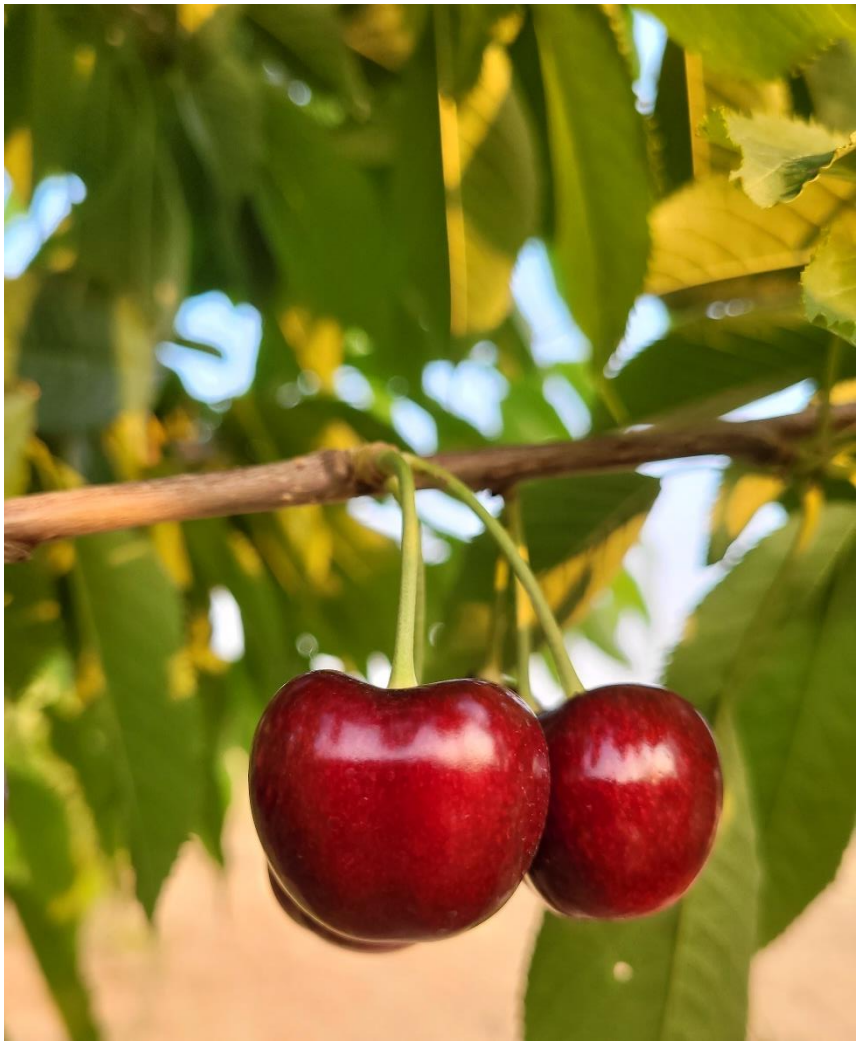
2024 NW Cherry and Stone Fruit Research Review



R3, an early maturing mahogany selection from the WSU cherry breeding program 5 days before harvest in a Phase 3 site near Zillah, WA on June 15, 2023.

Photo credit: Ines Hanrahan

November 7, 2023

Hybrid

Yakima, WA

Project/Proposal Title: Understanding little cherry disease pathogenicity.

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Cooperators: Washington cherry growers and extension agents.

Report Type: Continuing Project Report

Project Duration: 3 Years + No-Cost Extension

Total Project Request for Year 1 Funding: $155,882
Total Project Request for Year 2 Funding: $153,942
Total Project Request for Year 3 Funding: $148,198

Other funding sources: None

Budget 1
Primary PI: Scott Harper
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Contract Administrator: Anastasia Mondy
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Item	2020	2021	2022
Salaries[1]	60,528	62,950	65,468
Benefits	23,034	23,956	24,915
Wages[2]	4,650	4,836	5,030
Benefits	745	775	805
Equipment	0	0	0
Supplies	64,850	59,350	49,905
Travel	1,500	1,500	1,500
Miscellaneous	0	0	0
Plot Fees	575	575	575
Total	155,882	153,942	148,198

Footnotes: 1. Salaries and benefits are for a 1.0 FTE Postdoctoral Associate and a 0.25 FTE Technician to conduct research on this project. 2. Wages and associated benefits are 180 hours for a summer student research assistant to assist with sample collection, and for 80 hours for a field maintenance technician.

Objectives:

1. Establish and inoculate a field plot of representative cherry germplasm to screen for little cherry disease induction and potential sources of disease resistance/tolerance.

2. Identify the physiological effects of little cherry disease of different cherry cultivars from experimental plots and field collected samples to determine a) whether there are different symptom patterns, and b) what effect these have on fruit quality and tree health using a physiological and metabolomics approach.

3. Examine the underlying genetic basis of little cherry disease through examination of transcriptomic changes during disease induction and identify potential effectors or interacting genes/proteins at the host level to develop a method to screen germplasm for tolerance/susceptibility.

Significant Findings for 2023:

- We have identified and partially characterized the reaction of five distinct strains of the X-disease phytoplasma on sweet cherry, sour cherry, and peach.

Methods:

Objective 1. We established a 1-acre test block at WSU-IAREC consisting of 30 different cherry varieties, including commercially grown varieties, as well as cherries reported to have some level of tolerance or resistance to LChV2 or X-disease, and several accessions that represent more unique genetic backgrounds. To promote early fruiting, scions were budded on the precocious rootstock Gisela-6. In mid-late spring of 2020 (and 2021 for replacements), the budded trees were transplanted to the field. Inoculation with LChV2 or the X-disease phytoplasma was performed by chip budding of highly infected material in late summer of 2021. Orchard maintenance, including pruning, fertilization, pesticide application, and weed control, will be conducted according to current horticultural practices. *Note: Due to the low graft inoculation success rates and subsequent natural infection of the field block, this will be maintained as an observation block for monitoring disease onset.*

Objective 2. Knowing how different cultivars respond to both LChV-2 and X-disease phytoplasma is essential to developing an accurate field guide for growers. Therefore, we collected symptom development observations and physiological data from grower fields throughout the state, focusing on recording of symptoms present on known infected trees as the fruit developed from fruit set to harvest, collecting data on fruit size, color (both skin and pulp) and shape, and correlated this data with cultivar type. Second, we examined the sugar and secondary metabolite content of infected fruit at harvest, including sucrose, fructose, glucose, and sorbitol content as well as citric acid, malic acid, and total phenolic contractions, and compared these to fruit from healthy, uninfected trees to determine the effects of infection of fruit quality.

Objective 3. The underlying genetic basis of X-disease development was examined in parallel with the physiological studies. Samples of fruit, pedicel, and leaf tissue were collected from Bing grown in commercial orchards from bloom to harvest, depending on availability; due to removal of key orchards during this study, samples were collected from multiple sites. Tissue was macerated and total RNA extracted for library preparation and RNAseq. The resulting data was analyzed to identify genes that were differentially expressed to determine which pathways may be altered in cherry when infected with the '*Ca*. P. pruni' and associated with the expression of X-disease. *Note: Sample collection was performed from 2019 to 2023, and analysis will be ongoing to provide relevant information for subsequent WTFRC (and other granting agency) projects by the PI's and collaborators at USDA-ARS.*

Results and Discussion

Objective 1

The test plot was established in May 2021 at the WSU Pear Acres field site. Scions were grafted onto Gisela-6 rootstock in the greenhouse during 2020 and early 2021, with failures re-grafted in the field after planting in August 2021 and bark-chip grafted with Little cherry virus-2 genotype Rube-74 or '*Ca*. P. pruni' genotype 3 in September of 2021. Replacement scion grafts were made in late spring of 2022. Between 2021 through 2023 there was significant mortality in the block due to winter injury and vole and deer damage, reducing the total number of trees and/or scion combinations. A total of 31 trees were lost in 2022, and 37 in 2023, leaving 198 trees from the initial 276. Of these 119 have the grafted scion/rootstock combination, the remainder are Gisela 6 rootstocks without scions, and only 4 infected plants remain after the attrition of the past two years. In 2023 12-foot-high netting was erected around the block to exclude leafhoppers from entry, because this has been a problem in the past, and to prevent escape phytoplasma from inoculated plants.

As so many of the inoculated scion/rootstock combinations have been lost, we are reducing the scope of this experiment, and will maintain the orchard with other funds. It should be noted that we have observed LChV2 and/or '*Ca*. P. pruni' on a range of different cultivars in the field, with variance in symptoms being attributed to variance in infecting strain and environmental conditions rather than the host cultivar. In addition, commercially grown *P. avium* cultivars likely lack the genetic diversity to be resistant to '*Ca*. P. pruni', as they are all highly interbred, and the only tolerant, let alone resistant species of *Prunus* that have been identified either by our research or others are *P. domestica, P. armeniaca*, and wild ornamentals such as *P. serotina*. A search for resistance will require an experiment of different scope (see McCord proposal for 2024).

Objective 2

This objective was largely completed in 2021, with the description of symptom patterns and effects on fruit quality in multiple cherry cultivars. The discovery of multiple distinct genotypes of '*Ca*. P. pruni' present in WA during the SCBG project '*Epidemiology of the X-disease phytoplasma*' caused us to re-examine this data and put the disease development timeframe and symptoms patterns into a new context.

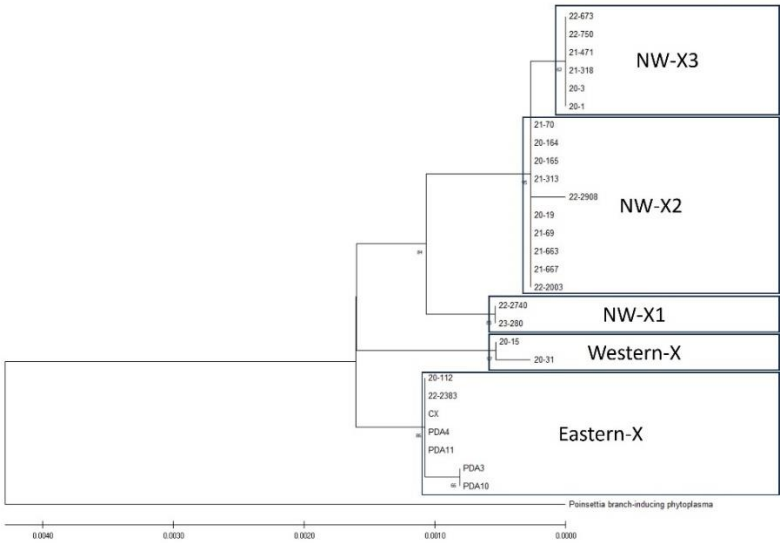

Figure 1. A phylogeny of sequences of the five strains of X-disease-inducing '*Ca*. P. pruni' that have been identified to date.

We have identified and characterized five genetically distinct strains of '*Ca*. P. pruni' that induce X-disease, named, in order of where they were first reported and/or where they are most widely distributed as 'Eastern-X', 'Western-X', 'Northwestern-X1', 'Northwestern-X2', and 'Northwestern-X3' (Figure 1). 'Northwestern-X3' is the dominant strain in Washington state, where it has largely replaced the 'Northwestern-X2' strain that was more common pre-2018. Oregon has primarily 'Northwestern-X2' while in both states what we are terming 'Eastern-X' and 'Western-X' are rare. For reference 'Western-X' includes the 'Green Valley' strain that was a major problem in California in the 1980s.

From the beginning of this epidemic there has been confusion between Little Cherry and X-disease symptoms because both caused a reduction in fruit size, pale skin and pulp color, and lower sugar content. Many of the symptoms reported in previous epidemics, such as in California in the 1970s, and earlier (1930s on the east and west coasts), such as the 'buckskin' mottle on fruit skin, along with fruit distortion, enlarged stipules, foliar chlorosis and/or anthocyanosis and tree decline reported in previous epidemics have not been observed. We now know that this was because the dominant strains in the PNW (Northwestern-X2 and Northwestern X3) do not cause these symptoms during the course of a normal in-field infection (Table 1). Below is a table describing what symptom patterns the different strains produce of major citrus hosts.

Table 1. Symptom patterns of different '*Ca* P. pruni strains on commercially grown *Prunus* species.

Genotype	**Symptoms Observed**		
	Sweet cherry	Sour cherry	*Peach/Nectarine*
Eastern-X	• Fruit: Small, pale. • Leaves: Witches' broom, enlarged stipules. • Other: Stunted growth, dieback, abnormal flowering.	• Not observed	• Fruit: Small, distorted. Delayed maturation. • Leaves: Epinasty, chlorosis, shot holing. • Other: Decline & dieback.
Western-X	• Fruit: Small, pale • Leaves: Normal.	• Fruit: Small, pale. • Leaves: Enlarged stipules.	• Not observed
Northwestern-X1	• Fruit: Small, pale. • Leaves: Witches' broom & enlarged stipules. • Other: Normal.	• Not observed	• Not observed
Northwestern-X2	• Fruit: Small, pale • Leaves: Normal. • Other: N/A	• Fruit: Small, distorted. • Delayed maturation.	• Fruit: Small, distorted. Delayed maturation. • Leaves: Epinasty, chlorosis, shot holing. • Other: Decline and dieback
Northwestern-X3			

Northwestern-X3 is the dominant strain in Washington state, while Northwestern-X2 is in Oregon. These strains are largely biologically similar in terms of disease, though from our other collaborative work, Northwestern-X3 is known to be more efficiently transmitted by the major leafhopper vector in Washington, *Colladonus montanus reductus* whereas we suspect that Northwestern-X2 may be more efficiently transmitted by *C. geminatus*. Fortunately, there are very few sites in Washington or Oregon that have the Eastern- or Western-X strains, and they do not appear to be spreading naturally by leafhopper transmission; it is likely that they are adapted to other leafhopper species that are rare or absent in the Pacific Northwest but common in the Eastern/Midwestern states or California, such as *Scaphytopius acutus, Fieberellia florii, C. clitellarius,* and *Paraphlepsus irroratus*.

Objective 3

Last year we reported on the differential expression of genes in fruiting tissues as compared to leaves and wood/bark, identifying significant changes early in the fruit development timeline at shuck fall and pit hardening. For 2023 we aimed to collect a final set of samples as well as additional control tissues to finalize the dataset and analyze together to smooth out environmentally driven inter-site and inter-year variation in expression levels.

However, there have been delays in hiring a replacement for the postdoctoral researcher, Dr. Alice Wright, who was working on this objective of the project, and while a postdoctoral researcher was hired in July of 2023, progress has been impacted. In addition, the characterization of the strains present in Washington has 1) caused us to evaluate the data collected thus far and 2) created opportunities for more in-depth research.

Therefore in 2023, we collected, extracted, and have sent for sequencing additional samples from Bing infected with strain Northwestern-X3 for the core analysis of disease expression in fruit; at time of writing the sequence data has not been returned. The PIs of this project have also met with experienced bioinformatics researcher Dr. Stephen Ficklin in WSU's Department of Horticulture to collaborate in performing detailed pathway analysis, which is intended to occur during November-January. We also collected samples from fruiting tissues from Bing infected Northwestern-X1 strain to see whether this strain impacts the same pathways as or dominant Northwestern-X3 strain, as well as tissue samples from emerging and expanding leaf buds to study the effect of the 'older' phenotype on tree growth and development pathways. These samples have been prepared and will be sent to sequencing during fall 2023.All samples from 2023 will also be analyzed to examine the levels of expression of phytoplasma genes as well.

Cumulatively this data will be used by pathology researchers at WSU and USDA-ARS to study disease expression and potential control measures and by the WSU cherry breeding program to screen lines for disease susceptibility.

Summary

The discovery and identification of the different strains of this phytoplasma is critically important for understanding the spread and induction of disease. We now know why it spread under the radar for so long (because the local strains are only fruit-affecting) and how (it is likely adapted to spread by *C. m. reductus*). This is also important to the industry because it allows state regulators to track and identify new or introduced strains, and take action to prevent further spread.

Goal for 2024 – No Cost Extension

- Our goal for the NCE is to analyze sweet cherry and phytoplasma gene expression data from 2019-2023 samples as a complete dataset, focusing on pathways analysis to inform the breeding program's screening efforts and future collaborate research between WSU/OSU and USDA-ARS.

Publications:

- Harper SJ, Northfield TD, Nottingham LR, DuPont ST, Thompson AA, Sallato BV, Serban CF, Shires MK, Wright AA, Catron KA, Marshall AT, Molnar C, Cooper WR. (2023) Recovery plan for X-disease in stone fruit caused by '*Candidatus* Phytoplasma pruni'. *Plant Health Progress 24(2): 258–295*.

Project Title: Isolation and in vitro culturing of the X-disease pathogen

Report Type: Final Project Report

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Cooperators: Alexandra M. Johnson (WSU Postdoctoral Research Associate)

Project Duration: 3-Years (NCE)

Total Project Request for Year 1 Funding: $ 29,000
Total Project Request for Year 2 Funding: $ 30,000
Total Project Request for Year 3 Funding: $0

Other related/associated funding sources:
Funding Duration: 2019 - 2021
Amount: $539,661
Agency Name: WTFRC/OSCC
Notes: "Supporting a robust PNW sweet cherry breeding and genetics program". PI: Per McCord. Co-PIs: Cameron Peace, Bernardita Sallato, Mateus Pasa.

Funding Duration: 2020 - 2021
Amount: $28,000
Agency Name: USDA Germplasm Evaluation Funds, *Prunus*
Notes: "Germplasm evaluation for sweet cherry genetic diversity and disease resistance". PI: Cameron Peace. Co-PIs: John Preece, Stijn Vanderzande, Alexandra Johnson.

WTFRC Collaborative Costs:

Item	2021	2022	2023
Salaries	$15,250.00	$15,950.00	$0.00
Benefits	$2,750.00	$2,900.00	$0.00
Wages	$5,850.00	$6,050.00	$0.00
Benefits			
RCA Room Rental			
Shipping			$0.00
Supplies	$2,150.00	$2,100.00	$0.00
Travel	$2,000.00	$2,000.00	$0.00
Plot Fees	$1,000.00	$1,000.00	$0.00
Miscellaneous			$0.00
Total	$29,000.00	$30,000.00	$0.00

Footnotes:
Graduate student support for Alexandra Johnson
Single use, disposable materials for sample collection and laboratory assays
Pullman-Prosser return for approx. 4-5 multi-day trips during spring and summer each year

Budget 1
Primary PI: Cameron Peace
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RECAP OF OBJECTIVES

Identify optimum growing conditions for generating and maintaining '*Candidatus* Phytoplasma pruni' colonies, the pathogen responsible for X-disease in sweet cherry

1. Develop a rapid and reliable method for culturing '*Candidatus* Phytoplasma pruni'
2. Optimize the culture medium for year-round live growth of '*Candidatus* Phytoplasma pruni'

SIGNIFICANT FINDINGS

- Bacteria were successfully cultured in a liquid medium and on a solid medium from cherry tissues known to be infected with '*Candidatus* Phytoplasma pruni' (*Ca.* P. pruni)
- Five colonies were isolated from the solid medium that were identified phenotypically as possible *Ca.* P. pruni in 2022; these isolates did not survive re-culturing in 2023 to be genotyped via PCR testing to putatively determine if they were Ca. P. pruni
- One isolate in 2021 was determined by DNA sequencing to not be *Ca.* P. pruni
- Year-round in vitro culturing of the X-disease pathogen is likely possible, although the bacteria are fastidious
- The information gained could be used by plant pathologists to inform and assist in devising new methods to combat the bacterial pathogen behind this devastating disease

METHODS

Three-year plan: **2021** – Collect infected plant material and begin isolating and identifying colonies produced. Maintain pure isolates of colonies found to respond to current genetic detection methods.
2022 – Collect additional plant material for bacterial isolation and identification. Maintain pure isolates of colonies found to be '*Candidatus* Phytoplasma pruni'. **2023** – collect additional plant material for isolation and identification. Confirm colonies putatively identified as *Ca*. P. pruni via PCR testing.

Experimental material: Field work utilized accessible material from sites throughout central Washington known to be infected with X-disease as determined by WSU plant pathologists. Twenty trees in 2021, a different set of 20 trees in 2022, and another different set of 10 trees in 2023 were used in this study. All trees were growing in commercial and research orchards, under standard management practices. In 2021, 2022, and again in 2023, stems, spurs, and fruit from trees with high to moderate levels of infection were harvested and processed as described below. Additionally, stems, spurs, and fruit from asymptomatic trees were collected and processed. Briefly, infected and asymptomatic plant materials were transported back to the laboratory for surface disinfestation (tissues were soaked in a 10% NaOCl solution for 10 minutes, and then thoroughly rinsed with distilled, sterile water). Once rinsed, plant materials were placed in a sterile laminar flow hood to dry and then dissected. Five 25 mm transverse sections were cut from each stem and spur using a sterile razor blade. Additionally, 25 mm sections of cambium were also harvested from stems. Five symptomatic, as well as five asymptomatic fruit from each infected tree were sliced sagittally into 10 mm sections. Each dissected piece of tissue was placed in a separate, sealable 15 mL vial of liquid phytoplasma growth medium and incubated to room temperature (26 °C) for at least 48 hours.

Bacterial cultures: Stems, spurs, and fruit from infected, symptomatic trees were collected and numerous colonies were produced on phytoplasma-selective medium (Contaldo and Bertaccini 2019) and in a selective environment. Generation of selective medium in 2022 was significantly delayed due to equipment failure, but was resumed before the end of the 2022 growing season. Both liquid and solid phytoplasma growth media contained the broad-spectrum antibiotic ampicillin (25 µg/mL) as well as the antimycotic nystatin (50 µg/mL), which inhibited growth of most bacterial and fungal species. Additionally, cultures were kept in an oxygen-restricted environment, which further limited growth of both obligate anaerobic and aerobic bacterial species. The pH reactive pigment phenol red was added to each vial of phytoplasma liquid growth medium. Tubes inoculated with dissected plant material were observed to turn yellow after 24 or more hours, indicating acidification of the medium by bacterial metabolic processes, and were considered positive for bacterial growth. These tubes were quantified for degree of color change and 1 mL of broth was removed from each vial and spread aseptically across a new plate of solid phytoplasma growth medium. Plates of solid medium inoculated with bacterial broth were then incubated for at least 48 hours at room temperature (26 °C) in a low oxygen environment. Isolated colonies observed growing after incubation were picked using a sterile loop, introduced to new liquid growth medium, cultured until indication of pH change, and streaked onto a new plate of solid growth medium. Isolated colonies were then sampled for DNA testing.

DNA testing: The existing PCR-based assay described by Kogej et al. (2020) that detects *Ca.* P. pruni-specific DNA sequences was used to putatively determine which colonies were likely *Ca.* P. pruni. Such colonies with the same genotypic signature as *Ca.* P. pruni were subject to targeted genome sequencing (i.e., specific genomic regions) to confirm identity.

RESULTS & DISCUSSION

Summary: In 2021, 2022, and 2023, bacteria were isolated from plant tissues known to be infected with *Ca.* P. pruni, the causative organism in X-disease. For all three years, most colonies generated were determined to not be *Ca.* P. pruni. However, in 2021, four colonies were putatively identified as *Ca.* P. pruni by PCR-based DNA assays. Genome sequencing of one of these colonies indicated it was not *Ca.* P. pruni. The remaining three colonies that were also putatively identified as *Ca.* P. pruni were transferred to a fresh liquid medium and then a solid growth medium, to generate additional pure colonies. These colonies were maintained in pure culture, in growth medium over 2021 and 2022, but did not survive additional culturing in 2023. Phenotypic observation in 2022 identified five additional colonies as possible *Ca*. P. pruni. These newly identified colonies were isolated and maintained in pure culture until 2023, but did not survive re-culturing on solid growth medium. In 2023, liquid cultures were generated but growth of colonies on solid medium was limited. Of the few colonies produced in 2023, none were phenotypically similar to previously reported *Ca*. species and thus not considered for additional culturing as *Ca*. P. pruni. Issues in 2023 with culturing isolates was determined to be from a defective water purification system introducing ionic contamination which negatively impacted bacterial growth.

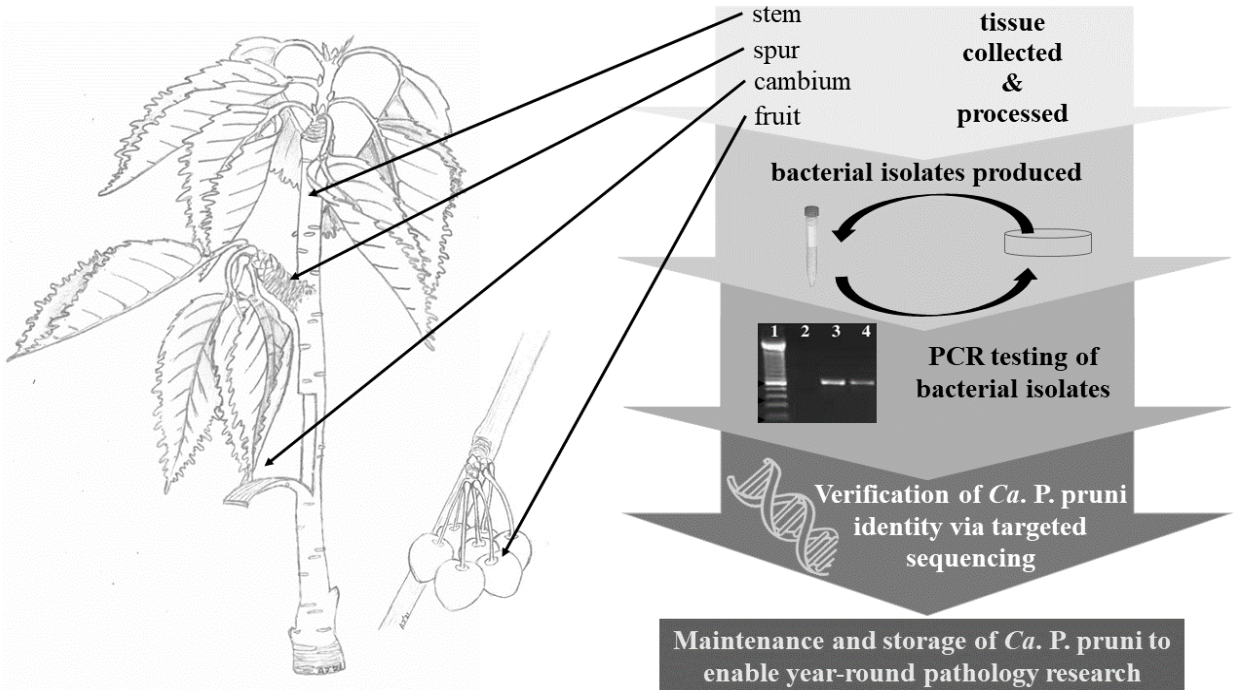

Figure 1: Collection, generation, and identification of bacterial isolates found in trees infected with *Ca.* P. pruni

Bacterial isolation: In 2021, 2022, and 2023, initial plates generated from broth cultures produced individual colonies on solid growth medium. Five to six species of bacteria were identified morphologically in 2021 and 2022, and two species of bacteria were identified morphologically in 2023. Broths from asymptomatic tissues did not produce any bacterial colonies, as expected. Colonies of different bacterial species cultivated in 2022 were phenotypically compared with those produced in 2021 to investigate the hypothesis that a specific subset of other bacteria must be present with *Ca.* P. pruni for X-disease symptoms to appear in sweet cherry trees; however, due to colonies failing to grow on medium produced in 2023, no definitive conclusions regarding presence of other bacterial species could be made.

Bacterial identity confirmation: From PCR-based assays of 60 colonies in 2021, four were putatively identified as *Ca*. P. pruni. One was sequenced to date and was found to be a contaminant. PCR testing of additional colonies produced over all three years was not conducted due to media contamination resulting in colonies failing to grow in 2023.

Bacterial storage: The eight colonies identified as possible *Ca.* P. pruni were maintained on solid phytoplasma growth medium in a low-oxygen environment until 2023. Medium contamination in 2023 rendered additional, long-term storage studies impossible. Colonies grown in 2021 that were putatively determined to be *Ca*. P. pruni indicate that in vitro culturing of this pathogen might be possible. However, results gathered from 2023 also

indicate that *Ca*. P. pruni is a particularly fastidious bacterial species and sensitive to ionic contamination.

EXECUTIVE SUMMARY

PROJECT TITLE: Isolation and in vitro culturing of the X-disease pathogen

Key words: microbiology, '*Candidatus* Phytoplasma pruni', sweet cherry

The objective of this project was to identify optimum growing conditions for generating and maintaining '*Candidatus* Phytoplasma pruni', the causative bacteria responsible for X-disease in sweet cherry, in vitro. Specifically, this project aimed to develop a rapid and reliable method for culturing *Ca*. P. pruni, and to optimize culture medium for year-round live growth of this bacterial species.

Over three growing seasons, different organs, including stems, leaves, and fruit, were gathered from trees known to be infected with *Ca*. P. pruni and from trees determined to not harbor the pathogens that were growing in orchards in Washington state. Organs were transported to a clean laboratory, where they were surface sanitized and dissected into small, ~10 mm sections. Plant sections were then placed in liquid growth medium and incubated for 7-14 days before an aliquot of broth was plated onto solid growth medium, then placed in a reduced oxygen growth chamber. Colonies morphologically similar to previously reported *Candidatus* colonies were subsequently genotyped using a published PCR assay. Of the colonies produced over 2021 and 2022, seven were putatively determined to be *Ca*. P. pruni. Targeted genomic analysis revealed one of these colonies was not *Ca*. P. pruni. Ionic contamination of media in 2023 from a defective water purification apparatus resulted in death of colonies kept from 2021 and 2022. Two colonies produced in 2023 on the contaminated medium were phenotypically dissimilar to previously reported *Candidatus* species and thus not maintained.

While in vitro culturing and maintenance of *Ca*. P. pruni was not successful, useful information was gained from this project. Results from putatively identified colonies produced in 2021 indicate that in vitro culturing of *Ca*. P. pruni is likely possible. Furthermore, colonies maintained from 2021 to 2023 using culturing techniques that alternate between liquid and solid growth mediums demonstrate that *Ca*. P. pruni can likely be grown in vitro year-round. Finally, colony growth failure in 2023 due to ionic contamination underscores the fastidious nature of this bacteria and the need for contaminant-free growth media when culturing *Candidatus* species. The collective information gained from all three years of this project could be used by plant pathologists studying *Ca*. P. pruni to inform and assist in devising new methods to address this bacterial pathogen which threatens the PNW sweet cherry industry.

Project/Proposal Title: Studying the infection progression of LCD pathogens in young trees.

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Total Project Request for Year 1 Funding: $65,656
Total Project Request for Year 2 Funding: $62,017

Other related/associated funding sources: None.
WTFRC Collaborative Costs: None.

Budget 1
Primary PI: Scott Harper
Organization Name: Washington State University
Contract Administrator: Samantha Bridger
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Item	2022	2023
Salaries	24,916	25,913
Benefits	9,079	9,443
Wages		
Benefits		
Equipment		
Supplies	31,661	26,661
Travel		
Miscellaneous		
Plot Fees		
Total	65,656	62,017

Footnotes:

Objectives:

1. Determine how rapidly diverse isolates of LChV-1, LChV-2, and/or the X-disease phytoplasma can infect young trees and establish a systemic infection after inoculation.

2. Determine the effect of coinfection with LChV-1, LChV-2, and/or the X-disease phytoplasma on infection progression and plant health.

Significant Findings:

- Tissue from low-titer (weak) trees may be readily grafted and used to infect trees by propagation, while tissue from high-titer (strong) trees has a low survival and infection rate.
- When high-titer transmission succeeds, systemic infection occurs rapidly and plants more rapidly become symptomatic.
- The presence of other vascular damaging pathogens (*Pseudomonas*, *Cytospora*, etc.) hamper systemic infection by the phytoplasma.
- Infected planting stock continues to be a risk factor that needs to be accounted for in new plantings and experiments.

Methods:

Objective 1

a) Identification and establishment of inoculum sources

Tissue from potential inoculum sources were collected from commercial orchards in Washington state in late 2021 and early 2022, total nucleic acids extracted as per established protocols, and the samples were tested for LChV1, LChV2, and *Ca*. P. pruni using validated assays (Katsiani et al. 2018; Kogej et al. 2020; Shires et al. 2022). Those samples positive for LChV-2 were further tested by an assay capable of discrimination between the 'LC5' and 'Rube-74' genotypes (Shires et al., unpublished), while '*Ca.* P. pruni' positives were genotyped using a high-resolution melt marker system developed during the SCBG project '*Epidemiology of the X-disease phytoplasma*' (Shires et al., unpublished).

b) Inoculation of seedlings for pathogen accumulation trials

Budwood from selected inoculum sources was collected in April, and again in July (due to the cold spring weather altering patterns of pathogen accumulation), and two rounds of grafting performed onto 12-month-old *P. avium* cv. 'Mazzard' seedlings. In each case, a single bud from the virus or phytoplasma inoculum source was t-grafted at approximately halfway up the stem of the rootstock, and bud survival assessed at 4- and 12-weeks post-grafting.

c) Testing of new planting stock

Combined root and cuttings from the top of the main stem/trunk of tree were collected from between 50-72 individual trees from 3 new cherry and 4 new peach orchards before the trees were planted in the spring of 2022. In 2023, samples were collected from 20 new cherry plantings, although sample size per-site was reduced. Samples were extracted and tested by qPCR for the presence of '*Ca*. P. pruni' (Wright et al. 2021) and LChV2 (Shires et al. 2022).

d) Risk of seed transmission of pathogens into planting stock

As *P. avium* cv. 'Mazzard' seedlings obtained from a commercial nursery in the Pacific Northwest were being used as receptor plants to be inoculated in these experiments, we examined whether seed transmission was a potential risk that could disrupt the experiments. Seeds collected from known '*Ca*. P. pruni' or LChV2 positives trees showing different degrees of symptom severity and

pathogen titer during the 2021 field season were surface sterilized in 10% bleach, dried and processed as follows:

i) Subsets from each seed lot were dissected, separating the seed coat and embryo, and total nucleic acids were extracted from each and tested separately. While LChV2 samples were tested for virus directly using a published assay (Shires et al. 2022), *Ca*. P. pruni samples were first tested for phytoplasma DNA presence using the standard assay (Kogej et al. 2020), then to determine whether the phytoplasma was alive, the samples were DNAse-treated and tested using an RNA-based assay targeting one of the highest-expression genes of the phytoplasma (Harper et al., unpublished).

ii) The majority of the seeds were cold stratified and germinated in moist vermiculite for a period of six months, survival assessed, and viable seeds planted in soil. Seedlings were grown on a mist-bench for three months, with gradual reduction in watering, then transferred to larger pots and moved to a greenhouse environment. Plants were tested for pathogen presence as above at three months and six months post-germination.

Objective 2

Since this project was proposed we have determined that the diversity of *Ca*. P. pruni genotypes is greater than was previously known. In lieu, in 2022 we examined mixed infections of '*Ca*. P. pruni' with LChV-2, or the two LChV2 genotypes together, in orchard trees. Sample collection, nucleic acid extraction, and PCR were performed as described above.

Results and Discussion

Objective 1

a) In 2023 efforts have been ongoing to capture and preserve LChV2 and '*Ca*. P. pruni' strains for experimentation. Based on experimental inoculations from July 2022, plus retrieval and replanting of field sources we have pure isolates of LChV2 strains LC5 and Rube-74, as well as X-disease inducing '*Ca*. P. pruni' strains Northwestern-X2 and X3 as well as Eastern-X. Leafhopper transmissions are underway to capture Northwestern-X1 and make additional plants of Eastern-X.

b) Last year we reported that we had inoculated a graft total of 67 mazzard seedlings with the LChV2 Rube-74 strain and 75 with '*Ca*. P. pruni' strain Northwestern-X3 in July of 2022. In October of 2022, very few of these plants were positive, and we assumed that the graft inoculations had largely failed but we kept a subset whose grafts had survived into 2023, repotting them from the 4" root training pots into larger 2-gal pots in May 2023 and maintaining them in greenhouse conditions (Table 1). Current-year wood and petiole tissue was retested in early September of 2023 by qPCR (Wright et al. 2021) and we found that most of those plants retained were positive for LChV2 and/or '*Ca*. P. pruni'. Cumulatively, 11 were positive for '*Ca* P. pruni alone, 4 for LChV2 alone, and 9 had mixed infections of both pathogens.

Table 1. Graft inoculation success and outcomes of inoculation after 14 months.

Source Inoculum	No. Graft Inoculated in July 2022	No. Grafts survived	No. Positive Oct. 2022	No. Positive September 2023*	No. Dissected for Quantification
LChV2 strain Rube-74 w/ weak '*Ca*. P. Pruni'	67	35	1	22	10
'*Ca*. P. pruni' strain NW-X3 w/ weak LChV2	75	10	0	7	2

Of these, 14/22 and 5/7 had established pathogen titers comparable to the initial included after 14 months, the remainder were lower. Quantification of the pathogen levels in these plants (Figure 1) indicated that high-titer inoculum resulted in higher resulting pathogen levels in the inoculated plants within 14 months, and similarly low-titer inoculum resulted in lower resulting pathogen levels in the same time period.

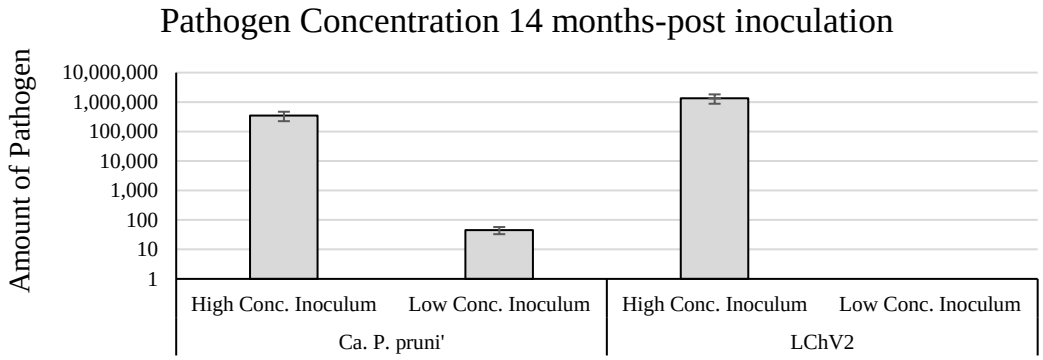

Figure 1. Levels of '*Ca*. P. pruni' and LChV-2 detected at 14 months post-inoculation in cherry seedlings graft-inoculated with high vs. low concentrations of the pathogens.

Seven plants with high titers of the target pathogens were preserved to contribute to the genotype collection for further research and to act as inoculation sources, while eight LChV2 and/or '*Ca*. P. pruni' positives with differing pathogen contractions were donated to the 'Canine Detection' project so that progress could be made there (along with twenty negative control plants). This left a total of twelve plants to be dissected for quantification and localization of pathogen titer. We found that while both pathogens had established a systemic infection in the seedling trees, there were patterns in the pathogen distribution and concentration (Figure 2)

For LChV2 inoculations, high-titer inoculum produced a high titer systemic infection throughout the plant, although the highest concentrations were above and just below the graft, suggesting in-season acropetal movement and accumulation. This is to be expected as the virus readily infects newer tissues, while older tissues are less accessible due to fewer plasmodesmatal connections between the sieve elements and companion cells where the virus replicates. The virus was at markedly lower concentration in the root tissues. No low-titer LChV2 infected trees were dissected due to low sample size.

The phytoplasma inoculations showed a similar low-titer vs. high-titer inoculation pattern, though in both cases a systemic infection occurred. In contrast to the virus, the phytoplasma was concentrated in the lower tissues of the plant, below the graft, and not in the upper sections of the stem of side branches. For both high- and low-titer inoculum the resulting pattern was the same, differing only in concentration (Figure 2).

Position on Seedling	*Ca*. P. pruni'		LChV2	
	Low Conc.	High Conc.	Low Conc.	High Conc.
Shoot Tip	99 ± 10	N/A		1,155M ± 509K
Side branches above graft	99 ± 9	N/A		1,747M ± 1,025M
Main stem above graft	179 ± 10	994 ± 0		1,764M ± 1,485M
Graft	105 ± 7	63K ± 0		1,105M ± 760M
Side branches above graft	158 ± 11	2,248M ± 1,196M		730K ± 384K
Main stem below graft	268 ± 48	6,607M ± 5,132M		1,991M ± 1,189M
Crown	235 ± 34	2,568M ± 0		892K ± 799
Main stem below soil	235 ± 22	1,283M ± 0		223K ± 279K
Lateral roots	131 ± 11	5,208M ± 0		128K ± 76K

Figure 2. Distribution and concetration of '*Ca*. P. pruni' and LChV2 in cherry seedlings at inoculated with high and low titers of the pathogen, at 14 months post-inoculation.

We also examined field grown peach trees that were found to be positive during the 2022 planting period testing (see previous report). Fourteen trees were removed and sequentially dissected at 6 months post-planting (September 2022), and twenty trees at 18 months post planting (September 2023). AT six months, the two plants though to be negative remained so, while 7 showed scattered low concentration distribution in one lime or leader, or the roots, while the rest of the tree remained negative (Figure 3a). Two trees were systemically infected from the roots and into the leaders, though concentration was low.

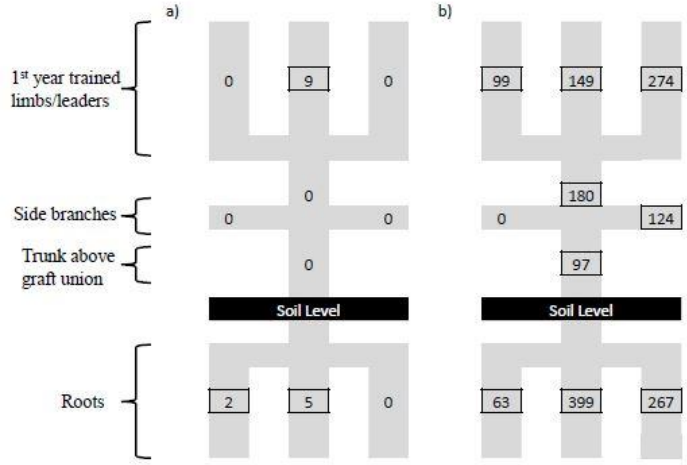

Figure 3. Distribution maps of a) lightly infected and b) heavily infected peach trees at six months post-planting. Numbers at each point are the concentration of phytoplasma cells found.

At eighteen months two trees had systemic infections (Figure 4a) and had started to produce classic X-disease foliar symptoms, and eight of the trees sampled had scattered, low titer infections that could be indicative of a 2023 in-season inoculation by leafhoppers or were infected at planting but failed to establish due to the heavy bacterial canker (*Pseudomonas syringae* pv. *syringae*) and wood-rot fungal infections (*Cytospora leucostoma* or *Eutypa lata*) that appear to have entered the young tree the graft union and spread systemically up the tree. These secondary pathogens also appear to have hindered the systemic infection of two trees in

which infection had become established (Figure 4b), damaging the phloem system or killing limbs such that the pathogen could not move. One tree appears to have a localized infection indicative of 'superspreader' in season-inoculation (Figure 4c); all three of the trees that tested negative at planting remained negative in September.

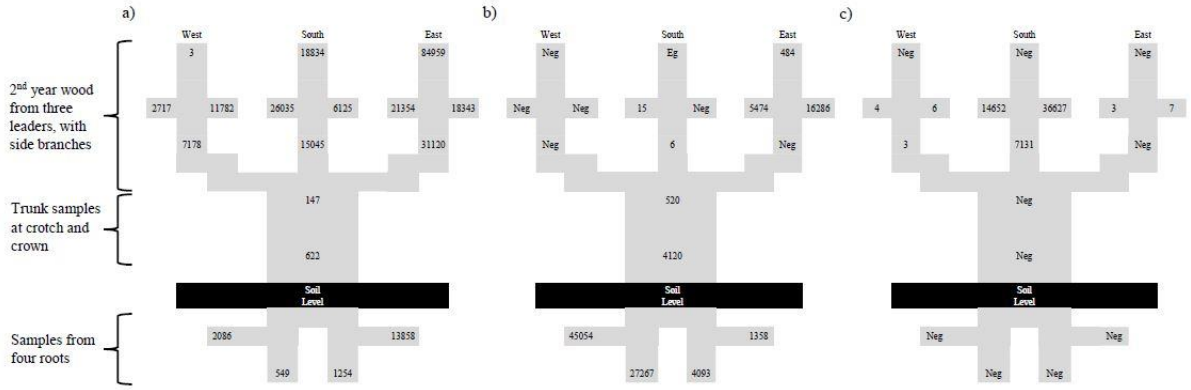

Figure 4. Distribution maps of a) heavily infected, b/c) lightly-infected peach trees at eighteen months post-planting. Numbers at each point are the concentration of phytoplasma cells found.

c) In 2023, to continue monitoring the potential presence of '*Ca*. P. pruni' and LChV2 in new planting stock, we collected and tested samples from a total of 20 cherry plantings, with samples sizes ranging from 7 to 48 trees sampled per site (Table 1). These were tested and confirmed by a highly specific and accurate assay developed to determine whether the '*Ca*. P. pruni' pathogen was replicating. Positives were found in 10 of the plantings, with incidence ranging from 2% to 60% (the latter was from a small sample size), and an average 8%. Positives were concentrated in specific cherry varieties from multiple propagators which suggests that there may be problems with source material, and from propagators in specific geographic areas. These will not be named to preserve confidentiality. Genotyping samples collected at planting allowed us to identify also allowed us to identify patterns of spread and introduction or movement of strains not common in Washington state, including Eastern and Western-like strains from sites or states that our other projects have indicated are present there.

d) This experiment was completed in 2022 and results described in the progress report for that year.

Objective 2

Field observations were reported in 2022, and an inoculation series to test the hypotheses generated from that data will be performed in 2024.

Summary

Cumulatively these data suggest that a systemic infection requires basipetal movement of the '*Ca*. P. pruni' to the roots, and after overwintering there, root-upwards recolonization of the tree limbs and colonization of the emerging leaves in the following season. Within-season movement in a tree is largely local, which correlates with the general patterns of source-sink photoassimilate flow in the phloem. This also agrees with our previous work mapping infections in heavily infected cherry (Wright et al. 2021). Other factors, including the environmental conditions and other pathogens (i.e., bacterial canker) can determine the extent or rate at which a systemic infection occurs, or why in some cases, it doesn't for several years. Also of note is that between September 2022 and September 2023, titer in the systemically infected peaches increased by 1-2 orders of magnitude (i.e. from tens/hundreds of cells to

thousands/tens of thousands of cells). The other point that needs to be made here is trees that are graft-inoculated or made from infected propagative material will likely have a higher and more detectable titer earlier in the infection cycle, or become systemically infected faster because unlike leafhoppers which are a one-time inoculation, the grafted tissue acts as a longer-term source of phytoplasma because not only can it unload into the attached phloem, but also replicate in the graft itself.

What does this mean for detection and diagnosis? As previously stated, sample collection is best made from limbs low down, close to the trunk. However, in large trees with no symptoms to guide sampling, it is nearly impossible to predict where the pathogen will be, and it is very easy to get inconsistent results when sampling from even the same leader or limb when the infection is in its early stages. The second finding, that the level of inoculum put into the plant is reflected in the titer a year later, is also important. Based on our current understanding of phytoplasma titer in leafhoppers, most deliver low numbers (<100) of cells, which will result, at least in the early stages of infection, low concentration levels of phytoplasmas that are scattered throughout the plant and difficult to detect. So-called 'superspreaders' containing orders of magnitude more phytoplasma are rare, but will result in a higher concentration, and thus more detectable, early infection, similar to plants made from infected propagative material.

<u>Goals for 2024 – No Cost Extension</u>

- Our goal for the NCE is to repeat the inoculation series with both graft-inoculation and using leafhoppers now that we know the upper limit for systemic infection of young plants (~12-14 months)

  We have already begun the leafhopper trials attempting to inoculate both cherry and peach. Testing of leafhoppers indicated that ~60% of leafhoppers were carrying phytoplasma, with concentrations ranging from hundreds to millions of phytoplasma cells. Within 12 days of inoculation, phytoplasma was detected in leaves fed on by the leafhoppers. Dissections are planned for a subset at periodic intervals.

- Mixed infection inoculations are planned for 2024 as we have single-isolate sources of LChV2 and X-disease genotypes.

Project Title: Evaluating Replant Strategies for X-disease Infected Orchards

Report Type: Final Project Report

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Project Duration: 3 Year

Total Project Request for Year 1 Funding: $43,388
Total Project Request for Year 2 Funding: $45,207
Total Project Request for Year 3 Funding: $27,494

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Station Manager/Supervisor: Nicole Strong

Item	2021	2022	2023
Salaries			
Benefits			
Wages[1]	3,864	3,864	3,864
Benefits[2]	2,704	2,704	2,704
Equipment			
Supplies[3]	11,480	6,000	6,000
Travel	660	660	660
Miscellaneous			
Plot Fees			
Total	$18,708	$13,228	$13,228

Footnotes: [1]Wages for a Bioscience Technician II (10 hours a week x 24 weeks x $16.10). [2]OPE calculated at 20%. [3]Testing new plants (160 plants x $25), testing soil, roots, and suckers (160 samples x $25), testing plants in year two (80 x $25), trees (40 x $12), and netting. [4]Travel to and from research plots ($0.54 a mile).

Budget 2
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Organization Name: Washington State University
Contract Administrator: Katy Roberts
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Station director: Naidu Rayapati

Item	2021	2022	2023
Salaries			
Benefits			
Wages[1]	5,400	5,616	5,841
Benefits[2]	522	543	565
Equipment			
Supplies[3]	17,500	24,560	6,600
Travel[4]	700	700	700
Plot Fees[5]	560	560	560
Miscellaneous			
Total	24,682	31,979	14,266

Footnotes: [1]Wages for a non-student temporary hire (24 weeks x 15 hours/week x $15). [2]Benefits for non-student temporary hire calculated at 9.7%. [3]Testing new plants (320 plants x $25), testing soil, roots, and suckers (320 samples x $25), testing plants in year two (160 x $25), trees (80 x $12), and netting. [4]Travel to and from research plots ($0.54 a mile). [5]Fees for using the Roza block at IAREC.

The Little Cherry Disease epidemic in Washington and Oregon is largely attributed to the X-disease phytoplasma (XDP). Currently, there are no known treatment for XDP, instead, orchardists must control the spread of the disease through timely tree removal. Growers are investing in several practices for tree removal, to reduce the risk of spreading XDP in replanted sites, without certainty of the cost benefit associated with these practices. These practices include the application of herbicide to the tree trunk in different ways, utilizing excavators to remove trees and large roots, replacing soil in the planting hole, fumigation and or leaving the ground fallow for one or more seasons. Despite these measures, orchardists have reported X-disease infections in newly planted blocks.

Our initial objective was to determine if the remaining infected roots could transmit the disease to newly planted trees, and how different practices might prevent the re-infection. To answer these questions, we 1) Evaluated the survival of infected roots after tree removal under different removal practices, 2) Assessed the infection rate in re planted trees followed different replanting strategies. To isolate infection risk due to leafhopper versus replanting strategy, trees were tested throughout qPCR test, and trees with no infection detected were netted.

In 2022, results for WA sites suggested that the qPCR test of these young trees did not identify low levels of infection. Thus, in 2023 we modified the objectives for WA sites to 3) better understand the progression of XDP under different case studies.

OBJECTIVES:

1. *Evaluate the survival of roots in the soil under three common replant strategies.*
2. *Evaluate the rate of infection of replanted trees under three common replant strategies*.
3. ***Better understand the progression of XDP and assess different sampling timing and tissue (Y3)***
4. *Provide orchardists with science-based replant strategies for X-disease infected orchards.*

SIGNIFICANT FINDINGS:

1. *Evaluate the survival of roots in the soil under three common replant strategies.*
    - Removal of individual trees did not kill all the infected roots, in both states, regardless of the application of herbicide in the cut stump.
    - However, root suckers were not observed at Washington or Oregon sites.
    - In WA, no live roots were found in sites where the entire orchard was removed, regardless of the method used, herbicide in the stump, with or without fumigation, with or without a period of fallow.
    - In Oregon, 70 roots were found where the entire block was removed. The majority of these roots were under 0.5 inches in diameter, and 90% of the roots were found in a state of decay.

2. *Evaluate the rate of infection of replanted trees under three common replant strategies*.
    - Infection reported by qPCR method was inconsistent throughout the season and tissue sampled, likely due to reduced titer level expected in young trees.
    - Given the above, it was not possible to attribute infection to the nursery stock or new infection of the block following the removal strategy, and assessing netting control could not be ground truthed.
    - There was no relationship between the number of practices and the rate of infection: Example; the lowest infection levels were in WA 7 (40%) where trees were removed by excavator (no herbicide applied), fallow for a year, and fumigated prior to planting. While the highest levels were in WA4 and WA5 (100%), where each tree received herbicide to the

stump, stumps and roots were removed with excavator, the ground was fallow for a year, and received fumigation prior to planting.

- The higher risk of infection could be attributed to the nursery stock, early infection in high pressure areas with infected neighbor blocks and high leafhoppers pressure.

3. Better understand the progression of XDP and assess different sampling timing and tissue (2023).

Washington:

- In 2021 initial sampling, there was 5% overall XDP infection, in trees from one to three years old.
- In 2022, 47% of the trees reported positive to XDP. With higher rate of infection reported in July (47%) compared to September (38%)
- In 2023, the detection rate was higher in September (87%) compared to June (57%), opposite to 2022.
- If an XDP infected tree is considered one with at least one positive (< 40 Ct) value, the overall infection reached 90%, vary between 60% and 100% across sites.
- If false negative corresponds to a non-detection (> 40 Ct) value, following a previous positive test at any given time, there were 58% false negative test, 56% corresponding to dormant testing of roots and stems.
- The sudden high level of infection (below Ct 36), detected in WA 1, 2, 4 and 5, in the second year after planting, suggests that trees were infected prior to planting.
- These results coincide with several other ongoing projects where the tissue or timing of sampling of asymptomatic trees can lead to false negatives.

Oregon:

- During our initial sampling in late summer, only 1 tree at a whole orchard removal site tested positive for XPD.
- In 2022, 47% tested across all OR sites positive for XDP. The single tree removal site had the greatest number of XDP infections (55%). The whole orchard removal sites had 40 and 45% infection rates respectively.
- In 2023, 68% across all OR sites tested positive for XDP when we included trees that tested positive in 2022. The single tree removal site had the greatest number of XDP infections (75%). The whole orchard removal sites had 65 and 55% infection rates respectively.
- XDP testing had a 40% false negative (non-detection) rate at The Dalles 3, and 10% false negative rate at The Dalles 4 in 2023
- In some instances, the CT value was higher in 2023 than it was in 2022.

METHODS:

1. Evaluate the survival of roots in the soil under three common replant strategies.

Eleven newly planted sites, with different removal and replanting methods after confirmed XDP infection, were selected for out three-year study (Table1). Two of the sites were blocks with individual tree removal: WA 1 and The Dalles 2. All other sites consisted of complete block removal of confirmed XDP infected blocks.

At each site, one gallon of soil was collected from random locations withing each block and sieved to separate soil from roots. Site that contained live roots were then sent to Harper's lab for XDP test. Similarly, in Oregon, a 1ft x 1 ft x 1ft volume of soil was excavated approximately two feet from each

tree. Soils were sieved through a 10 mm mesh soil sieve to separate the roots from the soil, and roots were brought back to the lab and measured using a caliper. Any root that appeared to be living was tested for LCD infection by Dr. Harper.

2. Evaluate the rate of infection of replanted trees under three common replant strategies.

In 2021, an initial quantitative polymerase chain reaction (qPCR) test was conducted in 10 trees per site to ensure trees were free of XDP or viruses and selected for netting. Trees with number of cycles (Ct) below 40, were considered infected, now on positive trees. These initially positive trees were discarded immediately by the orchardist. Molecular qPCR test with values above Ct 40 were considered not infected trees, now on negative to XDP. On each site, half of the negative trees were netted between May 27 and June 2 using a shade net (7% shade). The system for netting varied according to the orchard systems, tree high and grower needs (Figure 1).

Table 1. Removal and replanting conditions for Washington (WA) and Oregon (The Dalles) sites.

Location-Cultivar/Rootstock (previous root) Planting year	**Herbicide/Tree cut**	**Removal**	**Fumigation**	**Period to replant**
WA 1* Skeena/Gi.12 (G.12) 2019 - 2021	August - Stump herbicide	Spring - Stump removal. Replace soil in the planting hole	NO fumigation	Next spring
WA 2 Suitenote/Gi.12 (Mazzard) 2021	August - Stump herbicide.	Winter- excavator	Fall Fumigation	One year fallow
WA 4 Chelan/Mazzard 1 2021	August - Stump herbicide.	Fall -excavator	Spring Fumigation 1rate	One year fallow
WA 5 Chelan/Mazzard 2 2021	August - Stump herbicide.	Fall -excavator	Spring Fumigation x2 times	One year fallow
WA 3 Chelan/Gi.12 (Cherry) 2020	No herbicide	Fall-excavator	Spring Fumigation	Next spring
WA 7 Chelan/Gi.12 (Peach) 2020	No herbicide	Fall-excavator	Spring Fumigation	One year fallow
WA 6 Coral/Mazzard (K5) 2018	No herbicide	Fall -excavator	Spring Fumigation	Next spring
The Dalles 1 (Mazzard)	Stump herbicide	Fall- Ripping and large root removal	Cover cropping	Undecided
The Dalles 2* Bing/K.6	No herbicide	Fall - large root removal	NO fumigation	Following spring

(Mazzard)				
The Dalles 3 Benton/K.6 (Mazzard)	August- Stump herbicide	Fall-excavator. Ripped.	NO fumigation	Three years fallow
The Dalles 4 Suite Note/K.6 (Mazzard)	Herbicide applied to root suckers for two years	Fall-excavator. Removed large roots. Ripped.	NO fumigation	Two years fallow

* Individual tree removal in a block with high pressure and confirmed X-phytoplasma.

In WA, nets were removed after leaf drops to prevent damage by snow or wind and allow management winter pruning and training. In 2022, nets were re-installed during the spring (May – June). In Oregon, nets remained in place over winter in years 1 and 2, but were removed in winter of year 3 due to snow. Yellow sticky traps were installed inside the netting to monitor leafhoppers, and the effectiveness of the netting system, as well as root suckers and weeds.

Figure 1. Examples of netting strategies depending on tree height and system.

Sampling method and timing

In 2022, aerial tissue samples were collected following WSU standard sampling of five-inch wood with spurs, leaves and fruit stems when present, from 10 netted and 10 unnetted controls, during July 7th and September 25th. In Oregon, woody tissue was collected on August 28, 2022 and July 28, 2023 from near the base of the netted and controlled trees following the WSU standard protocol.

In 2023, roots and stems were collected from the same trees during the dormant season (February), and again on June 6th and September 3rd following the standard sampling. All samples were tested for little cherry disease (XDP, LChV 1 and LChV2) using qPCR testing method.

RESULTS AND DISCUSSION

1. Evaluate the survival of roots in the soil under three common replant strategies.

In WA sites, only the individual tree removal sites; WA 1 had live infected roots the following spring after removal, with root size larger than ¼ inch. In WA 1 site, 100% of the roots collected from the removal area were positive to XDP, suggesting that while herbicide application killed the aerial part of the tree, trunk and large stump, roots further from the removed area were still alive. In contrast, all WA sites with entire block removal (W2 – WA 7) live roots or root suckers were not found, indicating a successful tree and root removal, regardless of the method utilized and fallow period. These higher root death in whole block removal, versus the individual tree removal, can be attributed

to the limiting conditions that follow entire block removal; no irrigation, drying of the soils and consequently nutrients, having a direct impact on root survival. These results suggest the importance of early tree removal to increase the period with limiting conditions; heat, water, nutrients, and the higher risk with individual tree removal, as continue management will likely promote root survival and potential suckering.

In Oregon, we did not observe live roots at the individual tree removal site in 2022. We did excavate nine roots that appear to be alive from The Dalles 3 location, which was fallow for three years following whole block removal. The live roots were small in diameter but averaged 5 inches in length. The Dalles 3 is the only site where an excavator was not used with the explicit intent to remove large roots from the soil. We did not observe root suckers at OR sites.

2. Evaluate the rate of infection of replanted trees under three common replant strategies.

In Washington, the initial prospection of LCD infection in trees prior to netting showed that three out of six replanting sites had 10% XDP positive trees, while all trees were negative to LChV2. In WA 1 (individual tree replanting), despite the presence of live roots positive to XDP near the planting whole, all new planted trees (n=10) were negative LCD pathogens when sampled in August 2021. The three replanting sites with one out of ten XDP positive trees corresponded to WA 3, WA 4 and WA 6, where trees were immediately removed.

In 2022, the overall infection reported to XDP in WA sites ranged between 0 - 100% in July, where all sites, except WA 6, had at least 15% infection (Table 2). To our surprise, in September of the same year, the range of infection reported were lesser, ranging between 5% and 88%, in sites with infected trees, while WA 1 and W6 reported no XDP infection. In 2022, netting has no influence in the results (data reported in 2022), while there were no leafhoppers detected in yellow sticky traps placed inside the nettings. The detection level in July 2022 ranged between Ct 16.5 and 40, were values below Ct 36 indicate early infection, and values below Ct 22 are of serious risk (Serban and Harper, 2022).

Sites	Jul-22	Sep-22	Mar-22 Roots	Mar-23 Stems	June-23	Sep-23	Total %
WA 1 (n=20)	5 (30.8 - 37)		1 (37)		7 (36.1 - 38)	15 (34.7 - 39)	95%
WA 2 (n=20)	12 (32.0 - 39)	1 (39.5)	3 (32.3 - 35)	1 (35.6)	19 (28.1 - 38)	18 (34.8 - 40)	100%
WA 3 (n=20)	3 (39.4 - 40)	9 (36.4 - 38)	1 (34.2 - 35)	2 (33.5 - 34)	3 (32.0 - 38)	14 (21.5 - 39)	85%
WA 4 (n=8)	7 (20.6 - 38)	7 (20.5 - 37)	3 (33.8 - 38)	4 (30.5 - 32)	8 (14.2 - 38)	7 (17.5 - 34)	100%
WA 5 (n=8)	8 (16.5 - 38)	7 (20.3 - 38)	1 (38.2 - 38)	3 (30 - 32)	7 (15.6 - 39)	8 (18.4 - 33)	100%
WA 6 (n=10)					3 (38.2 - 39)	5 (34.9 - 38)	60%
WA 7 (n=20)	5 (37.5 - 40)	9 (33.4 - 38)			2 (37.4 - 38)	8 (34.3 - 39)	90%
Total %	**47%**	**38%**	**10%**	**12%**	**57%**	**87%**	**90%**

Note: Total % consider the percentage of trees that reported positive to XDP in one or more sampling dates.

In 2023 we removed the netting (as there were no differences between netting and un-netted trees) and changed the scope of the project for WA sites towards identifying the progression of the XDP in the monitored blocks, compare tissue and timing of sampling. Below we analyze case by case.

WA 1: Individual tree removal of Skeena on Gisela 12. The grower monitors individual trees prior to harvest, marking symptomatic trees, that are then cut to approximately 3 ft of the ground and the stump is immediately painted with herbicide (Roundup). The following spring, the stump, soil and roots, are removed by hand (aprox 2ft x 2ft x 2 ft), and the new trees are planted with fresh soil from a clean area (a video of the replanting process can be found in Good Fruit Grower online here). In this site selected trees vary in age between 2nd to 3rd leaf. In July 2022, two trees were positive to XDP in the control and three were positives under the netting (data not shown), with Ct values ranging between 31 – 37 (Table 3). Three other trees were positive to LChV2 with Ct values above 36 (data not shown). In September of the same year, none of the trees reported XDP infection. Given the lack of relation of infected trees with and without netting and the difficulty for the grower to conduct standard operations, in 2023 the nettings were not re-installed, however we continue monitoring leafhoppers in each sample tree with yellow sticky traps.

Dormant sampling of roots and stems reported only one XDP infected tree (Ct 37), being a different tree from the previously reported positive trees, while none of the previously positive trees reported XDP infection (Table 3). During June (2023), two trees were confirmed XDP positive (T18 and T20), five were new positive, while four were false negative. In September (2023) the detection rate increased (opposite to 2022) where 15 trees were XDP infected, six of them were confirmed positives, eight were new XDP positives and four were infected while not detected (Table 3)

Table 3. *Candidus Phytoplasma pruni* (XDP) molecular (qPCR) test results for WA 1. Ct values below 40 were considered XDP infected trees. N: negative to XDP.

Sampling Time	Number of cycles (Ct) values for XDP																			
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Jul-22		35		34		31								36				37		
Sep-22																				
Dormant 23 (Root)																				37
Dormant 23 (Stems)																				
Jun-23	36									37			38			37		36	37	37
Sep-23	39	36	37		38		37		36	38	38	38	35	38	36	37	38	38		
Observations[1]		4		5		5		**N**						4				3	1	2

[1] Observations: values correspond to the number of test reporting false negative, considered if a test reported non-detection or Ct value above 40, followed a previous positive test at any given time. N: indicate non-detected across the three years of study.

WA 2. An entire sweet cherry block was removed after confirmed XDP infection. Trees were removed in 2018, by cutting each tree to a stump, each treated with herbicide, and removed during the summer. Stumps and large roots were removed with an excavator during the fall, then smaller roots were removed by hand. The ground was left fallow for one year and fumigated before replanting in 2021 (Table 1). In 2021 all trees were negative to XDP. In 2022 July sampling, twelve trees came back positive to XDP with Ct values ranging between 34 and 39 (Table 4). Higher infection were reported in the caged trees (80%) compared to the controls (40%), and two trees were positive to LChV2 with Ct values above 32 (data reported in detail in 2022). Similarly, dormant sampling of roots and stems reported less infected trees, one of three sampled roots was a new positive tree, which

was also detected in the dormant stems and next two sampling times (T11). During June 2023, 95% of the trees reported XDP infection with Ct values ranging between 28 and 38, most of which were confirmed in September, with two samples being false negatives (Table 4). If we consider infected tree those with at least one positive test, this site had 100% infection after 2 years of planting.

Table 4. *Candidus Phytoplasma pruni* (XDP) molecular (qPCR) test results for WA 2. Ct values below 40 were considered XDP infected trees. N: negative to XDP.

Sampling Time	**Number of cycles (Ct) values for XDP**																			
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Jul-22		35		32	38	39	39			35		39		36	36	37		34		38
Sep-22							39													
Dormant 23 (Root)							32				35			34						
Dormant 23 (Stems)											36									
Jun-23	36	34	34	32	31	31	32	36	28	35	34		36	31	33	38	30	38	34	31
Sep-23	38	39	38	37	39	36	39		38		37	35	37	38	36	39	35	38	36	40
Observations[1]		3		3	3	3	1	1		4		4		2	3	3		3		3

[1] Observations: values correspond to the number of test reporting false negative, considered if a test reported non-detection or Ct value above 40, followed a previous positive test at any given time. N: indicate non-detected across the three years of study.

WA 3: An entire sweet cherry block with confirmed XDP infection was removed in 2018, by removing entire trees with excavator during the fall, with no herbicide applied to the stumps, fumigated, and replanted the following spring (Table 1). In 2022, none of the control trees (un-netted) reported XDP infection while three netted trees reported XDP infection, and four trees were positive to LChV2 (data reported in 2022). The Ct values for XDP were 39.4, suggesting a recent infection. In contrast with the other sites, the September sampling reported more infected trees, one being a confirmed positive (T18), eight new positives, and two false negatives (Table 5). The dormant sampling of roots and stems confirmed two previously positive trees, T4 and T12 respectively, plus an additional positive with Ct values of 34. Like 2022, in 2023 the early sampling during June confirmed only three XDP infected trees, while in September, 70% of the trees reported infection with Ct values ranging between 22 (high risk) and 39. With only three trees being negative to XDP across the three years (15%).

Table 5. *Candidus Phytoplasma pruni* (XDP) molecular (qPCR) test results for WA 3. Ct values below 40 were considered XDP infected trees. N: negative to XDP.

Sampling Time	**Number of cycles (Ct) values for XDP**																			
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Jul-22				39						39								40		
Sep-22	38	38			38			38				36	38				38	38	38	
Dormant 23 (Root)				34																
Dormant 23 (Stems)											34	34								
Jun-23				32							36		38							
Sep-23	37	38		22	38		38	38	39	37	38			39	39	39	37	37		
Observations[1]	3	3	**N**	2	3	**N**		3		4		3	3				3	3	4	**N**

[1] Observations: values correspond to the number of test reporting false negative, considered if a test reported non-detection or Ct value above 40, followed a previous positive test at any given time. N: indicate non-detected across the three years of study.

Sites WA 4 and 5. Both sites are in the same orchard, with fumigation rate as the main difference between replanting method (Table 1). Trees were XDP confirmed with high incidence level in the block and area. Here, each tree was cut to a stump, painted with herbicide, and removed with excavator in 2018. After one year of fallow ground, both sites were fumigated in the spring, WA 4 receiving a single fumigation in the fall, and WA 5 receiving a second fumigation during the spring prior to planting. The number of fumigations did not affect the rate of XDP infection; thus, we combined the analysis of both site into one. As indicated in Obj 1. one of 16 trees reported XDP infection prior to establishing the trail, and the tree was removed immediately. In 2022, the July sampling all trees were XDP positive, except for one (T10), two were also positive to LChV2 (data not shown). The Ct values ranged between 16.5 (very high risk) to 39, (11 below Ct 35 and 7 below 30), with no differences between netted and un-netted trees (data reported in 2022). The following sampling during September 2022 most trees were confirmed positive, one was a new positive and two were false negatives. Suggesting 100% infection after one year of planting. Dormant sampling of roots and stems were less precise with four XDP detections in the roots, and seven in the stems.

In 2023, one sample reported a false negative in the June (T3) and one in the September sampling (T1) (Table 6). The Ct values reported in 2023 ranged between 14 to 39. In these blocks, weed and root suckers were controlled intensively through the season, and there was no cover crop during the period of study. PI Harper reported that in 2022 the orchard had no XDP inoculum pressure (by measuring weeds and trees from a neighboring block). Thus, given the low Ct values (high infection levels) detected in the second year after planting, it is likely that the trees were infected prior to planting.

Table 6. Candidus Phytoplasma pruni (XDP) molecular (qPCR) test results for WA 4 and WA 5. Ct values below 40 were considered XDP infected trees. N: negative to XDP.

Sampling Time	Number of cycles (Ct) values for XDP															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Jul-22	36	38	34	37	21	21	23	38	38		22	38	37	16	21	21
Sep-22	33	32	35		31	21	21	38		37	20	33	38	21	33	24
Dormant 23 (Root)	36				38	34							38			
Dormant 23 (Stems)					31	31	30				31		35		32	32
Jun-23	38	33		16	20	14	21	16	37	19	21	33	39	16	23	25
Sep-23		32	33	24	18	18	19	18	34	19	22	33	33	21	19	24
Observations[1]	2	2	3	3			1	2	3	2	1	2		2	1	1

[1] Observations: values correspond to the number of test reporting false negative, considered if a test reported non-detection or Ct value above 40, followed a previous positive test at any given time. N: indicate non-detected across the three years of study.

WA 6. This site was removed in 2017 due to XDP confirmed infection utilizing an excavator during the fall (no herbicide to the trees). The following spring (2018), trees were planted after a spring fumigation (Table 1). In 2021, initial sampling reported one XDP infected tree and discarded. In 2022, none of the sampling dates reported XDP infection in the block, however, two trees were positive to LChV2, with Ct values above 36 (data not shown). In 2023, dormant sampling of roots and stems were also negative to XDP in all samples (data not shown). In 2023, in June sampling time three trees were positive to XDP. In September, two trees were confirmed positive, three were new positives and one was false negative. (Figure 2).

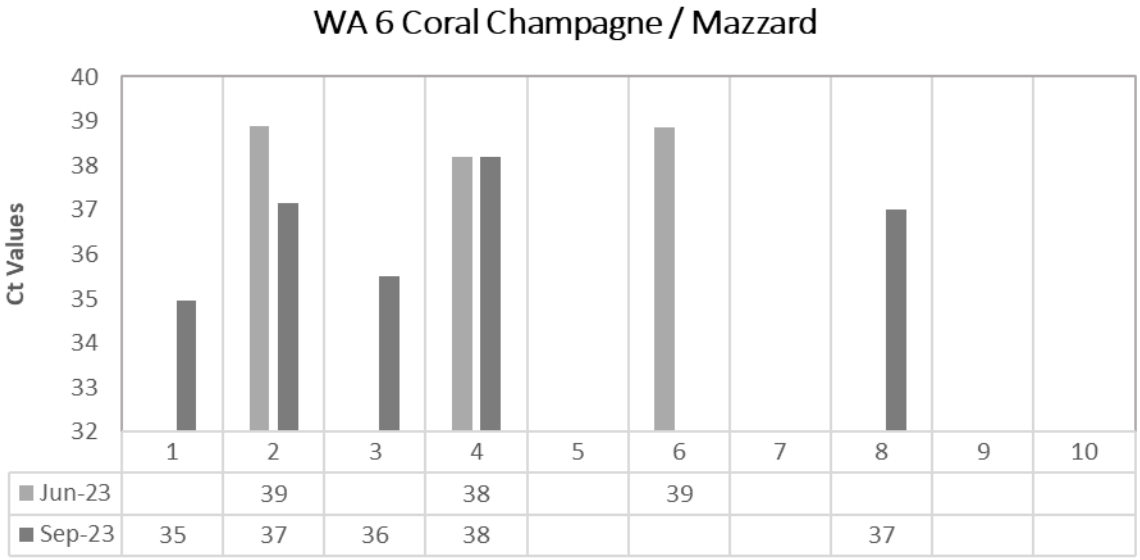

Figure 2. Ct values for XDP on individual trees at different sampling time.

WA 7. A replanted site after peaches, with extensive XDP infection. The trees were removed in the fall of 2018 with an excavator, and the site was left fallow for a year. Trees were replanted in 2020 after fumigation (Table 1). There were no XDP or LChV2 reported in the initial sampling. In 2022, the July sampling reported five XDP infected trees, and one was also LChV2 positives (data not shown), with Ct above 37. In September, four trees were confirmed positive, five were new XDP positives and one was a false negative. The Ct values ranged between 33.4 to 40.6. Similar to the other sites, dormant sampling of roots and stems was less precise, here with no positive trees. In June only one tree was confirmed positive, and one was a new XDP infection. In September 2023, three trees were confirmed positives and five were new XDP infected trees (Table 7). When considering at least one or more positive test throughout the season, after three years of planting this block reported 90% of infection.

Table 7. *Candidus Phytoplasma pruni* (XDP) molecular (qPCR) test results for WA 7. Ct values below 40 were considered XDP infected trees. N: negative to XDP.

Sampling Time	**Number of cycles (Ct) values for XDP**																			
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Jul-22		37		39				38										38		40
Sep-22		34		33					37		35		38	37			38	38		34
Dormant 23 (Root)																				
Dormant 23 (Stems)																				
Jun-23		37					38													
Sep-23			38			37	37			38	36		34			39			38	
Observations[1]	**N**	3		4	**N**			5	4		3	**N**	3	4	**N**		4	4		4

[1] Observations: values correspond to the number of test reporting false negative, considered if a test reported non-detection or Ct value above 40, followed a previous positive test at any given time. N: indicate non-detected across the three years of study.

3. Provide orchardists with science-based replant strategies for X-disease infected orchards.

Project details and preliminary results were shared by the PIs at different meetings, field days, newsletter and trade journal articles. For example, Co-PI Serban presented at the Wilbur Ellis Grower Meeting, with more than 65 participants, the Cherry Institute Annual Meeting, to more than 100 participants and at the Northwest Wholesale Grower Meeting, in Wenatchee WA for more than 80 participants. PIs Thompson and Sallato presented at the Little Cherry Disease Day, February 16, 2022, in Ellensburg WA to 178 participants and Sallato presented in person at the Roza Field day in 2021 and at the Little Cherry & X-disease Field Day, 2022, to more than 87 participants. An update was given at the Columbia Basin Tree Fruit Club meeting, along with other LCD projects, organized by PI Sallato. In 2023 Sallato shared the results in a Hispanic Field Day at the Roza farm to 15 participants.

Three newsletter articles and two videos were published in the Good Fruit Grower, and one article summarized the results of the project in Fruit Matters 2023. In addition, PI Serban was interviewed by the YaktriNews.Com.

Throughout August 2023, we conducted a survey to identify practices utilized in removal and replanting of XDP infected orchards. Of 62 respondents, 32.3% indicated removing trees and large roots, 29% apply herbicide to the stump, 11.3% spray the infected trees with insecticide prior removal, 12.9% fumigate pre planting, and 9.7% dry the ground after removal. When asked about replanting with sweet cherries, 61.5% indicated they have not replanted orchards after removing infected sites. For those that have replanted the orchards, 25% indicated they leave the ground fallow for at least one year, 19.4% request clean plants from nurseries, 16.7% control weeds that could host the pathogen, and 25% control vectors. None of the respondents use reflective sprays or fabrics, use netting around the perimeter of the blocks, and only 6% test the trees at planting.

When respondents were asked about the new priorities for LCD management; the three most listed priorities were 1. Clean plants from the nursery, 2. Best nursery practices and 3. Area wide management.

Outputs

Sallato, B., A. Thompson, S. Harper. Updates on Evaluating Replant Strategies for X-disease Infected Orchards. In: https://treefruit.wsu.edu/article/evaluating-replant-strategies-for-x-disease-infected-orchards/

Sallato. B. Little cherry disease: Removing the root risk — Video. Good Fruit Grower. In: https://www.goodfruit.com/removing-the-root-risk/

Sallato. B. Risk mitigation strategies for little cherry-driven removal and replant — Video. Good Fruit Grower. In: https://www.goodfruit.com/risk-mitigation-strategies-for-little-cherry-driven-removal-and-replant/ (In English and Spanish)

Serban, C. and S. Harper. 2023. What does the X-disease/Little Cherry Disease Test Results Numbers Means in Terms of Disease Progression?. In: https://treefruit.wsu.edu/article/what-does-the-x-disease-little-cherry-disease-test-results-numbers-means-in-terms-of-disease-progression/

Thompson, A. Not just little cherry. Good Fruit Grower. In: https://www.goodfruit.com/not-just-little-cherry/ (In English and Spanish)

Executive Summary
Project Title: Evaluating Replant Strategies for X-disease Infected

Abstract: This study investigates the efficacy of common replant strategies in mitigating X-disease phytoplasma (XDP) infections in orchards in Washington and Oregon. Removal of individual trees, even with herbicide application to the stump, did not eradicate all infected roots. Root suckers were absent at all sites regardless of rootstock or tree removal strategy. Entire orchard removal in Washington resulted in no live roots, while in Oregon, 70 roots were found, mostly states of decayed. Replanted tree infection rates were inconsistent, hindering attribution to nursery stock or removal strategy. Factors like nursery stock quality, early infections in high-pressure areas, and leafhopper presence posed higher infection risks. XDP progression analysis revealed a rise in infections over time, emphasizing the importance of careful sampling timing and tissue selection. These findings provide insights for orchard management practices and strategies to counteract XDP spread, offering a comprehensive perspective on root survival, tree infection dynamics, and XDP progression.

Key words: orchard removal, replant strategy, disease progression

Project Title: Identifying sources of X disease in cherry orchards

Report Type: Final Project Report.

Primary PI: Tobin Northfield
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Cooperators: Garrett Bishop, Scott Harper, Tianna DuPont

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $ 58,400
Total Project Request for Year 2 Funding: $ 55,849
Total Project Request for Year 3 Funding: $ 53,707

Other related/associated funding sources: Awarded
Funding Duration: 2020 - 2022
Amount: $249,360
Agency Name: WSDA/USDA Specialty Crop Block Grant
Notes: USDA SCBG funding to identify strains of phytoplasma in cherries and other stone fruit as well as weedy plants, and to conduct molecular gut content analysis on X-disease vectors. PI: Harper, co-PI's: Northfield, Cooper, DuPont

Other related/associated funding sources: Awarded
Funding Duration: 2021 - 2023
Amount: $244,750
Agency Name: WSDA/USDA Specialty Crop Block Grant
Notes: USDA SCBG funding to evaluate selective broadleaf herbicides as a management option for X-disease vectors. PI: Northfield, co-PI: Harper.

Other related/associated funding sources: Awarded
Funding Duration: 2022 - 2024
Amount: $295,376

Agency Name: USDA Crop Protection and Pest Management
Notes: USDA funding to develop phenology models for phytoplasma prevalence in plants and vectors to integrate into phenology models for leafhopper abundance (WTFRC project led by Nottingham). PI: Northfield, co-PI: Nottingham (WSU), Harper (WSU), Adams (OSU), Galimba (OSU).

Other related/associated funding sources: Awarded
Funding Duration: 2021 - 2023
Amount: $164,765
Agency Name: USDA AFRI
Notes: USDA postdoctoral fellowship awarded to Adrian Marshall (mentors: Northfield, Harper, and Cooper) to precisely estimate the time between acquisition to transmission for leafhoppers to better inform timing of control measures.

Other related/associated funding sources: Awarded
Funding Duration: Ongoing
Amount: $2 million per year indefinitely
Agency Name: USDA ARS congressional appropriations
Notes: Cooperative research project between USDA ARS and WSU to better understand little cherry disease (caused by X-disease phytoplasma and Little cherry virus). USDA PI: Cooper, WSU PI: Northfield.

WTFRC Collaborative Costs: $0

Budget 1
Primary PI: Tobin Northfield
Organization Name: WSU-TFREC
Contract Administrator: Anastasia Mondy
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Station Manager/Supervisor: Chad Kruger
Station manager/supervisor email address: cekruger@wsu.edu

Item	2020	2021	2022
Salaries[1]	$39,629.00	$41,214.00	$42,863.00
Benefits[2]	$4,478.00	$4,657.00	$4,844.00
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies[3]	$7,000.00	$4,000.00	$4,000.00
Travel[4]	$2,000.00	$2,000.00	$2,000.00
Plot Fees			
Miscellaneous			
Total	$53,107.00	$51,871.00	$53,707.00

Footnotes:

[1] new student position

[2] 11.3%

[3] Research consumables (e.g., cages, pots, soil), + molecular tests for disease presence

[4] In state travel

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

Budget 2
Co PI 2: W. Rodney Cooper
Organization Name: USDA-YARL
Contract Administrator: Mara Guttman
Telephone: 509-510-5619
Contract administrator email address: Mara.Guttman@usda.gov
Station Manager/Supervisor: Rodney Cooper
Station manager/supervisor email address: Rodney.Cooper@usda.gov

Item	2020	2021	2022
Salaries			
Benefits			
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies[1]	$5,293.00	$3,978.00	
Travel			
Plot Fees			
Miscellaneous			
Total	$5,293.00	$3,978.00	$0.00

Footnotes:

[1] molecular supplies for gut content analysis

Objective Recap, Goals, and Anticipated Accomplishments:

Objectives

1. Conduct oviposition tests and life cycle analysis on leafhoppers on five host plants (cherry, clover, dandelion, peach, alfalfa).

We sequenced key species-barcoding genes of *Colladonus reductus* and *Colladonus montanus* and have determined that the two "species" are highly similar for the genes sequenced, supporting a 1957 USDA bulletin (Nielsen 1957) suggesting they may be the same species or very closely related (identified as subspecies in the bulletin). Importantly, Nielson (1957) noted that what was referred to in California life history studies as *C. montanus* was actually the *reductus* subspecies, allowing us to use the detailed life cycle description provided by Severin and Klostermeyer (1950) to inform *C. m. reductus* management (Table 1). Similarly, an unpublished Oregon State University MS thesis (Marsh 1965) with Nielson as an advisor (along with Knud Swenson) shows that *C. m. reductus* overwinters as eggs in the Pacific Northwest. We find that the life cycles conducted by researchers on *C. m. reductus* and *C. geminatus* in the 1940s in California are quite similar to those conducted in Oregon in the 1950s, providing confidence in the values.

Table 1: Life cycle analysis conducted by Mervin Nielson (Nielson 1968), or Severin and Klostermeyer (Severin & Klostermeyer 1950) for *C. geminatus* and *C. montanus reductus*.

Life stage	***C. geminatus*** (days) (Nielson 1968, peach host, OR)	***C. geminatus*** (days) (Severin & Klostermeyer 1950, Celery host, CA)	***C. m. reductus*** (days) (Severin & Klostermeyer 1950, Celery host, CA)
Egg	20	17.6	14.3
1st instar larva	4.0	7.1	5
2nd instar larva	5.0	4	4.6
3rd instar larva	8.0	4.3	4.3
4th instar larva	6.0	3.5	3.6
5th instar larva	9.0	7.4	5
6th instar larva	N/A (Only 5 instars)	N/A (Only 5 instars)	7.5
Total nymph stages	32.0	26.6	27.6
Total egg, nymph	52	44.6	42
Pre-oviposition stage	8	7-13 (only range given)	13.9
Total generation time	60 days	~55.6 days	55.9 days

Despite detailed life cycle numbers, prior to this study it was unclear what plants leafhoppers feed on or how other hosts affect their growth and reproduction. In particular, due to its low abundance at the time, *C. m. reductus* was not included in the extensive *C. geminatus* study by Mervin Nielsen study in Oregon (Nielson 1968) but is typically far more abundant than *C. geminatus* in Washington orchards. Here, we originally set out to build on this research by evaluating the generation time for *C. m. reductus* and *C. geminatus* on 5 plant species: cherry, white clover (*Trifolium repens*), dandelion (*Taraxacum officinale*), peach and alfalfa. Understanding host plant use will help inform management plans. In our surveys of cherry farms in the Wenatchee and Yakima regions in this project and in the project title,

Figure 1. Nymph deformation in colony. *Example of leafhopper deformation (left) compared to healthy (right) C. m. montanus in leafhopper colonies. It is not clear what is causing these abnormalities in colonies.*

"Field evaluation of leafhopper controls for X-disease management" we rarely observed *C. geminatus*, with *C. m. reductus* being >95% of *Colladonus* spp. individuals collected by sweep nets and sticky traps, and even fewer in 2021. In response to the abundance of *C. m. reductus* and lack of knowledge, we focused our trials on this species. Furthermore, when collecting leafhoppers, we noticed they were commonly found on mallow plants, so we included mallow in our trials. In two initial attempts to start a colony of *C. m. reductus* with a diverse offering of plants (attempt 1: pea plants, clover, alfalfa; attempt 2: alfalfa, clover, mallow) the leafhoppers died as older nymphs or newly emerged adults, often with deformations (Figure 1). The cause of this mortality appears to be related to high humidity, and we now have *C. m. reductus* colonies in Wenatchee (WSU, Northfield) and Wapato (USDA ARS, Cooper) using humidity-controlled growth chambers (WSU) and rooms (USDA). The Wenatchee colony is raised on a diverse range of broadleaf weeds, and the Wapato colony is raised on celery root. Given the presence of a diverse diet in wild caught leafhoppers (according to molecular gut content analysis led by Co-PI Cooper), we have focused trials on feeding behavior, and used an oviposition test to determine the number of generations per year for *C. m. reductus*, which is unknown (2 reported for *C. geminatus* in the 1950s) and was unclear from sticky trap data. The findings from this study have also led to broadleaf-weed specific herbicide trials funded by a specialty crop block grant.

2. Evaluate incubation time and acquisition probability for leafhoppers feeding on each, cherry and peach trees and transmission likelihood to cherry, clover, dandelion, peach, and alfalfa.
In our evaluation of acquisition and transmission studies we had planned to follow the methods of previous studies (Jensen 1971, Suslow and Purcell 1982), with the addition of molecular techniques to better evaluate acquisition and transmission success. While leafhoppers have long been known to acquire X-disease from infected cherry trees, a 1951 study was unable to get *C. geminatus* to acquire X-disease in 17 symptomatic peach trees, leading to the thought that it might be a dead-end host (Nielson and Jones 1954). However, research and discussions over the course of the current study suggests these peach trees may have actually been infected with peach yellow leaf roll phytoplasma (*Candidatus* phytoplasma pyri, a.k.a. pear decline, transmitted by pear psylla), rather than X-disease, given the common misidentification at the time. Indeed, we found a study in 1950 in Wenatchee conducted by Homer Wolfe that demonstrated that X-disease vectors can readily acquire X-disease phytoplasma from peach trees at similar rates to cherry (Wolfe et al. 1951). In light of this information, we have focused on acquisition from cherry trees, due to higher number of available trees and to take advantage of trees with high pathogen titers, and the lack of need to demonstrate that acquisition from peaches can occur. To evaluate acquisition in year 2 of the project we planned to identify cherry and peach trees exhibiting X-disease symptoms during harvest, and place *C. geminatus* and *C. m. reductus* leafhoppers in sleeve cages on the diseased trees. After 1 week of feeding (the maximum time needed according to previous research) we planned to cut the branch off the tree, keeping the sleeve cage intact and place the sleeve cage and branch immediately into a cooler with ice for transport back to the WSU TFREC without allowing leafhopper escape. The leafhoppers were to be collected from cherry trees will then be transferred to greenhouse cages containing one of five potential host plants: cherry, peach, alfalfa, dandelion, or white clover, and replicated 8 times (40 total cages). Each cage was to include 3 *C. geminatus* and 3 *C. m. reductus*

leafhoppers, to focus on the potential of the plant to host the disease and allow for either leafhopper species to transfer the disease. We have adapted these methods slightly to take advantage of highly infected cherry trees in a commercial orchard, and to focus on *C. m. reductus*, given how abundant it is relative to *C. geminatus*.

3. Use molecular analysis on leafhoppers raised on different host plants to evaluate the reliability of gut content analysis to identify previous hosts of leafhoppers collected in orchards.

Research conducted by co-PI Rodney Cooper and colleagues on purple top disease in potatoes (Horton et al. 2018, Cooper et al. 2019), caused by a phytoplasma vectored by beet leafhoppers has included the development of molecular methods to identify previous plant hosts of leafhoppers collected from crops. While the methods have been focused on beet leafhoppers, rather than the *Colladonus* spp. that vector X-disease, we expected the methods to be directly applicable to identifying non-cherry plants as sources of leafhoppers. Here, we used leafhoppers arising from experiments described in objective 1 as a cost-effective evaluation of such methods for cherry-X-disease research. These data can then be used as pilot research justifying federal funding identifying alternative leafhopper hosts and their potential importance for disease transmission in cherry orchards. Thus, at the end of the life cycle analysis in year 1 we planned to send leafhoppers from the field trials to the USDA lab in Wapato for molecular analysis to identify the host plant within the insect's gut. Assuming identification success in year 1, in year 2 we planned to collect adult leafhoppers from the end of experiments and place them on cherry seedlings, raised separately for each host plant. We will then collect 5 leafhoppers from each seedling at 0, 1, 2, and 3 weeks to identify the timeframe in which the previous host plant can be detected.

Objectives timeline

Objective	Y1	Y2	Y3
1 Life history tests	x	x	
2 Transmission tests		x	x
3 Gut content analysis	x	x	

Significant Findings from this project:

- We have conducted oviposition studies on second and third generation leafhopper adults, and they readily laid eggs on broadleaf weeds and grasses. However, in each of two trials, all adults feeding on grass-only died (n = 6 cages), while adults feeding on broadleaf weeds alone or with grasses survived (n = 12 cages).
- Leafhopper eggs developing in grasses and broadleaf weeds did not survive when cut from the plant. Further studies are needed to see if this suggests mowing after oviposition reduces egg survivorship.
- We have developed and published methods for molecular gut content analysis for leafhoppers, including *C. m. reductus* and *C. geminatus* (Cooper et al. 2022).
- Field-collected *C. m. reductus* successfully transmitted X-disease phytoplasma to each, mallow (*Malva neglecta*) and alfalfa (*Medicago sativa*). These have not previously been reported as hosts. The other broadleaf plants (dandelion and white clover) did not test positive, but were less preferred feeding hosts and have been reported as phytoplasma hosts elsewhere (https://www2.ipm.ucanr.edu/agriculture/cherry/X-disease-cherry-buckskin/)
- Potted alfalfa plants infected in fall feeding trials were left outside for the winter, and tested positive for X-disease phytoplasma the following spring, suggesting they can host the phytoplasma from year to year.

- *C. m. reductus* and *C. montanus montanus* are genetically similar and may be the same species. Furthermore, literature at the time suggests that *C. montanus* in California in the 1950s was most likely *C. montanus reductus*, allowing us to use early *C. montanus* research on life histories and incubation period to inform management of *C. m. reductus*
- Of the plants included in the trials (cherry, peach, mallow, alfalfa, white clover, and dandelion), *C. m. reductus* have a strong affinity for mallow and alfalfa. Given how common these plants are in orchard groundcover, these hosts should be considered in management strategies. *C. m. reductus* may also benefit from a diverse diet, that includes tree feeding.
- Leafhopper feeding rates on cherry trees ranged from 14% to 51% of the observed feeding observations, depending the available herbaceous plants, with highest feeding when mallow was not present. Cherry feeding rates were highest when mallow was not present.
- Leafhopper feeding rates on peach trees ranged from 22% to 41% of the observed feeding, depending on the available herbaceous plants. Peach feeding was highest when mallow was not present.
- We conducted molecular gut content analysis on 5 *C. m. reductus* and 5 *C. geminatus* leafhoppers from a commercial orchard in Wapato and found all *C. m. reductus* had fed primarily on dandelion, with little else in their guts. Four of the five *C. geminatus* had fed on dandelion as well, demonstrating the importance of ground cover broadleaf weeds as leafhopper feeding hosts. Dandelion was the dominant weed at the location and time sampled. Subsequent analysis of leafhoppers over a much wider geographic range supports dandelion and mallow as an important host, amongst other common broadleaf weeds.
- In the second generation (August) leafhoppers in growth rooms deposited eggs on the underside of the leaves of cherry, mallow, and clover.
- *C. m. reductus* leafhoppers collected during the final generation (late September) laid eggs on grassy weeds in potted plants, but not broadleaf weed or trees, suggesting they may overwinter as eggs on tall grasses.

Key findings from federal funding leveraged by this project:

- Molecular gut content analysis led by co-PI Cooper continues to reveal the importance of broadleaf weeds in orchard ground cover as important X-disease vector hosts.
- Surveys led by cooperator Harper for X-disease phytoplasma hosts continue to highlight the importance of broadleaf weeds in orchard ground cover as hosts for phytoplasma
- Surveys led by PI Northfield suggest that X-disease phytoplasma transmitted in the field in October can overwinter in broadleaf weeds and be detectable in the plant the following spring.
- Ongoing broadleaf-specific herbicide trials to control leafhopper vectors in groundcover suggest promise for reducing leafhopper numbers.
- Ongoing electropenetragraph research suggest X-disease vector leafhoppers readily feed on each, phloem and xylem and it may be possible to use this technique to quantify feeding on each in a variety of plant types to determine when and where phytoplasma can be acquired.

Methods:

Feeding trials

We initiated feeding trials in 24in × 24in × 56in (w × w × h) cages with a combination of white clover, alfalfa, dandelion, mallow, Early Red Haven peach trees, and/or Bing cherry trees, with each plant in a separate pot (Figure 1). Each trial lasted 5 days and each cage contained 10-15 leafhoppers, depending on mortality after collection. In the first trial, we conducted observations every two hours from 8am to 11pm. However, leafhoppers rarely moved in the span of the two-hour intervals and did not appear active in observations made at 9pm and 11pm, which were in the dark and made with red headlamps to avoid disturbing insects. Therefore, in subsequent trials, observations were made at 8AM, 1PM, and 6PM, doing 3-minute time searches in each cage. Trials were conducted in environmentally controlled growth rooms set at 75F, with a 16:8 L:D daylength. During each observation, we counted how many leafhoppers were on each plant, what plants they were on and if actively feeding or not by visually observing stylets piercing the plant. We present data only on actively feeding leafhoppers summarized across the insects within a cage.

Figure 1 Feeding trial cages in the growth room.

The trials included the following treatments:

- 2 trials of cherry, alfalfa, clover, dandelion; each with 2 cages
  - Initiated June 11 and August 3, 2020
- 2 trials of peach, alfalfa, clover, dandelion; each with 2 cages
  - Initiated June 11 and August 3, 2020
- 1 trial of cherry, clover, mallow, dandelion; each with 2 cages
  - Initiated September 22, 2020
- 1 trial of peach, clover, mallow, dandelion; each with 2 cages
  - Initiated September 22, 2020
- 1 trial of peach, alfalfa, mallow, dandelion; each with 3 cages
  - Initiated August 22, 2020
- 1 trial of cherry, alfalfa, mallow, dandelion; each with 3 cages
  - Initiated October 6, 2020

Transmission tests

After the completion of the feeding trials, Scott Harper's team tested the plants for X-disease phytoplasma.

Field oviposition test

Based on yellow sticky card data, in the Pacific Northwest *Colladonus* species leafhoppers typically have three periods of abundance: May, late July/early August, and October. However, it is difficult to determine the number of generations per year from yellow sticky card data. This is because the October generation may be the same generation as the August generation, just moving into orchards after loss of alternative host plants. Because leafhoppers overwinter as dormant eggs, we evaluated the potential for eggs laid in field conditions in August to hatch into nymphs. Development of these eggs would then suggest that the August adults represent a distinct generation that gives rise to the adults collected in October. Therefore, during the first week of August 2020 we collected *C. m. reductus* and placed them in cages 24in × 24in × 24in mesh cages with combinations of herbaceous plants next to the

Brunner building at the WSU Tree Fruit Research and Extension Center. The cages were monitored periodically to identify the emergence of nymphs and/or adults.

Second generation oviposition test

2021 field season. Second generation oviposition trials took place in 2021 within two growth rooms under two different temperature regimes: 60°F, 30% relative humidity (RH) and 80°F, 30%RH with a 16:8 L:D daylength. Due to a growth room malfunction in the first replication, the first room fluctuated around 75-80°F with about 70% RH in the beginning. High humidity was corrected in subsequent trials by placing a dehumidifier in the rooms and set to 30%RH, but the temperature could not be corrected. This took place from July 22nd to July 29th 2021. For both temperatures, four rearing observation cages (24x24x56"; BioQuip) were set up individually with two cages with Bing cherry, two cages with Early Red Haven peach, and each with Dutch white clover (*Trifolium repens*), alfalfa (*Medicago sativa*), dandelion (*Taraxacum sp.*), and common mallow (*Malva sp.)*. Two additional cages of only clover, alfalfa, dandelion, and mallow were set up to test preference without the presence of fruit trees. Two rearing observation cages (24x24x56"; BioQuip) were placed in field conditions outside of the lab with clover, alfalfa, dandelion, and mallow as well to serve as a control. To each cage we introduced 5 male and 5 female field-collected *C. m. reductus*. Sex determination was conducted by anesthetizing them with CO2 using a modified sparkling water maker (SodaStream Inc.), and a microscope for identification. Two days were given before the start, and timed checks happened twice a day at 8-9AM and 5-6PM for 3 minutes. Leafhoppers were counted and we recorded what plants they were on, and if they were actively feeding. We made oviposition observations using the Simplified Leafhopper Egg Detection by Autofluorescence method, also known as the Blue Light Detection Method, to detect eggs within the plants (Hermann and Boll 2003; Yao et al. 2020). Using a blue LED flashlight with a 455-460nm wavelength (LEDwholesalers; Amazon) and wearing blue light blocking computer glasses (UVEX; Amazon), we scanned each plant for eggs. Plants that had eggs were recorded as well as where on the plant they were laid.

2021 Overwintering oviposition test

To see if eggs laid by the third generation that overwinter as eggs are laid in a different location, we set up an oviposition test that was similar to the "second generation" oviposition test. These trials took place from September 20th to September 24th, 2021, and then repeated again from September 28th to October 1st, 2021. The two growth rooms were maintained under two different temperatures: 70°F, 30% RH and 80dF, 30%RH with a 16:8 L:D daylength. For both temperatures in both replicates, two rearing observation cages (24 × 24 × 56"; BioQuip) were set up with two cages of Bing cherry, each with Dutch white clover (*Trifolium repens*), alfalfa (*Medicago sativa*), dandelion (*Taraxacum sp.*), and common mallow (*Malva sp.)*. Two additional cages of only clover, alfalfa, dandelion, and mallow were set up to test preference without the presence of fruit trees. Two rearing observation cages (24 × 24 × 56"; BioQuip) were placed in field conditions outside of the lab with clover, alfalfa, dandelion, and mallow as well to serve as a control. To each cage we introduced 10 females and at least 5 field-collected *C. m. reductus*. The leafhoppers that were placed in the second replication were put into the same cages as the first. Additionally, half of the cages (one with weedy hosts in the growth rooms and the field, and one with cherry in the growth rooms) were used to test a method of inducing oviposition in leafhoppers (Tipping *et al*. 2005). To do this, we placed the 10 females and around 5 males in a plastic tube with mesh secured on both ends to allow airflow and ran a hairdryer through both ends on cool for 2 minutes, flipping the side halfway through. Sex determination for both replicates was done by anesthetizing them with CO2 using a modified sparkling water maker (SodaStream Inc.), and a microscope for identification. Two days were given before the start of the first replicate, and checks happened once a day over a 5-day period for however long was needed for a thorough search of the plants (around 5-10 minutes). For the second replicate we allowed 24 hours for leafhopper acclimation before observations were initiated,

which included one check for the same amount of time, and halfway through, barley was added for additional observations. Egg detection was conducted using the Blue Light Detection (Simplified Leafhopper Egg Detection by Autofluorescence) method by using a blue LED flashlight (LEDwholesalers; Amazon) and wearing blue light blocking computer glasses (UVEX; Amazon) (Herrmann and Boll 2003; Yao et al. 2020). Plants were scanned for eggs using this method and plants with eggs were recorded.

2022 field season

We conducted the same experiment from August 9-12th, from September 1-9, and from October 28 – November 7 2022. For each experiment, we set up a total of 9 cages to evaluate oviposition in 3 treatments: broadleaf plants only (2 pots of alfalfa, 2 pots of clover), broadleaf plants and grasses (1 pot with alfalfa or clover, 1 pot containing perennial ryegrass or creeping red fescue), or grass only (2 pots containing perennial rye grass and two creeping red fescue), with 3 replicates per treatment. The key difference between the August and September experiments is that in the August experiment we realized there was too much plant material to search for eggs, making them difficult to find. Therefore, in the September and October experiments, we switched to seedling trays to reduce the amount of plant material in cages and improve egg identification. Cages were kept in a growth room set to 16:8h Light:Dark, 70°F (21°C), and 30%RH (controlled with a dehumidifier). To each cage, we added 5 females, field-collected *C. m. reductus* leafhoppers and began observations same day. We searched for eggs daily over 7 days, scanning each plant using a blue light and blue light filtering classes to find eggs. On each day, we removed plant material containing eggs when found and place in small deli cup on top of soil labeled by cage number and what plant it was found on. Because the blue light detection method can also confuse thrips feeding with eggs, we confirmed eggs in plant material under microscope. For each egg, we recorded the number of eggs, when they were found, and what plant they were on. Deli cups with eggs were kept in the same growth room that cages were in previously (16:8 L:D, 70°F (21°C), 30%RH) to monitor for nymph emergence. We also monitored each plant in separate cages and checked for nymph emergence after observations were finished.

Gut content analysis

C. reductus and *C. geminatus* were collected from orchards *C. geminatus* and *C. reductus* were collected in a commercial cherry orchard near Wapato, WA on 22 May 2020 from herbaceous ground cover between orchard rows using a sweep net and stored in -80C freezer for processing. Methods for molecular gut content analysis were similar to those previously described for psyllids (Cooper et al. 2019). Briefly, DNA was extracted from leafhoppers using a commercial kit, and plant DNA was amplified by polymerase chain reaction. The plant DNA amplicons were then sequenced and used to identify the dietary history of leafhoppers.

Results & Discussion:

Feeding trials. We observed active feeding on all plants offered during the feeding trials (Figure 3). In the feeding trials that included cherry trees, the order of *C. m. reductus* preference appeared to be: mallow, alfalfa, cherry, white clover, and dandelion. Indeed, when offered mallow, alfalfa and a cherry tree we did not observe feeding on dandelion. In the feeding trials that included peach trees, the order of preference

appeared to be: mallow, alfalfa, peach, white clover, and dandelion. However, interestingly, when offered mallow, alfalfa and peach together they fed more on peach than alfalfa. The fact that leafhoppers always fed on cherry or peach trees, regardless of what herbaceous plants were there begs the question of whether there is something important about feeding on trees that provide important nutrients to leafhoppers. However, future research is needed to determine whether this is the case.

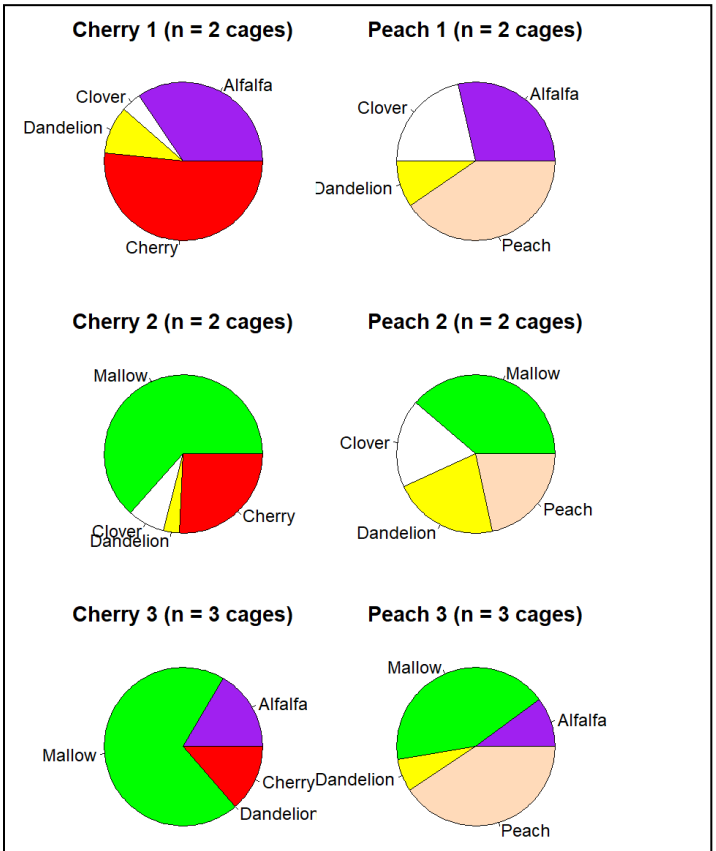

Figure 3 *Proportion of feeding observations made on each of the different plant species in cages: cherry (red), white clover (white), dandelion (yellow), alfalfa (purple), peach (peach), or mallow (green) during feeding observations.*

Field oviposition tests: Adult *C. m. reductus* leafhoppers collected in the first week of August and introduced to outside cages with mallow and clover readily laid eggs that hatched into nymphs and began reaching the adult stage in October, suggesting that the August generation is a separate generation from the first generation that emerges in May from overwintering eggs and from the October generation that lays eggs that remain dormant for the winter. Given that these two later generations typically occur after cherry harvest, leafhopper control after harvest is likely critically important.

Transmission test

Of the plants from the feeding trials, alfalfa and mallow tested positive for X-disease phytoplasma following the experiment. One of the two alfalfa plants tested positive with a Ct score of 36.82, and two of the three mallow plants tested positive with Ct scores of 38.71 and 38.29. In addition, one alfalfa, one mallow, and one dandelion plant was kept outside all winter and tested again the following April to see if the phytoplasma could survive the winter in the roots. Of those, the alfalfa tested positive with a 39.31 Ct score. Therefore, we found that alfalfa and mallow can host X-disease, and that it can survive the winter in broadleaf roots.

Second generation oviposition test

2021 experiment. During this experiment, most of the leafhoppers died within the acclimation period so there were fewer feeding results. From the data collected, there were no records of feeding on dandelion, cherry, or peach, but they did feed on (in order of preference) clover, mallow, and alfalfa. In the cages without a fruit tree, there were more observations on clover than there were on mallow, alfalfa, and dandelion. Overall, the feeding proportion for clover was 53%, for mallow was 37%, for alfalfa was 11%, with no feeding observations on either dandelion and cherry/peach. During this experiment we were able to find some eggs deposited in the first growth room (75-80°F conditions) despite the lack of feeding data. In the cages that had fruit trees, we found eggs deposited on the underside of the leaves of mallow, and clover, and did not observe any eggs on alfalfa and dandelion. Although no eggs were found in the cages without fruit trees, we were able to observe young instar nymphs on clover, as well as other nymphs on cherry, mallow, and clover within the fruit tree cages. Due to lack of leafhoppers in the field by the end of this experiment (being at the end of the second generation), we were not able to replicate this experiment before the final generation began.

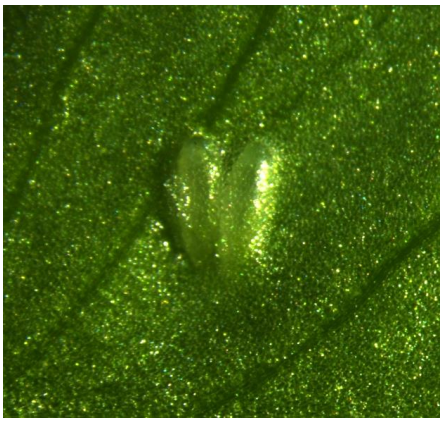

Figure 4. Leafhopper eggs in alfalfa (approximately 1mm long)

2021 Final generation oviposition test. No eggs were laid in any of the broadleaf plants in any of the cages. However, one of the pots with the dandelion plants had grass growing from the pots and we found 100 leafhopper eggs deposited in the grass. While it was a single cage that had eggs deposited, it suggests that leafhoppers may lay eggs in grass in the final generation to overwinter. This would make logical sense, given that they may be more likely to persist throughout the winter than tree leaves, or even leaves on perennial broadleaf weeds. With this observation on grass, barley was added halfway through the experiment, finding that they would feed on the barley, but no eggs were laid on it in any of the cages.

2022 experiments. In the August experiment, no eggs were found using the blue light detection method. At the end of the observation period, all plants were taken out of the cages and searched for eggs. The first set of plants were sorted through for about 1 hour and found 4 total eggs (cage B1: 1 clover, 2 alfalfa; cage BG2: 1 clover). Due to the large amount of plant material to sort through, egg searching was reduced to 15 minutes per plant, and no other eggs were found. Plants were then separated into individual cages and checked for nymph emergence, but no nymphs were found. This could be because the nymphs were too small to observe, the plants died before finding nymphs, or no/very few eggs were actually laid.

In the September experiment, plants were planted in seedling trays together for each cage to reduce amount of plant material needed to go through. Eggs were first found on 9/6, mostly with plain sight. On the last day, all trays were removed, and plants were sorted through to find eggs with no time restrictions. Overall, 48 eggs were found on barley, 28 on perennial ryegrass, 20 on creeping red fescue, 5 on clover, and 2 on alfalfa. A majority of the eggs were found in the cages that had only grass in them, and the eggs found in the broadleaves were found in the mixed broadleaf/grass cage. At the end of the trial, it was noticed that the leafhoppers in the grass cages had all died, and the leafhoppers in the cages with broadleaves had mostly survived. In our October/November trial we were more careful to document adult survivorship over the course of the experiment. When summed across the treatments, we observed 61 eggs laid on clover, 89 eggs laid on alfalfa, 69 eggs on perennial rye grass, 36 laid on red fescue, and 60 eggs laid on barley. However, all of the adults in the three grass-only cages died by the end of the 9-day experiment. In contrast, all cages in the treatments that included broadleaf weeds had live adults at the end of the experiment, with an average of 3.3 adults in the broadleaf only cages, and an average of 2.3 adults in the broadleaf-grass treatment cages. This consistent mortality in grass-only treatments suggests

that *C. m. reductus* relies on broad-leafed weeds as feeding hosts and only uses grass as reproductive hosts. In the September trial all of the eggs removed by cutting leaves from plants, but none of them emerged, and either developed partially (exhibiting eye spots in developing nymphs) before dying or had eye spots beginning to form.

Gut content analysis:

We conducted gut content analysis on 5 *C. m. reductus* and 5 *C. geminatus* collected on May 22, 2020 from our control blocks in the WTFRC project "Field evaluation of leafhopper controls for X-disease management." The block had many dandelions in the ground cover, and dandelion comprised the vast majority of plant DNA in all five *C. m. reductus*. Dandelion species included both common, and red-seeded dandelion. We did not detect cherry in the guts, but did identify small amounts of clover, alfalfa, and chickweed. Dandelion also dominated the plant species within guts of *C. geminatus* but they also had a more diverse group of plants in their guts, including mallow, chickweed, an *Oxybasis* species, and cherry. These findings support the feeding trial data that ground cover is an important part of the diet of these key X-disease vectors. These results will be built on by a WSDA/USDA Specialty Crop Block Grant to Scott Harper (PI), Tobin Northfield (co-PI), Rodney Cooper (co-PI), and Tianna DuPont (co-PI) that includes gut content analysis for known vectors. Furthermore, these results are documented in a recently published scientific publication describing methods for leafhopper gut content analysis (Cooper et al. 2022).

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EXECUTIVE SUMMARY

Title: Identifying sources of X disease in cherry orchards

Keywords: X-disease, *Colladonus montanus reductus*, leafhoppers

Abstract:
At the start of this project it was unclear where the X-disease vectors reproduced, or for *Colladonus montanus reductus* basic life history information or the number of generations of this key X-disease vector. Over the course of the study, we identified broadleaf weeds as a key reproductive host for *C. m. reductus*, identifying management of X-disease vectors within orchard ground covers as a key part of management. In feeding trials, *C. m. reductus* fed extensively on mallow and alfalfa, as well as potted peach and cherries trees. In oviposition trials, *C. m. reductus* readily laid eggs on broadleaf weeds and grass, but only survived when there was access to broadleaf weeds. Preliminary molecular gut content analysis results support the importance of broadleaf weeds as feeding hosts for X-disease vectors. Furthermore, we found that X-disease phytoplasma can survive the winter within broadleaf weeds, and that there are 3 generations of the key X-disease vector in the Pacific Northwest, *C. m. reductus*. Molecular analysis and further literature review provided detailed life cycle data on this species, conducted previously in California, and identification of eggs as the overwintering stage. This project provided preliminary data for a wide range of federally funded projects that 1) identified broadleaf weeds in orchard ground cover as commonly hosting X-disease phytoplasma, 2) used molecular gut content to demonstrate broadleaf weeds as common leafhopper hosts, 3) used molecular tests to identify multiple X-disease phytoplasma genotypes, 4) evaluated broadleaf-specific herbicide as a management strategy, 5) evaluated entomopathogenic fungi sprayed in groundcover to kill nymphs feeding on broadleaf weeds, 6) seasonal sampling of weeds, leafhoppers, and trees to better understand phenology of phytoplasma within host plants to determine the time points in which it can be acquired, and 7) use of electrical penetration graphs, which thus far has provided detailed information on leafhopper feeding and suggests that X-disease vectors may feed on each, xylem and phloem. This last project is important, as better distinguishing xylem versus phloem feeding using this technique may distinguish plants on which they phloem feed and potentially transmit X-disease phytoplasma from xylem feeding where water is accessed, but transmission cannot occur.

Project Title: X-disease Vector Identification and Acquisition From Low Titer Trees

Report Type: Final Project Report

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Cooperators: Rodney Cooper, Louis Nottingham, Garrett Bishop

Project Duration: 2-Year, no-cost extension

Total Project Request for Year 1 Funding: $ 55,266
Total Project Request for Year 2 Funding: $ 55,304
Total Project Request for Year 3 Funding: $0

Other related/associated funding sources: Awarded
Funding Duration: 2020 - 2022
Amount: $249,360
Agency Name: WSDA/USDA Specialty Crop Block Grant
Notes: USDA SCBG funding to identify strains of phytoplasma in cherries and other stone fruit as well as weedy plants, and to conduct molecular gut content analysis on X-disease vectors. PI: Harper, co-PI's: Northfield, Cooper, DuPont

Other related/associated funding sources: Awarded
Funding Duration: 2021 - 2023
Amount: $244,750
Agency Name: WSDA/USDA Specialty Crop Block Grant
Notes: USDA SCBG funding to evaluate selective broadleaf herbicides as a management option for X-disease vectors. PI: Northfield, co-PI: Harper.

Other related/associated funding sources: Awarded
Funding Duration: 2022 - 2024
Amount: $295,376
Agency Name: USDA Crop Protection and Pest Management
Notes: USDA funding to develop phenology models for phytoplasma prevalence in plants and vectors to integrate into phenology models for leafhopper abundance (WTFRC project led by Nottingham). PI: Northfield, co-PI: Nottingham (WSU), Harper (WSU), Adams (OSU), Galimba (OSU).

Other related/associated funding sources: Awarded
Funding Duration: 2021 - 2023
Amount: $164,765
Agency Name: USDA AFRI
Notes: USDA postdoctoral fellowship awarded to Adrian Marshall (mentors: Northfield, Harper, and Cooper) to precisely estimate the time between acquisition to transmission for leafhoppers to better inform timing of control measures.

Other related/associated funding sources: Awarded
Funding Duration: Ongoing
Amount: $2 million per year
Agency Name: USDA ARS congressional funding
Notes: Cooperative research project between USDA ARS and WSU to better understand little cherry disease (caused by X-disease phytoplasma and Little cherry virus). PI: Cooper, co-PIs: Northfield, others.

Other related/associated funding sources: Awarded
Funding Duration: 2021-2022
Amount: $40,000
Agency Name: WSU BioAg grant
Notes: Coordinate efforts with this project to test X-disease vectors for three potential biological control agents: a parasitic fly, a parasitic wasp, and an entomopathogenic fungus using molecular methods. This collection, by Cesar Reyes Corral has been conducted alongside the collection in this grant to share resources and gain synergistic insights. PI: Northfield, co-PIs: Harper, Cooper.

WTFRC Collaborative Costs: None

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Item	2021	2022	No-cost extension
Salaries	$28,260.00	$29,390.00	
Benefits	$10,206.00	$10,614.00	
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies	$13,362.00	$11,862.00	
Travel	$3,438.00	$3,438.00	
Plot Fees			
Miscellaneous			
Total	$55,266.00	$55,304.00	$0.00

Footnotes:
[1] New postdoctoral researcher position (50% FTE)
[2] 36.1% (postdoctoral researcher)
[3] Fieldwork consumables, X disease tests, and extension supplies
[4] Domestic travel for research and extension

Objectives

1) Evaluate leafhoppers as potential X-disease phytoplasma vectors.

While all known leafhopper vectors of X-disease phytoplasma are in the subfamily Deltocephalinae, worldwide there are 6,683 species in the subfamily (Zahniser and Dietrich 2013), and more than 20,000 species of leafhoppers across the 30 leafhopper subfamilies. Therefore, we will narrow down our search to leafhoppers that commonly occur in Pacific Northwest cherry orchards, to limit the number of leafhoppers tested. As part of our Specialty Crops Block Grant project we will conduct surveys of phytoplasma strains in leafhoppers, and here we will pair these surveys with molecular analyses of salivary glands to evaluate phytoplasma presence. We will conduct surveys of ground cover and cherry trees using sweep nets and insect vacuums (D-vacs), as each method may collect different leafhoppers better (Purcell and Elkinton 1980). Sampling trees and ground cover will account for our findings that leafhoppers often spend a great deal of time in the groundcover, but regularly move into the trees to feed (TD Northfield, personal observation). We will sample from 20 orchard blocks in each period of leafhopper abundance: May/June, and August/September. During the two-year survey period, a total of 1000 (non-*Colladonus*) leafhoppers feeding on groundcover and surrounding extra-orchard vegetation will be screened for phytoplasma presence, and if found to be positive, the phytoplasma will be genotyped. Because many of the leafhoppers will test negative, we will combine 10 leafhoppers of the same species into one sample, such that if a single leafhopper carries the phytoplasma the entire sample will be identified as positive. It is possible that non-vector leafhoppers have fed on the phytoplasma, but the phytoplasma is not able to make it through the leafhopper gut and to the salivary glands to be transmitted during feeding. Therefore, we will dissect and evaluate the presence of the phytoplasma only in the salivary glands to determine which leafhoppers have the ability to transmit rather than just acquire the phytoplasma.

2) Assess potential for vectors to acquire X-disease phytoplasma from trees with low titer levels.

To evaluate the effects of low titer levels on acquisition rates of X-disease phytoplasma, we will capitalize on within-season and between tree variation in X-disease phytoplasma titer (i.e. concentration) levels. Co-PI Harper's research suggests that phytoplasma titers increase over the course of the year. Therefore, we will place 5 phytoplasma-free *Colladonus* sp. leafhoppers from a laboratory colony in a sleeve cage on known X-disease infected trees at three periods: April (low phytoplasma levels), July (high phytoplasma levels), and September (lower phytoplasma levels). After 1 week of allowing the leafhoppers to feed on the branch, we will store the leafhoppers for molecular phytoplasma detection, and use qPCR to evaluate titer level within the branch. This molecular measure of phytoplasma titer level will allow us to ensure that we do have seasonal differences over the course of the year, as well as evaluate the effect of variation between trees within a given time point on the acquisition of phytoplasma by the leafhopper. We will set up 10 leafhopper sleeve cages at each time point, for a total of 30 sleeve cages and 150 leafhoppers per year. To analyze the data we will conduct a regression of phytoplasma titers (combining all data points) and acquisition rate.

Note: Due to problems rearing leafhoppers in colony to ensure uninfected leafhoppers to evaluate these tests, we were unable to conduct these experiments in 2021. Initial trials in 2022 were also unsuccessful in leading to acquisition, and therefore, we changed our methods for 2023.

3) Develop a website at treefruit.wsu.edu updating the list of known leafhopper vector status, organized by subfamily.

A gallery of leafhoppers will be created, which will list vector status on the treefruit.wsu.edu website. High quality images will be taken of leafhopper species screened using microscope camera and 2x macro-lens with image stitching technology. Images will also be obtained from existing resources. Images will be marked with a easy to read symbol to indicate vector status. The gallery will be organized by subfamily in order for viewers to be able to view the relationship between leafhoppers with known vector status and leafhoppers with negative vector status. For each leafhopper a description will be

included which designates vector status and other relevant details. Untested common leafhoppers will also be included prior to testing.

Significant findings

- 82% of the leafhoppers collected from our 22 sites in August 2021 were not *Colladonus* spp. leafhoppers
- We found no evidence that any other leafhoppers were transmitting X-disease phytoplasma. In contrast, we did identify *C. m. reductus* and *C. geminatus* collected from the same sites with high X-disease phytoplasma titers in their salivary glands/mouthparts.
- *Euscelidius variegatus* can transmit X-disease in the laboratory, but with a ~50% longer latency period than *Colladonus* species. Here we tested more than 300 *E. variegatus* over the course of two seasons and never found evidence that X-disease phytoplasma was replicating in their mouthparts.
- In the first generation (May/June) some *Colladonus* spp. (*C. m. reductus* and *C. geminatus*) leafhoppers had X-disease phytoplasma in their guts during the first generation, but none had phytoplasma in their heads. This suggests they can acquire phytoplasma in the first generation, but the long latency prohibits transmission.
- The likelihood that a *Colladonus* spp. leafhopper had X-disease phytoplasma replicating in its mouthparts was highest in the second (August) and third generation (October).
- Evaluation of X-disease acquisition with highly infected cherry trees for 3 days led to short latency periods, suggesting that the latency period is dose dependent.

Methods

1) Evaluate leafhoppers as potential X-disease phytoplasma vectors.

2021 Sampling. We collected leafhoppers from 22 sites from 8 different Central Washington orchards, ranging widely in management regime from the Wenatchee, Yakima, and Mattawa regions. We also collected from another site in Pasco, but did not find any leafhoppers. We collected the leafhoppers by sweep netting during the peak of the second generation of leafhoppers to determine leafhopper abundance and species composition. We targeted three time periods each focusing on a different generation of *Colladonus* adults (May 18-26, August 4-17, and September 13-October 7) to focus on the time when phytoplasma titers are highest, providing the greatest change of collecting phytoplasma in leafhopper salivary glands. Samples consisted of 20 sweeps in 10 rows at each site (200 sweeps per site). The contents of the sweeps were transferred to mesh bags and brought back to the lab for sorting and recording by species (*C. m.. reductus*, *C. geminatus*, *Scaphytopius acutus*, other leafhoppers, and nymphs). Other leafhoppers primarily consisted of a small unidentified brown species, and the larger *Euscelidius variegatus*. We primarily targeted cherry and stone fruit blocks, but we also included apple blocks in the vicinity of cherry/stone fruit blocks to broaden the range of leafhoppers we could find.

2022 Sampling. In the 2022 field season we expanded our range to 30 sites for generation 1, and 24 sites in *C. m. reductus* generations 2 and 3. For generations 2 and 3 we prioritized the 22 locations that had higher leafhopper numbers in our first round of sampling and added in 2 sites in Tonasket to increase our sample range. Compared to 2021 sampling in 2022 we were able to obtain more information, targeting blocks with high X-disease prevalence, and reaching farther north, to include 2 sites in the Chelan region, 2 sites in the Omak region, and 2 sites in the Tonasket region. We also included 6 sites in the Cashmere to Rock Island region, one in Mattawa, and the rest in the corridor from Yakima to Pasco. In 2022, because we were better at identifying *E. variegatus*, we tested them individually, analyzing entire bodies and heads together.

2) Assess potential for vectors to acquire X-disease phytoplasma from trees with low titer levels.

To develop methods, on Aug 1, 2022 branches were collected from an X-disease-infected cherry tree (CT 28 [tested in 2021], equivalent to less than 5,293 phytoplasma cells/sample) in the Rock Island

area. Branches were returned to the lab and placed in a deli cup with water in a mesh cage with an infected (CT 35, equivalent to less than 51 phytoplasma cells/ sample) dandelion plant in the greenhouse. The next day, we collected *C. m. reductus* leafhoppers from an apple block. Leafhoppers were sorted at the lab and all adults were placed in the mesh cage with the infected plant material. After one week of feeding, we collected the remaining alive adults (11) and transferred them into ethanol and whole bodies were tested by qPCR for X-disease phytoplasma. However, no X-disease phytoplasma was detected in any of the leafhoppers.

2023 study. On August 1st 2023, we evaluated X-disease acquisition using colony *C. m. reductus* leafhoppers. A test of 29 of the colony leafhoppers found no X-disease presence in the colony. We collected X-infected cherry branches from trees that were highly infected in a block in the Yakima region and placed the branches in three cages with colony *C. m. reductus*. Infected branches were removed after 3 days and replaced with clean celery root, and the branches were tested for X-disease. After 3 days of feeding on celery root, 10 *C. m. reductus* were collected, dissected in half, and tested for X-disease using qPCR. A second group of 12 *C. m. reductus* were collected, dissected, extracted, and tested for X-disease ten days after switching to celery root. Finally, a third group of 15 leafhoppers was collected 17 days post feeding and were dissected, extracted, and tested.

3) Develop a website at treefruit.wsu.edu updating the list of known leafhopper vector status, organized by subfamily.
We are using a microscope with a camera attachment to carefully photograph each type of leafhopper being evaluated.

Results and Discussion

1) Evaluate leafhoppers as potential X-disease phytoplasma vectors.

Of the 1844 adult leafhoppers collected from the 22 sites in August 2021, 257 (14%) were *C. m. reductus*, 4 were *C. geminatus*, and 64 (3.5%) were *Scaphytopius acutus*. The remaining were a combination of *Euscelidius variegatus* and a diverse group of brown colored leafhoppers that resemble *E. variegatus*, but appear to be different species (Figure 1). In addition, we collected 406 nymphs for which the species is unknown. We have extracted DNA from the "other" species to determine phytoplasma presence. Because *E. variegatus* has proven to be a vector in laboratory experiments, but has a 50% longer incubation period than *Colladonus* species (Jensen 1969), we will also measure the proportion of *E. variegatus* that have phytoplasma in their salivary glands. We have also been using a microscope with a camera attachment to take photographs of the various leafhoppers that we are testing so they can be shared in a webpage. We present some of those photographs in Figures 2 and 3.

2021 Sampling. In 2021 we tested 28 groups of 10 *E. variegatus* (280 total whole-bodies tested) and never detected X-disease phytoplasma from them. We also tested 6 other leafhopper species that look similar to *E. variegatus* and never found X-disease phytoplasma in their heads. These were tested in 5, 6, 5, 34, 2, 3, 1, and 1 groups of 10, respectively. All batches of leafhoppers tested included the same species from the same site. We also tested 17 groups of 10 green leafhoppers (comprising 4 species) and found no distinguishable X-disease.

For other known X-disease vectors (*C. m. reductus*, *C. geminatus*, and *S. acutus*), we tested them individually, evaluating their heads and guts. In some cases, we identified very low titers (*Ct* scores > 38, or less than 7 phytoplasma cells per sample) in the heads with no phytoplasma present in the rest of the body, likely representing a tiny amount of phytoplasma passing through the mouthparts. Four out of the 332 (1.2%) *C. m. reductus* heads tested positive, with *Ct* scores 35 or less (equivalent to less than 51 phytoplasma cells/ sample), all of which had positive tests for the bodies too, suggesting phytoplasma had successfully integrated through the body. 15 of the 332 *C. m. reductus* leafhoppers had *Ct* scores greater than 35 (equivalent to 51or more phytoplasma cells per sample), mostly with the body testing negative, suggesting they were not infective. All *C. m. reductus* that tested positive were collected in August and

October, with none collected in May or June coming back positive. The other key vector, *C. geminatus* was rarely collected, and the mouthparts of two of the 38 collected tested positive with *Ct* scores less than 35 (51 or more phytoplasma cells per sample). Again, these leafhoppers had phytoplasma in their bodies as well, whereas the two other leafhoppers with mouthpart *Ct* scores greater than 35 did not. We also collected and tested 42 *S. acutus*, which is a known vector, but no leafhopper heads tested positive with a *Ct* score of 35 or less.

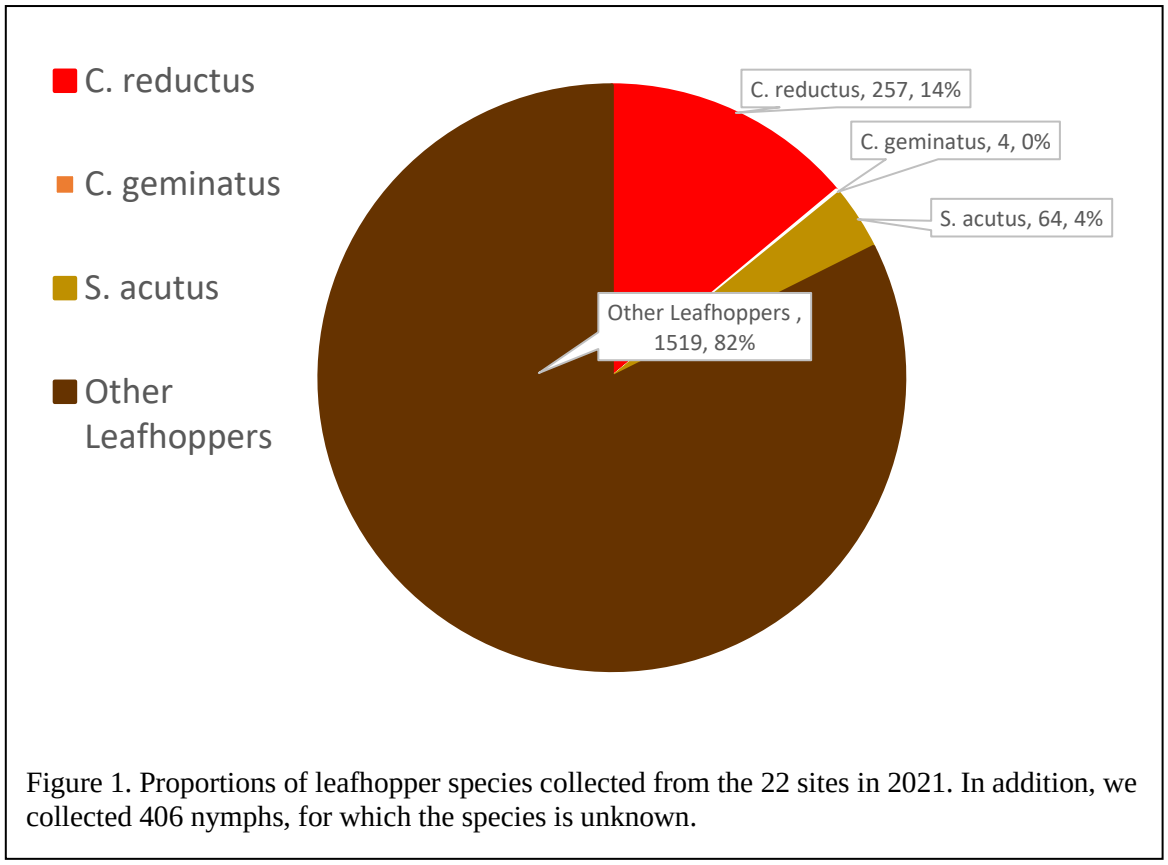

Figure 1. Proportions of leafhopper species collected from the 22 sites in 2021. In addition, we collected 406 nymphs, for which the species is unknown.

2022 Sampling. Interestingly, we found very few *Colladonus* spp. leafhoppers in northern growing regions, with only a few *C. geminatus*, and 1 *C. m. reductus* across our 6 sites in Chelan, Omak, and Tonasket. In the first generation 18% (13/71) of the *C. m. reductus* bodies and 5% (2/44) of *C. geminatus* bodies tested positive, but none appeared to be actively transmitting (high titers in mouthparts). In the second generation 9% (7/75) of the *C. m. reductus* bodies and 0% (0/6) *C. geminatus* bodies tested positive. Only 1 of the 75 tested *C. m. reductus* had high titers in the mouthparts, suggesting it was actively transmitting phytoplasma. In the third generation 25% (13/53) of the *C. m. reductus* bodies and 24% (4/17) of *C. geminatus* bodies tested positive, with only 1 *C. m. reductus* and no *C. geminatus* having high titers in the mouthparts. The low detection in mouthparts in 2022 may be influenced by the cold spring weather that year.

In the first generation, 2 of the 55 *E. variegatus* bodies tested positive for X-disease phytoplasma with *Ct* scores less than 35 (51 or more phytoplasma cells per sample), but the phytoplasma was not present in their mouthparts. We have continued to evaluate all collected leafhoppers, including 2 other brown species and 3 green species of leafhopper, but none of these have tested positive for X-disease phytoplasma. We also detected high levels of phytoplasma in the body of a treehopper at a site with highly infected trees, but no phytoplasma in the mouthparts, suggesting it was not able to transmit. In addition, because we did not find white apple leafhoppers in our cherry or stone fruit blocks, we placed white apple leafhoppers on

infected trees to see if they could acquire X-disease. However, all leafhoppers died within a matter of hours, suggesting they starved and did not feed on the cherry trees.

Figure 2. Variability in *E. variegatus* collected. Research on Italian populations suggested pigmentation can vary with sex and infection with other phytoplasmas (Galetto et al. 2018)

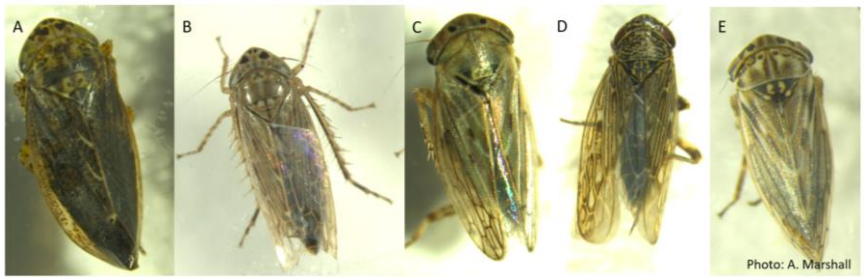

Figure 3. *E. variegatus* (A), and four other types of leafhoppers commonly collected in surveys. Leafhoppers are shown in order of abundance, with A being the highest abundance, and E being the least abundant. Note especially variation in the head shape and patterns, as well as the pronotum (the pirate hat-shape just behind the head). Pictures are not to scale: *E. variegatus* is larger than the other four leafhoppers.

2) Assess potential for vectors to acquire X-disease phytoplasma from trees with low titer levels.

Preliminary evaluations of adult leafhoppers acquiring X-disease phytoplasma from low titer trees in 2022 proved unsuccessful (all leafhoppers tested negative), and we retooled our approach to evaluating acquisition in 2023.

When caging leafhoppers to highly infected cherry branches (CT 22, equivalent to approximately 279,000 phytoplasma cells/sample) for 3 days, we found much shorter latency periods than previously reported, suggesting that the latency period may be dose-dependent. At 6 days post initial access (3 days post switch to celery root) 50% of leafhopper bodies tested positive with low titers and no heads tested

positive, showing they acquired the phytoplasma but were not transmissive. At 13 days post access (10 days post switch) ~25% of leafhopper bodies tested positive with high titers and ~20% of leafhopper heads tested positive with low titers. This demonstrates accumulation and replication of phytoplasma in leafhopper gut and the beginning of colonization of salivary glands and possible transmission. This was supported by the finding that all 3 celery roots had a positive testing leaf; however, with very low titers. At 20 days post access (17 days post switch) 50% of leafhopper bodies tested positive with high titers, and 50% of leafhopper heads tested positive with high titers. At this point the phytoplasma appeared to have colonized and replicated in the leafhoppers gut and salivary glands and they have become fully transmissive. This was shown by all three celery roots testing positive with slightly higher titers, and one leaf in particle having extremely high titers. In conclusion, if given only access to a high titer plant for 3 days, *C. m. reductus* can acquire and begin transmitting two weeks after initial access with a high likelihood of transmitting after 20 days post initial access. Now that the methods are worked out these tests need to be conducted with varying titers to determine the effect on acquisition and transmission time.

3) Photos and website development. A gallery of leafhoppers known to transmit X-disease was developed at https://treefruit.wsu.edu/vector-gallery/ This gallery contains images of the seven known vectors for X-disease phytoplasma.

A gallery of known leafhopper vectors and non-vectors has also been developed as part of a downloadable App for Iphone and Android. The app can be previewed here https://littlecherryguide.treefruit.wsu.edu/leafhopper-vectors/ The App is available in both English and Spanish. In 2023 leafhoppers tested as negative for X-disease phytoplasma vectors were added to the gallery. Currently 18 examples of non-vectors are included which allows growers to see some of the diversity of leafhoppers that are easily confused with vectors.

Figure 4. Examples of photos from the vector gallery of *C. geminatus* (top) and *C. m. reductus* (bottom).

A summary of known vector information was also updated at the main X-disease page https://treefruit.wsu.edu/crop-protection/disease-management/western-x/ receiving 1703 pageviews from 1032 individuals over the past year. We have used the app to train growers and consultants at field days in July 2022 and 2023 and September 2022. In 2023 we also distributed 50 business-card sized leafhopper identification cards with a QR code leading to the app for further information.

References

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EXECUTIVE SUMMARY

Project Title: X-disease Vector Identification and Acquisition From Low Titer Trees

Keywords: X-disease, leafhoppers, vectors

A large part of X-disease management has been focused on controlling X-disease vectors. In the 1950s in Wenatchee, WA Homer Wolfe tested the vector capacity of 200 species of leafhoppers before identifying *Colladonus geminatus* as the key vector in Washington, and later *Colladonus montanus reductus* was identified as a key vector in California. However, given the time that has past since that research, it is important to search again for unknown vectors. In our survey of 22 (in 2021) and 30 (in 2022) sites throughout the Washington cherry growing region, the vast majority of leafhoppers were not *Colladonus* spp. leafhoppers. However, we found no evidence of any unknown vectors transmitting X-disease phytoplasma. Previous research has demonstrated that *Euscelidius variegatus* can transmit X-disease in the laboratory, but with a longer latency period than *Colladonus* species. Here we tested more than 300 *E. variegatus* over the course of two seasons and never found evidence that X-disease phytoplasma was replicating in their mouthparts. In the first generation (May/June) some *Colladonus* spp. (*C. m. reductus* and *C. geminatus*) leafhoppers had X-disease phytoplasma in their guts, but none had phytoplasma in their heads. This suggests they can acquire phytoplasma in the first generation, but the long latency prohibits transmission then. The likelihood that a *Colladonus* spp. leafhopper had X-disease phytoplasma replicating in its mouthparts was highest in the second (August) and third generation (October). Evaluation of X-disease acquisition by leafhoppers feeding on highly infected cherry trees for 3 days led to shorter time to transmission than previously reported, suggesting that the latency period is dose dependent. Further analysis of leafhoppers feeding on plants varying in phytoplasma titer and for different time periods will help to better understand the latency period in different scenarios.

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

Report Type: Continuing Project Report

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Project Duration: 3 Year

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

Other related/associated funding sources: Awarded
Funding Duration: 2020–2023
Amount: $36,000
Agency Name: Corteva Agriscience, Gowan Co, Brandt Co., Nichino America, Kemin Industries**,** GroPro

Notes: Insecticide trials contribute costs for travel to field sites, 10% balance in postdoc's salary and other nominal expenditures.

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

Budget 1
Primary PI: Dr. Robert Orpet
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Station Manager/Supervisor: Chad Kruger
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Item	2022	2023	2024
Salaries	$47,727.00	$49,636.00	$51,621.00
Benefits	$17,498.00	$18,198.00	$18,926.00
Wages	$5,760.00	$5,990.00	$6,230.00
Benefits	$551.00	$573.00	$596.00
RCA Room Rental	$0.00	$0.00	$0.00
Shipping	$0.00	$0.00	$0.00
Supplies	$0.00	$0.00	$0.00
Travel	$0.00	$0.00	$0.00
Plot Fees	$0.00	$0.00	$0.00
Miscellaneous	$0.00	$0.00	$0.00
Total	$71,536.00	$74,397.00	$77,373.00

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

Budget 2
Co PI 4: Dr. Christopher Adams
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Item	2022	2023	2024
Salaries	$0.00	$0.00	$0.00
Benefits	$0.00	$0.00	$0.00
Wages	$5,760.00	$5,875.00	$5,993.00
Benefits	$634.00	$646.00	$659.00
RCA Room Rental	$0.00	$0.00	$0.00
Shipping	$0.00	$0.00	$0.00
Supplies	$0.00	$0.00	$0.00
Travel	$0.00	$0.00	$0.00
Plot Fees	$0.00	$0.00	$0.00
Miscellaneous	$0.00	$0.00	$0.00
Total	$6,394.00	$6,521.00	$6,652.00

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

Objectives

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

Deviations: Scouting occurred weekly in 2022 and 2023.

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

Deviations: None.

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

Deviations: Phenology models will be drafted and validated in 2024 at the earliest, so commercial timing tests facilitated by the research team in commercial orchards are unlikely.

Significant Findings

- Of the three potential X-disease vector leafhopper species (*Colladonus reductus, Colladonus geminatus,* and *Euscelidius variegatus*), the species *C. reductus* was usually most abundant with sticky card monitoring in 2022 and 2023. The species *E. variegatus* was sometimes more abundant, particularly at Oregon sites.
- With vacuum sampling, *E. variegatus* was the most abundant of the three species in Oregon orchards, but Washington vacuum samples for 2022 and 2023 are still undergoing major quantification.
- *C. reductus* adults had 2–3 peaks in seasonal sampling on sticky cards in Washington (June–July, August–September, and sometimes a small increase in October). Generational peaks of *E. variegatus* showed a similar pattern from Oregon vacuum sampling.
- There was little activity of leafhopper adults of any species early in the season on sticky cards until mid or late May in Washington. However, with in vacuum sampling, *E. varietgatus* were present in large numbers at the start of sampling in April in Oregon.

Methods

Site selection. In 2022, 10 Washington and three Oregon orchards were selected (Table 1) in consultation with industry collaborators Bishop, Voelker, and Smith. Orchards were chosen where large numbers of leafhoppers were expected. The orchards were either apricot, cherry, nectarine, apple, or pear with organic management, or conventional management with limited use of broad-spectrum sprays. In 2023, the same sites were used, except Richland 1 was replaced with Richland 2 because few leafhoppers were found at Richland 1 in 2022.

Leafhopper monitoring. In 2022 and 2023, leafhoppers were monitored weekly from May through October using sticky card and vacuum methods. Variable methods were used in 2022 between Washington and Oregon, and methods were standardized in 2023.

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

For vacuum samples, in 2022, Washington site sampling was conducted in a standardized area based on nine 0.81-m (32-inch) diameter circular areas per site whereas Oregon sites standardized sampling

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

Temperature monitoring. At sites not close to AgWeatherNet or AgriMet temperature sensors, METER Group ZL6 Advanced Cloud Data Loggers with ECT/RT temperature sensors were installed to record air temperature every 15 minutes.

Table 1. Site names, fruit crops, and years sampled for leafhoppers in 2022–2023.

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

Model development. An analytical algorithm is being evaluated and tested by co-PI Northfield to parameterize a predictive model for *C. reductus* sticky card counts using degree day accumulation. The algorithm estimates minimum and maximum temperature thresholds directly from the field data. The algorithm predicts cumulative leafhopper count data as a function of cumulative degree-days calculated from weather data and the estimated temperature thresholds. Then, the program determines parameters for a gamma cumulative density function for generations 1 and 2. Generation 3 may be possible as well, depending on fall leafhopper abundance in our data set. The analysis is still being conducted to allow optimization of temperature developmental thresholds, comparison of alternative cumulative density functions and error distributions, and utilization of all data across sites for a single best-fit model. Additional analysis is planned to compare sticky card vs. vacuum data and the effect of sampling resolution on conclusions.

Insecticide trials. Bioassay methods for leafhoppers were developed on a related project entitled "Insecticidal control of leafhoppers in cherries" led by co-PI Nottingham funded by the Washington Tree Fruit Research Commission. The same methods were applied in 2023 to test two rates of a new chemical, Wrath (geraniol 30%, peppermint oil 1%, cottonseed oil 0.1%, and rosemary oil 0.01%; manufactured by GroPro) in comparison with PyGanic.

Leafhoppers (*C. reductus*) were collected on August 24 from weeds in organic apple orchards using the vacuum. Leafhoppers were transported to the laboratory in a cage. Because of low catch, healthy adult leafhoppers from a laboratory colony were used to supplement. More than 70% of the total insects used in this assay were field-collected. In the laboratory, leafhoppers were sorted into 16-oz clear plastic deli cups with moist soil on the bottom, a cherry leaf in a tube of water, and a lid with a

circular plastic mesh cutout (5-cm diameter). Five leafhoppers were placed into each cup and lidded. Treatments (Table 2) were sprayed to the leaf, leafhoppers, and soil through the mesh cutout on the deli cup lid using a hand-pump aluminum spray bottle. Treatments included Wrath (high and low rates), PyGanic EC 1.4 (pyrethrins), and a water control. Tap water was used for insecticide mixtures and the water control. All applications, including the water control were mixed with Oroboost, and Brandt buffer-ten was used to adjust pH to 7 ± 0.1 (Table 2). Applications were made to the point of runoff, or approximately three pumps of the hand spray bottle. Five replicates per treatment were completed. Once treated, assay arenas were moved to a greenhouse and leafhoppers were exposed to treatments for 24 hours (1 day after treatment, DAT) before mortality was evaluated through the plastic deli cup. Mortality was evaluated again at 4 DAT. Within an exposure time, differences in mean mortality among treatments was assessed by ANOVA using the aov() function in R followed by Tukey's test using the TukeyHSD() function ($\alpha = 0.05$).

Additional bioassay plans are under development with BASF in 2024.

Table 2. Bioassay Treatments

Trt.*	Per acre rate	Mean spray weight per trt. (g ± SEM)
Water	-	2.78 ± 0.13
PyGanic	64 fl oz	2.78 ± 0.01
Wrath, low rate	32 fl oz	2.67 ± 0.16
Wrath, high rate	48 fl oz	2.79 ± 0.08

**all plus 0.4% v/v Oroboost and 0.1% Brandt buffer-ten for pH correction*

Results and Discussion

Leafhopper monitoring. Most leafhoppers found on sticky cards in 2022 (Figure 1) and 2023 (Figure 2) in Washington were species other than the three main leafhopper X-disease vectors. Among the three focal species, *C. reductus* was the most abundant. Sicky card data indicate that 2–3 adult generations of the species occur in Washington (Figures 2 & 3). The other X-disease vector species, *C. geminatus* and *E. variegatus*, were found in much lower numbers and more sporadically on sticky cards.

Vacuum samples from 2022 and 2023 are still being quantified for Washington and quantification for Oregon is complete only for 2022. Vacuum samples contain large amounts of insect bycatch and duff which require laborious sorting. Oregon vacuum samples show high abundance of *E. variegatus* throughout the season (Figure 3). Quantification of Washington samples will allow us to determine whether this is a regional difference or difference in effectiveness of sticky cards vs. vacuum for different leafhopper species.

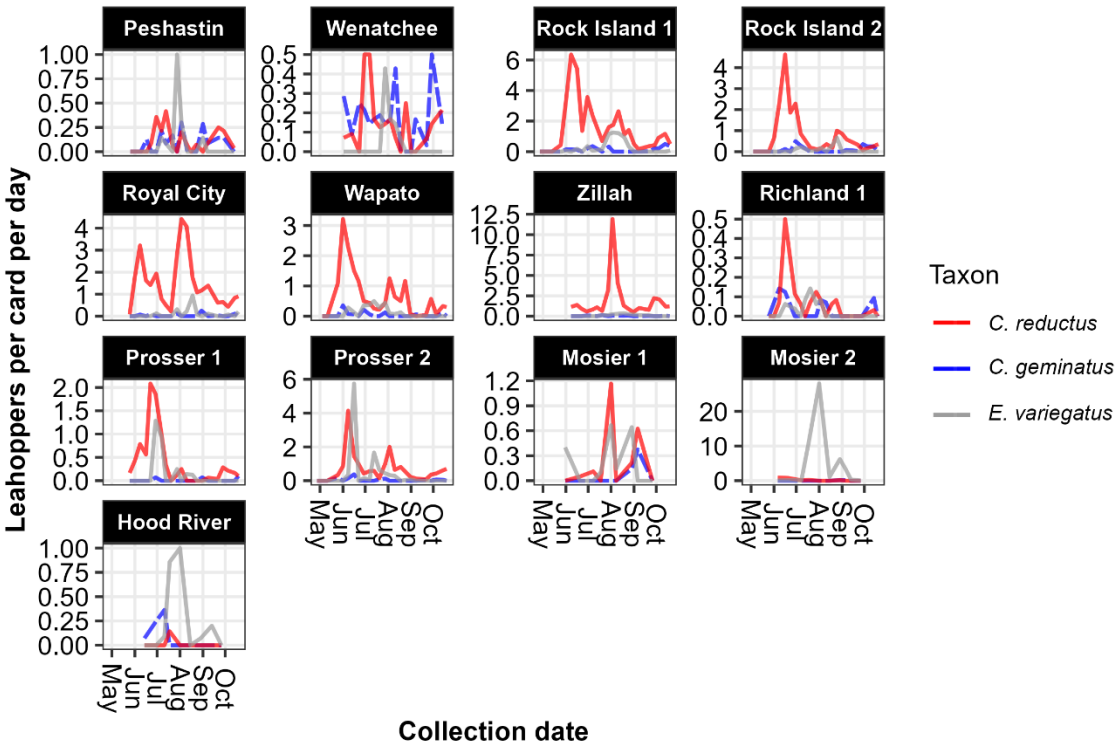

Figure 1. Leafhopper phenology from weekly (Washington) or biweekly (Oregon) sticky card monitoring in 2022.

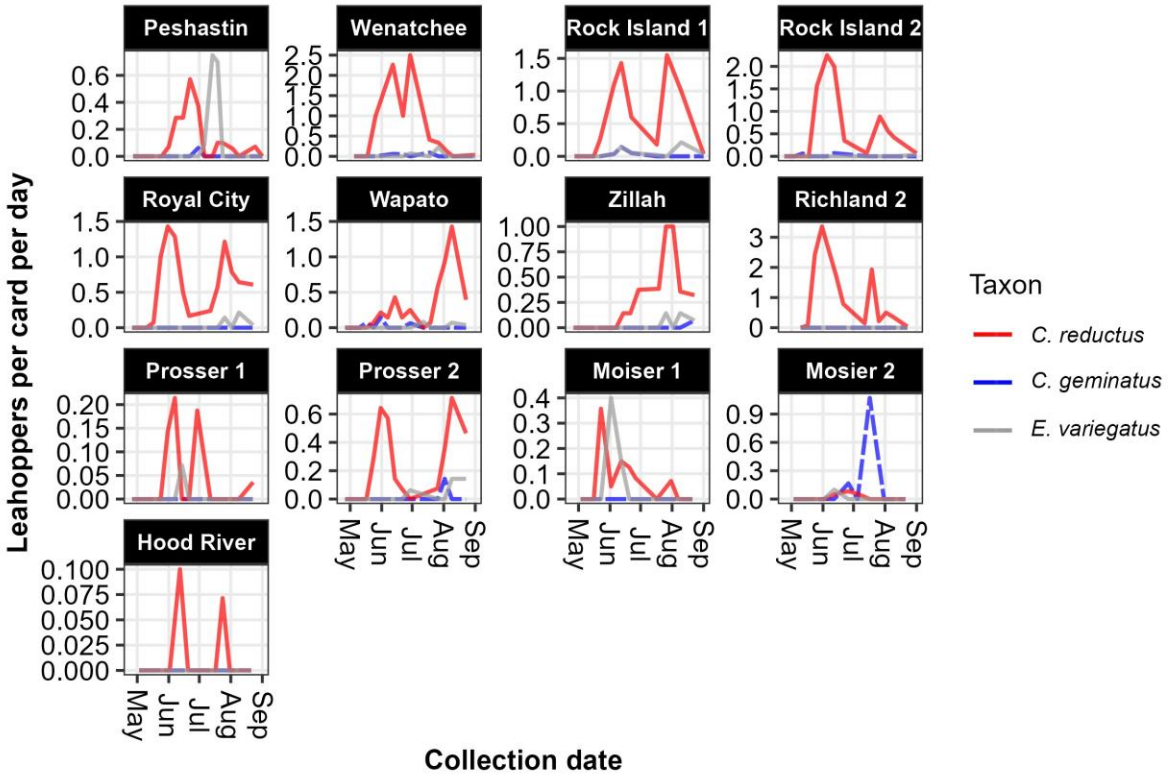

Figure 2. Leafhopper phenology from weekly sticky card monitoring in 2023 (partial data; monitoring and data entry is ongoing this season).

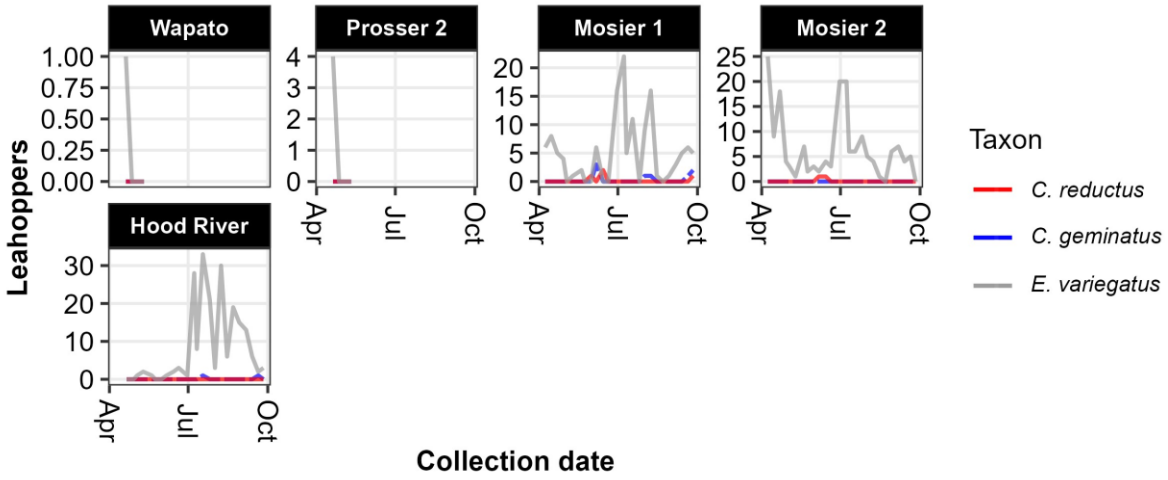

Figure 3. Partial leafhopper phenology from three Oregon orchards (Mosier1, Mosier 2, and Hood River) with complete data and two Washington orchards (Wapato and Prosser 2) with partial data quantified during 2022 using weekly vacuum sampling.

Temperature monitoring. Temperature data was collected at all sites where leafhoppers were monitored in 2022 and is ongoing in 2023.

Phenology model development. Until the models can be completed, all summary raw population dynamics data are available on PI Orpet's laboratory website (https://cahnrs.wsu.edu/tfrec-orpet/leafhopper-phenology-study/).

Insecticide trials. Significant differences in *C. reductus* mortality were found among treatments at both 1 and 4 DAT (Figure 4). At 1 DAT, mortality in the PyGanic treatment (92%) was significantly higher than in the control treatment (16%) and both Wrath treatments (low rate 21%, high rate 39%). Neither rate of Wrath treatment resulted in significantly higher mortality than the control at 1 DAT. At 4 DAT, mortality in all three insecticide treatments was significantly higher than control mortality (44%), with PyGanic reaching 100%, the low rate of Wrath reaching 84%, and the high rate of Wrath 92%. At 4 DAT, there was no significant difference in mortality between the low and high rates of Wrath.

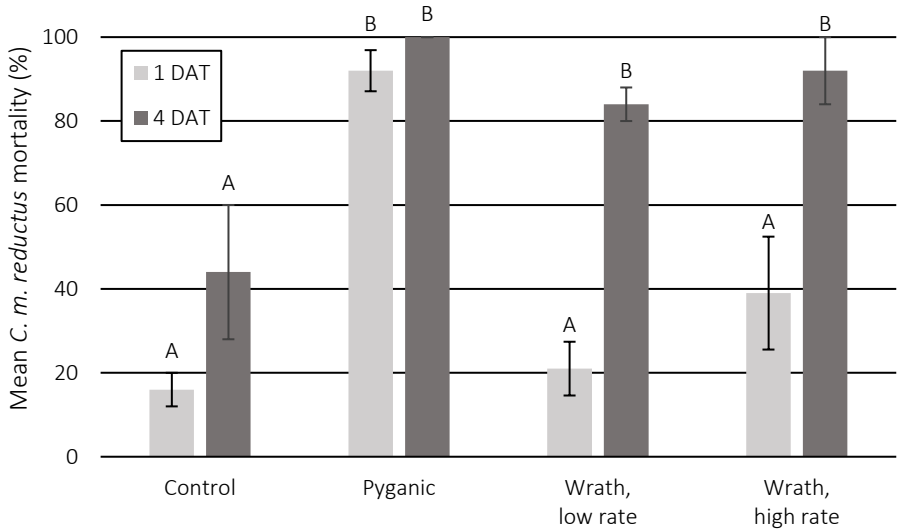

Figure 4. Mortality of C. reductus *adults after exposure to insecticide treatments in a contact bioassay. Mortality at 1 (lighter bars) and 4 (darker bars) days after treatment (DAT) Error bars are standard error of the mean. Bars headed by the same letter indicate significantly different means (Tukey test) within a DAT.*

The pyrethrins in PyGanic acted quickly and resulted in significantly higher mortality than the control or Wrath treatments at 1 DAT. However, after 4 days of exposure, the botanicals in the Wrath treatments produced mortalities statistically comparable to the industry standard. The mortalities achieved by both the low and high rates of Wrath were not significantly different. Considerable control mortality by 4 DAT was higher than desired and reflects the difficulty in keeping leafhoppers alive in the lab setting. Overall, the bioassay demonstrated toxicity of Wrath to *C. reductus*.

Conclusion. The team expects to catch up on sample backlogs for laboratory quantification this winter. Technical assistant Cody André, who performed most of the field work in 2022 and 2023 for Washington has been recruited for winter work and next field season. Dr. Northfield is developing the phenology model drafts. In 2024 and 2025, the team will have additional funds from a new Washington State Department of Agriculture Specialty Crop Block Grant project entitled "Leafhopper phenology model development and habitat assessment to improve cherry X-disease management" awarded to PI Orpet and co-PI Northfield. This new grant will support an additional project assistant and expansion of sampling to three-point transects within each orchard: point 1 will be adjacent vegetation, point 2 will be the orchard edge, and point 3 will be the orchard center. This expanded sampling will help us understand the role of orchard-adjacent habitat as a potential

leafhopper source and its role in phenology of the leafhoppers. Additional pesticide tests are also being planned. Altogether, the project is on target for its intended outcomes of permitting better-timed and effective sprays within a leafhopper management program.

Project Title: Dispersive distance of cherry X-disease vector leafhoppers

Report Type: Continuing Project Report

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Cooperators:

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $22,477
Total Project Request for Year 2 Funding: $23,210
Total Project Request for Year 3 Funding: $22,864

Other related/associated funding sources: None

WTFRC Collaborative Costs:

Budget 1
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Item	2022	2023	2024
Salaries[1]	$7,975.00	$8,215.00	$8,461.00
Benefits	$5,575.00	$5,742.00	$5,914.00
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies[2]	$4,500.00	$4,500.00	$4,500.00
Travel[3]	$1,000.00	$1,000.00	$1,000.00
Plot Fees			
Miscellaneous			
Total	$19,050.00	$19,457.00	$19,875.00

Footnotes:
[1]Adams lab Faculty Research Assistant at 0.15 FTE, with 3% increase in years 2 and 3; OPE 70%
[2]Research consumables - sticky cards, fluorescent powered,
[3]Travel to field plots

Budget 2
Co PI 2: Kelsey Galimba
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Station manager/supervisor email address: sturatz.rietz@oregonstate.edu

Item	2022	2023	2024
Salaries[1]	$1,000.00	$1,000.00	$1,000.00
Benefits			
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies[2]	$2,127.00	$2,453.00	$1,679.00
Travel[3]	$300.00	$300.00	$300.00
Plot Fees			
Miscellaneous			
Total	$3,427.00	$3,753.00	$2,979.00

Footnotes:
[1]Galimba lab FRA at 0.01 FTE
[2]Research consumables for ELISA testing
[3]Travel to field plots

Objectives

We looked at two approaches to marking leafhoppers that would allow us to successfully track their movements and understand the dispersive distance of this important insect vector. These two techniques have different strengths and weaknesses. The Protein marking method would allow us to mark insects where they are in the field using a sprayer. In this way we could mark thousands of insects without capturing and handling them. Challenges of this technique are that it requires a multi-step chemical process to detect the marking. Another challenge is that individual insects must be kept separate, or they risk cross contaminating one another, so insects must be processed individually. The DayGlow powder marking is much easier to detect, requiring only illumination with a black light to identify color marking, and several dozen insects can be counted on a sticky card without much effort. The challenge of this technique is that insects must first be captured, brought back to the lab, separated from all other insects, then transported back to the field, marked, released, then recaptured. We explored both of these techniques.

1) Develop methods for consistently marking vector leafhoppers that does not impede movement and allows for positive identification upon recapture.
2) Describe dispersive distance and rate of movement over time of key leafhopper vector species, within cherry orchards.
3) Describe rate of movement relative to prevailing wind direction and outside orchard habitat.

In year **one** we worked to establish the most viable technique for permanently and effectively marking and capturing leafhoppers with proteins that allowed for consistent detection of trace amounts of protein.

In year **two** we focused on using dayglow powder to mark insects and conducted mark release recapture experiments.

Proposed Timeline

Objective	Y1	Y2	Y3
1. Develop methods of marking	X		
2. Describe dispersive distance inside orchard		X	X
3. Describe dispersive distance outside orchard		X	X

Protein Marking.

Details worked on in year one includes how glue from sticky-traps will impact our ability to detect markers and overcoming contamination issues for successful amplification and detection of various protein markers.

Protocols for testing milk and egg proteins in a greenhouse setting were developed to answer the following questions related to Objective 1: Develop methods for marking leafhoppers.
1. Do both egg whites and milk work as protein markers for grass and leafhoppers?
2. Does trapping with sticky cards work? i.e. can we get a positive signal when insects are collected this way (on glue)?
3. Because some insect parts might be left behind, can we hole punch and test insect + card?
4. Will samples still test positive after sitting on a sticky card for 1 week?
5. Does trapping by other means (sweep netting/vacuum) and allowing the hoppers to comingle with unmarked insects, cause them to cross-contaminate unmarked insects?
6. Will marked insects still test positive for protein markers, after 1 week of living on unmarked vegetation, and does method of collection (sticky card or net) differ after this amount of time?

DayGlow powder

In year two we transitioned to using DayGlow powder for marking leafhoppers. Early work showed that they can be lightly dusted with powder without impacting survival. This work addressed Objective 2

1. Measure the proportion of marked leafhoppers that can be recaptured.
2. Measure the distance over which leafhoppers move within the orchard over one week.

Significant Findings of Protein Marking

1. **Do both egg whites and milk work as protein markers for grass/leafhoppers?**
Milk seems to work better than egg whites. The milk ELISA exhibited no false positives, for empty buffer, unsprayed grass, or unmarked leafhoppers but the egg white ELISA exhibited multiple false positives (Table 1). Additionally, while the rates of total positive leafhoppers after 24 hours of exposure to marked grass was the same between both proteins (63%), the milk protein appears to last longer – with greater numbers of positive leafhoppers after 1 and 2 weeks on a sticky card or on clean grass.

2**. Does trapping on sticky cards work? i.e. can we get a positive protein signal when insects are collected this way?**
Yes. There were multiple samples taken from sticky cards that were positive, both from insects that were removed from sticky cards with forceps and from insects left on cut-outs of sticky cards.

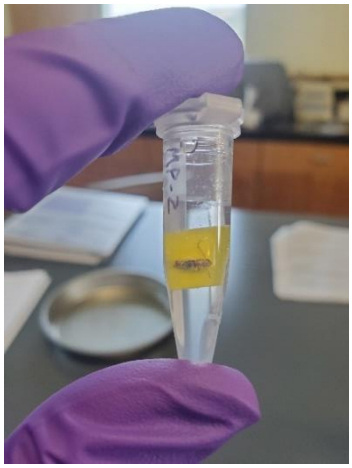

Figure 2. leafhopper + sticky card

3. **Can we just cut sticky card and wash insect + card?**
Yes, though sample sizes were small, results indicate that this method and aspirator collection were similar in the number of positive, marked insects. The cut outs had the assumed added benefit of keeping the hydrophobic insect bodies submerged in buffer during the extraction phase.

4. **Will samples remain positive after sitting on a sticky card for 1 week**?
Yes. It also appears from these data that milk lasts longer than egg white.

5. **Does trapping by other means (sweep netting/vacuum) and allowing the hoppers to comingle cause them to cross-contaminate unmarked insects?**
When 4 marked insects were allowed to comingle with 4 unmarked insects, we never saw cross-contamination. This is likely due to the low concentration of protein that the insects pick up from the marked grass.

6. Will marked insects still test positive for protein markers, after 1 week of living on unmarked vegetation? and does method of collection (sticky card or net) affect results?
As in the 24-hour tests, there is no clear superior method of collection – rates do not vary wildly between the two. After 1 week of exposure to unmarked grass after the initial 24 hours on marked grass, positive rates are lower for both proteins, but milk seems to hold up the longest.

Results table

		Milk		Egg Whites	
Sample	Total #	Positive #	Rate	Positive #	Rate
Negative control: empty extraction buffer	18	0	0%	1	6%
Negative control: unmarked leafhopper	4	0	0%	1	25%
Negative control: unmarked grass	3	0	0%	2	67%
Total negative control	**25**	**0**	**0%**	**4**	**16%**
24 hours - sticky card - removed with forceps	2	2	100%	0	0%
24 hours - sticky card - cut off, card included	2	1	50%	2	100%
24 hours - 4 caught off marked grass and comingled with 4 clean leafhoppers for 2 hours	8	2	50%*	3	75%*
Total after 24 hours	**12**	**5**	**63%**	**5**	**63%**
1 week - sticky card - removed with forceps	2	1	50%	1	50%
1 week - sticky card - cut off, card included	2	1	50%	0	0%
Kept on unmarked grass 1 week - sticky card - removed with forceps	2	1	50%	0	0%
Kept on unmarked grass 1 week - sticky card - cut off, card included	2	0	0%	0	0%
Kept on unmarked grass 1 week - 4 caught of marked grass and comingled with 4 clean leafhoppers for 2 hours	8	1	25%*	0	0%*
Total after 1 week	**16**	**4**	**50%**	**1**	**13%**
Kept on unmarked grass 2 weeks - sticky card - removed with forceps	2	0	0%	0	0%
Kept on unmarked grass 2 weeks - sticky card - cut off, card included	2	1	50%	0	0%
Kept on unmarked grass 2 weeks	3	0	0%	0	0%
Total after 2 weeks	**7**	**1**	**14%**	**0**	**0%**
Positive control: grass marked with milk	3	3	100%	3	100%
Positive control: grass from marked milk cage 1 week after being sprayed	3	3	100%	3	100%
Positive control: grass from marked milk cage 2 weeks after being sprayed	3	3	100%	3	100%
Total positive control	**9**	**9**	**100%**	**9**	**100%**
Grass from unmarked milk cage 1 week after introduction of marked leafhoppers	3	0	0%	0	0%

Table 1. Results from ELISA testing protocol to determine efficiency of milk and egg protein as markers for leafhopper dispersal research. Red numbers indicate false positives. Asterisks indicate that the positive percentage is out of 4, the total number of marked insects before comingling.

Methods

Set up: one replicate consisted of:
4 Cages

1. Grass with milk application.
2. Grass with egg white application.
3. Unmarked grass.
4. Unmarked grass.

Figure 1. Grass sprayed with milk protein marker

Four grass plants in cage 1 were sprayed with 100% whole milk, to saturation. Four grass plants in cage 2 were sprayed with 25% egg white, to saturation. Grass was allowed to dry for one hour. Thirty leafhoppers were added to cages 1 and 2, one hour after milk or egg application, and held for 24 hours. At 24 hours, eight leafhoppers were caught on a sticky trap. Two were removed with forceps and two were removed by cutting out the sticky card around them, and immediately frozen. Four were left on the stick card for one week in the greenhouse, and then removed in the same way. Four leafhoppers were also caught by aspirator and held in a small container for two hours with four unmarked leafhoppers. All eight of these were frozen after two hours. After this 24-hour period, eighteen leafhoppers were transferred to the unmarked (clean) grass cages 3 and 4 and allowed to live for one week. After *one* week on the unmarked grass, the exact same sticky card and aspirator collections were made. After *two* weeks, four leafhoppers were caught on a sticky trap, and two were removed with forceps and two were removed by cutting out the square of card around them. Three leafhoppers were collected from the cage via aspirator. All seven were frozen for processing. Sprayed grass samples were taken at 24 hour, one, and two weeks. Grass samples were collected at one week from the unmarked grass cages 3 and 4.

Controls (for milk protein)

- Extraction buffer negative control was always negative.
- Grass that was sprayed was always positive, up to 2 weeks later.
- Unsprayed grass was always negative.
- 4/4 leafhoppers with no exposure to milk tested negative.
-

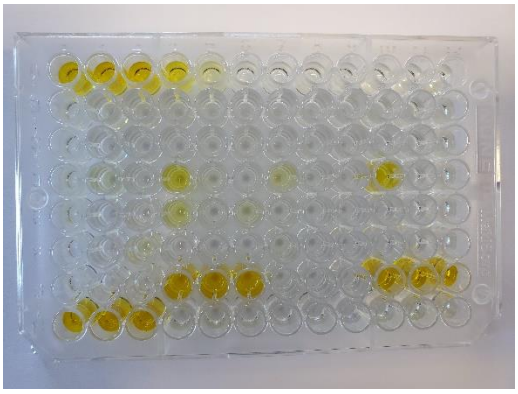

Figure 2. ELISA tray control results

Milk Samples

- 63% (5/8) of leafhoppers allowed to behave on sprayed grass, then collected 24 hours later tested positive.
- There was no transference of protein markers to clean leafhoppers in the aspirator.
- After a week on a sticky card, 50% (2/4) leafhoppers caught at 24 hours still tested positive.
- 25% (2/8) of leafhoppers allowed to behave on sprayed grass for 24 hours and then allowed to live on clean grass for one week tested positive, with no transference to clean hoppers.

Egg Whites

Control (for egg protein)

- One extraction buffer negative control was strongly positive. (false positive)
- Grass that was sprayed was always positive, up to 2 weeks later.
- 66% (2/3) unsprayed grass samples were positive. (false positive).
- 50% (2/4) of leafhoppers with no exposure to milk tested positive. (false positive).

Egg Whites

- 63% (5/8) leafhoppers allowed to behave on sprayed grass and then collected 24 hours later tested positive.
- There was no transference of egg protein to clean leafhoppers in the aspirator.
- After a week on a sticky card, 25% (1/4) of leafhoppers caught at 24 hours still tested positive.
- None (0/8) of the leafhoppers allowed to behave on protein marked grass for 24 hours and then allowed to live on clean grass for one week tested positive, no transference to clean hoppers.

DayGlow Powder Marking

Concurrently to protein marking we marked leafhoppers with DayGlow powder. This method involved several extra steps. Leafhoppers (and other insect) were captured from inside commercial orchards in The Dalles using sweep nets. Insects were transferred to screened insect cages within coolers kept cold with several ice packs. These cages were transported to the lab and leafhoppers were sorted from all other non-target insects. Leafhoppers were then collected, counted, and placed into cups with freshly cut bouquet of grass. Cups were designated as 1, 2, 3, of 4 meter release and held in the lab at room temperature overnight. The following day cups of leafhoppers were transported in a cooler back out to the field for release. A single yellow stick card was placed at the center of the releases. Each release distance was marked with a unique color. The experiment was replicated 4 times and each experiment used approximately 2,000 leafhoppers.

Figure 3. experimental layout of single trap multiple release experiment. Marked leafhoppers were released from four distance in four directions (approx. north, south, east, west).

Figure 4. populations of leafhoppers marked unique colors for each relase distance.

Conclusions and Future Directions

Protein marking

We accomplished a lot in this first season. Although our sample size is small, a 63% positive rate after 24 hours is relatively low, and likely not adequate for use in dispersal research, indicating that this method (spraying proteins on grass and allowing the insects to pick it up through contact) is likely not the most ideal use of these markers. A much more efficient use of proteins like milk might be to spray insects directly, in a mark-release-recapture study. When leafhopper cadavers are sprayed with milk or egg white, they test positive 100% of the time (12/12).

We are now performing additional experiments to address the following questions:

1. Will marked leafhoppers still be positive after a week on a sticky card, exposed to the orchard environment (sun and irrigation water)?
2. Will marked leafhoppers contaminate unmarked leafhoppers on a sticky card in the presence of irrigation (proteins washed onto unmarked insects)?
3. Will being sprayed directly kill or impair leafhoppers?
4. Will live marked leafhoppers be positive a week later or can they clean the protein off?

DayGlow powder

While protein marking may still be used, Dayglow powder making has worked well to effectively mark insects. Handling time to capture, transport, and separate leafhoppers from non-target insects is a bottleneck in the system but has been manageable. Initial ideas about leafhopper movement were that these insects might move over tens or hundreds of meters. Work in year one indicated that movement was much less. DayGlow powder marking allows us to uniquely mark several close distances, recapture them on yellow sticky card and easily identify the distance of release.

Significant Findings

The recapture rate of the thousands of marked leafhoppers on yellow sticky cards for these replicates was around 2%. While small. this is in line with previous mark-release-recapture experiments. The dispersive distance of DayGlow powered marked Euscelidius variegatus is only around 10 meters. This suggests that transmission of cherry-x disease around an infected tree may be quite slow, allowing growers to respond and prevent further infection within the orchard. Further work is needed to confirm recapture from the further distance (e.g. 5, 6, & 7 plus meters). This work will be carried out early next summer.

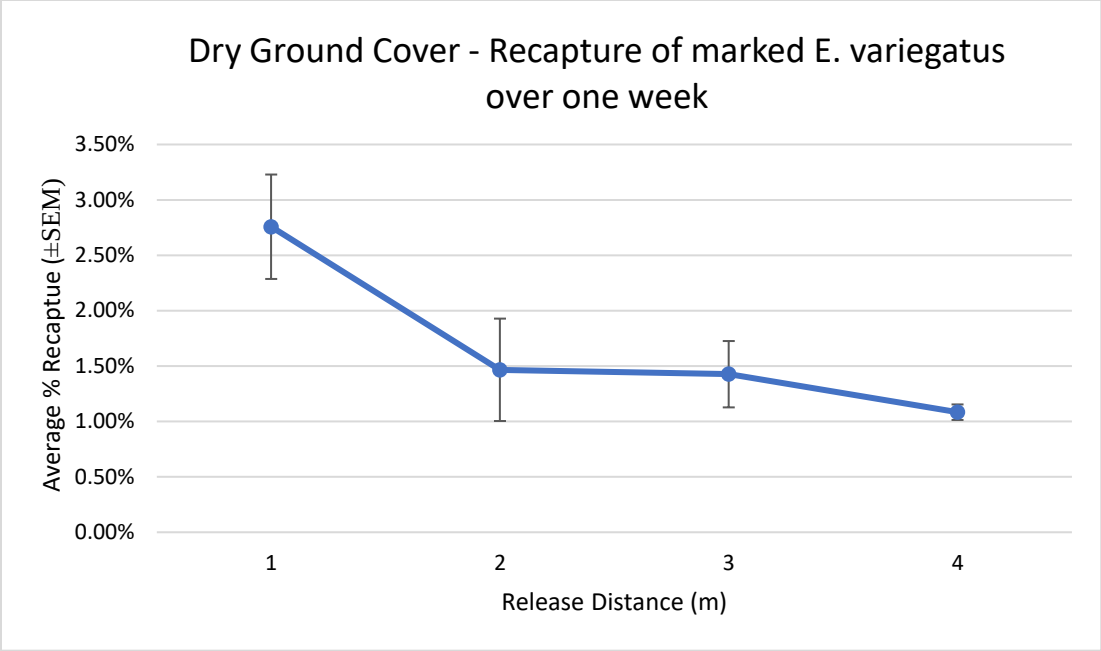

Figure 5. Proportion of released population recaptured over distance.

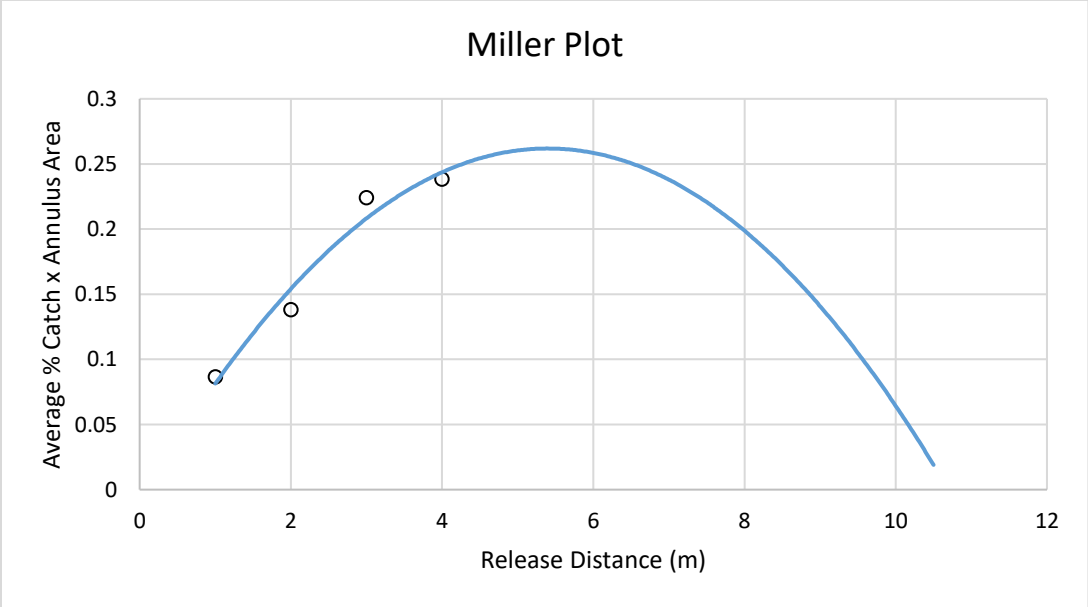

Figure 6. Miller Plot transformation of capture data reveals maximum dispersive distance of the released population.

Project Title: Cas12a-based rapid method for early detection of X-disease phytoplasma

Report Type: Continuing Project Report

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Project Duration: 2-Year

Total Project Request for Year 1 Funding: $ 85,800
Total Project Request for Year 2 Funding: $ 89,232

Other related/associated funding sources: Awarded (Harper)
Funding Duration: 2022 - 2023
Amount: $79,740
Agency Name: USDA-ARS
Notes:

WTFRC Collaborative Costs: None

Budget 1

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Item	1/3/2023	1/3/2024
Salaries[1]	$20,925.00	$21,762.00
Benefits[1]	$7,421.00	$7,718.00
Wages		
Benefits		
RCA Room Rental		
Shipping[2]	$254.00	$316.00
Supplies[3]	$14,300.00	$14,940.00
Travel	$3,000.00	$3,000.00
Plot Fees		
Miscellaneous		
Total	$45,900.00	$47,736.00

Footnotes: 4% inflation for year 2. [1]Postdoc salary for 4.5 months and postdoc benefit rate at 35.5%.[2]Shipping materials to PSU. [3]Including budget for co-PI Scott Harper: $6000 for year 1 and $6240 for year 2.

Budget 2

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Item	1/3/2023	1/3/2024
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

Co-PI 3: Dr. Scott Harper
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Footnotes: Scott's budget is included in PI Zhao's budget 1.

Objectives:

1. To establish and optimize a Cas12a-based method for early and rapid detection of cherry X-disease phytoplasma;

2. To apply the Cas12a-based method for field sample diagnosis (cherry, insects, weeds);

3. To train and promote the Cas12a-based method for diagnosis of X-disease phytoplasma.

Significant Findings

- XDP-specific RPA primers and crRNAs have been identified and synthesized based on the DNA sequence alignment and analysis of the *SecY* genes from the phytoplasma 16SrIII subgroups.
- An XDP-specific primer pair has been selected and validated for its high efficiency of RPA.
- A specific crRNA was selected for the XDP Cas12a detection assay.
- A two-pot RPA/Cas12a assay has been successfully developed for highly sensitive and specific detection of different XDP strains.
- A simple protocol of the RPA/Cas12a assay has been established for rapid detection of XDP.
- The RPA/Cas12a method has been demonstrated to be capable of specific and rapid detection of XDP-infected samples from the field.

Methods:

Procedures and Methodology. Since the initiation of this project in January 2023, we have successfully established an RPA/Cas12a method for highly specific, supersensitive, and rapid detection of cherry X-disease phytoplasma (XDP).

The DNA sequences of *SecY* genes from the XDP strains and other 16SrIII subgroups were obtained and aligned using sequence analysis tools. RPA primers and crRNAs were designed, chemically synthesized, and experimentally tested in RPA and Cas12a assays, respectively. They were evaluated for their specificity, amplification or cleavage efficiency, compatibility, and reproducibility. In the subsequent two-pot assay, both RPA and Cas12a reactions were performed under the isothermal condition at 102ºF (39ºC) (see **Fig. 1**). The fluorescent signal released from the oligo reporter due to Cas12a trans-cleavage was detected and quantified by a fluorescence microplate reader using following setting: excitation 485/20; emission 530/25nm. The RPA/Cas12a assay for XDP detection can be completed within one hour.

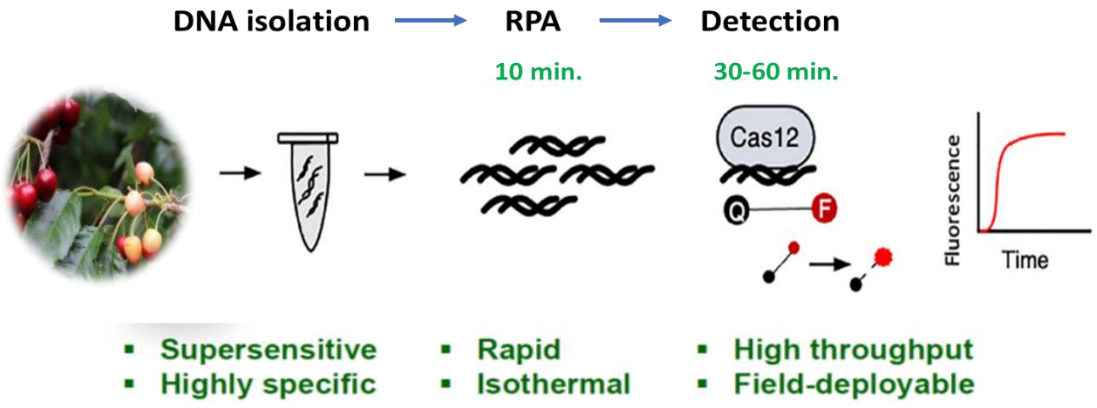


Fig. 1. Illustration of the RPA/Cas12a assay for XDP detection, which includes total DNA extraction, target DNA amplification by RPA and subsequent Cas12a detection with a fluorescence microplate reader based on trans-cleavage of oligonucleotide reporters.

Once successfully established our assay, we validated our assay specificity by using pure DNA fragment and DNA from infected cherry samples and determined the sensitivity of the assay by 10x serial dilution of pure DNA samples and infected cherry DNA samples (see Figs. 4 and 5 below).

Results from these experiments help us finalize our step-by-step protocols and instructions for diagnostic assay of X-phytoplasma.

Types and timing of anticipated results. The step-by-step protocol for the RPA/Cas12a-based diagnostic assay for cherry XDP was finalized, including RPA primer pairs, specific crRNA, and FAM reporter. We have also determined the detection limit and sensitivity of the assay. We will then optimize the times of RPA reaction, Cas12a detection procedure and signal detection by fluorescent microplate reader, which will significantly shorten the procedure and obtain results within an hour.

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

Results and Discussion:

Findings during the current year of the project. Based on the DNA sequence alignment of *SecY* genes from the XDP strains and other 16SrIII subgroups, five RPA primer pairs were designed, synthesized, and experimentally tested for specificity, amplification efficiency and reproducibility. The best pair of primers (FP1/RP1) was selected for highly efficient and specific amplification of XDP (**Fig. 2**). Importantly, the FP1/RP1 primer pair did not amplify DNAs from cherry plant or other phytoplasmas such as Phytoplasma solani and Phytoplasma mali.

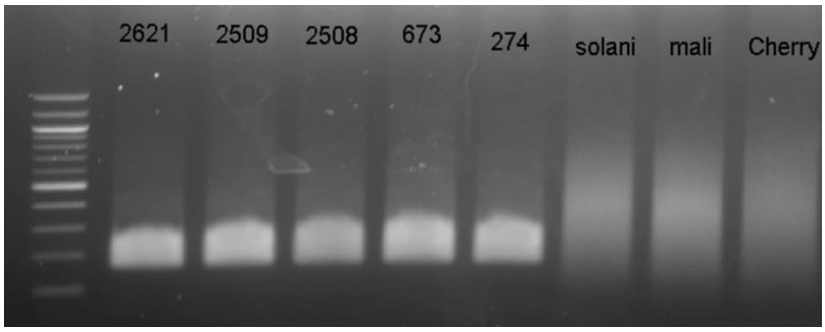


Fig. 2. Specificity and amplification efficiency of the FP1/RP1 primer pairs. Amplification of 185 bp fragment by the RPA assay only occurred with the XDP DNA samples (2621, 2509, 2508, 673, 274), but not with the DNA samples from Phytoplasma solani, Phytoplasma mali or cherry plant.

In addition, three crRNAs were designed, chemically synthesized, and tested for specificity, cleavage efficiency and compatibility with RPA. One specific crRNA was selected for the XDP Cas12a detection assay.

After the establishment of individual RPA and Cas12a detection assay, a two-pot RPA/Cas12a assay was successfully developed in Co-PI Dr. Yang's lab at Penn State for highly sensitive and specific detection of XDP DNA. Preliminary data showed that the RPA/Cas12a assay can detect XDP DNA at the sensitivity level of at least 100 aM (**Fig. 3**). We believe the assay sensitivity can be significantly improved once we further optimize the assay reagents and conditions.

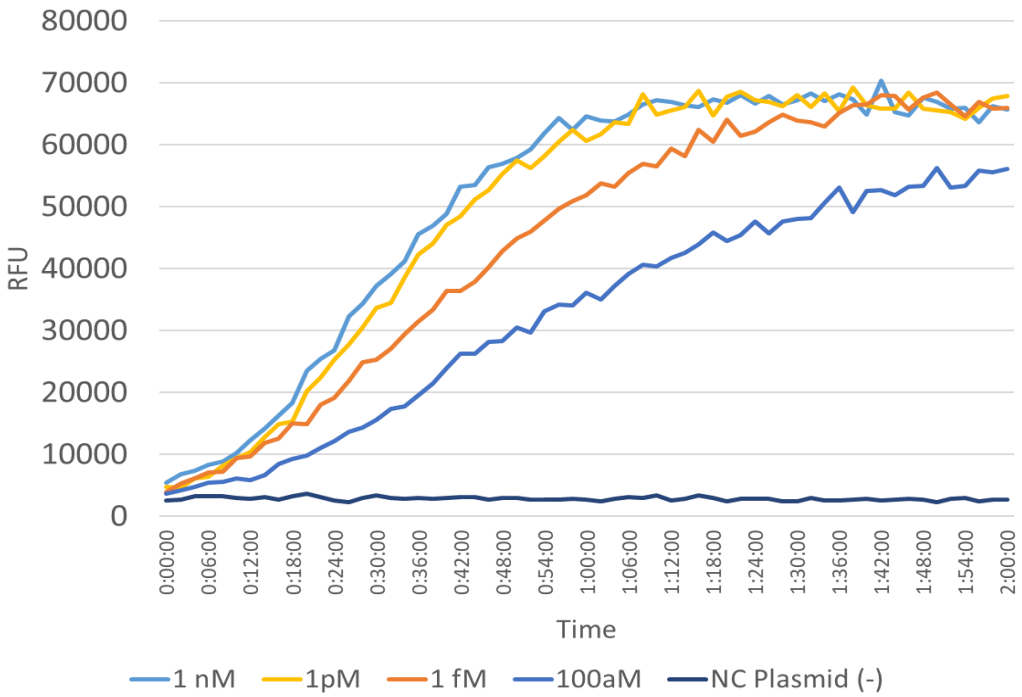
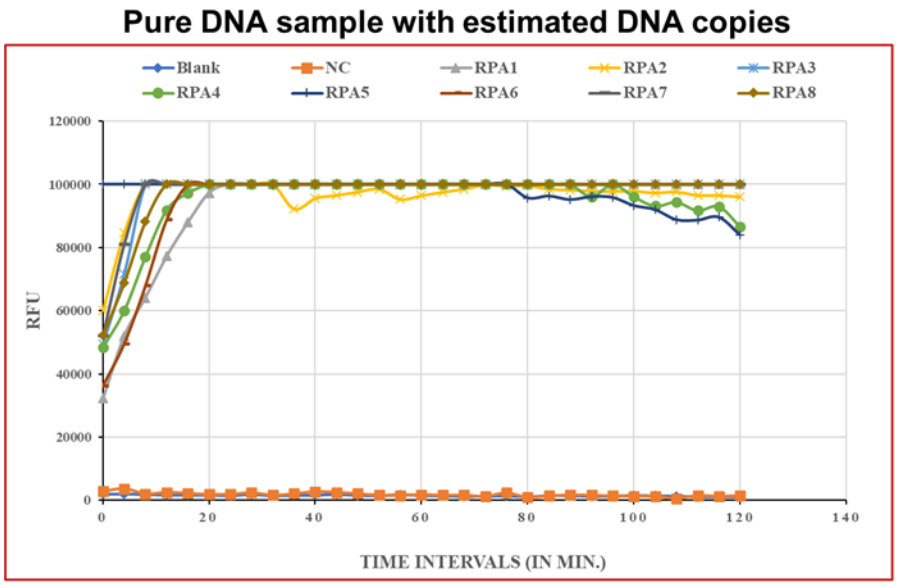


Fig. 3. The sensitivity test of the RPA/Cas12a assay for XDP DNA detection. A dilution series (1 nM, 1 pM, 1 fM, 100 aM) of plasmid DNA containing the *SecY* gene target region were used along with negative control plasmid. XDP DNA at 100 aM level could be readily detected by fluorescence microplate reader within 12 minutes of Cas12a reaction.

To validate the RPA/Cas12a assay for XDP detection of field samples, we used the assay protocol provided by Co-PI Dr. Yang to detect XDP at Washington State University in Prosser. We were able to successfully replicate and validate the RPA/Cas12a assay for XDP detection (**Figs. 4-6**). We could detect one to two copies of DNA by the RPA/Cas12a assay within 10 min using purified DNA and cherry DNA samples, respectively (**Figs. 4-5**). Furthermore, the two-pot RPA/Cas12a assay was demonstrated to be capable of specific and rapid detection of XDP-infected samples from the field with various concentration of XDP, ranging from 300 copies to 1 million copies of DNA (with Cq values 18 to 31) (**Fig. 6**). These results showed very higher sensitivity of RPA/Cas12a assay for XDP detection.

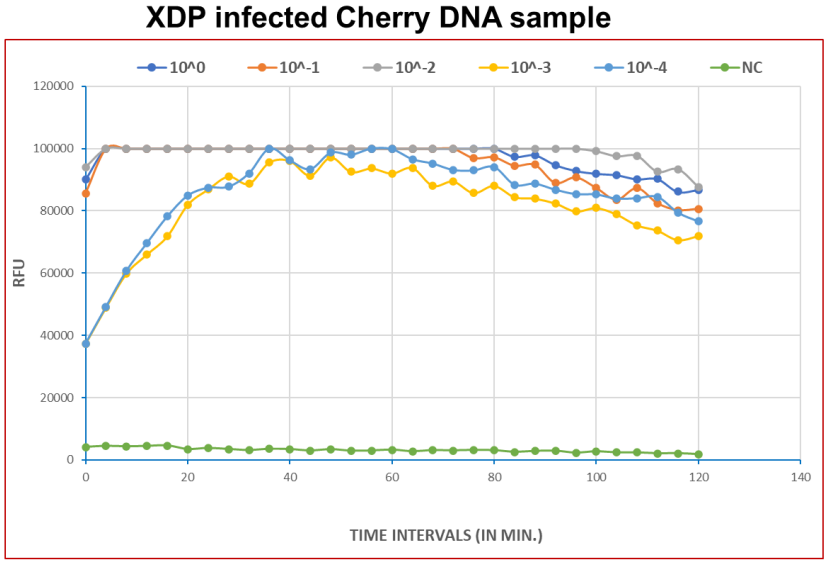


Sample	Copy no.
RPA1	1
RPA2	10
RPA3	100
RPA4	1000
RPA5	10000
RPA6	100000
RPA7	1000000
RPA8	10000000

NC: Negative control (non-infected sample); **Blank:** Water.

The max fluorescence reading is 100000 RFU.

Fig. 4. Detection of X Phytoplasma from pure DNA samples using Cas12a by serial dilutions.

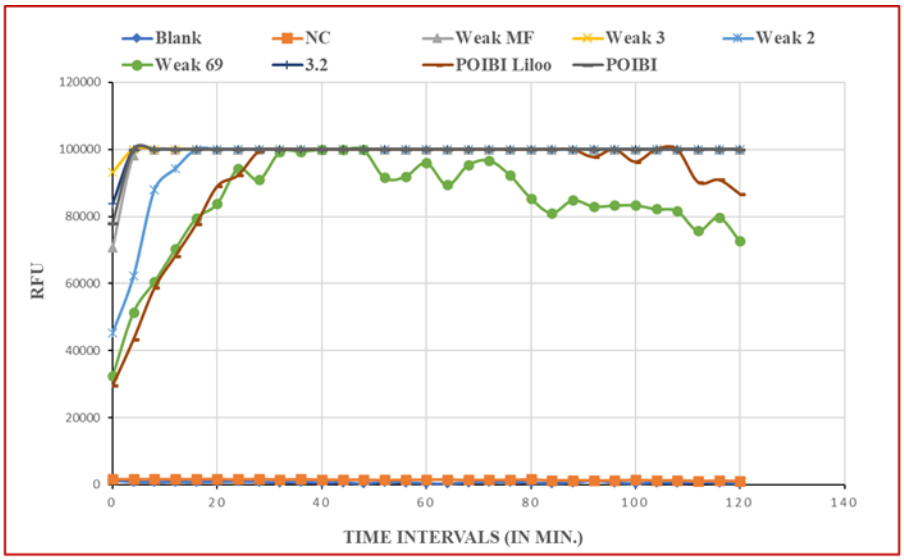


Template	Copy no.
10^{0}	20000
10^{-1}	2000
10^{-2}	200
10^{-3}	20
10^{-4}	2

NC: Negative control (non-infected sample)

The max fluorescence reading is 100000 RFU.

Fig. 5. Detection of XDP in an extract from an infected cherry tree.



NC: Negative control (non-infected sample); **Blank:** Water.

The max fluorescence reading is 100000 RFU.

Fig. 6. Detection of X-phytoplasma from infected cherry samples and other phytoplasmas.

Significance to the industry, and potential economic benefits. The cherry industry has been in an uncharted territory due to the cherry X-disease, which causes significant economic losses to growers and nurseries in the past several years. One major challenge in effectively managing the X-disease phytoplasma (XDP) is methods for early disease detection for non-fruit-bearing trees and for non-symptomatic trees/rootstocks in both orchards and nurseries. However, early detection of XDP is particularly challenging due to unculturable nature, low titer, and uneven distribution of the pathogens within infected plants as well as the ever changing genotypes of the pathogen. The RPA/Cas12a assay for XDP detection method developed in the current study specifically addressed the industry's need by providing a platform for early rapid detection of XDP; i.e. a) sensitive, detecting one to two copies of DNA within a sample, b) highly specific, detecting the target with no false positives or negatives, c) very rapid and have results within an hour, and d) can detect all current known genotypes of the pathogen. On the other hand, current laboratory-based testing by real-time PCR is the industry's standard for diagnosing an XDP infection not expressing symptoms, which could cost $60 per sample. The method we currently developed costs way less than that, which will provide the industry with an alternative inexpensive method for disease diagnosis. Therefore, the significant and potential economic benefits to the cherry industry of the current study are multifaced. It not only provides the industry with much-needed tool for fast, supersensitive, and accurate diagnosis, but it will also save time and money for both growers and nurseries.

Goals for year 2:

- Examine different sample extraction methods for compatibility with the assay.
- Determine dynamic range of the reaction sensitivity as compared to extant methods.
- Determine assay specificity with panels of different X-disease and other phytoplasma strains.
- Develop dual crRNA-based Cas12a assay, which is more sensitive than the single crRNA Cas12a assay.
- Develop one -pot RPA/Cas12a assay.

Towards the identification of Little cherry disease linked volatile biomarkers

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Researcher: Gajanan Kothawade, PhD Candidate, BYSE, Washington State University

Cooperators: Garrett Bishop, GS Long
Collaborator: Lynda Pheasant; Bernardita Sallato; Jacqueline Serrano; Sindhuja Sankaran
Equipment cost match: $60,000 (FAIMS-Lonestar VOC Analyzer, Owlstone Medical, UK)
WTFRC Budget: none

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

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

Item	2022	2023	2024
Salaries	46,200	48,048	
Benefits			
Wages			
Benefits	16,014	15,120	
Equipment			
Supplies	15,120	16,655	
Travel	2,697	2,697	
Miscellaneous			
Plot Fees			
Total	80,031	82,520	0

Footnotes: Year-1: 11-month salary support ($46,200 plus $16,014 benefits) for a postdoctoral researcher is requested. Postdoc will work closely with the PIs in planning and conducting experiments, data analytics and reporting. $1,500 requested to procure FAIMS sampling experiment lab consumables such as PTFE tubing, sampling glass jars, gloves, chem-wipes, headspace trapping cling films and N2 carrier gas. $7,120 requested to procure Tedlar bags ($178/ pack of 10 bags x 40 pack) for plant volatile trapping, and $5,000 towards PI-Ruddell's GC/MS consumables and maintenance. $2,697 is requested for field sampling related

travel (150 miles /trip x 20 trips) as well as GC/MS analysis travel from Prosser to USDA-ARS Wenatchee, WA (5 nights per diem + 270 miles/trip). PI Serban requests $1,500 towards extension outreach activities. **Year-2**: request is similar to Year 1 expect salary rate is adjusted by 4% per WSU policies. **Year-3.** Approved No Cost Extension until 6/30/2024.

Objectives

1. Volatile biomarker-based early X-disease and LCD infection detection for 'Bing' and 'Skeena' cultivars using FAIMS technique,
2. Develop a comprehensive understanding of associated volatile biomarkers release using GC/MS technique, and
3. Conduct pertinent extension education and technology demonstrations.

Significant Findings

Objective 1

- As reported in 2021 and 2022, similar but inconsistent results were observed for 2023 data during preliminary data analysis. However, further statistical analysis to validate use of FAIMS technique in early X-disease and LCD infection detection is being done. The results will be shared in the final report.

Objective 2

- The 'in-situ' plant tissue based volatile headspace analysis for 'Skeena' cultivar Z-2-Hexenal and 3-Hexenal (E) were predominantly distinguishable and seem to be linked with LCD infection (2022 Season). As reported in 2022, the key volatile compounds for the 'Bing' cultivar were Z-3-Hexenal and Z-2-pentenal.
- The validation for these compounds is under progress for data collected in 2023 season.

Objective 3

- The project results were shared with stakeholders during 'LCD Field Day' held at Buena, WA (June 21, 2022; Attendance: ~70 and June 14, 2023; Participants: ~34), Mattawa, WA (June 7, 2023; Participants: ~46). The project outcomes were also shared to the research community as a session talk at The American Society of Agricultural and Biological Engineers-AIM 2022 held at Houston, TX.

Methods

Sample collection and preparation. As a continuation of 2021 and 2022 field season field sampling, samples, including flowers, leaves, and fruits, were collected in 2023 field season from the lower canopy zones of the field-grown trees in Washington State (Wright et al., 2021; 2022). The samples were collected at the vital growth stages: flowering, shuck fall, pit hardening, first straw, harvest, and post-harvest for the

cultivars “Bing” and “Skeena” (Table 1; Fig. 1). Trees in the experiment were selected based on the preliminary infection results provided by WSU Clean Plant Network (CPN), Prosser, WA. Trees and branches were labeled with colored tape and labels for consistent data collection throughout the season. The root tissue analysis was conducted at the post-harvest growth stage for both cultivars.

Table 1. Field sites and sampling details for volatile analysis using FAIMS and GC/MS technique.

Season	Site	Cultivar	Growth stage	Method*	Samples
2021-22	Site 1	Bing	Flowering	1,3	Infected: 6 Negative: 3
			Shuck fall	1	
			Pit hardening	1	
			First straw	1	
			Harvest	1,3	
			Post-harvest	-	
	Site 2	Benton	Post-harvest	1,3	Infected:12 Healthy: 12
2022-23	Site 3	Bing	Flowering	1,2,3	Infected: 5 Negative: 4 Unknown: 3
			Shuck fall	1,3	
			Pit hardening	1,3	
			First straw	1,2,3	
			Harvest	1,2,3	
			Post-harvest	1,3	
	Site 4	Skeena	Flowering	1,2,3	Infected: 4 Negative: 4 Unknown: 3
			Shuck fall	1,3	
			Pit hardening	1,3	
			First straw	1,2,3	
			Harvest	1,2,3	
			Post-harvest	1,3	

*1: FAIMS; 2: GC/MS; 3: qPCR technique

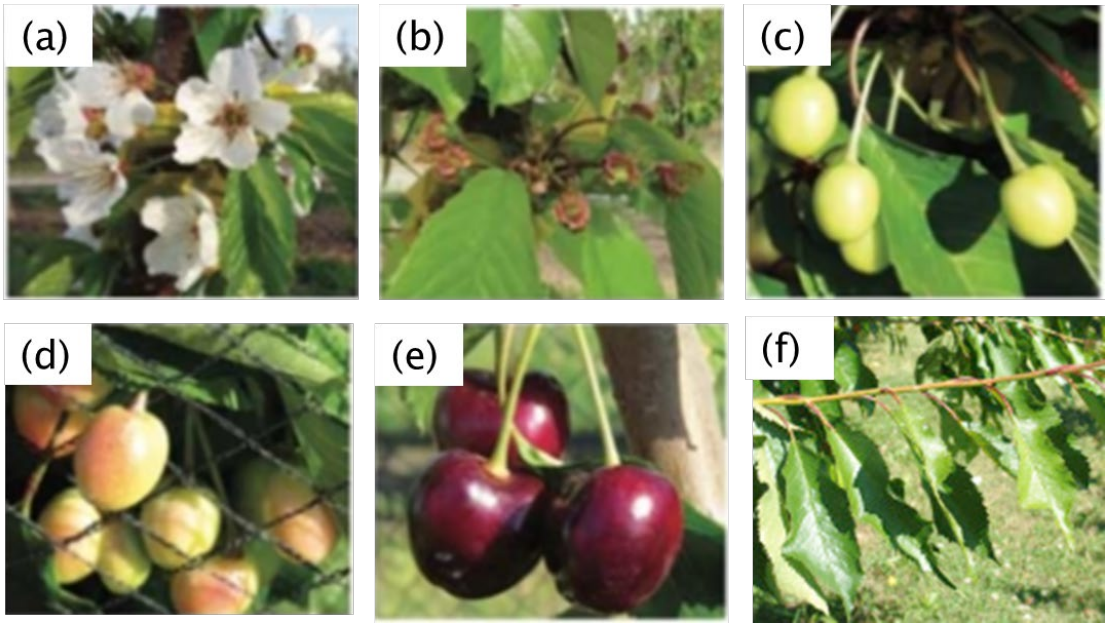

Figure 1. Typical field samples at different growth stages, (a) flowering; (b) shuck fall; (c) pit hardening; (d) first straw; (e) harvest, and (f) post-harvest.

All three methods reported in Table 1 for each field season are described below:

FAIMS. Field collected samples were stored in 1-gallon glass (sterilized) jars. Each sample jar was covered with a food-grade cling film for aerobic storage conditions (Fig. 2). Samples were then analyzed using a portable FAIMS system using a custom-developed unit (Arasaradnam et al., 2016; Kothawade et al., 2021). The glass jars were covered by a Teflon lid having two stoppers with two holes, one as an inlet for the carrier gas source (nitrogen air) and another to flush out the volatiles to the FAIMS ionization chamber. A total of six scans were conducted for each sample jar at the optimized operation parameters (flow rate: 1.5 L min^{-1} and pressure: 60 kPa).

Figure 2. A portable FAIMS system analyzing cherry leaves volatile profile.

The FAIMS scans output is an ion current spectrum that is proportional to the mass of distinct volatile organic compounds (VOCs) under a range of dispersion fields (DF: 0 to 100%) and compensation voltages (CV: -6 to 6V). The ion current spectrums from all scans generate three-dimensional data consisting of 51 DFs, 512 CVs, and resultant ion currents (arbitrary units, AU). The system needed to be purged for about 40 minutes using nitrogen air before scanning the next sample jar to remove the residues from the previous sample. A blank jar was also examined as a reference in data analysis for each sampling day.

GC/MS. The in-situ plant tissue based volatile analysis was conducted at the first straw and harvest stage. For the sample collection, 50 ml falcon tubes were used to store the samples (5 leaf/replicate). Liquid nitrogen was used to freeze (flash freeze) the samples and stored them in dry ice until the samples were moved to the -80 °C facility. The plant tissue was minced using liquid nitrogen and kept at an -80 °C facility. The scaling of ground plant tissue (0.5g) in 20 ml glass vials was performed in a box filled with liquid nitrogen to avoid thawing the samples at room temperature.

HPLC water, 5-Hexen-1-ol, and Isopropyl butyrate were used to prepare an internal standard (ISTD) for further extraction for volatile headspace sampling. A polystyrene box was then filled with liquid nitrogen (1" covering the bottom), and an aluminum block with samples was placed in the box. Next, 10 µl ISTD and 1 ml NaCl saturated solution were added to the samples, and all samples were incubated at room temperature, followed by sonication and agitation. An aluminum tray with labeled glass vials was placed on the GC/MS system. Volatile headspace was adsorbed onto glass traps packed with Tenax TA porous polymer (TDU tubes, Gerstel, Linthicum, MD, USA) (Lee et al., 2002; Hewavitharana et al., 2019). Thermally desorbed analyte was injected and analyzed using Agilent (Santa Clara, CA, USA) 6890/5975 GC/MS equipped with a Gerstel (Baltimore, MD, USA) Multipurpose Sampler (MPS), Dynamic Headspace Sampler (DHS), and Thermal Desorption Unit (TDU) (Rudell et al., 2009; Hewavitharana et al., 2019).

Samples including leaf tissue were collected from the same sites as reported above and stored in the 1-gallon glass jars for the partially destructive volatile analysis method. The volatile headspace from the jars was trapped on the adsorbent Tenax TA packed in glass collectors using a vacuum pump. The glass traps were then analyzed on the GC/MS as reported in the above paragraph.

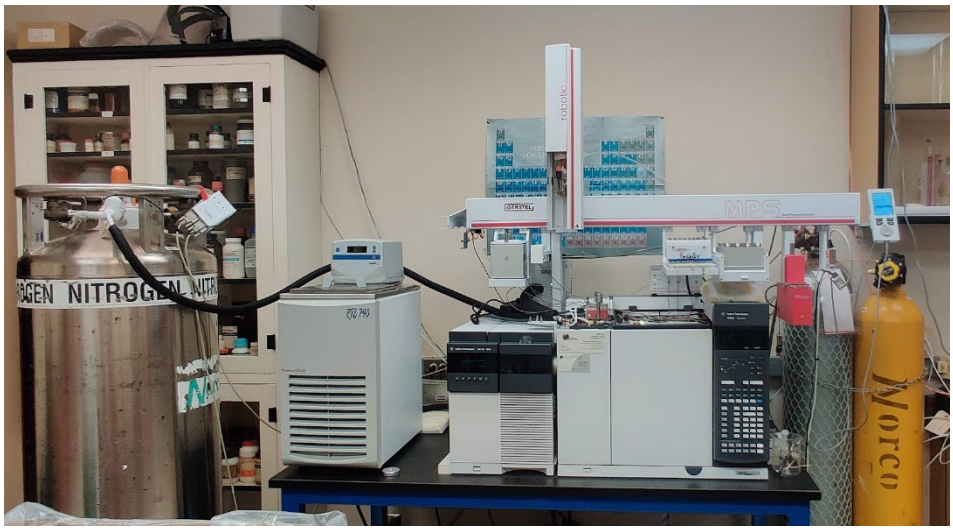

Figure 3. A GC/MS unit used for in-situ volatile headspace analysis.

qPCR (Molecular analysis). For all sites, samples were collected within a similar time frame (see table 1). Post-sample collection, a small section of stem from each branch was used for phloem tissue extraction. The extracted tissue was chopped using a razor blade and stored in a bead-beating tube. For further processing, 0.1 g tissue was scaled in labeled tubes. Similarly, the plant tissue stored at -80 °C was also weighed in labeled tubes (GC/MS samples). The Nucleic acids from these samples were extracted using the CTAB extraction method. Resulting samples were stored at -20 °C. The amplified samples were used as the template for the qPCR reaction. All the samples were diagnosed for X-disease phytoplasma (XDP), Little Cherry Virus-2 (LChV-2), Prunus Necrotic Ringspot Virus (PNRSV) and Prunus Dwarf Virus (PDV). The bacterial canker symptoms were observed based on visual scouting of the trees at the time of sampling. The results of the molecular analysis were used for comparative analysis.

Data analysis

FAIMS. The raw FAIMS scans from the volatile's headspace analysis were extracted to the convenient file format ('.txt' and '.csv'). After preprocessing, relevant ion current peaks were used for further analysis. The third ion current peak was observed (2021-22 data) as a representative of the ion current response associated with the volatiles released from the infected samples, which was not consistently present in the negative samples. Because of the seasonal and growth stage-specific variations in the results, statistical analysis of 2023 season is being reconsidered based on preliminary findings observed in the pre-processed data.

GC/MS. The key LCD-associated volatile biomarkers were identified by matching mass spectra to the Wiley/NIST [National Institute of Standards and Technology] library in the software MassHunter (Agilent, Santa Clara, CA, USA). The extracted peak responses for the volatile compound were analyzed using different dimensionality reduction methods like Principal component analysis (PCA) and Orthogonal partial least squares discriminant analysis (OPLSDA). The key volatile compounds were identified using statistical analysis and OPLSDA based VIP scores assessment. OPLSDA-based VIP scores were preferred to identify key volatile compounds. All the analysis were performed in MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/).

Analysis to be conducted. Key volatile compounds for 'Bing' and 'Skeena' observed in 2022 season will be validated on 2023 season data using similar methods.

Results and Discussion

Objective 1. Volatile biomarker-based early X-disease and LCD infection detection for 'Bing' and 'Skeena' cultivars using the FAIMS technique.

The visual assessment of the FAIMS data collected in 2023 indicated a variations in ion current peaks throughout the season. Also, most of the samples that were positive for XDP were also positive for PDV. A few samples were also infected with LChV-2 and PNRSV. These observations signified that analysis for 2023 season data needs better statistical understanding. Results will be reported in the final report.

Objective 2. Develop a comprehensive understanding of the associated volatile biomarkers release using the GC-MS technique.

For 'Skeena', the GC-MS analysis detected more than 100 compounds in the volatile headspace generated from the leaf tissues. After preliminary analysis using chromatograms, 55 compounds which were present in all the samples in varying concentration were selected for further analysis. Orthogonal PLSDA revealed the distinguished patterns between healthy and infected samples at harvest stage (Fig. 4).

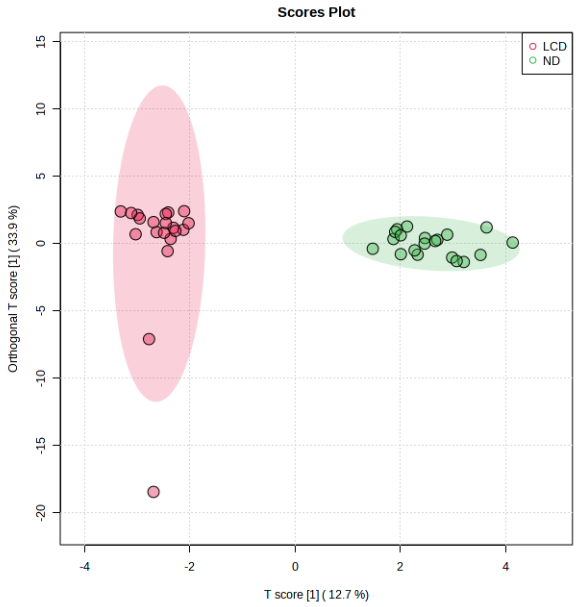

Figure 4. Orthogonal Partial Least Squares-based pattern separation of the GC/MS data for selected peaks at harvest growth stage for 'Skeena' cultivar (2022 season).

The VIP scores from the OPLSDA analysis indicated Z-2-Hexenal and 3-Hexenal (E) as key volatile compounds for 'Skeena' cultivar at harvest growth stage. The compounds with the highest VIP scores from OPLSDA analysis. Although VIP score for 2-ethyfuran was higher than Z-2-Hexenal and 3-Hexenal (E), its contribution towards LCD detection is not well understood (Fig.5).

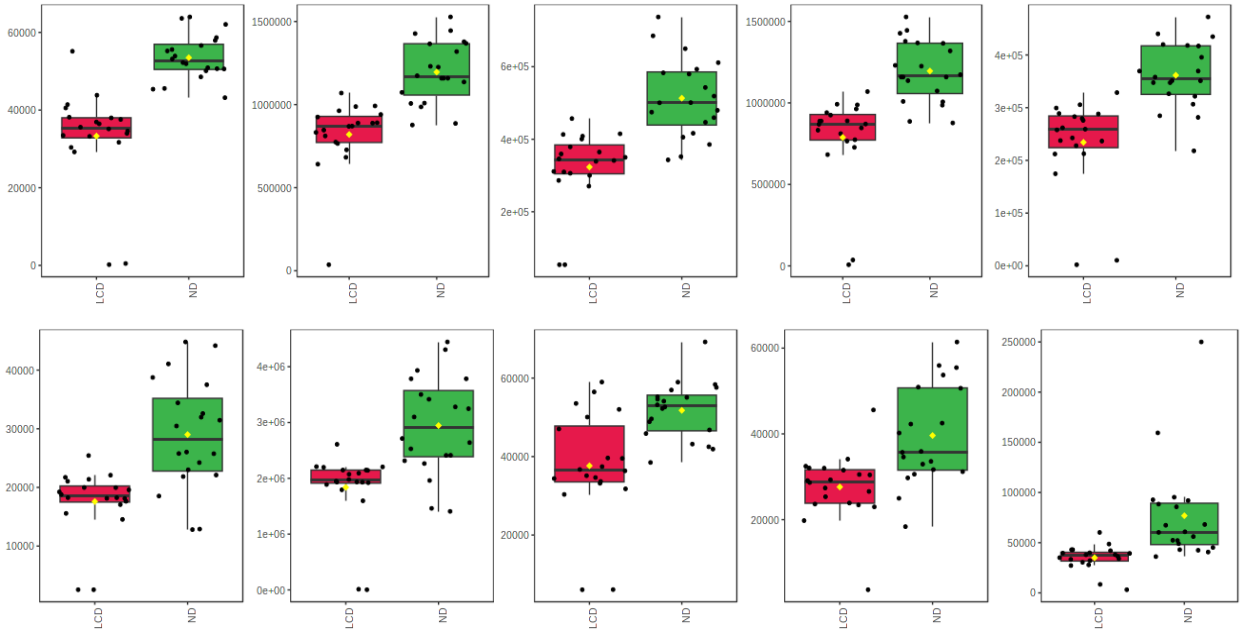

Figure 5. Orthogonal Partial Least Square Discriminant Analysis top ten VIPs for VOCs extracted using destructive method for cv., Skeena at harvest. (a)2-ethylfuran; (b)3-Hexenal (E); (c)Z-2-Hexenal; (d)(E)-3-Hexenal; (e)(E)-2-Pentenal; (f)3-Pentanol; (g)Hexanal, (S)-; (h)2-ethylacrolein, (E, E)-; (i)2-Methylbutanal and (j)Hexadienal [from left to right (a-e: first row; f-j: second row)].

Objective 3. Conduct pertinent extension education and technology demonstrations.

The project results were shared with stakeholders during 'LCD Field Days' held at Mattawa, WA (June 7, 2023; Participants: ~46) and Buena, WA (June 14, 2023; Participants: ~34). The project findings will also be presented in the NW Hort Show 2023 as Flash Talk.

Proposal Title: Determining Sweet Cherry Rootstock Sensitivity to X-Disease
Report Type: Continuing Project Report

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

Project Duration: 3 Year

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

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

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

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

Budget 2
Co PI 2: Ashley Thompson
Organization Name: Oregon State University
Contract Administrator: Charlene Wilkinson
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. X-disease infections without hypersensitive response symptoms have been verified in Gisela 6, Gisela 12, Krymsk 5, Krymsk 6, and Mazzard, indicating these rootstocks are susceptible.

2. Rootstocks that have been acquired or are scheduled for delivery include Cass, Clinton, Crawford, Gisela 3, Gisela 5, Colt, MaxMa14, Weigi 2 and Weigi 3. Clare, Lake and Crawford are pending availability.

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. Unfortunately, many tests are performed without recording scion data, and very few have records regarding rootstock. Data for the following rootstocks and scion cultivars were acquired:

Rootstock	**Scion**
Mazzard	Ann
Gisela 6	Attika
Gisela 12	Bing
Krymsk 5	Black Pearl
Krymsk 6	Burgundy Pearl
	Chelan
	Coral Champagne
	Lapins
	Rainier
	Regina
	Skeena
	Starletta
	Suite Note
	Van

Table 1. Cherry rootstocks and scions recorded in X-Disease testing data from OR and WA.

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

Rootstocks have been ordered from multiple nurseries. The following have arrived or will be arriving early 2024: Cass, Clinton, Crawford, Gisela 3, Gisela 5, Colt, MaxMa14, Weigi 2 and Weigi 3. Inoculations should be initiated in 2024.

Results and Discussion

Objective 1

Of the five rootstocks we identified in X-disease testing records, none had 0% positives. 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 almost certainly biased. We are considering any positives to be evidence that a particular rootstock is susceptible. We will focus on obtaining as many more X-disease records as we can throughout the project, but for now, Gisela 6, Gisela 12, Krymsk 5 and Krymsk 6 will be omitted from Objective 2.

Rootstock	Notes	Number Tested	% Positive
Mazzard	*P. avium*. Negative Control: susceptible to X-Disease.	58	22%
Gisela 6	*P. cerasus* x *P. canescens*	40	8%
Gisela 12	*P. cerasus* x *P. canescens*	100	60%
Krymsk 5	*P. fruticosa* x *P. serrulata.* Higher chance of hypersensitive response to X-Disease based on parentage.	31	3%
Krymsk 6	*P. cerasus* x *P. maackii*	61	64%

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

Scion	Number Tested	% Positive
Ann	4	50%
Attika	10	10%
Bing	18	33%
Black Pearl	71	13%
Burgundy Pearl	30	0%
Chelan	3	33%
Coral Champagne	27	4%
Lapins	1	100%

Rainier	1	100%
Regina	2	50%
Skeena	12	33%
Starletta	2	0%
Suite Note	10	30%
Van	2	100%

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

Report Type: Continuing Project Report

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

Project Duration: 3 Year

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

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

Footnotes:

[1] Estimated salary for one FRA to perform sample collection, testing and data analysis + 2 weeks of PI summer salary.

[2] Field testing equipment for NIR and Ca2+.

[3] Lab supplies and reagents.

Budget 2
Co PI 2: Ashley Thompson
Organization Name: Oregon State University
Contract Administrator: Charlene Wilkinson
Telephone: 541-737-3228
Contract administrator email address: charlene.wilkinson@oregonstate.edu

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

Budget 3
Co PI 2: Corina Serban
Organization Name: Washington State University
Contract Administrator: Stacy Mondy
Telephone: 916-897-1960
Contract administrator email address: arcgrants@wsu.edu

Item	2022	2023	2024
Salaries			
Benefits			
Wages			

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

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

Objectives

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

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

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

Significant Findings

Objective 1

2022

- Five collections of leaves from infected and uninfected trees in three locations were made and samples were frozen for lab testing.

2023

- New infections were identified for testing after removal of trees used in 2022. Three sites with positive trees in The Dalles, OR that are being tested for phytoplasma titer dynamics (USDA-CPPM project led by T. Northfield) were collected from throughout the growing season.
- Infected trees were identified late in the season at MCAREC, Hood River, OR

Objective 2

2022

- All leaves sampled in Objective 1 were scanned with a hand-held spectrometer to gather spectral reflectance data for >2,000 individual wavelengths.
- Testing method using small plastic bags, sandpaper and iodine solution doesn't show significant visual differences between infected and non-infected leaves.
- Conflicting with previous published research, non-infected sweet cherry leaves appear to contain substantial amounts of starch.

- Starch content of cherry leaves appears to by cyclical, with quantities and spatial distribution varying throughout the day.

2023

- All leaves sampled in Objective 1 were scanned with a hand-held spectrometer to gather spectral reflectance data for >2,000 individual wavelengths.

Objective 3

2022

- Collections of phloem sap from infected and uninfected trees in Objective 1 were made and frozen for further analysis.

Methods

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

In 2022, X-disease infections were identified and verified using qPCR (OSU Plant Clinic, Corvallis OR) at three separate locations in The Dalles, OR. Cultivars included 'Bing', 'Benton', and 'Royal Ann'. Collections of leaves from 3-5 infected and 3-5 non-infected trees were made at five dates throughout July and August. Collections were taken preferentially from limbs that bore symptomatic fruit, and from lower on the tree. For each tree, samples from 10 leaves were weighed and flash frozen for further processing to analyze starch content. A colorimetric Starch Assay Kit was purchased from Cell Biolabs. Testing was initiated in spring, 2023, but was paused after key personnel resigned shortly after. Sampling continued in 2023, but in three new locations following the removal of trees (Fig. 1). These orchards are associated with a growing/packing organization that plans to leave infected trees in the ground for the foreseeable future. Trees that were collected from were originally identified using qPCR, and are currently being tested every three weeks for titer level (USDA-CPPM project led by T. Northfield).

Figure 1. Example of infected cherry tree included in 2023 sampling, exhibiting strong symptoms.

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

In 2022, simultaneous collections were made from the same trees to use for both iodine testing and for spectral analysis. For the spectrometer, a midpoint between the distal and proximal ends and between the midvein and leaf margin was scanned for each leaf. Data is currently being organized for analysis. Communications with a statistician (Dr. Clark Kogan,

statistician on the Cherry Cold Hardiness project) have been initiated to determine whether modeling will be necessary to develop spectral signatures for X-Disease.

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

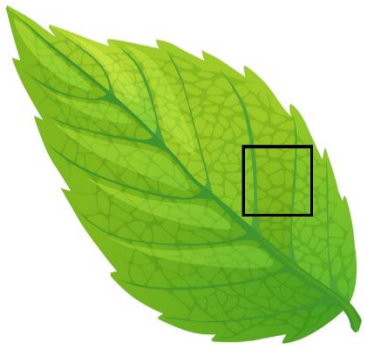

Figure 2. Leaf area that was used to clear and stain with iodine.

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

In 2023, iodine testing was paused because of key personnel resignation. Scanning in the field still occurred, in the trees described in Objective 1. Leaves from one branch with verified infections were made every three weeks.

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

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

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

Problems/Limitations Encountered

- Staffing was a major issue in 2022. We were able to hire a full-time research technician at the beginning of August, 2022 but she unfortunately resigned two months later. Out of five prospective summer students, only one followed through with applying and taking the position. The majority of all research is being performed by one full-time FRA and one part-time technician.
- Staffing was also a major issue in 2023, after the resignation of the lab's full-time FRA and a failed search to replace the full-time technician. Recruiting and retaining personnel at MCAREC has been challenging for all of the labs here, with three major resignations

this spring/summer between the labs and ongoing empty director and office manager positions.

- Obtaining materials and supplies necessary for research has been challenging because of supply chain issues. One example: Potassium Iodide ordered in September didn't ship until January, 2022.
- The majority of infected trees we used in 2022 from growers' orchards were pushed out, requiring us to find all new sample sites in 2023. This highlights the need for infected trees at MCAREC that we can use for experimentation. We are currently designing and planning for a screen house that will be able to house and contain them. In the meantime, we are attempting to infect turnip and other herbaceous species used as model systems for X-Disease in the past, using cherry material from The Dalles and the leafhopper *E. variegatus*. Recent discovery of X-disease-positive trees in a current planting at MCAREC may lessen the need for containment and provide readily-available material for testing, but management of these trees is still being determined.

Results and Discussion

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

All leaves that were frozen for starch content analysis were scanned with a hand-held spectrometer to gather spectral reflectance data. Data for each leaf consists of the reflectance value for 2,048 individual wavelengths ranging from 140 – 1334 nm. Data analysis is pending.

In 2022, iodine testing was attempted in two different ways. The sandpaper + baggy method that was shown to work in HLB infections in citrus has yet to show significant visual differences between infected and non-infected leaves. When the method is used on potato tubers, black cells can be seen floating in the iodine solution (Fig. 3). While darker leaf material can be seen floating in solution containing sandpaper from both infected and non-infected samples, it is never as dark as the starch-filled cells from the potato. Additionally, the solution doesn't appear to differ between the infected and non-infected leaves. This testing was performed at five different dates and on different cultivars with similar results.

The clearing and iodine staining method using leaves or portions of leaves gave unanticipated results. Previous research done at WSU in the 1980's asserts that normal (i.e. uninfected) sweet cherry leaves contain no, or very little starch. This would mean that the extra starch we hope is being accumulated in X-Disease infected leaves should be easy to distinguish. However, what we found after clearing leaves from X-Disease infected and non-infected trees is that both seem to contain starch, and in some cases (Fig. 4) uninfected leaves contain much more starch. This led us to expand our investigation. Additional testing suggests that starch does occur in cherry, and that its metabolism is dynamic, changing in response to circadian rhythms and possibly to light/solar exposure. Uninfected and infected eaves that had been kept in a dark refrigerator overnight showed no starch in mesophyll cells, but instead obvious starch granules in the guard cells surrounding the abaxial stomata (Fig. 5). Leaves from uninfected (presumably) 'Bing' trees at MCAREC showed that while starch is present in both leaves exposed and shaded from the sun, it appears more uniform and more concentrated in shaded leaves (Fig. 6). These results will be verified by starch assay. Altogether, the results from iodine testing in 2022 indicate a strong need for additional research into starch metabolism in sweet cherry leaves, with

strong evidence that former conclusions need to be re-explored before starch can be used as a marker for X-Disease infection. Having infected trees present at MCAREC within walking distance of the lab space should allow for less complicated sampling and testing, if starch content is as dynamic as previous testing implies.

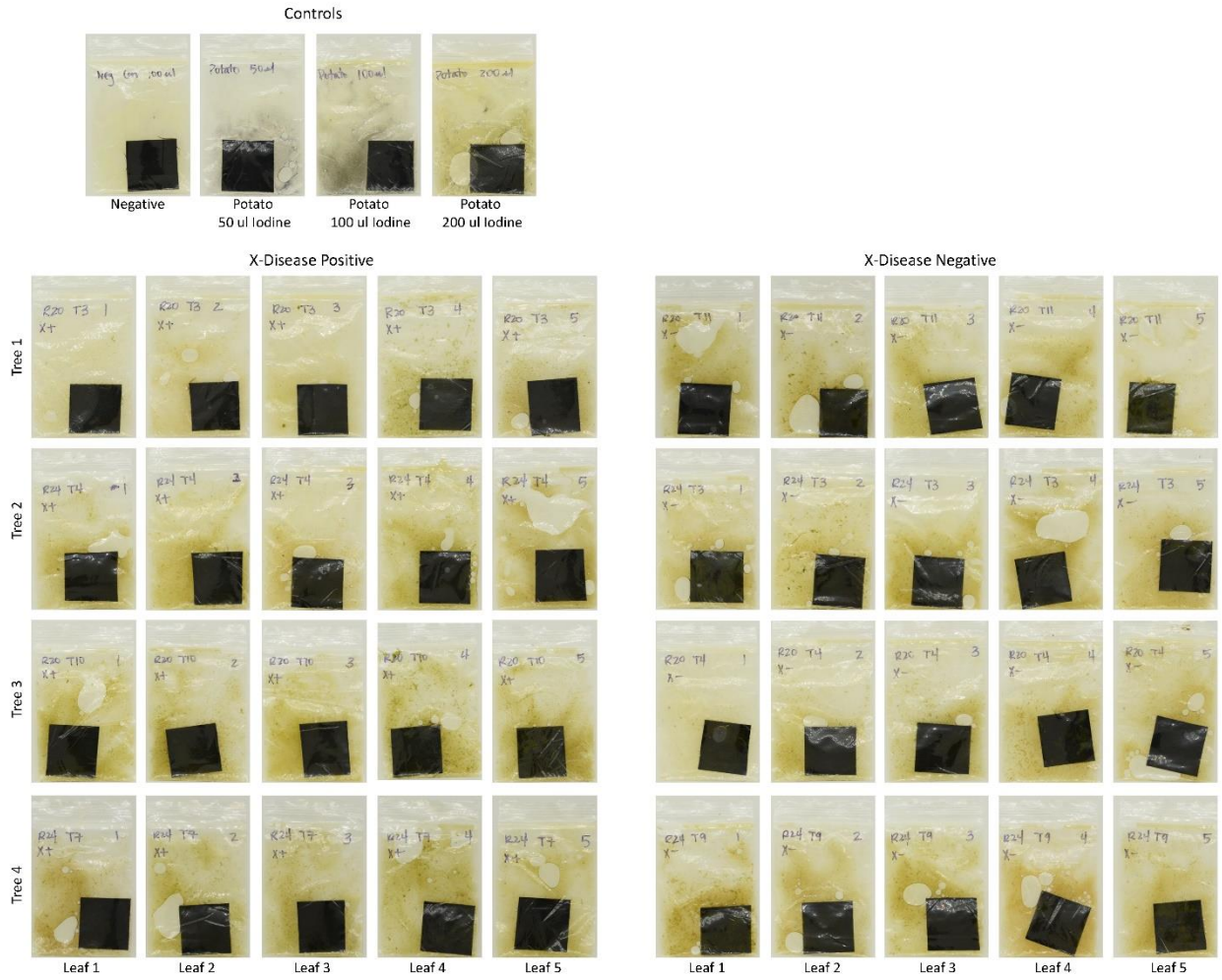

Figure 3. Baggy iodine method to detect starch in infected leaves. Sandpaper squares were used to accumulate leaf tissue from infected (left) and non-infected (right) leaves. 100 ul of iodine and 1 ml of water were added and color change was observed. No apparent difference between infected and non-infected leaves is present.

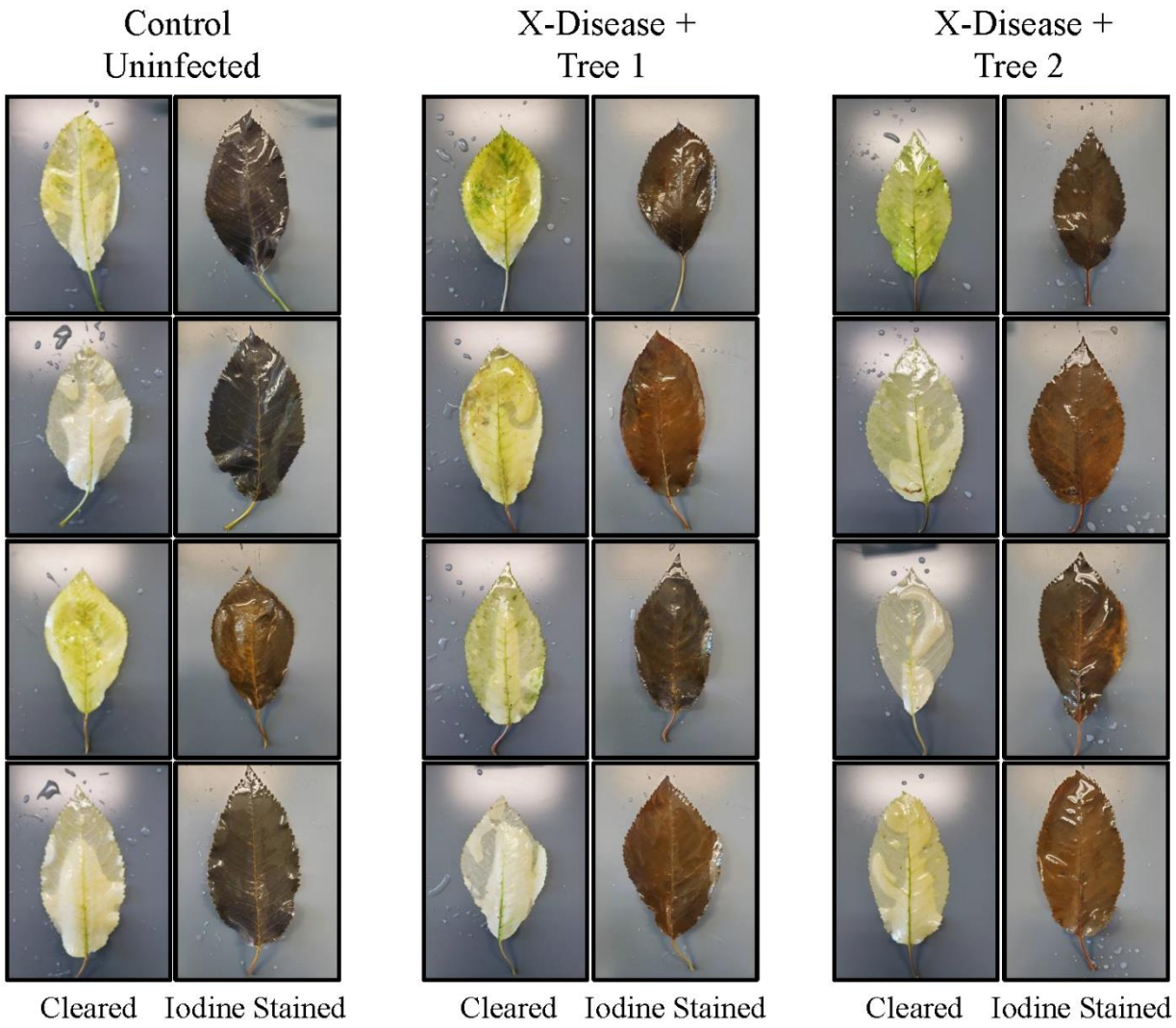

Figure 4. Clearing iodine method to detect starch in infected leaves. Leaves were cleared using boiling ethanol and stained with iodine. Uninfected leaves appear to contain higher levels of starch than infected leaves.

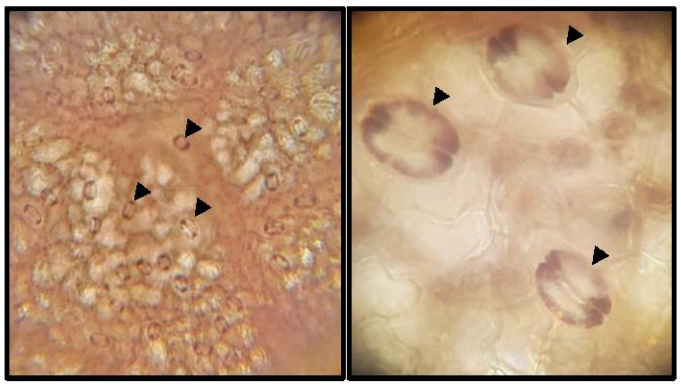

Figure 5. Leaves kept in the dark overnight prior to clearing and staining showed redistribution of starch to the guard cells of the stomata, indicated with black arrows.

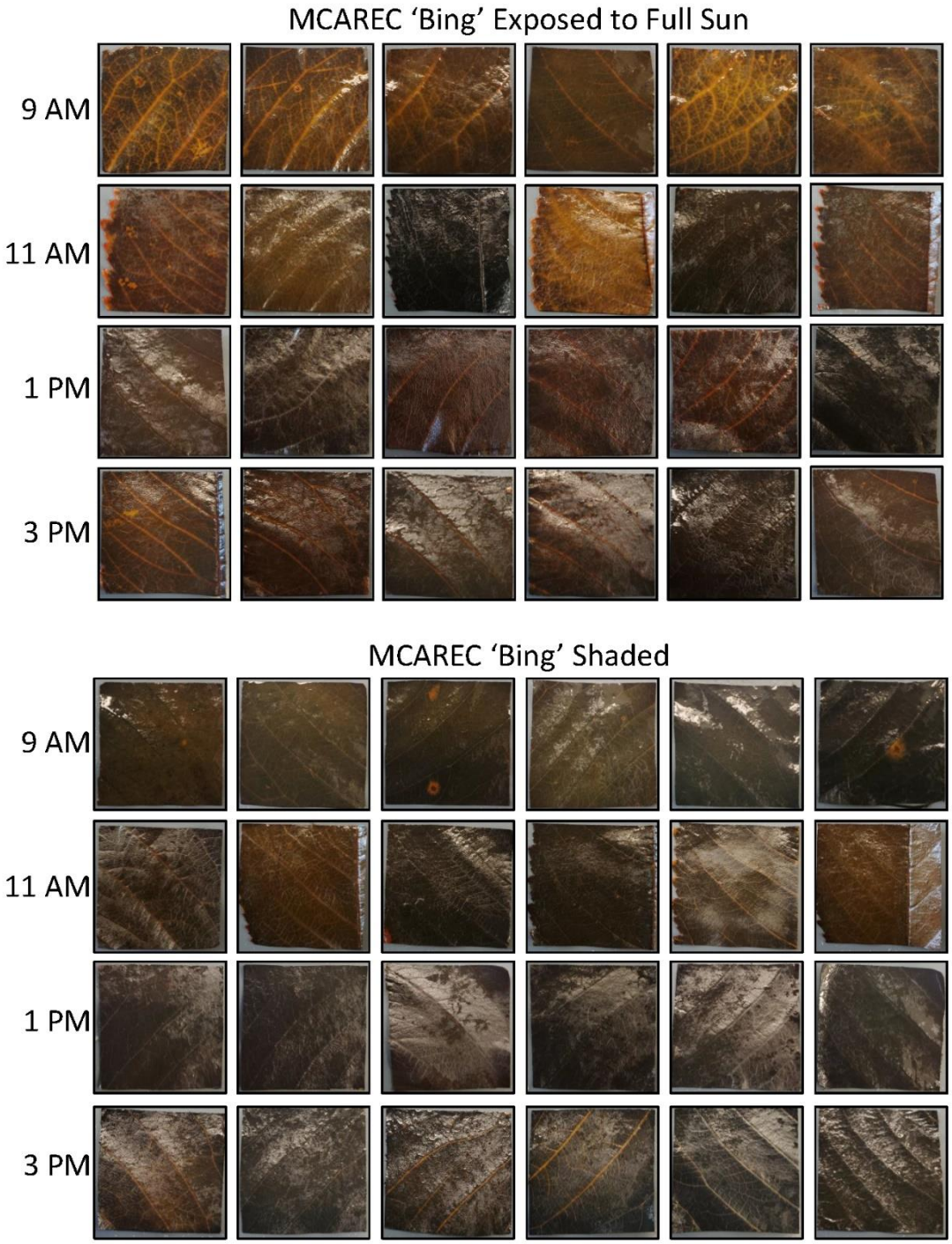

Figure 6. Clearing and staining method used on uninfected leaves, from exposed and shaded portions of the canopy. The presence of high levels of starch in uninfected leaves was unanticipated, as previous published research states that starch is absent in sweet cherry leaves. The higher concentration of starch in shaded leaves supports the new hypothesis, that starch is present in cherry leaves, but is dynamic, changing throughout the day and with different solar exposure.

Proposal Title: Real-Time Detection of Little Cherry Disease using Detector Canines

Report Type: Continuing Report

Primary PI: Jessica Kohntopp
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Cooperators: Cody Molnar (WSU), Hannah Walters (Stemilt Growers LLC), Teah Smith (Zirkle Fruit Company), Craig Harris (Harris Farms), Bill Howell (NNII), Todd Cameron (Cameron Nursery)

Project Duration: 2-Year

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 LLC
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 (34%)	$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	$64,578.00	$66,376.00

Footnotes: 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. Supplies for boxes/tins, harnesses, booties, canine training supplies, camera for videos, tripod, mini freezer etc. Travel to cover 5 trips of 5 days each to bring the canines to WA for blind studies, mock orchards, demonstration and outreach days. **Year-2** travel costs were doubled because canines will be in WA more times for in depth studies.

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

Item	2023	2024
Salaries		
Benefits		
Wages	$6,233.00	$43,631.00
Benefits		
RCA Room Rental		
Shipping		
Supplies	$955.80	$3,538.20
Travel		
Plot Fees		
Miscellaneous		
Total	$7,188.80	$47,169.20

Footnotes: Year-1: Co-PI Harper requests $315 for propagation costs, $640.80 for testing costs, and $6233 for labor cost to create and provide training materials for the canines. **Year-2:** Nursery Study: $105 for propagation costs, $6233 for grafting labor cost. Temporal Study: $390 for propagation costs, $3043.2 for testing costs, and $37,390 (0.5FTE) for labor costs.

Budget 3 : Removed until further notice.
Co-PI: Corina F. Serban
Organization Name: Washington State University
Contract Administrator: Anastasia (Stacy) Mondy
Telephone: (509)-335-2885
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Item	2022	2023
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	$2,500.00	$2,500.00

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

Objectives:

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

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

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

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

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

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

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

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

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

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

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

Objective Deviations: As of right now, there has not been any deviations from the objectives listed above, timelines have changed a bit but all objectives should still be finished on time.

Significant Findings:

- **Both canines have odor discrimination of the 8 positive plants we currently have,** distractor plants are now needed to confirm canines are sitting on LCD odor and not a different component.
- **Aika** has in total ran **3,873 plants** with **90.30% accuracy** (from initial stages til present time)
- **Humma** has in total ran **3,936 plants** with **91.88% accuracy** (from initial stages til present time)
- After moving outdoors in the ‘true training stages’ both canines have been above **98% accuracy**

Methods:

Canine Selection (May 29 - June 1):

Two canines were selected from a personal contact in Mexico. Ms. Kohntopp spent three days in Mexico testing over 15 canines to find the two that would work best for this job. The canines were evaluated based on two main criteria; hunt drive and play drive. Hunt drive: the want and ability to continually hunt for odor without getting discouraged by not finding the target odor right away. This ensures natural endurance for long searches. Play/ball drive: the want to do anything to get the toy (reward). This ensures the quick trainability of the canine. Other factors that were also considered during this time were environmentals (how the dog reacts in different environments), personality, and general overall health. The two canines selected for this project were a 13 month old Belgian Malinois named Aika (Left) and a 15 month old Dutch Shepherd named Humma (Right).

Training Material Set One (June 28):

The first set of training material was acquired on June 28th. This consisted of two Western X positive trees and 10 healthy trees (negatives). It should be noted that there were many factors different between the positive and negative trees, not just the infection. Positive trees were in 3 gallon pots whereas negatives were in 4" pots, positives were older than the negatives, taller, and more full of leaves. The soil, fertilizer, tagging, and potentially pot material were not the same. When training from live plants, it is imperative that all factors are the same except for the disease. Canine noses are extremely sensitive therefore they could easily be alerting on any number of the differences between those trees, not just the disease itself.

Delay to Training (July 9 - 24):

Both canines were taken in for a full veterinary examination on June 7th, a full blood panel and fecal exam was conducted but they missed tapeworms. Both canines were put on a dewormer July 10. Three days later Humma was taken into the vet again for an ear and respiratory infection. Humma was placed on antibiotics for 10 days. Odor training was not done during this time period because

canine's health always comes first. However this time was spent doing obedience training and working on canines' release of the toy.

Initial Training: Part One (July 26 - Sept 10):

Canines' odor training started up again on July 26th after Humma's health was back to 100%. Canine training was done in a controlled environment and consisted of verbal reinforcement paired with reward (brief play with toy) when the canine alerted on the correct target odor via self-discovery, whereas an incorrect alert was not reinforced. Training was started with one box with a hole cut out containing one positive plant. The trainer taught the canines to search the box by putting their nose in the hole and to alert by sitting. Once the canine started repeating this process on their own, another box containing a negative plant was added to the line up. The canine would search the two boxes and when the canine was proficient at alerting on the correct box, more boxes containing negative plants were added to the line up. The trainer would have liked the canines to search the physical plant much sooner, however, with the large visual differences, the plants had to stay in the boxes until new training material was received.

Noteworthy in Dog Training

Heat cycles in canines have been known to affect odor training in a handful of ways. I have seen canines that completely forget what they are supposed to do, others give up searching easily, some canines are thrown off by the odor of a different female in heat, the list goes on. However, both canines had their heat cycles and thankfully neither of them were affected.

Humma in heat: Aug 30 - Sept 17 Aika in heat: Sept 9 - 30.

Training Material Set Two (Sept 11):

The second set of training material was acquired on September 11th. This consisted of three Western X positive trees, three Little Cherry Virus 2 positive trees and 12 healthy trees (negatives). This time around all trees, both positive and negative, had similar characteristics. There were little to no visual differences between plants i.e. same pots, potting media, fertilizer, and were tagged the same. I was also given extra pots to re-pot the positives and negatives that I currently had along with the same potting media, fertilizer, and tags. This brings the training material tally up to 8 positive plants and 22 negatives.

Delay to Training (Sept 13 - 24):

Unfortunately shortly after picking up the plants from Prosser, Ms. Kohntopp acquired a stomach bug that took her out for 11 days.

Initial Training: Part Two (Sept 24 - 28):

Typically speaking, there is not usually a part two to the initializing steps. However, due to the multiple different factors in the first set of plants, I wanted to make sure the canines had a solid foundation on the 'correct' odor profile. The canines progressed quickly because they already had an understanding of the 'game' but it was apparent that canines had not imprinted on the LCD odor until they were re-initialized.

Training Grid (Sept 29 - Present):

Canines were moved outside to the training grid within five days of seeing the new training material.

It is important to get canines outside sooner than later because there are several distractions they have to learn to work through and overcome. The current training grid consists of 4 rows of 6 plants (24 plants total); we are limited to this size grid due to the number of plants we currently have access to. Both canines adjusted very well to being outside and are progressing better than expected. Outside distractions the canines have encountered: Wind, cows, insects, vehicles and ATVs driving by, birds, loud noises, additional outdoor odors, etc.

Distractor Training:

This is the next main step for the canines. 'Distractor Plants' are young live potted plants that are infected with other diseases that are commonly found in cherry orchards. This could also include environmentally stressed plants i.e. water deprived, nutrient deficient etc. This step ensures the canines are only alerting on the Target Odor and not another pathogen or generally sick plant. We are currently waiting for the distractor plants to be ready for training. Once the trainer believes canines are proficient on Little Cherry Disease discrimination, a blind study will be performed.

Blind Studies

This will be conducted to test the accuracy, sensitivity, and specificity of the canines in the training grid. The blind study will include 100 LCD positive plants and 900 negative plants for a total of 1000 plants per canine. Since there will not be enough plants for one run of 1000, it will be divided into multiple different runs of the number of plants we have available to us at the time. This study was originally planned to be conducted in Idaho but due to one of the Co-PIs current status being unknown, the canines might be brought up to WA instead. Each run will be completely randomized via a computerized generated program, and the trainer will not know the location of the true positive plants. When canine alerts, the trainer will raise their hand to call the alert. Observer will verbally confirm/deny the alert so the canine can be rewarded/pulled off alert and the run will continue. Canines will alternate between runs and plants will be moved after each canine. The canine responses will be evaluated as a true positive rate (TPR; sensitivity), true negative rate (TNR; specificity) and overall accuracy (TP+TN/n) as defined below and described by Gottwald et al., 2020. The definitions and formulas for the evaluation of canine performance have been utilized for previous successful efforts to train canines to detect pathogens causing plant diseases and are listed

below.

Where n = total population assessed.
True Positive (TP) correct canine alert on positive sample
True Negative (TN) correct rejection, no alert on negative sample
False Positive (FP) false alert on negative sample, Type I error
False Negative (FN) missed positive target, Type II error

Sensitivity (SEN) or true positive rate, = TP/(TP + FN)
Specificity (SPE) or True Negative Rate = TN/(FP + TN)
Precision or Positive Predictive Value (PPV) = TP/(TP + FP)
Negative Predictive Value (NPV) = TN/(TN + FN)
False Positive Rate (FPR) = FP/(FP + TN)
False Negative Rate (FNR) = FN/(FN + TP)
False Discovery Rate (FDR) = FP/(TP + FP)
Accuracy (ACC) = (TP + TN)/n

The goal is to get the canines to **>95% accuracy, >90% sensitivity, and >90% specificity**.

Mock Cherry Orchard Training

If the blind study is conducted in WA, this would be a perfect time to introduce canines to their first orchard. There is a cherry block at WSU IAREC in Prosser, WA, where there are known LCD positive and negative trees. Although the orchard is the same concept as young trees in the training grid, it can sometimes not be as straightforward to the canines. In the initial stages, canines might need a little extra help with verbal reinforcements, quick rewards, and possibly actively presenting the cherry trees. Once canines begin understanding the task at hand, the trainer will allow the canine to be independent again only reinforcing positive alerts. Canines may also struggle due to the time of year. There might not be enough odor present for canines to properly alert. Either way, it will be good exposure for the canines so when they come back to truly train in an orchard setting, it will not be brand new.

Results and Discussion:

Both canines have made tremendous strides since the initial stages of training each with their own hiccups along the way. Some important points to note from the datatables below, both canines have seen over 3,000 negative plants and well over 400 positive plants from the beginning of training. These numbers, however, are not from 3,400 new or different plants. They all stem from the same 8 positives and 22 negatives. There is always concern with running the canines that many times on the same plant for fear they are memorizing the specific plant rather than alerting on an odor. I am not concerned about this with these two canines. In their beginning stages they were trained with the same 2-3 positive plants. Anytime I introduced a new positive plant both canines alerted without hesitation or confusion. Had they passed up the new hot plant on their first run then I would be concerned about memorization. It should be noted that the misses and falses seen in Total Runs, Box Runs, and Indoor Plant Runs includes the timeframe the canines were still learning. It was not until the plants were moved outside that the canines had a solid

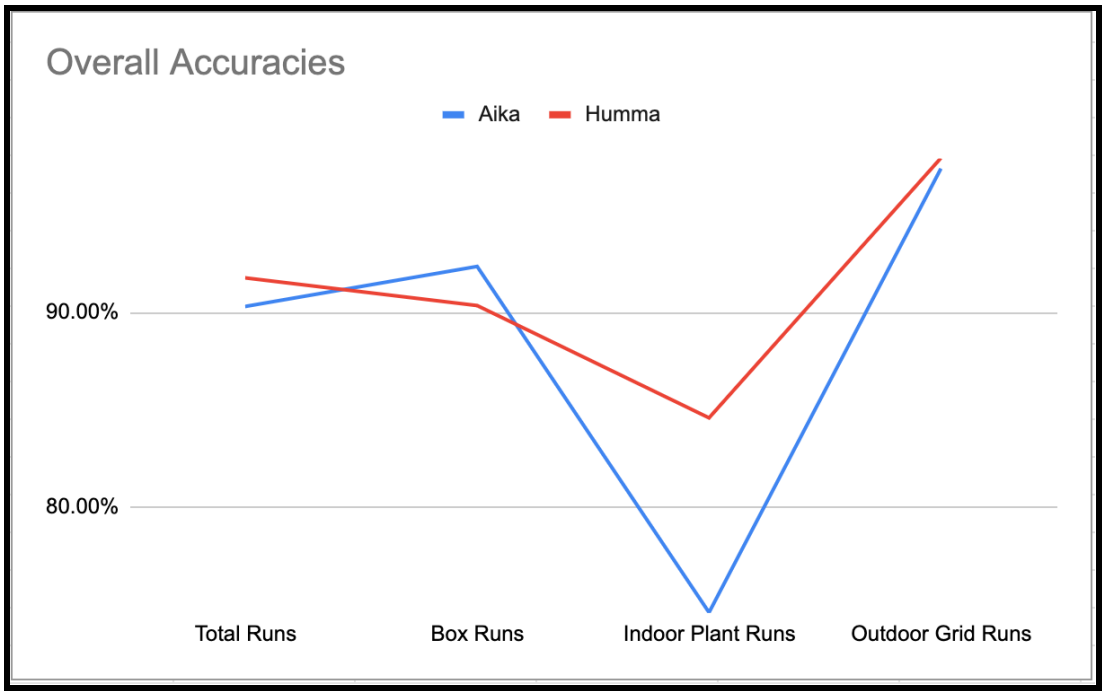
understanding of the game. From Sept 29th on, we will be getting more accurate data on what the canines are capable of doing which is shown in the highlighted column. A very interesting point to note is the dip in the accuracy for both canines during the Indoor Plant Runs. This is largely due to the inconsistencies in the first set of plants. When I introduced the second group of plants, both canines continually passed up the hot plant and we had to start from the beginning stages of odor initialization again. This tells me that during the Box Runs neither canine was imprinted on LCD odor. I have more confidence that the canines are closer to being imprinted on the correct odor since training with the second group of plants. However, distractor plants are a crucial point in determining what the canines are alerting on. Both canines are showing great promise and have great commitment when they alert on a positive plant. The canines cannot be pulled off nor coax away once they have their mind set.

AIKA	Total Runs	Box Runs	Indoor Plant Runs	Outdoor Grid Runs
Number of Runs	421	214	116	91
Total # of +	495	214	116	165
Total # of -	3378	823	692	1863
Canine Alerts	447	198	87	162
Canine False Alerts	15	3	12	0
Canine Misses	48	16	29	3
Accuracy	90.30%	92.52%	75.00%	98.18%

Aika Table: Total run data is taken from the first day canine recognized 'hot' odor on 8/7 til the last training day 10/15. Box data is taken from when plants were still in boxes between 8/7 - 9/23. Indoor plants data is taken from plant training days in a controlled setting between 9/24 - 9/28. Outside data was taken from 9/29 - 10/15.

HUMMA	Total Runs	Box Runs	Indoor Plant Runs	Outdoor Grid Runs
Number of Runs	430	228	109	93
Total # of +	505	228	109	168
Total # of -	3431	876	647	1908
Canine Alerts	464	206	92	166
Canine False Alerts	18	7	11	0
Canine Misses	41	22	17	2
Accuracy	91.88%	90.35%	84.40%	98.81%

Humma Table: Total run data is taken from the first day canine recognized 'hot' odor on 8/7 til the last training day 10/15. Box data is taken from when plants were still in boxes between 8/7 - 9/23. Indoor plants data is taken from plant training days in a controlled setting between 9/24 - 9/28. Outside data was taken from 9/29 - 10/15.



Overall Accuracies: It is important to note the dip when it came to the Indoor Plant Runs. This could be due to many factors, but it shows neither canine was initially imprinted on LCD odor.

All training records and videos are available upon request

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. This has caused a large economic impact on growers of over $115 million. 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. The hopes of this project is not only to prove that canines can find and alert on Little Cherry Disease odor but that they could be the year-round, real-time, non-destructive diagnostic tool that is needed in this industry to help mitigate the spread of LCD.

Project Title: Coordinating SWD and X Disease Management

Report Type: Continuing Project Report

Primary PI: Elizabeth H. Beers
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Cooperators: Ash Sial, Rufus Isaacs, Kent Daane, Hannah Burrack, Joanna Chui, Frank Zalom

Project Duration: 3 Years

Total Project Request for Year 1 Funding: $ 24,865
Total Project Request for Year 2 Funding: $25,800
Total Project Request for Year 3 Funding: $26,772

Other related/associated funding sources: Awarded
Funding Duration: 2020- 2024
Amount: Beers: 18,634, 17,751, 16,890; Northfield: 72,197, 73,313, 73,817
Agency Name: USDA ScRI
Notes: This USDA-SCRI project is a national collaboration of SWD researchers covering sweet cherries and berries. Funding is for 4 years (Sept. 1, 2020 through August 31, 2024).

WTFRC Collaborative Costs: none

Budget 1
Primary PI: Elizabeth H. Beers
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Item	Year 1: 2021	Year 2: 2022	Year 3: 2023
Salaries[1]	13,752	14,302	14,874
Benefits[2]	4,839	5,033	5,234
Wages[3]	3,900	4,056	4,218
Benefits[4]	874	909	946
RCA Room Rental			
Shipping			
Supplies	$1,500.00	$1,500.00	$1,500.00
Travel			
Plot Fees			
Miscellaneous			
Total	$24,865.00	$25,800.00	$26,772.00

Footnotes: [1]Salaries: 0.25 FTE post-doc; [2]Benefits (salaries): 35.2%; [3]Wages: $15/hr, 20 hr/week, 13 weeks/yr; [4]Benefits (wages): 22.4%.

Objectives:
(Objectives 1-5 are the objectives in the leveraged SCRI proposal covering a broad range of US regions and crops affected by SWD; Objective 6 is an additional objective solely for Washington cherry and is the main focus of this continuing report.)

1. Implementation of best management programs for sustainable management of SWD in collaboration with grower influencers.
2. Develop economics-based decision aid tools to support the identification and implementation of profit-maximizing SWD management strategies.
3. Evaluate sustainable alternatives to insecticides for long-term SWD management.
4. Assess and reduce the risk of insecticide resistance development.
5. Develop and disseminate actionable recommendations that enable producers to optimize pest management decisions, and evaluate their impact.
6. Determine the impact of SWD controls on leafhopper vectors of X-disease.
    a. As the sustainable alternatives to SWD insecticides (Obj. 3) are currently being tested and adapted for the unique climate and growing conditions that defines Eastern Washington cherry production, Obj. 6 was altered to assess the impacts of X-disease leafhopper vector management on SWD populations in Eastern Washington cherry orchards.
    b. Given the potential horticultural benefits of Extenday and Surround WP kaolin clay foliar application and recent findings on the potential for these products to control leafhopper vectors of X-disease phytoplasma, the aim of the revised objective was to assess these products as part of an integrated approach to SWD management in Eastern Washington cherry production.

Significant Findings:
Objective 6
- There is preliminary evidence that biorational control of leafhoppers and SWD will be complementary

2023:
- Extenday and weed mat do not significantly reduce SWD oviposition in comparison to the control.
- Over a 24 hour exposure period, Extenday and weed mat did not significantly reduce egg viability (lab-emerged adults post experiment) compared to the control.

2022:
- At Cashmere 1, Extenday reduced SWD adult trap counts by 58.2% in comparison to the control.
- At Cashmere 2, Extenday applied postharvest reduced SWD adult trap counts by 67.6% in comparison to the control.

2021:
- At the Wenatchee site, Extenday applied postharvest reduced SWD adult counts by 65.83% while Surround reduced SWD adult counts by 66.60% in comparison to the control. Mowed blocks were comparable or hosted more SWD than the control.
- At the Wapato site, Extenday applied postharvest reduced SWD adult trap counts by 47.34% while Surround reduced SWD adult trap counts by 37.32%.

Methods:
Objective 6
Current Year: We hypothesized that the observed reduction in SWD trap capture in Extenday blocks was due to reflected light in those blocks disrupting host orientation behavior. This mechanism has been observed for other orchard pests such as pear psylla (Nottingham and Beers 2020, Nottingham et al. 2022). We speculated that Extenday induced disruptions in host orientation behavior would reduce ovipositions by female SWD into cherry fruit. In 2023, we conducted a mesocosm experiment to

assess the potential impact of Extenday and black weed mat barrier on SWD oviposition in cherry. This trial was conducted in late September to allow for environmental conditions conducive for improved fly survival in our experimental cage setup.

Three treatments (Extenday, weed mat, and uncovered control) were assessed in mesocosm cages (56 in x 23 in x 23 in screen cages, Raising Butterflies LLC, Salt Lake City, UT). Five replicate cages for each treatment were set up across three adjacent rows in a randomized complete block design at the TFREC pear orchard (15 ft tree by 20 ft row spacing). The pear orchard provided an environment with the shade normally associated with mature trees, along with the temperature and RH of an orchard floor. Within each row, cages were spaced 10 ft apart (Plate 1). Each cage contained two trays (20 in x 10 in plastic growing trays) of bluegrass sod (Harmony Outdoor Brands, Lakewood Ranch, FL) (Plate 2). The sod in the Extenday and weed mat cages were covered with a 56 in x 23 in rectangle of Extenday reflective groundcover or black polypropylene landscape fabric (Greenscapes Inc, Calhoun, GA) respectively (Plates 3, 4). In each cage, a one gallon jug holding four cherry branches was placed between the two sod trays in the center of the cage. Cherry branches (~1-1.5 ft in length) were sourced from an unsprayed cherry orchard at WSU Sunrise Research Orchard. Each cherry branch had five 'Sweetheart' cherries attached with a binder clip resulting in 20 cherries per cage. No other food sources or oviposition substrates were present. Adult female SWD (100/cage + 20 males) were released into each cage at 12 pm on Sept 21. They were allowed to oviposit for 24 hours before adults were removed and ovipositions were counted. The fruit was kept for 3 weeks in the lab to assess SWD emergence.

Plate 1. Mesocosm cages in the TFREC pear orchard.

Plate 2. Control cage with cherry setup between sod trays

Plate 3. Extenday cage with cherry setup over Extenday covering sod trays.

In each treatment, one cage hosted the environmental sensors and dataloggers. HOBO Pro v2 dataloggers (Onset Computer Corporation, Bourne, MA) continuously measured temperature and relative humidity. The HOBO datalogger was hung from the south facing cherry branch. Reflected light (measured as Photosynthetic Photon Flux Density) was

continuously measured with SQ-520 Full Spectrum quantum sensors (Apogee Instruments Inc, Logan, UT). The quantum sensor was fixed to the inner ceiling of the cage with a binder clip. Due to an unexpected power loss, the control cage's quantum sensor was only able to record for the first 21 hours of the experiment. In addition, a Lighting Passport Essence Pro spectrometer (Asensetek Inc, New Taipei City, Taiwan) took point measurements of the relative intensity of the different wavelengths of light reflected by each surface at 2 pm on Sept 21 and then at 9 AM and 12 PM on Sept 22.

Plate 4. Weed mat cage with cherry setup on top of weed mat covering sod trays.

Previous Years: The impacts of post-harvest X-disease vector leafhopper management on SWD populations was assessed in 2021 at two cherry orchard sites near Wapato and Wenatchee respectively. The Wapato cherry orchard consisted of 28 acres of 'Sweetheart' cherries (12 ft tree by 18 ft row spacing) and was conventionally managed. The Wenatchee cherry orchard consisted of ~25.9 acres of 'Coral Champagne' cherries (10 ft tree by 15 ft row spacing) and was in the first year of transitioning from conventional to organic management.

At the Wenatchee orchard, four treatments were assessed: Extenday groundcover (Extenday USA Inc, Union Gap, WA), Surround kaolin foliar application (NovaSource, Phoenix, AZ), weekly mowing, and an untreated control. Each treatment was repeated in a randomized block design with 4 replicate blocks for each treatment. Each block was 200 ft long and 12 rows wide. Treatments were maintained from mid-July until early November with adult SWD trapped throughout this period.

Each replicate had two traps, one at 50 ft and one at 150 ft from the block 's edge in the middle (seventh) row. The traps consisted of a modified 32 oz plastic jar with a Scentry SWD Lure (Scentry Biologicals Inc., Billings, MT) suspended from the lid in front of screened holes to allow the odor of the lure to diffuse, and the flies to enter the trap. Flies were retained and preserved in 300 mL of drowning solution (5 L water, 50 g sodium benzoate, 50 mL unscented dish soap). Drowning solution was changed and trap contents collected every two weeks. Trap contents were assessed under a dissecting microscope (Leica Microsystems, Wetzlar, Germany) and the number of SWD males and females was noted. The Scentry SWD lures were replaced every 6 weeks.

At Wapato, three treatments were assessed; Extenday groundcover, Surround kaolin foliar application, and an untreated control. Each treatment had 2 replicate blocks. Each block was 200 ft long and 12 rows wide. Treatment blocks were maintained from mid-July until mid-October when the treatments were removed in preparation of the block's removal in November (due to high prevalence of X-disease). Sampling for adult SWD was conducted as described above, except there were four traps per block (at 50 feet in row 5, 100 feet in rows 6 and 8, 150 feet in row 7).

The Wapato site's cherry trees were removed in October 2021 due to the ongoing X-Disease epidemic, preventing this site from being surveyed in 2022. As the Wenatchee site was transitioning to certified Organic management, it could not participate in the 2022 experiments given the addition of the conventional herbicide treatment.

In 2022, we conducted an assessment of two cultural control practices (Extenday and herbicide) compared to an untreated control at two conventionally managed orchard sites near Cashmere, WA. The first orchard (Cashmere 1) consisted of 5.37 acres of 'Rainier' sweet cherries (9 ft tree by 15 ft row spacing), and the second orchard (Cashmere 2) consisted of 3.13 acres of 'Rainier' sweet cherries (10 ft tree by 18 ft row spacing).

At each orchard, the three treatments (Extenday, herbicide, and untreated control) were repeated as two replicate blocks. Each block was 130 ft long and 6 rows wide. Treatment blocks were setup in late May and maintained until the end of October. The herbicide treatments consisted of 1 preharvest groundcover application of SPUR (Clopyralid, Albaugh LLC, Ankenny, IA) on May 20th and 1 postharvest groundcover application of Venue (Pyraflufen ethyl, Nichino America Inc, Wilmington, DE) on 25 July. These herbicides were applied to row middles to control broadleaf weeds. At Cashmere 1, the Extenday Block was maintained for the duration of the experiment. At Cashmere 2, Extenday was maintained by the grower collaborator across all 3 treatment blocks from 30 May 30-27 June due to concerns of ripening. The Extenday was removed from all Cashmere 2 blocks by June 2 for harvest and was then re-applied solely to the designated Extenday Blocks on 15 July. As such, our Cashmere 2 analyses consist only of post-harvest comparisons.

Surveys to collect and identify SWD adults was conducted as described for 2021, except the drowning traps were changed weekly. Each replicate had two traps, one in the second row at 30 ft from the block's edge and another in the third row at 65 ft from the block edge.

Results and Discussion:
Current Year: In the 2023 mesocosm experiment, the effect of treatment on SWD ovipositions/cage was not significant. SWD oviposition in Extenday and weed mat cages was comparable to control cages (Table 1, Figure 2a) The effect of treatment on lab-emerged adult flies/arena was not significant (Table 1, Figure 2b).

There was more reflectance from Extenday than from the weed mat or the control (Figure 3). The grass control also reflected more light than the weed mat.

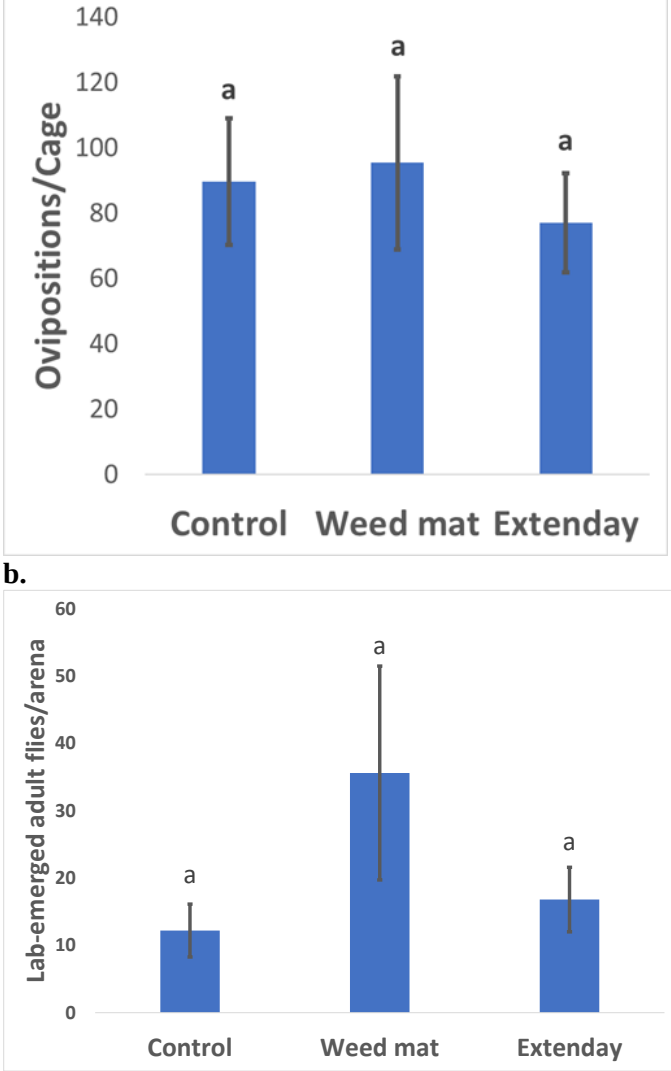

Fig. 2. a. SWD ovipositions/cage by treatment, **b.** lab-emerged adult flies/arena by treatment, 2023.

The relative intensity of red, orange, yellow, green, blue, violet, and ultraviolet light reflected by Extenday was greater than those same wavelengths reflected by the weed mat or control (Figure 4).

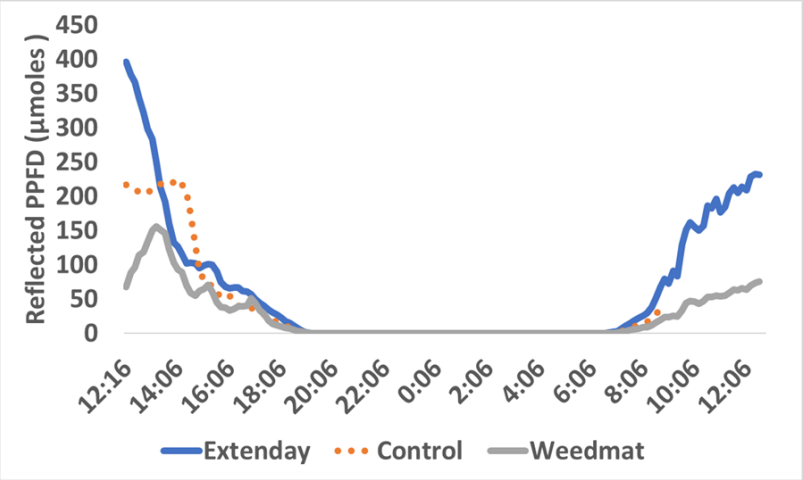

Fig. 3. Reflected photosynthetic photon flux density (PPFD, µmoles of light) by treatment, 2023.

Although recorded temperatures were similar among the three treatments (Figure 5a), the relative humidity in the Extenday and weed mat cages was lower than in the control (Figure 5b). Although the amount and intensity of light was greater in Extenday than in the other blocks, the reflected light did not interfere with SWD egg laying behavior. The results of the 2023 mesocosm experiment suggest that mechanisms other than reduced oviposition are responsible for the suppression of SWD in Extenday blocks. Although the viability of eggs did not differ among the treatments, it is possible that the 24 hour exposure period utilized in our trial is too short to assess egg viability and subsequent larval development in fruit on the trees. Prior studies with plastic mulches in raspberry noted that plastic mulches (metallic, black, and white) significantly reduced SWD larval counts in fruit as well as adult trap capture compared to the control (McIntosh et al. 2023, McIntosh et al. 2021). Those studies noted that reduced larval development in fruit might be due to plastic mulch induced changes in canopy microclimate. It is also possible that the Extenday serves as a physical barrier that prevents SWD larvae and pupae from completing their development in the soil. In raspberry, dropped larvae and pupae suffered increased mortality after 4 hours on plastic mulches compared to a grass control (C. Guédot, personal communication). With X-disease leafhoppers, Extenday is primarily thought to reduce trap capture by preventing access to broadleaf weeds and other groundcover hosts (Marshall et al. 2023).

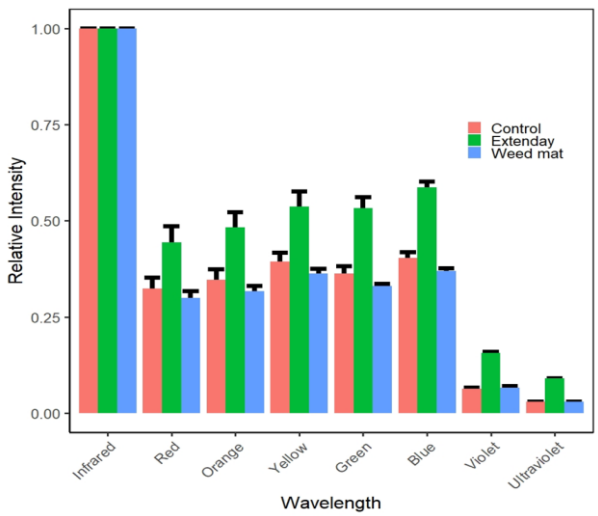

Fig. 4. Relative intensity of light wavelengths reflected by each treatment, 2023.

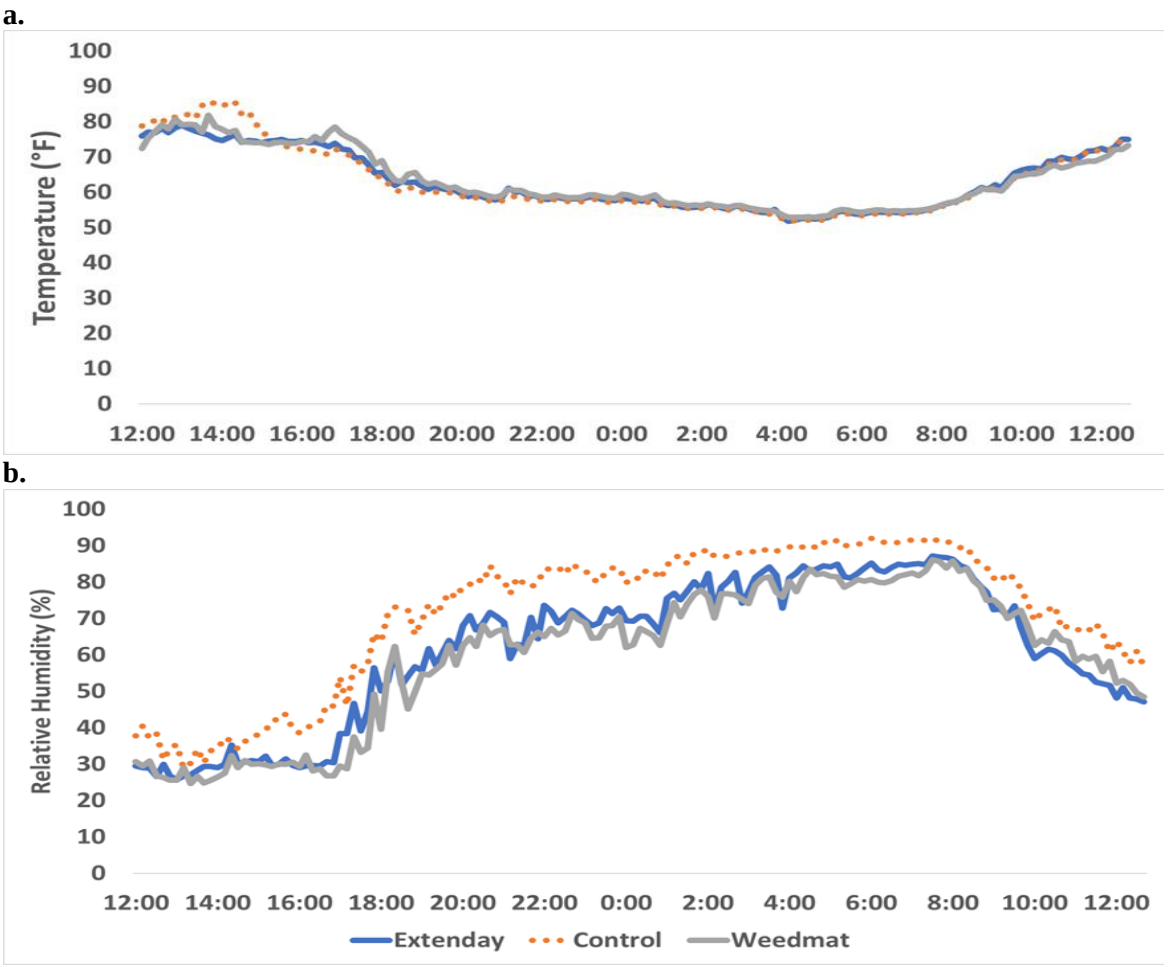

Fig. 5. a. Cage temperature (°F) and **b.** relative humidity (%) by treatment, 2023.

Previous Years: SWD counts across all treatments were initially low, potentially due to the extreme heatwave events that the Pacific Northwest experienced during the summer of 2021. At Wapato, SWD counts in traps started to increase in late August while at Wenatchee, SWD counts remained low until late September (Figure 6a, 6b). At the Wenatchee site, there was a significant effect of treatment ($X^2 = 175.7$, df = 3, $P < 0.0001$) on SWD collected per trap. Extenday applied postharvest reduced SWD adult counts by 72% while Surround reduced SWD adult counts by 71% in comparison to the control (Figure 6c, Table 2). Mowed blocks hosted more SWD than control blocks. At the Wapato site, there was a significant effect of treatment ($X^2 = 18.919$, df = 2, $P < 0.0001$) on SWD collected per trap. Extenday applied postharvest reduced SWD adult counts by 47.9% while Surround reduced SWD adult counts by 41.3% (Figure 6d, Table 2).

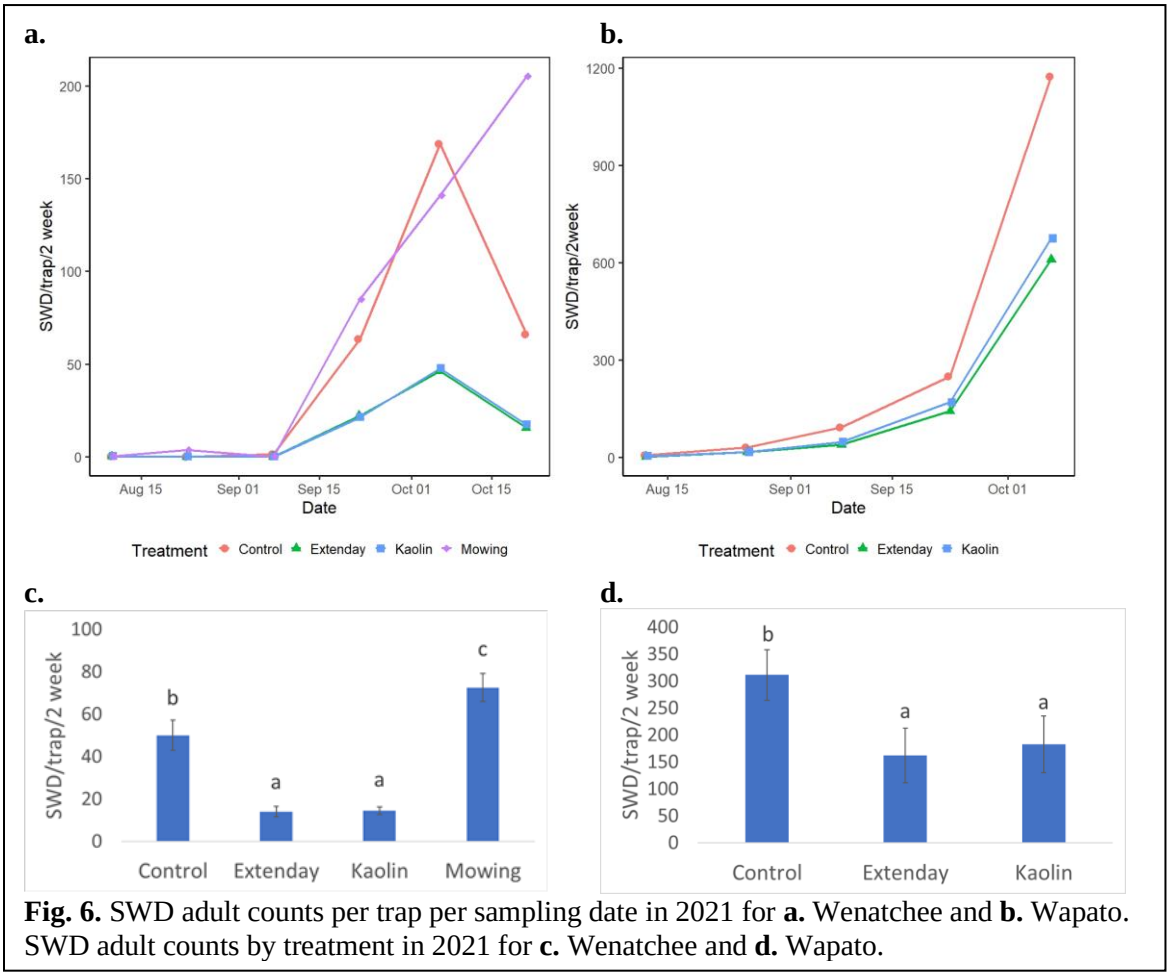

Fig. 6. SWD adult counts per trap per sampling date in 2021 for **a.** Wenatchee and **b.** Wapato. SWD adult counts by treatment in 2021 for **c.** Wenatchee and **d.** Wapato.

In 2022, SWD counts in traps at both Cashmere sites remained low until mid-September (Figure 7a, 7b). At Cashmere 1, there was a significant effect of treatment ($X^2 = 9.6809$, df = 2, $P < 0.01$) on SWD collected per trap. Extenday reduced SWD adult counts by 58.2% while herbicide reduced SWD adult counts by 17.8% in comparison to the control (Figure 7c, Table 3). At Cashmere 2, there was a significant effect of treatment ($X^2 = 37.179$, df = 2, $P < 0.001$) on SWD collected per trap. Extenday applied postharvest reduced SWD adult counts by 67.6% while herbicide treated blocks increased SWD adult counts by 37% (Figure 7d, Table 3).

The results from 2021 and 2022 suggest that postharvest canopy and groundcover-based management of leafhopper vectors of X-disease phytoplasma may also reduce SWD populations in cherry orchards. The reduced counts of SWD observed for Extenday and Surround treated blocks were observed in orchards representative of high pressure and low pressure situations for both SWD and X-disease leafhoppers. The 2022 trials support the use of Extenday to suppress SWD. These preliminary

results suggest that these integrative management options may be viable under a wide scale of potential pest pressure.

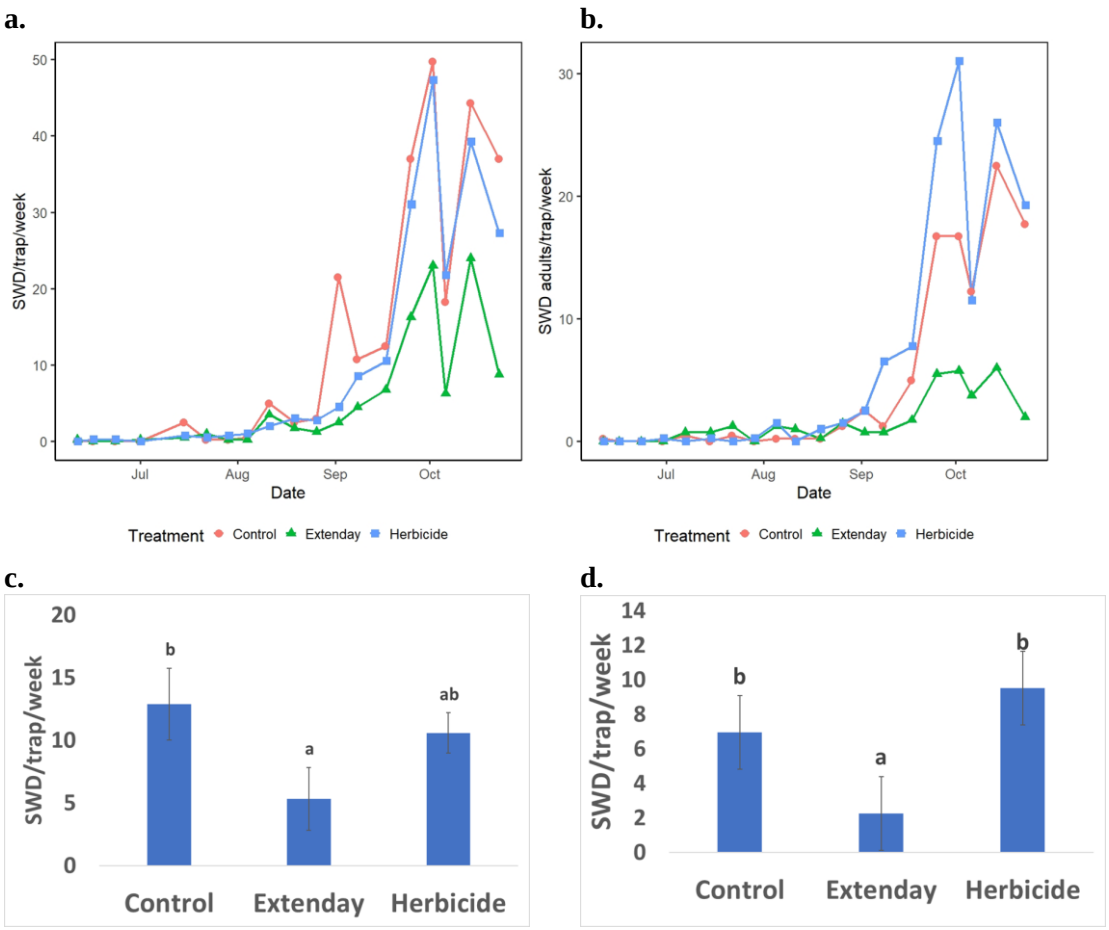

Fig. 6. SWD adult counts per trap per sampling date in 2022 for **a.** Cashmere 1 and **b.** Cashmere 2. SWD adult counts by treatment in 2022 for **c.** Cashmere 1 and **d.** Cashmere 2.

References

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 175, 106445.

McIntosh, H., C. Guédot, and A. Atucha (2022). Plastic mulches improve yield and reduce spotted-wing drosophila in primocane raspberry. Scientia Horticulturae 320, 112203.

McIntosh, H., A. Atucha, P.A. Townsend, W. Beckett Hills and C. Guédot (2021). Plastic mulches reduce adult and larval populations of *Drosophila suzukii* in fall-bearing raspberry. Journal of Pest Science 95, 525-536.

Nottingham, L.B., R.J. Orpet, and E.H. Beers (2022). Integrated pest management programs for pear psylla, *Cacopsylla pyricola* (Förster) (Hemiptera: Psyllidae), using kaolin clay and reflective plastic mulch. Journal of Economic Entomology 115, 1607-1619.

Nottingham, L.B. and E.H. Beers (2020). Management of pear psylla (Hemiptera: Psyllidae), using reflective plastic mulch. Journal of Economic Entomology 113, 2840-2849.

Table 1: SWD ovipositions/cage by treatment, mesocosm cage trial, 2023

Treatment	n	Ovipositions/cage	Emerged adult flies/arena
1. Control	5	89.6 a	12.2 a
2. Extenday	5	77.0 a	16.8 a
3. Weed mat	5	95.4 a	35.6 a

Table 2: SWD catch by treatment and percentage reduction from the control, field trial, 2021

	Wenatchee[a]		**Wapato**[a]	
Treatment	SWD/trap/2 wk	% Reduction	SWD/trap/2 wk	% Reduction
1.Control	50.00 b	-	311.10 b	-
2. Extenday	14.00 a	72.0%	162.08 a	47.9%
3. Kaolin	14.48 a	71.0%	182.70 a	41.3%
4. Mowing	72.54 c	-45.1%	NA[b]	NA[b]

[a]Treatment blocks were replicated four times at Wenatchee and two times at Wapato.
[b]Mowing treatment was not conducted at Wapato.

Table 3: SWD catch by treatment and percentage reduction from the control, field trial, 2022

	Cashmere 1[a]		**Cashmere 2**[a]	
Treatment	SWD/trap/wk	% Reduction	SWD/trap/wk	% Reduction
1.Control	12.89 b	-	6.95 b	-
2. Extenday	5.31 a	58.2%	2.25 a	67.6%
3. Herbicide	10.59 ab	17.8%	9.52 b	-37.0%

[a]Treatment blocks were replicated two times at Cashmere 1 and 2.

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

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

Cooperators:
Mike Omeg, Stacey Cooper, Brian Nix

Project Duration: 3 Year

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

Other related/associated funding sources: N/A

WTFRC Collaborative Costs:

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

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

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

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

Objectives

1) Establish and increase a colony of *Ganaspis brasiliensis* wasps (year 1)
2) Conduct releases at selected orchards (years 2 & 3)
3) Measure establishment of wasps (year 3)

Significant findings

- We have established a colony of *Ganaspis brasiliensis* wasps

Proposed Timeline

Objective	2023	2024	2025
1. Establish and increase colony of wasps	X	X	X
2. Conduct releases		X	X
3. Measure establishment			X

Methods

1. **Establish a colony of *Ganapsis brasiliensis* wasps**. Rearing protocols have been published by several authors (Rossi-Stacconi et al. 2022) and I have visited with ODA to see their colony rearing procedures (Figure 1). We will spend year one trying to get sufficient numbers of wasps to conduct effective releases at several orchards in The Dalles

Potential pitfalls

Rearing these wasps is very labor intensive and requires daily maintenance including providing a supply of SWD larvae of specific age to adult wasps to parasitize. Mass rearing has not been perfected and lots of wasps die even with daily attention. Development time is slow and female wasps only produce a few dozen eggs in their lifetime.

Anticipated results

We have already started a colony of *G. brasiliensis*, and it has been slowly growing. Through collaboration and communication with other researchers in the region, we expect to grow this colony over the coming months.

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

Potential pitfalls

Releases done near managed orchards are at risk of pesticide sprays. We will work hard to find location that are near, but not within the possible drift, of managed sweet cherry orchards. Several patches of blackberry were removed or impacted by wildfires last year. We will communicate with growers to insure that habitat loss for the wasp is avoided. Excessive heat in the summer could also put this insect at risk as it does not like extreme heat.

Anticipated results

As long as there are wild SWD populations, and we don't have another heat dome, we anticipate that *G. brasiliensis* will establish where released. Over time (many years) we should see SWD numbers be reduced in these habitats around managed orchards. Future research will be needed to measure that long term impact.

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

Potential pitfalls

There is an element of chance involved with sampling for tiny insects. Establishment may occur but we may not be able to measure that success.

Anticipated results

This insect has demonstrated the ability to become established in the PNW. I expect that we will demonstrate successful establishment of this beneficial wasp.

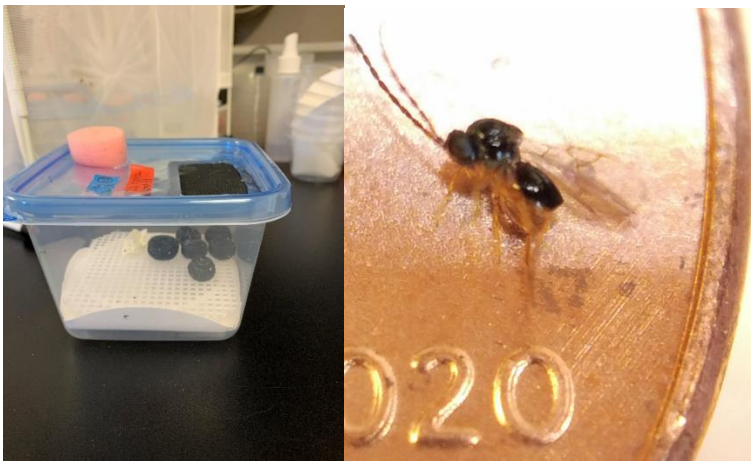

Figure 1. *Ganapsis brasiliensis* rearing container (left). Blueberries are placed in SWD colony to allow flies to lay eggs. Blueberries with eggs and young SWD larvae are placed in containers with newly emerged wasps, *Ganapsis brasiliensis* (right) on a penny for scale.

Results and Discussion

Although labor intensive we have successfully started a colony of this parasitic wasp. Using fresh blueberries is expensive and a major bottleneck for this project. We also struggled with mold, despite our best efforts to keep the insectary clean. A regional working group is working on ways to improve rearing techniques and to develop an artificial diet to replace the need for fresh fruit.

The invasive spotted wing drosophila (SWD) has disrupted integrated pest management in sweet cherry across the Pacific Northwest. Currently control of this insect pest can only be accomplished through regular applications of broad-spectrum insecticides such as organophosphates, pyrethroids, and neonicotinoids. The current management programs are costly, unsustainable, and a recipe for future pesticide resistance. Alternative management tools are critical for long term control of this key pest. Even the best pesticide program cannot control pest populations outside the orchard. Because SWD can reproduce on a wide range of host plants including Himalayan blackberry, there is a constant threat of re-invasion from just outside the boarders of every orchard. These boarder habitats would be an ideal habitat for a biocontrol tactic. In 2020 Betsey Beers (Beers et al. 2020) reported the first known incidence of this parasitoid occurring here in the PNW. Recently USDA released an environmental assessment for the release of a biological control agent, *Ganaspis brasiliensis*, to control SWD. This larval parasitoid is tiny, measuring only 1.5 mm in length (Figure 1). USDA APHIS has determined that, because of its host specificity, *G. brasiliensis* would be an excellent biological control tool against SWD, “with no negative impacts to non-targets expected” (USDA APHIS). In its native range, spotted wing drosophila is <u>not</u> considered a crop pest, and *G. brasiliensis* parasitism rates can be as high as 75% in fruit samples. While there are likely other factors keeping SWD in check (in Asia), this is an extremely promising biological control for this invasive pest. The discovery of this wasp is encouraging because it means it can survive here on its own. Any additional effort we invest in building of the wasp population should have a high probability of success.

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

Report Type: Continuing Project Report

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

Cooperators:
M3 Agriculture Technologies

Project Duration: 3 Year

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

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

WTFRC Collaborative Costs:

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

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

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

Objectives

1) Collect data on overwintering flies and temperature data. (Year 1 beginning fall/winter 2022)

   Progress: We established 4 orchard blocks in The Dalles and installed 20 remote temperature and humidity sensors in each, connected to a wireless network, paired with 20 insect traps. Blocks were between 5 and 10 acres. Because of a late start and early snow last year we did not catch many flies. The second season of data collection has just begun, and those numbers are much higher.

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

   Progress: data analysis will occur in years 2 & 3.

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

   Progress: Unique non pesticide winter control tactic has been applied on separate experimental block. Data is currently being collected.

Significant findings

- We established 4 orchard blocks in The Dalles and installed 20 remote temperature and humidity sensors in each, with wireless a network, paired with 20 insect traps. (Figure 2)

Proposed Timeline

Objective	2022	2023	2024
1. Winter Trapping	X	X	X
2. Correlate temperature and terrain		X	X
3. Apply Winter control sprays		X	X

Methods

1) Expand preliminary overwintering trapping data to include more orchards with variable border habitats. (year 1)

The inspiration for this research came from visiting an orchard in The Dalles (Figure 1) and observing that there were no wild resources outside of the managed cherry orchard. The conventional wisdom is that SWD leave cherry orchards after harvest to find suitable overwintering sites. In addition, this area experiences high winds most of the year, so flies should need to seek shelter. At the center of the orchard is a valley with some wild blackberry habitat. Our hypothesis was that overwintering SWD would move to the valley at the center of the orchard to seek shelter for the winter. In an effort to capture the microclimate across the orchard we equipped the orchard with 20 temperature and humidity readers and connected them to a central hub modem with cell phone connectivity for remote data access (Figure 4). We then placed around 30 SWD traps across the orchard in an effort to capture this theoretical movement. While catch data from the traps in the valley was the highest (>4000), other traps still caught SWD at very high levels (1000s). This would suggest that flies are not moving out of the orchard and not seeking shelter within the orchard. Many questions remain to be answered abut winter movement. We propose to expand this research project to two other orchards and try to replicate these results.

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

A key question of this research is: Are temperature, wind, or habitat (or all of the above) correlated with successful SWD overwintering here in the PNW, or are flies just remaining in orchards regardless of these factors. In selecting additional orchards, we will look for locations with variable off-site habitat and different topography. Catch data will be tested with multi-variant statistical analysis to look for correlation with factors such as elevation, temp, humidity, habitat, etc.

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

Depending on what we learn from objective 2 we will develop a management plan to attack SWD where they are. We could spray them only in targeted locations, i.e. where they have hunkered down in protected valleys or blackberry patches, or we will plan on full orchard sprays. We have plans for two approaches. One approach would be a conventional insecticide spray such as pyrethroids and organophosphates. A second approach is to spray a non-caloric sweetener (Erythritol) that has been shown to be lethal to SWD. We will set up randomized complete block design for each and try to standardize block size and planting.

Figure 1. Orchard in The Dalles were preliminary research was conducted, showing extreme topography within orchard. There is 80 m (262 ft) of elevation difference at this orchard, with a valley in the center. Note lack of habitat outside orchard.

Results and Discussion

Research plots are set up and collecting data. We experienced considerable delay in the first season trying to get appropriately sized solar panels and modems that could communicate across some of the topography in The Dalles. Last year cold temperatures and early snow set in and reduced fly populations to near zero, so we don't have any SWD data to correlate with last year's temperature data. We are still working with M3 Agriculture Technologies to synthesize temperature data and produce graphics to display the range of temperatures across these blocks over the entire winter (Figure 3).

This year we have SWD traps up early and are currently collecting insect data well ahead of potential snow fall. We are hopeful that the SWD catch continues through November and December to reveal movement in response to the changing temperature.

Challenges: This system is still being Beta tested. Some data sensors are not communicating with the modem again this year and I will need to do some trouble shooting to ensure the system is working as we are going into the coming season. Lack of insect data in year one is frustrating but variable seasonal weather is always a challenge.

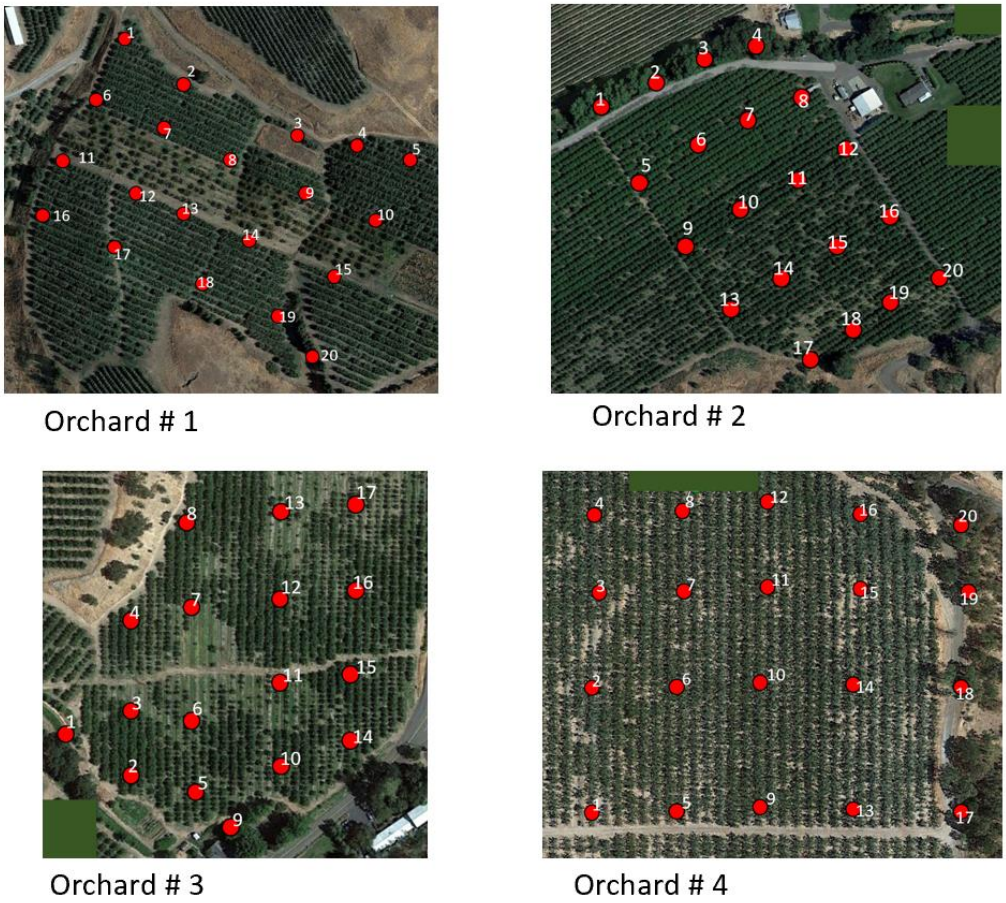

Figure 2. Experimental design of 4 orchard blocks. Red dots indicate locations of temperature sensor and SWD trap. Blocks are between 5 and 10 acres with elevation change of 5 – 20 meters.

Figure 3. Variable temperatures across a cherry block .

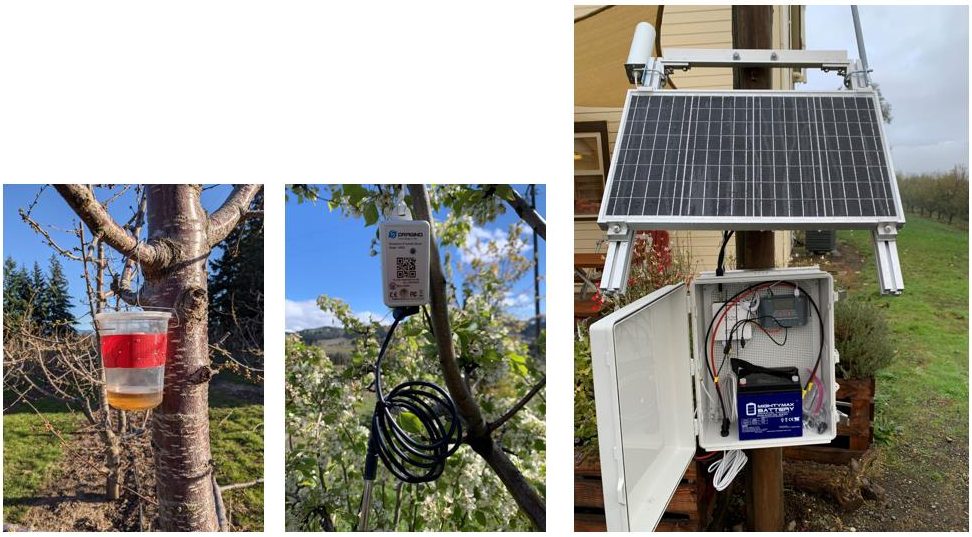

Figure 4. SWD trap, Temperature sensor and solar panel powered modem for data collection.

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

Primary PI: Tory Schmidt
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Cooperators: Gerardo Garcia (WTFRC), Northwest Horticultural Council, Pacific Agricultural Labs (Sherwood, OR), Orondo Orchards

Project Duration: 3 Years

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

Other related/associated funding sources: Most chemical products donated by registrants

Primary PI: Tory Schmidt
Organization Name: WTFRC
Contract Administrator: Paige Beuhler
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Contract administrator email address: paigeb@treefruitresearch.com

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

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

2023 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/.

TRIAL DETAILS

Harvesting treated cherries

- Mature 'Skeena'/Mazzard multiple leader open vase trees on 10' x 16' spacing near Orondo, WA
- 9 insecticides/acaricides & 6 fungicides applied at or near maximum rates and minimum pre-harvest and re-treatment intervals
- Applications made by Rears PakBlast PTO-driven airblast sprayer with 8 oz non-ionic surfactant (Regulaid)/100 gal water at 200 gal water/acre
- A total of roughly ½" of rain fell on the trial block on 4 separate days during the study, primarily on June 9 (0.38 in) and June 27 (0.11 in)
- Parka was applied by the commercial grower twice during the study (6/7, 6/22) to reduce rain cracking, which may have helped preserve residues of pesticides applied by WTFRC staff
- Samples submitted overnight to Pacific Agricultural Labs (Sherwood, OR) for chemical 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 the same. With that approach, **all 2023 residues complied with domestic tolerances but some exceeded foreign MRLs for important export markets**:

Insectides/acaricides: Bexar, Asana XL, Carbaryl 4L, Onager

Fungicides: Torino, Miravis, Cevya

Even though 2023 residue levels were largely consistent with findings from previous years, astute followers of these reports may note relatively fewer chemistries produced results which exceed key MRLs; this is primarily because several foreign markets recently have either relaxed their tolerances or posted official MRLs for these products. These positive developments are due in part to the efforts of the Northwest Horticultural Council to encourage regulators around the world to adopt and publish reasonable pesticide residue standards for imported Northwest cherries. MRLs are known to change frequently and cherry producers should routinely monitor the most current information (https://nwhort.org/export-manual) to facilitate compliance with constantly evolving foreign standards.

Dried residues on fruit at harvest

Measured residue levels vs. MRLs for pesticides applied to cherry fruit at 200 gal water/acre. 'Skeena'/Mazzard, Orondo, WA. WTFRC 2023.

Common name	Trade name	Application rate[1]	Application timing(s)	Measured residue	US tolerance[2]	Lowest export tolerance[3]
		per acre	*days before harvest*	*ppm*	*ppm*	*ppm*
tolfenpyrad	Bexar	27 oz	28, 14	**0.79**	2	0.01 (many)
abamectin	Agri-Mek SC	4.25 oz	21	0.014	0.09	0.06 (KOR)
thiamethoxam	Actara	5.5 oz	21, 14	0.21	0.5	0.5 (many)
esfenvalerate	Asana XL	14.5 oz	21, 14	**0.20**	3	0.01 (THAI)
chlorantraniliprole	Altacor	4.5 oz	21, 10	0.14	2.5	0.5 (KOR)
cyclaniliprole	Verdepryn 100SL	11 oz	14, 7	0.19	1	0.7 (many)
cyflufenamid	Torino	8 oz	14, 7	**0.22**	0.6	0.01 (THAI)
carbaryl	Carbaryl 4L	96 oz	10, 3	**2.0**	10	0.01 (THAI)
flutianil	Gatten	8 oz	10, 3	0.052	0.4	0.4 (many)
zeta-cypermethrin	Mustang Maxx	4 oz	10, 3	0.12	2	1 (KOR)
propiconazole	Orbit	4 oz	10, 1	0.24	4	2 (many)
thiophanate-methyl*	Topsin 4.5FL	30 oz	10, 1	1.06	20	3 (JPN)
pydiflumetofen	Miravis	5.1 oz	10, 1	**0.18**	2	0.01 (JPN)
mefentrifluconazole	Cevya	5 oz	10, 1	**0.53**	4	0.01 (THAI)
hexythiazox	Onager	24 oz	7	**0.47**	1	0.2 (KOR)

[1] All materials were applied by Rears PakBlast sprayer with 8 oz Regulaid/100 gal water

[2] 25 July 2023. http://mrldb.nwhort.org/#top_markets

[3] Major export markets for Pacific Northwest cherries; 25 July 2023. http://mrldb.nwhort.org/#top_markets

* Reported thiophanate-methyl values reflect sum total of thiophanate-methyl and carbenzadim 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.

Project Title: Etiology of Cherry Cankers and Dieback in the Pacific Northwest

Report Type: Continuing Project Report (**NCE**)

Primary PI: Gary Grove
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Cooperators: Garrett Bishop, GS Long

Project Duration: 1-Year

Total Project Request for Year 1 Funding: $ 9960 (**NCE**)

Other related/associated funding sources: None

WTFRC Collaborative Costs: None

Budget 1
Primary PI: Gary Grove
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Wages[1]	$591.00
Benefits	$59.00
RCA Room Rental	
Shipping	
Supplies[2]	$3,720.00
Travel[3]	$3,590.00
Plot Fees	
Miscellaneous[4]	$1,000.00
Total	$8,960.00

Footnotes: [1] = timeslip labor for media preparation; [2] = petri plates, growth media (2 types), antibiotics, and orchard tools; [3] overnight (Grove) and local travel (Grove and DuPont); [4] = autumn canker/ dieback workshop

Budget 2
Co-PI 2: Ashley Thompson
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Station Manager/Supervisor: Nicole Strong
Station manager/supervisor email address: nicole.strong@oregonstate.edu

Salaries	
Benefits	
Wages	
Benefits	
RCA Room Rental	
Shipping	
Supplies[1]	$500.00
Travel[2]	$500.00
Plot Fees	
Miscellaneous	
Total	$1,000.00

Footnotes: [1] = lab, field, outeach costs; [2] = local travel

Objectives

A. Sample symptomatic cherry trees in multiple orchards in Eastern WA and OR, where dieback has previously documented and include additional orchards in other regions in the PNW (no more than 5 per
orchards per county).
B. Remove bark on and adjacent to diseased tissue and (using magnification) search for fungal fruiting
bodies that could enable field identification.
C. Isolate, purify, morphologically characterize and store fungal and bacterial isolates from symptomatic
tissue using standard sterile microbiological techniques. Photographically document canker morphology
and isolate growth habit/color on growth medium.
D. Summarize known information about cherry dieback in the PNW and distribute the information to industry.

Methods

Over 70 orchards were sampled in 2023 (Figure 1). Samples were collected in Adams, Benton, Grant, Okanogan, Chelan, Yakima, Franklin, Walla Walla, Klickitat, Douglas, and Wasco Counties. Cankers and dieback were found in most locations. Bark on and adjacent to diseased tissue was removed and (using magnification) observed for fungal fruiting bodies that in some cases facilitated identification (Figures 7 and 8). Six to eight fragments (~8 mm; 0.31") from the margin between healthy and necrotic tissue (Figures 2 and 3) were be taken from each sample. Fungal isolation was performed by surface sterilization using 95% ethanol or 0.055 NaOCL followed by immediate flaming and subsequent placement onto Potato Dextrose Agar (PDA) and Malt agar (MA) amended with 100 ppm (0.1g/L) tetracycline and 2.5 ml / l lactic acid. Pure cultures of each fungal isolate were obtained using single hyphal tip sub-culturing methods on PDA and MA (Figure 4). Cultures were incubated at ambient temperature conditions in darkness and then photographed to document colony color and morphology.

Hyphal transfers from pure cultures were made to glass microscope slides and observed for conidia and conidiophore morphology at 40 - 250 X. If fruiting bodies were observed in pure cultures, representative samples were transferred to glass slides observed at 100 X to determine fruiting body and ascospore or conidia morphology.

Significant findings:

- Canker / dieback issues are widespread and quite severe
- Bacterial, Leucostoma (=Cytospora), Eutypa, and Calosphaeria cankers were documented in the region
- Bacterial canker and Leucostoma cankers were the most frequently encountered
- Calosphaeria canker was documented in 4 orchards (as if 10/11/23).
- Multiple instances of mixed infections were documented
- > 30 fungi distinct from those listed above were collected from diseased tissue and many await positive identification and proof of pathogenicity
- Fungal fruiting bodies were easier to detect during late summer /fall *in situ*

Results and Discussion

Leucostoma (=Cytospora) and bacterial cankers were documented in the late 20th century as the primary canker issues facing cherry growers in the Pacific Northwest (PNW). However, cankers of dieback of cherry have become more prevalent over the last decade and in some cases appear to different from known diseases. A canker survey was conducted during the 2023 growing season: samples from >70 (at the time of writing) orchard sites (Figure 1). Plant material was observed for disease signs (fungal reproductive structures; Figures 7 and 8) and then tissue was removed from the edges of cankers (Figure 2) and cultured on potato dextrose agar (PDA) and malt agar (MA). Mycelium from the edges of colonies were transferred to PDA and MA and incubated 14-21 days in darkness at 22 C (71.6 F) to purify cultures (Figure 4).

Canker and dieback was widespread and in some sites *quite* severe, far more than what was apparent in similar surveys conducted in late 20th century. Both bacterial and Leucostoma cankers were quite common but cankers caused by Eutypa (Figures 2 and 3) and Calosphaeria (Figures 7 and 8) were also documented in the region. Mixed infections (bacterial canker + Leucostoma, Leucostoma+ Eutypa, and Leucostoma + Calospshaeria; were also discovered (Figures 5-8) in multiple regions. However, over 30 fungi different from *L. cinctum*, *E. lata*, and *C. pulchella* were isolated from diseased tissue, cultures purified, and are undergoing morphological identification on PDA and MA (Figure 4). The pathogenicity of these isolates is unclear as are their respective roles in the cherry canker complex. No discernable geographic patterns (aside from *C. pulchella* detection in only Walla Walla, Yakima, and Wasco Counties) were observed for the distribution of various fungal pathogens. Canker/dieback causation varied significantly within production regions (Figure 5).

Preliminary information regarding the project was distributed via treefruit.wsu.edu, *Fruit Matters*, and trade publications.

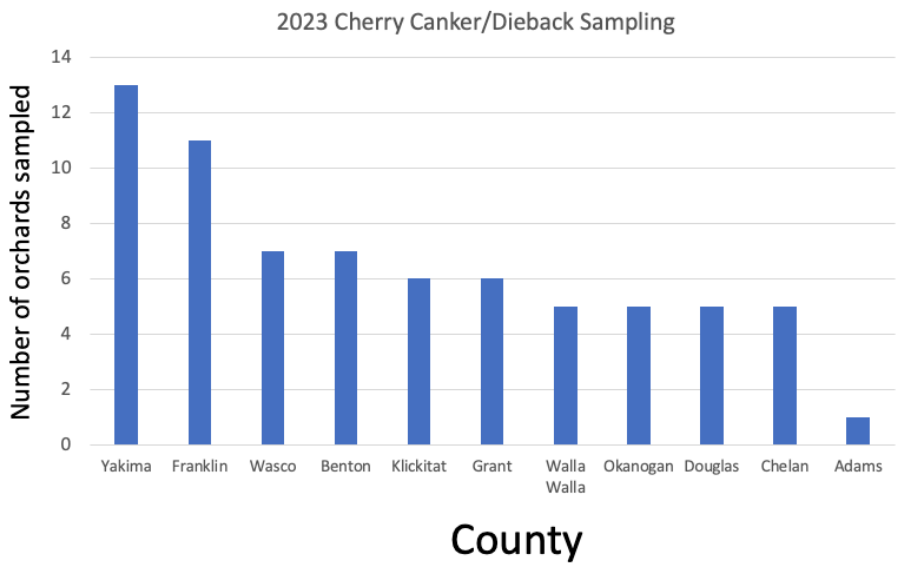
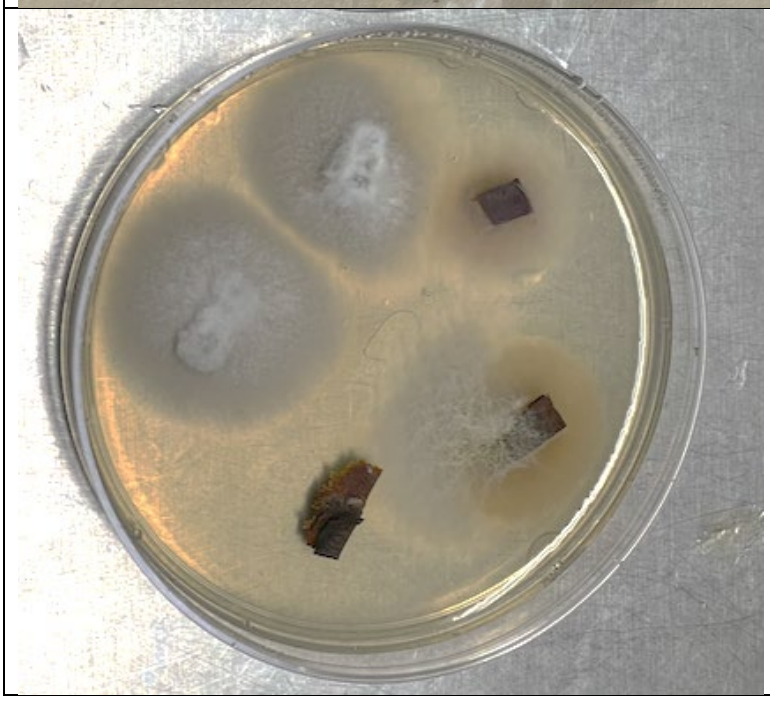


Figure 1. 2023 (as of October 10) cherry dieback/canker collections in the PNW. Collections will continue in October and November.

		**Figure 2.** Tissue was taken from the margins of diseased tissue and plate on potato dextrose (PDA) and malt (agar) amended with oxytetracycline and lactic acid.
		**Figure 3**. *Eutypa lata* growing from diseased cherry wood. Tissue was taken from the margins of diseased tissue and plate on potato dextrose (PDA) and malt (agar) amended with oxytetracycline and lactic acid.

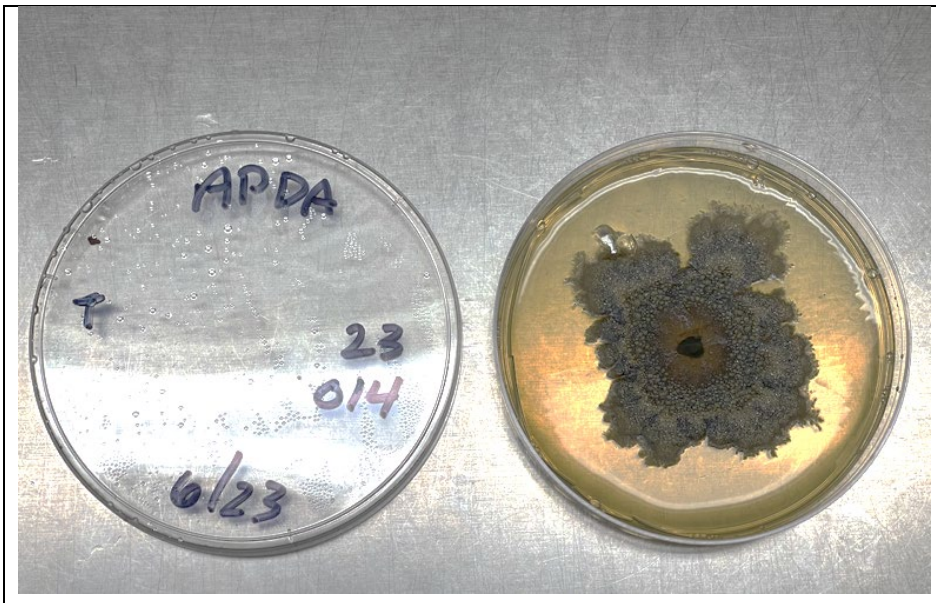


Figure 4. Leucostoma (=Cytospora) spp. Isolated from symptomatic "Chelan" trees near Wallula, WA. Isolate was obtained from a tree infected by both *Calosphaeria pulchella* and this isolate. See Figs. 6 and 7 below.

Figure 5. Results from various orchard sites in Franklin and parts of Adams and Walla Walla counties. Note that variation in causal agents and mixed infections.

	**Figure 6 (mixed infection).** Diseased Chelan tree near Wallula, WA. Tree is infected by both *Calosphaeria pulchella, Leucostoma* (=Cytospora) *cinctum,* and a second undetermined Leucostoma species (see Figure 4).
	**Figure 7**. Signs of *Calosphaeria pulchella*. Black areas are clusters of the sexual state (perithecia) of this pathogen.

Figure 8. Signs of *Calosphaeria pulchella.* Black-gray area is a clustes of the sexual state (perithecia) of this pathogen at higher magnification than in Figure 7. Each club-like structure is a perithecium (source of ascospores).

Extension. Current information on cankers of cherry was distributed via oral presentations at The Columbia Basin Tree Fruit Club (April 26), OSU Wasco County Preharvest Cherry Tour (June 6), and the Okanogan Horticultural Association Summer Field Day (August 3) and publications on the Tree Fruit Web Site / Fruit Matters:

Dupont, T., and Grove, G., and Thompson, A. 2023. Fungal Canker and Dieback Pathogens of Stone Fruit. *Fruit Matters*, August 2023. https://treefruit.wsu.edu/fungal-canker-and-dieback-pathogens-of-stone-fruit/. (also an information portal that will be updated as knowledge is gained)

Grove, G.G., and Sallato, B. 2023. The Fungi Among Us. *Fruit Matters*, August 2023. https://treefruit.wsu.edu/article/the-fungi-among-us/.

Project Title: A robust PNW sweet cherry breeding and genetics program, 2022-2024

Report Type: Continuing Project Report

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Cooperators: Allan Bros. Fruit, Custom Orchards, Inc. Orchardview Farms, Stemilt Growers, Thompson Hill Orchards, Breeding Program Advisory Committee (BPAC) members

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $ 183,524
Total Project Request for Year 2 Funding: $ 182,948
Total Project Request for Year 3 Funding: $ 201,863

Other related/associated funding sources:

Awarded
Amount: $458,022
Funding Duration: 2020-2024 (No-cost extensions in 2022, 2023)
Agency Name: WTFRC/OSCC
Notes: "Understanding little cherry disease pathogenicity". PI. Scott Harper. Co-PIs: Alice Wright, Per McCord

Awarded
Amount: $599,807
Funding Duration: 2022-2025
Agency Name: USDA NIFA—AFRI
Notes: "Improving grading methods to infer eating quality in sweet cherries under different cold chain scenarios". PI: Carolina Torres. Co-PI's: Rene Mogollon, Per McCord

Pending
Amount: $45,962
Funding Duration: 2024-2026
Agency Name: WTFRC/OSCC
Notes: "Screening cherry rootstocks for resistance to X-disease phytoplasma". Co-PIs: Scott Harper, Rodney Cooper, Adrian Marshall

Pending
Amount: $23,466
Funding Duration: 2024-2025
Agency Name: WTFRC/OSCC
Notes: "Identifying sources of resistance to *Pseudomonas* and powdery mildew". Co-PI: Cameron Peace

Pending
Amount: $649,971
Funding Duration: 2024-2027
Agency Name: USDA AFRI Foundational and Applied Science
Notes: "Facilitating Machine Harvestability Of Sweet Cherry Through Plant Growth Regulators And Genetics". Co-PIs: Matt Whiting, Amit Dhingra

WTFRC Collaborative Costs: None

Budget 1
Primary PI: Per McCord
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Station manager/supervisor email address: naidu.rayapati@wsu.edu

Item	2022	2023	2024
Salaries	$52,744.00	$54,854.00	$57,048.00
Benefits	$17,375.00	$18,070.00	$18,793.00
Wages	$39,426.00	$41,003.00	$42,643.00
Benefits	$9,514.00	$9,894.00	$10,290.00
RCA Room Rental			
Shipping			
Supplies	$29,561.00	$31,605.00	$33,181.00
Travel	$6,100.00	$6,100.00	$6,100.00
Plot Fees	$8,700.00	$10,656.00	$12,080.00
Miscellaneous	$2,500.00	$2,500.00	$2,500.00
Total	$165,920.00	$174,682.00	$182,635.00

Footnotes: Salaries includes 1.0 FTE research technician. Wages includes temporary labor for crossing, harvesting, seed extraction/transplanting, plus farm crew wages. Supplies includes costs for fruit evaluation, DNA extraction/genotyping, embryo rescue, propagation supplies/services, orchard maintenance, and equipment maintenance. Travel includes fuel, insurance, vehicle maintenance, and lodging/per diem costs (the latter during pollination season).

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

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

Budget 2
Co PI 2:
Organization Name: Kelsey Galimba
Contract Administrator: Charlene Wilkinson
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Station Manager/Supervisor: Stuart Reitz
Station manager/supervisor email address: stuart.reitz@oregonstate.edu

Item	2022	2023	2024
Salaries	$3,655.00	$2,718.00	$5,198.00
Benefits	$2,637.00	$1,946.00	$3,723.00
Wages	$3,439.00	$865.00	$4,034.00
Benefits	$625.00	$87.00	$690.00
RCA Room Rental			
Shipping			
Supplies	$4,599.00	$1,000.00	$3,057.00
Travel			
Plot Fees	$2,649.00	$1,650.00	$2,526.00
Miscellaneous			
Total	$17,604.00	$8,266.00	$19,228.00

Footnotes:
1. Salary: for one FRA to perform PGR applications, training, thinning, netting and data collection.
2. Wages: for hourly employees and students to assist with orchard activities and quality tests.
3. Supplies: include irrigation, trellising, block maintenance, and training supplies.
4. Research plot fees ($3,500/acre).

Objectives

1) Continue to generate seedlings, and rigorously evaluate seedlings and selections at all phases of the breeding program, including those now in Phase 3.
   a) Develop protocols for fruit evaluation via a small-scale commercial grade optical sorter (externally funded).
2) Test the effects of plant growth regulators on selections that have been advanced to Phase 3.
3) Increase the number of targeted cross made, seeds germinated, and seedlings transplanted
   a) Continue to utilize DNA information for superior and complementary parent selection and seedling screening.
   b) Deploy newly developed DNA tests for cracking susceptibility and fruit firmness.
4) Continue to implement timely and proper practices for orchard management (training/pruning, pest and disease monitoring and control, nutrient management).

Significant Findings

- 354 P1 seedlings were evaluated in the fruit quality laboratory. One late-ripening mahogany selection was advanced to Phase 2.
- Multi-location data was collected from 5 selections in the new Phase 2 trial (planted 2021)
- Phase 3 selections ‘R19’ and ‘R3’ (both early season) were harvested for the first time.
    - ‘R19’ displayed excellent firmness and Brix, but poor color and small size (possibly due to short growing season)
    - ‘R3’ showed good size response to GA and has a meaty texture. Test block has a high incidence of X-disease

- A single-lane Tomra InVision 2 optical sorter was installed in May 2023, and used to grade fruit from Phase 2 and 3 trials. Research is ongoing to optimize the sorter maps for both harvest and postharvest grading
- Though not significant, trends in fruit size and firmness for Phase 3 selection 'R29' follow reported effects of GA_3 application (larger, firmer, less mature) However, darker color in treated fruit opposes reported delay of coloration usually caused by GA_3. These numbers are non-significant when a t-test is performed, likely due to small sample size and variation between trees.
- Seed production from crossing program was acceptable (nearly 6,300) with more than 60% being produced in the crossing greenhouse. Bumblebees used successfully in making greenhouse crosses.
- 1178 seedlings from 2022 crosses were planted in 2023. DNA tests for self-fertility, powdery mildew resistance, low cracking, and high firmness were used to eliminate 303 seedlings prior to planting.
- PCR testing of 105 trees for Little Cherry Disease did not detect any trees infected with Little Cherry Virus 2 but did detect 10 trees infected with X-disease (all in the same block), which will be removed this fall.

Methods

Fruit evaluation

As in prior years, breeding program advisory committee (BPAC) members were invited to inspect P1 seedlings during the harvest. Walkthroughs were conducted generally twice per week (once with BPAC members). Selection criteria in the field were based on fruit size, firmness, and flavor. Fruit from field-selected P1 seedlings and P2/P3 standards and selections were evaluated in the laboratory for defects (harvest and post-harvest), weight, diameter, firmness (via Firmtech), stem pull force (P2/P3 only), color, sweetness (°Brix), and titratable acidity. Total harvest weight was measured for P2 selections and standards on a per-tree basis. P1 selections with an average fruit weight less than 9 g and firmness less than 270 g/mm were generally not evaluated for downstream traits.

An induced cracking test was performed (4-hour soak in deionized water) for P2/P3 selections and P1 seedlings with enough fruit. Prior to storage, P2/P3 samples were treated with a fungicide soak (Shield-Brite® FDL-230SC). Fruit sampled for post-harvest analysis was placed in modified-atmosphere packaging and stored in a walk-in cooler for 4 weeks.

Optical Sorter

A single-lane Tomra InVision2 optical cherry sorter was installed in a renovated space of the West Building in May 2023. Tomra trainers provided 1 week of training at their facility in Visalia CA, and also several days of onsite training. The sorter was used to analyze fruits from P2 and P3 plots, including postharvest samples. While optimizing the sorter map, we captured and saved images from each fruit in a given plot. These images are being re-analyzed with the optimized map generated during the season, and compared with the traditional analysis data generated in the fruit lab. We are also working to develop a post-harvest map that can evaluate pitting, decay, skin luster, and stem color.

Plant Growth Regulators

Four 'R29' trees were divided into treated and untreated scaffolds, and these were either sprayed with ProGibb 4% or with water at straw stage. Fruit was harvested when at least 60% of fruit was at a 4 on the CTIFL scale. Individual fruits were weighed, measured for diameter using digital calipers, measured for firmness using a Firmtech, and graded for color using the CTIFL scale. Values from four trees were averaged, and a student's unpaired t-test was used to determine significant differences.

Four 'R19' trees were divided into Retain®-treated, Parka™-treated and untreated scaffolds. Trees were treated with Retain® at full bloom.

'R3' was included in PGR trials in 2022, but was omitted in 2023 due to loss of key personnel (the Galimba Lab Research Assistant resigned during bloom). It will be included again in 2024.

Crossing and DNA tests

Crosses were made in the field at the Roza orchard, and in the hoop house at IAREC Headquarters. Crosses were made (a) by hand with emasculated blossoms and a small repurposed makeup applicator for pollination, (b) by hand with non-emasculated blossoms (crossing greenhouse only), and (c) by caging entire trees and utilizing bumblebees (crossing greenhouse) or mason bees (field) for pollination. The major criteria for crossing included early/late ripening, large fruit size, firmness, and self-fertility. Other criteria included powdery mildew resistance, crack resistance, and enhancing genetic diversity, the latter including use of interspecific crosses with 'Akebono' flowering cherry (*Prunus* x *yedoensis*) and Western sand cherry (*P. besseyi*).

Seedlings from 2022 crosses were germinated in cold storage and transplanted to the greenhouse in late fall 2022-early winter 2023. Prior to being transplanted in the field, the seedlings were sampled for DNA extraction. Based on the traits expected to segregate in particular crosses, seedlings were DNA-tested via PCR for powdery mildew resistance, self-fertility, cracking resistance, and fruit firmness.

Embryo Rescue

For crosses targeting early ripening and interspecific hybrids, embryo rescue was used as in prior years (since 2019). Based on prior experiments, Woody Plant Medium (WPM) was used exclusively, and fruit were harvested no earlier than straw phase. Fruit were sterilized in 70% ethanol with a few drops of surfactant, and cut open with a pair of anvil pruners to extract the seed. The seed coat was removed prior to placing the seed on the growth medium. Embryos were then stored in a walk-in cooler (39-46 °F) until germination. Once germination occurred, embryos were transferred to the growth cart under LED lights until true leaves and roots had formed (approximately 2 weeks). At this point, the embryos were transplanted into potting mix and placed in a clear plastic storage box in a greenhouse for 2 weeks' acclimation. The storage box lid was kept closed for the first week and opened for short periods each day for the second week, after which acclimation was considered complete.

Disease Screening and Orchard maintenance

The female parents (mother trees) of 46/54 crosses were screened via quantitative reverse transcription PCR (qRT-PCR) for *prune dwarf virus* (PDV) and *Prunus necrotic ringspot virus* (PNRSV), which are both pollen- and seed-transmissible. In addition, 13 seedling trees with virus-like symptoms were also tested. A total of 105 seedling and parental trees in the field that were symptomatic for Little Cherry Disease were screened via qPCR or qRT-PCR for X-disease phytoplasma and Little Cherry Virus 2, respectively. Orchards were sprayed, on average, every 2 weeks from 25 March to 13 October. Pre-harvest target insects were aphid, cherry maggot, cherry fruit fly and spotted-wing *Drosophila*. Powdery mildew was also targeted in parental and P2 blocks. Post-harvest (July 15th onwards), the target pests were leafhoppers and two-spotted spider mites. Orchard irrigation was managed by the IAREC farm crew, and most mechanical weed control and pruning were handled by breeding program employees. Plastic tree guards were placed around younger seedlings to allow for herbicide application, and a particular focus was made on summer pruning to control vigor in the younger trees. Vigorous leaders in the 2021 P1 planting were headed back, and the 2019 and 2020 P1 plantings were mechanically hedged to a height of 10 feet. Soil samples taken from each block in late spring were used to guide fertilizer applications. 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

A total of 354 P1 seedlings passed field selection criteria and were evaluated in the lab. This was the first year for evaluation of seedlings from crosses made since the crossing program restarted in 2018. One late-ripening selection, CR20T046, was advanced to Phase 2 for planting in 2025. The mother tree will be monitored for foliar and fruit symptoms of powdery mildew in 2024.

The newly planted (2021) Phase 2 trial is comprised of 4 mahogany (R45, R46, R47, CR01T078) and 1 blush (R35) selections, along with 'Benton', 'Bing', 'Rainier', and 'Skeena' as standards. Table 1 summarizes the characteristics of the new selections based on first-year data. R46 and R47 are the earliest and have a similar ripening time (6-12 d before 'Bing'). R46 is the larger of the two, whereas R47 has higher firmness and had excellent crack resistance. CR01T078 ripened slightly later than 'Bing' this year. Its characteristics were very similar to 'Skeena', but with slightly larger (and firmer) fruit. R45 is the latest mahogany selection, ripening several days after 'Bing'. Larger than 'Skeena', with similar firmness, R45 had very low stem pull force (a maximum of 348 g). R35 ripened several days later than 'Rainier' and had larger and much firmer fruit (though size could be affected by crop load). Brix and acidity are similar to 'Rainier'.

2023 was also the first year for obtaining harvest data from the new Phase 3 trials (Table 2). The R19 trial was hosted by Stemilt Growers near Mattawa, and R3 was hosted by Thompson Hill Orchards near Zillah. Although 'R19' continued to have good firmness and Brix compared to 'Chelan', fruit size was smaller and color was poor, with a mix of light and medium-colored fruits. Early-season cherries in particular had a shorter development period in 2023 due to late bloom and subsequent warm temperatures, and 'R19' may be more sensitive to these weather conditions. We will continue to evaluate 'R19' at Mattawa, and also expect a small crop in 2024 from the P3 trial at Dallesport. 'R3' performed well at Zillah, showing good response to GA application. Informal taste tests were very positive. A high percentage of trees at the Zillah location were found to be infected with X-disease (and have been removed). Whether this is due to locally high disease pressure (a neighboring block had very high incidence of XDP) or a high sensitivity to infection is unknown. We will continue to monitor the 'R3' trial at Zillah for XDP, as well as a newer planting at the Mattawa site that will come into fruiting in 2024.

Table 1. Characteristics of five Phase 2 selections based on the first year of data. Results are averaged (when available) across 3 locations [IAREC (Prosser), Sagemoor (Pasco), and MCAREC (Hood River)].

ID	Color (CTIFL)	Timing (vs. Bing)	Fruit weight (g)	Firmness (g/mm)	°Brix/TA	Notes
R47	5.28	-13	8.8	386	24.2/0.54	Low cracking
R46	5.74	-11	10.4	291	22.2/0.43	
Rainier	Blush	-3	9.4	226	24.6/0.40	
R35	Blush	-1	11.6	323	24.7/0.34	No data from Hood River
Benton	6.24	-3	8.9	174	26.4/0.53	No data from Sagemoor
CR01T078	5.68	+2	10.2	300	26.3/0.71	
Bing	5.93	N/A	8.1	282	27.9/0.47	
Skeena	5.4	+3	9.3	266	25.9/0.59	
R45	5.67	+3	10.3	297	26.6/0.52	Low stem pull force; Self-fertile

Table 2. Characteristics of two Phase 3 selections, R19 and R3, and 'Chelan'.

ID	Location	Color (CTIFL)	Timing	Fruit weight (g)	Firmness (g/mm)	°Brix/TA	Notes
Chelan	Mattawa	5.4	6/16	7.2	337	21.3/0.64	
R19	Mattawa	2.5	6/14	5.9	372	23.2/0.42	Self-fertile
Chelan	Zillah	6.2	6/14	8.5	219	19.6/0.44	
R3 (w/GA)	Zillah	N/A	6/23	10.2	249	20.8/0.40	
R3 (w/o GA)	Zillah	5.4	6/20	7.7	274	18.7/0.48	

Optical Sorter

The comparisons between the optical sorter and fruit lab measurements for cracking, blemishes, doubles, and pitting demonstrated the challenges of using the optical sorter in a research setting where multiple varieties are being analyzed. In general, the sorter detected fewer cracks, more general blemishes, fewer doubles, and less pitting vs. lab-analyzed fruit (Table 3). We will continue to optimize our sorter maps and set points during the off-season, including a map for analyzing postharvest traits such as stem color and skin luster.

Table 3. Percentages of defects (per plot) detected by the Tomra optical sorter and by manual analysis in the fruit lab.

Trait	Sorter (average 154 fruit/plot)	Fruit lab (average 50 fruit/plot)
Cracking	8.6	16.8
General blemishes	57.1	29.6
Doubles	0.3	1.6
Pitting	0.9	7.1

Plant Growth Regulators

When averaged across R29 trees, GA_3 applications did not significantly change fruit size (weight, diameter, or row size), firmness, or color (Table 4). Results for individual trees varied greatly. Ex: Treated and untreated fruit differed in color by only .04 CTIFL units for Tree #11, but Tree #26 fruit had a difference of 1.56 units. Though not significant, trends in fruit size and firmness follow reported effects of GA_3 application (larger, firmer, less mature) However, darker color in treated fruit opposes reported delay of coloration usually caused by GA_3. These numbers are non-significant when a t-test is performed, likely due to small sample size and variation between trees.

R19 trees were treated with Retain®, but fruit were completely gone by straw stage. This is likely due to ground squirrel activity, but may be attributed to birds as well. R19 has been observed to be extremely attractive to birds in the past, though damage usually isn't nearly as heavy or as early.

Table 4. Results of GA_3 application on R29 at Hood River (MCAREC).

R29	Fruit Weight (g)	Fruit Diameter (mm)	Fruit Row Size	Color (CTIFL)	Firmness (g/mm)	Stem Retention Force (g)
GA_3-Treated	10.71	27.51	9.53	5.37	255.39	-0.31
Untreated	10.25	26.35	9.92	5.01	240.59	-0.24

Crossing & Seedling generation

In 2023, the breeding program made 54 crosses, producing an estimated 6,267 seed. This is considerably less than the amount produced in 2022, despite more favorable pollination weather for field crosses. Due to the sudden onset of warm weather, the bloom period was highly compressed, limiting the number of field crosses that could be made. Mason bee pollination was also generally not as effective as in 2022, for unknown reasons. However, bumblebees were successfully used for the first time in the crossing greenhouse. For the first time, most seed produced (66%) came from the crossing greenhouse. We will expand the use of bumblebees in 2024, as well as utilizing more bee-proof cages to reduce or eliminate mis-pollination from escaped bees. In line with breeding program goals, the vast majority of seed (95%) came from bi-parental crosses.

In 2023, 867 embryos were rescued. Prior research suggests that maintaining warmer temperatures during embryo development may reduce abortion and the need for rescue. From crosses made in the hoop house we split some early ripening families into two groups, one rescued and the other stratified conventionally. As germination is ongoing it is too soon to tell whether the hoop house environment reduces the need for embryo rescue, but preliminary observations indicate it will still be required for certain crosses.

In 2023, 1178 seedlings (from 2022 crosses) were transplanted to the field, the 2nd-highest since 2018. An additional 303 seedlings were eliminated prior to transplanting based on DNA test results. We continued to have challenges with our seed cleaning protocol, with some batches of seed being drowned due to not being dried properly after cleaning. Fortunately seed from 2023 crosses received better care in cleaning, and we expect higher germination percentages.

Disease Screening & Orchard Maintenance

The incidence of LCD was reduced in breeding program seedling blocks in 2023 vs. 2022, despite an increase in the number of trees tested. In 2022, 21 out of 47 trees tested positive, including 7 infected with little cherry virus 2 (LChv-2). In contrast, out of 105 trees tested in 2023, none were positive for LChv-2, and only 10 tested positive for XDP. These trees were all in one block (B53) at the Roza orchard, will be removed by the IAREC farm crew. It is interesting to note that these trees are infected with the 'Eastern X' genotype of the phytoplasma, which is currently rare in the Pacific Northwest. It likely came from a highly infected 'Brooks' tree that was removed from the B53 block the previous year, suggesting a localized spread which will be easier to control. The breeding program regularly tests for the presence of PDV and PNRSV in breeding materials. Two mother trees (out of 10) at the Roza orchard tested positive for PDV (none for PNRSV). In the hoop house, two mother trees (out of 40) tested positive for PDV, and two for PNRSV (one tree was positive for both viruses). Several trees had inconclusive results and will be retested, but known positive trees will be removed.

Project Title: Sweet Cherry Bud Cold Hardiness Model
Report Type: Final Report

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Project Duration: 1-Year

Total Project Request for Year 1 Funding: $ 87, 341

	Kelsey Galimba OSU	**Gwen Hoheisel WSU**	**Lav Khot WSU**
Salaries	$12,000	$8779	$8750
Benefits	$8592	$4748	$3950
Wages	$5760	$8120	
Benefits	$576	$1626	
Equipment			
Supplies	$200	$500	
Travel	$500	$3240	
Miscellaneous	$20,000[1]		
Plot Fees			
Total	$47,628	$27,013	$12,700

1. Fee for the now-independent (previously WSU) statistician (C.K.) to continue the modeling.

OBJECTIVES

The goal of this proposed project is to replicate the data collection performed in 2020-2021, providing an additional year of data to both improve and validate the constructed models. By the conclusion of this project, growers will have access to cold hardiness models for the four cultivars on AgWeatherNet, and will have characterizations of both how accurate the models are, and how they could potentially improve with more data collections in the future.

Objective 1: Gather an additional season of weekly phenology and lethal temperature data using Bing, Chelan, Sweetheart and Regina buds gathered in Washington and Oregon to improve the current model.

Objective 2: Quantify the uncertainty in the model after the two years of data collection, validate the model, and predict if and how additional collections would improve model accuracy.

Objective 3: Make the sweet cherry cold hardiness models publicly available on AgWeatherNet.

SIGNIFICANT FINDINGS

- Phenology is not the most accurate predictor of bud lethal temperature, especially in the early stages of dormancy to bud swell where internal development can occur without external appearances changing.

- Between dormant to first swell (stage 0 to 1), there is significant bud development internally where cold hardiness is lost, yet the external phenology appears unchanged. Some orchardists have noted this and look for yellow pollen development by cutting open dormant buds. Any yellow typically means some loss of lethal temperatures compared to the traditional Critical Temperature Chart.

- Between the two cherry cold hardiness proposals (2021 and 2022) we collected from eight field locations for Bing, Chelan and Sweetheart and five field locations for Regina.

- We define a full season's worth of data as a dataset (fall-spring). In the model, we are using seven full and one partial dataset for Bing, six full and two partial for Chelan and Sweetheart, and two full and three partial for Regina. Partial datasets were either a result of missing weather data or from the 2021 sampling that was initiated in the middle of the season for Oregon collections.

- Error in the mean LT is currently roughly ±1.8^{o}F/~1^{o}C Analysis of sample size indicates that an additional eight datasets, effectively doubling the size of the data collection, would be needed to reduce error in the mean LT to roughly ±1.2^{o}F/~0.7^{o}C. Collecting more freezer and weather data per cultivar decreases the error around the mean LT, but there is also error associated with the model.

- There are two sources of error inherent in this model. The first is error around the mean LT (e.g. 28 ± 4^{o}F). This is used to set wind machines and heaters. Additionally, there is error in damage because not every field will experience 10, 50, or 90% damage exactly, the error in lethal temperature (LT± 4^{o}F) does not directly translate to error in predicted loss. Some fields

may experience much greater or less than 10% damage. Therefore, our model prediction of LT is conservative and predicts an LT in which the aggregate damage across fields will average 10% damage. Meaning, mitigation at the predicted LT10 will ensure that a majority of fields experience 10% or less mortality. But it is likely that many fields will be more cold-hardy and could modify their mitigation practices if they understand their specific orchard hardiness.

- Discussions with other researchers in this field have indicated that there are currently two cold hardiness models being developed. Our traditional scientific techniques and modeling are one effort. Additionally, Dr. Paola Pesantez Cabrera under the AgAID project has developed a cold hardiness model with AI techniques. Data from Dr. Whiting's lab is used in that model. Both teams are keen on collaborating together for the best outcome.

RESULTS AND DISCUSSION

Objective 1: Collect more LT data.

In the 2020-2021 season, collaborators from OSU and WSU evaluated all available previously-gathered data while also collecting new data, with the goal of constructing and validating a sweet cherry cold hardiness model. Ultimately, data gathered prior to 2020 was deemed unreliable, with various concerns ranging from labeling inconsistencies to apparent early LT shifts that are biologically improbable. This caused the priority to shift to constructing a model using current-season data, and prompted an increase in the amount of data that was gathered, starting in early February.

Through the 2022-2023 winter season, cherry buds were collected in Washington and Oregon (Fig. 1). In the Yakima Valley, WA, three separate orchards for Bing, Sweetheart, and Chelan provided weekly samples. In The Dalles, OR, three orchards for Bing, Chelan, Sweetheart, and Regina were used, and one collection for each of these cultivars was also made in Hood River, OR. Both hobo dataloggers and commercial weather stations were used to collect temperature. The maximum number of samples that fit in the freezers were collected in the 2022-2023 season.

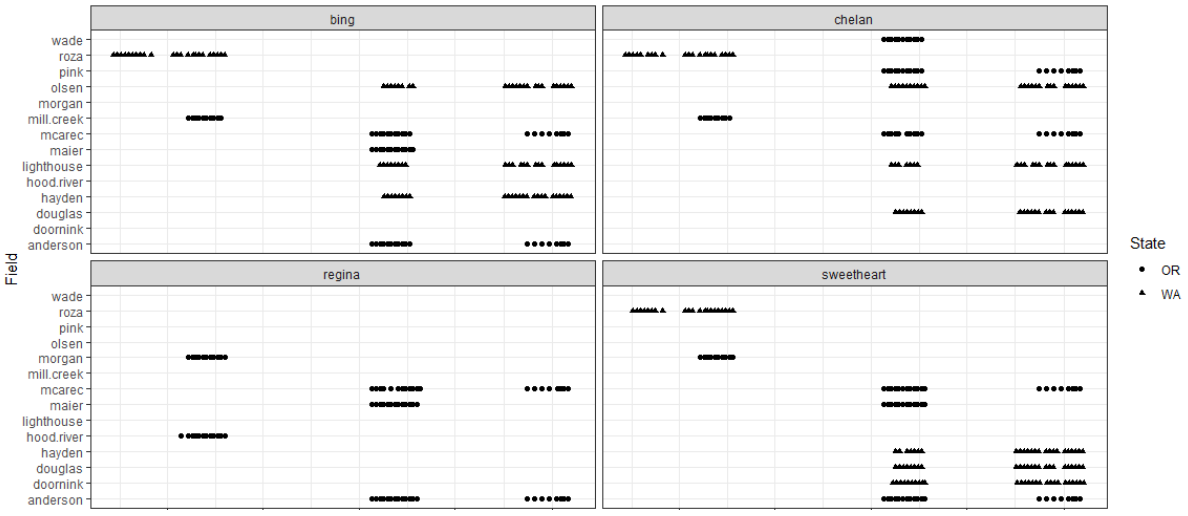

Figure 1: Depiction of the collection points aligned with reliable weather data for four different cultivars over the 2021 and 2022 grant proposals. Farms with two distinct data sets (ex. Olsen) indicate a full dataset of fall and spring collection. Some farms have partial datasets due to loss of weather data, but all viable data was included in the model to improve predictions.

Objective 2: Model development, uncertainty, and future needs
With any model, there is an analysis of uncertainty and assessment of improvements in the model.

1) Analysis of Sample Size:

From two grant proposals, we have collected lethal temperature data on four cultivars. In the first year, we collected one full dataset and one partial dataset each for Bing, Chelan and Sweetheart, and two partial datasets for Regina. In 2022-2023 we collected six full datasets for all four cultivars. In the model, we are using seven full and one partial dataset for Bing, six full and two partial for Chelan and Sweetheart, and two full and three partial for Regina. Partial datasets in the 2020-2021 season were due to Oregon being included in the project after sampling had already been initiated. Partial datasets from 2022-2023 are due to data being excluded because weather data was either missing or untrustworthy.

Two questions can be asked about the current datasets: 1) If we collect more data, can we better predict lethal temperature (LT)? 2) How much data are needed to predict LT? Figure 2 shows how much we would expect the mean LT25 to vary across replicate experiments comprised of a fixed number of datasets, suggesting that the improvement in the precision of the mean is minimal as we move past 16 datasets. **To sufficiently reduce the error in the mean for, we recommend that a total of 16 datasets are created to reduce the standard error in the LT25 to under ±0.7ºC/~1.2ºF.** As we reduce the standard error in the mean, the prediction error gets reduced. Currently for three cultivars, we have nearly six complete datasets and while an error ±1.8F is low, any error in the mean is going to systematically increase prediction error. Collecting an additional 2 years of data would help improve the standard error and model.

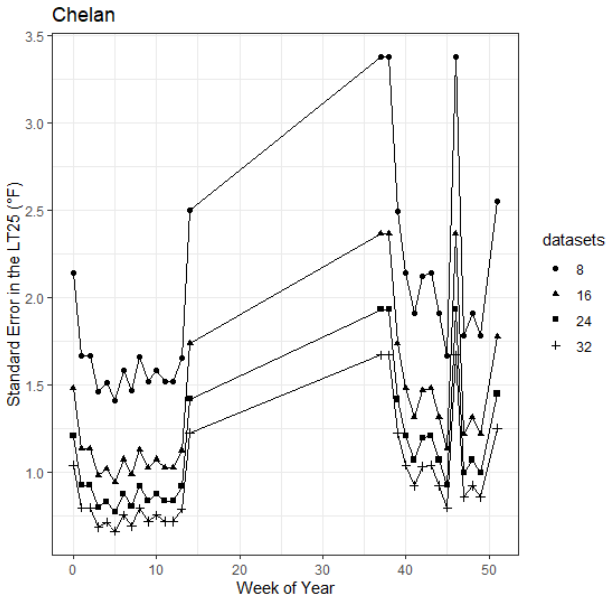

Figure 2: Standard error of the mean lethal temperature (LT25) for Chelan for a hypothetical project that is 1x – 4x times the scale of this project. Other varieties have similar characteristics. Week 0 = Jan 1. Weeks 15-36 are spring-summer months with no measure of cold hardiness, thus ignore the line. Notice that the more samples, the lower the error.

2) Cherry Cold Hardiness Model:

The Cherry Cold Hardiness Model (CCHM) was developed in similar fashion to the Blueberry Cold Hardiness Model (BCHM), however, in blueberries there are twice the number of datasets, meaning this co-analysis offers the opportunity to draw on similarities between the cold hardiness models. The

CCHM was developed to weight weather data that causes damage that could have occurred earlier in the season (Fig. 3). Furthermore, the LT predictions compute "population-level lethal temperature". To do this we created a code library that takes bud death data from both years of cold hardiness experiments, fits Bayesian Generalized Linear Mixed Models (GLMM), extracts population-level lethal temperatures. We plan to perform model approximation via lookup tables and uses AWN data to forecast lethal temperatures, similar to what is done with the BCHM.

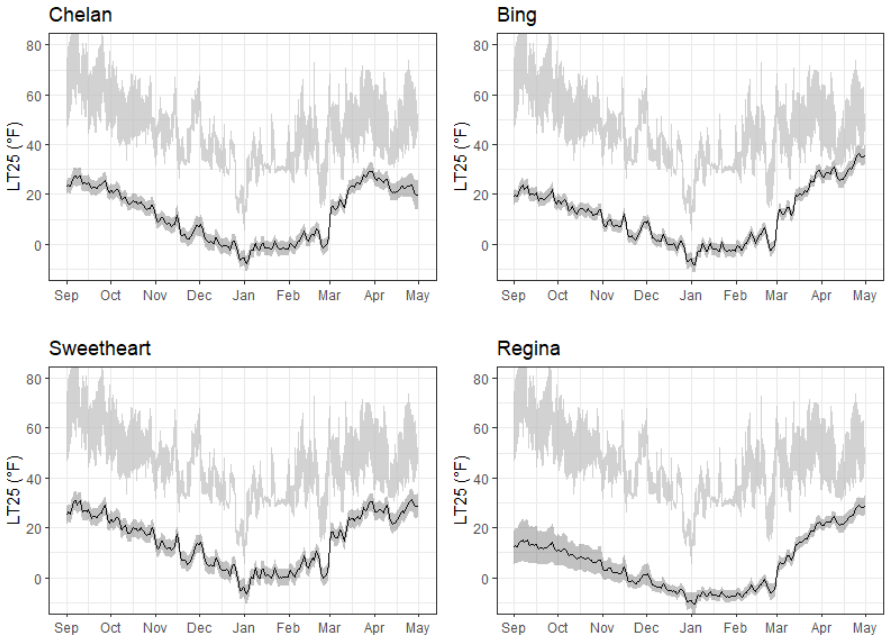

Figure 3: Depiction of the LT25 for four cultivars. The solid black line is the predicted LT25 with 95% confidence intervals around the mean (darker grey). The upper shaded line is the maximum and minimum daily temperatures from a weather station. Note higher error around the mean in fall months for Regina, an effect of less data from those months for that cultivar.

Predicting LT from "population level lethal temperatures" is key to accurately representing true damage across all fields. There are two ways of characterizing prediction error. The error around the mean LT shown as a temperature ± error in °F. This is used to set wind machines and heaters. Additionally, there is error in damage. **Because not every field will experience 10, 50, or 90% damage exactly, the error in lethal temperature (LT± error in °F) does not directly translate to error in predicted loss.** Grower financial loss is better characterized by error in predicted loss (e.g., an LT10 is predicting 10% loss but some fields my experience 40% loss), rather than just using the error in lethal temperature (e.g., if the predicted LT10 is 10°F then LT can range from 6-14°F actual is 12°F) (Fig. 4). The variability in predicted loss might be translated directly into revenue loss (e.g., losing 50% instead of 10% of the buds might be estimated as a $0.5M revenue loss, depending on size of farm) and errors in the positive direction (meaning less damage from a less sensitive field) could correspond to a potentially loss in labor and fuel for unnecessary mitigation. Averaging and minimizing lost revenue due to prediction error across growers is one way to try and minimize the overall loss experienced by growers. While more complex modeling, "population level lethal temperatures" estimates the lethal temperature in a manner that targets better grower decision making for cold mitigation.

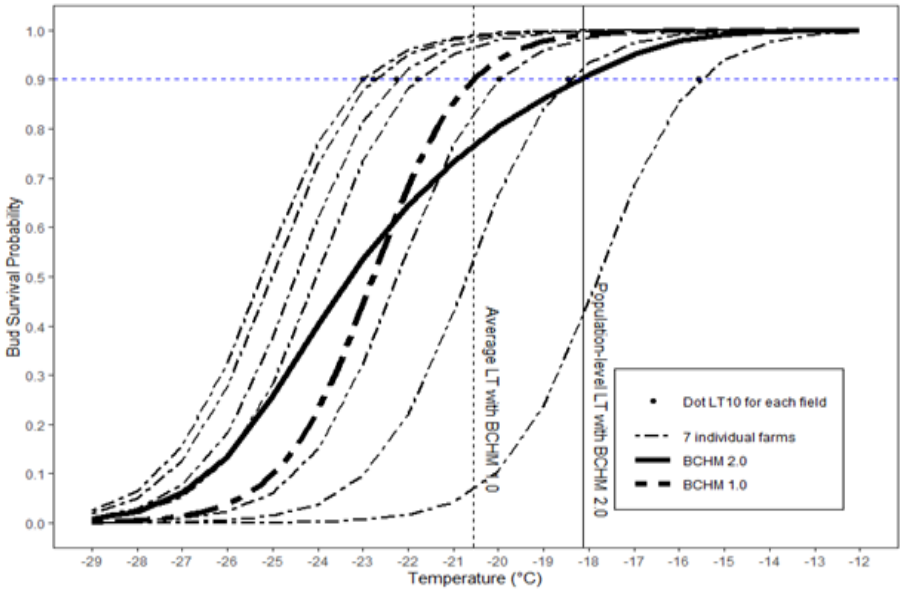

Figure 4: This is example is of a blueberry analysis as it is very clear, but similar results are seen in cherries. These are survival curves for seven blueberry fields on any one day. 90% survival equates to 10% mortality, LT10 (dots). In prior blueberry studies, a simple mean LT (dashed line at –20.5ºC) can often overestimate hardiness on some farms. Because large farm-to-farm variability, seen from the wide range of different LT10s per farm, management (heaters/wind machines) with a simple mean LT10 would cause damage in the 3 farms with curves to right of the average LT line because they are less cold hardy than the predicted LT10. The industry as a whole would experience 24% mortality with some farms faring better (0% damage) and others fairing much worse (40+%). Using the 'Population-level LT' in CCHM results in a higher LT10 (-18.2ºC) because it accounts for more of the farm-to-farm variability and averages across a larger simulated dataset. But **essentially most of the farms will not suffer severe loss at this LT10. The expected loss across the industry is 10% for CCHM. However, there are many farms that are far more cold-hardy than the LT and could save in mitigation expenses. Monitoring how in individual field is responding to cold could lead to tailored field management decisions that allow savings in propane and other expenses.**

In addition to this project, there is a separate, but cooperative project with AgAID. Scientists are creating cold hardiness models in cooperation with Dr. Markus Keller's and Dr. Matt Whiting's labs grape and cherry, respectively. The grape cold hardiness model will be live on AWN this winter for industry review and use. The AgAID team uses traditional models such as the one in this proposal to validate the reliability of AI techniques being used.

Objective 3: Cold hardiness model available on AWN

While the CCHM is developed it is still being ported to AgWeatherNet (AWN). There needs to be a conversation and a clear understanding with industry on the limitations and accuracy of the model (objective 2). Our team will be speaking with the cooperating growers and other interested partners. However, in general, **we expect the model and outreach information to be very similar to the information presented on the blueberry AWN portal, seen below:**

Estimating Lethal Temperatures and Damage

Mitigating freeze or cold injury is challenging. There is a balance between applying protective measures and economic losses from failing to do so. There are three key factors that a grower needs to understand to determine total risk from a cold event;

1. *Knowing the average lethal temperature*
2. *Knowing the range of damage that can occur*
3. *Assessing how your fields vary from the average; meaning is there more or less damage than expected compared to the average prediction (e.g., 10, 50, or 90% mortality). Then cold mitigation can be adjusted.*

Interpreting the Graph

- *LT10: Estimates the temperature in which 10% of flowers within a bud will die*
- *LT50: Estimates the temperature in which 50% of flowers within a bud will die*
- *LT90: Estimates the temperature in which 90% of flowers within a bud will die*
- *Tmin: Represents the minimum temperature for the day.*
- *Lethal temperatures are determined with forecasted weather data, shown as FCLT. Care should be taken as actual temperatures can vary from the forecasted weather.*

How to use LT

The critical lethal temperatures (LT) shown in the graph are predicted values from separate cold hardiness models created with years of data collected in multiple locations across the state. The cultivar can be changed across the top menu bar.

The model accurately predicts LT50 within +/- 3.6-4.1°F (2.0-2.3°C). The LT can be used to determine cold mitigation strategies (e.g., when to initiate wind machines and heaters). However, fields vary greatly due to location, fertility programs, plant vigor, and pruning practices. This leads to some fields experiencing more (or less) damage than the 10, 50, or 90% average in the LT.

Range of damage

Because there is high variability, we recommend looking at this table showing the range of damage that occurred across fields and years at any given lethal temperature. This indicates that actual damage in a field will often differ substantially from model predictions. We strongly encourage growers to assess damage in their field(s) after freezing events and record the damage. Learning this will give an understanding of the field(s) vigor and the ability to adjust mitigation temperatures in the future.

	Majority (90%) of the fields will experience this range of bud mortality.			
LT associated with bud survival	*'Duke'*	*'Liberty'*	*'Draper'*	*'Aurora'*
LT10	*1% - 34%*	*0% - 44%*	*1% - 32%*	*2% - 28%*
LT50	*17% - 87%*	*15% - 89%*	*14% - 84%*	*20% - 79%*
LT90	*65% - 99%*	*48% - 99%*	*63% - 98%*	*70% - 97%*

Examples:

1. *If temperatures drop to a predicted level of LT10 in a 'Duke' field, then we would expect 10% of the flowers within buds to die on average across all fields. But any single field can vary from the average so some fields will experience no damage (1%) while others may have 34% death.*
2. *If a catastrophic temperature decline occurs associated with a LT90 in a 'Draper' field, then we estimate across all blueberry fields that 90% of the flowers will die. However, individual fields will experience somewhere between 63% and 98% death.*

How will your field differ from the model?
A model is a highly educated estimate. Actual field conditions can vary. If lethal temperatures presented in the model are used to inform cold mitigation in a field or on a farm (e.g., heaters or wind machines), then it is helpful to understand whether and how much your field temperatures differ from the AgWeatherNet station(s) you are using. The actual cold hardiness values at your site will vary depending on preceding local environmental conditions. In general, if the temperatures in your field have been colder than those at the AgWeatherNet weather station used to run the model, then your blueberry buds may be more hardy than the stated temperatures. Conversely, if the temperatures in your field have been warmer than those at the AgWeatherNet weather station, then your buds may be less hardy.

EXECUTIVE SUMMARY

Project title: Sweet Cherry Bud Cold Hardiness Model

Key words: lethal temperature, dormancy, bud loss, winter kill, cherry

Abstract:

Spring frost is a significant production hazard to all temperate fruit tree species. Because sweet cherry (*Prunus avium*) is among the earliest to initiate development in the spring, it is particularly susceptible to late frost events. In order to avoid frost damage to buds, growers use a variety of mitigation practices throughout the critical spring period, such as orchard heating, irrigation, frost fans, and spray applications. These strategies are expensive and require that growers make time-critical decisions based upon the current bud lethal temperature (LT), a parameter that changes as trees transition from dormancy to full bloom. Currently, the LT of a given orchard is estimated based on phenology charts that correlate developmental stage with LT, requiring the grower to accurately characterize the bud stage most prevalent in their orchard. This is complicated by the fact that the available phenology charts vary in their listed LTs, and by the fact that a remarkable amount of internal development (and LT changes) occur without a visible change to the outside of the bud in early stages. These issues highlight the need for a weather-related decision-support tool based on Growing Degree Days (GDD) to guide sweet cherry growers in their response to spring cold weather events.

One of the objectives of this grant was to collect more field data. Both WA and OR collected 'Bing', 'Chelan', 'Sweetheart', while 'Regina' was collected only in OR. The analysis in sample size indicates that the error around the mean LT would be minimized with at least 16 data collections. As we reduce the standard error in the mean, the prediction error gets reduced. Currently for three cultivars, we have at least six complete datasets and while the resulting error of ±1.5°F is low, any error in the mean is going to systematically increase prediction error. Collecting an additional two years of data, increasing datasets to 16, would help improve the standard error and the model.

There is also error associated with the prediction and field variation. Assessing how grower fields vary from the average; meaning is there more or less damage than expected compared to the average prediction (e.g., 10, 50, or 90% mortality) is critical. Then cold mitigation can be adjusted. Because not every field will experience 10, 50, or 90% damage exactly, the error in lethal temperature (LT± 4°F) does not directly translate to error in predicted loss. An average LT will be predicted according to the closest public weather station. In general, if the temperatures in a field have been colder than those at the weather station, then those buds may be more cold-hardy. Conversely, if the temperatures in your field have been warmer, then your buds may be less hardy. Additionally, fertility, vigor, crop load all influence hardiness. We strongly encourage growers to assess damage in their field(s) after freezing events and record the damage. Learning this will give an understanding of the field(s) vigor and the ability to adjust mitigation temperatures in the future.

Project/Proposal Title: Nutrient management for high quality sweet cherries

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Report Type: Continuing report

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $ 14,716
Total Project Request for Year 2 Funding: $ 15,138
Total Project Request for Year 3 Funding: $ 15,576

Other related/associated funding sources: Root Growth Management to Reduce Ca Deficiency Disorders in Apples and Cherries. P.I. B. Sallato. Awarded.
Funding Duration: 2019 - 2022
Amount: $152,938
Agency Name: Washington State USDA- Specialty Crop Block Grant
Notes:

Budget 1

Organization Name: Washington State University
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Item	2021	2022	2023
Salaries			
Benefits			
Wages[1]	9,600	9,984	10,384
Benefits	928	966	1,004
Equipment			
Supplies[2]	3,888	3,888	3,888
Travel	300	300	300
Miscellaneous			
Plot Fees			
Total	14,716	15,138	15,576

Footnotes: [1] Wages for two temporary support at 15 USD/hour for Sallato's and Torre's lab for 310 hours each (9.4% benefits), plus 600 hours of technician at Sallato's lab at 15 USD/hour and 68.3% benefit. [2] Supplies include laboratory supplies and nutrient samples at 18 USD/sample.

OBJECTIVES

The goal of this project is to improve nutrient management strategies from an understanding of the nutritional composition of good and poor-quality fruit. We proposed to undertake a prospective analysis of orchard growing conditions and fruit nutrient levels and their relationship with key quality parameters: size, firmness, and storability. This research approach permits an in-depth analysis of fruit nutritional content and fruit quality, identifies predictors, determines nutrient extraction, and begins to develop fruit-specific nutritional management strategies for sweet cherry.

1) Identify adequate nutrient conditions for fruit quality in sweet cherry.

2) Determine nutrient demand of different sweet cherry varieties.

3) Identify key conditions leading to better fruit quality and storability in sweet cherry.

4) Develop outreach and educational materials and workshops.

SIGNIFICANT FINDINGS

- Year differences explained 12% and 15% of fruit firmness and size variability, respectively. In 2022, fruit firmness was 16% higher, ranging between 269 and 388 gr $\cdot$ mm^{-1} across varieties and sites.
- Variety differences explained only 5% of firmness and size variability, when compared across year, while the interaction of year x cultivar, explained 20% of firmness variability and 23% of size variability.
- Firmness and size were very variable within sites. An example is provided where firmness ranged between 89 and 480 g $\cdot$ mm^{-1} on a Skeena site. Similarly for fruit size.
- Cultivar and Cultivar x Year interaction had the greatest influence in fruit nutrient variability, being highest for K.
- Nutrients vary by site and year, however with no clear relation with fruit firmness and size differences.

- Fruit size and weight were significantly and negatively correlated with N, P, K, Ca, Mg dry concentration and with P, K, Ca, and Mg fresh concentration, however also the correlations were weak.
- When comparing means between big and small, or firm and soft, across all years and cultivars. Macronutrient concentrations were always higher in the small fruit, while no nutrient differences were found between firm and soft fruit.
- Only when dividing all fruit samples, across cultivars, years, and sites, into three firmness levels, we found that soft fruit (firmness < 200 $g \cdot mm^{-1}$) had reduced N and S concentration.
- The lack of relationship between fruit quality parameters and nutrient concentrations can be attributed to the high levels of nutrients found in all samples, being within or above the critical values reported for sweet cherry in the literature.
- Nutrient extraction was determined for Skeena, Coral Champagne, and Chelan. Given the consistency of the results across sites, years and cultivars, these values are likely representative of most sweet cherry cultivars grown in Washington.

METHODS

This project takes an observational approach to better understanding the relationships between cherry fruit quality/storability, and fruit nutrient content. There are no imposed treatments, instead, we collected fruit from four commercial warehouses around the state and worked with the natural variability in quality that exists.

The relationship between fruit quality and storability was analyzed for Chelan, Coral Champagne from three commercial orchards and five commercial orchards of Skeena. For each cultivar and orchard, we obtained four replicate bulk fruit samples of at least 5 lbs of the largest and smallest fruit size from the packing house (typically 12-row and 9-row+), in order to have sufficient fruit for storage and nutrient testing from each size category. Each replicated sample from each size category was divided in half (ca. 2.5 lbs). One set of samples were sent to Torre's laboratory at TFREC for storage evaluation test, and the other half were taken to Whiting's laboratory for harvest analysis at IAREC. In Whiting's laboratory, fruit were analyzed individually for weight, size (mm) and firmness (Firmtech II). Further, for each sample unit (ca. 100 fruit each), the 10^{th} and 90^{th} percentile ranking of firmness testing were selected for nutritional analysis (minimum 15 fruit per category) (Figure 1). To determine fruit nutrient content, each fruit sample were separated into pulp, stems and pits to determine fresh and dry weight ratios. Dried tissue samples were homogenized and sent for chemical analysis of nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), manganese (Mn), cupper (Cu), zinc (Zn) and boron (B). To ensure representative and consistent nutrient analyses, samples were sent to Soil Test laboratory (Moses Lake) for total nutrient. Soil Test laboratory is a certified laboratory by the Soil Science Society of America and the North American Proficiency Test Program (NAPT) for plant program assessment (visit https://www.naptprogram.org/about/participants?ssoContinue=1). The laboratory incorporates blind certified sample to monitor nutrient accuracy by utilizing certified material from NAPT program.

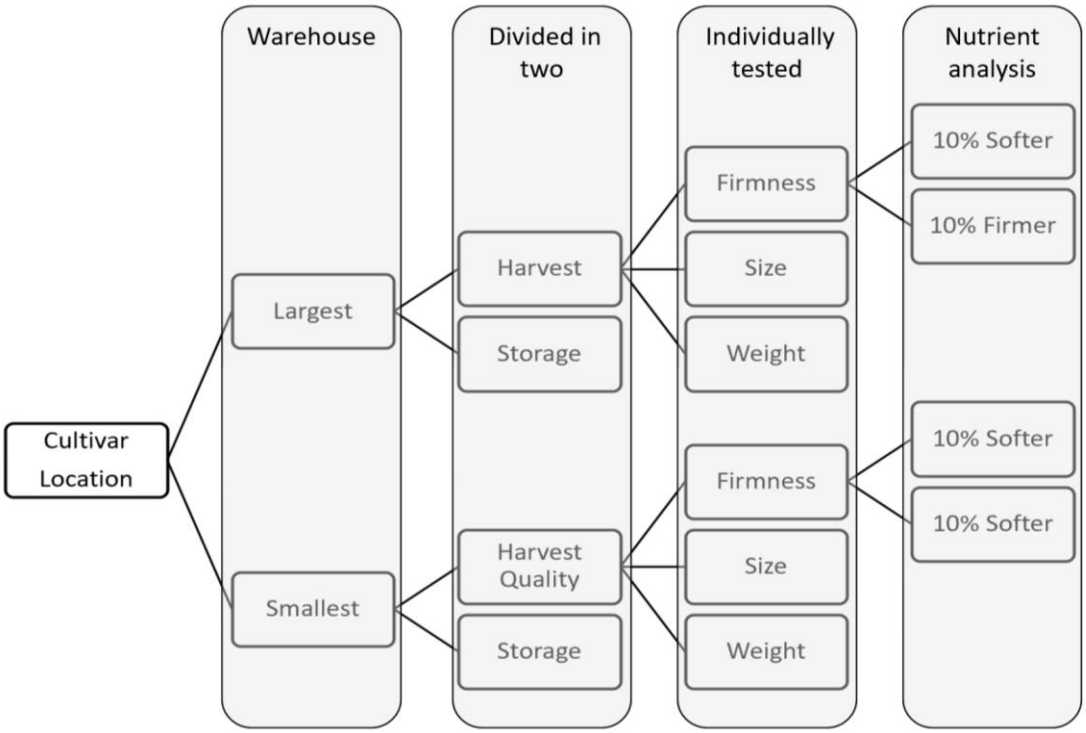

Figure 1. Fruit sampling scheme for nutrient and storability analyses.

In Co-PI Torres's laboratory in Wenatchee, fruit were stored for four weeks in cold storage, and analyzed fruit weight, color, size and firmness, plus storage disorders including decay, stem browning, or pitting.

RESULTS AND DISCUSSION

Fruit quality summary by year, cultivar, and site

Fruit quality varies widely among years, cultivars, and sites (Table 1). When evaluating all the fruit received from the packing houses, year differences explained 12% and 15% of fruit firmness and size variability, respectively ($p<0.001$). Fruit firmness was 16% higher in 2022, ranging between 269 and 388 $g \cdot mm^{-1}$ across cultivar and sites. Fruit size was also 3% and 14% higher in 2022, compared to 2021 and 2023, respectively (Table 1). The variety, on the other hand, explained only 5% of firmness and size variability, when comparing across year ($p<0.001$), while the interaction of year and cultivar, explained 20% of firmness variability and 23% of size variability.

Skeena fruit was consistently larger than Coral Champagne and Chelan (4 to14% larger), and Chelan was larger than Coral Champagne in 2021 and 2023, but smaller in 2023. In relation to fruit firmness, Coral Champagne was always softer ($238 – 292\ g \cdot mm^{-1}$) than the other two varieties, while Chelan was firmer than Skeena in 2021 and 2023, but not in 2022 (Table 1). The impact of site on fruit firmness and size was also significant ($p<0.05$), however among the explanatory variables, year and cultivars were the most influential. Across all years, the variability in firmness among sites, cultivars and years is very high. Figure 1 represents firmness and size variability among orchards for Skeena in 2022, underscoring the importance of managing variability in orchards to maximize the proportion of the higher quality fruit.

Table 1. Fruit firmness and diameter differences by year, cultivar, and site. Different letters indicate statistical differences between years (bold capital), cultivars within years (bold) and among sites within year and cultivar (small letters) (Tukey test and $p < 0.05$)

			Firmness ($g \cdot mm^{-1}$)				Diameter (mm)			
Year	Variety	Site	Mean	Min	Max	StdDev	Mean	Min	Max	StdDev
2021	Chelan	1	295b	139	427	51.5	25b	20	33	3.4
		2	301a	159	444	49.4	26b	20	30	3.3
		3	255c	140	367	43.8	29a	25	35	2.9
	Chelan		**285.1a**	**139**	**444**	**52.4**	**26.6b**	**20**	**35**	**3.7**
	Coral	1	233b	123	377	44.8	25b	19	32	4.3
		2	236b	131	382	43.6	27a	22	32	2.8
		3	247a	140	360	36.2	25b	20	32	4.1
	Coral		**238.6c**	**123**	**382**	**42.1**	**25.6c**	**19**	**32**	**3.9**
	Skeena	1	304a	176	422	43.6	27b	24	32	2.1
		2	278c	161	394	36.7	28a	22	32	1.9
		3	289b	172	419	42.9	26c	22	30	2.0
		4	260d	146	394	40.4	26c	22	31	2.3
		5	260d	164	378	33.4	28a	25	31	1.2
	Skeena		**277.3b**	**146**	**422**	**42.5**	**26.8a**	**22**	**32**	**2.1**
2021			**269.3B**	**123**	**444**	**49.5**	**26.4B**	**19**	**35**	**3.2**
2022	Chelan	1	269c	134	434	48.3	25c	20	32	3.5
		2	350a	150	613	60.8	27b	21	34	3.6
		3	313b	154	544	58.7	29a	22	35	3.3
	Chelan		**310.9b**	**134**	**613**	**65.6**	**26.9b**	**20**	**35**	**3.8**
	Coral	1	275b	130	457	62.3	26b	21	31	3.4
		2	302a	199	636	45.2	27a	22	34	4.0
		3	298a	140	448	47.3	26b	20	34	4.5
	Coral		**292.0c**	**130**	**636**	**53.3**	**26.0c**	**20**	**34**	**4.0**
	Skeena	1	309c	134	537	54.9	29c	22	34	2.5
		2	388a	197	614	62.9	30a	25	33	1.0
		3	324b	205	527	49.1	28d	23	32	2.4
		4	305c	170	440	43.4	29b	23	33	1.5
		5	322b	133	539	49.9	28d	22	32	2.5
	Skeena		**326.5a**	**133**	**614**	**58.2**	**28.6a**	**22**	**34**	**2.3**
2022			**311.4A**	**130**	**636**	**60.9**	**27.3A**	**20**	**35**	**3.6**
2023	Chelan	2	267b	139	478	47.0	22a	18	30	2.4
		3	303a	169	628	57.0	22a	18	26	2.1
	Chelan		**285.0a**	**139**	**628**	**55.1**	**22.2c**	**18**	**30**	**2.3**
	Coral	1	245b	156	401	37.2	23a	17	31	4.1
		3	265a	137	408	37.1	24a	17	30	2.3
	Coral		**255.1c**	**137**	**408**	**38.6**	**23.5b**	**17**	**31**	**3.3**
	Skeena	1	268a	101	455	55.4	25b	21	32	2.3
		3	265ab	97	480	54.7	26a	20	31	2.1
		4	261b	89	468	47.0	25b	21	31	2.2
	Skeena		**264.8b**	**89**	**480**	**52.6**	**25.4a**	**20**	**32**	**2.2**
2023 Total			**268.2B**	**89**	**628**	**51.2**	**23.9C**	**17**	**32**	**2.9**

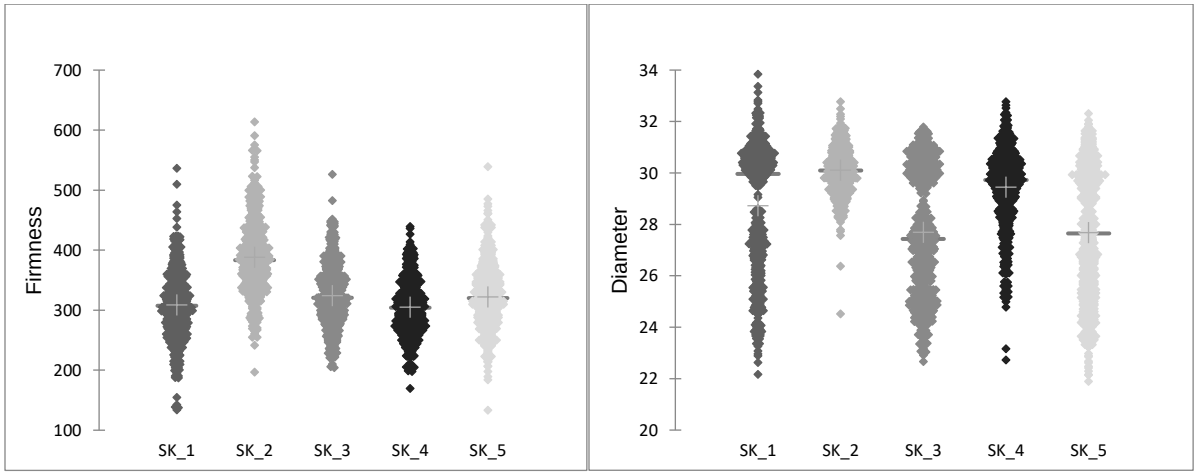

Figure 1. Firmness (left) and fruit size (right) variability across Skeena orchards in 2022. Middle cross indicates mean value.

Nutrient differences of segregated fruit by year, cultivar and site

Within each size category, fruit from the 10th and 90th percentile ranking of firmness were selected for individual fruit quality analyses and then were combined for nutrient analyses. By the time of this report, we have not received the nutrient results for 2023 Skeena, thus information and conclusions are based on 2021, 2022 and 2023 Chelan and Coral Champagne.

When combining all categories and sites, fruit concentration of N, K, Mg and S was different among years, and all macronutrients were different among cultivars (Table 2).

Table 2. Fruit firmness, size, weight, dry matter (DM) and macronutrient differences among years, variety. Different letters in the same column indicate significant differences within year and variety based on Tukey test ($p<0.05$). R^2 indicates the percentage of the variability in nutrient concentration (%) explained by the interaction of year and variety, shown only for factors with significant p value.

Factor		Firmness ($g.mm^{-1}$)	Size (mm)	Weight (g)	DM	N	P	K	Ca	Mg	S
						mg $\cdot 100g^{-1}$ (fresh)					
Year	2021	273.0 b	26.7 b	9.0 b	19 a	189.0 b	32.1	261.0 a	19.5	16.8 a	12.9 a
	2022	316.0 a	29.9 a	10.8 a	19 a	201.4 a	30.8	234.3 b	19.6	15.3 b	13.2 a
	2023	275.0 b	22.8 c	8.2 b	18 b	174.6 b	32.1	216.7 c	20.3	15.0 b	11.0 b
p value		<0.001	<0.001	<0.001	0.01	0.003	0.18	<0.001	0.702	<0.001	0.001
Variety	Ch*	295.5 a	26.5 b	8.3 c	19	212.6 a	33.5 a	273.4 a	23.0 a	17.7 a	13.2 a
	CC	268.2 b	26.7 b	9.1 b	19	180.5 b	29.4 b	226.8 b	20.2 b	14.9 b	11.6 b
	Sk	306.5 a	28.7 a	11.1 a	19	184.0 b	31.9 a	231.1 b	16.4 c	15.2 b	13.2 a
p value		0.000	<0.001	<0.001	0.51	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Year*Variety		0.89	0.22	0.007	0.99	0.000	0.270	0.001	0.642	0.188	0.000
	R^2	-	-	0.24	-	0.15	-	0.27	-	-	0.12

*Ch; Chelan, CC; Coral Champagne, Sk; Skeena.

Nitrogen was 6 and 15% higher in 2022 when compared with 2021 and 2023 respectively, which aligned with 2022 having firmer and bigger fruit. However, K and Mg were higher in 2021, and S lowest in 2023,

Table 3. Firmness, size and nutrient concentration by cultivar and year. Different letters in the same column indicate significant differences based on Tukey test ($p<0.05$).

Cultivar	Year /Site		Firmness ($g.mm^{-1}$)	Diameter (mm)	Nutrient concentration fresh ($mg \cdot 100g^{-1}$)					
					N	P	K	Ca	Mg	S
Chelan	2021	1	277	25.3 b	227 ab	36.1 ab	303.2	25.5	18.9	12.4 b
		2	303	25.0 b	272 a	39.4 a	323.9	22.4	20.0	15.7 a
		3	253	29.8 a	187 b	29.6 b	274.7	20.9	19.9	15.4 ab
	p value		0.110	0.000	0.010	0.013	0.147	0.411	0.829	0.073
	2022	1	271 b	28.4	184 b	31.9 b	255 b	25 ab	16.0 b	10.1 c
		2	359 a	30.4	229 a	35.9 a	312 a	27 a	18.5 a	12.6 b
		3	317 ab	29.1	200 b	26.5 c	251 b	21 b	15.7 b	15.1 a
	p value		0.042	0.254	0.003	<0.0001	<0.0001	0.050	0.006	<0.0001
	2023	2	272	22.1	191	35.2	234.9	23.4	16.7	11.5
		3	312	22.2	211	33.2	232.4	19.4	15.5	12.7
	p value		0.237	0.934	0.105	0.430	0.788	0.097	0.051	0.061
Coral Champagne	2021	1	240	25.5	193.1	35.0 a	261.3 a	23.2	17.2	12.1
		2	251	27.0	167.3	26.0 b	205.2 b	18.2	14.2	10.4
		3	244	25.5	171.8	28.3 b	235.2 ab	20.4	16.4	11.4
	p value		0.864	0.482	0.151	0.002	0.039	0.184	0.170	0.127
	2022	1	283	29.2	194.6	27.1	231.7	22.0 a	14.6 ab	11.4 b
		2	311	30.6	228.0	31.3	255.2	16.1 b	13.6 b	15.4 a
		3	300	29.0	192.6	27.4	226.5	23.2 a	15.7 a	12.7 b
	p value		0.641	0.426	0.101	0.046	0.074	0.001	0.049	0.006
	2023	1	249	23.5	127.5 b	31.4	213 a	20.3	13.6	10.9 a
		3	267	23.4	169.1 a	28.6	186 b	18.2	14.1	8.6 b
	p value		0.453	0.949	0.000	0.068	0.002	0.058	0.481	0.001
Skeena	2021	1	317	27.6 ab	218.4 a	29.9 ab	255 ab	15.2 bc	17.1 a	17.4 a
		2	283	27.8 a	203.0 a	34.4 a	240 ab	19.5 a	15.6 ab	12.2 b
		3	300	26.3 ab	143.3 b	33.7 ab	276 a	17.9 ab	16.0 ab	11.1 b
		4	266	26.1 b	136.6 b	33.3 ab	268 ab	18.6 a	16.7 a	11.5 b
		5	269	27.7 ab	159.9 b	28.0 b	227 b	12.7 c	13.2 b	11.9 b
	p value		0.081	0.017	<0.0001	0.030	0.042	<0.0001	0.011	<0.0001
	2023	1	313	31.2 a	180.3 b	32.9 a	196	16.8	15.0 ab	13.5 ab
		2	400	32.6 a	246.5 a	37.0 a	207	16.0	16.9 a	16.3 a
		3	329	30.7 a	194.4 ab	31.5 ab	204	16.3	13.4 b	12.7 b
		4	308	30.7 a	207.0 ab	32.8 ab	215	15.1	15.4 ab	14.3 ab
		5	327	28.0 b	181.4 b	27.8 b	211	15.9	13.7 b	12.3 b
	p value		0.132	<0.0001	0.029	0.006	0.809	0.748	0.011	0.029

with no relation to fruit firmness or size. Among cultivars, Chelan had more than 15% higher N concentration, with no differences between Coral Champagne and Skeena, and no relationship with fruit quality (i.e., Chelan and Skeena were the firmest and Skeena were the largest). Coral Champagne had the lowest P and S, while highest K, Ca, Mg, again, with no relation with fruit firmness and size (Table 2). Overall, the cultivar and cultivar*year interaction had a greater influence in fruit nutrient variability (R2 value) being highest for K (27%).

Nutrients also varied by site and year (Table 3), however with no clear relation with fruit firmness and size differences. For example, Chelan site 2 had higher firmness fruit in 2022, with also higher N, P, K, Ca and Mg concentration. Site 3 had no nutrient concentration differences with site 1, despite being 18% larger. For Coral Champagne, there were no differences in fruit firmness and size among sites across all years, while nutrient concentration was different among sites. For example, N levels were higher in site 3 only in 2023, P and K levels were higher in site 1 in 2021 and 2023, and Ca levels were higher in site 1 and 3, but only in 2022. Similarly, for Skeena, during 2021 and 2022 there were no differences in firmness, while size differences were agronomically irrelevant and only significant between site 4 (26 mm) and site 2 (28 mm) in 2021, and site 5 having the smallest size in 2022 (28 mm). In Skeena, N levels differences were inconsistent in 2021 and 2022 (Table 3), with site 5 being consistently lowest in P.

Nutrient relationship with fruit quality

To better understand the relation between firmness, diameter and weight with nutrient concentrations, we evaluated the correlations across all samples (n=408). Firmness correlated significantly ($p<0.001$) with N and S concentrations in dry and fresh weight, and with P in fresh weight, however the correlations were weak (R2 below 0.23). Fruit size and weight were significantly and negatively correlated with N, P, K, Ca, Mg dry concentration and with P, K, Ca, and Mg fresh concentration, however again the correlations were weak (R2 below 0.5) (Table 4).

Table 4. Pearson correlation between fruit quality indicators and dry nutrient concentration (%) and fresh nutrient concentration (mg/100g). Bold values indicate significance level of $p< 0.05$.

Variables	Firmness ($g \cdot mm^{-1}$)	Diameter (mm)	Weight (g)
N %	**0.183**	**-0.107**	**-0.124**
P %	0.023	**-0.343**	**-0.236**
K %	-0.059	**-0.331**	**-0.389**
Ca %	-0.073	**-0.485**	**-0.509**
Mg %	-0.059	**-0.425**	**-0.440**
S %	**0.177**	0.043	0.010
Dry Matter %	**0.153**	**0.193**	**0.089**
N mg/100g	**0.236**	-0.006	-0.066
P mg/100g	**0.116**	**-0.156**	**-0.134**
K mg/100g	0.035	**-0.156**	**-0.257**
Ca mg/100g	-0.002	**-0.363**	**-0.424**
Mg mg/100g	0.039	**-0.234**	**-0.306**
S mg/100g	**0.228**	**0.124**	0.040

Given the significance of years and cultivar in fruit quality, we evaluated the correlation after grouping by year or cultivar. For firmness, the correlations with nutrients were either not significant ($p>0.05$) or weak ($R2 < 0.37$) across all years and cultivars (data not shown). When grouping by year, fruit size (diameter or

weight) was strongly and negatively correlated Ca concentration (R2 > -0.70) and Ca content (R2 > -0.69), but only in 2021 (data not shown). While there were no strong correlations between fruit quality and nutrients when grouping by cultivar. The relationship between Ca and fruit size appears to be a consequence of higher concentration of Ca in small fruit, rather than a cause effect relationship.

While there were no correlations between nutrients and fruit quality, when comparing means between categories; big and small, or firm and soft, across all years and cultivars, macronutrient concentrations were always higher in the small fruit, while no differences were found between firm and soft fruit (Table 5). The interaction of fruit quality categories firmness x size, was a secondary factor for nutrient levels, being significant ($p<0.05$) for P, K, Ca, Mg and S (Table 4), however the percentage explained by the interaction was generally low, except for Ca. Here, size and firmness explained 24% of Ca variability, attributed largely to fruit size.

Table 5. Fruit nutrient concentration differences between size, firmness, and the interaction of size x firmness categories across all three years and cultivars. Different letters in the same column indicate significant differences within size and firmness category based on ANOVA test ($p<0.05$). R^2 indicates the percentage of the variability in nutrient concentration (%) explained by the interaction of fruit size and firmness.

Fruit Quality Category			Nutrient concentration dry (%)[1]						
		Unit[2]	Dry Matter	N	P	K	Ca	Mg	S
SIZE	Small	24.6 b	18%b	1.05 a	0.17 a	1.36 a	0.12 a	0.09 a	0.07 a
	Big	30.0 a	19%a	0.98 b	0.16 b	1.22 b	0.09 b	0.08 b	0.07 b
	p value	<0.0001	0.004	0.002	<0.0001	<0.0001	<0.0001	<0.0001	0.006
FIRMNESS	Firm	361 a	0.19	1.02	0.17	1.28	0.10	0.08	0.07
	Soft	221 a	0.19	1.00	0.17	1.29	0.10	0.08	0.07
	p value	<0.0001	0.683	0.482	0.474	0.462	0.373	0.361	0.299
SIZE x FIRMNESS	p value		0.595	0.098	0.002	0.040	0.067	0.034	0.013
	R^2		0.02	0.03	0.08	0.11	0.24	0.15	0.04

[1]Means of 208 fruits/category. [2]Unit of category, being diameter (mm) for size, and force ($g.mm^{-1}$) for firmness.

The distribution of nutrient concentration across sites and years by cultivars also was highly variable (Figure 2). And despite the differences among cultivars (Table 2), nutrient levels reported for sweet cherry in our study are either within or above the critical levels reported in the literature (Figure 2).

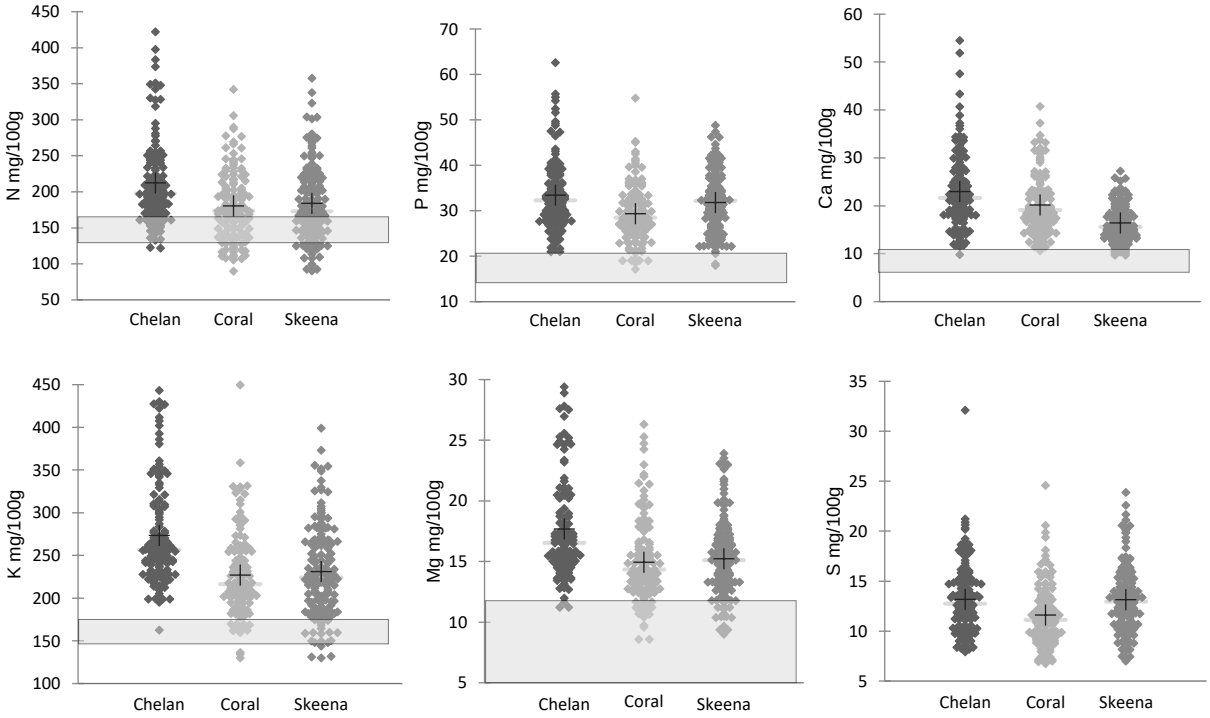

Figure 2. Fruit macronutrient distribution by cultivar. The gray box corresponds to the critical range reported in the literature for sweet cherries.

To further assess the relationship between firmness and nutrient levels, we combined all data; cultivars, sites and years, and categorized them in three firmness levels, defining firm (> 300 g·mm^{-1}), middle (200 – 300 g·mm^{-1}) and soft (< 200 g·mm^{-1}). Consistent with 2022 results, only N and S concentration were significantly lower in the soft fruit (Table 6).

Table 6. Nutrient and dry matter differences between firmness categories for all sites, cultivars and years. Different letters indicate statistical differences between columns ($p<0.05$)

Firmness	Nutrient concentration fresh mg.100g^{-1}					
	N	P	K	Ca	Mg	S
Middle	193.3 a	31.7	244.9	19.2	16.1	12.8 a
Firm	194.8 a	31.8	241.4	19.1	15.8	13.0 a
Soft	163.0 b	29.9	229.2	20.4	15.3	10.9 b
p value	0.000	0.201	0.228	0.399	0.410	0.000

Nutrient extraction

Nutrient extraction was determined for 2021 and 2022, as we are still waiting for 2023 nutrient tests for Skeena. The extraction of nutrients was determined for the best quality fruit (big and firm fruit) for all sites, as a guide to calculate rate of fertilizer needed for a ton of fruit produced. It should be noted, that regardless the differences in fruit quality and yields between years, the extraction of nutrients by the fruit were not different, nor there were differences between cultivars. The ranges of extraction determined in

our study are equivalent to values reported in the literature, however with narrower ranges, providing a more precise estimation of nutrient demand by harvested crop.

Table 7. Nutrient extraction in pounds per ton of fruit produced by cultivar for 2021 and 2022.

	N	P	K	Ca	Mg	S
Nutrient / Cultivar	Lb/USTon					
Chelan	4.33	0.76	5.77	0.38	0.33	0.27
2021	4.34	0.78	5.97	0.33	0.35	0.27
2022	4.32	0.73	5.54	0.43	0.32	0.27
Coral Champagne	4.52	0.72	5.04	0.36	0.30	0.28
2021	4.30	0.74	4.97	0.36	0.31	0.26
2022	4.75	0.71	5.11	0.36	0.29	0.30
Skeena	4.57	0.83	4.97	0.33	0.32	0.28
2021	4.63	0.86	5.79	0.37	0.34	0.29
2022	4.51	0.79	4.15	0.30	0.29	0.27
Range	4.3 - 4.8	0.71 - 0.86	4.15 - 5.97	0.3 - 0.4	0.29 - 0.35	0.27 - 0.30
Literature*	2.7 - 11.7	1.50	7.60	0.40	-	-

We requested a no cost extension to provide in-depth analysis of all data collected in this three-year study. Given that nutrient results have not been returned by the collaborating laboratory, we can’t draw further conclusions. In our final report, we will also include results from post-harvest analysis and the outreach and extension efforts develop with the information gained.

Project Title: Precision Nutrient Management for sweet cherry orchards

Report Type: Continuing Project Report

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Cooperators: Douglas Fruit, Cameron Nursery, Dory Linneman and Dana Sirota (PNNL), Lav Khot.

Project Duration: 3 Year

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

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

Item	2023	2024	2025
Salaries			
Benefits			
Wages[1]	7,190	7,478	7,777
Benefits	734	763	793
RCA Room Rental			
Shipping			
Services	56,680	6,680	6,680
Supplies	4,400	4,400	4,400
Travel	1,000	1,000	1,000
Miscellaneous			
Plot Fees			
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 objectives are to investigate new technology for determining biomass partitioning and root growth, through an integration of existing resources, expertise, and technology. This proposal addresses the priority listed in the 2022 RFP. This study will provide information to validate (or not) the current premise for calculating nutrient demand based on fruit extraction only, determine the differences between growing conditions and rootstocks, and provide an estimation of nutrient demand for sweet cherry.

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

All proposed sites and two additional commercial sites were selected for objective 1.
These sites are: 1) A 5th leaf Benton on MxM14, Gisela 12 and Gisela 5. 2) 12th leaf Skeena and Selah on G.12. 3) A first leaf Skeena G.6, G.12 and Mazzard.

Sites 1 and 2 are the only ones bearing fruit, and fruit yield, biomass partitioning of the fruit and nutrient levels were determined after harvest. Sites 3 correspond to new plantings, where 3 trees per rootstock were selected for complete destructive analysis.

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

Root growth was evaluated in sites 1 and 2. Root windows were installed on one of each rootstock (Example of the root windows can be found in Good Fruit Grower, link https://www.goodfruit.com/a-window-to-the-roots/). Root growth was monitored starting at bloom and continued on a biweekly basis until leaf fall. Images were captured on a 1 ft grid for the root window.

The minirhizotron (CI-600 In-Situ Root Imager, CID-Bioscience) was received in the summer, so the contrast between tools will start in year 2. Similarly, the Electrical resistivity tomography (*ERT*) to be monitored by our collaborators at PNNL, was not installed due to administrative delays and changes in leadership for the project. This system will be installed during the fall of year 1.

3. Soil Nutrient Availability and Leaching

To determine the availability and potential leaching of nutrients throughout the season, nutrient levels were evaluated at three depths throughout the season; bloom, active shoot growth, end of shoot growth, leaf yellowing, after leaf drop.

4. Deliver outreach and extension of new findings.

No outreach or extension activities to report for year 1.

SIGNIFICANT FINDINGS

- Biomass partitioning varied, depending on the rootstock, with higher biomass allocated to underground tissue (big and fine roots) in the more vigorous rootstocks Mazzard, compared to G12 and G6.
- Fruit fresh partitioning and dry matter also varied across rootstocks.
- Root growth started approximately a month after full bloom, when soil temperatures were above 70 F.
- In both Gisela rootstock, roots had a second flush of growth around September 19th, however it averaged less than 45 cm per square foot and lasted less than a month.
- Nitrate levels in sites 1 and 2 were within normal ranges, with consistently higher levels in the first 8 inches, declining in depth, suggesting little to no leaching between June and September.

METHODS

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

Biomass partitioning is being evaluated in the following conditions:

- 5th leaf Benton on MxM14, Gisela 12 and Gisela 5. Steep leader training
- 12th leaf Skeena and Selah on G.12. UFO
- 1st leaf Black Pearl on G.6, G.12 and Mazzard

At harvest, sites 1 and 2 were harvested, and to determine yield and nutrient extraction of fruit from four replicates per rootstock / cultivar. After harvest, a subsample of leaves was collected to determine nutrient levels in recently mature leaves to monitor nutrient status.

During the fall we will collect all leaves, weigh them, determine leaf area and nutrient levels at leaf drop. During the winter, three trees per site and rootstock will be excavated for whole tree partitioning and biomass allocation: total weight of each tree component, dry matter of each component, and total macronutrients N, P, K, Ca, Mg and micronutrient B.

During the winter we will utilize vision system technology to assess growth and monitor additional environmental and growing conditions (AgWeathernet, soil moisture content, among other).

2. Timing: Identify strategies to monitor root growth

Root growth was evaluated in sites 1 and 2, with different rootstocks and systems. During year one, root windows were installed on each rootstock (Example of the root windows can be found in Good Fruit Grower, link https://www.goodfruit.com/a-window-to-the-roots/). Root growth was monitored starting at bloom, on a biweekly basis until leaf fall. Images were captured on a 1 ft grid for the root window.

In year 2, we will contrast the information with two newer technologies: a minirhizotron (CI-600 In-Situ Root Imager, CID-Bioscience) and an Electrical resistivity tomography (*ERT*). Both are non-destructive root monitoring systems that can help monitor root growth in established orchards. The minirhizotron is a 360 scanner that can be inserted in clear plastic tubes buried around the trees to scan roots throughout the season, while the ERT uses electrical sensors to monitor growth, a

technology yet to be validated in orchard settings. The latter will be evaluated by our collaborators at the Pacific Northwest National Laboratory located in Richland, WA.

All images will be analyzed using the WinRHIZOTRON image analysis software (Regent Instruments Canada, Quebec, Canada).

5. Soil Nutrient Availability and Leaching

To determine the availability and potential leaching of nutrients throughout the season, nutrient levels were evaluated in the root zone and below the root zone, six times through the season; bloom, active shoot growth, end of shoot growth, leaf yellowing, after leaf drop. This will inform fertilization timing to increase efficiency in nutrient uptake.

RESULTS AND DISCUSSION

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

Bearing sites
Biomass partitioning was determined in fruit on two sites: 1) a 5 year old sweet cherry 'Benton' block, grown on three different rootstock; MxM14, Gisela 12 and Gisela 5, and 2) A 12 year-old block of Skeena and Selah on Gisela 12.

The average fruit size for site 1 ranged between 8.5 and 11 g with no statistical differences between rootstocks. While fruit diameter ranged between 18.7 and 29.5 mm (equivalent to rows 12 to 9), being 8.5% and 3.5% smaller in Gi5 and Gi 12 respectively, compared to MxM14 ($p < 0.001$) (Figure 1)

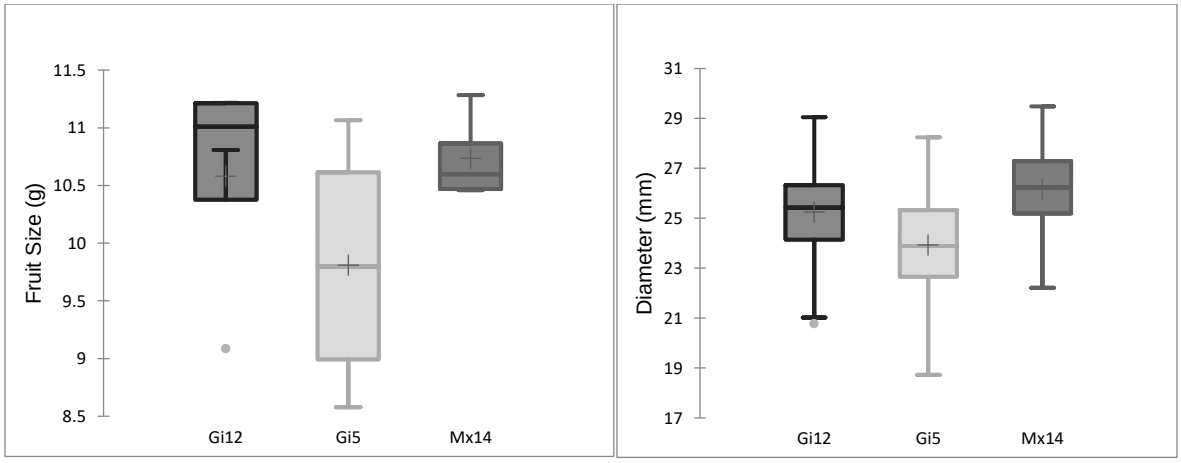

Figure 1. Fruit size (grams) box plot for Benton sweet cherry on Gisela 12 (Gi12), Gisela 5 (Gi5) and MxM 14 (Mx14).

Table 1. Fruit fresh partitions into flesh, pit and stems, and tissue dry matter by rootstock. Different letters indicate statistical differences within columns.

Rootstock	Fruit Weight (g)	Fresh (%)			Dry Matter (%)		
		Flesh	Pit	Stems	Flesh	Pit	Stems
Gi5	9.8	5.1	1.0a	0.10	23	58a	52
Gi12	10.6	4.8	1.0a	0.05	23	59a	47
MxM14	10.7	5.6	0.7b	0.10	22	52b	48
Pr > F(Model)	0.352	0.182	0.001	0.671	0.379	0.003	0.262

The average fruit size for site 2 ranged between 6.4 and 9.5 g with no statistical differences between cultivars. Fruit diameter ranged between 15.5 and 27.4 mm (equivalent to rows 12 to 9.5), being 5.7% smaller in Selah compared to Skeena ($p < 0.001$). (Figure 2)

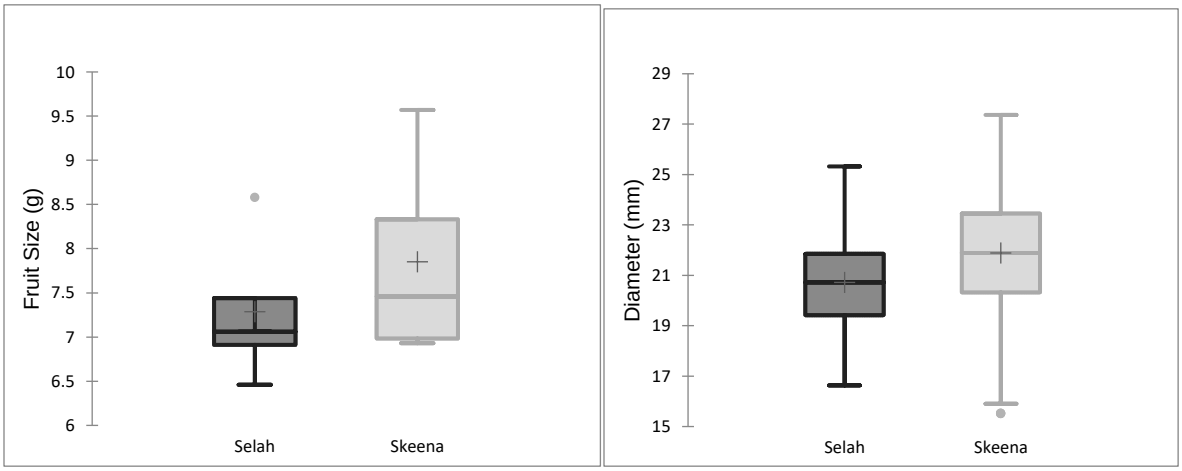

Figure 2. Fruit size (grams) box plot chart for Selah and Skeena on G12.

Partitioning work for Selah and Skeena fruit is underway. Whole tree partitioning for all sites have not been determined for year 1, as it will be carried out during the dormant season (after natural leaf drop).

Non-bearing site

Black Pearl trees grown on three different rootstocks were selected prior to planting to determine initial biomass partitioning when grafted into three different rootstocks: G.6, G.12 and Mazzard. An additional nine trees per rootstock were planted in IAREC research center to monitor and evaluate over the next two years.

Initial fresh partitioning varies among rootstocks (Table 2), with Mazzard having more than double the allocation of fresh weight partitioned towards big and fine roots compared to the Gisela rootstocks. Between the two Gisela rootstocks, the only difference was the proportion of fine roots, which were almost double in Gi6 compared to Gi12 (Table X)

Table 2. Fresh weight partitioning and dry matter of different tree structures of Black Pearl grown on G6, G12 and Mazzard.

Rootstock	Fresh Partitioning (%)					Dry Matter (%)				
	Leader	Laterals + Buds	Trunk	Big Roots	Fine Roots	Leader	Laterals + Buds	Trunk	Big Roots	Fine Roots
Gi6	48a	15a	15.6	16a	4.9b	54	48a	49	54	51
Gi12	55a	15a	16.5	10a	2.5c	52	47ab	61	43	48
Mazzard	33b	7.9b	15.6	33b	10.0a	53	39b	60	35	47
Pr > F(Model)	0.006	0.050	0.858	0.018	0.000	0.676	0.055	0.195	0.145	0.773

The biomass (dry weight) partitioning was equivalent to fresh partitioning, due to the consistency in dry matter content for each tissue across rootstocks, with the exception of the laterals+buds, where Mazzard had 8% less dry matter compared to both Gisela rootstocks (Table 2), leading to significant differences in biomass (dry) partitioning (Figure 3).

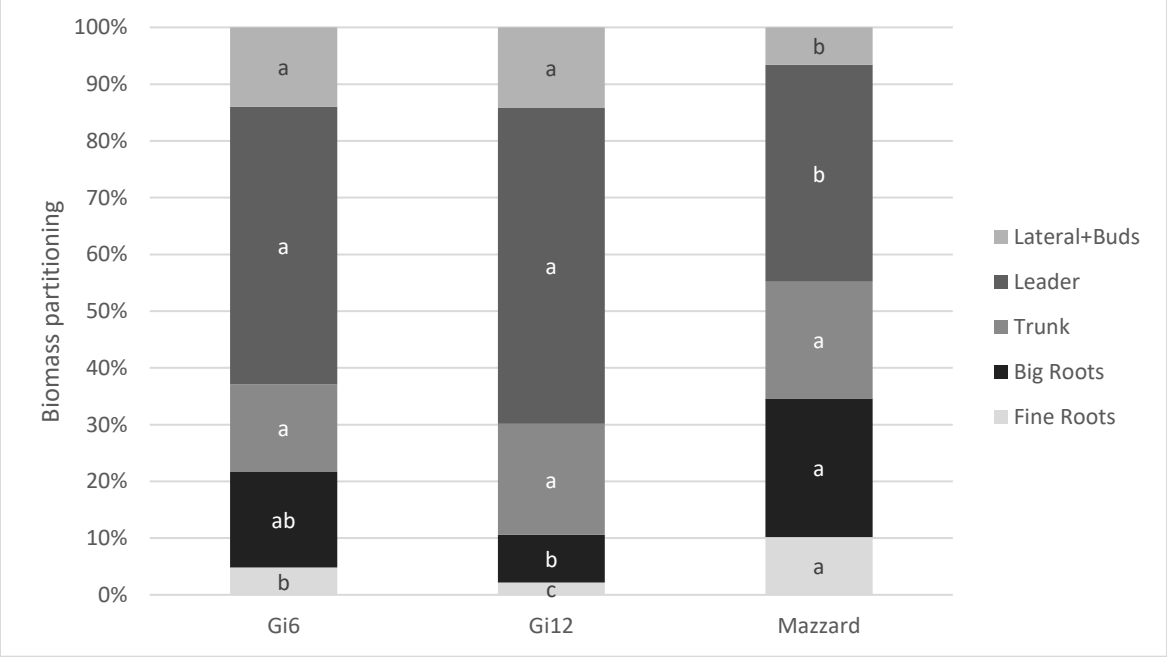

Figure 3. Whole tree biomass partitioning of Black Pearl on Gi6, Gi12 and Mazzard nursery stock prior to planting.

Nutrient partitioning and nutrient content of fruit and new trees for year one is still under evaluation. In year 2, we will include data from two additional commercial sites, to determine differences across growing conditions.

2. Timing: Identify strategies to monitor root growth

Root windows were installed on Sites 1 and 2, in three replicates per treatment and monitored throughout the season starting May 24th (Figure 4).

Figure 4. Root growth images collected on June 6th for Gisela 5, Gisela 12 and MxM 14 (left to right), with different colors associated to different dates.

Roots from all rootstocks started to grow around the middle of May, approximately a month after full bloom, as soil temperature at 8-inches were above 70 F. The average growth of individual roots during the first two weeks was less than 2 cm (data not shown), while higher root growth rate was observed between June 6th and the end of July (Figure 5), overlapping with higher shoot growth and stage III of fruit growth. Trees were harvested June 26th and the roots continued to grow until July 31st, however after this date, roots started to decay and turnover. In both Gisela rootstocks, roots started a new flush of growth around September 19th (data not shown), however it averaged less than 45 cm per square foot and lasted less than a month.

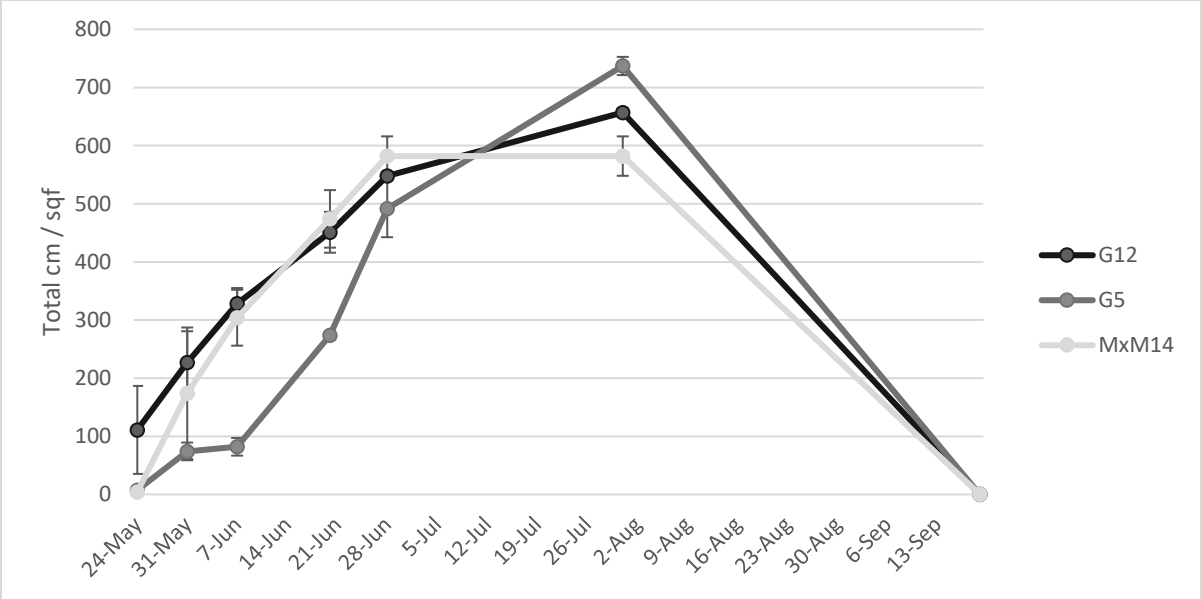

Figure 5. Total root cumulative growth (cm) observed in one square foot two-dimensional section, on Benton sweet cherry grown on Gisela 12, Gisela 5 and MxM14.

On site 2, we did not observe root growth in the root windows until August - September. Here the root windows were established around bloom, which might have impacted the area around the root window, preventing us from observing the growth of roots. This has been previously reported, where it is suggested that measurements be collected after soil and roots have settled for at least a couple of months.

In years 2 and 3, we will install the minirhizotron (CI-600 In-Situ Root Imager, CID-Bioscience) and the Electrical resistivity tomography (*ERT*) to contrast technologies.

3. Soil Nutrient Availability and Leaching

Nutrient levels were measured during June, July, August and September at three depth: 8, 12 and 24 inches. Soil nitrate (NO_3) levels where low ranging between 0.2 and 12 mg/kg, with higher levels in the surface across all dates, suggesting higher mineralization and availability. It appears that there was little or no leaching as we saw nitrate levels decrease with increasing soil depth. We will continue to measure mineral nitrogen throughout the year in additional sites during year 2 and 3.

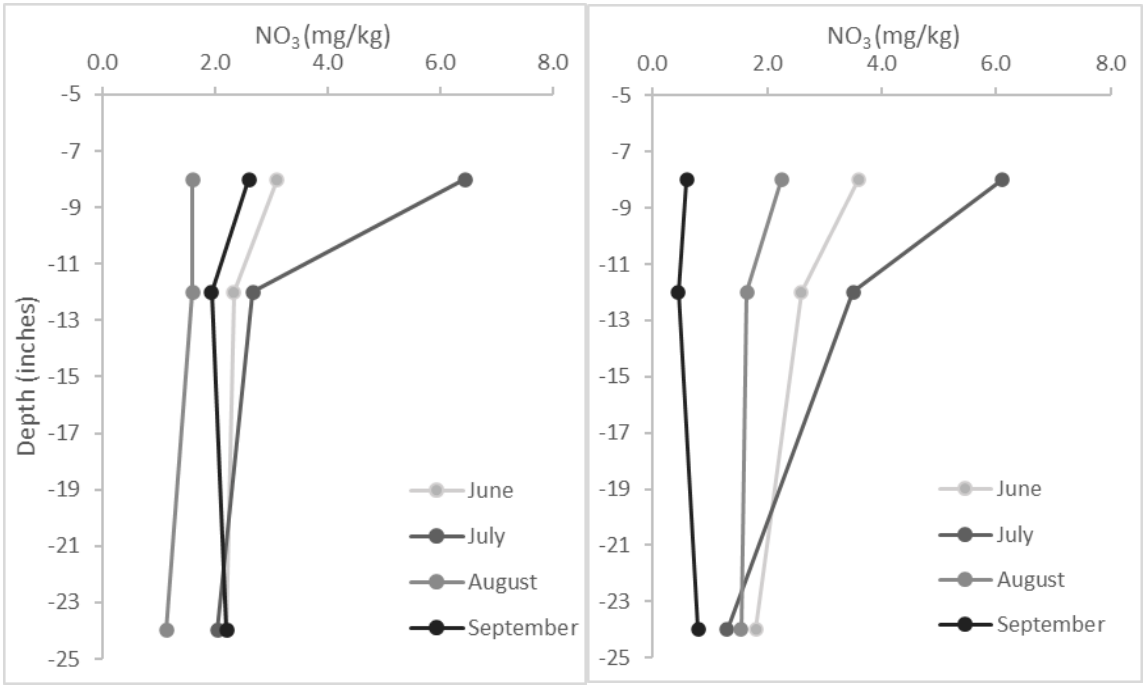

Figure 6. Soil nitrate (NO_3) throughout the season at three depths on site 1 (left) and site 2 (right).

Project Title: Targets and tools for post-bloom thinning

Report Type: Continuing Project Report

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Cooperators: Haydn Farms

Project Duration: 2 Year

Total Project Request for Year 1 Funding: $ 43,135
Total Project Request for Year 2 Funding: $ 45,310

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
Salaries	30,434	31,651
Benefits	4,678	4,865
Wages	6,146	6,392
Benefits	627	652
Equipment		
Supplies	250	250
Travel	1,000	1,500
Miscellaneous		
Plot Fees		
Total	43,135	45,310

Footnotes: salaries will fund M.S. student, wages for summer timeslip assistance with trial setup, fruit harvest, and fruit quality analyses; supplies for fruit quality testing and field trials, travel is to research plots in WA and to attend industry meetings for presenting results.

OBJECTIVES

1. Develop practical balanced cropping targets
2. Better understand the effects of timing of thinning
3. Investigate efficacy of Ethephon and Accede® as post-bloom thinners
4. Summarize and disseminate key findings to stakeholders

In this first year we were unable to recruit and hire a graduate student in time to lead the project. As a result, we were able to complete three PGR trials, assessing the efficacy of Ethephon and Accede in 'Coral Champagne', 'Chelan', and 'Benton'. We are requesting a no-cost extension and will treat the 2024 season as year 1 and 2025 as year 2.

SIGNIFICANT FINDINGS

- Ethephon and ACC are variable in their efficacy as post-bloom thinners
- Thinning efficacy may be cultivar dependent – treatments were largely ineffective for 'Chelan' but nearly thinned all fruit in 'Benton'

METHODS

Investigate efficacy of Ethephon and Accede® as post-bloom thinners

We previously screened several candidate PGRs for their efficacy at post-bloom thinning of sweet cherry. This research revealed potential for Ethephon as an effective tool when applied soon after shuck fall. *None of the other PGRs were effective*. In addition, there were no observed negative effects of Ethephon at this application timing and rate (much lower rates than those used to induce abscission near harvest). We did not observe any reduction in fruit firmness nor the induction of gumming from applications made shortly after shuck fall. These preliminary results were promising and we propose to further investigate the efficacy of Ethephon as a post-bloom thinner, specifically evaluating the role of application rate and timing on fruit load and fruit quality.

Based on our previous findings, we will focus efforts on the period between shuck fall and 7 – 10 days afterward. We will evaluate the effect of Ethephon 2 (21.7% 2-chloroethyl phosphonic acid) and Accede® applied at 100 and 200 ppm on fruit set and fruit quality. Treatments will be made to heavily-set trees at shuck fall or shuck fall + 7 – 10 days (orchard blocks to be identified each Spring). We will conduct both small- and large-scale trials using a pressurized backpack sprayer and commercial airblast equipment, respectively. Applications will be made to entire trees, with a minimum of 10 replicate branches (2/tree) selected for determining fruit set/density and fruit quality.

Within a day of application, we will flag two limbs in every tree (1 east-facing and 1 west-facing) and count fruitlet density (fruitlets/limb cross-sectional area and length), measuring limb caliper at the same time. In addition, we will measure fruit diameter on 10 fruit per limb to record fruitlet size at the time of treatment – this will facilitate comparisons among cultivars with respect to timing and final fruit size. A photo journal will be collected as well to visually document application timings and crop densities. At commercial fruit maturity we will make fruit counts to the same limbs and assess thinning efficacy as % fruitlet removal. Fruit subsamples (minimum 100 fruit per replication) will be collected and analyzed for quality attributes including color, weight, diameter, and firmness, at the facility in Prosser. Each treatment will also be evaluated for gummosis and/or other symptoms of phytotoxicity at harvest.

RESULTS & DISCUSSION

'Coral Champagne'

This trial was conducted in a 'Coral Champagne'/Mazzard block north of Pasco. Trees were trained to a Y-trellised, Tatura-type architecture with 7 horizontal fruiting tiers. Treatments were made on 10 May by backpack sprayer just to drip.

Fruit set in untreated control was just under 70%. Fruit set reported here is the number of fruit harvested from selected limbs compared to the number of fruit present at the time of treatment (i.e., not overall fruit set as a % of available flowers). The inverse represents fruit drop and was ca. 32% for the control. Fruit density of untreated control limbs was not particularly high at ca. 36 fruit per foot. By other metrics that we evaluated, fruit density in control was ca. 8.0 fruit/spur or 0.86 fruit/cm^2 branch cross-sectional area. Treatment with ACC did not reduce fruit set (i.e. did not increase fruit drop) though fruit density was reduced by ca 40% with ACC applied at 400 ppm (Table 1). Ethephon at 100 ppm was ineffective as a thinner, however, Ethephon at 200 ppm reduced fruit set by about 36% (P=0.12) compared to the control. Fruit density was significantly reduced by applications of Ethephon 200 compared to the control – 14.7 vs. 35.5 fruit/foot, respectively.

Table 1. Effects of PGR treatments on fruit drop, fruit density, and fruit quality in 'Coral Champagne'. Data with different letters are significantly different at P>0.1.

Treatment	Fruit set (%)	Fruit/ft	Color (CTIFL)	Soluble solids	Firmness (g/mm)	Fruit weight (g)
Control	67.9 a	35.5 a	4.2 ab	13.4 a	246.8 bc	9.2 a
ACC 200	64.9 a	25.8 ab	3.6 b	12.7 a	255.3 abc	7.1 cd
ACC 400	58.2 a	20.8 b	3.8 ab	14.1 a	270.1 a	6.7 d
Ethephon 100	52.7 a	18.8 b	4.3 a	13.6 a	241.1 c	8.5 ab
Ethephon 200	43.7 a	14.7 b	4.0 ab	13.3 a	260.8 ab	7.9 bc
Pr > F	0.119	0.001	0.031	0.21	0.001	<0.0001

No treatment improved fruit quality, with the exception of an increase of about 10% of fruit firmness in response to treatment with ACC at 400 ppm (Table 1). Interestingly, treatment with ACC reduced fruit weight by about 25% compared to the control. The higher rate of Ethephon also reduced fruit weight by about 14%. Only Ethephon at 100 ppm did not have a negative effect on fruit weight. It is also interesting to see that no treatment had any significant effect on fruit color. Therefore, these treatments did not hasten maturity – a concern that growers have expressed.

'Chelan'

This trial was conducted in a 'Chelan'/'Gisela6' block, in Tricities. Trees were trained to a Y-trellised architecture with 7 horizontal fruiting tiers. Treatments were applied at ca. 10 days after shuck fall.

Fruit set in the untreated control was much lower than in 'Coral' at 37% (Table 2). This means that between the time of treatment and harvest, nearly 63% of the fruit dropped without any treatment. This is unusually high and affected this experiment. Ethephon treatments did not affect the drop significantly. Treatment with 100 or 200 ppm exhibited fruit drop rates of ca. 67% and 59%, respectively. ACC treatments were similarly ineffective at inducing fruit drop. Applications of 200

and 400 ppm ACC resulted in drop of 67% and 74%, respectively, statistically similar to the untreated control. Similarly, no treatment had any effect on fruit density as fruit/foot was similar among all treatments, and relatively low overall (only ca. 16 for control).

Table 2. Effects of PGR treatments on fruit drop, fruit density, and fruit quality in 'Chelan'. Data with different letters are significantly different at P>0.1.

Treatment	Fruit set (%)	Fruit/ft	Color (CTIFL)	Soluble solids	Firmness (g/mm)	Fruit weight (g)
Control	36.7 ns	15.9 ns	4.04 ab	12.1 ab	269.1 ns	7.0 ns
ACC 200	32.6	15.5	4.13 ab	12.7 a	262.4	6.9
ACC 400	26.3	9.9	3.84 b	11.5 b	262.8	7.0
Ethephon 100	33.9	11.6	4.93 a	11.9 ab	286.6	7.7
Ethephon 200	41.4	14.6	3.93 ab	12.0 ab	275.8	7.3
Pr > F	0.64	0.59	0.088	0.12	0.38	0.31

Fruit quality was largely unaffected by treatment. Soluble solids were reduced slightly with ACC at 400 ppm, and treatment with Ethephon at 100 ppm increased fruit color (though treatment with Ethephon at 200 ppm did not). Firmness and weight were similar across all treatments.

'Benton'

This trial was setup at the WSU-Roza experimental farm in a 'Benton'/'Gisela5' block. Trees were trained to a modified steep leader architecture. Treatments were applied about 12 days after shuck fall.

Table 3. Effects of PGR treatments on fruit drop, fruit density, and fruit quality in 'Benton'. Data with different letters are significantly different at P>0.1.

Treatment	Fruit set (%)	Fruit/ft	Color (CTIFL)	Soluble solids	Firmness (g/mm)	Fruit weight (g)
Control	40.3 a	17.2 a	5.0 ns	20.0 ns	213.2 a	9.2 a
ACC 200	17.8 b	6.6 b	5.3	20.2	196.4 ab	8.4 ab
ACC 400	14.1 b	2.8 b	5.4	19.4	194.5 ab	9.2a
Ethephon 100	12.5 b	3.2 b	5.1	19.5	219.4 a	7.5 b
Ethephon 200	0.31 b	0.6 b	5.5	19.7	187.3 b	7.4 b
Pr > F	<0.0001	<0.0001	0.19	0.74	0.047	0.003

For 'Benton', fruit set of control limbs was 40% (i.e., about 60% of the fruit present on application of treatments dropped by harvest time). This translated to about 17 fruit/foot at harvest, which again is not a very high crop load. Control limbs had ca. 3 fruit/spur on average. In this trial each thinning treatment was effective (Table 3). Application of ACC at 200 and 400 ppm reduced fruit set by 56% and 66%, respectively. These treatments reduced fruit density to 6.6 and 2.8 fruit per foot. Treatment with ACC200 and ACC400 reduced crop load to 1 and 0.5 fruit/spur, respectively (data not shown). Ethephon applications were even more effective at reducing crop load. Fruit set was reduced by 69% and nearly 100% with applications of Ethephon at 100 and 200 ppm, respectively. This translated to 3.2 and 0.6 fruit/foot.

Despite the significant reductions in crop load with all treatments, there was no increase in fruit size nor soluble solids. This is likely due to the low crop load in untreated limbs – fruit had sufficient carbohydrate resources. ACC treatments were similar to the control in fruit weight, but the Ethephon treatments reduced fruit weight (Table 3). This is similar to the reduction in 'Coral Champagne' fruit weight we observed under treatment with 200 ppm Ethephon.

We assessed the relationships between fruit quality attributes and fruit density (as fruit/foot, fruit/limb cross-sectional area, fruit/spur) and found little to no correlations. For example, in 'Coral Champagne', there was no clear relationship between fruit/foot and any key fruit quality attribute (Fig. 1). These experiments need to be conducted in blocks that have higher crop load in order to better assess these relationships.

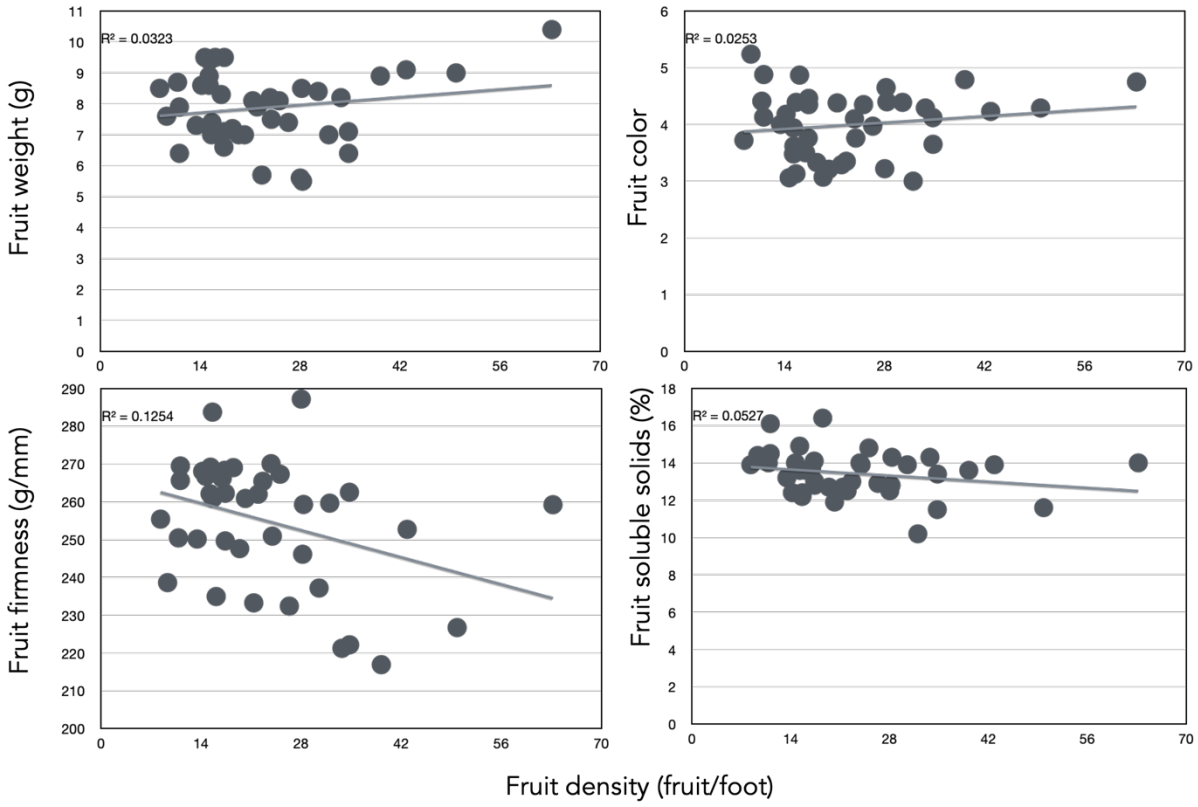

Figure 1. The relationships between fruit quality and fruit density (fruit/foot) for 'Coral Champagne'.

Project Title: Sweet cherry cultivar-specific export suitability
Report Type: Continuing Report

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1. USDA-ARS Tree Fruit Research Laboratory – Hood River Worksite
2. USDA-ARS Tree Fruit Research Laboratory – Wenatchee, WA
3. Oregon State University Extension Service – Hood River and Wasco Counties

Project Duration: 2-year
Total Project Request for Year 1 Funding: $ 54,944
Other related/associated funding sources: Awarded
Funding Duration: 2023
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Agency Name: USDA-ARS
Notes: Support for part of a base-funded research assistant and travel research reviews.

Budget 1
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	Item	2023
1	Salaries	$25,329
2	Benefits	$9,042
3	Wages	$7,208
4	Benefits	$551
5	RCA Room Rental	$1,319
6	Shipping	
7	Supplies	$4,000
8	Travel	$2,000
9	Plot Fees	
10	Miscellaneous	
11	Indirect cost	$5,494
	Total	$54,944

Footnotes:

1. **Salary for part-time research assistant pay grade GS-8 (0.425 full-time equivalent [FTE])**
2. **Benefits for part-time research assistant pay grade GS-8 (0.425 full-time equivalent [FTE])**
3. **Wages for summer research assistant pay grade GS-5 (approximately 0.5 FTE for 4 months each year)**
4. **Benefits for pay grade GS-5 (approximately 0.5 FTE for 4 months each year)**
5. **Cold room rental is per Oregon State University-Mid Columbia Research and Extension Center (OSU-MCAREC) rates.**
6. **-**
7. **Supplies include data loggers and sensors, reagents, packing supplies, shipping costs, laboratory consumables.**
8. **Travel is for collecting samples.**
9. **NA**
10. **NA**
11. **Indirect cost at 10%.**

Objectives

1. **Determine transport stresses and corresponding effects on sweet cherry fruit quality.** In Year 1, project goals were to obtain data on temperature and drop/shock affecting fruit in transit, in order that these conditions could be simulated in a laboratory setting. Several devices to collect this data were explored; most were prohibitively expensive ($1000 - $1800; these devices will likely make a one-way journey if transported overseas). One company, which sells a low-cost device (~$40) that makes temperature and GPS coordinates available in real time, refused to sell to the USDA. Industry contacts were reluctant to provide data. Plans for this goal will be discussed with Oregon Sweet Cherry Commission and Tree Fruit Research Commission board members.
2. **Postharvest cultivar comparison under potential export conditions.** Goals are to evaluate respiration rates and fruit quality, including firmness, stem color, soluble solids, pitting, and postharvest decay, before and after simulated export handling (4-week hold) for present or potential Pacific Northwest export cultivars, in both air and modified atmosphere (MA) bags. After discussion with industry stakeholders, a mix of both traditional cultivars and newer sweet cherry cultivars were selected for Year 1: **Coral Champagne, Black Pearl, Chelan, Bing, Santina, Skeena, and Regina**.
3. **The influence of transport stresses on sweet cherry quality**. Primary potential transport stresses evaluated in Year 1 were temperature (40 °F) and duration of holding (4 weeks). The effects of regular and MA bags were also contrasted.

Significant Findings

- Initial quality corresponds to quality after storage. As one project advisor said, any cultivar can be exported if initial size and quality are optimal.
- Modified atmosphere (MA) bags (LifeSpan®, Amcor Inc.)* prolonged fruit quality longevity irrespective of cultivar or temperature. Differing respiration rates according to cultivar suggest that a packinghouse could tailor MA technology to cultivar for better outcomes.
  **use of a specific product does not imply endorsement*
- **<u>As this summary represents one season of data, not all cultivars had multiple lots in the study, and many factors influence fruit and stem quality, cultivar-specific results should be viewed as preliminary:</u>**
  - High temperature storage (40 °F) affected quality attributes differently according to cultivar, e.g. Black Pearl (three lots in the study) retained a high percentage of stems at both optimal (31 °F) and high (40 °F) temperatures, while Cristalina (only one lot evaluated) retained stems at 31 °F and lost a relatively higher portion at 40 °F.
  - Respiration rates (carbon dioxide [CO_2] production) vary over the course of storage and are higher at higher temperatures. Regina had relatively high respiration rates,

while Black Pearl and Skeena had lower; Bing respiration was mid-range. Respiration is not reported for all cultivars. High respiration can indicate fruit are using stored carbohydrates, decreasing quality potential. Fruit quality at 4 weeks postharvest generally supports the hypothesis that lower respiration in storage corresponds to superior quality outcomes.

- A preliminary cultivar summary is available on the last page of this report.

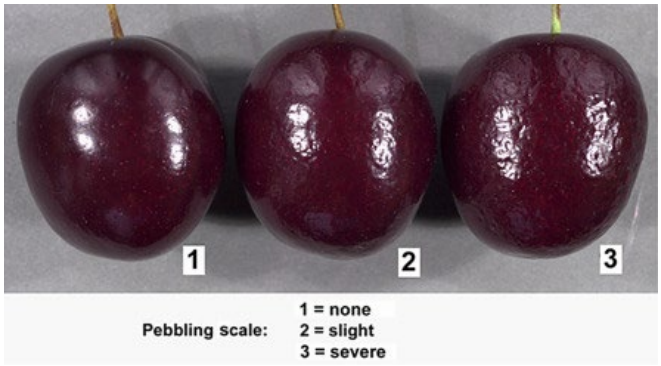
Methods

The primary project goal was to determine sweet cherry **cultivar-specific** differences in fruit and stem quality (**Table 1**) post 4-weeks of storage at optimal and less-than optimal conditions. Most sweet cherries were obtained within a day of packing from commercial partners (**Table 2**). Upon receipt, fruit were transferred from packinghouse-specific materials to MA bags (LifeSpan) and regular polyethylene bags. In total, four combinations of temperature and bag were evaluated: 31 °F + regular bag, 31 °F + MA bag, 40 °F + regular bag, 40 °F + MA bag. Respiration rates were evaluated at pickup, 2 d, 4 d, 1 wk, 2 wk, 3 wk, and 4 wk. for a subset of fruit stored in regular polyethylene bags (opening MA bags would alter the atmosphere). Like respiration, bag atmosphere for fruit held modified atmosphere bags was evaluated at 2 d, 4 d, 1 wk, 2 wk, 3 wk, and 4 wk. At pick-up and after a 4-week storage, sweet cherry fruit and stem quality were evaluated (**Table 1)**. Characteristics recorded and summarized below were stem presence/absence, stem browning, fruit firmness, stem pull force, soluble solids (Brix), titratable acidity, color (both CTIFL [Cerise Interprofessional Technical Center for Fruits and Vegetables] and lightness, hue, and chroma via colorimeter; colorimeter data is not presented in this report), as well as pitting, cracking, rot, pebbling (a fruit symptom of desiccation), stem doublets (more than one stem attached to a single cherry – implies another cherry has lost its stem), and sliced stems. Statistics were performed in a statistical software program (SAS, SAS Institute Inc, Cary, NC) with the programs PROC GLM (continuous data) and PROC FREQ or PROC LOGISTIC (binary data); principal components analysis was performed in multivariate statistical software (PAST; Hammer et al., 2001).

Table 1. *Sweet cherry quality attributes evaluated in this study.*

Visual quality		**Indirect sensory**	**Physiological**
Stem browning	Pitting	Firmness	Respiration
Stem presence	Pebbling	Brix (soluble solids) (sweetness)	Bag atmosphere
Stem shrivel	Cracking	Titratable acidity	
Color	Rot	Stem pull force (pedicel rupture force)	

Figure 1. *Pebbling (Toivonen and Manganaris, 2020). Pebbling is thought to be caused by water loss.*



Results and Discussion

Sweet cherry cultivar quality post-packing/pre-storage

Table 1 summarizes fruit quality post-pickup from packinghouse cooperators or harvest from orchard. **Stem browning** was lowest for the fruit packed by hand (Santina, and fruit from orchard P), followed by Skeena (two packinghouses), Chelan (one packinghouse), and Regina (two packinghouses); differences in stem browning according to cultivar have been documented previously (Kupferman, 2004). At this stage post-harvest, most stem browning appeared to originate from abrasion, presumably on the cluster cutter. The extent to which this type of stem browning is influenced by stem characteristics versus cluster cutter type or settings has not been extensively examined. **Fruit firmness** was highest for one lot each of Black Pearl, Bing, and Cristalina (each from a different packinghouse). Chelan and Skeena had consistently mid-range firmness, and Regina had consistently the lowest firmness. Note that, overall, Black Pearl had inconsistent firmness (three packinghouses). Many factors influence firmness, including pre-harvest gibberellic acid application, harvest temperature, and preharvest water status (Schrader, 2006). **Stem pull force**, the force required to separate the stem from the fruit was highest for hand-packed Regina, and Bing (one packinghouse), Chelan (two packinghouses), and Black Pearl (two packinghouses).

Table 2. *Receipt dates and fruit quality at pickup/harvest from packinghouse/orchard (pre-storage).*

Pickup /harvest Date (2023)	Packing-house/ Orchard[a]	Cultivar	Stem browning (1 = 0-25%, 2 = 26-50%, 3 = 51-100%)	Firmness (g/mm)	Color (CTFIL)	Stem pull (kg force)	Stem length to width ratio[c]	Brix (soluble solids)	Titratable acidity (% malic acid equivalents)
6/19	L	Coral Cham.	1a	295efg	5.3fg	0.366fgh	351efg	23.6b	0.85def
6/22	L	Black Pearl	0.9a	275fgh	5.9cd	0.477cdef	485ab	20.5cd	0.61i
6/26	Z	Black Pearl	0.8abc	247ijk	6.5ab	0.15k	449abc	18.5hij	0.66hi
6/27	Q	Black Pearl	0.9ab	357a	5.5ef	0.526bcde	500a	19.5efgh	0.86def
6/27	Q	Chelan	0.3def	309cde	6.6a	0.546bcd	389def	24.7a	0.68h
6/28	N	Chelan	0.8abc	340ab	6cd	0.435defg	353efg	20.6cd	1.12b
6/28	N	Cristalina	0.8abc	297def	6.6a	0.592bc	336fg	19.5efg	0.71h
6/29	M	Santina[b]	1.1a	345ab	5g	0.634b	457abc	24ab	0.89de
6/29	L	Bing	0g	257hij	6.2bc	0.323ghi	323fg	20.9c	0.7h
7/5	Q	Bing	0.1eg	238jkl	5.3f	0.183jk	387def	18.4hi	1.24a
7/10	P	Skeena[b]	0g	300cdef	4.2i	0.215ijk	347fg	19.6defg	0.86de
7/12	N	Bing	0.8abc	326bc	4.6h	0.174jk	492ab	18.8ghij	0.99c
7/12	N	Skeena	0.2feg	323bcd	5.5ef	0.338ghi	437bcd	19.4fghi	0.84efg
7/14	L	Regina	0.6bcd	228l	5.4f	0.294hij	407cde	18.4hi	0.92d
7/19	Q	Regina	0.5cde	227l	6.1c	0.442defg	363efg	19.2fghi	0.79fg
7/19	Q	Skeena	0.2eg	270gh	5.6ef	0.405efgh	359efg	17.9i	0.79g
8/1	P	Regina[c]	0g	188m	5.8de	0.796a	327fg	20.1cdef	0.82efg
Overall model Pr > F			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Cultivar			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Packinghouse			0.0001	<0.0001	<0.0001	<0.0001	0.0012	<0.0001	0.0095
Cultivar*Packinghouse			<0.0003	<0.0001	<0.0001	<0.0001	0.0028	0.0051	<0.0001

Values in a column followed by differing letters are statistically different and represent cultivar x packinghouse interaction.

a. Random letters assigned to packinghouses for anonymity.
b. Packed in-lab
c. Lower numbers indicate thicker stems.

Stem length-to-width ratio is an indirect measure of stem thickness, which could be influenced by both cultivar-specific stem characteristics or stem desiccation: a lower value indicates an overall thicker stem (potentially preferrable). Hand-packed fruit had among the lowest values for this measure, supporting a hypothesis that this measure can indicate desiccation status, e.g., fruit that did not undergo a commercial harvest and packing process, and which were evaluated within hours of harvest had the least-desiccated stems. Black Pearl (three packinghouses) and Bing (two packinghouses) had among the highest stem length-to-width ratio, suggesting there also is a cultivar-specific component to this value. Bing had the among the highest **Brix** (top three out of first four lots); other cultivars were less consistent. Bing also had relatively high **titratable acidity (TA)** while Black Pearl (three packinghouses) had the lowest. Previous studies have linked high TA to storage potential (Dong, 2018; Wang and Long, 2014). "Packinghouse/Lot" had statistically significant influence for many fruit quality measures (**Table 2**), but, with the possible exception of stem browning, one could expect numerous factors (e.g. pre-harvest management, environment) to influence fruit quality; these factors were not evaluated in the present data, and are included in "packinghouse/lot" statistically. Pitting, stem doublets, and sliced stems pre-storage are summarized in **Table 3**. Stem doublets are when two or more stems are attached to a single cherry, and sliced stems are when the thickened distal end of the stem is sliced off, leaving the stem more susceptible to desiccation. Neither stem doublets nor slice stems differed according to cultivar in this first year. Pitting incidence differed according to cultivar (**Table 3**); stem doublets and sliced stems did not. Pitting can vary among cultivars (Kupferman, 2004) and harvest maturity can influence incidence (Ross et al., 2020); harvest maturity cannot be accounted for in the present study.

***Table 3**. Fruit quality at pickup, continued. No data were collected from Santina and data were removed for fruit that did not go through a commercial packingline.*

Cultivar	Pitting incidence (%)	Stem doublets (%)[a]	Sliced stems (%)[a]	Number of packinghouses or orchards
Bing	15e	15	43	3
Black Pearl	15e	11	36	3
Chelan	21b	15	30	2
Coral Champagne	33a	16	0	1
Cristalina	16d	13	20	1
Regina	20c	16	46	3
Skeena	3f	16	36	3
Wald's Chi-sq test stat[a]	13	2	8	
Degrees of freedom	6	6	6	
total n	420	420	420	
n missing	0	0	0	
p-value	0.0500	0.9566	0.1951	

Post hoc separations are according to pairwise contrast statements at $p<0.05$. Values in a column followed by differing letters are statistically different (pitting only).

a. The overall model was not statistically significant, e.g., there is too much variability in the data to determine if these numbers represent differences among the cultivars.

Respiration

Carbon dioxide production is an indicator of respiration rate and may reflect how quickly fruit may be using carbohydrate reserves. Researchers have suggested that a higher respiration rate could indicate decreased storage potential as fruit that are respiring more quickly may utilize reserves more quickly (Tapia Garci-a, 2017; Wang, 2014). After about 1 week in storage, respiration rates were typically at their lowest and would begin to increase thereafter (data not shown). Holding temperature influenced respiration rates and irrespective of temperature, Regina had the highest respiration rate, followed by Bing, and then Skeena and Black Pearl at 1 week (**Table 4**); data is not shown for cultivars with less than three different lots in the study.

Table 4. *Sweet cherry respiration rates 1 week after harvest.*

1 week resp.	Temperature	mg CO_2 / kg fruit•hr	
Bing	31 °F	23.1	B
	40 °F	37.6	
Black Pearl	31 °F	16.4	C
	40 °F	23.3	
Regina	31 °F	28.2	A
	40 °F	44.7	
Skeena	31 °F	17.6	C
	40 °F	27.9	
Model Pr > F		0.0002	
Packinghouse		0.0911	
Cultivar		<0.001	
Temperature		0.0002	
Cultivar*Temperature		0.0054	

Values in a column followed by differing letters are statistically different and represent the main effect cultivar.

Four weeks postharvest fruit and stem quality following optimal and less-than-optimal storage

Present results are consistent with previous studies: modified atmosphere (MA) bags and storage temperatures of 31 °F extend fruit quality relative to warmer storage temperatures and regular polyethylene bags (Wang, 2014). After 4 weeks, irrespective of sweet cherry cultivar or packinghouse, stem retention is improved, and cracking, pitting, pebbling, shrivel, and rot lower in 31 °F + MA bag, relative to the other handling combinations tested (31 °F + regular bag, 40 °F + MA bag, 40 °F + regular bag) (**Table 5**).

Table 5. *Summary of temperature and modified atmosphere (MA) bags influence on fruit quality at 4 weeks postharvest.*

Overview: MA and temperature at 4 wk		Stem retention (% with stem)	Cracking (% cracked)	Pitting (% with pitting)	Pebbling (% with pebbling)	Shrivel (% with shrivel)	Rot (% with rot)
31 °F	**MA bag**	85a	12b	50b	51b	6b	3b
	Regular	74b	19a	54a	55a	10a	7a
Chi-sq test stat		76	47	7	4	28	32
Degrees of freedom		1	1	1	1	1	1
n		3996	4080	4080	4080	3996	4080
n missing		84	0	0	0	84	0
p-value		<.0001	<.0001	0.0077	0.0378	<.0001	<.0001
40 °F	**MA bag**	68b	13b	47b	50b	10b	9b
	Regular	72a	21a	55a	66a	12a	19a
Chi-sq test stat		8	52	26	94	2	89
Degrees of freedom		1	1	1	1	1	1
n		3705	4080	4080	4080	3996	4080
n missing		375	0	0	0	84	0
p-value		0.0056	<.0001	<.0001	<.0001	0.1800	<.0001

Bag type comparison at 31 °F and 40 °F performed at separate Chi-Square Goodness of Fit Tests. P-values less than 0.05 indicate that percent of fruit (proportions) are significantly different.

In near-optimal holding conditions (31 °F+MA), Chelan, Skeena, and Black Pearl had the highest stem retention (**Tables 6a and 6b).** Pitting was lowest for Coral Champagne, Chelan, Skeena, Bing, and Black Pearl. Pebbling was lowest for Coral Champagne and Cristalina (one lot/packinghouse each), followed by Regina and Bing (**Table 6b)**. Slight pebbling may not be a market-limiting defect; see **Figure 1**. Average stem browning was lowest for Cristalina, Coral Champagne, and Regina (**Table 6b**). Pitting severity was highest for Bing, Regina, and Skeena. Firmness was highest for Coral Champagne, Black Pearl and Chelan. For these three cultivars, and for most of the other

cultivars as well, firmness values were nominally higher than pre-storage samples (**Table 2**), which has been said to be an indicator of export potential (Ross et al., 2020), although the firmness measuring device used in this study does not capture texture nuances nor indicate relative acceptability to consumers. Color differed among cultivars, with fruit in MA bags retaining the lightest color (data not shown). Stem pull force was highest for Chelan and Black Pearl, and stem-length-to-width ratio was lowest for Coral Champagne and Regina, indicating they generally had the thickest stems.

Table 6a. *Sweet cherry cultivar quality after 4 weeks in near-optimal holding conditions (31 °F, modified atmosphere packaging.*

Optimal holding: 31°F+ MA bag for 4 wks.	Stem retention (% with stem)	Cracking (% cracked)	Pitting (% with pitting)	Pebbling (% with pebbling)	Shrivel (% with shrivel)	Rot (% with rot)	Number of packing-houses or orchards
Bing	78f	9d	30e	39e	12b	1d	3
Black Pearl	87c	13b	32d	70c	8c	11b	3
Chelan	90b	4e	14f	93a	18a	3c	2
Coral Champ.	79e	0f	9g	0f	0e	0e	1
Cristalina	73g	8d	70a	48d	0e	12a	1
Regina	87c	15b	50d	25f	1d	1d	3
Santina	80d	37a	69b	87b	0e	2d	1
Skeena	93a	12c	30e	51d	1d	0e	3
Wald's Chi-sq	54	75	326	301	52	63	
Degrees of freedom	7	7	7	7	7	7	
n total (n =120 lot)	2010	2040	2040	2010	2010	2040	
n missing	30	0	0	30	30	0	
p-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	

Values in a column are significant different at p <0.05 according to contrast statements in proc logistic.

Table 6b*. Sweet cherry cultivar quality after 4 weeks in near-optimal holding conditions (31 °F, modified atmosphere packaging.*

Optimal holding: 31°F+ MA bag for 4 wks.	Stem browning (1 = 0-25%, 2 = 26-50%, 3 = 51-100%)	Pitting severity	Firmess (g/mm)	Color (CTIFL)	Stem pull force (kg)	Stem length to width ratio[d]	Brix (soluble solids)	Titratable acidity (%)	Number of lots evaluated
Bing	1.7a	1.6a	344c	6.7a	0.2599cd	628a	23.5a	0.84a	3
Black Pearl	1.2c	1d	389b	6.2b	0.4266b	633a	18.3c	0.53d	3
Chelan	0.9d	1d	423a	6.2b	0.6946a	469b	18.0c	0.73b	2
Coral Champagne	0f	1d	409ab	6.1b	0.2963c	436b	19.4bc	0.73b	1
Cristalina	0.8de	1cd	359c	5.6c	0.1595d	480b	17.9c	0.51d	1
Regina	0.7e	1.5b	291d	5.5c	0.278c	467b	21.1b	0.60c	3
Santina	1.2c	1.2cd	291d	5.5c	0.2153cd	572a	17.6c	0.70b	1
Skeena	1.4b	1.3c	353c	5.1d	0.293c	500b	19.3c	0.67b	3
Overall model Pr > F	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	
Cultivar	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	
Packinghouse	<.0001	<.0001	0.0012	0.0137	<.0001	0.0058	<.0001	<.0001	
Cultivar*Packinghouse	<.0001	<.0001	<.0001	<.0001	<.0001	0.0439	<.0001	<.0001	

Values in a column followed by differing letters are statistically different and represent cultivar main effect cultivar.

Table 7a. *Fruit quality after 4 weeks when fruit were held at 40 °F and in regular perforated bags.*

High temp: 40 °F, regular bag	Stem retention (% with stem)	Cracking (% cracked)	Pitting (% with pitting)	Pebbling (% with pebbling)	Shrivel (% with shrivel)	Rot (% with rot)	Number of lots evaluated
Bing	63e	13d	74b	53d	24b	8e	3
Black Pearl	83a	9f	21f	76c	7c	8e	3
Chelan	81c	15c	42d	77b	31a	13c	2
Coral Champ.	64d	0g	14g	0e	0c	0e	1
Cristalina	20g	7g	28e	55d	0c	18b	1
Regina	58f	36a	77a	35e	0c	30a	3
Santina	77c	11e	44c	86a	23b	15c	1
Skeena	82b	22b	60c	60d	0c	12d	3
Chi-sq test stat	370	218	719	304	147	181	
Degrees of freedom	7	7	7	7	7	7	
n	3705	4080	4080	3706	3708	4080	
n missing	375	0	0	374	372	0	
p-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	

Values in a column are significant different at p <0.05 according to contrast statements in proc logistic.

Table 7b. *Stem browning and pitting severity for affected fruit 4 weeks postharvest for fruit held at 40 F and in a regular perforated bag.*

High temp: 40 °F, regular bag	Stem browning (1 = 0-25%, 2 = 26-50%, 3 = 51-100%)	Pitting severity	Firmness (g/mm)	Color (CTIFL)	Stem pull force (kg)	Stem length to width ratio[d]	Brix (soluble solids)	Titratable acidity (%)	Number of lots evaluated
Bing	2.8a	1.4b	329b	5.4d	0.3109b	727a	23a	0.78a	3
Black Pearl	1.4d	1c	314b	6.7a	0.3696b	674a	17.9bc	0.43d	3
Chelan	1.1e	1c	362a	6.9a	0.6182a	554bc	17.6cd	0.75a	2
Coral Champagne	0.2g	1c	306bc	n.d.	0.1615c	527bc	n.d.	n.d.	1
Cristalina	0.4f	1c	282cd	6.7ab	0.1454c	464d	17.5cd	0.39d	1
Regina	1.1e	1.5a	239e	6.5b	0.2243c	497cd	19.0b	0.58bc	3
Santina	2.2b	1c	198f	6.9a	0.1485c	707a	16.9d	0.53c	1
Skeena	2c	1.4b	265d	6.1a	0.2083c	577b	17.9bc	0.61b	3
Overall model Pr > F	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	
Cultivar	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	
Packinghouse	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	
Cultivar*Packinghouse	<.0001	<.0001	<.0001	<.0001	<.0001	0.0003	0.6418	<.0001	

Values in a column followed by differing letters are statistically different and represent cultivar main effect cultivar.

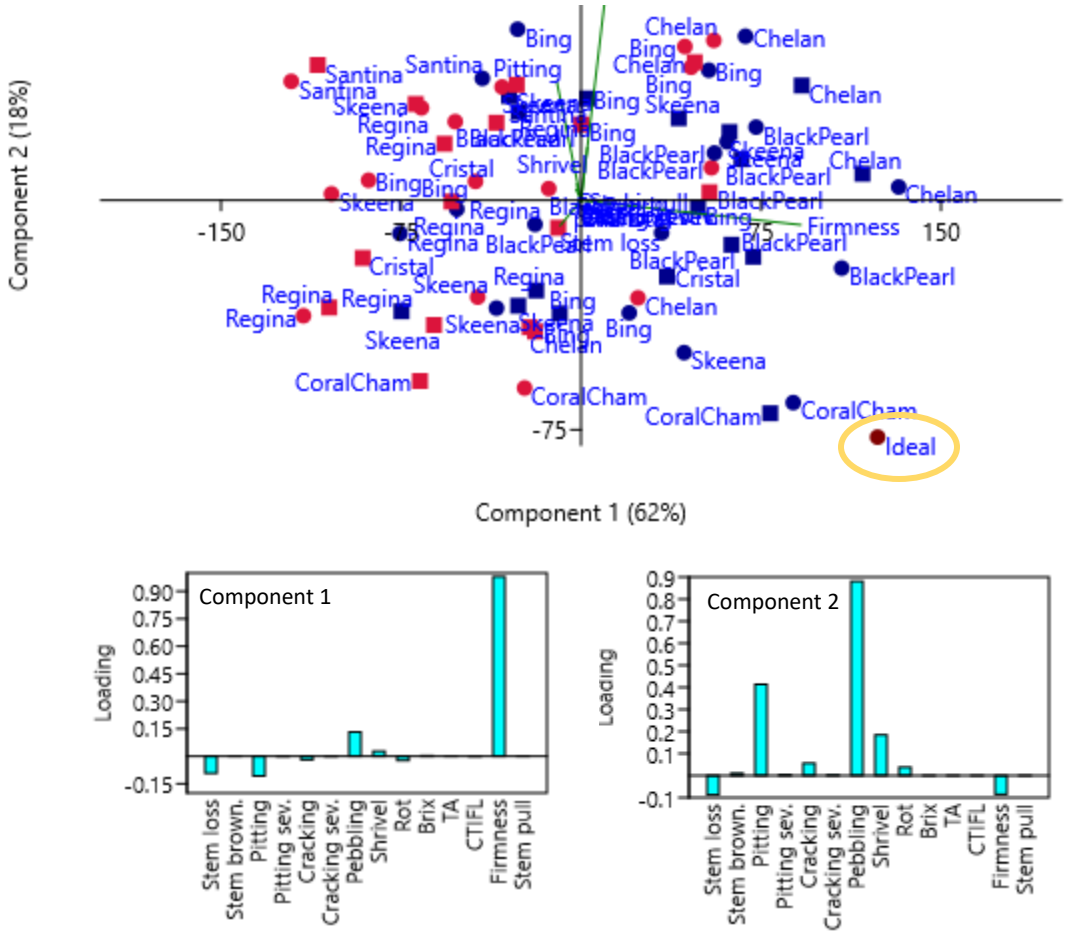
Fruit quality attributes that could indicate cultivar specific resilience to less-than-optimal storage conditions (4 weeks in a regular perforated bag, 40 °F) were also examined (**Tables 7a and 7b**). Black Pearl, Skeena, and Chelan had the highest stem retention. Coral Champagne, Cristalina, and Black Pearl had the lowest cracking (recall that this is cracking either not found during packing or that occurred post-packing). Coral Champagne, Black Pearl, and Cristalina had the lowest pitting. Coral Champagne had virtually no pebbling, and Regina had low pebbling as well. Stem browning

(**Table 7b**) was highest for Bing and Santina, and lowest for Coral Champagne and Cristalina. Pitting severity was highest for Bing, Regina, and Skeena. Firmness was highest for Chelan, Bing, and Black Pearl. Stem pull force was highest for Chelan, Black Pearl, Bing, and Regina. Stem length-to-width ratio was lowest for Cristalina, Regina, and Coral Champagne.

The ideal export cherry

For modelling purposes, the ideal export sweet cherry would, after 4 weeks of holding, have no pitting, cracking, pebbling, shrivel, nor rot, and have a thick green stem, high Brix, high titratable acidity, dark red color, high firmness, and strong stem retention. In a multivariate analysis called principal components analysis (PCA), **firmness**, **pebbling**, and **pitting** were the characteristics measured that contributed most to the model containing this ideal cherry (**Figure 2**); in short, in this data set, these were the measures that differed the most among the fruit evaluated. Coral Champagne (one lot represented in the project), stored in either regular or MA bags at 31 °F were the closest to the simulated ideal, followed by one lot of Skeena (three total lots represented in the project), one lot of Cristalina (only one lot in the project) and several lots of Black Pearl; all were fruit stored at 31 °F. One lot of Chelan performed well under high temperature conditions (40 °F), as did the single lot of Coral Champagne; with only one lot in the project, it is uncertain whether this lot truly represents the cultivar characteristics of Coral Champagne. Fuit size, which can vary with cultivar, is not included in the analysis, as fruit were sized on packinglines. The poorer performance of Santina in this model may be attributed to its relatively lower firmness, which is a factor that both pre- and post-harvest management can alter; note that Santinas in this project were not harvested commercially and were hand-packed (no commercial packingline). Firmness at harvest (**Table 1**) for Santina was relatively high, suggesting that the lack of proper commercial handling in-lab reduced this element of storage potential.

Figure 2. *Modelling the ideal export cherry. Circles indicate regular bag storage; squares indicate modified atmosphere bag storage; blue indicates 31 °F and red indicates 40 °F.*



Conclusion

Initial quality generally corresponded to quality after storage; for example, Reginas had relatively low firmness entering storage, and this attribute remained relatively low. Interestingly, two out of three lots Black Pearl had the lowest titratable acidity pre-storage but this was not linked to relatively poorer quality post-storage in other measures, e.g. firmness or pitting; firmness was high and pitting were low in Black Pearls after 4 weeks of storage. Bings had high Brix and high titratable acidity entering storage and retained this post-storage. However, some quality attributes differed among cultivars in relation to temperature; e.g. deliberate temperature mis-management (40 °F) may indicate which characteristics and which cultivars are more resilient in this condition. To clarify potential cultivar differences, Year 1 of sweet cherry cultivar comparison under export conditions is summarized in **Table 7**.

Table 7*. Sweet cherry cultivar summary post-storage, Year 1. Season-specific and lot-specific factors can influence quality, and for some cultivars, only one lot was present in the study. This summary should be viewed as preliminary. Optimal temperature for storage is 31 °F; high temperature (not optimal) was 40 °F. Fruit color was consistently optimum in fruit stored at 31 °F in MA bags and is not discussed here.*

Cultivar	Number of packing-houses or orchards in study	Harvest timing relative to Bing (days)[a]	Notes from PNW 604[a]	Summary from this project, Year 1, 2023	
				Stem quality after a 4 week hold	Fruit quality after a 4 week hold
Bing	3	--	Moderate fruit size, high rain cracking susceptibility	Mid-range stem retention; high stem browning; high temp. increases both loss and browning; mid-range stem pull force and relatively thin stems	Mid-range firmness; relatively high pitting; pitting was nominally lower in high temp. storage. High Brix and titratable acidity
Black Pearl	3	-9.5	Low pitting potential; productive variety.	Excellent stem retention at optimal and high temperatures. Mid-to-high stem browning. Good stem pull force, but relatively thin stems	Relatively low pitting, especially at higher temperature storage; good firmness at both optimal and high temperature storage. Low titratable acidity
Chelan	2	-10	Can overset, flavor is mild; successful export cultivar.	Excellent stem retention at optimal and high temperatures. Low-to-mid range stem browning. Excellent stem pull force and mid-range stem thickness	Relatively high pitting. Excellent firmness, at both optimal and high temperature storage. Relatively high acidity
Coral Champagne	1	-4	Potentially weak stem attachment; productive tree	Mid-range stem retention and stem loss increased under high temp. conditions. Extremely low stem browning under both optimal and high temp. conditions. Low stem pull force and medium-thick stems	High firmness; low pitting under both optimal and high temperature storage; Relatively high acidity at 31 °F; no data for 40 °F
Cristalina	1	-3.2	No information	Low stem retention but extremely low stem browning. Mid-range stem pull force. Excellent stem thickness.	Medim-high firmness at optimal temperature storage; medium-low in high temperature storage. High pitting in optimal storage temp; low in high temperature storage. Low titratable acidity
Regina	3	+10.9	Large and firm fruit; many reports indicate this is one of the best shipping cherries. Naturally low in productivity.	Excellent stem retention at optimal storage temperature but mid-range to low at high temps. Low stem browning in optimal storage but relatively high in high temps. Good stem pull force and excellent stem thickness.	Relatively low firmness under either temperature regime. Mid-range pitting and mid-range titratable acidity
Santina	1	-6	Large size, self-fertile	Good stem retention. Mid-range stem browning at optimal temperatures but relatively high stem browning at high temps. Low stem thickness and low stem pull force. Note that no fruit from this cultivar underwent commercial packing in this study, and no postharvest treatments were applied.	Relatively low firmness; relatively low pitting. Note that no fruit from this cultivar underwent commercial packing in this study, and no postharvest treatments were applied. Relatively high titratable acidity at optimal temps; relatively low titratable acidity at high temps.
Skeena	3	+11.4	Good export history; self-fertile; large firm fruit, rain cracking can be a problem	Excellent stem retention. Mid-to-high stem browning. Mid-range stem pull force and stem thickness.	Mid-range firmness and pitting; mid-range titratable acidity

a. Long et al., 2021

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