

Project Title: Experimental Orchard for X-Disease and Little Cherry Disease Research

Report Type: Final Project Report

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Total Project Request for Year 1 Funding: \$ 65,586

Other related/associated funding sources: None

Budget 1

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Item	2022	2023	2024
Salaries	\$35,265.00		
Benefits	\$2,821.00		
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies	\$26,500.00		
Travel			
Plot Fees			
Miscellaneous			
Total	\$64,586.00	\$0.00	\$0.00

Footnotes:

Objectives

1) Develop and maintain an experimental cherry orchard for X-disease/little cherry disease research.

Significant Findings

A cherry orchard consisting of 37 Bing trees and 38 Gabrielle trees was planted at the USDA Experimental Farm near Moxee, WA. The new orchard will be used exclusively for research on Little Cherry Disease/X-disease by researchers at the USDA and Washington State University. The farm is located about 5 miles from the nearest commercial cherry orchard, so presence of infected cherry trees will not be a major threat to commercial production.

Results and Discussion

The current epidemic of X-disease or little cherry disease is causing substantial economic losses to the cherry production in the Pacific Northwest. Symptoms often don't manifest for several years after initial infection. Visible disease symptoms begin with development of small, off-color, bitter-tasting cherry fruits, eventually leading to tree death (Uyemoto et al. 1991). Other stone fruits, including peach, nectarine, and plum, are similarly affected, but development of symptoms and tree death occurs more rapidly in other stone fruits than in cherry (Marcone et al. 2014).

X-disease is caused by a phloem-limited bacterium called "Candidatus Phytoplasma pruni" (16SrIII). This pathogen is transmitted by several different leafhopper species, but *Colladonus reductus* (=montanus) and *C. geminatus* appear to be among the most important vectors in the Pacific Northwest (Wolfe et al. 1950, Kaloostian 1951, Wolfe et al. 1951, Harper et al. 2020, Prengaman 2020). Both leafhopper vectors feed on a wide range of weedy host plants within at least 14 families (Severin and Frazier 1945, Severin and Klostermeyer 1950, Jensen 1953, Nielsen 1957). The X-disease phytoplasma also has a wide host range that includes many herbaceous weeds that the leafhoppers develop on as nymphs. The leafhoppers acquire the pathogen from these weedy herbaceous hosts or from infected cherry trees as nymphs, then transmit the pathogen to uninfected cherry trees. There are no cures for X-disease, so growers rely primarily on regular use of insecticides to reduce vector populations and culling of infected trees or entire orchards (DuPont 2020, Harper et al. 2020).

Previous research on X-disease and associated leafhopper vectors was conducted nearly half a century ago in California, leaving many unknowns related to the current X-disease epidemic in the Pacific Northwest. It is still unclear which leafhoppers, other than those already identified, are vectors of the X-disease phytoplasma in the Pacific Northwest, or from which weedy host species leafhoppers primarily acquire the pathogen. It is also unclear how development of disease symptoms is affected by cherry cultivar or rootstock. Identification of factors related to the spread of X-disease phytoplasma in the Pacific Northwest is considered high priority. A major challenge to the conduct of this research is the lack of a suitable research orchard where infected trees can be maintained for experiments without risking infection of commercial orchards.

Research is currently underway to identify methods to cure trees of X-disease phytoplasma or to prevent trees from becoming infected. This work is being conducted in cooperation with funded research on a similar group of pathogens called "Candidatus Liberibacters". Like phytoplasmas, Liberibacters are phloem-limited bacteria that are transmitted by insect vectors. *Liberibacter asiaticus* is the pathogen that causes citrus greening disease (Huanglongbing) resulting in substantial economic losses for citrus growers in Florida, Texas, and California. *Liberibacter solanacearum* is a related pathogen that infects potato, tomato, and other crops and weeds within the Solanaceae. PI Cooper collaborates with citrus researchers on the development of novel therapies for Liberibacters (Shatters and Heck 2020). Some of these therapies developed for Liberibacters may be effective against X-disease Phytoplasma.

The development of a designated orchard to conduct research on X-disease and little cherry disease where trees can be infected without threatening commercial orchards was listed as a high research priority for 2022 by the Washington Tree Fruit Research Commission and Oregon Sweet Cherry Commission.

The objective of our project was to plant an experimental orchard at the USDA experimental farm near Moxee, WA to support new and ongoing research on management of X-disease. The experimental orchard is located about 5 miles from the nearby commercial cherry orchard (Figure 1). This isolation reduces the likelihood that the experimental plot will threaten commercial orchards with X-disease.

We established an 80-tree block of cherry trees in spring of 2022. The orchard consists of 37 Bing and 38 Gabriel trees. Bing trees were certified disease-free, but Gabriel is a new variety and was not certified. The orchard is irrigated with sprinkler irrigation. Broadleaf weeds were managed with herbicides and orchard row middles were planted with perennial rye grass and fescue to reduce populations of the vector within the orchard. All trees survived and are available for research in spring of 2023.

A few challenges were encountered that required modification of the original plan. Desired cherry cultivars were not available at the time of purchase, so we purchased trees of the Gabriel variety. Bing trees became available prior to planting, so we established a mix block of 37 Bing on Mazzard rootstock and 38 Gabriel on GIS-12 rootstock. The orchard also includes 5 Attika on Mazzard rootstock as pollinator trees. Our proposal included cages to confine and protect trees from vectors. Our source of cages – BioQuip – went out of business in early spring of 2022, and we were unable to find an alternative source of cages until late summer. We have purchased 21' x 328' roll of Protek Net-Insect Exclusion netting and 1" snap clamps to build A-frame cages with 1" PVC pipe in spring of 2023. Browsing by deer and porcupines were a constant challenge in summer of 2022. Four porcupines were removed from the orchard, and the gate and perimeter fence has been repaired to exclude deer in 2023.



Figure 1. Site of the proposed research orchard (top), and general vicinity of the research farm (bottom). The star marks the orchard location while the circle shows a 5-mile radius.



Figure 2. Newly planted experimental cherry plot to be used for research on biology and management of Little Cherry Disease/X-disease.

All trees were tested for presence of X-disease phytoplasma using real time PCR. Two trees – 1 Bing and 1 Gabriel – were infected with X-disease phytoplasma. Titer levels were relatively high, suggesting that the trees were infected at the nursery level, not infected after planting. Both trees will be caged and maintained to determine how soon symptoms are observed in nursery-infected trees.

The USDA-ARS Temperate Tree Fruit and Vegetable Research Unit budget was increased by \$2 million in 2022 to support research on Little Cherry Disease/X-disease. These funds are to be used in part to maintain the experimental orchard at the Moxee Farm. ***No new funds for the orchard are requested from Washington Tree Fruit Research Commission in 2023.*** We plan to use USDA funds to increase our well capacity so that the orchard size can be increased as needed. We also plan to install new wind machines at the orchard site to provide frost protection and to purchase and install a modular workspace to provide USDA and WSU researchers with a clean and air-conditioned place to meet, sort samples, and take lunch breaks. Initial funds from WTFRC to establish the orchard provided evidence for stakeholder support helps us justify to ARS Administration the infrastructure improvements at the USDA experimental farm.

Executive Summary

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Key words: Little Cherry Disease, X-Disease, Phytoplasma pruni, experimental orchard

Abstract

The pathogens that cause X-disease and little cherry disease are primary threats to stone fruit production in the Pacific Northwest. There are currently no methods to directly control these pathogens, so management of X-disease and little cherry disease relies upon removal of infected orchards and the use of insecticides to suppress populations of the insect vectors. Very little is known of the basic biology of these pathogens and their insect vectors. The development of a designated orchard to conduct research on X-disease and little cherry disease where trees can be infected without threatening commercial orchards was listed as a high research priority for 2022 by the Washington Tree Fruit Research Commission and Oregon Sweet Cherry Commission. We planted an experimental cherry orchard at the USDA experimental farm near Moxee. The orchard consists of 37 Bing trees on Mazard rootstock 38 Gabriel trees on GIS-12 rootstock, and Attika trees on Mazard rootstock for pollinators. The USDA experimental farm is located at least 5 miles from the nearest commercial cherry orchard, so the presence of infected cherry trees would not be a major threat to commercial production. Real time PCR revealed that two trees – 1 Bing and 1 Gabriel – were infected with X-disease phytoplasma at planting. Both trees will be caged and maintained to determine how quickly disease symptoms are observed in trees that are infected at nurseries. This new orchard will be dedicated to basic and applied research on X-disease and little cherry disease USDA and WSU researchers and will support ongoing research to better understand epidemiology of X-disease and to screen experimental products to reduce pathogen infection in trees.