FINAL REPORT **YEAR**: 2

Project Title: Native hosts of the X-disease phytoplasma

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Cooperators: Washington cherry growers

Total Project Request: $75,999 **Year 1:** $38,107 **Year 2:** $38,336

Other funding sources

None.

Budget

Organization Name: Washington State University **Contract Administrator:** Katy Roberts
Telephone: 509-335-2885 **Email address:** arcgrants@wsu.edu

Item	2018	2019
Salaries[1]	$12,106	$20,145
Benefits	$4,152	$6,910
Wages[2]	$9,840	$0
Benefits[3]	$932	0
Equipment	0	0
Supplies[4]	$9,577	$10,281
Travel[5]	$1,000	$1,000
Miscellaneous	0	0
Plot Fees	0	0
Total	$38,107	$38,336

Footnotes:

1. 0.25 FTE Salary for Dr. Wright the first year, and 0.4 FTE for the second.
2. Wages for a field worker for sample collection.
3. Benefits calculated at standard WSU rates.
4. Supplies include: Field sampling supplies and laboratory consumables and reagents
5. Travel covers mileage to reach field sampling sites.

OBJECTIVES

1. *Survey plants both within and in the vicinity of cherry orchards for X-disease phytoplasma.*
Plants were collected around central and eastern Washington, both within cherry orchards afflicted with X-disease phytoplasma and from locations distant from orchards. Plants were photographed for later identification. Tissue was harvested for DNA extractions and screened for the presence of phytoplasma using a generic phytoplasma qPCR assay. Positive plants were further screened for the presence of X-disease using a qPCR assay. A total of 923 plants, including trees, shrubs, broadleaf weeds, and grasses, were tested for the presence of X-disease. Only 10 tested positive for phytoplasmas and of those 4 were positive for X-disease.

2. *Identify potential reservoirs and sources of spread for X-disease phytoplasma in Washington.*
This objective was initially proposed to examine leafhopper spread of X-disease from herbaceous weeds. However, the low incidence of X-disease found in weeds during the survey indicates that weeds alone cannot explain the incidence and severity of X-disease in cherry orchards. Given that weeds are unlikely to be the main source of this pathogen, studying leafhopper spread from weeds would not be informative. Therefore, this objective was refocused on three alternative reservoirs: 1) Cherry planting stock, for symptoms are only visible on fruit and nursery stock is rarely, if ever, tested for the presence of this phytoplasma, 2) Other *Prunus* species, for we have recently detected X-disease in peach and nectarine orchards in Washington, and 3) Pome fruit orchards in the vicinity of cherry orchards, for X-disease has been reported to infect apples in other states.

SIGNIFICANT FINDINGS

1. Less than two percent of plants tested for the presence of phytoplasma were positive and only a subset of those contained X-disease phytoplasma, suggesting that non-crop plants in and around orchards may not be the source of X-disease phytoplasma.

2. Samples from three large nurseries were screened for the presence of X-disease phytoplasma. From the first nursery, one of the 66 samples screened was positive for X-disease. In the second, one of 107 samples was positive. In the third, none of the 25 samples were positive. The presence of the pathogen in two of the three nurseries demonstrates that X-disease is present in nurseries at low numbers and has the potential to be amplified and spread.

3. X-disease phytoplasma was detected in peaches and nectarines in both Wapato and Pasco. X-disease was also detected in plums. The phytoplasma was present in these trees in very high numbers indicating that they may serve as a reservoir for spread of the pathogen. Apricots have not yet tested positive for this specific phytoplasma.

4. While X-disease phytoplasma has been reported to infect apple in both Pennsylvania and the Czech Republic, survey results in Washington apple orchards were negative.

RESULTS AND DISCUSSION

This project was developed to identify reservoirs for the X-disease phytoplasma as this pathogen has severely increased in incidence in eastern and central Washington. The intention was that identifying the environmental reservoirs would allow for another control strategy in that the reservoirs, once known, could be removed and thereby reduce spread of the disease. In the first year of the project a survey was conducted of weeds and non-crop perennials in and around orchards to identify plants that may serve as a reservoir for the X-disease phytoplasma. Plants were collected March through September and multiple

individuals for several species were sampled, including plants from the sagebrush, non-crop trees and shrubs, and annual weeds (Tables 1-3). DNA was extracted from each plant and screened for the presence of phytoplasma using a generic phytoplasma qPCR assay. Plants that were positive were subsequently screened by the more specific X-disease phytoplasma qPCR assay.

Table 1. Abbreviated list of annual weeds screened for phytoplasma.

Plant	Number Sampled	Locations	Phytoplasma Positive
Tumble mustard	45	17	2
Common mallow	24	11	0
Kochia	22	11	0
Puncturevine	22	9	1
Prickly lettuce	21	12	0
Redroot pigweed	20	9	0
Common lambsquarters	19	6	0
Perrenial pepperweed	17	9	0
Western yarrow	17	9	0
Common mullein	16	9	2
Field bindweed	16	10	0
Russian thistle	16	10	1
Scurf-pea	15	8	0
Canada thistle	13	7	0
Dandelion	13	7	0
Blue mustard	12	5	0
Flixweed	12	4	2
Salsify	12	6	0
Showy milkweed	12	7	0
White clover	12	5	0
Fiddleneck	11	11	0
Poison hemlock	11	5	0
Prostrate knotweed	11	6	0
Scotch thistle	11	6	0
Bushy wallflower	9	6	0
Common purselane	9	4	1
Shepherd's purse	4	2	1

Table 2. List of sagebrush plants screened for phytoplasma.

Plant	Number Sampled	Locations	Phytoplasma Positive
Sand sagebrush	12	7	0
Big sagebrush	6	6	0
Hopsage	3	3	0
Greasewood	2	1	0
Antelope bitterbrush	1	1	0
Douglas rabbitbrush	1	1	0
Gray rabbitbrush	1	1	0
Green rabbitbrush	1	1	0

Table 3. Abbreviated list of perennial species screened for phytoplasma.

Plant	Number Sampled	Locations	Phytoplasma Positive
Russian olive	11	7	0
Narrowleaf willow	10	7	0
English walnut	8	3	0
Wood's rose	8	6	0
Eastern cottonwood	7	6	0
Himalayan blackberry	6	4	0
Siberian pea tree	6	2	0
Elderberry	5	4	0
Apple	3	1	0
Netleaf hackberry	3	1	0
unknown perrenial	3	3	0
White alder	3	3	0
Golden currant	2	2	0
Oregon ash	2	1	0
Red birch	2	1	0
Nootka Rose	2	2	0

A total of 923 plants representing at least 173 species were screened for phytoplasmas (Tables 1, 2, and 3). Only ten plants were positive for phytoplasma and these were all annuals. The phytoplasma positive plants included flixweed, puncturevine, mullein, tumble mustard, shepherd's purse, Russian thistle, and common purselane. These ten were screened further using an X-disease specific qPCR assay. Only four, one flixweed, one puncturevine, and two tumble mustards, were positive for X-disease (Figure 1). This is less than 0.5% of the plants screened. The four positive plants had only low levels of X-disease present (Figure 1D). Of the weeds that were positive, these were among the most sampled species. Forty-five tumble mustards from 17 locations, 22 puncture vines from 9 locations, and 12 flixweeds from 4 locations were tested. This would suggest a low incidence of phytoplasma infection among these species. Furthermore, it is not known if the insect vector(s) transmitting X-disease feed on these plants and if,

particularly at such low concentrations, they are likely to acquire the pathogen. Also of note, no plants native to the sagebrush were positive for the pathogen. If X-disease is present in the sagebrush, it is likely to be at such low numbers that it is not a significant source of the pathogen. Collectively, these data indicate that the main source of X-disease lies elsewhere.

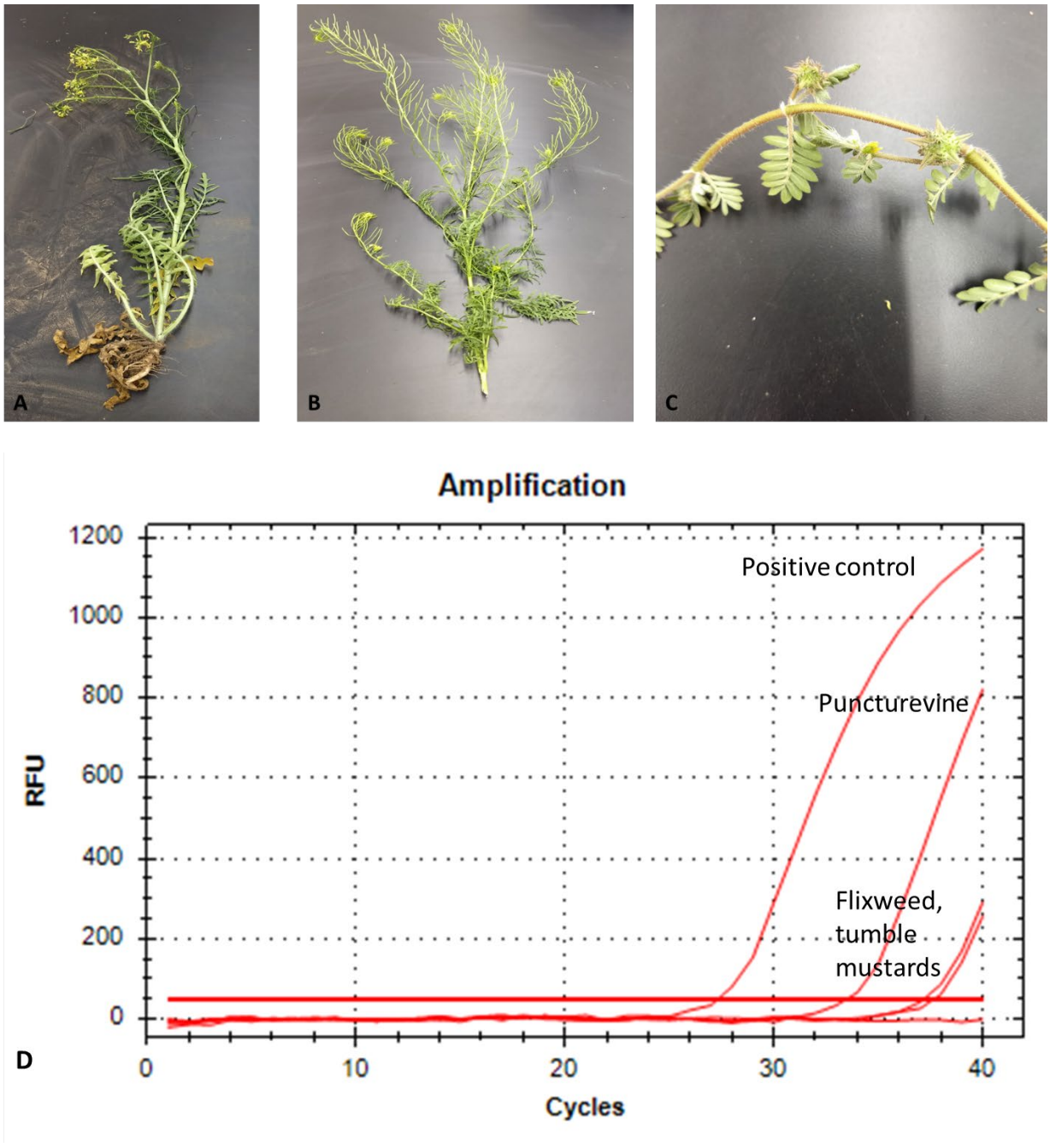

Figure 1. Representatives of plant species that tested positive for X-disease phytoplasma: A) tumble mustard, B) flixweed, and C) puncturevine. D) Quantitative PCR results for the X-disease phytoplasma assay. The red lines show amplification for, from left to right, the positive control, puncturevine, and the flixweed and tumble mustards.

While the survey does not rule out non-crop plants in and near orchards as a potential source of X-disease phytoplasma, it does indicate that the greatest reservoir for this pathogen may be located elsewhere. For the second year of our project, we chose to rewrite objective two to look for other sources of this pathogen. That included surveying nurseries and examining other crops, specifically other stone fruit and pome fruit.

Samples were collected from three large nurseries. In the first 66 samples were collected. In the second, 107 samples were collected. In a third, 25 samples were screened. For the first two, one sample each was positive for the X-disease phytoplasma while in the third, none were positive. While this may seem to be a small number of positives, given the number of trees produced by nurseries, this is a concern. If one of those positives served as a source of budwood, that would contribute to the spread of the disease.

Given that other stone fruit are susceptible to X-disease, we suspected that they may be serving as a reservoir to spread this disease. We found that peaches, nectarines, and plums in Washington are infected with this phytoplasma (Figure 2). The phytoplasma is at far higher concentrations in these trees than what was observed in the four weeds that were positive in the first year (Figures 1 and 3). Given the amount of pathogen present in these stone fruit, it is unlikely that they are a dead end host for this pathogen. Even if the insect vector has a poor acquisition rate from these reservoirs, the number of insects out there paired with the concentration of pathogen in the tree and how widespread it is throughout the Columbia River and Yakima Valley region make peaches, plums, and nectarines likely contributors to the spread of this pathogen.

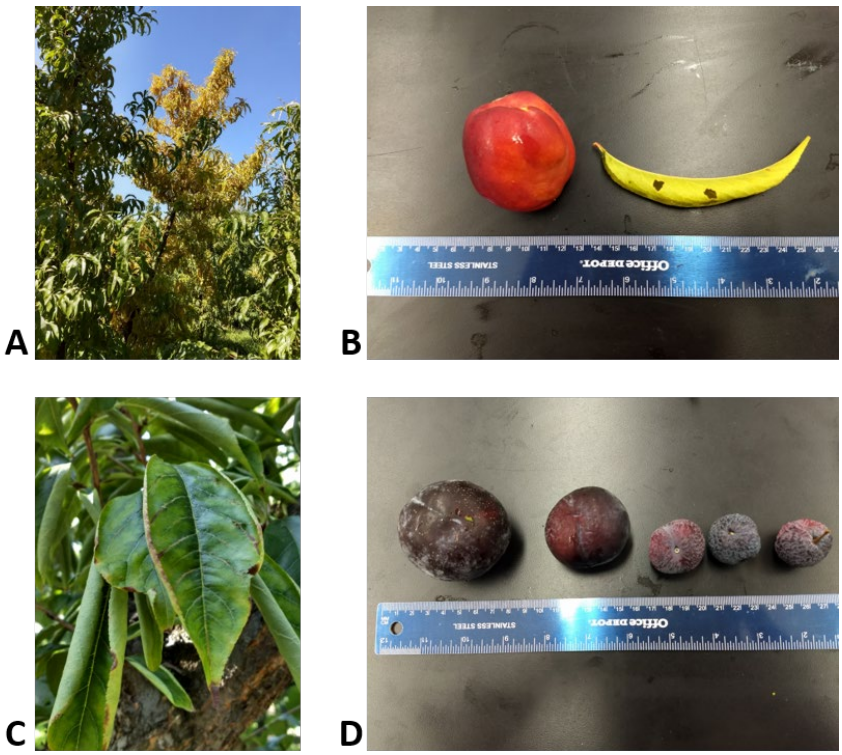

Figure 2. Trees infected with both X-disease and pear decline. A) Nectarine tree exhibiting yellowing symptoms. B) Misshapen fruit and a leaf showing shot-holing, characteristic of X-disease infection in nectarines. C) Plum tree infected with both X-disease and pear decline. D) Misshapen fruit from infected plum tree.

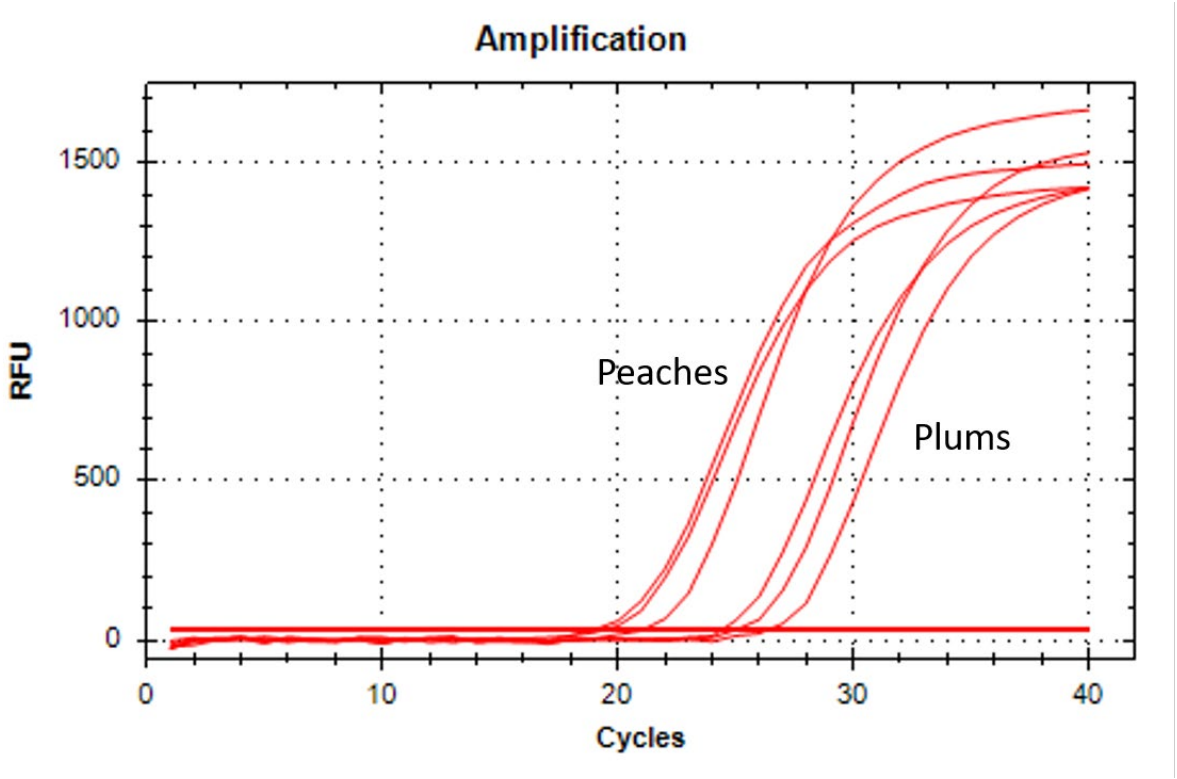

Figure 3. Detection of X-disease phytoplasma by qPCR.

Lastly, pome fruit were investigated. In tree fruit production, apples are often grown next to or near cherries. While it has been demonstrated that X-disease phytoplasma can infect apples in both Pennsylvania and the Czech Republic, we did not detected it in a survey of 69 apples samples in Washington State. This is encouraging as it suggests that apples are an unlikely reservoir for this pathogen.

In screening nearly a thousand non-crop plants it was found that less than 0.5% were positive for X-disease phytoplasmas. While this does not rule out weeds and other non-crop plants as a reservoir, it makes it very unlikely that these plants are the primary reservoir. Infection rates of X-disease among cherries are extremely high meaning that within orchard pathogen load is probably sufficient for tree to tree spread either by an insect vector or root grafting. As nurseries are often located near orchards, it is not surprising to see even a low incidence of X-disease presence in nurseries through random insect 'strikes' transmitting the pathogen. Furthermore, peaches, nectarines, and plums, which are also experiencing high rates of X-disease infection, are often grown next to or near cherries, raising the possibility that there is movement of the pathogen between these crops and cherries.

Moving forward, we recommend that nurseries be pro-active with regards to X-disease phytoplasma. Any mother trees that produce fruit should be inspected for little cherry disease symptoms. At harvest and through August would be ideal times for testing suspect trees or random testing to check for X-disease presence. Growers should also be aware that this pathogen infects other stone fruit and should be on the lookout for symptoms in peach, nectarine, and plum blocks. Most importantly, any infected trees should be promptly removed to reduce spread of this pathogen. In the long term, there is a need for testing or inspection of all Prunus material moving in to the state, for the X-disease phytoplasma is found throughout the country, and preventing its re-introduction will be critical to management.

EXECUTIVE SUMMARY

Project Title: Native hosts of the X-disease phytoplasma

Keywords: Little cherry disease, X-disease phytoplasma, nursery, stone fruit

Objectives

1. Survey plants both within and in the vicinity of cherry orchards for X-disease phytoplasma.
2. Identify potential reservoirs and sources of spread for X-disease phytoplasma in Washington.

Significant Outcomes

1. In a survey of weeds and non-crop perennials in and around orchards, less than two percent of plants tested for the presence of phytoplasma were positive and only a subset of those contained X-disease phytoplasma, suggesting that non-crop plants in and around orchards may not be the source of X-disease phytoplasma.

2. Samples from three large nurseries were screened for the presence of X-disease phytoplasma. From the first nursery, one of the 66 samples screened was positive for X-disease. In the second, one of 107 samples was positive. From the third, none of the 25 samples were positive. These data demonstrate that X-disease is present in nurseries at low numbers and has the potential to spread from nurseries.

3. X-disease phytoplasma was detected in peaches and nectarines in both Wapato and Pasco. X-disease was also detected in plums. In all three crops, the phytoplasma was present in very high numbers, indicating that these trees may serve as a reservoir for spread of the pathogen.

4. While X-disease phytoplasma has been reported to infect apple in both Pennsylvania and the Czech Republic, survey results in Washington apple orchards were negative.

Future Directions

Weeds in and around orchards are unlikely to serve as a major reservoir for X-disease. X-disease positive plants were not detected in the sagebrush – if any sagebrush species can harbor this pathogen, it is likely not at an incidence high enough to significantly impact spread of the pathogen. Instead, our work has shown that X-disease is most commonly found in stone fruit, specifically cherries, peaches, nectarines, and plums and is present at a high concentration in these trees. Infected stone fruit trees are likely serving as a reservoir, allowing for transmission of the pathogen to healthy trees via an insect vector or root grafting. Nurseries are not immune to X-disease. A short survey of three nurseries revealed the presence of X-disease at low numbers in two of the three nurseries. Given the propagation potential of a single nursery tree, nurseries should be on their guard against X-disease. Random testing and monitoring for symptom development in trees old enough to produce fruit is advised. Growers should watch for symptoms in cherries and other stone fruit and remove infected trees. As insect control is difficult to achieve, removal of reservoirs such as infected trees is essential to reducing the spread of the disease.