.7 c	32.0 bc	9.8 b	13.6 c	60.0 b	5.9 c	4.14
WSU Black Pearl/Gi12	9.6 bc	36.6 bc	8.5 b	16.9 bc	73.0 b	7.2 c	3.77
WSU Black Pearl/Mazzard	7.9 c	26.6 c	6.5 b	16.8 bc	61.1 b	6.1 c	3.48
Commercial Chelan/Mazzard	17.6 ab	77.2 ab	12.2 ab	44.4 ab	80.1 b	13.2 b	4.43
Commercial Black Pearl/Mazzard	23.1 a	110.8 a	17.0 a	57.5 a	155.3 a	21.5 a	4.75
p value	<0.001	<0.001	0.005	0.001	<0.001	<0.001	0.060

On a per-acre basis, the differences change (Table 6). Because the WSU IAREC trees were planted at almost four times the density of the commercial blocks (1,089 vs 290 trees/acre), N accumulated in the trees per acre did not differ among blocks (49–88 lb N/acre; p = 0.33), nor did K, Mg or S. P and Ca per acre were highest at WSU IAREC.

Cumulative N inputs, however, differed widely: about 34 lb/acre at WSU IAREC, and 75 lb/acre for 'Black Pearl' and 300 lb/acre for 'Chelan' at the commercial site. The 'Chelan' block received the most N but stored the least per acre (49 lb N/acre, plus about 5 lb N/acre in fruit). Although direct comparisons with the commercial sites cannot be made, the results show that N applied above young-tree demand was not stored in the trees and was likely exposed to loss.

Table 6. Nutrients accumulated in 3rd leaf trees per acre (lb/acre) at the actual planting density of each block, and cumulative N applied since planting. Different letters within a column indicate significant differences (Tukey's HSD, p < 0.05).

Block	Trees/acre	N applied[a] (lb/acre)	N	P	K	Ca	Mg	S
			(lb/ac)					
WSU Black Pearl/Gi6	1,089	34	76.8	23.5 a	32.6	144.1 ab	14.2	4.1
WSU Black Pearl/Gi12	1,089	34	87.8	20.5 a	40.6	175.1 a	17.3	5.4
WSU Black Pearl/Mazzard	1,089	34	64.0	15.7 ab	40.4	146.6 ab	14.6	4.2
Commercial Chelan/Mazzard	290	300	49.4	7.8 b	28.4	51.3 b	8.4	3.0
Commercial Black Pearl/Mazzard	290	75	70.9	10.9 ab	36.8	99.4 ab	13.7	4.4
p value			0.327	0.012	0.823	0.024	0.083	0.266

[a]*WSU: 10 g MAP/tree at planting + 10 g urea/tree in 2024 + 20 lb N/acre in 2025. Commercial: 100 ('Chelan') and 25 ('Black Pearl') lb N/acre per year for three years.*

For young orchards, these results support estimating N demand from expected biomass growth (about 4 g N per kg of new fresh biomass) at the actual planting density, rather than applying fixed annual rates. N stored in the tree was similar across blocks receiving 34 to 300 lb N/acre, so growth and density, not N rate, determined how much N the trees took up.

2. Timing: Identify strategies to monitor root growth and nutrient availability.

2.1 Root growth monitoring. Root windows, the minirhizotron and ERT were compared over 2023–2025, and detailed results were presented in the 2024 and 2025 reports.

Monitoring methods. Overall, root windows installed between trees provided the most direct and simplest method to monitor the timing of root growth and to compare relative root growth among sites (Figure 4).

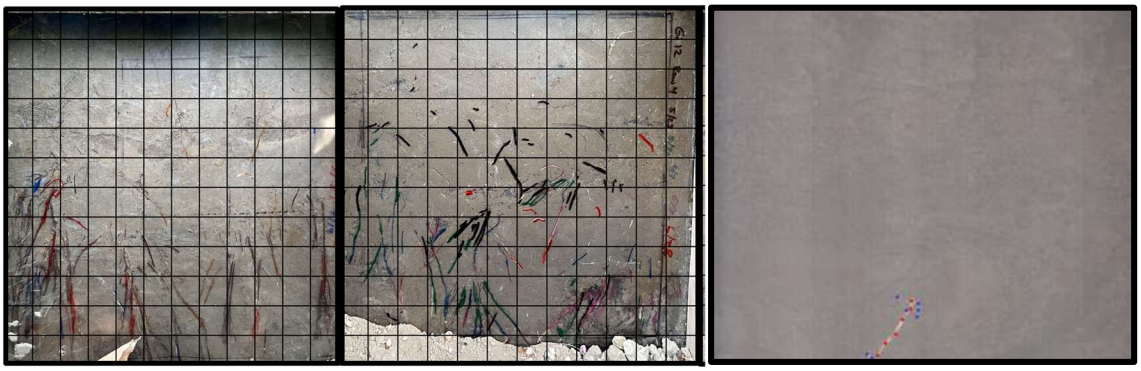


Figure 4. Root growth images collected on June 6, 2023, through root windows for Gisela 5 (left) and Gisela 12 (middle), and with the CID Bio-Science minirhizotron for Gisela 12 (right). Different colors correspond to different monitoring dates.

The minirhizotron did not provide useful information for this study. Although installation and image collection were simple (CI-600, CID Bio-Science), roots were difficult to detect and quantify in the images (Figure 4, right).

Detailed ERT findings were presented in the 2025 report. In summary, time-lapse images of bulk electrical conductivity obtained with ERT were sensitive to root presence, but also to changes in temperature, irrigation, water conductivity and saturation, which made them difficult to interpret without additional soil sensors and controlled environment.

Timing of root growth. Root growth in sweet cherry, regardless of rootstock, began 3 to 4 weeks after full bloom in all years, when the minimum soil temperature at 8 inches exceeded 49 °F. Growth was slow (less than 14 mm per day) during the cell-division period of fruit growth and increased during stage III of fruit growth and after harvest (early June to the end of July; see 2024 report). A few new roots also appeared in late summer (end of September), but they were short-lived.

2.2 Nitrogen leaching after post-harvest urea application.

Before treatment (September), ammonium and nitrate levels were low at both sites (Figure 5). At Roza (silt loam), one replicate had high pre-existing NH_4-N (31–73 ppm from 2 to 18 inches), which explains the positive baseline difference for NH_4-N at that site (Figure 5).

Mattawa (sand). NH_4-N increased at 2 inches after urea application (19.8 ppm above the control in November and 13.1 ppm in December; Figure 5). By April, NH_4-N at 2 inches had declined by 60% (+8.1 ppm), while it increased slightly at 6 inches (+7.4 ppm). Nitrate remained very low, with no change through December (differences ≤0.5 ppm) and background levels at 18 and 24 inches (Figure 5). In April, nitrate increased slightly at 2 and 6 inches (+3.0 and +1.7 ppm), and the NO_3-N share of surface mineral N rose from 8% to 28%, suggesting slow nitrification of the remaining ammonium as the soil warmed in spring. Losses below 24 inches in the weeks immediately after application, before urea hydrolysis, cannot be excluded, because urea moves freely with water until it is converted to ammonium.

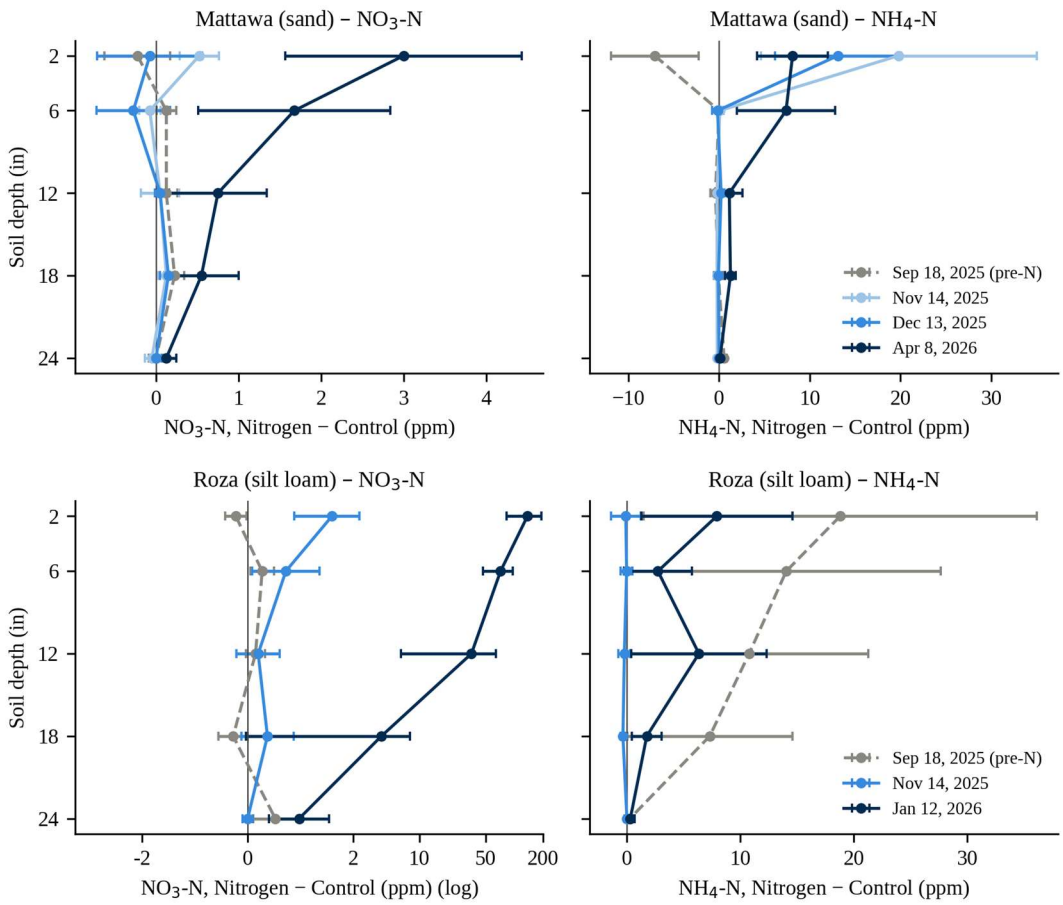


Figure 5. Net effect of urea on soil nitrate (left) and ammonium (right) by depth at Mattawa (sand, top) and Roza (silt loam, bottom). Values are Nitrogen − Control means ± SE of the difference (n = 4). September was sampled one day before application. Roza nitrate is shown on a logarithmic scale.

Roza (silt loam). No increase in NH_4-N or NO_3-N was detected in November, eight weeks after application (Figures 5 and 6). By January, nitrate in the Nitrogen plots was 138 ppm (2 inches) and 72 ppm (6 inches) above the control, making up 95% of surface mineral N. Nitrate was also 35 ppm higher at 12 inches and 4 ppm higher at 18 inches. These deeper increases came mainly from one replicate (123 and 17 ppm), while the other replicates stayed below 14 ppm at 12 inches. Nitrate at 24 inches remained near background (+1.0 ppm). The absence of change in November, followed by a large nitrate increase in January, supports the hypothesis that, with no irrigation after the application set, the urea granules dissolved and hydrolyzed slowly during the fall, and the resulting ammonium was converted to nitrate in early winter, when trees take up little N. Granular placement also created strong spatial variability, reflected in the large standard errors.

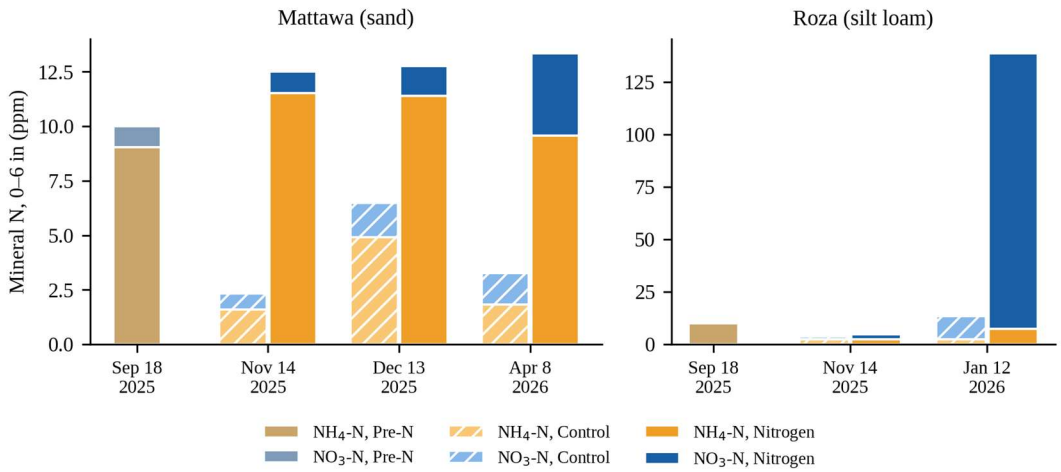


Figure 6. Ammonium (NH_4-N) and nitrate (NO_3-N) in the top 6 inches (mean of 2 and 6 inch samples) at Mattawa and Roza. September values pool Control and Nitrogen plots (pre-treatment, n = 8); other dates n = 4 per treatment.

In the sandy soil, most of the post-harvest urea was not recovered in the 0–24 inch profile during fall and winter. We attribute this to the coarse texture and, probably, to the long irrigation set before dormancy that is commonly applied to refill the soil profile and reduce the risk of frost damage. In the silt loam, mineral N remained in the upper soil and had been converted to nitrate by January; the slower infiltration and greater water-holding capacity of this soil kept the N within the sampled profile.

In both soils, nitrate, whether accumulated over winter (silt loam) or newly formed from ammonium in spring (sand), is mobile and at risk of moving below the root zone with the first spring irrigations or precipitation. Because new roots do not form until 3–4 weeks after full bloom, N applied after leaf fall or early in spring remains exposed to loss before roots can take it up. Applying N during periods of active uptake (from root growth onset in spring through shoot growth, and post-harvest only while leaves are still active), in smaller doses through fertigation, would reduce the time that mineral N remains exposed to leaching.

EXECUTIVE SUMMARY

Project title: Precision Nutrient Management for sweet cherry orchards

Key words: sweet cherry, nutrient demand, biomass partitioning, rootstock, root growth, nitrogen leaching, urea

Nutrient recommendations for sweet cherry are usually based on fruit removal, while there is little information regarding demand prior to full production, during tree establishment. Over three seasons (2023–2025), we quantified biomass and nutrient partitioning by whole-tree excavation in a mature 'Benton' orchard (Gi5, Gi12 and MxM14; 6th–8th leaf) and a young 'Black Pearl' orchard at WSU IAREC (Gi6, Gi12 and Mazzard; 1st–3rd leaf), and in 2026 we compared the young orchard with two commercial blocks of the same age. We also evaluated methods to monitor root growth and evaluated the risk of leaching with post-harvest urea applications in a sandy and a silt loam soil.

Fruit removed 3.1–4.0 lb N, 0.8–1.0 lb P and 4.0–5.8 lb K per ton, with small differences among rootstocks and among cultivars (when compared with our previous project). In sweet cherry, analyzing fruit flesh alone is enough to estimate removal. Whole-tree N in 'Benton' did not increase from the 6th to the 8th leaf, and new growth (1-year shoots, spurs and fine roots) held only 9% of tree N, most of which is recycled within the orchard. Annual N demand of mature orchards is therefore driven by yield: about 31–40 lb N/acre for a 10 tons/acre crop.

In young orchards, nutrient demand is driven by biomass growth. Third-leaf 'Black Pearl' at WSU IAREC accumulated 64–88 lb N/acre (1,089 trees/acre), about twice the N applied over three years (34 lb N/acre). Commercial trees of the same age were 2–3 times larger, yet N stored per acre was similar in all blocks (49–88 lb N/acre) despite cumulative inputs of 34 to 300 lb N/acre. N concentration in tree biomass was consistent (about 4 g N per kg of fresh weight), so the N demand of new orchards can be estimated from expected growth and planting density rather than fixed annual rates.

Root windows were the most practical monitoring tool; minirhizotron images were difficult to quantify, and ERT was confounded by soil moisture and temperature. In our study, new roots formed 3–4 weeks after full bloom, once the soil at 8 inches exceeded 49 °F, with the most growth from early June to the end of July. Scheduling N applications based on root growth should be considered for improving nitrogen use efficiency.

Post-harvest urea applied in September was largely not recovered in the sandy soil profile, while in the silt loam it was converted to nitrate during dormancy (72–138 ppm above the control in the top 6 inches by January), when trees cannot take it up.

Recommendations. Base N rates on expected yield in mature orchards and on expected biomass growth at the actual planting density in young orchards. Apply N while roots and leaves are active, from root growth onset in spring through shoot growth and post-harvest only before leaf senescence, in split fertigation doses, to improve efficiency and reduce leaching.

Project Title: Optimizing the use of Gibberellic Acid (GA) to Improve Fruit Quality in Sweet Cherry

Report Type: Continuing Project Report

Primary PI: Kelsey Galimba
Organization: Oregon State University
Telephone: (541) 386-2030
Email: kelsey.galimba@oregonstate.edu
Address: 3005 Experiment Station Dr
City/State/Zip: Hood River, OR 97031

Co-PI 2: Ashley Thompson
Organization: Oregon State University
Telephone: (541) 296-5494
Email: ashley.thompson@oregonstate.edu
Address: 400 E. Scenic Dr.
City/State/Zip: The Dalles, OR 97058

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $ 33,571
Total Project Request for Year 2 Funding: $ 34,278
Total Project Request for Year 3 Funding: $ 35,005

Other related/associated funding sources: None

WTFRC Collaborative Costs: None

Budget 1
Primary PI: Kelsey Galimba
Organization Name: Oregon State University
Contract Administrator: Charlene Wilkerson
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	2026	2027	2028
Salaries[1]	$9,391.00	$9,581.00	$9,776.00
Benefits	$5,163.00	$5,283.00	$5,407.00
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies	$2,000.00	$2,000.00	$2,000.00
Travel			
Plot Fees	$3,500.00	$3,500.00	$3,500.00
Miscellaneous[2]	$304.00	$304.00	$304.00
Total	$20,358.00	$20,668.00	$20,987.00

Footnotes: [1] Partial summer salary for Galimba and salary for one month of salary for one FRA.
[2] Cold storage rental fees.

Budget 2
Co PI 2: Ashley Thompson
Organization Name: Oregon State University
Contract Administrator: Charlene Wilkerson
Telephone: (541) 737-3228
Contract administrator email address: charlene.wilkinson@oregonstate.edu
Station Manager/Supervisor:
Station manager/supervisor email address:

Item	2026	2027	2028
Salaries	$8,969.00	$9,238.00	$9,515.00
Benefits	$4,244.00	$4,371.00	$4,502.00
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies			
Travel			
Plot Fees			
Miscellaneous			
Total	$13,213.00	$13,609.00	$14,017.00

Footnotes: [1] Partial summer salary for Thompson salary for one month of salary for one FRA.

Objectives

1. Compare the effects of different GA types (GA_3 and $GA_{4+7)}$ on harvest and post-harvest quality, including fruit size, firmness, and return bloom. Cultivars: Bing, Regina

2. Determine the benefits of different GA_3 application timings, including early (cell division), middle (pit hardening), and late (cell expansion) on harvest and post-harvest quality. Cultivars: Bing, Regina

3. Determine the quality benefit of single vs split applications of GA_3 on self-fertile cultivars. Cultivars: Skeena, Lapins

4. Develop and disseminate an up-to-date roadmap for GA use, including comparisons of products and updated timing recommendations.

Significant Findings

All findings refer to treatment vs. no-spray control and indicate statistical differences.

Objective 1: Different Products (GA_3 and GA_{4+7})

<u>Regina</u>

Harvest

- GA_3 caused increase in size and firmness.
- GA_3 caused decrease in sugars, acids and color.
- GA_{4+7} had no effect on size, firmness, or quality.

Post-harvest

- GA_3 caused increase in size and firmness.
- GA_3 caused decrease in sugars and acids.
- GA_{4+7} had no effect on size or firmness but reduced stem pull.

<u>Bing</u>

Harvest

- GA_3 did not affect size, but increased firmness.
- GA_3 had no statistically significant effect on quality.
- GA_{4+7} had no effect on size, firmness, or quality.

Post-harvest

- GA_3 did not affect size, but increased firmness.
- GA_3 had no effect on quality.
- GA_{4+7} had no on size, firmness, or quality.

Objective 2: GA_3 Timing (first white, translucent, beginning of reddening, ~10 days prior to harvest)

<u>Regina</u>

Harvest

- Middle (translucent green) application increased size and firmness.
- Late (beginning of reddening) and very late (~10 day prior to harvest) applications increased stem pull.
- Early (first white) application increased acidity, middle and late decreased acidity.
- Late application decreased color.

Post-harvest

- Middle and late applications increased size.
- Late and very late applications increased stem pull.

- Middle application decreased sugars.
- Middle and late applications decreased acidity.

Bing
Harvest

- None of the applications affected size.
- Middle, late, and very late applications increased firmness.
- Late and very late applications increased stem pull.
- No applications affected sugars, acids, or color.

Post-harvest

- None of the applications affected size.
- Late application increased firmness.
- Late and very late applications increased stem pull.
- No applications affected sugars, acids, or color.

Objective 3: Single (translucent) **vs. Split** (translucent and straw) **GA_3**
Lapins
Harvest

- Neither single or split applications affected size.
- Single and split both increased firmness – split increase was higher.
- Split increased stem pull.
- Single and split both decreased color – split decreased it more.

Post-harvest

- Both single and split applications increased size (specifically weight).
- Single and split both increased firmness – split increase was higher.
- Split increased stem pull.

Skeena
Harvest

- Neither single or split applications affected size.
- Single and split both increased firmness.
- Both single and split decreased acidity and color.

Post-harvest

- Both single and split decreased acidity.

Methods

Objective 1. Compare the effects of different GA types (GA_3 and GA_{4+7}) on harvest and post-harvest quality, including fruit size, firmness, and return bloom. Cultivars: Bing, Regina

In 2025, field studies were conducted at experimental orchard at Oregon State University's Mid-Columbia Agricultural Research and Extension Center (MCAREC) in Hood River, OR. Treatments included two products ProGibb® LV Plus (5.7% w/w) as GA_{4+7} and ProVide® 10SG (10.0% w/w) as GA_3 (Valent USA Corp., Walnut Creek, CA) applied to 'Bing' and 'Regina'. A single application of 5 ppm GA_{4+7} made at first white (>50% flowers with white petals) was compared with single 20



Figure 1. GA_{4+7} spray application to 'Regina' sweet cherry trees at the first-white stage using a backpack sprayer.

ppm GA_3 applications made when pit hardening began (the beginning of Stage II fruit development) i.e., at the translucent green, and also when pits were fully hardened (the end of Stage II) i.e. at straw with red color beginning to develop. Treatments were applied to the whole canopy. Each experimental unit was replicated three times, with three trees per replication, and treatments were arranged in a randomized complete block design (RCBD). A nonionic surfactant (Simulaid; Genesis Agri Products Inc., Yakima, WA) was added at 0.1% (v/v), and treatments were applied until uniform and complete canopy coverage was achieved, with spray reaching the point of runoff. Applications were made using an SR 450 gasoline-powered backpack sprayer (STIHL Inc., Virginia Beach, VA). The six-nozzle backpack sprayer was calibrated using a SpotOn Sprayer Calibrator (Innoquest, Inc., Woodstock, IL, resulting in an output of 0.45 gal min^{-1}. At a target spray volume of 200 gal $acre^{-1}$ (0.386 gal $tree^{-1}$), the spray time was approximately 50 seconds per tree, with 25 seconds allocated to each side of the tree. An unsprayed control was included in all studies and received H_2O + surfactant (0 ppm GA).

Objective 2. Determine the benefits of different GA_3 application timings, including early (cell division), middle (pit hardening), and late (cell expansion) on harvest and post-harvest quality. Cultivars: Bing, Regina



Figure 2. GA_3 spray coverage on ‘Lapins’ cherries at the straw stage, with visible droplets on fruit and leaf surfaces.

In 2025, field studies were conducted in an experimental orchard at Oregon State University’s Mid-Columbia Agricultural Research and Extension Center in Hood River, OR. Treatment included different timings of single GA_3 applications evaluated on ‘Bing’ and ‘Regina’. Applications were made at four timings: first white (>50% flowers with white petals), when pit hardening began (the beginning of Stage II fruit development) i.e., at the translucent green, when pits were fully hardened (the end of Stage II) i.e. at straw with red beginning, and at final fruit sizing (~10-14 days prior to harvest). The application method follows as in objective 1 methods.

Objective 3. Determine the quality benefit of single vs split applications of GA_3 on self-fertile cultivars. Cultivars: Skeena, Lapins

In 2025, field studies were conducted at experimental orchard at Oregon State University’s Mid-Columbia Agricultural Research and Extension Center in Hood River, OR. Treatments included a comparison of single and split applications of GA_3 using the self-fertile cultivars ‘Skeena’ and ‘Lapins’. For the single application, GA_3 was applied at 20 ppm when pit hardening began (the beginning of Stage II fruit development) i.e., at the translucent green. For the split application, 12 ppm GA_3 was applied at the translucent green followed by 8 ppm when pits were fully hardened (the end of Stage II) i.e. at straw stages.

For objective 1, 2, and 3, 100 fruits were picked during commercial harvest from each tree utilized during the study. Fruits were harvested from all sides of the tree that were accessible by hand, while avoiding fruit from the very top and bottom of the canopy. Following harvest, fruits were transferred to the cold room (36°F). The next day, 50 fruits were transferred to the laboratory. Fruit weight, diameter, firmness, °Brix, and titratable acidity were measured on 50 fruits at harvest and on another 50 fruit after 21 days of cold storage. Fruit color was measured only at harvest. Return bloom will be assessed the following spring.

Problems/Limitations Encountered

For objectives 1 and 2 with cultivar 'Bing', 40 out of 90 trees could not be included in the study because they were incorrectly identified on MCAREC maps, reducing the sample size. However, valid statistical methods were used to account for available datasets to evaluate the treatment effect.

Objective 4. Develop and disseminate an up-to-date roadmap for GA use, including comparisons of products and updated timing recommendations.

At the end of the three-year project results from Objectives 1-3 will be used to formulate recommendations for GA use. This will be included in the report and publication as an extension article.

Factor Tested	GA Type	Cultivar	Application Timing
Timing	GA_3	Bing	Early
GA Type, Timing			Mid
Timing			Late
Timing			Very Late
Timing		Regina	Early
GA Type, Timing			Mid
Timing			Late
Timing			Very Late
GA Type	GA_{4+7}	Bing	Early
GA Type		Regina	Early
Single/Split	GA_3	Skeena	Single
Single/Split			Split
Single/Split		Lapins	Single
Single/Split			Split

Table 1. Summary of applications used in this project.

Results and Discussion

Objective 1

At harvest: When comparing GA_{4+7} (at first white) vs GA_3 (at translucent green) along with no spray control, only GA_3 increased 'Regina' fruit size and firmness. Among quality parameters, GA_3 increased stem attachment but decreased sugar content and acids and caused fruit to be lighter color than control. GA_{4+7} had almost no effect, possibly because a single application was used (recommendation is 1-4). 'Bing' results had similar trends, though differences between GA_3 and control fruit were only significant for firmness.

When comparing GA_{4+7} vs. GA_3, only GA_3 increased 'Regina' fruit size and increased stem attachment. GA_3 also decreased sugar content and acids and caused fruit to be lighter color than control. GA_{4+7} had almost no effect, possibly because a single application was used (recommendation is 1-4). 'Bing' results had similar trends, though differences between GA_3 and control fruit were only significant for firmness, and as well increasing stem attachment.

Post-harvest: Post-harvest evaluation followed similar trend as at harvest for fruit size and firmness in both 'Regina' and 'Bing'. For quality parameters, GA_3 increased 'Regina' stem attachment but reduced sugar content and acids as general trend. For 'Bing', GA_3 had no effect on quality expect increasing stem attachment but only at straw state.

GA_{4+7} vs. GA_3			Size		Firmness	Quality			
			Fruit Weight (g)	Diameter (mm)	Firmness (g/mm)	Stem Pull (kgf)	Sugars (°Bx)	TA (%)	Color (CTIFL 1-7)
Regina	Harvest	Control	11.19 a	27.74 ab	301.81 a	-0.96 ab	23.31 b	0.88 b	6.22 b
		GA_{4-7}	10.88 a	27.38 a	294.87 a	-0.91 a	22.88 ab	0.86 b	6.34 b
		GA_3	12.09 b	28.54 b	326.08 b	-1.00 b	21.83 a	0.78 a	5.78 a
	Post Harvest	Control	11.07 a	27.47 ab	364.23 a	-1.16 b	22.75 b	0.77 b	
		GA_{4-7}	10.84 a	27.10 a	356.86 a	-1.00 a	22.03 b	0.75 b	
		GA_3	11.82 b	28.22 b	382.55 b	-1.24 b	20.69 a	0.66 a	
Bing	Harvest	Control	8.12 a	24.84 a	168.78 a	-0.66 a	21.90 a	1.05 ab	5.76 ab
		GA_{4-7}	7.87 a	24.38 a	174.31 a	-0.67 a	22.16 a	1.08 b	5.82 b
		GA_3	8.53 a	25.24 a	192.12 b	-0.69 a	20.49 a	0.98 a	5.41 a
	Post Harvest	Control	8.01 a	25.31 a	203.41 a	-0.567 a	21.36 a	1.07 a	
		GA_{4-7}	7.73 a	24.84 a	203.82 a	-0.585 a	20.62 a	0.96 a	
		GA_3	8.51 a	25.78 a	218.86 b	-0.574 a	19.79 a	0.97 a	

Objective 2

At harvest: For 'Regina', early (first white) GA_3 applications did not affect size or firmness but did increase acid content. Middle (translucent green) applications of GA_3 had the greatest effect on size and firmness. Late (start of reddening) and Very Late (~10 days prior to harvest) applications did not improve firmness but increased stem attachment. Middle and Late applications decreased acidity and lightened color.
'Bing' results had similar trend in size but lacked significant differences. Middle, Late and Very Late applications increased firmness in 'Bing'. Stem attachment increases were seen in Late and Very Late applications. No differences were observed in sugars, acids, or color.

Post-harvest: During postharvest, GA_3 timing affected several quality traits in 'Regina'. Middle and Late applications increased fruit weight, while Middle application increased fruit diameter. Middle application also resulted in the greatest firmness, whereas Very Late had the lowest firmness. Late and Very Late applications increased stem attachment. The middle application reduced titratable acidity.

In 'Bing', GA_3 timing did not affect fruit size, while Late application increased firmness. Late and Very Late applications increased stem attachment. No differences were observed in sugar content, while Middle and Very Late applications reduced titratable acidity.

	GA_3 Timing	Fruit Weight (g)	Diameter (mm)	Firmness (g/mm)	Stem Pull (kgf)	Sugars (°Bx)	TA (%)	Color (CITFL 1-7)
Regina	Harvest Control	11.19 a	27.74 a	301.81 ab	-0.96 a	23.31 ab	0.88 b	6.22 bc
	Harvest Early	11.28 a	27.74 a	281.43 a	-0.97 a	24.14 b	0.94 c	6.32 c
	Harvest Middle	12.09 b	28.54 a	326.08 c	-1 ab	21.83 a	0.78 a	5.78 ab
	Harvest Late	11.76 ab	28.1 a	303.74 b	-1.08 c	22.12 a	0.78 a	5.68 a
	Harvest Very Late	11.59 ab	28.08 a	292.14 ab	-1.06 bc	23.13 ab	0.9 bc	5.86 abc
	Post Harvest Control	11.07 a	27.47 ab	364.23 bc	-0.96 a	22.75 bc	0.77 c	
	Post Harvest Early	10.92 a	27.10 a	341.82 ab	-0.97 a	24.06 c	0.81 c	
	Post Harvest Middle	11.82 b	28.22 b	382.55 c	-1.00 ab	20.69 a	0.66 a	
	Post Harvest Late	11.81 b	27.92 ab	355.82 ab	-1.08 c	21.93 ab	0.69 ab	
	Post Harvest Very Late	11.37 ab	27.61 ab	339.11 a	-1.06 bc	22.47 bc	0.75 bc	

		Fruit Weight (g)	Diameter (mm)	Firmness (g/mm)	Stem Pull (kgf)	Sugars (°Bx)	TA (%)	CTIFL Color
Bing	Harvest Control	8.12 a	24.84 a	168.78 a	-0.66 a	21.90 a	1.05 a	5.76 a
	Harvest Early	8.13 a	24.75 a	170.41 ab	-0.70 ab	23.10 a	1.14 a	5.75 a
	Harvest Middle	8.53 a	25.24 a	192.12 c	-0.69 ab	20.49 a	0.98 a	5.41 a
	Harvest Late	8.26 a	24.92 a	205.93 d	-0.89 c	22.33 a	1.10 a	5.40 a
	Harvest Very Late	7.94 a	24.47 a	179.40 b	-0.78 bc	21.81 a	1.17 a	5.58 a
	Post Harvest Control	8.01 a	25.31 a	203.41 a	-0.57 a	21.36 a	1.07 ab	
	Post Harvest Early	8.14 a	25.29 a	209.82 a	-0.59 ab	22.54 a	1.15 b	
	Post Harvest Middle	8.51 a	25.78 a	218.86 a	-0.57 ab	19.79 a	0.97 a	
	Post Harvest Late	8.26 a	25.51 a	243.16 b	-0.71 c	21.90 a	1.13 b	
	Post Harvest Very Late	7.95 a	25.03 a	207.85 a	-0.66 bc	20.39 a	1.04 a	

Objective 3

At harvest: In ‘Lapins’, both single and split applications trend towards larger fruit, though the differences are not statistically significant, likely due to fruit-to-fruit variability. Both applications increased firmness, but the split application increased it more. The split application also increased stem attachment. Both applications caused lighter-colored fruit, but the split application fruit were lightest. ‘Skeena’ results were similar but had an increase in acidity with both applications and no effect on stem attachment.

Post-harvest: In ‘Lapins’, both single and split applications increased fruit weight, while neither affected fruit diameter. Both applications increased firmness, with the split application having the greatest effect. The split application also increased stem attachment. Neither application affected sugar or acid content. In ‘Skeena’, GA_3 applications had no effect on fruit size, firmness, stem attachment, or sugar content, but both applications reduced acidity.

GA_3 Single vs. Split			Size		Firmness	Quality			
			Fruit Weight (g)	Diameter (mm)	Firmness (g/mm)	Stem Pull (kgf)	Sugars (°Bx)	TA (%)	CTIFL Color
Lapins	Harvest	Control	9.39 a	26.12 a	203.61 a	-0.62 a	19.46 a	0.73 a	5.47 c
		Single	10.58 a	27.17 a	227.57 b	-0.7 a	19.85 a	0.73 a	4.93 b
		Split	10.53 a	27.29 a	242.91 c	-0.79 b	19.81 a	0.74 a	4.45 a
	Post Harvest	Control	9.14 a	26.26 a	255.35 a	-0.52 a	18.65 a	0.72 a	
		Single	10.42 b	27.41 a	273.25 b	-0.56 a	18.95 a	0.72 a	
		Split	10.44 b	27.42 a	291.58 c	-0.68 b	18.87 a	0.71 a	
Skeena	Harvest	Control	10.01 a	27.81 a	279.21 a	-0.71 a	18.37 a	0.76 b	5.76 b
		Single	10.04 a	27.92 a	307.20 b	-0.73 a	17.79 a	0.71 a	5.26 a
		Split	9.26 a	26.98 a	315.30 b	-0.72 a	17.54 a	0.69 a	5.14 a
	Post Harvest	Control	9.88 a	27.60 a	331.89 a	-0.71 a	17.95 a	0.70 b	
		Single	9.83 a	27.60 a	352.17 a	-0.69 a	17.28 a	0.64 a	
		Split	9.07 a	26.66 a	352.10 a	-0.68 a	16.91 a	0.64 a	

Objective 4

Results from the 2025 harvest data were presented at the MCAREC Field Day in July 2026.

Literature Review

Gibberellic Acid (GA) is a plant hormone that is involved in a number of aspects of plant growth and development. GA is used extensively as a Plant Growth Regulator (PGR) in the sweet cherry industry to increase fruit size and fruit firmness. Two different GAs are registered for use in improving sweet cherry fruit quality: GA_3 (Falgro®, ProGibb®) and GA_{4+7} (NovaGib®, ProVide®).[1–3] Direct comparisons between the effects of the two GAs are lacking, and timing and application number recommendations for each product are very different. GA_3 is typically applied at translucent green to straw timing, corresponding to the end of pit hardening for most cultivars. Application recommendations range from single to multiple sprays, with multiple sometimes being recommended for self-fertile or heavily-cropped trees.[4,5] GA_{4+7} recommendations consist of applications much earlier in development, with 1-4 applications every 7-10 days, starting when flowers are at first white. Multiple studies have shown that early timing for GA_3 might give more of a boost to fruit size than applications at straw,[6] but this has yet to be established. Additionally, it is widely accepted that late applications of GA can boost firmness, but the details related timing and benefit of late sprays are lacking. The goal of this project is develop up-to-date recommendations regarding GA applications in sweet cherry orchards. Here we focus on GA type, timing, and single vs. split applications. Future work beyond the scope of this project could potentially provide finer-tuned timings, application numbers, rates, or specific recommendations for additional cultivars.

Literature/Material Cited

1. ProVide® Plant Growth Regulator | Valent BioSciences. *Valent BioSciences - Global Agriculture* https://www.valentbiosciences.com/agriculture/products/provide/.

2. ProGibb® Plant Growth Regulator | Valent BioSciences. *Valent BioSciences - Global Agriculture* https://www.valentbiosciences.com/agriculture/products/progibb/.
3. Products. *Fine Americas* https://www.fine-americas.com/products (2014).
4. Thompson, A. Pest management guide for tree fruits: Hood River, The Dalles, White Salmon, Rogue Valley, Umatilla County. *Extension Communications* https://extension.oregonstate.edu/catalog/pub/em-8203-pest-management-guide-tree-fruits-hood-river-dalles-white-salmon-rogue-valley (2014).
5. Prengaman, K. Got gibberellins? *Good Fruit Grower* https://goodfruit.com/got-gibberellins/ (2019).
6. Zhang, C. & Whiting, M. D. Improving 'Bing' sweet cherry fruit quality with plant growth regulators. *Sci. Hortic.* **127**, 341–346 (2011).

Project Title: Cherry rootstock research and demonstration orchard

Report Type: Continuing Project Report

Primary PI: Bernardita Sallato
Organization: Washington State University
Telephone: 509-7869205
Email: b.sallato@wsu.edu
Address: 24106 North Bunn Rd
Address 2:
City/State/Zip: Prosser, WA 99350

Co-PI 2: Matthew Whiting
Organization: Washington State University
Telephone: 509-7969225
Email: mdwhiting@wsu.edu
Address: 24106 North Bunn Rd
Address 2:
City/State/Zip: Prosser, WA 99350

CO-PI 3: Antonia Sanchez Labbe
Organization: Washington State University
Telephone: 509-7869205
Email: a.sanchezlabbe@wsu.edu
Address: 24106 North Bunn Rd
Address 2:
City/State/Zip: Prosser, WA 99350

Cooperators: Variety International, NNII, Simplot, Olsen Brothers.

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $ 28969
Total Project Request for Year 2 Funding: $ 22384
Total Project Request for Year 3 Funding: $ 22939

Other related/associated funding sources: Sweet Cherry Demonstration Block. Awarded. PI Matthew Whiting, Co-PI Bernardita Sallato.
Funding Duration: 2022 – 2024
Amount: 12,000
Agency Name: Northwest Nursery Institute, Awarded
Notes: Funding for existing irrigation and weather station and trellis post.

Budget 1
Primary PI: Bernardita Sallato
Organization Name: Washington State University
Contract Administrator: Hollie Tuttle
Telephone: **509 7869226**
Contract administrator email address: prosser.grants@wsu.edu
Station Manager/Supervisor: Naidu Rayapati

Salaries	8967	9326	9699
Benefits	4382	4558	4740
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies	15500	7500	7500
Travel			
Plot Fees			
Testing services	120	1000	1000
Total	28969	22384	22939

Footnotes: Salary support (20% FTE) for research lab assistant plus benefits at 48.9% rate. Supplies include orchard establishment, soil preparation, fertilization, management, wiring, irrigation, lab supplies. Lab testing includes external testing for soil and tissue nutrient tests.

Objectives

Our aim is to develop a systematic and contrasting evaluation of new cherry rootstocks for Washington and Oregon growers. We have the following objectives:

1. Establish a public sweet cherry rootstock research and demonstration orchard in Prosser.
2. Evaluate key horticultural traits imposed by different rootstocks including precocity, bloom timing, yield and fruit quality.
3. Evaluate adaptation to environmental threats provided by new rootstocks, heat, drought and frost tolerance.
4. Deliver information to Washington and Oregon growers.

Significant Findings

- Of 360 planted trees, 19 died; losses were greatest in Alva (17.5%) and Rulan 8 (12.5%), while EC11 and Gisela 12 had no losses. The cause remains undetermined.
- There were significant differences among genotypes in root system size and root types (i.e, structural vs. fine feeder roots) in the nursery trees
- Larger root systems at planting did not consistently correspond to greater first-season growth.
- Alva and Mazzard had the highest image-based root-size scores, but MxM14 had the greatest trunk-diameter increase (14 mm), despite its smaller initial root system.
- Rulan 8 had large initial root size but limited tree growth. It had the lowest tree height (62 inches), significantly below Mazzard and MxM14, and the smallest reported trunk-diameter increase (3.8 mm).
- Rootstocks differed significantly in leaf Ca and Mg concentrations. Krymsk®7 had the highest levels (1.89% Ca and 0.49% Mg); MxM14 and Gisela 12 were below the recommended minimum for both nutrients.
- Leaf B differed substantially despite no B fertilizer application. Rulan 8 had the highest concentration (103.5 mg/kg), significantly above all other rootstocks; four rootstocks exceeded the 60 mg/kg reference limit.

Methods

1. Establish a public sweet cherry rootstock research and demonstration orchard in Prosser.

The sweet cherry rootstock orchard was established in April 15th, 2026, at the Washington State University Irrigated Agriculture Extension and Research Center in Prosser headquarters site, contiguous to the new sweet cherry demonstration orchard, supported by the Northwest Nursery Improvement Institute (NNII) (Lead PI -Whiting). The cultivar ‘Skeena’ was grafted onto nine rootstocks and planted in 4 rows, in blocks of 10 trees each, in a randomized block design. The rootstock selection includes the standards; Mazzard, Gisela 12 and MxM14, which will be contrasted with A9xVSL2 (Krymsk 5), Rulan 8, Krymsk®7, EC 11, EC 12 and Alva (RVL 4). We were expecting Weigi 3, but there were not enough trees, thus Weigi was not included in this year’s planting. These candidate rootstock selections were identified by members of the Northwest Nursery Improvement Institute.

Before planting, bare-root trees were photographed for an image-based root-size index. At planting, 10 g of monoammonium phosphate was placed in each planting hole and covered with soil before

positioning the trees. Trees were headed to approximately knee height and irrigated with two drip lines for seven hours.

Trees were trained as a vertical wall with multiple leaders in one plane, approximately 8–10 inches apart. Automated irrigation includes two drip lines and a sprinkler line. On-site weather observations and soil-moisture monitoring supported irrigation management.



Figure 1. Photos of the planting process at IAREC, Prosser farm (left), headed trees right after planting (right).

2. Evaluate horticultural traits imposed by different rootstocks, precocity, bloom timing, yield and fruit quality.

During the establishment phase (years 2026 to 2028), data collection will focus on tree survival, tree height, susceptibility to diseases when present, trunk cross sectional area, shoot growth, branching habit, leaf nutrient levels, and trunk growth. When possible, we will include assessments of precocity, bloom timing, yield, and fruit quality.

Root index evaluation: The six reference photographs illustrate increasing visible root size on a 1–6 ordinal scale. Scores describe the spread and apparent density of roots in two-dimensional photographs taken before planting. The index does not estimate absolute root volume.



Figure 2. Illustration of scoring rate for image base root size; 1 = smaller and short root systems, 6 = largest and denser root system.

3. Evaluate adaptation to environmental threats provided by new rootstocks, heat, drought and frost tolerance.

Trees will be evaluated for heat and cold tolerance measurements: bud hardiness, stem water potential during heat days, leaf photosynthesis, root growth. We will monitor other traits, such as bacterial canker, rain cracking, sunburn among others, when conditions permit.

4. Deliver information to Washington and Oregon Tree Fruit industry.

Starting in 2027, we will conduct a field tour for growers (Co PI Sanchez-Labbe), in close coordination with Oregon State University (A. Thompson). This will provide an opportunity for contrasting information between regions.

Results and Discussion

1. Establish a public sweet cherry rootstock research and demonstration orchard in Prosser.

The sweet cherry rootstock research and demonstration orchard was established at IAREC Prosser in April 15th, 2026. Following planting, nighttime air temperature reached approximately 30°F on April 15–17, however we observed no visible frost injury. The temperature record (Figure 3) extends from near-freezing conditions to approximately 99°F; 35 days above 90°F occurred mainly in July and August. This first-season exposure provides context for establishment and general conditions for the block.

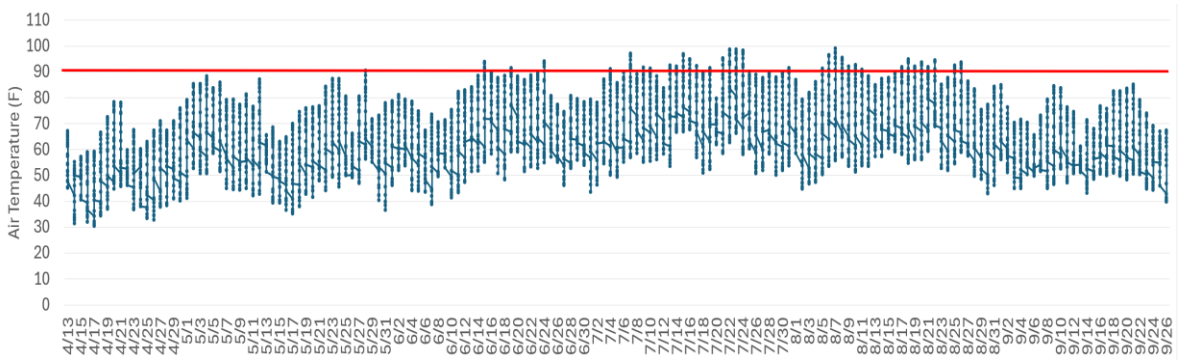


Figure 3. Hourly temperatures (F) recorded at IAREC Sweet Cherry Rootstock block.

The irrigation in the orchard is automated (Wiseconn Systems) and soil moisture was recorded throughout the season at five depths. The average series generally fluctuated within 30 to 50% soil moisture, with two short declines and recoveries.

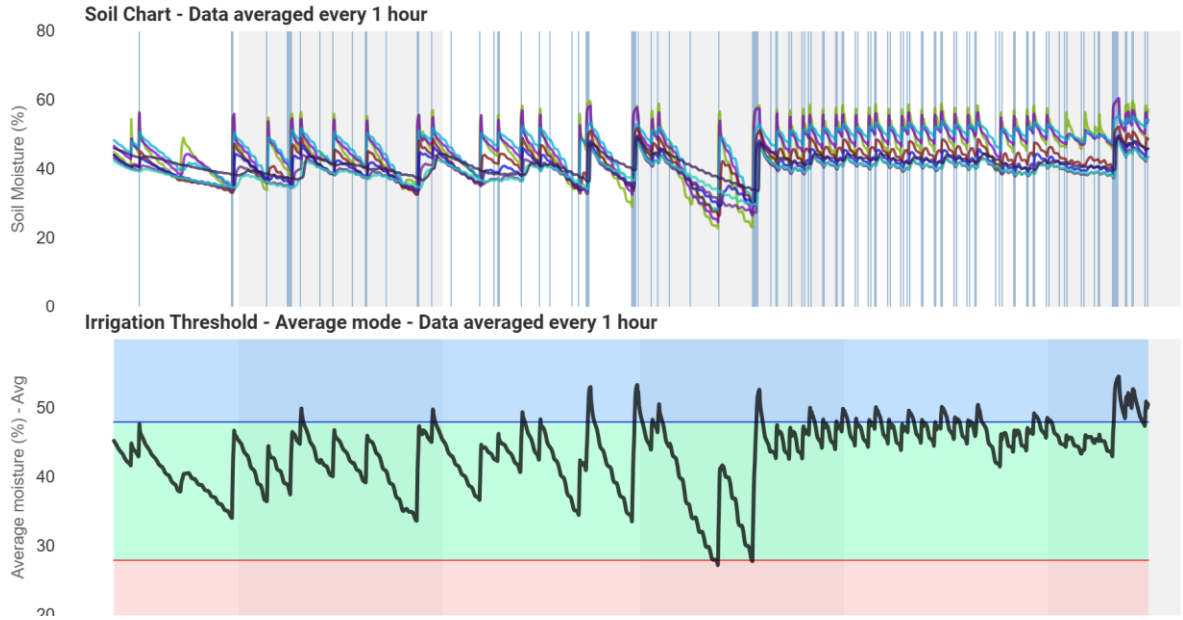


Figure 4. Daily irrigation at five depths (top) and average (bottom).

At planting, 10 g of monoammonium phosphate (MAP) was incorporated to the planting hole, covered with a layer of soil (to prevent root burning) before placing the trees. During the second week of June, an additional 20 lb of N and 20 lb of P_2O_5 per acre were added via fertigation (in-kind donation from Simplot).

2. Evaluate horticultural traits imposed by different rootstocks, precocity, bloom timing, yield and fruit quality.

2.1 Rootstock variability at planting

Root size ranking was higher for Alva and Mazzard compared to Krymsk®7, Gisela 12 and MxM14, while minimal differences were found with EC 11, EC 12, Rulan 8 and A9xVSL2 (Figure 5). The photographs below illustrate differences in lateral spread and fine-root density across rootstocks and

examples representing each rootstock. A wide, less dense root system can have a high visual score as was observed for Mazzard (Figure 6), that distinguished by having large and thicker root system, however with fewer fine roots, compared to the other high scored root systems; Alva, Rulan 8 and A9xVSL2 rootstocks (Figure 6)

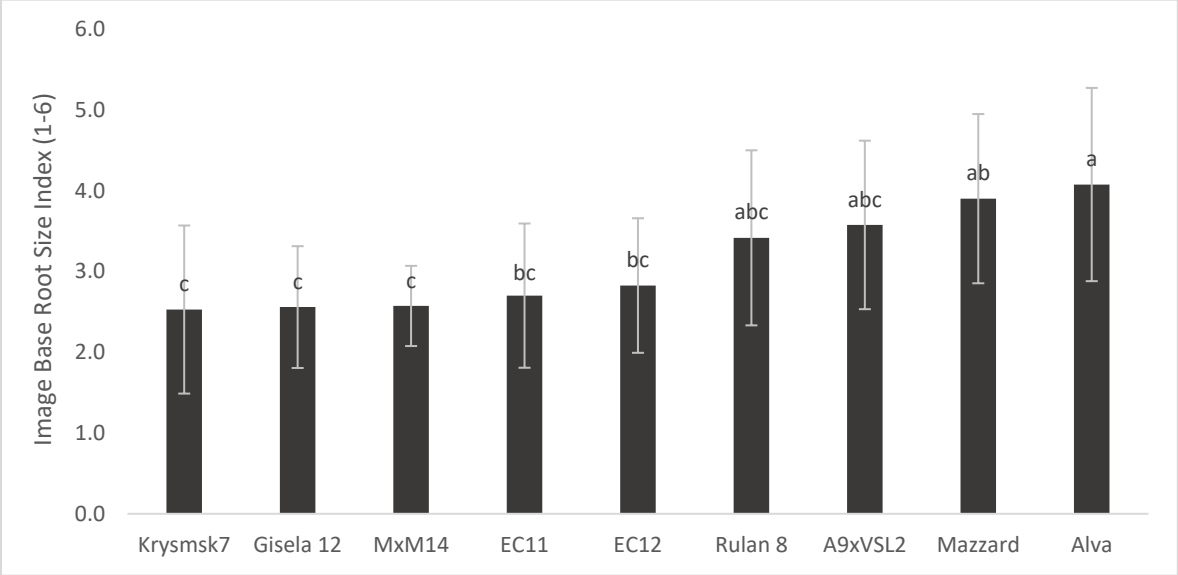


Figure 5. Image based root size index differences across rootstocks. Error bars represent standard deviation of the sample. Different letters above the bars indicate significant differences using Tukey test at $p < 0.05$.

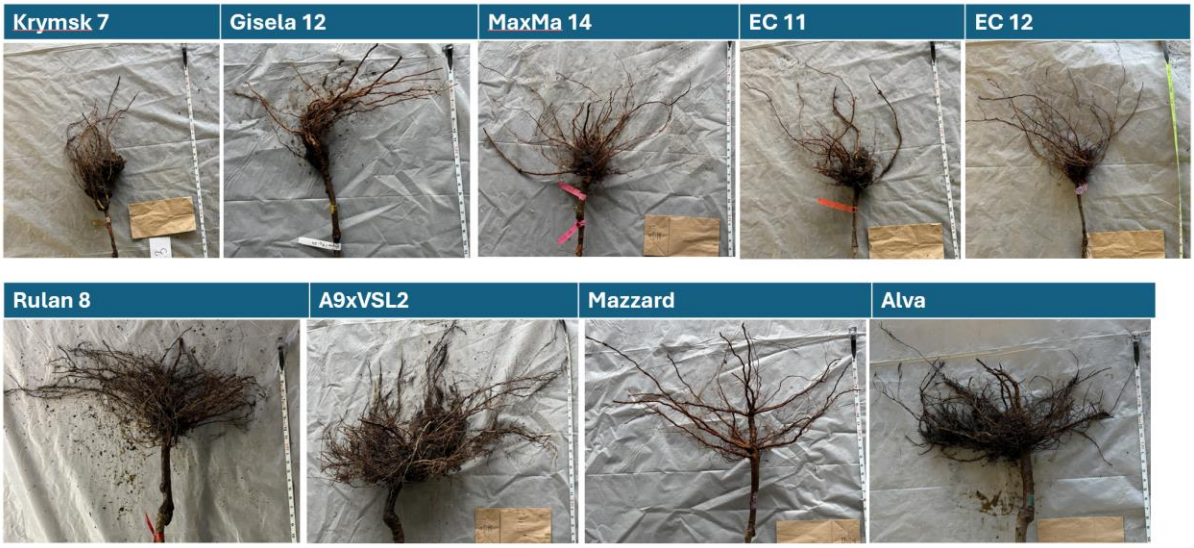


Figure 6. Image of a root system representing each rootstock.

2.2 Tree mortality

There were 19 dead trees by the end of the 2026 season. Alva had seven losses (17.5% of 40 planned trees) and Rulan 8 five (12.5%); A9xVSL2 and EC12 had two each (5%), and Krymsk®7, Mazzard

and MxM14 each had one (2.5%). With no losses in EC11 and Gisela 12. These trees will be replaced in 2027. The cause of mortality was unclear.

2.3 Tree growth

Total tree height was measured at the end of the growing season (September 29th), considering the tallest leader. The smallest trees were Rulan 8 at ca. 62 inches of height, significantly smaller than MxM14 and Mazzard, with close to 80 inches. Interestingly, there was no relationship between initial nursery tree root system characteristics and end of season tree height – rootstocks with the smallest root system; such as Krymsk®7, Gisela 12 and MxM14 were as tall as Mazzard and Alva (both with larger root systems).

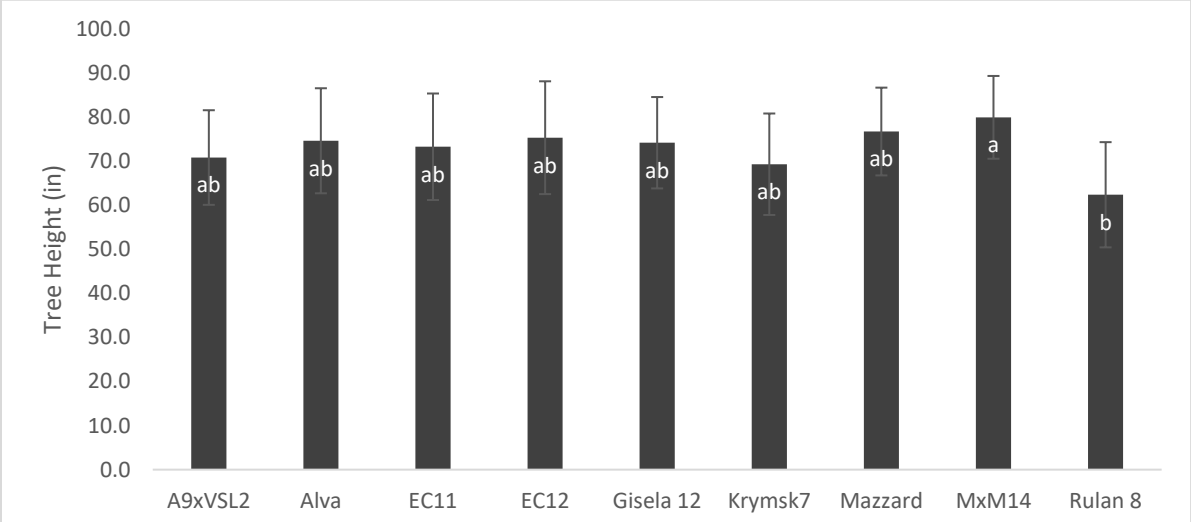


Figure 7. Tree height at the end of the growing season September 29th, 2026. Error bars represent standard error. Different letters above the bars indicate significant differences using Tukey test at $p < 0.05$.

Trunk diameter at planting ranged between 10.3 (0.41 inches) to 23.6 mm (0.93 inches) being larger in A9xVSL2, Alva and Rulan and smallest in MxM14, Mazzard and EC12, with the exception of Mazzard, there results were congruent with the larger root size ranking. By the end of the season, Alva remained highest, only compared to EC12, Krymsk®7 and Mazzard (Figure 8)

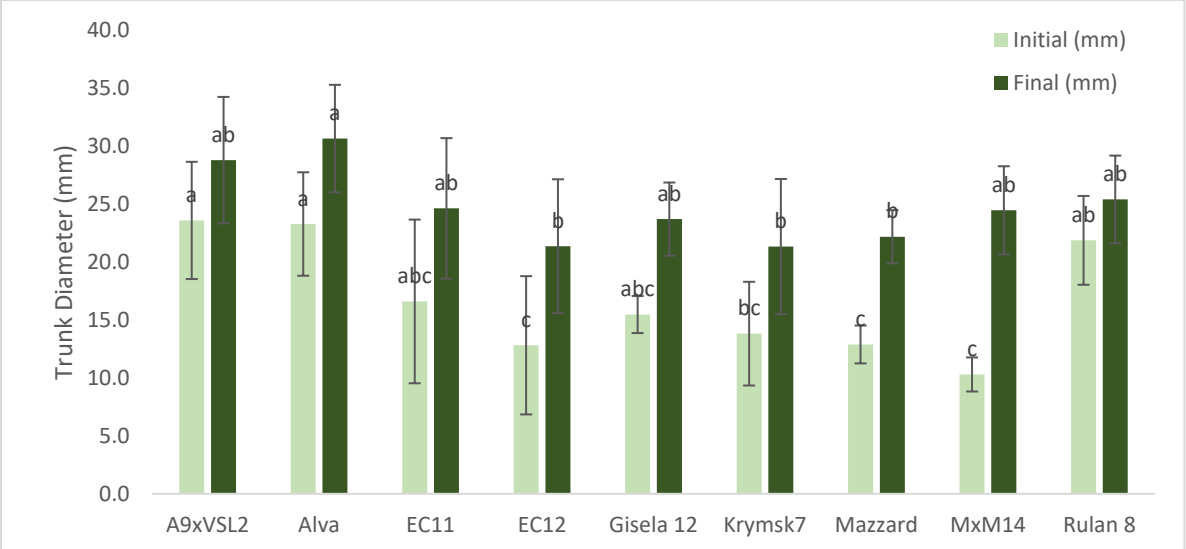


Figure 8. Trunk diameter (mm) at planting and at the end of the growing season by rootstock. Error bars represent standard deviation of the sample. Different letters above the bars indicate significant differences using Tukey test at $p < 0.05$.

The gained in trunk diameter was higher for MxM14 (14 mm), followed by Mazzard and EC12 (9.3 mm), all of them higher than Rulan 8 with only 3.8 mm trunk diameter gain.

2.3.1 Leaf nutrient concentrations

Compared to the leaf nutrient recommendation for cherries (Sallato et al., 2021), nitrogen (N) and phosphorus (P) levels were above the reference values of 3.0% and 0.3% respectively, for all nine rootstocks, with no differences among them (Table 2). Potassium (K) levels were within adequate ranges (1.8–2.3%), while sulfur (S) was in the lower range and deficient in MxM14 and Gisela 12, with no differences among rootstocks. Leaf calcium (Ca) was below the minimum standard (1.20%) in EC12, Alva, Mazzard, Gisela 12 and MxM14, all of them lower than in Krymsk®7. Leaf magnesium (Mg) was deficient (0.30%) in MxM14 and Gisela 12, both rootstocks along with Mazzard, were lower than Krymsk®7.

Regarding micronutrients, zinc (Zn) and Fe were not different across rootstocks, and levels were within standard values, except for Gisela 12. Leaf boron (B) exceeded 60 mg/kg in Krymsk®7, A9xVSL2, EC12 and Rulan 8, with the lowest values found in Gisela 12 and Alva, despite low levels of B in the soil and no added B this season. Leaf manganese (Mn) was lower in MxM14, Gisela 12, Mazzard, and EC12 compared to A9xVSL2, however all rootstocks were within adequate ranges. Similar with copper (Cu) where Mazzard had the lowest and Rulan 8 the highest, while all rootstocks were within adequate ranges (Table 2)

Table 2. Leaf nutrient levels by rootstock.

Nutrient / Rootstock	N	P	K	Ca	Mg	S	Zn	Fe	Mn	Cu	B
	%						mg/kg				
Krymsk®7	3.9	0.57	2.3	1.89 a	0.49 a	0.25	17.3	166.8	73.8 ab	7.3 ab	71.8 b
A9xVSL2	4.1	0.49	1.9	1.51 ab	0.42 ab	0.20	15.3	118.3	88.0 a	8.8 ab	62.3 bc
EC 11	4.0	0.48	2.0	1.28 abc	0.41 ab	0.21	13.3	138.3	52.8 ab	6.8 ab	54.5 bc

EC 12	4.0	0.52	2.1	1.03 bc	0.34 ab	0.22	15.8	91.3	44.5 b	6.5 ab	62.3 bc
Rulan 8	3.8	0.44	1.8	1.53 ab	0.42 ab	0.20	13.8	92.0	64.3 ab	10.3 a	103.5 a
Alva	3.7	0.47	2.1	1.18 bc	0.37 ab	0.21	16.0	116.0	59.0 ab	7.5 ab	40.3 c
MxM14	3.6	0.48	2.3	0.78 c	0.27 b	0.19	18.3	126.5	40.5 b	5.8 ab	47.3 bc
Mazzard	3.7	0.47	2.2	1.09 bc	0.33 b	0.20	12.3	111.0	46.3 b	5.3 b	49.5 bc
Gisela 12	3.8	0.37	2.0	1.13 bc	0.29 b	0.17	10.5	44.0	43.0 b	5.8 ab	39.0 c
p value	0.188	0.064	0.824	0.001	0.001	0.490	0.127	0.538	0.004	0.037	<0.0001

3. Evaluate adaptation to environmental threats provided by new rootstocks, heat, drought and frost tolerance.

3.1 Stem water potential

Stem water potential assessments were used to determine the water status of trees and can be used to assess water stress. Mean stem water potential ranged from approximately −0.39 MPa for MxM14 to −1.0 MPa for MxM14 for August 15 and 20, 2026. Stem water potential was generally more negative on August 20, when air temperature reached 85°F under clear skies (Figure 9), with no differences across rootstocks. During August 15th, when temperatures were lower, MxM14 had a third of the stem water tension of Rulan 8. Regardless of these differences, stem water potential values were never below -1.7 MPa, suggesting no stress during these measurements (Figure 9).

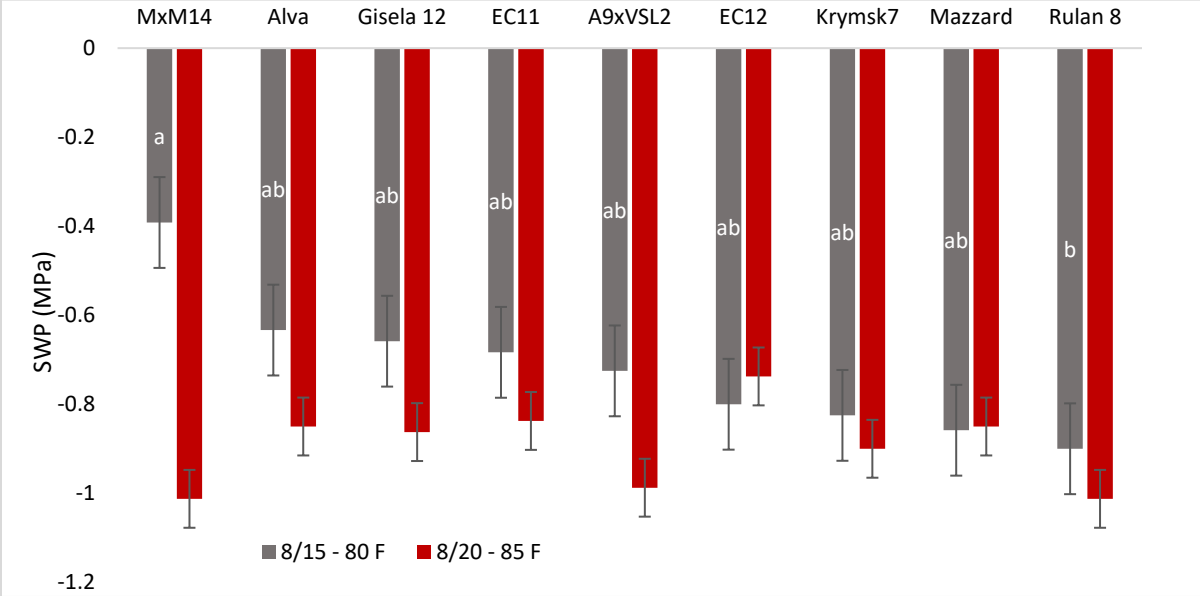


Figure 9. Mid-day stem water potential (MPa) recorded during August 15th, 2026, with air T 80 F (grey) and August 20th, 2026, with air T 85 F.

Plan for 2027

Our objective for 2027 is to continue focusing on Obj 2. Evaluating key horticultural traits imposed by different rootstocks, including tree growth, leaf area, leaf nutrient levels, gain in trunk diameter and root suckering if present. Obj 3. Evaluate adaptation to environmental threats provided by new rootstocks, heat, drought and frost tolerance. We will incorporate more frequent indicators of environmental threats; and possibly two additional indicators; autophagy in collaboration with Dr.

Smertenko and root microbiome in collaboration with Dr. Trivedi. We will monitor other traits, such as bacterial canker (Dr. Zhao), leaf sunburn, when conditions permit.
Starting in 2027, we will work on Obj. 4 to deliver information to Washington and Oregon growers. Conducting a IAREC field tour for growers (Co PI Sanchez-Labbe), in close coordination with Oregon State University (A. Thompson). This will provide an opportunity for contrasting information between regions.

Literature Review

Rootstocks affect sweet cherry vegetative growth, cropping and fruit quality, and their effects depend on crop load and training (Whiting and Lang, 2004; Whiting et al., 2005; Usenik et al., 2010). Early growth comparisons in this orchard should therefore be followed through bearing years rather than treated as fixed rankings. Koumanov et al. (2018) emphasize that irrigation, fertigation and management interact with dwarfing-rootstock performance.

Frost and summer heat can threaten Washington sweet cherry establishment and production. The 2026 Prosser weather record documents both exposures at this site, but visible absence of frost damage and a single season of growth do not establish rootstock-specific resilience. Later evaluations should pair weather and soil-water records with repeated physiological measurements and tree performance.

Rootstock can influence tree water relations, but stress responses depend on scion, age, environment and irrigation. In a severe postharvest drought experiment on four-year-old cherry trees in Hungary, MxM 14 had the most negative midday stem water potential among the studied rootstocks, while maintaining comparatively stable sap flow (Mohay and Lakatos, 2025). That result does not directly predict the first-season performance of Skeena trees in Prosser. The initial stem-water-potential observations here provide a baseline for repeated measurements during defined stress episodes; they do not establish drought tolerance.

This public replicated demonstration orchard permits side-by-side study of new and established rootstocks under the same management. Subsequent years are needed to relate survival, tree architecture and water relations to bloom, cropping and fruit quality, and to separate rootstock effects from establishment conditions.

References:

Courtney, R. 2021. Learning from the heat wave. Cherry industry loses 20 percent of the crop to heat and wonders about future impacts. Good Fruit Grower, September 2021. https://www.goodfruit.com/learning-from-the-heat-wave/

Usenik, V., Fajt N, Mikulic-Petkovsek, M., Slatnar A., Stampar, F., Veberic, R. (2010). Sweet Cherry Pomological and Biochemical Characteristics Influenced by Rootstock. J. Agric. Food Chem 58, 8, 4928–4933

Lang, G., Howell, W., Ophardt, D. and Mink, G. (1997). Biotic and abiotic stress response of interspecific hybrid cherry rootstocks. Acta Hortic. 451, 217-224 ActaHortic.1997.451.22

Whiting, M. D., and Lang, G. (2004). ‘Bing’ sweet cherry on the dwarfing rootstock ‘Gisela 5’: Crop load affects fruit quality and vegetative growth but not net CO2 exchange. J. Amer. Soc. Hort. Sci. 129:407 -415.

Whiting, M. D., Lang, G., and Ophardt, D. (2005). Rootstock and training system affect sweet cherry growth, yield, and fruit quality. *HortScience*, *40*(3), 582-586.

Sallato, B. 2021. Leaf Tissue Analysis. WSU Tree Fruit Extension. https://treefruit.wsu.edu/orchard-management/soils-nutrition/leaf-tissue-analysis/ (cherry leaf reference ranges and sampling guidance; accessed September 2026).

Mohay, B., and Lakatos, L. 2025. Impact of Severe Drought Stress on Water Relations of Young Cherry Trees Grafted onto Growth-Reducing Rootstocks. Horticulturae 11:997. https://doi.org/10.3390/horticulturae11090997

Koumanov, K. S., Staneva, I. N., Kornov, G. D., and Germanova, D. R. 2018. Intensive sweet cherry production on dwarfing rootstocks revisited. Scientia Horticulturae 229:193–200. https://doi.org/10.1016/j.scienta.2017.11.009

Project Title: Effects of maturity and weather on fruit quality

Report Type: Continuing Project Report

Primary PI: Lee Kalcsits
Organization: Washington State University
Telephone: 509-293-8764
Email: lee.kalcsits@wsu.edu
Address: 1100 N Western Ave.
Address 2:
City/State/Zip: Wenatchee/WA/98801

Cooperators: Barbara Blanco-Ulate (UC Davis)

Project Duration: 1-Year

Total Project Request for Year 1 Funding: $ 41,820

Other related/associated funding sources: Cherry Stem Retention II: Effects of Maturity, Weather, and Preharvest Treatment Timing on Fruit Quality
Funding Duration: 2026
Amount:
Agency Name: California Cherry Board

WTFRC Collaborative Costs: none

Budget 1
Primary PI: Lee Kalcsits
Organization Name: Washington State University
Contract Administrator: Kevin Rimes
Telephone:
Contract administrator email address: kevin.rimes@wsu.edu
Station Manager/Supervisor: Stephen Ficklin
Station manager/supervisor email address: stephen.ficklin@wsu.edu

Item	2026
Salaries	$12,046.00
Benefits	$4,818.00
Wages	
Benefits	
RCA Room Rental	
Shipping	
Supplies	$3,300.00
Travel	$1,460.00
Plot Fees	
Miscellaneous	
Total	$21,624.00

Footnotes:

Budget 2
Primary PI: Ashley Thompson and Brian Pearson
Organization Name: Oregon State University
Contract Administrator: Brian Pearson
Telephone:
Contract administrator email address: brian.pearson@oregonstate.edu
Station Manager/Supervisor: Brian Pearson
Station manager/supervisor email address: brian.pearson@oregonstate.edu

Item	2026
Salaries	$9,213.00
Benefits	$4,671.00
Wages	
Benefits	
RCA Room Rental	
Shipping	
Supplies	$5,398.00
Travel	$914.00
Plot Fees	
Miscellaneous	
Total	$20,196.00

Footnotes:
[1]Salary for 0.15 FTE for Faculty Research Assistant Niall Millar

[2]Fringe benefits calculated at 50.7% of salary

[3]Supplies include infield temperature, relative humidity sensors, and par sensors, a Nikon camera for color analysis, and miscellaneous lab supplies for field collection

[4]Travel to and from field collection sites over 8 weeks

Objectives

1. Validate maturity and fruit-quality relationships to define the optimal harvest window for ‘Coral Champagne.’

2. Evaluate the influence of environmental conditions on stem retention.

Significant Findings

Fruit quality data was acquired for all seven locations spanning 4-5 cherry fruit color stages

There were locations that lost stem retention force earlier than other sites even though fruit firmness progression was similar across all sites in OR and WA.

Fruit harvested at stage 6 for all locations had fruit with firmness above 300 g mm-1 and stem retention force of above 500 g force. However, warmer sites like Pasco South and Mattawa had higher proportions of fruit with low fruit retention force.

More locations will be sampled in 2027 including resampling of the same sites pending cooperator approval.

Methods

Cherry orchards. The study will focus on ‘Coral Champagne’ cherries. This cultivar was chosen to align with the proposed cultivar targeted by Dr. Blanco-Ulate and the California Cherry Board. WSU personnel will work with three commercial orchards located in Washington state with nearby WSU AgWeatherNetwork stations to capture meaningful differences in microclimate (heat load, canopy humidity, air movement, canopy light environment) while keeping sampling logistically feasible. Similarly, OSU will also work with three commercial orchards with nearby weather stations to measure weather data.

Orchard blocks were selected in collaboration with participating growers. Orchard identification and grower coordination will be supported by Dr. Blanco-Ulate and her UCCE collaborators (Dr. Giulia Marino and Dr. Mohammad Yaghmour). We will prioritize blocks that are commercially managed and representative of industry practice rather than experimental plots. For each selected block, we will document standard grower programs, including the use of growth regulators and nutritional sprays (e.g., gib products, Apogee, calcium programs, and any other preharvest sprays), so that these background practices can be considered during data interpretation.

State	County	Orchard / Site
Washington	Franklin	Pasco North
Washington	Franklin	Pasco South
Washington	Douglas	Mattawa
Washington	Grant	Orondo
Washington	Klickitat	Dallesport
Oregon	Wasco	Mosier
Oregon	Wasco	Skyline

Environmental monitoring. To understand how preharvest weather and microclimate influence stem retention, each orchard will be equipped with an in-orchard environmental monitoring unit. Stations will record air temperature and relative humidity and calculate VPD at frequent intervals. These measurements will allow us to characterize both seasonal conditions and short-term conditions in the ~10 days leading up to harvest. Weather data (temperature, RH, VPD) will be summarized daily and matched to biological data from each block. Growing degree accumulation from bloom to harvest will be calculated for each site to normalize developmental timing across orchards.

Tree selection and tagging. In each orchard, approximately 10 'Coral Champagne' trees of uniform vigor, canopy size, and crop load will be tagged for maturity and fruit-quality assessments.

Maturity and quality assessments. Cherry maturity was classified using a 10-stage color scale based on computational image analysis (Figure 1). This scale objectively quantifies skin hue and red coverage, allowing consistent maturity comparisons across orchards. Approximately 100 cherries per orchard were collected weekly from tagged trees as fruit progresses through the 10 stages, representing sun-exposed and shaded canopy positions. Data was collated across the different states and used to assess the impact of growing environment on fruit development and quality.

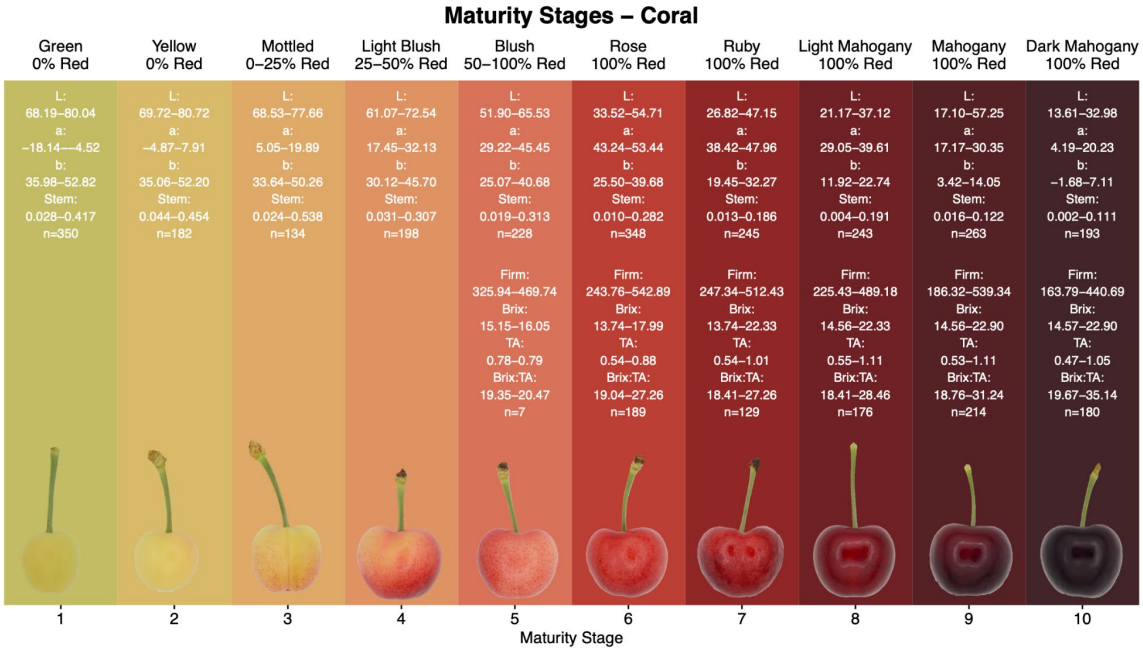


Figure 1. Maturity stages for 'Coral Champagne' cherry fruit (Blanco et al. 2025; unpublished)

Measurements included:

- Pedicel–fruit retention force (TA-XT texture analyzer)
- Firmness (FirmTech II, nondestructive)
- Soluble solids (°Brix)
- Titratable acidity (TA) and pH
- Skin and flesh color (image-based L*a*b analysis)

Results and Discussion

Fruit was picked based on fruit maturity stage to standardize comparisons across orchards. Figure 1 shows examples of maturity at stage 3, 6, and 9 for four different orchards.

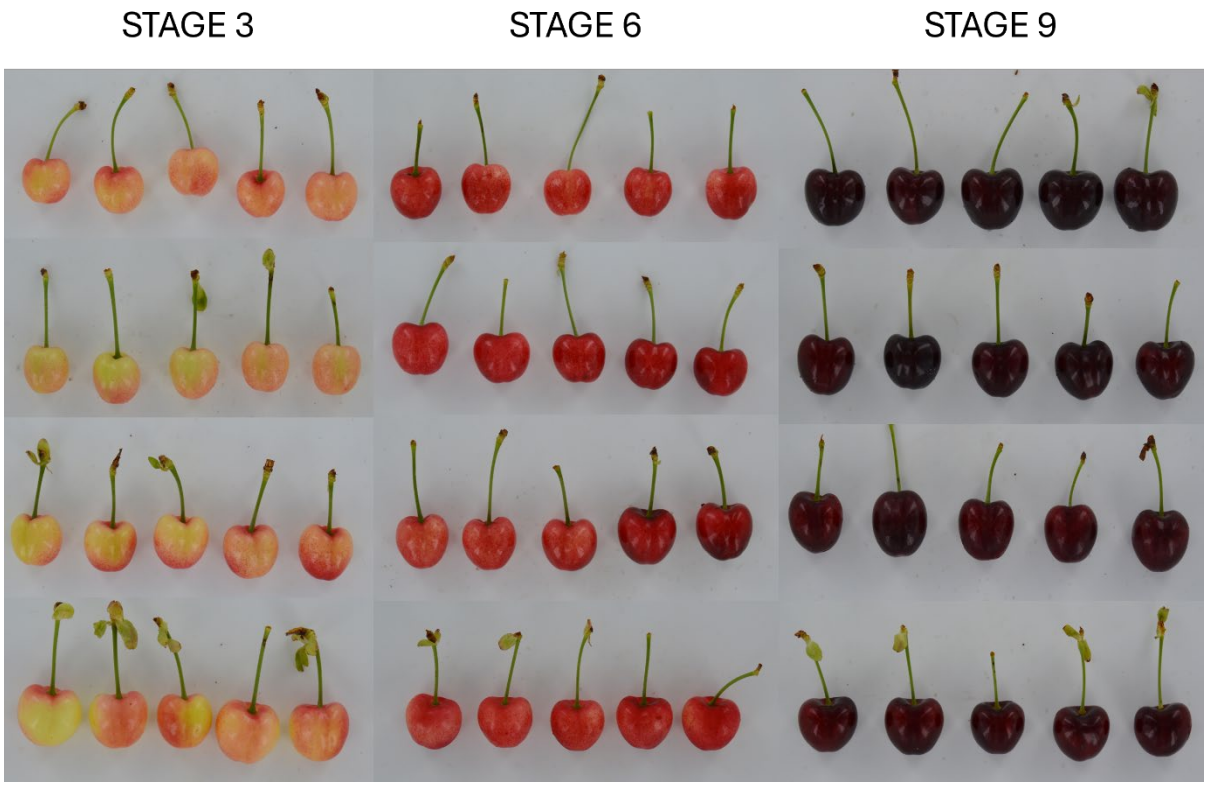


Figure 2. Photos of fruit at stage 3, stage 6, and stage 9 from Pasco North, Pasco South, Mattawa, and Orondo (Top to bottom). Note: fruit diameter not scaled for comparisons between photos (i.e. fruit at stage 3 are smaller than stage 9).

Environmental location appears to affect fruit growth more than fruit softening or stem retention. At comparable stages of development, fruit size differed among Pasco, Mattawa, and Orondo, with Orondo generally producing larger fruit later in development. In contrast, site differences in firmness and stem-pull force were greatly reduced after accounting for fruit developmental status, suggesting that much of their apparent environmental response reflects differences in maturity progression rather than a direct site effect.

Overall mean stem retention force dropper below 500 g force for three orchard locations when fruit maturity reached stage 9 and 10; Pasco North, Pasco South, and Mattawa all had low stem retention force. All sites had mean stem retention force of less than 650 g force when stage 10 was reached for fruit maturity. There was variability among fruit in the fruit retention force (Table 1). Minimal fruit had fruit retention force below 500 g force at stage 3 fruit maturity for all locations. However, there were differences among sites at stage 6, 9, and 10. It appears that the warmer sites had higher proportions of fruit with low fruit retention force compared to cooler sites.

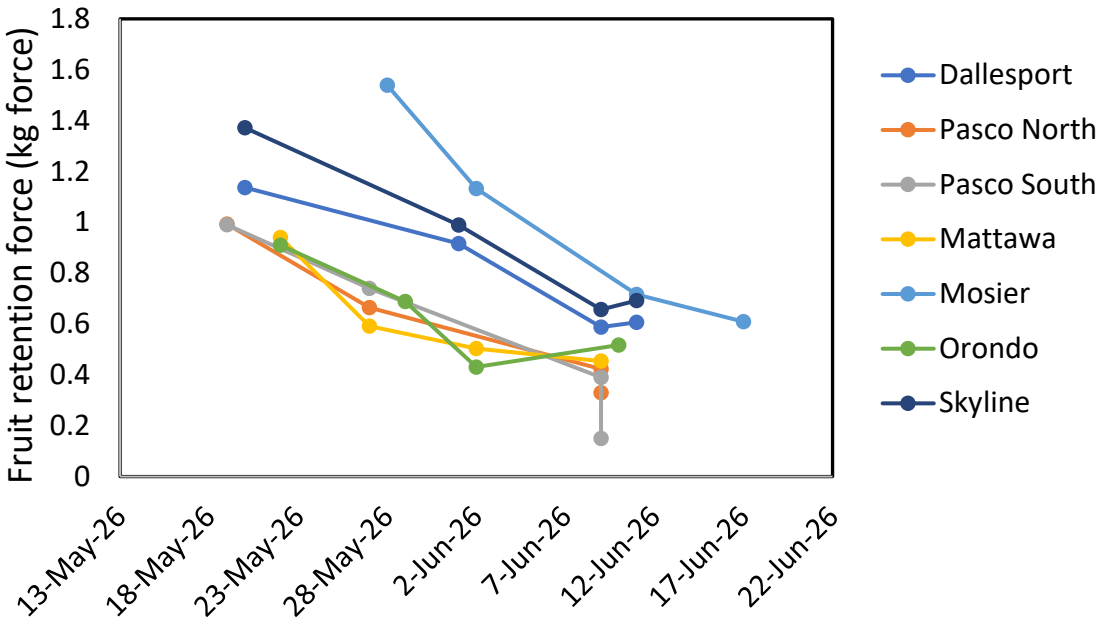


Figure 3. Fruit retention force (kg force) over time for seven orchard locations for 'Coral Champagne' cherry samples at stage 3, 6, 9, and 10 for each location.

Table 1. The percentage of fruit with fruit retention force of <500 g force for 'Coral Champagne' cherries sampled at four different maturity stages from seven orchard locations

	Maturity Stage			
Location	**S3**	**S6**	**S9**	**S10**
Dallesport	0.0%	3.0%	29.0%	22.0%
Pasco North	1.0%	6.0%	51.1%	80.9%
Pasco South	0.0%	20.0%	46.0%	100.0%
Mattawa	2.0%	30.0%	52.0%	54.2%
Mosier	0.0%	0.0%	6.0%	33.3%
Orondo	0.0%	14.3%	62.0%	42.0%
Skyline	0.0%	0.0%	12.2%	14.0%

Fruit firmness followed similar patterns across all orchard locations as fruit maturity progressed from stage 3 to stage 10 (Figure 4). None of the sites had fruit firmness fall below 200 g mm^{-1}, considered to be the minimum commercial threshold for marketable fruit (Sallato et al. 2025). Fruit was softer on average for Mosier, Dallesport, Pasco South, and Pasco North. Fruit diameter was between 26 and 28 mm in diameter for all seven sampled locations (Figure 5). Fruit was nearly finished sizing by stage 9 for most orchard locations but there were big sizing gains observed from stage 6 to stage 9 that corresponded to decreases in fruit retention force and fruit firmness (Figure 5). The influence of seasonal temperatures on fruit growth rates and final fruit size need to be analyzed and will require more locations in 2027 to have adequate sampling numbers.

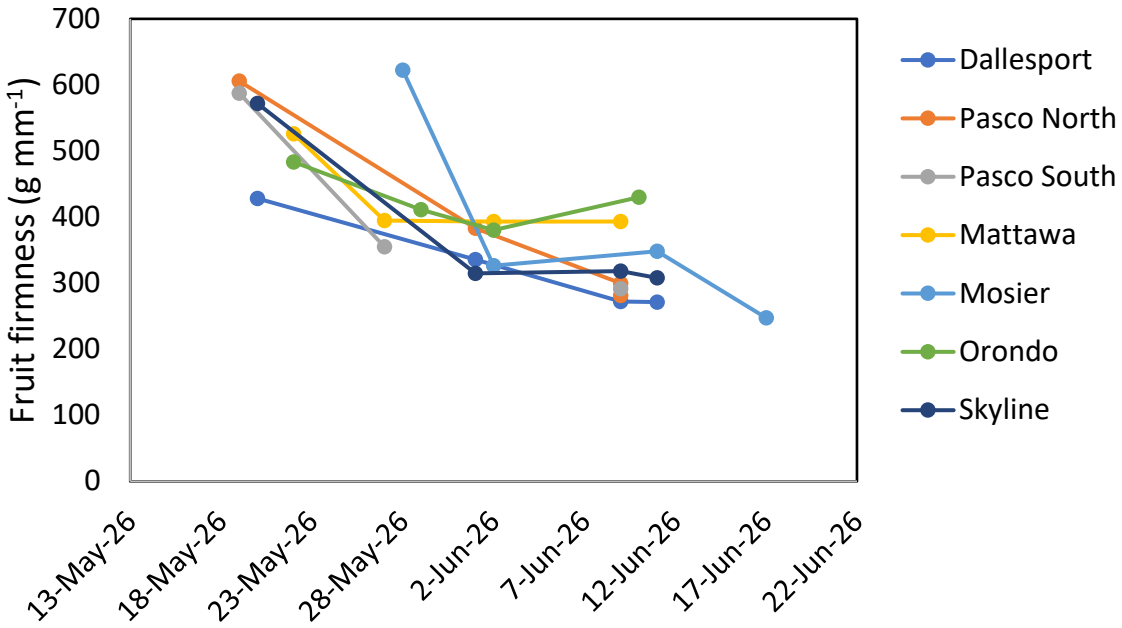


Figure 4. Fruit firmness (g mm^{-1}) over time for seven orchard locations for 'Coral Champagne' cherry samples at stage 3, 6, 9, and 10 for each location.

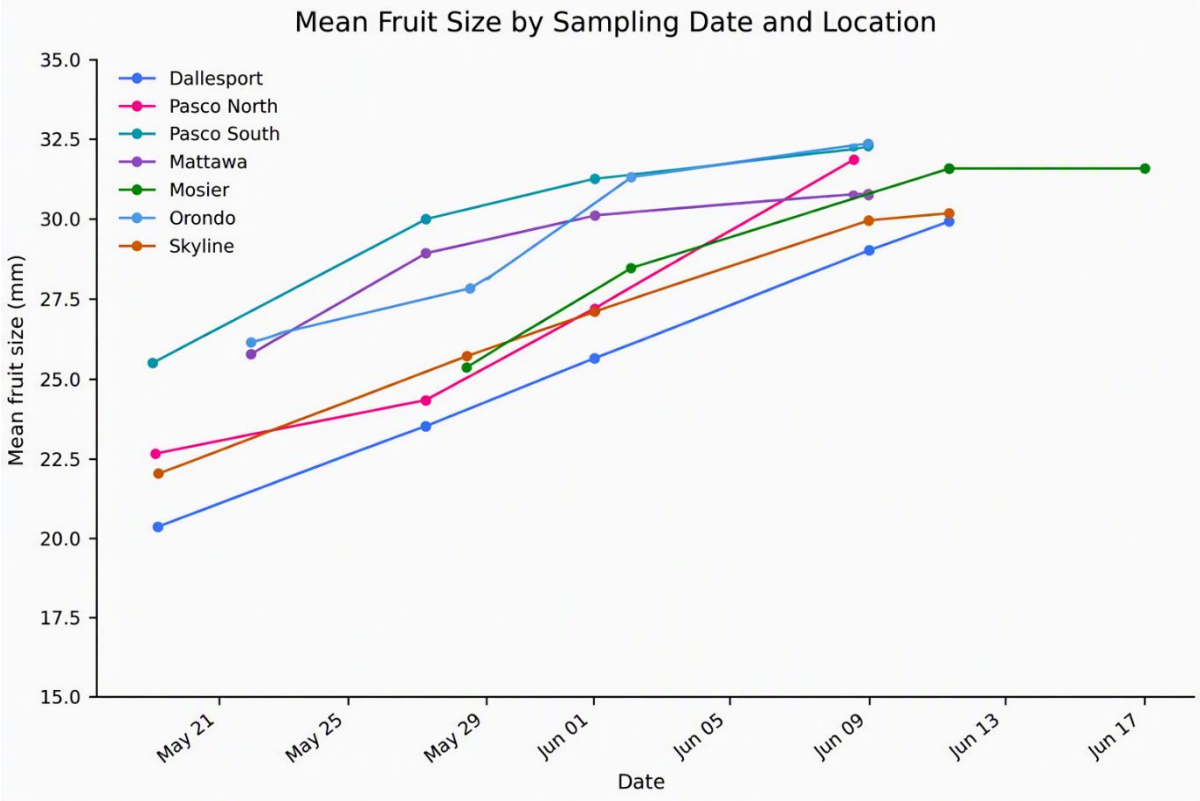


Figure 5. Fruit firmness (g mm^{-1}) over time for seven orchard locations for 'Coral Champagne' cherry samples at stage 3, 6, 9, and 10 (left to right) for each location.

Table 2. Monthly Growing Degree Day (baseline of 50 °F) accumulation for March to June at three of the Washington locations; Pasco, Orondo, and Mattawa. Environmental data from Oregon will be integrated into this dataset for 2027.

Month	Pasco	Orondo	Mattawa
March	84.5	50.4	68.6
April	173.9	147.7	137.2
May	484.3	475.2	462.4
June	600.1	597.0	584.9

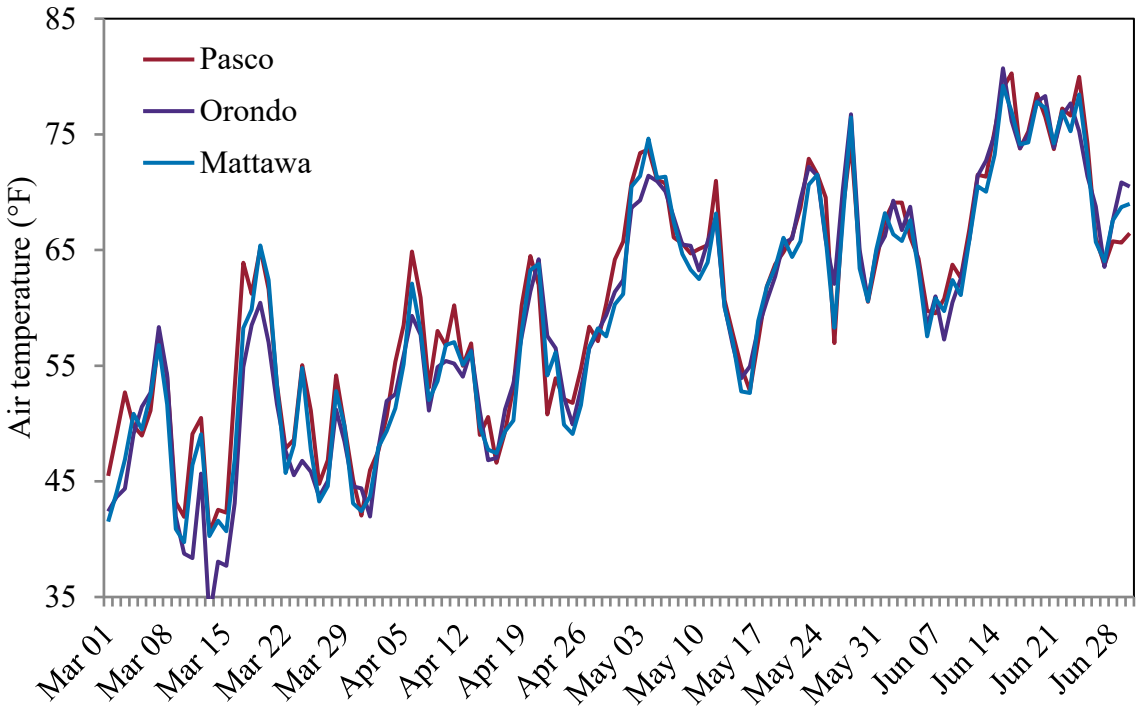


Figure 5. Air temperature from March 1 to June 30, 2026 for three locations in Washington state. Environmental data from Oregon will be integrated into this dataset for 2027.

Literature

Leisso, R, Thompson A. 2025. Sweet cherry cultivar-specific export suitability. WTFRC Cherry Review Final Report. https://www.treefruitresearch.org/wp-content/uploads/2025/02/25.-Leisso_SweetCherryExportCVs_FINAL.pdf

Sallato, B, Whiting MD, Torres C. 2025. Nutrient management for high quality cherries. WTFRC Cherry Review Final Report. https://treefruitresearch.org/report/nutrient-management-for-high-quality-sweet-cherries/

Project Title: Evaluating Heat Stress Response in Novel Cherry Rootstocks

Report Type: Continuing Project Report

Primary PI: Ashley Thompson
Organization: Oregon State University Extension Service
Telephone: (541) 296-5494
Email: ashley.thompson@oregonstate.edu
Address: 400 E Scenic Drive
Address 2: Suite 2.278
City/State/Zip: The Dalles, OR 97058

Co-PI 2: Kelsey Galimba
Organization: Oregon State University MCAREC
Telephone: (541) 386-2030
Email: kelsey.galimba@oregonstate.edu
Address: 3005 Experiment Station Dr.
City/State/Zip: Hood River, OR 97031

CO-PI 3: Brian Pearson
Organization: Oregon State University MCAREC
Telephone: (541) 386-2030
Email: brian.pearson@oregonstate.edu
Address: 3005 Experiment Station Dr.
City/State/Zip: Hood River, OR 97031

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 2026:

- EC 12 was excluded from the study due to an insufficient number of trees available to meet the replication requirements of the randomized complete block design.
- The soil moisture curve data we received from the UC Davis Analytical Laboratory is questionable and makes interpreting the volumetric water content (VWC%) difficult. Thompson and Pearson are working with the UC Davis Analytical Lab to determine a path forward. For now, we will report VWC% without this important context.

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

Deviation 2026: Initial TCSA was measured after planting in 2026. We will measure other horticultural traits 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 2026: 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".

Significant Findings:

- In the first three months after planting, we observed no differences in rootstock stress tolerance as measured by stomatal conductance. This is likely
- Tree mortality was relatively high in this block, with 19% of trees dying in year 1, likely due to bacterial canker. At this point, trees on all rootstocks in the trial have been affected, with no clear difference in susceptibility among rootstocks.

Methods:

Study location, Experimental Design, and Treatments

On May 6, 2026, trees were planted 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 four trees per experimental unit. Trees were planted on a 6' x 13' spacing. Each rootstock treatment was replicated five times. The rootstock treatments include A9 x VSL2, Rulan 8, Krymsk 7, EC 11, and RVL 4. Mazzard, Krymsk 5 and Gisela 12 serve as controls. Rootstocks were bud-grafted with the

cultivar ‘Skeena’. Trees were headed soon after planting and will be trained as a steep leader system. Irrigation and pest and disease management practices were standardized and applied uniformly throughout the experimental block to the best of the farmer’s abilities.

Measuring Rootstock Stress Tolerance Using Evapotranspiration (ET), Plant Available Water, and Stomatal Conductance

A soil moisture release curve was developed by UC Davis Analytical Lab following procedures outlined by Klute, 1986. This release curve was developed to assess the relationship between irrigation application volume and plant available water (PAW). Soil moisture sensors (EC-5) were 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 weather monitoring station (RX3000) was installed at the research site on June 5, 2026 to collect local, on-farm weather conditions (temperature, humidity, wind speed, and solar radiation) and to calculate evapotranspiration (ET) and vapor-pressure deficit (VPD) (Tables 1 and 2). A porometer (LI-COR LI-600) was used to record stomatal conductance (measurement of leaf gas exchange and transpiration), leaf temperature, chlorophyll fluorescence (an indicator of photosynthetic energy conversion), and leaf VPD weekly. Measurements were conducted at midday when plant water stress is highest. Exact dates of measurements were 6/30/2026, 7/8/2026, 7/16/2026, 7/23/2026, 8/10/2026, 8/20/2026, and 8/27/2026. Measurements began when the young trees developed fully mature leaves.

Table 1. Monthly mean temperatures from the weather station at 45.578146,-121.165315

Month	Temperature				
	Mean °C	Mean maximum °C	Mean minimum °C	Max °C	Min °C
June	20.13	26.67	12.97	35.98	7.03
July	22.82	29.75	14.92	37.44	10.50
August	22.71	30.09	14.71	36.22	8.98
September	16.85	23.89	10.75	30.79	5.89

Table 2. Monthly humidity, precipitation and reference evapotranspiration from the weather station at 45.578146,-121.165315

Month	Relative humidity	Precipitation	Reference evapotranspiration, ET_0
	RH, %	Total, mm	mm/day
June	48.59	264.00	162.58
July	47.68	0.00	185.39
August	46.91	24.00	168.51
September	61.80	500.00	114.60

Measuring Tree and Fruit Response

Tree growth of each tree in each experimental unit will be measured as trunk cross sectional area (TCSA) by measuring the trunk diameter 12 inches (30 cm) above the soil line. Initial TCSA measurements were taken on June 25, 2026. We will continue measuring TCSA annually. Trees were monitored for mortality during the growing season.

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 two 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.

Results and Discussion:

Rootstock Stress Tolerance

No differences in stomatal conductance were observed among rootstocks on individual dates (Fig.1). Weekly soil VWC remained between approximately 15 and 30% throughout the study period (Fig. 2). Although VWC varied visually among rootstocks during some weeks, soil moisture was generally similar across rootstocks. The only statistically significant difference occurred during week 27, when EC 11 plots had higher VWC than Krymsk 7 and Mazzard plots. A more complete characterization of soil moisture over time will improve interpretation of the stomatal conductance data in the future.

Most published stomatal conductance data is collected from cherry trees over two years old. Unlike established trees, newly planted trees, like those in this study, have a relatively limited root system and must simultaneously regenerate roots and support shoot and leaf growth. This can temporarily reduce the plant's ability to supply water to the canopy, even when irrigation is adequate (Jacobs et al. 2009). As a result, the relatively low stomatal conductance observed in in the first year of this study may reflect the physiological limitations associated with tree establishment rather than differences in soil water availability and plant stress among rootstocks.

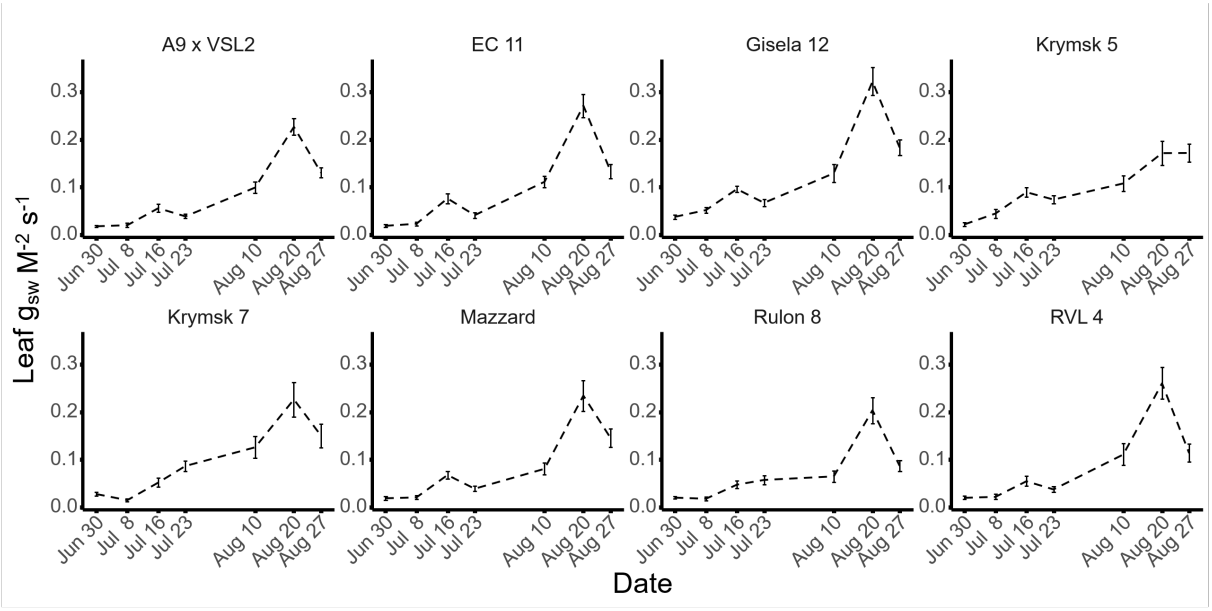


Figure 1. Mean stomatal conductance per rootstock on 6/30/2026, 7/8/2026, 7/16/2026, 7/23/2026, 8/10/2026, 8/20/2026, and 8/27/2026

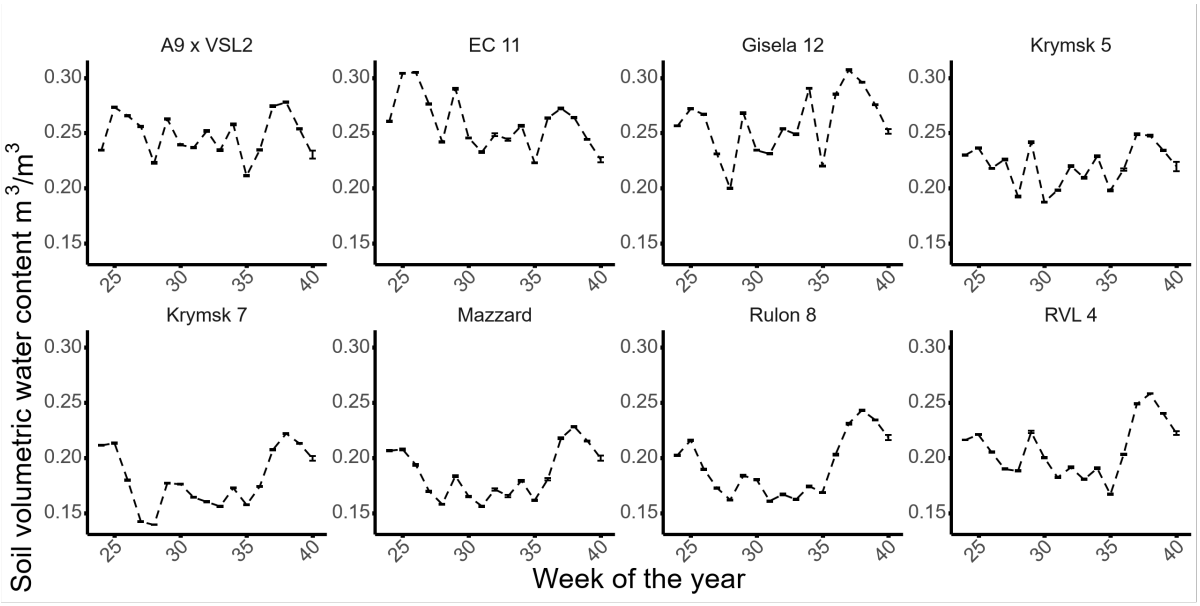


Figure 2. Mean soil volumetric water content per rootstock per week. Week 25 = June 25, 2026 though June 21, 2026.Week 40 = September 28, 2026 through October 4, 2026.

Tree mortality:

Total tree mortality for the first growing season was 19%. A 25% mortality rate was observed for the A9 X VSL2, RVL4, and Krymsk 7 rootstocks (Fig. 3). Lower mortality rates were observed in the control rootstocks, Gi. 12 (15%), Mazzard (15%), and Krymsk 5 (10%). Dead trees exhibited symptoms of bacterial canker (*Pseudomonas syringae*) infection, including cankers, dieback, and dead buds. It is unknown whether these infections began in the nursery or in the orchard following planting. We will continue to monitor tree mortality throughout the study period to determine if certain rootstocks have greater mortality than the controls.

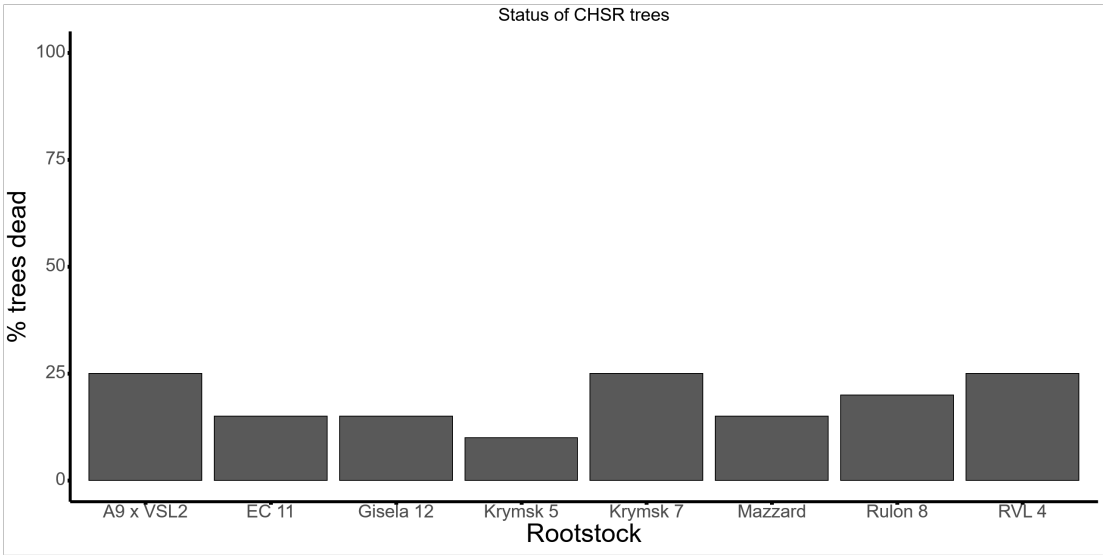


Figure 3. Tree mortality as of 29 Sept. 2026 was recorded for the ‘Skeena’ cherry trees grafted onto A9 x VSL2, Rulan 8, Krymsk 7, EC 11, RVL 4, Mazzard, Krymsk 5 and Gisela 12.

Tree Growth Response

Trees grafted to A9 x VSL2, Rulan 8, EC 11, RVL 4, were larger than the controls (Mazzard, Krymsk 5 and Gisela 12) and Krymsk 7 at planting (Fig. 4). It is important to note that the larger trees were sourced from a different nursery than the smaller trees. Moving forward, differences in mature tree size may affect stomatal conductance readings since size is often related to tree stress responses, such as stomatal conductance (Mohay and Lakatos, 2025).

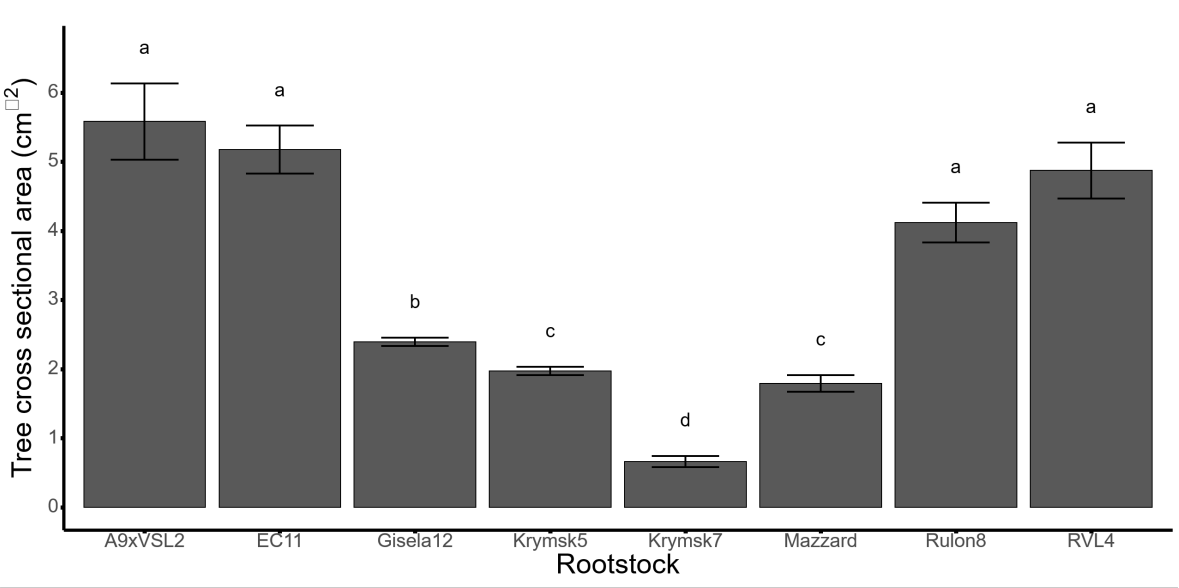


Figure 4. Trunk cross sectional area of the 'Skeena' cherry trees grafted onto A9 x VSL2, Rulan 8, Krymsk 7, EC 11, and RVL 4. Mazzard, Krymsk 5 and Gisela 12 measured 30 cm from the soil line one month after planting in The Dalles, Oregon. Different letters indicate a means separation of $p \leq 0.05$ level of significance. Error bars represent the standard error of the mean.

Literature Review:

Recent increases in summer temperatures are of great concern to the sweet cherry industry throughout the Pacific Northwest. The 2021 Pacific Northwest heat dome caused temperatures in The Dalles, OR to soar to 118°F and similar record setting temperatures were recorded across the cherry growing regions of Washington. Northwest Cherry Growers reported that the heat dome was responsible for a 20% reduction in cherry volume from the region (Courtney and Mullinax, 2021), and growers in The Dalles reported that 56% of cherry acreage suffered from some kind of heat damage. The climate of the Pacific Northwest has warmed substantially over the last 50 years, and climatologists project that the climate will warm 5°F over the next 25 years, and that extreme weather events like heat domes will become more frequent (O'Neill et al., 2023; Thompson et al., 2022). Recent conditions conform to these predictions; in 2021 and 2022, The Dalles experienced 65 days per year above 90°F. This is a significant increase compared to the previous 50-year average of 39.4 days per year above 90°F (O'Neill et al., 2023), Scientists predict that this climate trend will continue if the atmospheric concentration of greenhouse gases is not reduced. In fact, climatologists project that heat waves of similar magnitude to the June 2021 heat dome will recur one in every six years by 2100 (Thompson et al., 2022).

Most research focuses on the response of scion to heat stress; however, rootstocks play a clear role in regulating the scion heat stress response (Balfagon et al., 2022; Goncalves et al., 2005). Heat stress in

plants is characterized by increased transpiration and elevated water consumption. Transpiration is an important mechanism used by plants to cool leaves and transport mineral nutrients. This occurs when stomata open to allow evaporation of water, removing heat from the leaf surface. During periods of high heat, transpiration often increases to mitigate high leaf temperatures and avoid damage to plant tissue. If soil moisture is insufficient or plants cannot keep up with transpirational demands, stomata can close, reducing photosynthesis and exacerbating supra-optimal leaf temperature conditions. Scion-rootstock interactions influence plant water relations, leaf gas exchange, and water-use efficiency. A 2005 study (Goncalves et al.) observed significant differences in plant water stress among sweet cherry (*Prunus avium*) grafted on varying rootstocks. For example, 'Summit' had 35% greater water stress when grafted onto CAB 11E compared to Edabriz. Overall, authors found dwarfing rootstocks led to greater water stress compared to trees grafted onto invigorating rootstocks. Water stress leads to poor plant growth and development, as well as reduced quality and yield of fruit. Similar results have been observed in other tree fruit cropping systems, such as citrus (Balfagon et al., 2022), peach (Jimenez et al., 2020), and apple (Zhou et al., 2016). More information is needed to better understand the role new cherry rootstocks can play in mitigating scion stress response.

References:

Balfagon, D., J.L. Rambla, A. Granell, V. Arbona, and A. Gomez-Cadenas. 2022. Grafting improves tolerance to combined drought and heat stress by modifying metabolism in citrus scion. Environmental and Experimental Botany. 195

Courtney, R. and T.J. Mullinax. Learning from the Heat Wave. Good Fruit Grower. Sept 7, 2021

Dinny, S. Breeding the New Cherry. Good Fruit Grower. May 18, 2016.

Gonclaves, B., J. Moutinho-Pereira, A. Santos, A.P. Silva, E. Bacelar, C. Correia, and E. Rosa. 2005. Scion-rootstock interaction affects the physiology and fruit quality of sweet cherry. Tree Physiology. 26:93-104.

Jacobs, D.F., K. Francis Salifu, A.S. Davis. 2009. Drought susceptibility and recovery of transplanted Quercus rubra seedlings in relation to root system morphology. Ann. For. Sci. 66: 504

Jimenez, S., M. Fattahi, K. Bedis, S. Nasrolahpour-moghadam, J.J. Irigoyen and Y. Gogorcena. 2020. Interactional Effects of Climate Change Factors on the Water Status, Photosynthetic Rate, and Metabolic Regulation in Peach. Frontiers in Plant Science. 11. DOI: 10.3389/fpls.2020.00043'

Mohay, P., and T. Lakatos. 2025. "Impact of Severe Drought Stress on Water Relations of Young Cherry Trees Grafted onto Growth-Reducing Rootstocks" *Horticulturae* 11, no. 9: 997. DOI: 10.3390/horticulturae11090997

O'Neill, L., N. Siler, P. Loikith, and A. Arends in Fleishman, E., editor. 2023, Sixth Oregon Climate Assessment. Oregon Climate Change Research Institute, Oregon State University, Corvallis, OR.

Roper, T.R. and R.A. Kennedy. 1986. Photosynthetic characteristics during leaf developmenun un 'Bing' sweet cherry. Journal of the American Society of Horticultural Science. 11:6. 938-941.

Thompson, V., A.T. Kennedy-Asser, Y.T.E. Lo, C. Huntingford, O, Andrews, M. Collins, G.C. Hegerl, and D. Mitchell. 2022. The 2021 western North America heat wave among the most extreme events ever recorded globally. Science Advances. 8:18 DOI:10.1126/sciadv.abm6860

Wheeler, W. B. Black, and B. Bugbee. 2021. Water stress in dwarfing cherry rootstocks: Increased carbon partitioning to roots facilitates improved tolerance of drought. Horticulturae. 7:424.

Zhou, B., J. Sun, S. Liu, W. Jin, Q. Zhang and Q. Wei. 2016. Dwarfing apple rootstock responses to elevated temperatures: A study on plant physiological features and transcription level of related genes. Journal of Integrative Agriculture. 15:5. 1025-1033.

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

Report Type: Continuing Project Report

Primary PI: Per McCord
Organization: WSU Dept. Horticulture
Telephone:509-786-9254
Email: phmccord@wsu.edu
Address: 24106 N. Bunn Road
Address 2:
City/State/Zip: Prosser, WA 99350

Co-PI 2: Kelsey Galimba
Organization: Oregon State University
Telephone: 541-386-2030 X38218
Email: Kelsey.galimba@oregonstate.edu
Address: OSU MCAREC
Address 2:3005 Experiment Station Dr.
City/State/Zip: Hood River, OR 97031

CO-PI 3: Cameron Peace
Organization: WSU Dept. Horticulture
Telephone: 509-335-6899
Email: cpeace@wsu.edu
Address:
Address 2: PO Box 646414
City/State/Zip: Pullman, WA 99164

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: 2023–2027 (no cost extension)
Amount: $22,376
Agency Name: WSDA Nursery Research Funding
Notes: 'Evaluating new stone fruit cultivars for western Washington'.

Awarded
Funding Duration: 2025–2027 (no cost extension)
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-PIs Cameron Peace, Alexandra Johnson.

Awarded
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-PIs, including Per McCord, Bernardita Sallato, and Matthew Whiting from WSU.

Requested
Funding Duration: 2027–2031
Amount: $10.18 million
Agency Name: USDA NIFA Specialty Crops Research Initiative
Notes: 'Reducing economic risk in Prunus crops through enhanced environmental resilience and modernized management systems'. PI: Per McCord; co-PI's Matthew Whiting and Cameron Peace (WSU), multiple additional co-PIs.

Requested
Funding Duration: 2027–2028
Amount: $106,638
Agency Name: Washington Tree Fruit Research Commission/Oregon Sweet Cherry Commission
Notes: 'Marker-assisted breeding for better stem quality and late ripening'. PI: Per McCord; co-PI Cameron Peace.

Requested
Funding Duration: 2027–2028
Amount: $27,600
Agency Name: Northwest Nursery Improvement Institute
Notes: 'Establishing a rootstock trial for 'R19' sweet cherry'. PI: Per McCord; co-PI Rob Blakey

WTFRC Collaborative Costs: None

Budget 1
Primary PI: Per McCord
Organization Name: Washington State University
Contract Administrator: Kevin Rimes
Telephone:
Contract administrator email address: arcgrants@wsu.edu

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).

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)

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

- 'R19' (Cascade Daybreak™) released in 2026
    - First WSU cherry release since 2007
- Phase 3 selections R3 and R29 evaluated
    - **R29 continued strong performance observed** (2nd year of Phase 3). Excellent size and flavor, 'Bing' timing. Will evaluate for 3rd harvest in 2027.
    - **R3 dropped** from evaluation after uneven performance
- Phase 2 data collected for **five** selections. One dropped (CR11T019: 'Skeena' timing but not as firm)

- **Severe frost** in Prosser followed by bird predation eliminated almost all crop from seedlings (Phase 1), but **one** early-ripening seedling (PC-7) advanced to Phase 2 trials
- Utilized up to 10 different DNA tests to evaluate potential parents as well as seedlings
- 2026 crosses guided by DNA tests of parents for fruit size, firmness, cracking, self-fertility, and powdery mildew resistance
- Acceptable germination rate of 57% resulted in more than **2,500 seedlings** from 2025 crosses
- Eliminated **1275 seedlings (51%)** based on DNA test results (highest cull proportion since 2018)
- Canine testing of budwood samples for X-disease phytoplasma and Little Cherry Virus identified **six** positive samples (3 from Prosser); infected trees removed at Prosser; managers of other sites notified
- Implemented spray recommendations for control of aphids, mites, peach twig borer, Western cherry fruit fly and SWD, leafhoppers, and powdery mildew
- Weed control improved in seedling blocks

Methods

Fruit evaluation

A mild winter and warm spring weather resulted in early bloom (beginning in March 2026). Frost events in late March 2026 severely reduced fruit set at the Headquarters blocks (seedlings and Phase 2), despite the use of wind machines. Estimated crop loss in the seedling blocks in particular was >95%. Available fruit were analyzed as for 2025. 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. Phase 1 seedling selections were classified as either mahogany or blush. In the fruit quality lab, fruit from all selections (including Phase 1 seedlings) were photographed and evaluated for weight and firmness (via Firmtech). Standards and Phase 2/3 selections were also evaluated for SSC and titratable acidity. An induced cracking test (4-hour soak in deionized water) was performed for all samples 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 Variety Protection, additional data were collected for the Phase 3 selections R3 and R29 plus the standards 'Chelan' and 'Bing'. These data included bloom timing, growth habit, and detailed measurements of 1-year-old shoots, flowers, leaves, and fruit.

Crossing and DNA tests

The results of DNA tests for cracking, firmness, and fruit size were used to guide crossing combinations for the 2026 season. Additional criteria used in crossing decisions included bloom time, ripening time, powdery mildew resistance, 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 as well as interspecific crosses with Western sand cherry (*Prunus besseyi*). 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 2025 and spring of 2026, germinated seedlings from the 2025 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. We have switched from a 2L Proptek™ pot to a smaller (0.66L) D-40 Deepot™. These smaller pots still allow for adequate

root development but take up half the bench space in the greenhouse and utilize less than half of the potting mix compared to the larger pots. Seedling transplanting took place in February 2026 with 516 dormant seedlings left over from 2025 (2024 crosses), and again in May 2026 with approximately 800 current-year seedlings (from 2025 crosses).

Disease screening and orchard maintenance
We utilized canine screening to test budwood bundles for the presence of X-disease phytoplasma and/or little cherry virus. This screening was applied to approximately 40 budwood samples received from other breeding programs, as well as approximately 20 samples collected by WSU for internal propagation or distribution.

Orchards were sprayed beginning with a delayed dormant spray on 22 March 2026 targeting aphids and scale insects. Pre-harvest cover sprays (late April-mid June 2026) targeted Western cherry fruit fly, peach twig borer, and spotted-wing *Drosophila*. Powdery mildew was also targeted in the Phase 2 block. Post-harvest (to date being late July and late August 2026) spray applications targeted leafhoppers and spider mites. Two more post-harvest applications are scheduled October and November 2026. Orchard irrigation was managed primarily by the IAREC farm crew, who also winter-pruned the older parental block at the Roza and summer-pruned (via hedging) the younger parental block at the Roza as well as 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 and summer. In addition, the 2019 seedling planting at Headquarters (approximately 780 trees) was removed.

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, only 7 seedlings were selected in the field for laboratory evaluation, vs. 283 in 2025. This reduction was due to 2026's severe frost damage and bird predation of the few remaining fruit. One seedling was advanced to Phase 2 based on at least two years' data: **PC-7** (PSC2020019-023) is an early-ripening mahogany type.

For Phase 2, frost damage at Prosser, Sagemoor, and Valley View limited fruit availability. Data were collected from two selections in the 2021 cohort and four selections in the 2022-2024 cohorts (Table 1).

R35B, a mid-late ripening blush selection, had been advanced to Phase 3 in 2025. It was harvested 7 days after 'Rainier' in 2026, and continues to display larger, firmer fruit. Phase 3 trials of R35B will be planted in spring 2027.

R47, a large-fruited early mahogany selection, was slated for an extra year of evaluation in 2026, including tests with additional pollenizers and potentially Retain to improve fruit set. Frost destroyed the crop at Sagemoor and Prosser, so these experiments will be delayed until 2027.

R25 continues to demonstrate very early ripening, being harvested 6 days before 'Chelan' at Sagemoor in 2026. Fruit size and firmness are similar to 'Chelan', but R25 has higher SSC and is self-fertile.

FR31T011 is early-mid season with very large fruit. It uniquely has a medium-red skin and white flesh with very high SSC and low TA. Firmness may be an issue; we hope to get data from this selection in 2027 from Valley View, which utilizes GA.

CR11T019 was dropped. It ripens with 'Skeena' but is not superior to it.

The latest selection, **CR21T043**, ripens approximately two weeks after 'Skeena'. SSC was very high, and cracking very low, but firmness varied widely across locations (190 g/mm in Prosser vs. 282 in Valley View).

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]For blush, harvest timing relative to 'Rainier'; [1]PROS, Prosser; SAGE, Sagemoor; HRIV, Hood River (MCAREC); NACH, Naches (Valley View).

Selection ID	Market class	Harvest timing	Fruit weight (g)	Firmness (g/mm)	SSC/TA	Locations harvested[1]	Notes
R35B	Blush	Rainier + 7 days	14.6	356	25.5/0.96	HRIV	**Advanced to Phase 3**
Rainier	"	--	10.7	228	22.3/0.76	HRIV	Standard blush
R25	Mahogany	Chelan - 6 days	10.7	352	23.2/0.51	SAGE	
Chelan	"	--	9.8	266	20/0.55	PROS, SAGE, HRIV	Standard mahogany (early)
FR31T011	"	Skeena -11	13.1	267	26.1/0.43	PROS, HRIV	Light red skin, white flesh. Unique flavor.
R47	"	Skeena -13	12.8	276	25/1.47	HRIV	Light crops
Benton	"	Skeena -13	12.4	216	20.7/1.10	HRIV	Standard mahogany (mid)
Bing	"	Skeena -11	10.1	220	24.2/1.25	HRIV	Standard mahogany (mid)
Skeena	"	--	11.1	276	22.5/0.64	PROS, HRIV, NACH	Standard mahogany (mid-late)
CR11T019	"	Skeena +1	11.2	251	22.6/0.47	PROS, NACH	**Dropped**
CR21T043	"	Skeena +14	11.2	236	26.2/0.49	PROS, NACH	New

'R19' was released in 2026 (PVP applied for) and is being registered under the trademark name Cascade Daybreak™. **This is the first new WSU sweet cherry cultivar since 2007.** We evaluated it again in 2026 at Cheri Barn. We also completed the fourth year of Phase 3 evaluations for R3, and the second year for R29 (Table 2).

'R19' on Krymsk-6 rootstock continued to do well at the Cheri Barn location. According to Stemilt Growers, it had a commercial packout of 90%, and **yielded the highest grower returns for the first three weeks of the season**.

Due to its inconsistent performance, R3 was dropped from consideration for release for commercial PNW production. R29 continued to perform well at Dallesport, as well as at a smaller test planting near Desert Aire. It continues to yield very large fruit with long stems, good firmness, and low cracking. It ripens with or slightly later than 'Bing'. R29 will be evaluated again in 2027 before making a decision whether to release. It is currently undergoing therapy at CPCNW in Prosser for several viruses (Cherry necrotic rusty mottle virus, Grapevine line pattern virus, Prune dwarf virus, and Prunus necrotic ringspot virus).

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

ID	Location	Rootstock	Harvest date	Weight (g)/Row Size (peak)	Firmness (g/mm)	SSC/TA	Notes
R19	Cheri Barn	Krymsk-6	6/1	7.32/10	415	21.8/0.25	Released as Cascade Daybreak™
R19	Cheri Barn	Mazzard	6/8	10.0/10	340	27.1/0.12	Poor fruit set on Mazzard
Black Pearl	Cheri Barn	Krymsk-6	6/8	7.41/Under	286	20.57/0.14	'Chelan' (typical standard) trees were removed
R3	Cheri Barn	Krymsk-6	6/11	11.6/9.5	333	21.1/0.06	**Dropped**
Chelan	Buena	Gisela-12	6/9	9.5/10.5	200	22.3/0.49	Poor set
R3	Buena	Gisela-12	6/11	10.4/10	210	19.1/0.38	Poor set
Chelan	Dallesport	Gisela-12	6/4	5.81/Under	371	21.4/0.44	
R3	Dallesport	Gisela-12	6/10	12.2/9.5	400	23.0/0.1	
R29	Dallesport	Gisela-12	6/20, 6/22	12.0/9.5	329	22.6/0.21	Good vigor in cold site.
Bing	Dallesport	Gisela-12	6/18	8.6/11	281	24.2/0.35	
R29	Desert Aire	Gisela-12	6/17	12.8/9	253	22.9/0.19	Secondary site

Crossing and DNA tests

In 2026, the breeding program created 50 families, of which 46 were bi-parental. Total seed produced was estimated at 6,306, about 10% less than in 2025. Extensive use was made of DNA test results to guide parental combinations. For seeds from 2025 crossing, the overall germination rate of

57% resulted in more than 2,500 seedlings. DNA testing was used to cull inferior seedlings of 2025 families. A total of 1,275 seedlings were eliminated based on DNA test results, the highest number recorded to date. Additional seedlings were culled for stunted growth.

Disease screening and orchard maintenance

Out of approximately 60 budwood samples, six tested positive via canine detection for the causal agents of little cherry disease. Three of these were from IAREC Headquarters, one from a breeding program test plot in Washington, and two were received by WSU from out of state. The trees at Headquarters were cut down, and the responsible parties of the other material were notified.

The spray regime was effective at protecting trees from visible insect damage, and from powdery mildew in the case of the Phase 2 block. Weed pressure particularly in the youngest seedlings was higher in 2026, but a combination of spring and summer herbicide applications and weed-eating resulted in good control by the end of the season. A winter herbicide will be applied in December 2026.

Project Title: Pesticide residues of PNW cherries
Report Type: Continuing Project Report

Primary PI: Tory Schmidt
Organization: WA Tree Fruit Research Commission
Telephone: (509) 669-3903
Email: tory@treefruitresearch.com
Address: 1719 Springwater Ave.
City/State/Zip: Wenatchee, WA 98801

Cooperators: Gerardo Garcia (WTFRC), Northwest Horticultural Council, Pacific Agricultural Labs (Sherwood, OR), Gale Fource Orchards

Project Duration: 3 Years

Total Project Request for Year 1 Funding: $ 7000
Total Project Request for Year 2 Funding: $ 7250
Total Project Request for Year 3 Funding: $ 7500

Other related/associated funding sources: Most chemical products and some lab fees are paid for by registrants

Primary PI: Tory Schmidt
Organization Name: WTFRC
Contract Administrator: Paige Beuhler
Telephone: (509) 665-8271
Contract administrator email address: paigeb@treefruitresearch.com

Item	2026	2027	2028
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	$3,000.00	$3,050.00	$3,100.00
Total	$7,000.00	$7,250.00	$7,500.00

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 Portland area
3 Travel costs include hauling equipment to & from plots

2026 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/.

Dried residues on cherries at harvest



TRIAL DETAILS

- Trial conducted in mature 'Skeena'/K.6 central leader trees on 10' x 16' spacing near East Wenatchee, WA
- 14 insecticides/acaricides, 5 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
- Nearly 1" of rain fell on the trial block May 25-29, affecting only residues from the initial applications of Kudos 27.5WDG, Bexar, and Esteem
- Fruit samples shipped overnight to Pacific Agricultural Labs (Sherwood, OR) for residue analysis

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 so. With that approach, **all 2026 residues complied with domestic tolerances but some exceeded foreign MRLs for important export markets: Kudos 27.5WDG, Bexar, Esteem, Asana XL, Zivalgo, 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.

Spraying trial block at 200 gal/acre



Measured residue levels vs. MRLs for pesticides applied to cherry fruit at 200 gal water/acre. 'Skeena'/K.6, East Wenatchee, WA. WTFRC 2026.

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	**0.11**	0.4	0.01 (THA)
tolfenpyrad	Bexar	27 oz	28, 14	**0.22**	2	0.01 (many)
pyriproxyfen	Esteem	16 oz	28, 14	**0.24**	1	0.01 (THA)
thiamethoxam*	Actara	5.5 oz	21, 14	0.105*	0.5	0.5 (many)
esfenvalerate	Asana XL	14.5 oz	21, 14	**0.040**	3	0.01 (THA)
lambda-cyhalothrin	Warrior II	2.56 oz	21, 14	0.083	0.5	0.3 (many)
isocycloseram	Zivalgo	2.5 oz	21, 14	**0.049**	1	0.01 (JPN,KOR)
Indoxacarb	Avaunt eVo	6 oz	21, 14	0.075	1	0.1 (CAN)
cyflumetofen	Nealta	13.7 oz	21, 7	0.045	1.5	0.4 (THA)
quinoxyfen	Quintec	8.7 oz	21, 7	0.039	0.7	0.4 (many)
cyflufenamid	Torino	8 oz	14, 7	**0.040**	0.6	0.01 (THA)
flonicamid	Beleaf 50SG	2.8 oz	14, 7	0.19	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	10, 3	**1.2**	10	0.01 (THA)
spinosad	Entrust SC	6.8 oz	10, 3	0.043	0.2	0.2 (many)
difenoconazole	Inspire Super	20 oz	10, 3	0.16	2.5	0.2 (CN,VNM)
cyprodinil	Inspire Super	20 oz	10, 3	0.27	2	2 (many)
pydiflumetofen	Miravis	5.1 oz	10, 1	0.098	2	2 (many)
mefentrifluconazole	Cevya	5 oz	10, 1	0.18	4	1.5 (TWN)
hexythiazox	Onager	24 oz	7	0.11	1	0.2 (KOR)
pyrethrins	Pyganic 5.0EC	15.6 oz	3, 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; 24 August 2026. http://mrldb.nwhort.org/#top_markets
* Reported thiomethoxam values reflect the sum total of thiomethoxam and clothiandin 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.

WTFRC CHERRY PESTICIDE RESIDUE STUDIES 2012-2026



Since 2012, the Washington Tree Fruit Research Commission has conducted annual field studies to evaluate the harvest residues of numerous insecticides, acaricides, fungicides, and plant growth regulators commonly used in commercial cherry production in the Pacific Northwest. In order to provide a broad summary of all measured residues, this report summarizes all results regardless of application rates and timings or supplemental treatments such as hydrocooling or application of commercial rain protectants; values in **bold red font** highlight those residue levels which **exceed current maximum residue levels** (MRLs) in some key export markets. For results from specific years, please review annual reports of these studiesat www.treefruitresearch.org. For more information on MRLs or other regulatory issues, please consult the Northwest Horticultural Council at www.nwhort.org.

STUDY DETAILS



- All trials conducted on mature commercially farmed cherry trees:
  - 2012-2020: 'Bing'/Mazzard – Orondo, WA
  - 2021-2023: 'Skeena'/Mazzard – Orondo, WA
  - 2024-2026: 'Skeena'/K.6 – East Wenatchee, WA
- All applications made with 2 x 25 gal Rears Pak-Blast sprayer calibrated to either 200 or 400 gal water/acre, except for malathion (Fyfanon ULV AG), which was applied by helicopter as formulated (no water added)
- All pesticides except malathion (Fyfanon ULV AG) applied with 8 oz Regulaid/100 gal water/acre
- Pesticides were typically applied at the maximum concentration and minimum preharvest interval as allowed by their respective labels
- Additional treatments in some years included: application of rain protectants (RainGard or Parka), postharvest fruit rinsing in a commercial hydrocooler with a 120 second cycle, application in dilute (400 gal water/acre) vs. concentrated (200 gal water/acre) sprays
- Residue analyses conducted by Pacific Agricultural Labs (Sherwood, OR) or OMIC USA Laboratory (Portland, OR)

MAJOR FINDINGS



- Residues measured for all treatments in all years complied with domestic tolerances set by US EPA
- Most residues which exceeded foreign MRLs occurred in markets which set their tolerances at the limit of quantitation (LOQ), the smallest amount which standard laboratory instruments can accurately measure
- Residues of some pesticides were decreased on fruit which received hydrocooling, but results were too inconsistent and unpredictable to consider it a reliable strategy for reducing residue levels
- Application of the rain protectants RainGard and Parka consistently increased the persistence of pesticide residues, particularly for chemicals applied shortly before harvest
- Spraying concentrate vs. dilute (200 vs. 400 gal water/acre) had no consistent effect on residue levels
- All cherries treated with **Torino, Asana XL, Danitol 2.4EC, Zivalgo, Kudos 27.5WDG, Esteem, or Bexar** produced residues greater than 0.01 ppm, which exceed MRLs currently posted in some key export markets

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

WASHINGTON TREE FRUIT RESEARCH COMMISSION

Minimum, maximum, and median residues vs. MRLs of pesticides applied to 'Bing'/Mazzard or 'Skeena'/Mazzard cherries near Orondo, WA (2012-2023), or 'Skeena'/K.6. near East Wenatchee, WA (2024-2026), WTFRC.

Chemical name	Trade name	# years evaluated	# samples analyzed	Minimum residue	Maximum residue	Median residue	US MRL[1]	Lowest export MRL[1]
				ppm	*ppm*	*ppm*	*ppm*	*ppm*
abamectin	Agri-Mek 0.15EC	10	38	0	0.043	0.013	0.09	0.06 (Kor)
acetamiprid	Assail 70WP	9	32	0.083	0.93	0.445	1.5	1 (Twn)
azoxystrobin	Abound	5	26	0.021	0.87	0.23	2	1 (Twn)
beta-cyfluthrin	Baythroid XL	7	32	0	0.35	0	0.3	0.01 (Twn, Tha)
bifenazate	Acramite 50WS	4	22	0	0.27	0.0155	2.5	2 (many)
boscalid	Pristine	5	20	0.072	1.1	0.37	3.5	1.7 (Can, Twn)
buprofezin	Centaur	7	22	0	1.2	0.51	2	1.5 (Twn)
carbaryl	Carbaryl 4L	15	48	0	10	2.4	10	0.01 (Tha)
chlorantraniliprole	Altacor	4	10	0.084	0.18	0.13	2.5	0.5 (Kor)
cyantraniliprole	Exirel	3	12	0.1	0.54	0.2	6	4 (Twn)
cyclaniliprole	Verdepryn 100SL	4	8	0.059	0.19	0.116	1	0.6 (Twn)
cyflufenamid	Torino	5	12	0.031	0.27	0.1035	0.6	0.01 (Tha)
cyflumetofen	Nealta	2	4	0.035	0.15	0.0925	1.5	0.4 (Tha)
cyprodinil	Inspire Super	1	2	0.19	0.27	0.23	2	2 (many)
diazinon	Diazinon 50W	5	22	0	0	0	0.2	0.01 (Kor)
difenoconazole	Inspire Super	1	2	0.12	0.16	0.14	2.5	0.2 (Chn,VNM)
emamectin benzoate	Proclaim	3	6	0	0	0	0.09	0.005 (Tha)
esfenvalerate	Asana XL	4	8	0.038	0.2	0.087	3	0.01 (Tha)
etoxazole	Zeal	4	22	0	0.16	0.0335	1	0.01 (Tha)
fenpropathrin	Danitol 2.4EC	10	40	0.14	2.8	0.72	5	0.01 (Tha)
flonicamid	Beleaf 50SG	3	6	0.13	0.28	0.235	0.6	0.6 (many)
~~flubendiamide~~	~~Belt SC~~	~~5~~	~~26~~	~~0.039~~	~~0.81~~	~~0.071~~	~~na~~	~~na~~
fluopyram	Luna Sensation	3	18	0.021	0.25	0.0745	2	0.6 (Kor)
flutianil	Gatten	4	10	0.03	0.074	0.0455	0.4	0.4 (many)
flutriafol	TopGuard	9	36	0.087	0.64	0.215	1.5	0.8 (many)
fluxapyroxad	Merivon	3	12	0.23	0.59	0.285	3	2 (Twn, Vnm)
hexythiazox	Onager	6	26	0.087	0.47	0.21	1	0.2 (Kor)
imidacloprid	Nuprid 2SC	6	26	0.027	0.26	0.125	3	0.5 (many)
indoxacarb	Avaunt eVo	1	2	0.063	0.075	0.069	1	0.1 (Can)
isocycloseram	Zivalgo	1	2	0.028	0.049	0.0385	1	0.01 (Jpn,Kor)
lambda-cyhalothrin	Warrior II	6	26	0	0.17	0	0.5	0.3 (many)
malathion	Fyfanon ULV AG	3	14	0	0.012	0	8	0.5 (Twn)
mefentrifluconazole	Cevya	4	8	0.14	0.53	0.265	4	1.5 (Twn)
metconazole	Quash	4	22	0.011	0.083	0.028	0.2	0.2 (many)
metrafenone	Vivando	4	16	0	0.075	0	2	1 (Twn)
myclobutanil	Rally 40WSP	4	12	0.35	0.79	0.425	5	1 (Can, Twn)
penthiopyrad	Fontelis	3	18	0.046	0.22	0.11	4	4 (many)
permethrin	Perm-Up 3.2EC	9	36	0	1.2	0.275	4	0.1 (Can)
phosphite*	Tree Shield	3	10	7.6	31	15.52	?	?
prohexadione calcium	Kudos 27.5WDG	2	4	0.07	0.4	0.21	0.4	0.01 (Tha)
propiconazole	Orbit	11	42	0	0.53	0.225	4	2 (many)
pydiflumetofen	Miravis	4	10	0.073	0.18	0.12	2	2 (many)
pyraclostrobin	Pristine/Merivon	7	28	0.023	0.79	0.3	2.5	1 (HKG, Twn)
pyrethrins	Pyganic 5.0EC	3	6	0	0	0	1	0.01 (Tha)
pyriproxyfen	Esteem	3	6	0.22	0.48	0.295	1	0.01 (Tha)
quinoxyfen	Quintec	8	30	0.013	0.16	0.0395	0.7	0.4 (many)
spinetoram	Delegate WG	4	24	0	0.16	0.013	0.3	0.09 (Chn,Tha)
spinosad	Entrust	8	32	0.013	0.17	0.0525	0.2	0.2 (many)
spirodiclofen	Envidor 2SC	3	18	0	0.13	0.0515	1	0.8 (Twn)
spirotetramat	Ultor	3	10	0	0.041	0.013	4.5	2 (Chn)
sulfoxaflor	Closer/Transform	3	8	0.31	0.73	0.42	3	1.5 (many)
thiamethoxam**	Actara	7	16	0.082	0.434	0.21	0.5	0.5 (many)
thiophanate-methyl ***	Topsin 4.5FL	10	34	0	1.96	0.705	20	3 (Jpn)
tolfenpyrad	Bexar	10	26	0.16	1.2	0.48	2	0.01 (many)
trifloxystrobin	Luna Sensation	4	22	0	0.55	0.027	3	0.5 (Kor)
triflumizole	Procure 480SC	6	28	0.054	0.47	0.24	1.5	1 (Twn)
zeta-cypermethrin	Mustang MAX	11	42	0	0.34	0.115	2	1 (Kor)

[1] Major export markets for Pacific Northwest cherries; 10 September 2026. https://mrldb.nwhort.org/#top_markets

* EU tolerance for fosetyl-Al formerly defined as sum total of residue levels of fosetyl-Al, phosphonic acid and all salts including phosphite); no MRLs are published for fosetyl-Al or phosphite as of 10 September 2026

** Thiamethoxam values reported are sum totals of thiamethoxam and clothiandin residues

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

WASHINGTON TREE FRUIT RESEARCH COMMISSION

Project Title: Cherry Shaker Bagger Concepts

Report Type: Final Project Report

Primary PI: **Michael Corsetto**
Organization: Davis Mechatronics
Telephone: 650-218-6300
Email: mike@davismechatronics.com
Address: 27993 Mace Blvd
Address 2:
City/State/Zip: Davis, CA 95618

Cooperators: Mark De Kleine, TrellX

Project Duration: 1-Year

Total Project Request for Year 1 Funding: $ 40,000

Other related/associated funding sources: Davis Mechatronics investment

WTFRC Collaborative Costs:

Item	**(Type year of project start date here)**		
Salaries	$30,000.00		
Benefits			
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies	$10,000.00		
Travel			
Plot Fees			
Miscellaneous			

Total	$40,000.00		

Footnotes:

Budget 1
Primary PI: Michael Corsetto
Organization Name: Davis Mechatronics
Contract Administrator: Cedric Jeanty
Telephone: 615-584-7035
Contract administrator email address: cedric@davismechatronics.com
Station Manager/Supervisor: Optional
Station manager/supervisor email address: Optional

Item	**(Type year of project start date here)**		
Salaries	$30,000.00		
Benefits			
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies	$10,000.00		
Travel			
Plot Fees			
Miscellaneous			
Total	$40,000.00		

Footnotes:

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

Recap Original Objectives

The primary objective of this project is to automate the manual cherry bagging process. Currently, human operators must manually place individual bags onto shaker nozzles, creating a production bottleneck. The goal is to develop a mechanical solution to eliminate this manual effort, thereby providing substantial cost savings to cherry growers and packers.

Significant Findings

System testing achieved a success rate of nearly 90% across full operational cycles, with specific tests showing success in 94 out of 105 bags. We found that bag variance, differences in material stiffness, handle height, and dimensions, significantly impacts reliability. Doubling error rates between bag types from 7% to 14%.

Methods

Initial Research/Testing



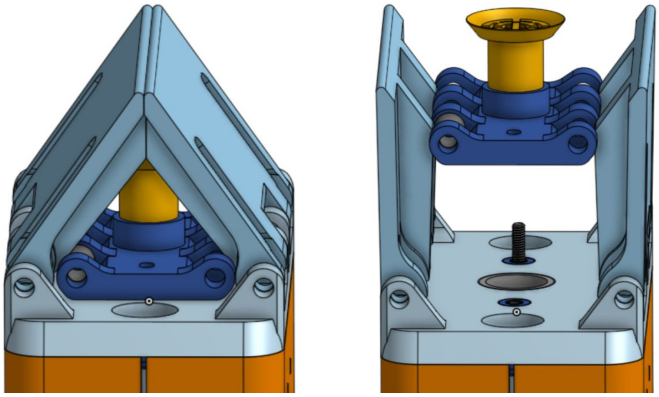
1. Outer bag grabber *2. Inner bag Pincher*

3. Inner 4 finger opener *4. Passive bag opener*

Initial Prototypes for bag grabbing concepts

These initial concepts were not pursued due to sizing and spacing constraints. For example, the outer bag grabber (idea 1) would physically hit the template after inserting the bag and would rub on neighboring bags when retracting pulling them off the template. Idea 4 (passive bag opener) couldn't exit through the template after bag placement without decreasing the final area and impacting its performance. Additionally, the initial aperture was too large to deal with zipped bags independently. The other prototype ideas were too inconsistent to move forward with.

Decided on Duckbill Concept



*

First prototype with duckbill concept

Following initial prototyping for multiple concepts, the Duckbill Concept was selected for further development. This design incorporates a retractable suction cup positioned beneath the bill to facilitate bag centering and catch points when inserting into a bag. We found this configuration maintained consistent pressure while providing an acceptable margin of error during operation.

Bag opener



Initial grabber design

The finalized bag-opening strategy involves a through-template grabber mechanism. This approach necessitated a pre-opening stage and a secondary hand-off mechanism. A four-bar linkage system was engineered to maintain parallel alignment between the bag faces and the insertion tool. In accordance with industry standards for food processing, suction cups were selected as the primary bag interface. We would eventually add more suction cups to the grabber but the geometry didn't change.



The duckbill with blower attached in the template

The integrated grabber assembly utilizes the previously validated duckbill and internal suction cup. To further open the bag for template insertion, a bottom-fed blower was integrated to inflate bags during the downward stroke, thereby expanding the aperture and improving template fit.

Full System Assembly

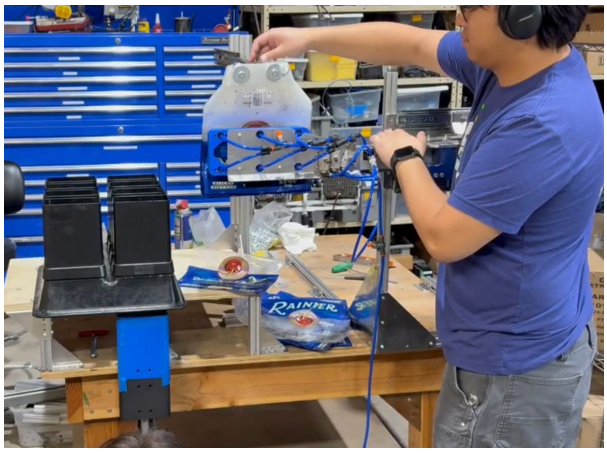


Initial Assembly of Architecture

The system architecture was formalized to isolate mechanical movements and eliminate variables associated with human handling. The test platform consists of a vertical rail for the insertion tool and horizontal rails for bag transport and grabber movement. Bags are indexed horizontally to the grabber before being positioned over the template for retrieval.

Recorded videos from initial system-wide testing is documented below:
https://drive.google.com/drive/u/0/folders/1iT3uurpS_gB6XPk9MzSeGzzQtoWmzhg_

Video of testing: "Initial system test”



Initial system wide test with human moved DOFs

To further eliminate human handling variables we fully automated the movement for each system. Pneumatic cylinders were implemented for all travel, and a motorized four-bar linkage provided multi-position control for the grabber. This configuration allowed for high-fidelity testing without human intervention, ensuring results representative of a production-ready system.



Architecture with Pneumatics

With the system fully mechanized, we could start identifying the catch points in the system. For the next month, most of our focus went into getting the bag onto the template. Here's one of the first test runs of the system, and you can see that even with the duckbill being slowly inserted, there were still many catch points that needed to be ironed out, especially when the bag first touched the template.

Video of testing:
"Pneumatic Actuated Testing"



Duckbill with expansion knobs

Our initial testing on the full system indicated that active inflation was actually unhelpful since it compromised suction force from the internal suction cup and made the bag less pliable while it was inserting on the template. To resolve this, the blower was replaced with mechanical expansion knobs integrated into the insertion tool. Additionally, the duckbill profile was reduced to improve centering across both axes. These modifications eliminated material interference during the upward stroke of the insertion tool.



Final knob placements

Final refinements involved relocating the mechanical flippers to the corners of the opening mechanism. This adjustment successfully prevented corner catching and improved system consistency to a success rate of nearly 90% across the full operational cycle. We ran a test of 105 bags from 2 variants and succeeded at 94 of them.

Results and Discussion

During the evaluation phase, we examined several cherry bag variants and observed that dimensional and material inconsistencies significantly impact grabber reliability. For the V2 iteration, we attempted to mitigate these effects by centering the grabber assembly relative to the bag aperture. Nonetheless, fluctuations in bag sizing and materials continue to present operational challenges across all parts of the operation. Differences in material stiffness can mess with the assertion step, and differences in sizing mess with every part of grabbing. Variations in bag type can double our error rate from 7% to 14%, depending on how different the bags are.

Bag Type	Success	Failure
Blue Rainer	51	4
Black Rainer	43	7
Loose Packed	10	2

Bag Type	Failue Mode	Notes
Blue Rainer	On Template	plunger didn't grab top gully
Blue Rainer	Grabber	bag extension didn't fully grab bag
Blue Rainer	On Template	plunger didn't fully grab/ bag handle caught on template
Blue Rainer	Grabber	Failed to pick up bag
Black Rainer	Bag	grabs bag right on zipper and rezips the bag
Black Rainer	Bag	Black bags hang slightly higher than blue bags have to adjust whole thing for the black bags so they don't reseal
Black Rainer	Bag	resealed again after adjustment
Black Rainer	On Template	hit top of template
Black Rainer	Grabber	grabber didn't pick up bag
Black Rainer	Grabber	opening sunction middle bottom failed
Black Rainer	Bag	Duck Bill caught on bag
Loose Packed	On Template	handle caught
Loose Packed	On Template	Handle caught

Notes from a test day with the amount of successful grabs for each bag in the top corner



Tested Bags(top) Black Rainer on top of light blue bag(left) Blue Rainier on top of light blue bag (right)

We found bag stiffness to be the main variable between bag styles. The stiffer light blue bag had more catch points when inserted into the template. Also, every bag we tested had different handle heights and bag sizes. The two Rainier bags that arrived on the wickets we tested were slightly different sizes, as were the wickets themselves and bag materials. Most importantly, you can see that the handles are at different heights. This handle area is crucial due to the nature of where the zipper is in proximity to it. The different handle sizes means that the mechanism may not grab the zipper area optimally and may have to be adjusted per bag to achieve consistent results. The light blue loose-packed bags we tested were half an inch bigger in width and height than the Rainier bags and had extra handle material on both sides of the bag instead of on one for the Rainier, increasing catchpoints when inserting into the template. While the system could be adjusted to use different bags, it was very time-consuming and had to be readjusted to the previous bag when switching back. Having a standardized bag or small selection of bags going forward would improve system reliability and significantly reduce the scope of the project.

Literature Review

Current Bagging Process

Every step in the described process is done using manual labor.



Figure 1. Shakers used to fill individual cherry bags.

Plastic liners are placed inside of the shipping box. The standard size box is 23” x 15.25” x 6”, corrugated, and packed to 20lbs.

Figure 2. Corrugated box with plastic liner.

Currently, plastic liners are placed within the bottom of the shipping box. Individual cherry bags are placed over the shaker nozzles, by hand, one at a time. Cherries are conveyed, by bulk, to the top of the shaker and hence into the individual bags.



Figure 3. Bags are placed individually over each shaker nozzle using human labor.

The shaker, complete with bags, is placed inside the shipping box to be bulk filled: https://www.youtube.com/watch?v=zxv_CzmQyQQ (2:22) shows bulk filling into the shaker trays. Not shown is how the bags are placed onto the shakers. This is the key automation step within the packaging process.



Figure 4. Shakers are placed inside the shipping box.



Figure 5. Shakers are raised and "shook" leaving cherries in their individual bags, inside the shipping box.

Executive Summary

This project addresses the manual labor bottleneck in the cherry packaging process by developing an automated bag-handling solution. We successfully prototyped and tested a mechanical system featuring a duckbill and grabber mechanism designed to position bags onto shaker nozzles. System testing validated a 90% success rate, confirming the technical feasibility of the design. While variances in bag materials and dimensions require further attention, this automated approach eliminates manual intervention, providing a pathway to substantial cost savings and increased throughput for cherry growers.

Project Title

Cherry Shaker Bagger Automation Concepts

Key words

Automation, Cherry Packaging, Grabber Design

Abstract

This report details the development and testing of automated concepts for the cherry bagging process. By replacing manual labor with a mechanized "duckbill" and "grabber" system, the project aims to increase efficiency for cherry growers. Testing achieved a 90% success rate, though significant challenges remain regarding bag material and size variance.

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

Report Type: Continuing Project Report -- No-Cost Extension

Primary PI: **Frank Zhao**
Organization: WSU-IAREC
Telephone: 509-786-9284
Email: Youfu.zhao@wsu.edu
Address: 24106 N. Bunn Rd.
Address 2:
City/State/Zip: Prosser, WA 99350

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:

Awarded (2026-51181-46407)
Funding Duration: August 1, 2026, to July 31, 2030
Amount: $5,853,999.
Agency Name: USDA-NIFA-Special Crop Research Initiative (SCRI)
Project title: Integrated strategies for managing bacterial canker and blossom blast disease of stone fruits
Leading and Participating Institutions: WSU (lead), MSU, OSU, UCR, Cornell, Clemeson

WTFRC Collaborative Costs: None

Budget 1
Primary PI: Dr. Frank Zhao
Organization Name: WSU-IAREC Prosser
Contract Administrator: Jamie Meek
Telephone: **(509)786-9231**
Contract administrator email address: jamie.meek@wsu.edu; or prosser.grants@wsu.edu
Station Manager/Supervisor: Naidu Rayapati
Station manager/supervisor email address: naidu.rayapati@wsu.edu

Item	1/3/2024	1/3/2025	1/3/2026	1/3/2027
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	0

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:

- Survey found that sprinkler irrigation resulted in outbreak of bacterial canker disease (BCD) in orchard in Yakima Valley in 2026, caused hundreds of tree death, leading to significant economic losses.
- Greenhouse experiments using spray inoculation replicated symptoms from those caused by sprinkler irrigation.
- Obtained complete whole genome sequences for seven *Pseudomonas syrinage* pv. *syrinage* (Pss) and five *P. amygdali* pv. *morsprunorum* (Pam) isolates from Washington orchards.
- Genome-based analyses, including phylogenomics and comparative genomics, were performed on 169 *Pseudomonas* genomes, including 92 BCD-associated genomes from major sweet cherry-producing regions around the world.
- The majority of Pss strains causing BCD were grouped with *Pseudomonas fragariae* (Pfr), which causes strawberry leaf spot disease.
- We proposed to reclassify Pss as *P. fragariae pv. pruni* pv. nov. for those causing BCD on stone fruits.
- Developed and optimized a multiplex PCR assay to differentiate Pfr from traditional Pss strains.

Methods

Procedures and Methodology:

1. Field survey in 2026

In 2026, we continued surveying newly established orchards for BCD due to severe weather conditions in April. Samples of diseased shoots (canker/ooze) were collected and were immediately put on ice and transferred to lab or stored in a cold room until further processing. Bacteria were isolated on King's B (KB) medium at 82°F (28ºC) and slow-growing fluorescent *Pseudomonas*-like colonies were selected. Bacterial isolates were subsequently cultured routinely on KB medium and single colony was purified for each isolate and stored in 15% glycerol at -112°F (-80°C).

2. Pathogenicity assay on cherry trees using spray inoculation in greenhouse

Greenhouse experiments were conducted at the Washington State University (WSU) IAREC in Prosser, WA, USA and 'Coral Champagne' sweet cherry (*Prunus avium* L.) trees was newly planted in a five-gallon garden pots. Trees were maintained under standard conditions for one month prior spray inoculation. For inoculum preparation, bacterial isolates were cultured in LB at 80°F with shaking at 200 rpm for 18 h. Bacterial cells were collected by centrifugation at 16,000 × g for 2 min, and the supernatant was discarded. Bacterial pellet was washed twice by resuspension in 1 mL of sterile PBS, followed by centrifugation under the same conditions. After the final wash, cells were resuspended in 1 mL of sterile PBS. The optical density of each suspension was measured at 600 nm using a GENESYS 150 UV-Visible spectrophotometer and adjusted to an OD_{600} of 0.1 in sterile PBS. Following inoculation, shoots were placed in sterile plastic bags lined with moistened sterile absorbent paper to maintain high relative humidity for 24 hours. Symptoms were monitored for 30 days post inoculation and PBS was used as control.

3. Genome sequencing and phylogenomics analysis

Twelve genomes (7 Pss and 5 Pam) were newly sequenced of strains isolated from sweet cherries during a disease outbreak in Washington State (BioProject PRJNA1309123). The remaining 157 genomes were retrieved from the National Center for Biotechnology Information (NCBI) GenBank Assembly database. A single colony was cultured overnight in LB broth prior to High Molecular Weight genomic DNA (HMW gDNA) extraction using the QIAGEN Genomic-tip 20/G kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. All library preparations and sequencing were performed at SeqCenter (Pittsburgh, PA, USA). For Illumina sequencing, libraries were prepared using the tagmentation and PCR-based Illumina DNA prep kit and sequenced on a NovaSeq X instrument (150-bp paired-end reads). For PacBio sequencing, gDNAs were sheared with a Megaruptor 3 (Diagenode Inc.) (6-10kb), and library preparation was performed with and without final size selection using the PacBio SMRTbell prep kit 3.0 followed by sequencing on a Revio 25M Flowcell using the Revio Polymerase kit following the manufacturer's instructions. The assembled genomes were classified using GTDB-Tk v2.6.0 and the Genome Taxonomy Database (GTDB) version 226 with default settings. The species-level designations of the 169 genomes were confirmed using the Type Strain Genome Server (TYGS), which performs whole-genome-based taxonomic analysis against type strain genomes. Digital DNA-DNA hybridization (dDDH) values were calculated using the Genome-to-Genome Distance Calculator (GGDC 3.0). Species boundaries were assessed using the established 70% dDDH threshold. Pairwise genomic similarity across the 169 genomes was determined using complementary average nucleotide identity (ANI) approaches. A threshold of 95% ANI was applied for species-level delineation. ANI thresholds of 90%, 95%, and 96% were used to evaluate genomic relationships among strains.

4. **Developing a multiplex PCR assay for differentiating Pfr from Pss**.

A total of 1906 publicly available *Pseudomonas* genome assemblies were retrieved from the National Center for Biotechnology Information (NCBI). Phylogroups were assigned by comparing the *cts* sequence of each genome with reference sequences. Comparative genome screening was then used to identify regions conserved within the PG02d or PG03 phylogroups but absent or sufficiently divergent in the other phylogroups. The Psm449 primer pair was designed within the previously described Psm1 SCAR marker region to generate a shorter product suitable for multiplex PCR (m-PCR). Primer-binding sites within the selected target regions were identified computationally using custom Python scripts executed on the Kamiak high-performance computing cluster at Washington State University (WSU). Candidate primer pairs were screened against all 1,906 genome entries using *in silico* PCR analysis. An *in silico* PCR result was considered positive when both primers aligned to the same genome in the correct orientation and generated a predicted product of expected size. Candidate primer pairs were retained when they produced the expected amplicon in the intended target group while remaining absent or sufficiently divergent in the non-target genomes.

5. Optimizing PCR conditions for multiplex PCR.

Each primer pair was initially evaluated independently in a PCR before being combined in the m-PCR assays. Each 20 µL reaction contained 2 µL of 10× DreamTaq Green Buffer (Thermo Scientific), 0.4 µL of dNTP mixture containing 10 mM of each dNTP (200 µM final concentration of each dNTP), 0.2-0.4 µL of each 10-µM primer (0.10-0.20 µM final concentration of each primer), 0.1 µL of DreamTaq DNA polymerase (0.5 U per reaction), 1 µL of genomic DNA, and nuclease-free water (NFW) to a final volume of 20 µL. All three primer pairs within each multiplex set were subsequently combined in a single reaction. The amount of each primer was adjusted between 0.2-0.4 µL (0.10-0.20 µM final concentration) to obtain products of comparable band intensity. During optimization, amplifications were performed using an S1000 thermal cycler (Bio-Rad Laboratories). Annealing temperatures were initially evaluated independently over ranges of 58-66 °C for the PG02d primer pair, 63-66 °C for the Psm449 primer pair, and 65-66 °C for the PG03 primer pair. Both multiplex sets were then evaluated at annealing temperatures ranging from 60 to 65 °C. The optimized cycling conditions consisted of an initial denaturation at 95 °C for 2 min, followed by 30-35 cycles of denaturation at 95 °C for 30 s, annealing for 30 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 5 min. Target-appropriate positive controls, known negative genomic DNA controls, and a no-template control were included in PCR and m-PCR run. PCR products were separated by electrophoresis using 10 µL of each amplification product on a 1.1% (w/v) agarose gel stained with SYBR™ Safe DNA Gel Stain (Invitrogen by Thermo Fisher Scientific) and run at 115V for 30 min in 1X TAE buffer, alongside a 1 kB DNA ladder (Thermo Scientific™ GeneRuler 1 kb Plus DNA Ladder). Gels were visualized and documented using the Azure 200 Biosystems gel documentation imaging system. Amplification was determined by the presence of an amplicon of the expected size.

Types and timing of anticipated results:

In 2026, we continued monitoring BCD development in newly established cherry orchards and conducted pathogenicity tests in greenhouse to collaborate field symptoms with greenhouse spray inoculation. We also performed genome sequencing, phylogenomic and comparative genomics analyses and further defined the identity of the causal agent of BCD in Washington orchards. In addition, we developed a multiplex PCR to differentiate Pfr from Pss, which will promote the accurate diagnosis of the disease.

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

Results and Discussion:

1. Sprinkler irrigation caused severe BCD outbreak and tree death

In the spring 2026, one orchard about 10 to 11 acres had severe BCD outbreak, leading to death of hundreds of trees. The trees were initially planted in 2025. The grower started sprinkler irrigation in early April, 2026 and by the time we visited in Mid-May, about 95% of trees showed severe symptoms of BCD (**Fig. 1**) and the grower had already removed more than 600 deed trees. The symptoms included severe infections on newly emerged buds on young shoots as well as on trunks, leading to wilting and death of the leaves and shoots (**Fig. 1AB**). Main trunks formed severe canker with ooze and cankers were still expanding, exhibiting copious gummosis, even in areas treated with paints (**Fig. 1 CDE**). We reasoned that the main reason for the outbreak was due to irrigation system used as the symptoms were shown in areas directly targeted by the sprinklers (**Fig. 1C**). This is a perfect example of BCD outbreak due to sprinkler irrigation similar to what we observed during the 2023 BCD outbreak. It is thus recommended that growers should use drop irrigation when you first establish a new cherry orchard to avoid BCD.



Fig. 1. Field observations of BCD outbreak in an orchard caused by sprinkler irrigation in 2026. a) Infections at the buds on several shoots, leading to wilting and death of the emerging leaves; b) enlarged photo showing infections on several shoots, leading to wilting and death of the leaves. c) sprinkler system used in the orchard and positions of the infection directly at the range of the sprinkler system. d) newly formed canker with gummosis on the trunk; e) Gummosis on the trunk treated with paint and copper.

2. Greenhouse experiments using spray inoculation replicated BCD symptoms.

As shown in Fig. 2, we spray inoculated first-year cherry trees in greenhouse (**Fig. 2)**. The symptoms caused by spray inoculation included infections at the young bud or junctions of shoots and leaves. Typical symptoms of BCD including water-soaking on shoots, necrotic lesions, wilting,

cankers and oozes were all replicated (Fig. 2), indicating that BCD pathogens could cause infections through bud, junctions and wounds; and the bacteria are spread by rain, water and irrigation systems.



Fig. 2. Symptoms obtained in greenhouse after spray inoculation with *Pseudomonas syringae* pv. *syringae* (Pss) and *P. amygdali* pv. *morsprunorum* (Pam) strains. a) Initial infections at multiple buds, leading to necrotic lesions; b) initial infections at the junctions, leading to water-soaking lesions which eventually will lead to wilting and death of the leaves; c) infections at the emerging bud on the trunk, leading to gummosis and canker and wilting; d) Infections at the bud, leading to death/wilting of the leaves.

3. Phylogeny and phylogenomics analysis.

Phylogenomic analysis based on ANI distances resolved distinct species-level clades across the 169 genomes (**Fig. 3**), which were consistent with genome-based reassignments. Genome-based analyses, including ANI and dDDH together with genome BLAST distance phylogenies generated through the Type (Strain) Genome Server (TYGS), revealed substantial discrepancies between previously deposited NCBI species designations and genome-inferred reassignments. Of the 169 genomes, 65 genomes maintained the same species designation as their existing NCBI submissions, whereas 87 were clustered with distinct species based on TYGS clustering and 17 could not be assigned to any known *Pseudomonas* species (**Fig. 3**). Among the 87 genomes with revised taxonomic assignments, 69 were clustered with the *P. fragariae* strain Pfr17. The remaining 18 were clustered with *P. amygdali* (n = 10), *P. cerasi* (n = 3), *P. avellanae* (n = 4), and *P. triticifolii* (n = 1). ANI analysis revealed that 69 genomes previously designated as *P. syringae* exhibited ≥98.6% similarity to the genome of *P. fragariae* strain Pfr17, substantially exceeding the species-level threshold. These results were strongly supported by dDDH values, which ranged from 88.1-90.1% relative to that of *P. fragariae* strain Pfr17. These results further supported the reassignment of the 69 genomes, which formed a distinct cluster with *P. fragariae* (**Fig. 3**).

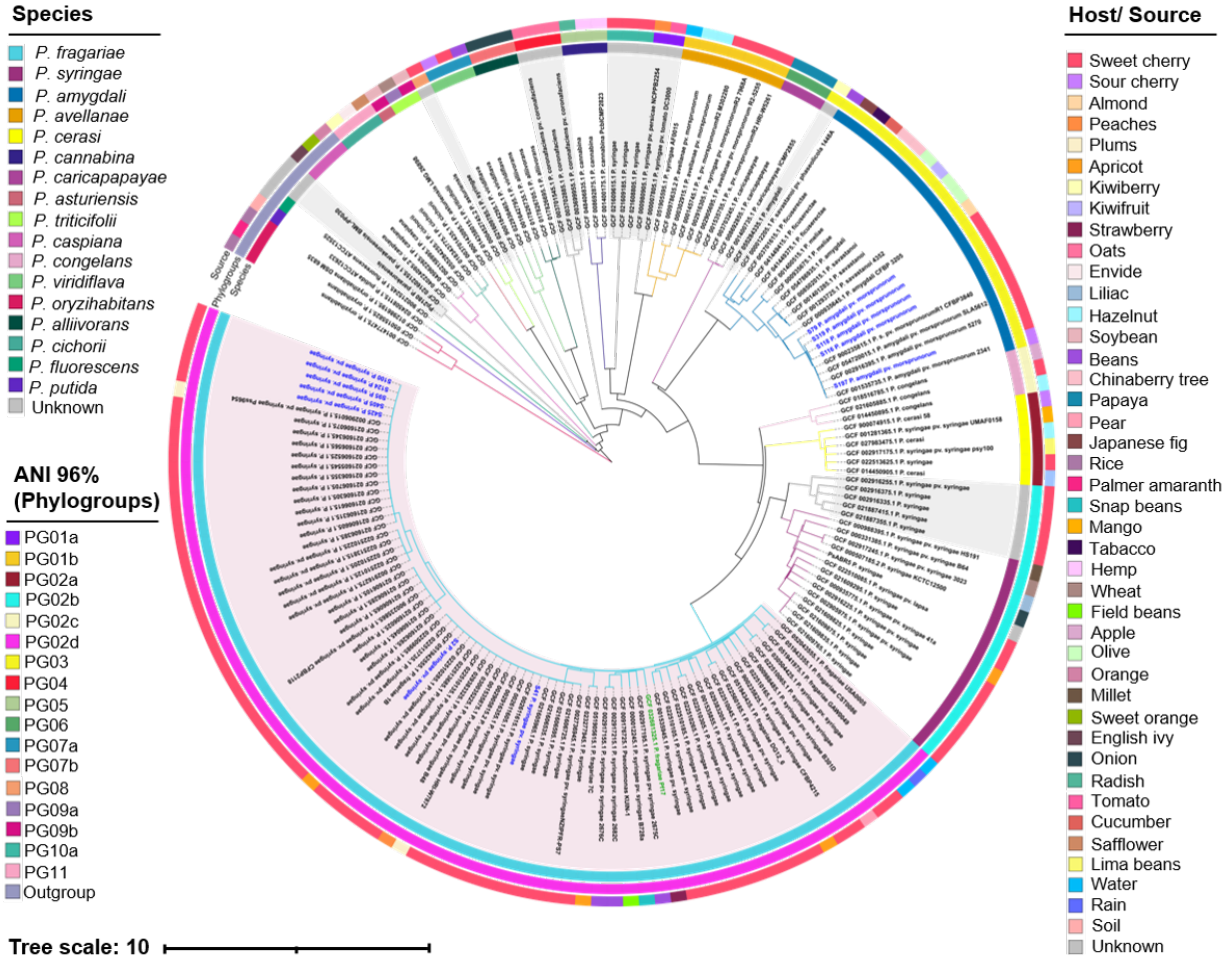


Fig. 3. Circular phylogenetic tree of the 169 *Pseudomonas* genomes with species, phylogroup, and host/source annotations. Three concentric annotation rings are displayed from inner to outer: (a) species identity, with *P. fragariae* highlighted in teal; (b) phylogroup assignment (ANI 96% phylogroups, PG01a-PG11) representing genomically coherent species-level clusters at the 96% ANI demarcation threshold; and (c) host/source of isolation, encompassing a broad range of plant hosts, environmental sources (water, rain, soil), and unknown origins. Within the *P. fragariae* clade, tip labels are color-coded to indicate strain origin and significance: blue labels denote strains from Washington, green labels indicate the reference strain *P. fragariae* strain 17, and black labels denote all other strains included in the analysis.

The *P. fragariae* phylogroup PG02d formed a monophyletic clade clearly separated from *P. syringae* phylogroup PG02b and other members of the PSSC. The *P. fragariae* clade (n=76) comprised of strains from diverse hosts and environmental sources, including *Fragaria* spp. (strawberry, n=1), *Prunus avium* (sweet cherry, n=60), *Prunus armeniaca* (n=3), *Prunus domestica* (plums, n=2), *Prunus persicae* (peaches, n=1), *Pyrus* spp. (pear, n=1), *Phaseolus vulgaris* (snap beans, n=4), and *Vicia faba* (field beans, n=1) as well as water (n=2) and rain (n=1) samples. The relationships among species, phylogroups, and host origins are illustrated in the Sankey plot (**Fig. 4**). Sixty BCD-associated Pss genomes were redesignated as *P. fragariae* phylogroup PG02d. On the other hand, the 92 BCD-associated genomes were distributed across six major phylogroups (PG01,

02, 03.1, 07, 08 and 10), comprising 10 phylogenetic lineages or subgroups**.** These results suggest that the major pathogen causing BCD, which previously recognized as Pss, belongs to *P. fragariae*.

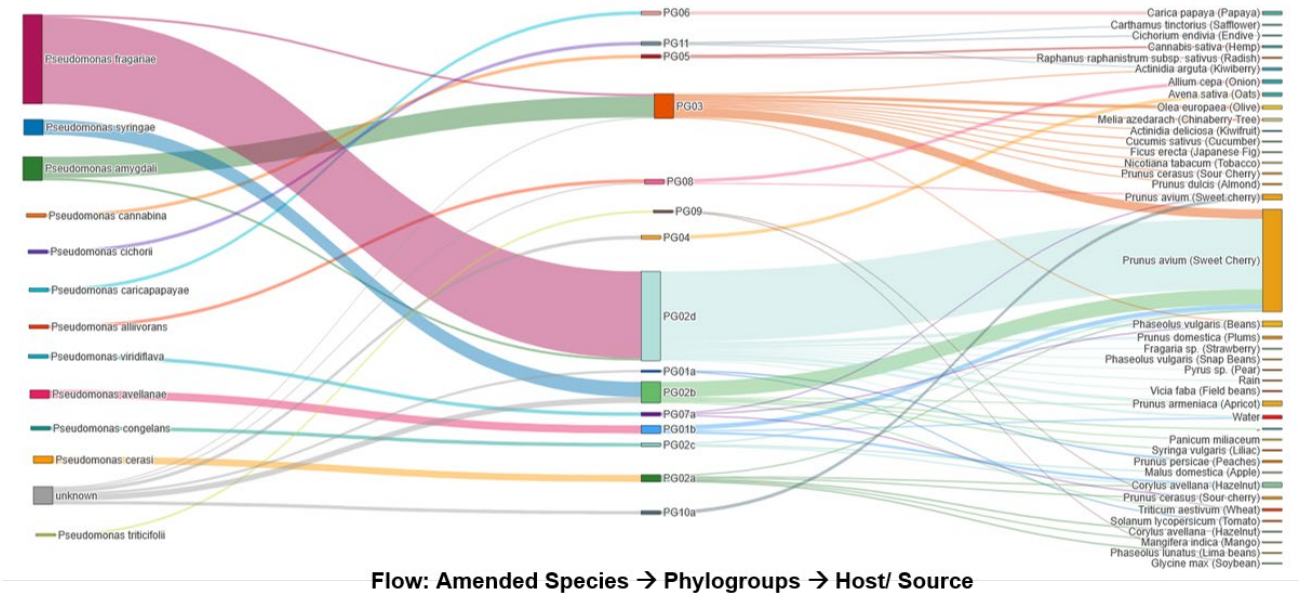


Fig. 4. **Sankey plot illustrates the relationships among species assignment, phylogroup, and host origin for the 169 *Pseudomonas* genomes.** Left nodes represent the amended species assignment based on concordant genome-based analyses (ANI, dDDH, TYGS, MLSA, and core-genome phylogeny); central nodes represent the phylogroup designations; right nodes represent host or environmental source of isolation. The majority of the cherry-associated (*Prunus avium*) genomes previously classified as *P. syringae* (PG02b) were grouped to *P. fragariae* (phylogroup PG02d). Genomes that could not be assigned to any recognized species by TYGS are labeled as “unknown."

4. Development of a multiplex PCR.

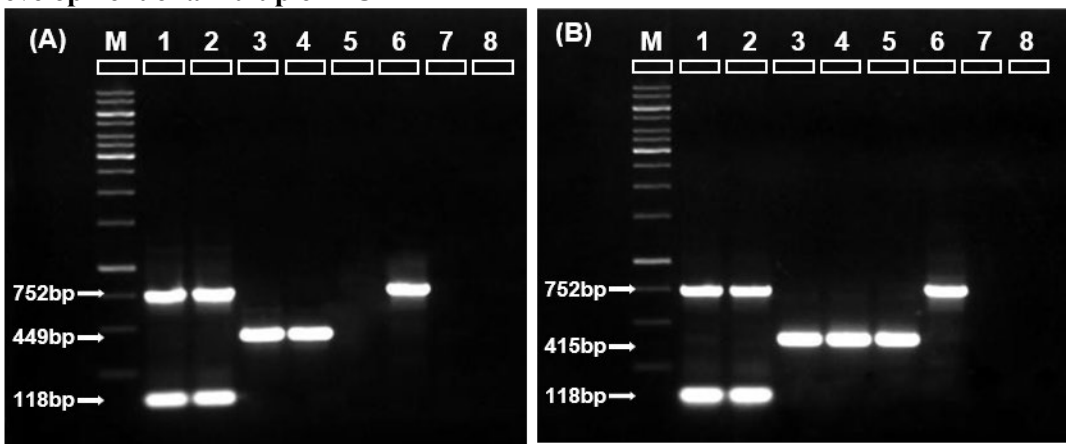


Fig. 5. PCR amplification profiles using optimized multiplex PCR assays. (A) Multiplex Set 1 containing the SyrB, Pfr118, and Psm449 primer pairs. (B) Multiplex Set 2 containing SyrB, Pfr118, and Pam415 primer pairs. M, 1 kb DNA ladder; lane 1, Pfr strain S2; lane 2, Pfr strain S124; lane 3, Pam strain S79; lane 4, Pam strain S197; lane 5, *P. amygdali* CFBP 1670, previously identified as *P. savastanoi* and representing a non-pv. *morsprunorum* member of PG03; lane 6, Pss strain FF5; lane 7, *Erwinia amylovora* Ea2160; lane 8, water. The expected amplicon sizes were 752, 449, 415, and 118 bp for the SyrB, Psm449, Pam415, and Pfr118 primer pairs, respectively.

Comparative genome analysis identified specific genes, which were used to design specific primer pair for the phylogroups PG02d (Pfr149) and PG03 (Pam415). For identification of Pam, Psm449 primer pair was developed from the previously described Psm1 SCAR marker to generate a 449 bp product. The syrB primer pair was used to produce a 752 bp amplicon for phylogroup PG02. Due to similar size produced by the Psm449 (449 bp) and the Pam415 (415 bp) primer pairs, two sets of multiplex primers were tested. Both sets contained the syrB (752 bp) and Pfr118 (118 bp) primer pairs, while multiplex PCR Set 1 and 2 contained Psm449 (449 bp) and Pam415 (415 bp) primer pairs, respectively. PCR conditions were optimized separately for each newly designed primer pair. Annealing temperatures of 64 °C for the Pfr118 primer pair and 65 °C for the Psm449 and Pam415 primer pairs were evaluated. A final annealing temperature of 62 °C was selected for both multiplex sets.

In Multiplex Set 1, Pfr strains S2 and S124 produced the expected 752 bp and 118 bp products (**Fig. 5A**, lanes 1 and 2). Pam strains S79 and S197 produced the expected 449 (lanes 3 and 4). *P. amygdali* strain CFBP 1670, but not to pv. *morsprunorum*, did not produce the Psm449 product (lane 5). Pss strain FF5 produced only the 752 bp product (lane 6), while *Erwinia amylovora* Ea2160 and the water control produced no amplification (lanes 7 and 8). In Multiplex Set 2, Pfr strains S2 and S124 produced the 752 bp and 118 bp products (**Fig. 5B**, lanes 1 and 2). Pam strains S79 and S197 produced the 415 bp PG03 product (lanes 3 and 4). The 415 bp product was also amplified from *P. amygdali* CFBP 1670 (lane 5). Pss strain FF5 produced only the 752 bp *syrB* product (lane 6), while *E. amylovora* Ea2160 and the water control produced no amplification (lanes 7 and 8). These results suggested that multiplex PCR could successfully differentiate Pfr from Pss and Pam as well as other members with the PSCC.

Significance to the industry and potential economic benefits:

Due to widespread copper resistance in *Pseudomonas* pathogens, growers rely more on culture control measures for BCD management. One critical element in establishing new cherry orchards to avoid BCD occurrence is using proper irrigation system. Survey conducted in 2026 again found that sprinkler irrigation resulted in severe BCD outbreak in young orchards like we have reported for the 2023 BCD outbreak. These field symptoms could be replicated by greenhouse spray inoculation, further indicating that *Pseudomonas* spread in orchards through irrigation water application. The plant-pathogenic *Pseudomonas syringae* species complex (PSSC) comprises one of the most taxonomically and epidemiologically challenging groups of phytopathogenic bacteria. Application of genome-wide taxonomic approaches has since revealed substantial discrepancies within the genus *Pseudomonas*. Taxonomic uncertainty has important implications for plant disease management, as unresolved species boundaries can complicate pathogen detection, epidemiological surveillance, and interpretation of host-pathogen interactions. During 2023 survey, we found for the first time that 5% isolates were *Pseudomonas amygdali* pv. *morsprunorum* (Pam) in WA to be the causal agent of BCD of sweet cherries; In addition, 75% isolates were classified as *Pseudomonas syringae* pv. *syringae* (Pss) as the main pathogen causing BCD of sweet cherries in WA. However, based on genome sequencing and phylogenomics analysis, we re-assigned Pss to *P. fragariae*. Furthermore, we developed and optimized a multiplex PCR assay to differentiate Pfr from Pss, thus enabling accurate diagnosis of the pathogens of BCD. Our findings are significant and could provide direct economic benefits to growers and the cherry industry by using proper irrigation system, thus reducing losses due to BCD and increasing profitability. Accurate species identification underpins pathogen monitoring, resistance screening, and disease management.

Literature Review.

The genus *Pseudomonas* comprises a diverse group of Gram-negative bacteria that occupy a wide range of ecological niches, including soil, water, plant, insect, and animal/human-associated environments**,** and exhibit remarkable metabolic versatility **(Gross and Loper 2009).** Among plant-

associated pseudomonads, many are important plant pathogens capable of infecting numerous hosts with broad host ranges and causing diverse diseases, including leaf spots, blights, cankers, galls, and wilting diseases **(Morris et al. 2019; Xin et al. 2018)**. This ecological and pathogenic diversity has made *Pseudomonas* one of the most intensively studied bacterial genera, including the plant-pathogenic *Pseudomonas syringae* species complex (PSSC). BCD of sweet cherry is a disease complex caused by at least five phylogenetically distinct lineages (phylogroups) within the PSSC, including *P. syringae* pv. *syringae* (Pss, PG02), Pam (PG03, formerly referred to as *P. syringae* pv. *morsprunorum* race 1), and *P. avellanae* pv. *morsprunorum* (Pav) (PG01, formerly referred to as *P. syringae* pv. *morsprunorum* race 2), *P. syringae* pv. *avii*, and *P. syringae* pv. *persicae* **((Iličić et al. 2026, Gomila et al. 2017; Hulin et al. 2020; Ruinelli et al. 2022)**. In addition, *P. cerasi* was consistently associated with canker formation **(Kałużna et al. 2016)** and *P. viridiflava* (PG07) exhibited variable pathogenicity on sweet cherries **(Maguvu et al. 2024)**.

Genome-informed population studies have substantially enhanced our understanding of diversity within the PSSC. Prior to that, DNA-DNA hybridization (DDH) and multi-locus sequence typing (MLST) studies reclassified the PSSC into at least nine genomospecies and 13 phylogroups, respectively (**Berge et al. 2014, Gardan et al. 1999**). Increasing availability of whole-genome sequences has substantially transformed bacterial systematics and enabled the adoption of genome-based metrics for species delineation, defined by the thresholds of ≥95-96% average nucleotide identity (ANI) and ≥70% digital DDH (dDDH). A comprehensive genome-scale reassessment by **Tran et al. (2017)** detected 207 erroneous species labels among 373 *Pseudomonas* genomes. Additional genome-based studies have also reassigned historically classified taxa into novel or amended genera. Thus, unresolved species boundaries can complicate pathogen detection, epidemiological surveillance, and interpretation of host-pathogen interactions, which could further impact disease management.

Among closely related members of the PSCC, accurate differentiation is difficult due to similar colony morphology, fluorescence, biochemical reactions, and pathogenicity characteristics. The *syrB* PCR detects strains carrying a gene associated with cyclic lipodepsipeptide production and has been used for the presumptive identification of Pss (**Sorensen et al. 1998**). The SCAR markers developed for the morsprunorum lineages provide more targeted and specific detection of Pam and Pav (**Kałużna et al. 2016**). A recent genomic study of sweet cherry-associated Pss populations in Chile alongside publicly available genomes belonging to phylogroup PG02 has further subdivided PG02 into PG02a, PG02b, PG02c, and PG02d subgroups, the later was found to be the predominant subgroup causing BCD among Chilean sweet cherry isolates **(Correa et al. 2026)**. Phylogenomic analyses further reassigned BCD pathogens within the PSCC into multiple species and comparative genomic studies of *Prunus*-associated strains have also shown that similar canker symptoms can be produced by genetically distinct lineages with different pathogenicity-associated gene repertoires. Thus, it is critical to develop new methods to differentiate multiple species (phylogroups) causing BCD of sweet cherries, especially those belonging to PG02d and PG02b. Multiplex PCR permits multiple diagnostic targets to be examined simultaneously as demonstrated for other bacterial plant pathogen complexes (**Potrykus et al. 2014**). This could also be developed for BCD by using genome-informed knowledge of the pathogens.

References:

1. Berge, O., Monteil, C. L., Bartoli, C., Chandeysson, C., Guilbaud, C., Sands, D. C., and Morris, C. E. 2014. A user's guide to a data base of the diversity of *Pseudomonas syringae* and its application to classifying strains in this phylogenetic complex. PLoS ONE 9.
2. Correa, F., Millas, P., Almada, R., Figueroa, F., Martinez, J. P., and Sagredo, B. 2026. Genomic characterization of *Pseudomonas syringae* pv. *syringae* populations affecting sweet cherry orchards in Chile reveals local adaptation and virulence signatures. Plants 15:552.
3. Gardan, L., H. Shafik, S. Belouin, R. Broch, F. Grimont, and P. A. D. Grimont. 1999. DNA Relatedness Among the Pathovars of *Pseudomonas syringae* And Description of *Pseudomonas*

tremae sp. nov. and *Pseudomonas cannabina* sp. nov.(ex Sutic and Dowson 1959). Int J System Evol Microbiol 49: 469-478

4. Gomila, M., Busquets, A., Mulet, M., García-Valdés, E., and Lalucat, J. 2017. Clarification of taxonomic status within the *Pseudomonas syringae* species group based on a phylogenomic analysis. Front. Microbiol. 8:2422.
5. Gross, H., and J. E. Loper. 2009. Genomics of secondary metabolite production by *Pseudomonas* spp. Nat Product Rep 2611: 1408-1446.
6. Hulin, M. T., Jackson, R. W., Harrison, R. J., and Mansfield, J. W. 2020. Cherry picking by pseudomonads: After a century of research on canker, genomics provides insights into the evolution of pathogenicity towards stone fruits. Plant Pathol. 69:962-978.
7. Hulin, M. T., Armitage, A. D., Vicente, J. G., Holub, E. B., Baxter, L., Bates, H. J., Mansfield, J. W., Jackson, R. W., and Harrison, R. J. 2018. Comparative genomics of *Pseudomonas syringae* reveals convergent gene gain and loss associated with specialization onto cherry (*Prunus avium*). New Phytol. 219:672-696.
8. Iličić, R., Scortichini, M., and Popović Milovanović, T. 2026. An overview of bacterial canker in stone fruits caused by different pseudomonads: *Pseudomonas syringae* species complex and related species. Agriculture 16:1358.
9. Kałużna, M., Albuquerque, P., Tavares, F., Sobiczewski, P., and Puławska, J. 2016. Development of SCAR markers for rapid and specific detection of *Pseudomonas syringae* pv. morsprunorum races 1 and 2, using conventional and real-time PCR. Appl. Microbiol. Biotechnol. 100:3693-3711.
10. Maguvu, T. E., R. J. Frias, A. I. Hernandez-Rosas, E. Shipley, G. Dardani, M. T. Nouri, et al. 2024. Pathogenicity, phylogenomic, and comparative genomic study of *Pseudomonas syringae* sensu lato affecting sweet cherry in California. Microbiol Spect 1210: e01324-24.
11. Morris, C. E., J. R. Lamichhane, I. Nikolić, S. Stanković, and B. Moury. 2019. The overlapping continuum of host range among strains in the *Pseudomonas syringae* complex. Phytopathol Res 11: 4.
12. Potrykus, M., Sledz, W., Golanowska, M., Slawiak, M., Binek, A., et al.. 2014. Simultaneous detection of major blackleg and soft rot bacterial pathogens in potato by multiplex polymerase chain reaction. Ann. Appl. Biol. 165:474-487.
13. Ruinelli, M., Blom, J., Smits, T. H., and Pothier, J. F. 2019. Comparative genomics and pathogenicity potential of members of the *Pseudomonas syringae* species complex on *Prunus* spp. BMC Genom 20:172.
14. Sorensen, K. N., Kim, K. H., and Takemoto, J. Y. 1998. PCR detection of cyclic lipodepsinonapeptide-producing *Pseudomonas syringae* pv. syringae and similarity of strains. Appl. Environ. Microbiol. 64:226-230.
15. Tran, P. N., M. A. Savka, and H. M. Gan. 2017. In-silico taxonomic classification of 373 genomes reveals species misidentification and new genospecies within the genus *Pseudomonas*. Front Microbiol 8: 1296.
16. Xin, X. F., B. Kvitko, and S. Y. He. 2018. *Pseudomonas syringae*: What it takes to be a pathogen. Nat Rev Microbiol 165: 316-328.

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

Report Type: Final Project Report

Primary PI: Claire Murphy
Organization: Washington State University Irrigated Agriculture Research and Extension Center
Telephone: 509-786-9201
Email: claire.murphy@wsu.edu
Address: 24106 N Bunn Rd
City/State/Zip: Prosser, WA 99350

Co-PI 2: Manoella Mendoza
Organization: Washington Tree Fruit Research Commission
Telephone: 509-665-8271
Email: manoella@treefruitresearch.com
Address: 1719 Springwater
Avenue City/State/Zip: Wenatchee, WA 98801

Cooperators: Between the 3 field objectives, 7 unique cherry packinghouses in Washington and 4 in California were cooperators across objectives 1 - 3. The identity of the operations serving in cooperator roles will be kept confidential.

Project Duration: 3 Year

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

Other related/associated funding sources: None
Funding Duration: NA
(Type Current Funding Year) Amount: NA
Agency Name: NA
Notes: NA

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: 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: naidu.rayapati@wsu.edu

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, 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

Recap Original Objectives

1. Evaluate the prevalence of *Listeria* spp., as an indicator for *L. monocytogenes*, on food contact surfaces on the packing line within commercial cherry packinghouses at three time points (beginning, middle, and end of the season) over two packing seasons.
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 cross contamination risks associated with hydrocooling.
4. Evaluate the survival of foodborne pathogens on cherries during storage and determine changes in pathogen populations over time.

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).
- Sanitizer concentration was not consistently related to any water quality examined, highlighting the need to directly monitor sanitizer levels during packing.
- Higher aerobic plate counts (APC) on incoming cherries were associated with higher APC in the water and on outgoing cherries. Water APC was also associated with APC on outgoing cherries, demonstrating the potential for cross-contamination.

Objective 3:

- Hydrocooling was not associated with an overall increase in microbial loads on cherries. Total coliform and HPC concentrations decreased by an average of 0.87 and 0.77 log MPN/cherry, respectively, from pre- to post-hydrocooling ($p < 0.001$ and $p = 0.001$).
- Each 1-log increase in hydrocooler water HPC was associated with a 0.59-log greater pre- to post-hydrocooling change in cherry HPC ($p < 0.001$), indicating that higher microbial loads in the water were associated with smaller reductions or increases in HPC on cherries.

Objective 4:

- *L. monocytogenes* demonstrated greater persistence on cherries during refrigerated storage, decreasing by approximately 2.2 log CFU/cherry over 14 days, compared with reductions of approximately 3.6 and 4.0 log CFU/cherry for *Salmonella* and STEC, respectively.

Methods

Objective 1:

Study Design: 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 premoistened 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:

Experimental design and processing: Four commercial cherry packinghouses (A–D) in Washington State were sampled during the 2025 season. Packinghouses A and B operated four sequential discrete water systems, while C and D operated three. Production water was treated with calcium hypochlorite, with sanitizer levels managed using oxidation-reduction potential (ORP) targets of 800–850 mV (A and B) or >750 mV (C and D). Water pH was adjusted using acid-based treatments to approximately pH 6.0–7.5. At each sampling time point, three individual cherries were collected before entering the discrete water systems and three after exiting the discrete water systems.

Sample collection: Triplicate 100 mL water samples were collected from each discrete water system into sterile bottles containing sodium thiosulfate to neutralize residual sanitizer. Sampling was repeated throughout the production day. Samples were transported on ice and stored at 4°C for up to 48 h before microbiological analysis. Water pH, turbidity, temperature, and free chlorine concentration were monitored throughout each sampling day. Turbidity was measured using a portable turbidimeter and reported as nephelometric turbidity units (NTU). Free chlorine was measured using a titration kit with 0.5 ppm increments.

Sample processing: Individual cherries were rinsed with 10 mL of 0.1% peptone using a standardized rub-shake-rub procedure. Cherry rinsates and water samples were serially diluted as needed and analyzed for aerobic plate counts (APC), *Enterobacteriaceae* (EB), total coliforms (TC), and generic *Escherichia coli* (EC) using Petrifilm methods. Colonies were enumerated according to manufacturer instructions following incubation. Limits of detection were 0.0 log CFU/mL for water and 1.0 log CFU/cherry for cherry samples.

Structural equation model: Structural equation modeling is a statistical approach that allows multiple relationships to be evaluated simultaneously, helping identify how factors such as water quality, sanitizer concentration, water hold time, and microbial populations may be related to one another and ultimately influence microbial populations on cherries (Figure 1). Separate SEMs were developed for each DWS using the same model structure to evaluate both direct and indirect relationships among water quality, microbial populations in the water, and microbial populations on cherries over the water hold period.

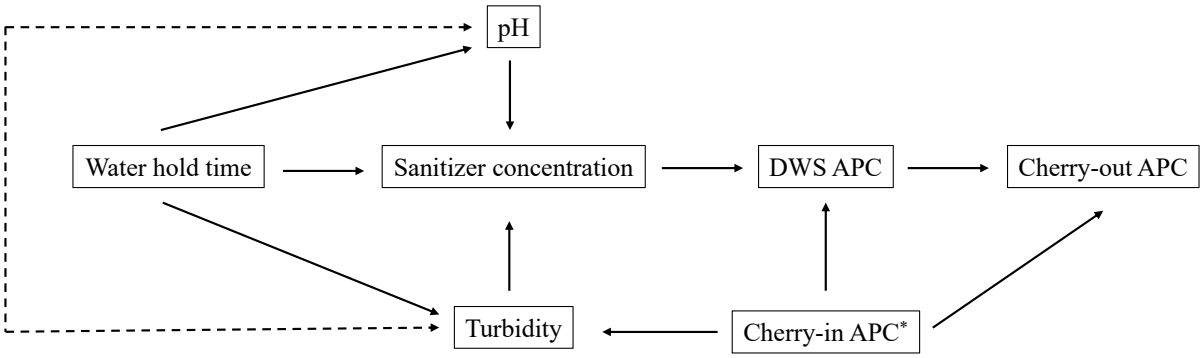


Figure 1. Graph illustrating the structural equation model structure applied to each discrete water system in the separate SEMs. The solid arrows represent direct effects, whereas the dashed arrow is representative of a covariance structure.

Objective 3:

Experimental design, sample collection and processing: Four commercial cherry packinghouses in Washington State were sampled during the 2026 season. Triplicate water samples (100 mL) were collected from hydrocooler water systems, and three individual cherries were collected before entering and after exiting the hydrocooler at each sampling time point throughout the total water hold period. Water samples were collected into sterile bottles containing sodium thiosulfate to neutralize residual sanitizer. All water and cherry samples were transported on ice and stored at 4°C for up to 48 h before microbiological analysis. Individual cherries were rinsed with 100 mL of 0.1% peptone using a standardized rub-shake-rub procedure. Cherry rinsates and water samples were analyzed for heterotrophic plate counts (HPC), total coliforms, and generic *Escherichia coli* using the IDEXX Quanti-Tray system according to manufacturer instructions. The limit of detection for water samples was 0 log CFU/100 mL and 0 log CFU/cherry for cherries. Water pH, turbidity, temperature, and sanitizer concentrations were monitored throughout the water hold period. Microbial trends were evaluated to assess changes over water hold time, potential cross-contamination between cherries and water, and physicochemical and operational factors associated with microbial water quality and post-hydrocooler cherry contamination.

Objective 4:

Experimental design, sample collection and processing: Fresh cherries were evaluated for survival of *Listeria monocytogenes*, *Salmonella*, and Shiga toxin-producing *Escherichia coli* (STEC) during refrigerated storage. Separate five strain rifampicin-resistant cocktails were prepared for each pathogen. Each pathogen cocktail was independently spot inoculated onto 10 g cherry samples at a ~ 5 log CFU/g. Inoculated cherries were dried for 1 h and stored at 4°C (40°F) for up to 14 days. Samples were collected at 0 and 4 h and on days 1, 2, 3, 5, 7, 10, and 14. At each sampling time point, 20 mL of 0.1% peptone water was added to each 10 g cherry sample, followed by a rub-shake-rub procedure. Samples were plated in duplicate on TSA supplemented with rifampicin and incubated at

35°C (95°F) for 48 h for *L. monocytogenes* and 24 h for *Salmonella* and STEC before enumeration. Survival and reductions of pathogens on fresh cherries were characterized.

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%; 9/144), 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%

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 during packing suggests that *Listeria* spp. may be introduced or redistributed during production, including through incoming fruit or movement within the packing environment. 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 4: 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.

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). 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. 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 relatively limited detection of generic *E. coli* and total coliforms suggests that these organisms alone may have limited utility for characterizing microbial dynamics and cross-contamination in cherry postharvest water systems.

Enterobacteriaceae was detected in 45% (175 /387) of water samples, with populations among positive samples ranging from 0.30 to 2.15 log CFU/mL. For cherries, *Enterobacteriaceae* was detected on 18% (95/518) of samples collected, with concentrations of positive samples ranging from 1.30 to 3.80 log CFU/cherry. *Enterobacteriaceae* is a bacterial family that includes many species commonly associated with environmental and fecal contamination, making it a useful indicator of sanitation effectiveness and cross-contamination evaluation. Among the indicator organisms tested, APC had the greatest prevalence rate and was therefore used as an indicator of microbial water quality and populations on cherries for structural equation models. APC measures the total number of viable bacteria capable of growing in oxygenated conditions, providing an overall estimate of

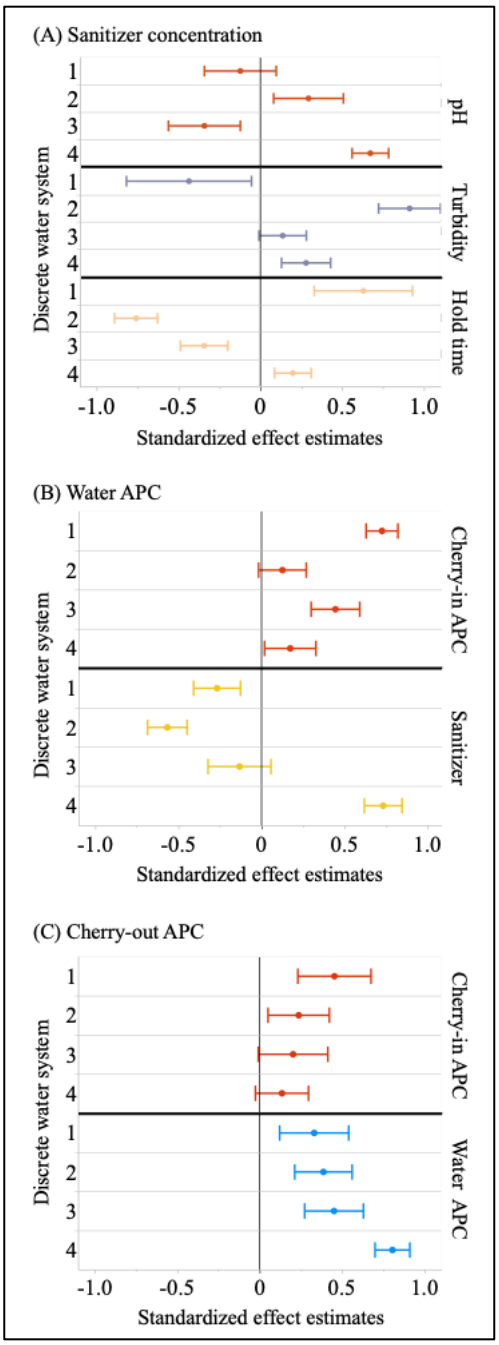


Figure 2. The association between explanatory variables and (A) sanitizer concentration, (B) water aerobic plate count (APC) populations, or (C) cherry-out APC levels. The effect estimates should be interpreted as the effect of a one standard deviation (SD) increase in the explanatory variable on the outcome variable.

microbial load. APC was detected in 88% (341/387) water and on 81% (419/518) of the cherry samples.

Four identical structural equation models were developed for each discreate water system. The relationship between pH and sanitizer concentration varied among water systems, with no consistent pattern observed across discrete water system (Figure 2a). Turbidity and sanitizer concentration also showed different relationships among systems. In discrete water system 1, higher turbidity was associated with lower sanitizer concentrations [Confidence intervals (CI) = -0.81, -0.06], while the opposite pattern was observed in discrete water system 2 (CI = 0.72, 1.10). Relationships were weaker in discrete water system 3 (CI = -0.01, 0.28) and discrete water system 4 (CI = 0.13, 0.43). Water hold time also showed different relationships with sanitizer concentration across systems, with positive associations in discrete water system 1 (CI = 0.33, 0.92) and discrete water system 4 (CI = 0.09, 0.31) and negative associations in discrete water system 2 (CI = -0.89, -0.63) and discrete water system 3 (CI = -0.49, -0.20). These results demonstrate that sanitizer concentration cannot be predicted from a single water quality factor across all packinghouse systems. The differences among water systems highlight the importance of individual packinghouses routinely monitoring sanitizer concentration and water quality during operation rather than relying on pH, turbidity, or water hold time alone to indicate whether sanitizer levels are adequate.

The cherry results further demonstrate that microbial levels can change as fruit moves through the packing process (Figure 3). The direction and magnitude of these changes varied among packinghouses and discrete water system, with some systems showing decreases in APC after entering the water system and others showing increases at later processing stages. The SEM results also showed a consistent relationship between the microbial levels on incoming cherries and in the process water (Figure 2b). Higher bacterial levels on incoming cherries were associated with higher bacterial levels in the water, indicating that incoming fruit can contribute to the microbial load in the water. Higher sanitizer concentrations were associated with lower bacterial levels in the water in discrete water system 1 (CI = -0.40, -0.13), discrete water system 2 (CI = -0.68, -0.45), and discrete water system 3 (CI = -0.32, 0.06). Higher bacterial levels on incoming cherries were also generally associated with higher bacterial levels on outgoing cherries (Figure 2c), with positive associations in discrete water system 1 (CI = 0.24, 0.66), discrete water system 2 (CI = 0.21, 0.56), discrete water system 3 (CI = -0.01, 0.41), and discrete water system 4 (CI = -0.03, 0.30). Water bacterial levels were also associated with higher bacterial levels on outgoing cherries, with associations

observed in discrete water system 1 (CI = 0.12, 0.54), discrete water system 2 (CI = 0.04, 0.43), discrete water system 3 (CI = 0.27, 0.63), and discrete water system 4 (CI = 0.70, 0.91). Together, these findings demonstrate the potential for microbial transfer from incoming fruit to process water and from process water back to fruit. The association between water microbial levels and outgoing fruit microbial levels further supports the importance of managing process water to reduce opportunities for cross-contamination. These results reinforce the value of monitoring incoming fruit, maintaining effective sanitizer concentrations, and monitoring water quality throughout the packing process.

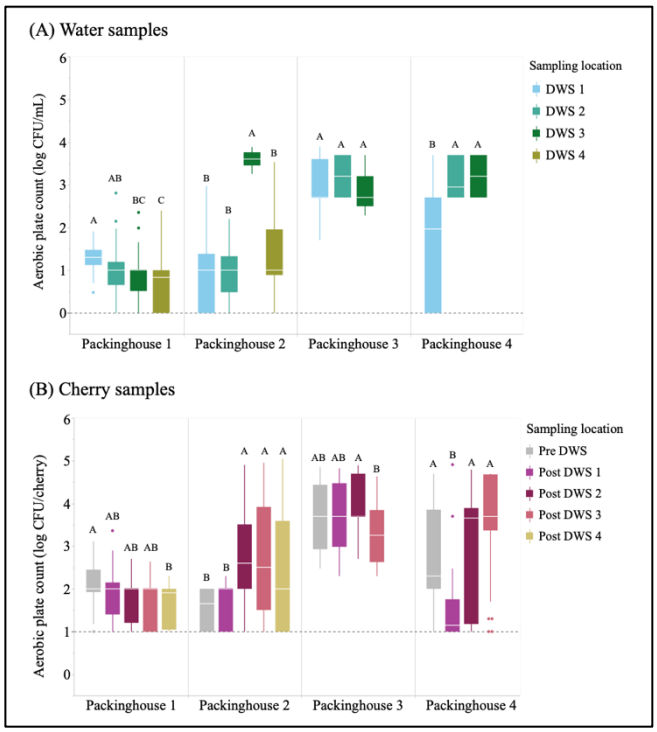


Figure 3. Aerobic plate count populations of (A) water, and (B) cherry samples by packinghouse and discrete water source (DWS). Different uppercase letters above box plots represent a significant difference in population means among sampling locations within individual packinghouses ($P < 0.05$).

Objective 3:

Generic *E. coli* was detected in 33% (30/90) of water samples, with concentrations among positive samples ranging from 1.16 to 3.38 log MPN/100 mL. For cherries, generic *E. coli* was detected on 8% (14/165) of samples collected, with concentrations among positive samples ranging from 0.30 to 2.81 log MPN/cherry. Total coliforms were detected in 61% (55/90) of water samples, with concentrations among positive samples ranging from 0.30 to 4.66 log MPN/100 mL. For cherries, total coliforms were detected on 80% (132/165) of samples collected, with concentrations among positive samples ranging from 0.30 to 5.19 log MPN/cherry. Heterotrophic plate counts (HPC) were detected in 98% (87/89) of water samples, with concentrations among positive samples ranging from 0.30 to 5.38 log MPN/100 mL. For cherries, HPC were detected on 100% (165/165) of samples collected, with concentrations ranging from 0.30 to 5.38 log MPN/cherry.

Across sampling events with paired pre- and post-hydrocooling samples (n = 27), total coliform concentrations decreased by an average of 0.87 log MPN/cherry (95% CI: 0.47, 1.27 log MPN/cherry; $p < 0.001$), while HPC decreased by an average of 0.77 log MPN/cherry (95% CI: 0.34, 1.21 log MPN/cherry; $p = 0.001$). Total coliform concentrations increased following hydrocooling during 6 of 27 sampling events, while HPC concentrations increased during 7 of 27 sampling events. Although HPC generally decreased following hydrocooling, the magnitude of the pre- to post-hydrocooling change was associated with HPC concentrations in the hydrocooler water. After accounting for packinghouse and sampling time, each 1 log increase in water HPC was associated with a 0.59 log greater change in HPC on cherries from pre- to post-hydrocooling (95% CI: 0.26, 0.92; $p < 0.001$; Figure 4). In contrast, water TC concentration was not associated with the pre- to post-hydrocooling change in TC on cherries ($\beta = 0.08$; 95% CI: −0.20, 0.37; $p = 0.568$). Among the

physicochemical water-quality parameters evaluated, turbidity was positively associated with total coliform concentrations in hydrocooler water (β = 0.020, $p < 0.001$). However, turbidity was not associated with post-hydrocooling total coliform concentrations on cherries ($P = 0.108$). Sanitizer concentration, temperature, and pH were not associated with total coliform concentrations in the water or on post-hydrocooling cherries ($p > 0.05$). None of the physicochemical water-quality parameters evaluated were significantly associated with HPC concentrations in the hydrocooler water or on post-hydrocooling cherries ($p > 0.05$).

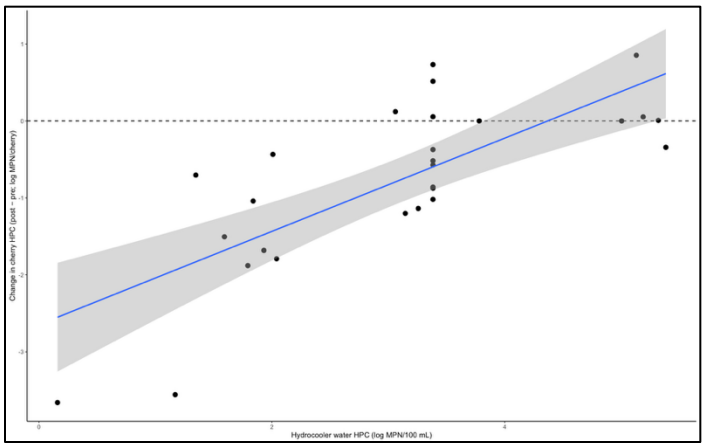


Figure 4. Association between HPC in hydrocooler water and the change in HPC on cherries following hydrocooling

These results suggest that hydrocooling under the conditions observed in the four packinghouses studied did not generally result in increased microbial loads on cherries, with both total coliform and HPC concentrations decreasing on average following hydrocooling. However, these decreases should not be interpreted as evidence that hydrocooling is a microbial reduction step. The relationship between HPC concentrations in hydrocooler water and the pre- to post-hydrocooling change on cherries indicates that the microbiological quality of recirculated water may influence microbial persistence on fruit. Importantly, increases in HPC following hydrocooling were observed during some individual sampling events despite the overall decrease, emphasizing that average reductions do not eliminate the potential for transfer under specific operating conditions. The association between turbidity and total coliform concentrations in water further suggests that accumulation of organic material or other suspended solids may coincide with declining microbiological water quality, although this relationship did not translate to increased total coliform concentrations on post-hydrocooling cherries. Maintaining water quality through appropriate sanitizer management, removal of accumulated organic material and debris, and routine monitoring throughout operation may help limit conditions that favor microbial persistence and transfer.

Objective 4:

Populations of all three foodborne pathogens decreased during refrigerated storage. *L. monocytogenes* showed the greatest persistence, decreasing from approximately 5.1 ± 0.5 log CFU/cherry at day 0 to 2.9 ± 0.1 log at day 14, a reduction of approximately 2.2 log. *Salmonella* decreased from approximately 5.3 ± 0.4 to 1.7 ± 0.5 log CFU/cherry, while STEC decreased from approximately 5.4 ± 0.3 to 1.4 ± 0.3 log CFU/cherry by day 14, corresponding to reductions of approximately 3.6 and 4.0 log CFU/cherry, respectively. These results demonstrate that none of the three foodborne pathogens grew under refrigeration and populations decreased over 14 days of storage. The greater persistence of *L. monocytogenes* highlights its ability to survive under refrigerated conditions and its continued importance in refrigerated produce and postharvest environments. Although substantial

reductions were observed for *Salmonella* and STEC, viable populations remained detectable after 14 days. These findings reinforce the importance of preventing contamination and maintaining appropriate sanitation and handling practices throughout postharvest operations.

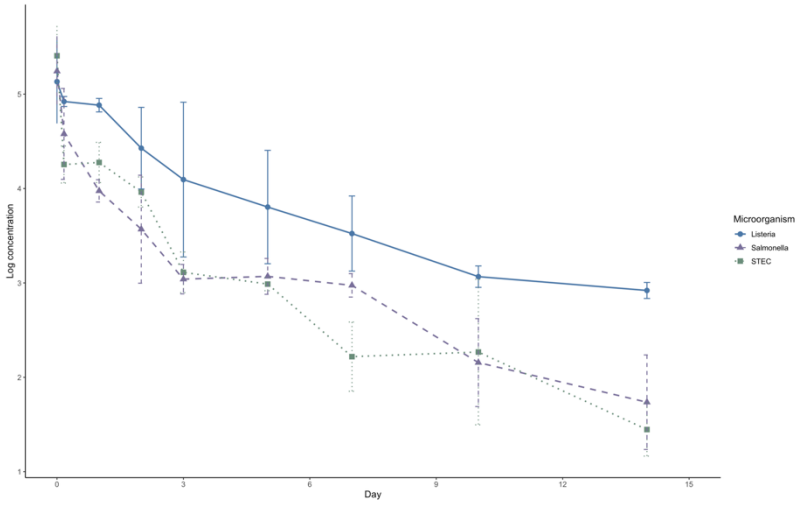


Figure 5. Change in *L. monocytogenes*, *Salmonella*, and STEC populations during 14 days of refrigerated storage

Literature Review

Limited research has evaluated food safety risks specifically associated with the postharvest handling of fresh cherries. However, studies of other fresh produce commodities demonstrate that packinghouse environments and food contact surfaces can provide opportunities for microbial introduction, persistence, and cross-contamination. *Listeria* spp., in particular, have been recovered from food contact surfaces in fresh produce packinghouses. Hamilton (2018) reported *Listeria* spp. on 10.9% (62/565) of food contact surfaces sampled from three tomato packinghouses in Tennessee, while Ruiz-Llacsahuanga et al. (2021) detected *Listeria* spp. on 4.6% (136/2,988) of food contact surfaces in Washington apple packinghouses. In apple packinghouses, polishing brushes and other surfaces containing bristle materials were among the sites with the greatest prevalence. Similarly, bristle flaps were among the most frequently contaminated food contact surfaces in the current cherry study, with *Listeria* spp. detected on 6.3% of samples in Washington and 4.6% in California. Given the potential for bristle materials to retain debris and moisture and serve as harborage sites for *Listeria* spp., the cherry industry should prioritize replacing these materials with more hygienic alternatives when feasible and, when replacement is not possible, implement routine cleaning, sanitization, and replacement when signs of wear are observed.

Postharvest water represents another potential route for microbial cross-contamination during fresh produce handling. Recirculated water can allow microorganisms introduced by incoming fruit to accumulate in the water and potentially transfer to subsequently handled fruit. The primary role of sanitizers in postharvest water is to control microorganisms in the water and limit cross-contamination. Luo et al. (2012) found that *E. coli* O157 cross-contamination during leafy green washing occurred when free chlorine dropped below 1 ppm but was not detected when ≥1 ppm was maintained. Similarly, Pabst et al. (2024) observed widespread *Salmonella* cross-contamination of tomatoes in the absence of PAA, while maintaining 10–80 ppm PAA prevented detectable transfer. These findings reinforce the importance of directly monitoring sanitizer concentrations and water quality during operation.

Additionally, traditional fecal indicator organisms may have limitations for characterizing microbial dynamics in packinghouses. Schneider et al. (2017) reported that 93.8% (1,498/1,597) of pre- and post-processed tomato samples had generic *E. coli* populations below the detection limit, while Strawn et al. (2026) detected generic *E. coli* in only 1.6% of pre- and post-flume apple samples and 1.3% of postharvest water samples. Similarly, generic *E. coli* was detected in only 1.5% of cherry

samples and 2.5% of water samples in data presented. In contrast, APC was widely detected and demonstrated relationships among incoming cherries, process water, and outgoing cherries.

Finally, few studies have evaluated the survival of foodborne pathogens directly on fresh cherries. Previous research demonstrated that *L. monocytogenes* did not grow on cherries during refrigerated storage, consistent with the current findings (Girbal, et al., 2021). This research here demonstrated that none of the three pathogens evaluated grew during 14 days of storage at 4°C, however, all remained detectable at the end of storage.

Collectively, these findings demonstrate that food safety efforts during cherry packing should focus on preventing contamination and limiting microbial transfer through effective equipment sanitation and process-water management, as neither hydrocooling nor refrigerated storage should be relied upon to eliminate contamination once it occurs.

References:

Food and Drug Administration (FDA)., 2022. BAM Chapter 10: Detection of *Listeria monocytogenes* in Foods and Environmental Samples, and Enumeration of *Listeria monocytogenes* in Foods. Available at: https://www.fda.gov/food/laboratory-methods-food/bam-chapter-10-detection-listeria-monocytogenes-foods-and-environmental-samples-and-enumeration

Girbal, M., Strawn, L. K., Murphy, C. M., Bardsley, C. A., & Schaffner, D. W. 2021. ComBase models are valid for predicting fate of Listeria monocytogenes on 10 whole intact raw fruits and vegetables. *Journal of food protection*, 84(4).

Hamilton A.M. 2018. Prevalence of indicator organisms, equipment assessment of risk, and lexicon development: an analysis of the tomato packinghouse environment. MS thesis. University of Tennessee, Knoxville, Tennessee.

Luo, Y., Nou, X., Millner, P., Zhou, B., Shen, C., Yang, Y., Wu, Y., Wang, Q., Feng, H. and Shelton, D., 2012. A pilot plant scale evaluation of a new process aid for enhancing chlorine efficacy against pathogen survival and cross-contamination during produce wash. *International Journal of Food Microbiology*, 158(2)

Pabst, C. R., Kharel, K., De, J., Bardsley, C. A., Bertoldi, B., & Schneider, K. R. (2024). Evaluating the efficacy of peroxyacetic acid in preventing Salmonella cross contamination on tomatoes in a model flume system. Heliyon, 10(10).

Ruiz-Llacsahuanga, B., Hamilton, A., Zaches, R., Hanrahan, I. and Critzer, F., 2021. Prevalence of *Listeria* species on food contact surfaces in Washington State apple packinghouses. *Applied and environmental microbiology*, *87*(9).

Schneider, K. R., De, J., Li, Y., Sreedharan, A., Schneider, R. G., Danyluk, M. D., Pahl, D.M., Walsh, C.S., Todd-Searle, J., Schaffner, D.W., Kline, W., & Buchanan, R. L. (2017). Microbial evaluation of pre-and post-processed tomatoes from Florida, New Jersey and Maryland packinghouses. Food Control, 73, 511-517.

Strawn, L. K., Tocco, P., Zeng, H., Lin, Y., Nam, J. H., Rump, E. S., ... & Murphy, C. M. 2026. In-Plant Validation of Postharvest Water Sanitation Practices to Prevent Cross-Contamination in Michigan Commercial Apple Packinghouses. *Journal of Food Protection*, 100795.

Executive Summary

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

Key Words: cherries, food safety, *Listeria*, postharvest water, hydrocooling, cross-contamination, sanitation

Abstract: This three-year project evaluated food safety risks associated with commercial postharvest cherry handling, with emphasis on *Listeria* spp. on food contact surfaces, microbial quality and cross-contamination in postharvest water systems, hydrocooling, and survival of foodborne pathogens on cherries during refrigerated storage. Commercial cherry packinghouses in Washington and California participated across the study objectives.

Listeria spp. was detected in all packinghouses evaluated, with an overall prevalence of 2.0% (38/1,920) on food contact surfaces in Washington and 2.4% (15/636) in California. Bristle flaps, solid conveyor belts, and singulators were among the most frequently contaminated surfaces. Only 3.9% of positive sampling sites were positive both before and during packing within the same sampling event, suggesting that most detections represented transient contamination rather than persistent harborage.

Evaluation of commercial postharvest water systems demonstrated that microbial populations on incoming cherries can influence microbial quality throughout the packing process. Generic *E. coli* was detected in 1.5% of cherries and 2.5% of water samples, while total coliforms were detected in 18.0% and 45.1%, respectively. Higher APC populations on incoming cherries were associated with higher APC populations in process water and on outgoing cherries, and higher water APC was also associated with higher APC on outgoing cherries. Relationships between sanitizer concentration and individual water-quality parameters varied among water systems, emphasizing the importance of directly monitoring sanitizer concentrations rather than relying on individual physicochemical parameters as indicators of sanitizer status.

Hydrocooling did not generally increase microbial populations on cherries under the commercial conditions evaluated. Total coliform and HPC populations decreased by an average of 0.87 and 0.77 log MPN/cherry, respectively, following hydrocooling. However, increases occurred during some individual sampling events, and higher HPC populations in hydrocooler water were associated with less favorable changes in HPC on cherries. These findings indicate that hydrocooling should not be considered a microbial reduction step and reinforce the importance of maintaining the microbiological quality of recirculated water.

Finally, *L. monocytogenes*, *Salmonella*, and STEC did not grow on cherries during 14 days of refrigerated storage, but viable populations of all three pathogens remained detectable. *L. monocytogenes* demonstrated the greatest persistence, decreasing by approximately 2.2 log CFU/cherry compared with reductions of approximately 3.6 and 4.0 log CFU/cherry for *Salmonella* and STEC, *respectively*.

Overall, the results demonstrate that effective cherry food safety management should focus on preventing contamination and limiting microbial transfer throughout postharvest handling. Priority practices include hygienic design and sanitation of difficult-to-clean food contact surfaces, particularly bristle materials; routine monitoring and management of process-water quality and sanitizer concentrations; control of organic material and debris accumulation; and recognition that incoming fruit can contribute microbial loads to recirculated water systems.

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

Report Type: Continuing Project Report

Primary PI: Per McCord
Organization: WSU Dept. Horticulture
Telephone:509-786-9254
Email: phmccord@wsu.edu
Address: WSU IAREC
Address 2: 24106 N. Bunn Road
City/State/Zip: Prosser, WA 99350

Co-PI 2: Cameron Peace
Organization: WSU Dept. Horticulture
Telephone:509-335-6899
Email: cpeace@wsu.edu
Address: WSU
Address 2: 1670 NW Wilson Mall
City/State/Zip: Pullman, WA 99164

CO-PI 3: Alexandra Johnson
Organization: WSU Dept. Plant Pathology
Telephone:
Email: alexandra.johnson2@wsu.edu
Address: WSU IAREC
Address 2: 24106 N. Bunn Road
City/State/Zip: Prosser, WA 99350

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.

Awarded
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-PIs, including Per McCord, Bernardita Sallato, and Matthew Whiting from WSU.

WTFRC Collaborative Costs: None

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

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

- Targeted sequencing of the *Pmr1* region conducted for 9 individuals
    - Additional sequencing to be completed Q4 2026

- Phenotypic screening completed for canker and powdery mildew
    - Canker: confirmed partial resistance of 'Regina'; key sources of susceptibility include 'Coral Champagne', 'Rainier', and potentially 'Lapins'
    - Mildew: confirmed resistance of 'Regina'; additional potential sources of resistance include 'Coral Champagne', 'Ambrunes', and FR09T032

Methods

Identify genetic factors
Completed in Year 1 (2025)

Targeted sequencing of Pmr1 *region*
In Year 1, we identified 27 genes with features involved in disease resistance that were located in the region of *Pmr1* on chromosome 5. In Year 2, we designed 23 CRISPR guide RNAs to target these genes. Following the protocol of Gilpatrick et al. (2020), DNA was treated first with phosphatase, and then with a combination of the CRISPR guide RNAs and the Cas-9 nuclease. The purpose of phosphatase treatment is to prevent the joining of sequencing adapter molecules to the DNA, except where the DNA has been cut by CRISPR/Cas9. Following the addition of sequencing adapters, the samples were sequenced using Oxford Nanopore MinION flow cells. 'Moreau' was sequenced by itself; the remaining samples were sequenced in two groups of four.

Screening assays
From June to September 2026, we conducted six rounds of bacterial canker screening and five rounds of powdery mildew screening. 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 with Tween surfactant, 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 10-µL 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. Plates were scored via a 0-4 quantitative scale, with 4 being the most infected.

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. Plates were scored for presence/absence of conidia (mildew reproductive structures).

Results and Discussion

Genetic factors for bacterial canker
Completed in Year 1. Two potential markers were found on chromosome 2.

Targeted sequencing
To date, we have performed targeted sequencing for nine cherry accessions (Table 1). We will perform two more rounds of sequencing (two more MinION cells) in Q4 2026. Initial analysis of the sequencing data suggests there are indeed DNA sequence differences in the *Pmr1* region, but these need to be further characterized. The data also suggest that the targeted sequencing method is not very efficient (because a significant amount of DNA from non-targeted regions is being sequenced). We have confirmed that the DNA is being cleaved by the CRISPR/Cas9 treatment, so for the follow-on sequencing we will optimize the phosphatase portion of the protocol.

Table 1. Sweet cherry accessions utilized for targeted sequencing of *Pmr1*.

Sample ID	***Pmr1* genotype**	**Comment**
Skeena	*Pmr1|Pmr1*	Susceptibility control
Schneiders	*Pmr1c|Pmr1*	Parent of Regina
Regina	*Pmr1c|Pmr1*	
Hedelfingen	*Pmr1b2|Pmr1*	Parent of Venus
Venus	*Pmr1b2|Pmr1*	
Moreau	*Pmr1a|Pmr1*	Parent of Chelan
Chelan	*Pmr1a|Pmr1*	
FR23T090*	*Pmr1a|Pmr1b1*	Two resistance alleles
FR27T050**	*Pmr1a|Pmr1b2*	Two resistance alleles

* FR23T090 = BB PMR-1 × 'Rainier') × Mildew Immune Mazzard 17
** FR27T050 = CC (PMR-1 × 'Rainier') × 'Venus'

Screening assays
In 2026 we screened a total of 292 unique individuals for canker, and 222 for powdery mildew. Combined with the initial screens in 2025, this totals 337 and 301 individuals for canker and mildew respectively. Based on the assays of individual accessions and/or their offspring, we have identified several sweet cherry cultivars that are key sources of resistance or susceptibility to bacterial canker. (Table 2). 'Lapins' was not directly tested, but it is a grandparent of many susceptible seedlings, indicating it is likely another susceptible source. Based on field observations, 'Bing' is known to be susceptible, but consistently scored resistant ("0") in our tests. False negatives are known to occur with this assay, but other possibilities include resistance to the pure bacterial strain of *Pseudomonas* used in our assay or a mis-identification of the sample labeled as 'Bing'.

Table 2. Key genetic sources of resistance and susceptibility to bacterial canker, based on a detached leaf disc assay. Reported score is the highest (worst) observed for each sample.

Sample ID	**Canker score (0-4, 4 = worst)**	**Comment**
CR20T046	1	In Phase 2 of breeding program
CR21T043	1	Phase 2

Regina	1	
Skeena	1	
Cascade Daybreak (R19)	4	
PC-1	4	Phase 2
PC-2	4	Phase 2
Rainier	4	
Sweetheart	4	
Tieton	4	
Coral Champagne	3	

For mildew, we confirmed several sources of resistance such as 'Chelan' and its relatives (via the original source 'Moreau') and 'Venus'. Although the *Pmr1c* allele carried by 'Regina' is not known to be a resistance allele, 'Regina' showed resistance in our assays; this could be due to the presence of a resistance allele at one or more other genes besides *Pmr1*. Screening of 'Ambrunes' and 'Coral Champagne' and their offspring also suggested these cultivars are sources of resistance. The breeding parent FR09T032 ('Lapins' × 'Krupnoplodnaya') was not screened directly but is also a potential source of resistance based on observations of its offspring.

New/refined DNA tests
In 2026, two pairs of PCR primers were designed for genotyping the two potential markers for canker resistance. Leaf tissue for DNA extraction was collected from 190 samples that were also screened via the detached leaf disc assay. These samples will be used to validate the potential markers in Q4 2026.

The analysis of the *Pmr1* sequencing data should be completed in Q1 2027. This will include the identification of critical sequence difference amongst the various *Pmr1* alleles and the development of refined DNA tests. These tests will be validated on the samples we screened in 2026.

Literature Cited

Timothy Gilpatrick, Isac Lee, James E. Graham, Etienne Raimondeau, Rebecca Bowen, Andrew Heron, Fritz J. Sedlazeck, Winston Timp 2020. Cas9 Enrichment for Nanopore Sequencing. protocols.io
https://dx.doi.org/10.17504/protocols.io.bmi5k4g6

Project Title: Evaluation of UV-C for management of cherry diseases and pests

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

Primary PI: Tamara Collum
Organization: USDA-ARS
Telephone:(304) 725-3451 x315
Email: tami.collum@usda.gov
Address: Appalachian Fruit Research Station
Address 2: 2217 Wiltshire Rd
City/State/Zip: Kearneysville, WV 25430

Co-PI 2: Tracy Leskey
Organization: USDA-ARS
Telephone: 304-725-3451 x329
Email: tracy.leskey@usda.gov
Address: Appalachian Fruit Research Station
Address 2: 2217 Wiltshire Rd.
City/State/Zip: Kearneysville, WV 25430

Cooperators: None

Project Duration: 3 Year

Other related/associated funding sources: USDA-ARS In-House Funding
8080-30500-001-000-D Advanced Production and Automation Systems for Temperate Fruit Crops Through Discovery and Integration.
Funding Duration: Feb 20, 2025 – Feb 19, 2030
Amount: 422,774
Agency Name: USDA ARS
Notes: In-house USDA ARS funding will be used to cover cost of permanent technician time and growth chamber costs.
WTFRC Collaborative Costs: None

Budget 1
Primary PI: Tamara Collum
Organization Name: USDA ARS
Contract Administrator: Laura Lehman
Telephone: 301-450-2439
Contract administrator email address: laura.lehman@usda.gov
Station Manager/Supervisor: Tracy Leskey
Station manager/supervisor email address: tracy.leskey@usda.gov

Item	2024	2025	2026	2027
Salaries	$6,544.00	$6,871.00	$7,215.00	
Benefits	$2,748.00	$2,886.00	$3,030.00	
Wages				
Benefits				
RCA Room Rental				
Shipping				
Supplies	$3,000.00	$3,000.00	$3,000.00	
Travel				
Plot Fees				
Miscellaneous				
Total	$12,292.00	$12,757.00	$13,245.00	$0.00

Footnotes:

OBJECTIVES

1) Evaluate cherry fungal pathogens and spotted wing drosophila (SWD) sensitivity to UV-C (254nm) or far UV (222nm) light in direct laboratory assays.
In this objective, cherry fungal pathogens and SWD will be exposed to UV-C (254nm) or far UV (222nm) in a laboratory setting to determine the minimum duration and dose required to kill the pathogen or pest.

2) Evaluate control of fungal rots and SWD on sweet cherry fruit after of UV-C (254nm) or far UV (222nm) light treatment.
In Objective 2, sweet cherry fruit will be harvested at commercial maturity and inoculated with fungal pathogens or fruit will be held with mature female SWD or infested with SWD eggs prior to treatment with UV-C or far UV. We will measure incidence of disease or SWD oviposition, and egg and larval survivorship after treatment to determine the optimal dosage and exposure time to eliminate disease and pests without damaging the fruit.

3) Evaluate UV-C or far UV-C treatments in combination with biological control products on sweet cherry fruit.
Antagonists or biocontrol products such as Bio-Save (*Pseudomonas syringae*) will be applied after UV exposure to fill in the microbial void left after irradiation. We will measure incidence of disease after treatment to determine if UV treatments are more effective when used in combination with biological control products.

Proposed Timeline

Objective	2024	2025	2026
1. UV-C and far UV direct laboratory assays	X	X	
2. Evaluate control of fungal rots and SWD on fruit	X	X	X
3. Evaluate UV in combination with biological control on fruit		X	X

Significant Findings

- UV-C treatment of *Monilinia fructicola* (brown rot pathogen), *Botrytis cinerea* (gray mold pathogen), *Penicillium expansum (*blue mold pathogen*)*, and *Colletotrichum fioriniae* (anthracnose pathogen) for 120 seconds significantly reduced the growth of the fungi in plate assays.
- Far UV treatment of *Monilinia fructicola* (brown rot pathogen), *Penicillium expansum (*blue mold pathogen*)*, and *Colletotrichum fioriniae* (anthracnose pathogen) for 45 seconds or more significantly reduced the growth of the fungi in plate assays.
- UV-C or far UV treatments did not inhibit the growth of *Alternaria alternata* (Alternaria rot pathogen) in plate assays.
- UV-C treatments of 15 seconds or more on SWD eggs significantly reduced emergence of adults to 9-25% in plate assays.
- Far UV treatments of 60 or 120 seconds on SWD eggs significantly reduced emergence of adults to 42 or 23% respectively in plate assays.
- UV-C treatments of 120 seconds reduced disease incidence for brown rot on sweet cherry fruit but did not significantly reduce gray mold disease incidence.
- UV-C or far UV treatments did not have any adverse effects on fruit quality.
- UV-C or far UV treatments of 120 seconds or less did not reduce adult SWD mortality or ability to produce offspring.

METHODS

Objective 1: Evaluate cherry fungal pathogens and spotted wing drosophila (SWD) sensitivity to UV-C (254nm) or far UV (222nm) light in direct laboratory assays.

Fungal Pathogens
M. fructicola (the causative agent of brown rot), *A. alternata* (the causative agent of Alternaria rot), *Botrytis cinerea* (the causative agent of gray mold), *Penicillium expansum (*the causative agent of blue mold*)*, and *Colletotrichum fioriniae* (the causative agent of anthracnose) were grown on potato dextrose agar (PDA) media and evaluated with direct UV-C exposure to assess pathogen sensitivity to UV treatment. UV-C treatment was conducted using a conventional germicidal UV-C lamp with maximum emission at 254 nm (55-W Phillips Model TUV PL-L, Phillips North America Corp., Andover, MA USA). The UV-C lamp was mounted 30 cm from the targeted irradiation surface. Conidia of each pathogen was harvested from 5 to 10-day old cultures grown on PDA with 0.05% Tween 20 by gently scraping culture surfaces with a glass rod and filtering through two layers of cheese cloth. The pathogen suspension was adjusted to a concentration of 1 x 10^4 conidia/ml using a hemocytometer. 10 ul of the conidia suspension was spotted in the center of a 100 mm diameter Petri dish containing PDA. Plates were randomized then placed on trays by treatment group, with 3 plates per treatment group. Plates were exposed to UV-C for 0, 30, 60, 90 or 120 seconds, placed in the dark for 4 hours, then incubated at 25°C (77°F) for seven days. The growth of the pathogen on each plate was measured at 5- and 7-days post treatment.

Far UV experiments were performed as described above using a 500-W lamp (Sterilray.com, Somersworth, NH USA) with a maximum emission at 222 nm mounted 30 cm from the targeted irradiation surface. Plates were exposed to UV-C for 0, 15, 30, 45, 60 or 90 seconds, placed in the dark for 4 hours, then incubated at 25°C (77°F) for seven days. Two additional exposure times of 180 and 300 seconds were added for experiments with *A. alternata*. The growth of the pathogen on each plate was measured at 5- and 7-days post treatment.

Spotted Wing Drosophila (SWD)
SWD were exposed to UV-C or far UV to assess the lethal dose requirement for adults. In this case, 5 mature adult females or males were held in each container. There was a total of 10 replicates (each with 5 SWD) per treatment group. SWD were exposed to UV-C for 0, 15, 30, 45, 60, or 120 seconds. Survivorship was assessed 4, 24, and 48 hours after treatment. Adults not exposed to UV treatments served as a control.

To test if UV light treatments affect SWD fecundity, SWD adults were exposed to UV-C or far UV light for 0, 15, 30, 45, 60, or 120 seconds then left in an egg laying substrate for 2 days. 3 males and 3 females were used per replicate. The original SWD adults were removed and then the presence of new adult emergence from the substrate was recorded over 13 days. A total of 27-30 replicates were conducted per exposure time for UV-C and far UV treatments. Adults not exposed to UV treatments served as a control.

Newly deposited SWD eggs on diet plates were exposed to UV-C or far UV for 0, 15, 30, 45, 60, or 120 seconds. There were 10-20 eggs per plate. The proportion of adults that emerged from the eggs was tracked over 2 weeks. Eight to ten replicates per exposure time were performed for UV-C treatments and five to ten replicates per exposure time were performed for far UV treatments. Eggs not exposed to UV treatments served as a control.

Objective 2: Evaluate control of fungal rots and SWD on sweet cherry fruit after of UV-C (254nm) or far UV (222nm) light treatment.

Fungal Pathogens

Organic dark sweet cherry fruit was purchased from a local market. For pathogen inoculations, fruit was surface sanitized with 70% ethanol for 1 min and rinsed three times with sterile water. Fruit was arranged on sterilized test tube racks with 10 fruit per treatment group and allowed to air dry in covered bins. Fruit was wounded with a sterile nail to a depth of 5 mm or sandpaper and inoculated with 10 µL of pathogen conidia suspension (1 x 10^4 conidia/mL) or sterile water for non-inoculated controls. One hour post inoculation, fruit was irradiated with UV-C for 120 seconds or with far UV for 60 seconds. Untreated fruit (0 seconds) were used as a control. After irradiation, fruit was placed in plastic fruit bin and kept in the dark for 4 hours. After the dark period, lids were removed, and approximately 200 mL of distilled water was dispensed into the bottom of each bin and then the fruit bins were covered with saran wrap to maintain a humid environment. Fruit bins were incubated at room temperature for 7 days under continuous light. Disease severity was determined after 4 or 7 days by measuring the diameter of the pathogen lesion. Disease incidence (the proportion of cherries showing disease symptoms) was also recorded. Pathogens used for experiments included *M. fructicola* (Brown rot), *Penicillium expansum* (Blue mold), *Botrytis cinerea* (Gray mold), and *Colletotrichum fioriniae* (anthracnose).

Spotted Wing Drosophila (SWD)

For SWD experiments, fruit were surface sanitized with 70% ethanol as described above then three female and three male SWD were placed per cherry fruit held in individual small, screened containers for 24 hours. The next day total eggs laid per cherry were counted and then fruit was irradiated for 0, 15, 30, 45, 60, or 120 seconds with UV-C or far UV light. Following the treatment, adult emergence was monitored for 14 days. Ten replicates were performed for UV-C and far UV treatments.

Objective 3: Evaluate UV-C or far UV-C treatments in combination with biological control products on sweet cherry fruit.

Dark sweet cherry fruit were wound inoculated with fungal pathogens as described in objective 2. Infected fruit was irradiated after infection for 120 seconds with UV-C light or 60 seconds with far UV light. Nontreated fruit (0 second UV exposure) was used as a control. After irradiation, fruit was subjected to a 4-hour dark period then dipped in Bio-Save (*Pseudomonas syringae*) for 2 minutes and placed on sterile racks in bins. Fruit bins were covered with saran wrap and incubated in a humid environment at room temperature for 7 days under continuous light. Disease severity was determined after 4 or 7 days by measuring the diameter of the pathogen lesion.

Problems/Limitations Encountered

- We were unable to hire a summer student as planned to contribute to this project this year. We are planning to begin recruiting this fall and have someone in place starting in Jan 2027.

RESULTS & DISCUSSION

Objective 1: Evaluate cherry fungal pathogens and spotted wing drosophila (SWD) sensitivity to UV-C (254nm) or far UV (222nm) light in direct laboratory assays.

Fungal Pathogens

In the direct plate assays, we saw a significant reduction of *M. fructicola* cherry isolate Ch-9 growth on PDA plates after UV-C treatment of 120 seconds compared to untreated (0 second) controls at high (1 x 10^4) concentrations of the pathogen (Figure 1A). In 2026, we tested an additional isolate of *M. fructicola* Mf-7 that is an aggressive isolate collected from a decayed peach in West Virginia. Mf-7 also had significantly reduced growth on PDA plates after UV-C treatment of 120 seconds (Figure

1B). Three additional fungal pathogens, *B. cinerea*, *P. expansum* and *C. fioriniae* were tested in plate assays in 2026 (Figure 1D-F). UV-C treatments were most effective on *P. expansum* (causative agent of blue mold) with an exposure time of 30 seconds significantly decreasing the growth of the pathogen. UV-C treatments of 120 seconds significantly reduced the growth of *B. cinerea* and *C. fioriniae*. This was consistent with previous reports of UV-C light being effective against these pathogens (Janisiewicz et al 2021). As reported in the previous year, UV-C treatment of up to 120 seconds did not significantly reduce the growth of *A. alternata* (Figure 1C).

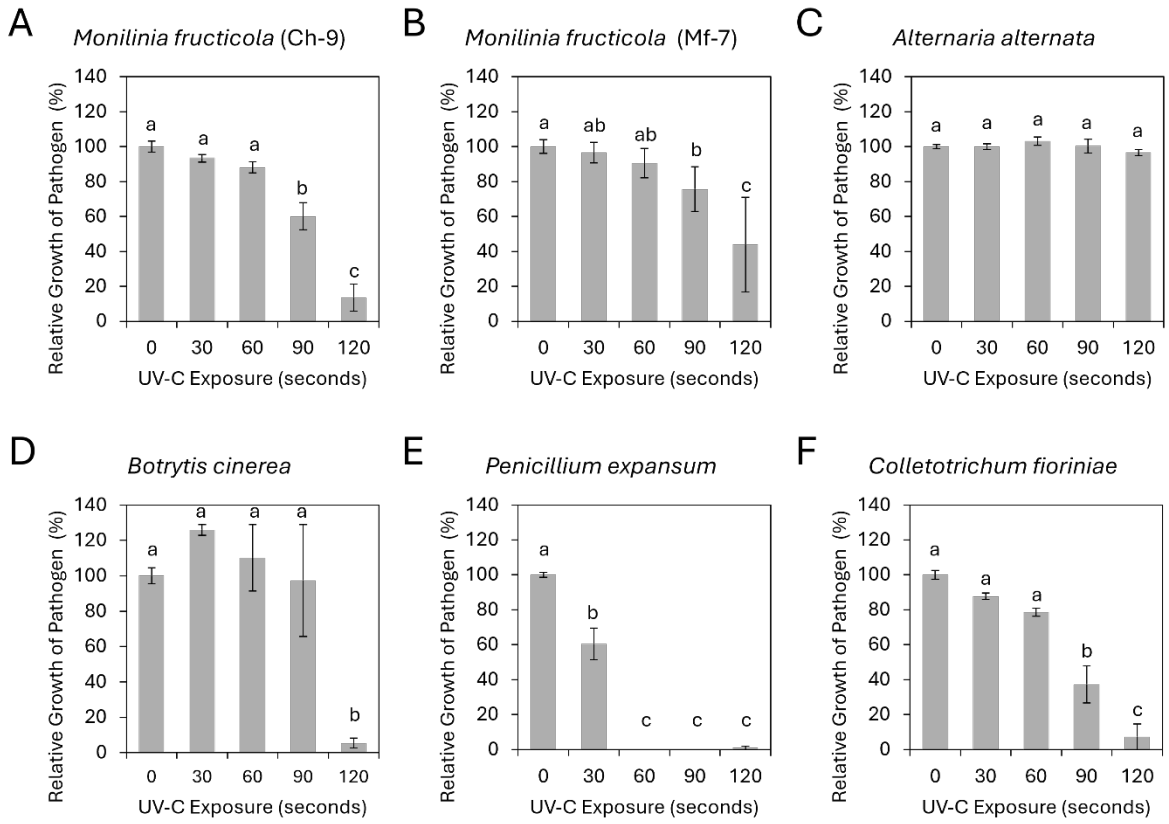


Figure 1: UV-C fungal pathogen plate assays. Bars represent the mean ± standard error. Growth of pathogens is shown relative to the control treatment of 0 seconds which was set at 100%. Different letters indicate a significant difference between treatments. A) *Monilinia fructicola* isolate Ch-9, B) *Monilinia fructicola* isolate Mf-7, C) *Alternaria alternata*, D) *Botrytis cinerea*, E) *Penicillium expansum*, F) *Colletotrichum fioriniae*.

M. fructicola was sensitive to far UV treatment, and exposure times of 45 seconds or more significantly reduced pathogen growth. We saw no growth of *M. fructicola* on plates after 60 seconds of far UV treatment (Figure 2A). In 2026, we tested additional fungal pathogens *P. expansum* and *C. fioriniae* which both had significantly reduced growth after far UV exposure times of 15 seconds or more, which was consistent with previous reports (Janisiewicz et al 2021). *A. alternata* growth was not reduced by far UV treatments, even when we extended the exposure time to 300 seconds (5 minutes). *A. alternata* is a pigmented darkly colored fungus which can contribute to its resistance to UV, however this protection can be wavelength dependent. Unfortunately, we found both the UV-C (254nm) and far UV (222nm) light treatments are not effective for control of *A. alternata.*

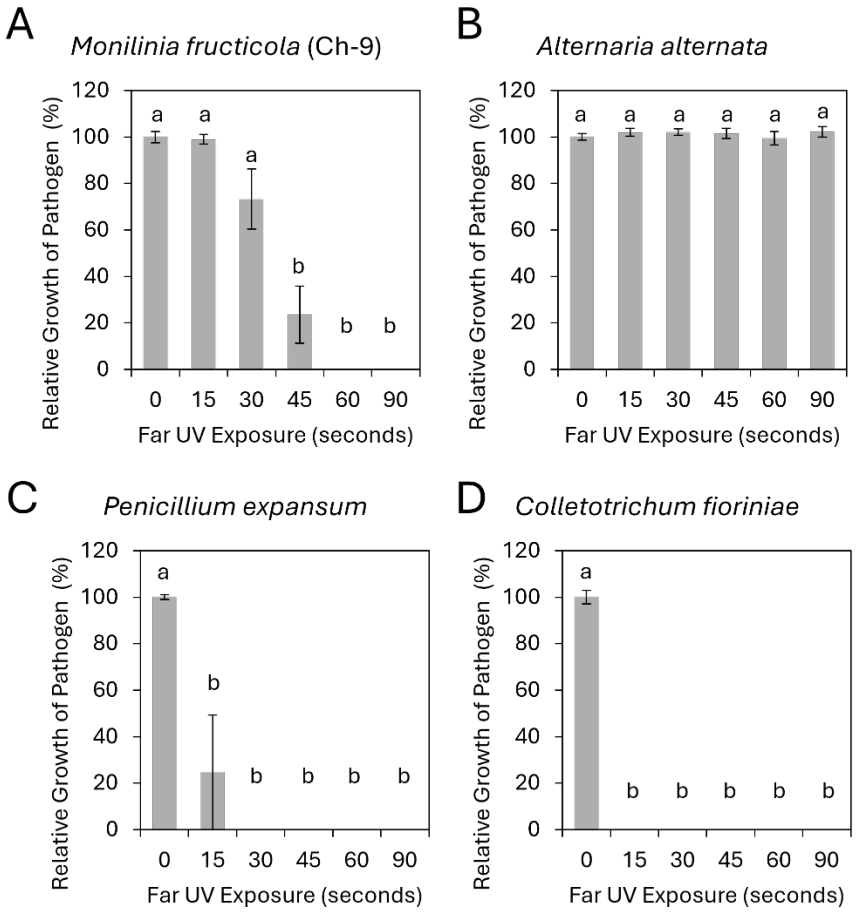


Figure 2: Far UV fungal pathogen plate assays. Bars represent the mean ± standard error. Growth of pathogens is shown relative to the control treatment of 0 seconds which was set at 100%. Different letters indicate a significant difference between treatments. A) *Monilinia fructicola* isolate Ch-9, B) *Alternaria alternata*, C) *Penicillium expansum*, D) *Colletotrichum fioriniae*.

Spotted Wing Drosophila (SWD)

Adult male or female SWD were exposed to UV-C or far UV treatments for 0, 15, 30, 45, 60, or 120 seconds and survivorship was assessed 48 hours after treatment. Adults not exposed to UV (0 seconds) served as a control. We did not see any significant changes to survivorship with either UV-C or far UV light treatments. To test if UV light treatments affect SWD reproduction, UV treated SWD adults were left in an egg laying substrate for 2 days. 3 males and 3 females were used per replicate. The original SWD adults were removed and then the presence of new adult emergence from the substrate was recorded over 13 days. Adults not exposed to UV treatments (0 seconds) served as a control. We observed no difference in the new adult emergence for any of the UV-C or far UV treatments.

We also evaluated the effect of UV treatments on SWD eggs. Newly deposited SWD eggs on diet plates were exposed to UV-C or far UV for 0, 15, 30, 45, 60, or 120 seconds. The proportion of adults that emerged from the eggs was tracked over 2 weeks. UV-C treatments of 15 seconds or more significantly reduced the emergence of adult SWD (Figure 3A). Far UV treatments were less effective, but we did observe a significant decrease in the emergence of adult SWD at 60 and 120 seconds (Figure 3B).

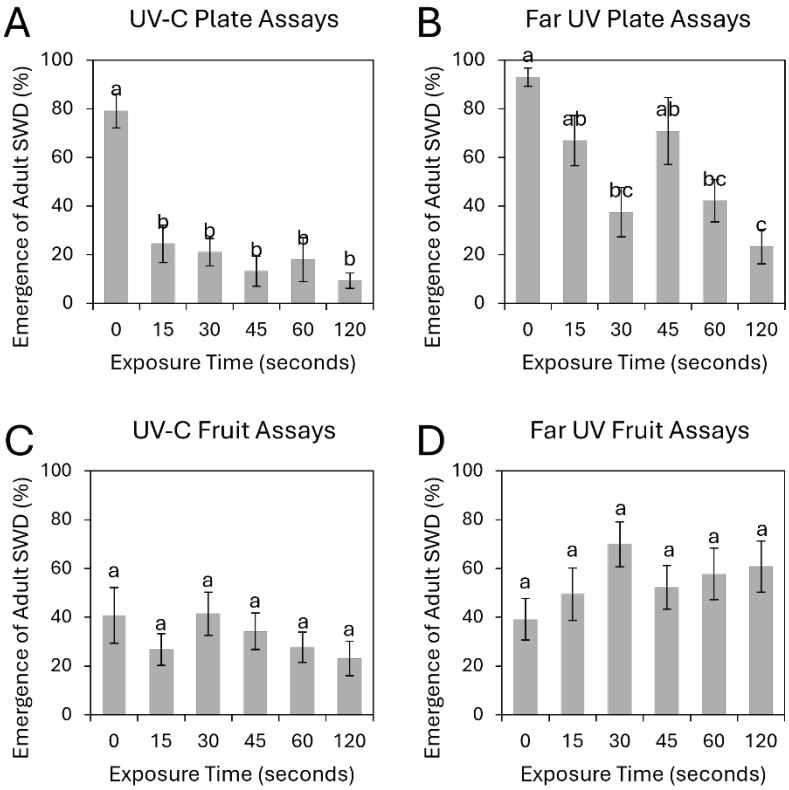


Figure 3: Spotted wing drosophila assays. Bars represent the mean ± standard error. Different letters indicate a significant difference between treatments. A) SWD eggs treated with UV-C light on diet plates. B) SWD eggs treated with far UV light on diet plates. C) SWD eggs treated with UV-C light on cherry fruit. D) SWD eggs treated with far UV light on cherry fruit.

Objective 2: Evaluate control of fungal rots and SWD on sweet cherry fruit after of UV-C (254nm) or far UV (222nm) light treatment.

Spotted Wing Drosophila (SWD)
For SWD experiments, fruit three female and three male SWD were placed per cherry fruit held in individual small, screened containers for 24 hours. The next day total eggs laid per cherry were counted and then fruit was irradiated for 0, 15, 30, 45, 60, or 120 seconds with UV-C or far UV light. Following the treatment, adult emergence was monitored for 14 days. Ten replicates were performed for UV-C and far UV treatments. We did not observe any significant differences in adult emergence for any of the UV treatments (Figure 3 C&D).

Fungal Pathogens
In the previous year, we found that when cherry fruit was challenged with one of three different rot pathogens, *M. fructicola* (brown rot), *P. expansum* (blue mold), and *B. cinerea* (gray mold) using a sterile nail wound inoculation method, UV-C and far UV treated fruit had a small but significant decrease in brown rot and blue mold disease severity (Figure 4A&B) but did not have a significant effect on gray mold disease (Figure 4C). However, in all cases we saw 100% disease incidence with all cherries becoming infected, just with reduced lesion development. We hypothesized that the UV lights could not penetrate deep into the wound caused by the nail. In 2026, we used a sandpaper inoculation strategy where more of the pathogen would remain on the fruit surface and perhaps be more susceptible to UV light treatments.

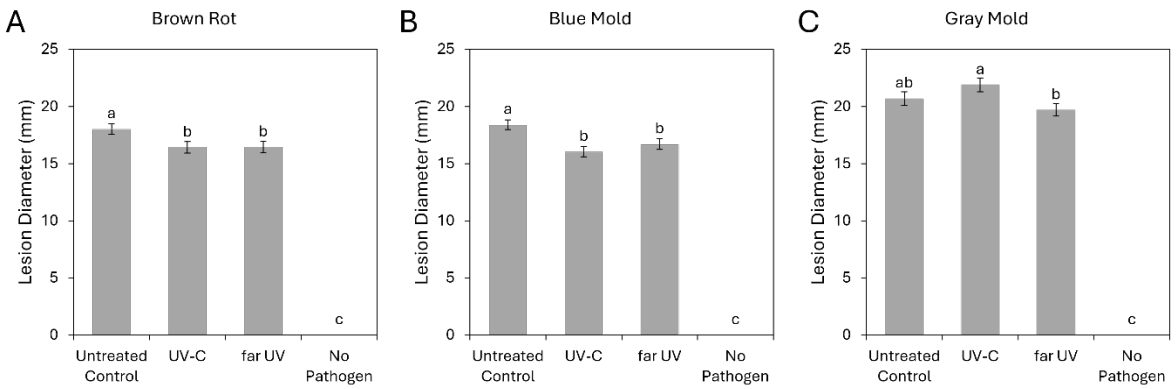


Figure 4: Lesion diameter of fungal pathogens on cherry fruit. Cherry fruit was inoculated using a nail wounding method**.** Bars represent the mean ± standard error. Different letters indicate a significant difference between treatments. A) Brown rot disease development on fruit treated with UV-C or far UV light. B) Blue mold disease development on fruit treated with UV-C or far UV light. C) Gray mold disease development on fruit treated with UV-C or far UV light.

In 2026, cherry fruit was challenged with either *M. fructicola* (brown rot) or *B. cinerea* (gray mold). After inoculation, fruit was treated with UV-C for 120 seconds or 0 seconds as a control. The exposure time was chosen based on the results from the direct plate assays in Objective 1. Fruit inoculated with sterile water (no pathogen) was used as additional controls. When cherry fruits were challenged with *M. fructicola* using the sandpaper inoculation method we saw 93.3% disease incidence in untreated controls after five days post inoculation and a significant decrease (36.7% disease incidence) in fruit that were exposed to UV-C light for 120 seconds. When cherry fruits were challenged with *B. cinerea*, we observed a 60% disease incidence in untreated controls and 40% disease incidence in UV-C treated cherries five days post inoculation. Although this was a numerical reduction it was not statistically significant.

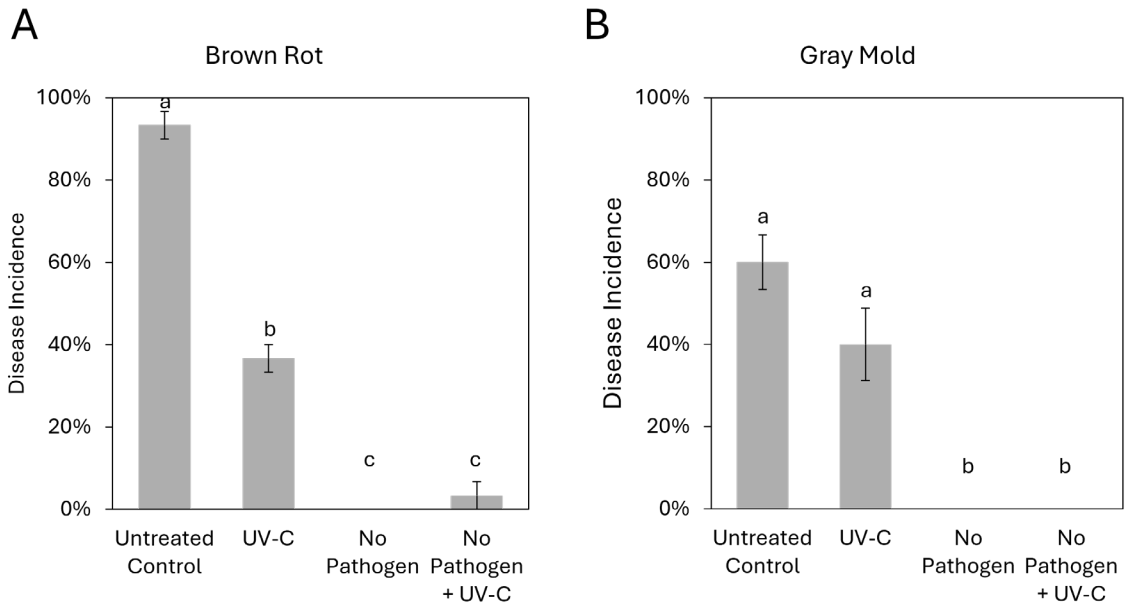


Figure 5: Disease incidence on cherry fruit. Cherry fruit was inoculated using a sandpaper method**.** Bars represent the mean ± standard error. Different letters indicate a significant difference between treatments. A) Brown rot disease incidence on fruit treated with UV-C light. B) Gray mold disease development on fruit treated with UV-C light.

We plan to repeat these experiments next year as well as test far UV treatments using the sandpaper inoculation method. Additionally, experiments were performed with a high (1 x 10^4 conidia/mL) concentrations of the pathogens. We plan to test an additional lower concentration (1 x 10^3 conidia/mL) of pathogens next year as it is possible that UV treatments will provide greater control when pathogen levels are low. UV-C or far UV light treatments did not have any effects on fruit weight or firmness.

Objective 3: Evaluate UV-C or far UV-C treatments in combination with biological control products on sweet cherry fruit.

In preliminary experiments we did not observe any significant differences between UV treatment + BioSave (active ingredient *Pseudomonas syringae*) compared to UV treatments alone, but these experiments were only conducted once in 2025. This year we have acquired additional biocontrol products including Serenade ASO (active ingredient *Bacillus subtilis*), Theia Fungicide (active ingredient *Bacillus subtilis*), Howler EVO (active ingredient *Pseudomonas chlororaphis*), and Botector WG (active ingredient *Aureobasidium pullulans*). Experiments will be performed next year to determine if UV light treatments in combination with biological control products improve control of fungal rots compared to either treatment alone.

Literature Cited

Janisiewicz, W., Takeda, F., Evans, B., and Camp, M. 2021. Potential of far ultraviolet (UV) 222 nm light for management of strawberry fungal pathogens. *Crop Protection* 150: 105791.