FINAL PROJECT REPORT

Project Title: The hunt for leafhopper vectors of Western X in Washington cherries

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Cooperators: Washington cherry growers, Stemilt Growers, G.S. Long

Other funding sources

Agency Name: National Clean Plant Network – Fruit Trees
Amount awarded: NCPN-FT pays land rental fees and maintenance costs of the virus research block where field experiments are conducted, sources of plant material for research and plant maintenance. The estimated cost associated with this project is $22,300 and is a portion of a larger NCPN grant to WSU-Prosser.
Notes: WSU is including this information on other funding available for the support of similar research undertaken by the faculty member proposing this research. These resources are listed to identify other support granted for this research and are not included as a commitment of cost-share by the institution

Total Project Funding: **Year 1**: $33,740 **Year 2**: $34,603 **Year 3**: $35,499

Budget History:

Item	2016	2017	2018
Salaries[1]	$15,601	$16,226	$16,875
Benefits[2]	$5,939	$6,177	$6,424
Supplies[3]	$12,200	$12,200	$12,200
Travel	$0	$0	$0
Miscellaneous	$0	$0	$0
Plot Fees	$0	$0	$0
Total	$33,740	$34,603	$35,499

Footnotes:
1. 0.25 and 0.10 FTE of Research Associates Ferguson and Wright, respectively.
2. Benefits calculated at standard Washington State rates.
3. Supplies include partial funding of:
- **Fuel to travel to research sites**
- **Leafhopper colony establishment and maintenance**
- **Field sampling supplies**
- **Acquisition/retention of inoculated plants over 2 years**
- **Sample extraction and PCR assays**

RECAP OF ORIGINAL OBJECTIVES

1. **Conduct survey of leafhoppers in Western X affected orchards.**
   As planned, we conducted surveys of leafhoppers in affected orchards in 2016 to 2018, beginning in March/April and concluding in October. While some blocks were sampled in three years, sampling had to be discontinued in other blocks removed by the grower. Molecular testing of leafhoppers to determine which species have the Western X (WX) phytoplasma was accomplished, concentrating on two abundant species, *Colladonus reductus* and *C. geminatus*.
2. **Conduct survey of host plants for leafhoppers and/or WX phytoplasma in affected orchards.**
   Potential host plants for leafhoppers were collected from field sites and identified; some were propagated in the greenhouse. Data from sticky card and sweep sampling inside and outside of the orchard also provided information on potential host plants.
3. **Examine the capability of selected leafhopper species to transmit WX phytoplasma.**
   Colonies of *C. reductus* and *C. geminatus* were established and maintained on celery seedlings. Acquisition and inoculation studies (n=75) were conducted in late season 2017 and 2018.

SIGNIFICANT FINDINGS

- Objective 1 (100% complete): Two leafhopper vector species, *C. geminatus* and *C. reductus*, were the most abundant species found on sticky cards in cherry tree foliage and in habitats outside of orchards in most sites.
- Objective 1 (100% complete): Molecular diagnostic testing showed that for *C. geminatus*, 40/519 samples (8%) from within affected orchards and 71/348 samples (20%) from extra-orchard habitats, were positive for Western X phytoplasma. Likewise, for *C. reductus*, 103/687 samples (15%) from within affected orchards and 20/204 samples (10%) from extra-orchard habitats were positive for WX. Our data show that there are two main time periods of WX-positive leafhopper activity in cherries—the spring and late summer into fall—although time periods may vary among orchards and year to year. Other infrequently collected potential vector species of leafhoppers tested for WX (number positive/sample size in parentheses) were: *Fieberiella florii* (1/12), *Osbornellus* sp. (1/23), *Scaphytopius* sp. (2/43), *Paraphlepsius* sp. (0/16), *Euscelidius variegatus* (1/35), *Macrosteles* sp. (0/15), *Circulifer tenellus* (0/22), and *Erythroneura* sp. (0/3).
- Objective 2 (100% complete): From collections of weed and sagebrush plants and data from sticky card and sweep samples, we completed our ecological survey and compiled a list of 33 potential host plant species for leafhoppers and/or Western X.
- Objective 3 (90% complete): In late season 2017 and 2018, we conducted a total of 75 acquisition and/or inoculation trials with *C. geminatus* and *C. reductus*. One transmission was successful: *C. reductus* collected from an infected cherry block transmitted WX to a previously WX-free *Prunus tomentosa* tree. Testing recipients for Western X is not complete.

RESULTS & DISCUSSION

Objective 1: Conduct survey of leafhoppers in Western X affected orchards.

Data from sticky cards and sweep net samples yielded information on leafhoppers present in cherry tree foliage as well as foliage in extra-orchard habitats (sagebrush, *Purshia* sp., clover, etc.). Overall, for the two most abundant species, 14% of *C. reductus* samples were WX-positive, and 13% of *C. geminatus* samples were WX-positive. We analyzed DNA samples of other potential vector leafhopper species for Western X (Table 1). One sample each of *Fieberiella florii*, *Osbornellus* sp., *Euscelidius variegatus*, and two samples of *Scaphytopius* sp. were positive for WX.

Table 1. Leafhopper species collected from sweet cherry orchards and extra-orchard habitats, 2016-2018. Overall percentages of WX-positive samples for each species are given here.

Leafhopper species	No. pooled samples	No. positive for Western X	Percent positive for Western X
Colladonus reductus	891	123	14
Colladonus geminatus	867	111	13
Euscelidius variegatus	35	1	3
Scaphytopius sp.	43	2	5
Osbornellus sp.	23	1	4
Macrosteles sp.	15	0	0
Paraphlepsius sp.	16	0	0
Fieberiella florii	12	1	8
Dikraneura sp.	10	0	0
Circulifer tenellus	22	0	0
Erythroneura sp.	3	0	0
Ceratagallia sp.	1	0	0

The Mattawa1 site was sampled in two different blocks because the 2016 block was taken out in late 2016. The same Mattawa1 block sampled in 2015 was studied in 2017. This location had the greatest abundance of *Colladonus* spp. leafhoppers with peak numbers occurring mainly in spring and late summer into fall (Fig. 1). *Colladonus* DNA samples showed a range of 4 to 14% positive for Western X, including orchard and sageland samples at the Mattawa1 site (Tables 2 and 3). Note that leafhoppers positive for WX were found mainly during spring and late summer into fall. See Figs. 2-5 for fairly similar results for Mattawa2, Mattawa3, Mattawa4, Granger, Benton City, and Selah.

For the Mattawa2 site, which was across the highway from Mattawa1, the orchard border was not sampled in 2016 (Fig. 2). The entire block was removed in late 2016 and apples were planted. The 2017 leafhopper sticky traps were in plants in the windbreak border. Less than 11% of DNA samples from leafhoppers collected at Mattawa2 tested positive for Western X (Tables 2 and 3)

The Mattawa4 site showed a greater percentage of *Colladonus* leafhopper samples positive for Western X for those from the neighboring sageland in 2016 (14 to 47%) compared with those collected in the orchard (0 to 8%; Tables 2 and 3; Fig. 3). In 2017, regular insecticide sprays kept leafhopper numbers low, and fewer leafhopper DNA samples were positive for Western X.

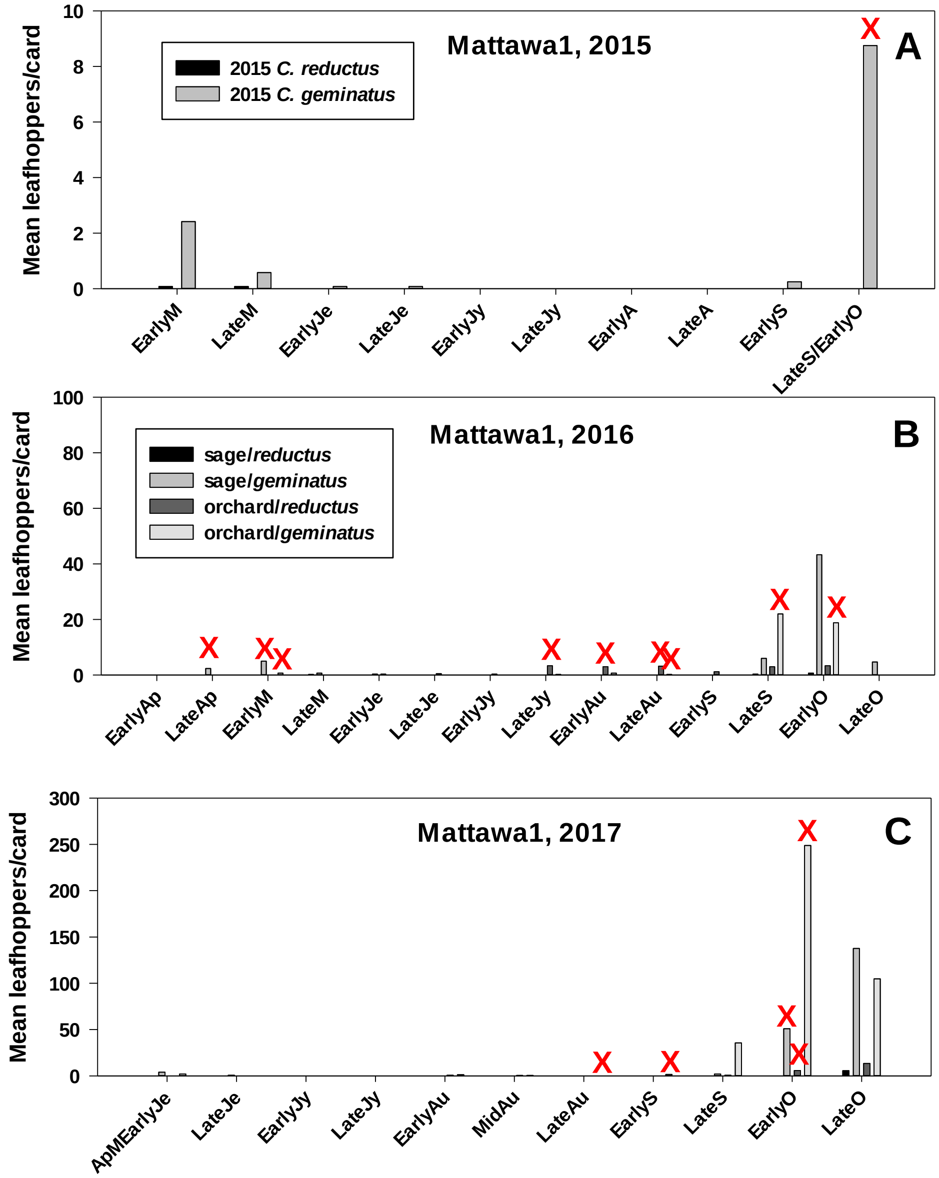

Figure 1. Seasonal abundance of and incidence of Western X (X over bar) in *Colladonus* spp. leafhoppers at the Mattawa1 site, 2015-2017.

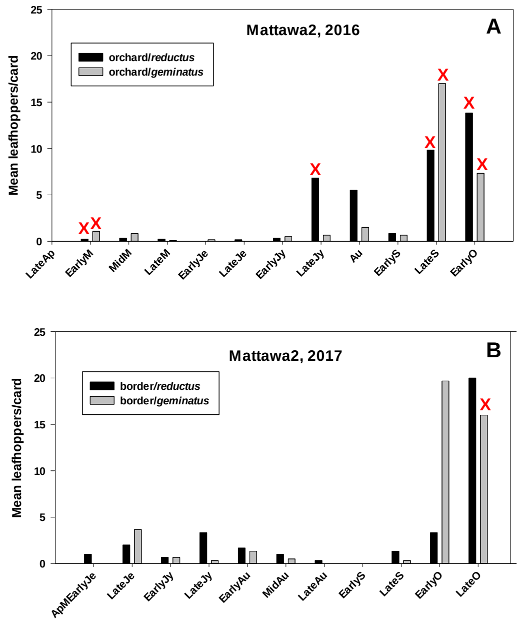

Figure 2. Seasonal abundance of and incidence of Western X (X over bar) in *Colladonus* spp. leafhoppers at the Mattawa2 site, 2016-2017.

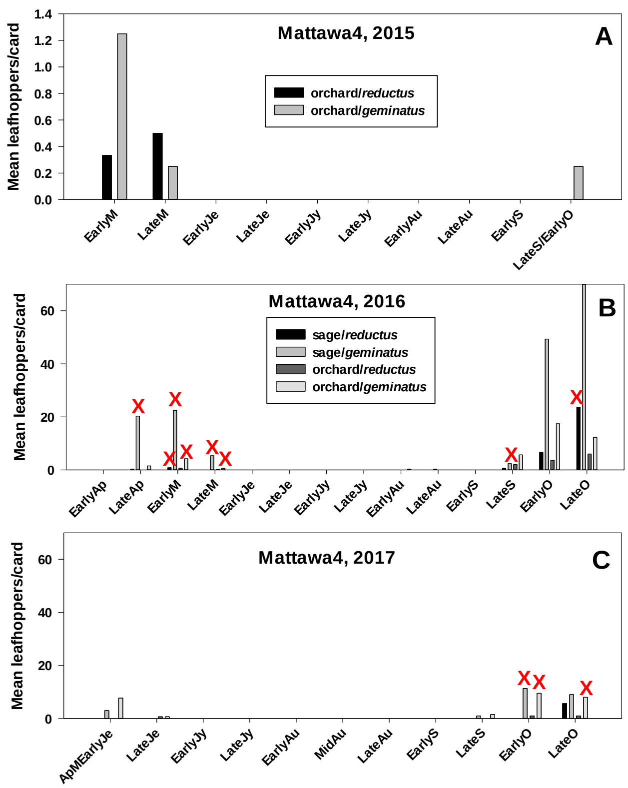

Figure 3. Seasonal abundance of and incidence of Western X (X over bar) in *Colladonus* spp. leafhoppers at the Mattawa4 site, 2015-2017.

While the Mattawa3 location had large *Colladonus* populations in fall of 2016, no leafhoppers were found to be positive for Western X in the fall samples from orchard and sageland collections (Fig. 4A and B, Tables 2 and 3). In 2017, frequent insecticide applications for Western cherry fruit fly made access to and sampling in those orchards difficult; three WX-positive samples came from the small sageland area outside of the orchard blocks. Only six WX-positive samples were found at the Benton City site; four of these samples were found in the sticky traps in border weeds (Fig. 4C). Orchard sampling at the Selah site had to be discontinued when the grower removed the trees in those blocks being sampled (Fig. 4D). A few weeks of orchard sampling took place in another block that was next to sageland recently burned off by a human-caused brush fire. Only one *Colladonus* sample taken from a *Purshia* sticky trap at the Selah site was found to be WX-positive (Tables 2 and 3).

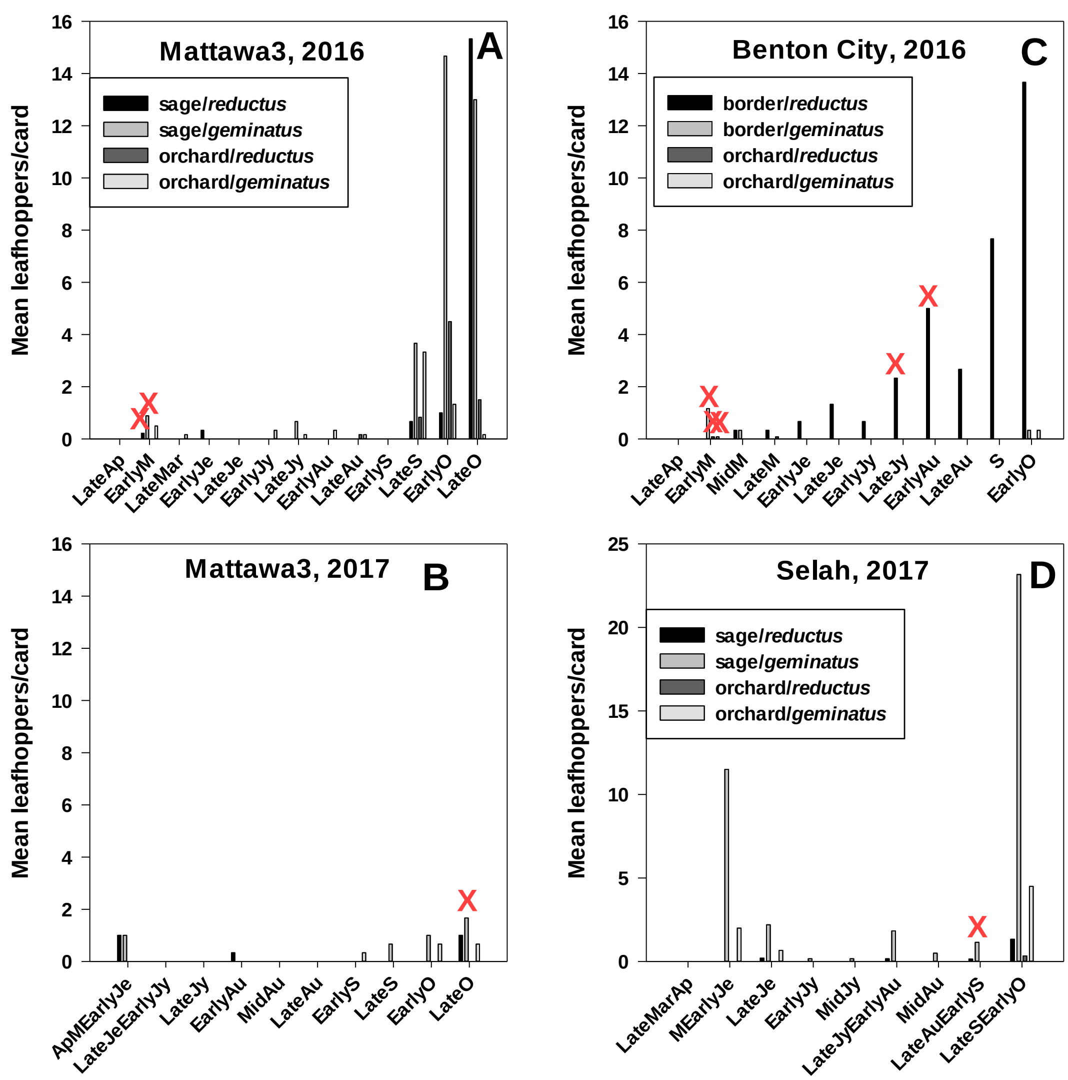

Figure 4. Seasonal abundance of and incidence of Western X (X over bar) in *Colladonus* spp. leafhoppers at the Mattawa3 site, 2015-2017; Benton City site, 2016; and Selah site, 2017.

Compared with other sites, the Granger site showed greater percentages of *Colladonus* leafhoppers positive for Western X in those that came from within the orchard (19 to 30%) as well as those collected from the neighboring alfalfa field (8 to 21%; Tables 2 and 3; Fig. 5). This site is a good example of an orchard with minimal pest and disease management.

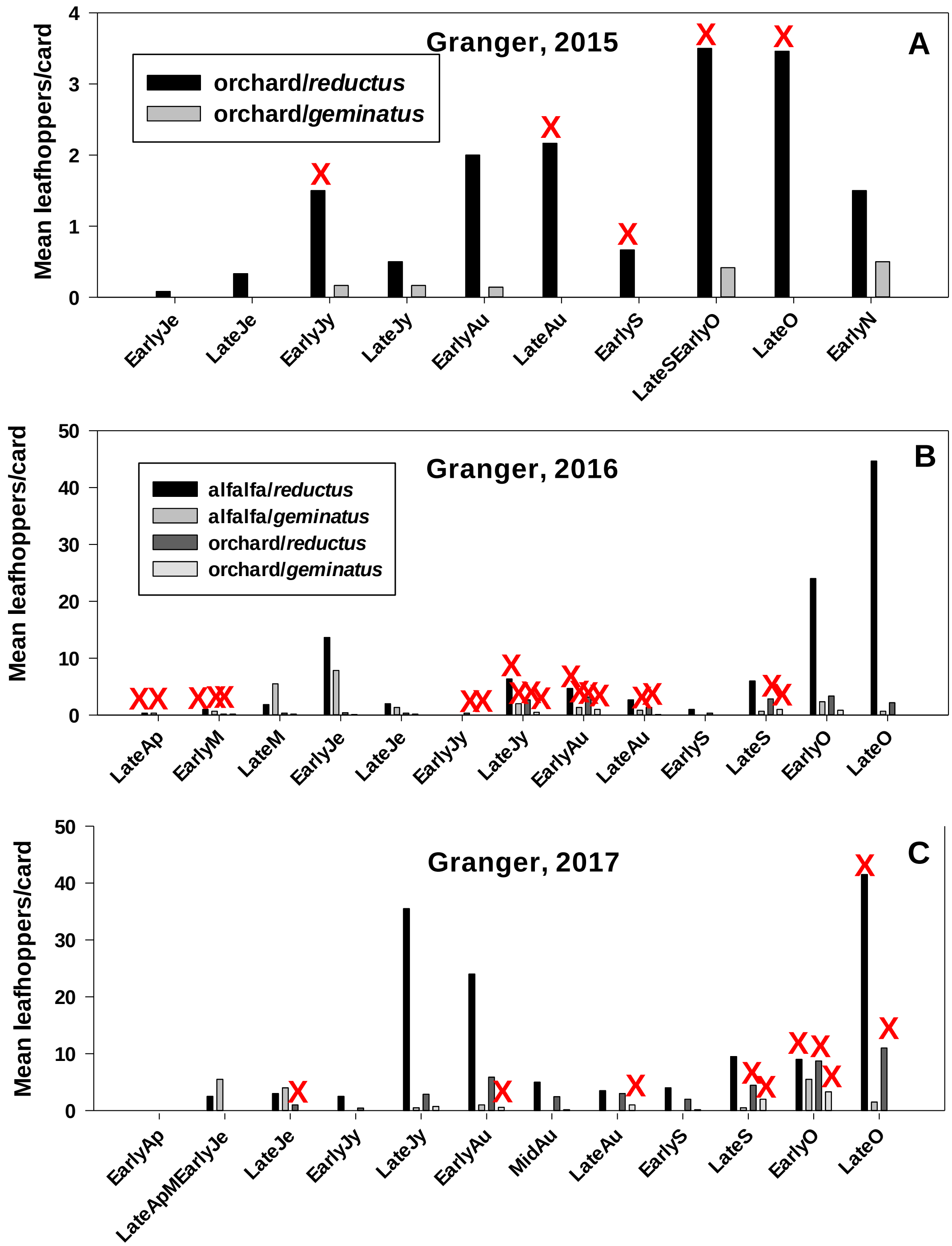

Figure 5. Seasonal abundance of and incidence of Western X (X over bar) in *Colladonus* spp. leafhoppers at the Granger site, 2015-2017.

For the Wapato site, *C. reductus* appeared to be relatively dominant over *geminatus*, and a greater percentage of *reductus* samples were positive for Western X (18% vs. 4%, Fig. 6, Tables 2 and 3). The drops in leafhopper numbers in early June and mid August 2018 were due to insecticide sprays. With G.S. Long collaboration, we continue to sample in the Wapato block through October and November. Note that in 2018, an additional study was conducted to compare sticky trap captures near ground versus tree trap captures. This would give us additional information on leafhopper movement among host plants.

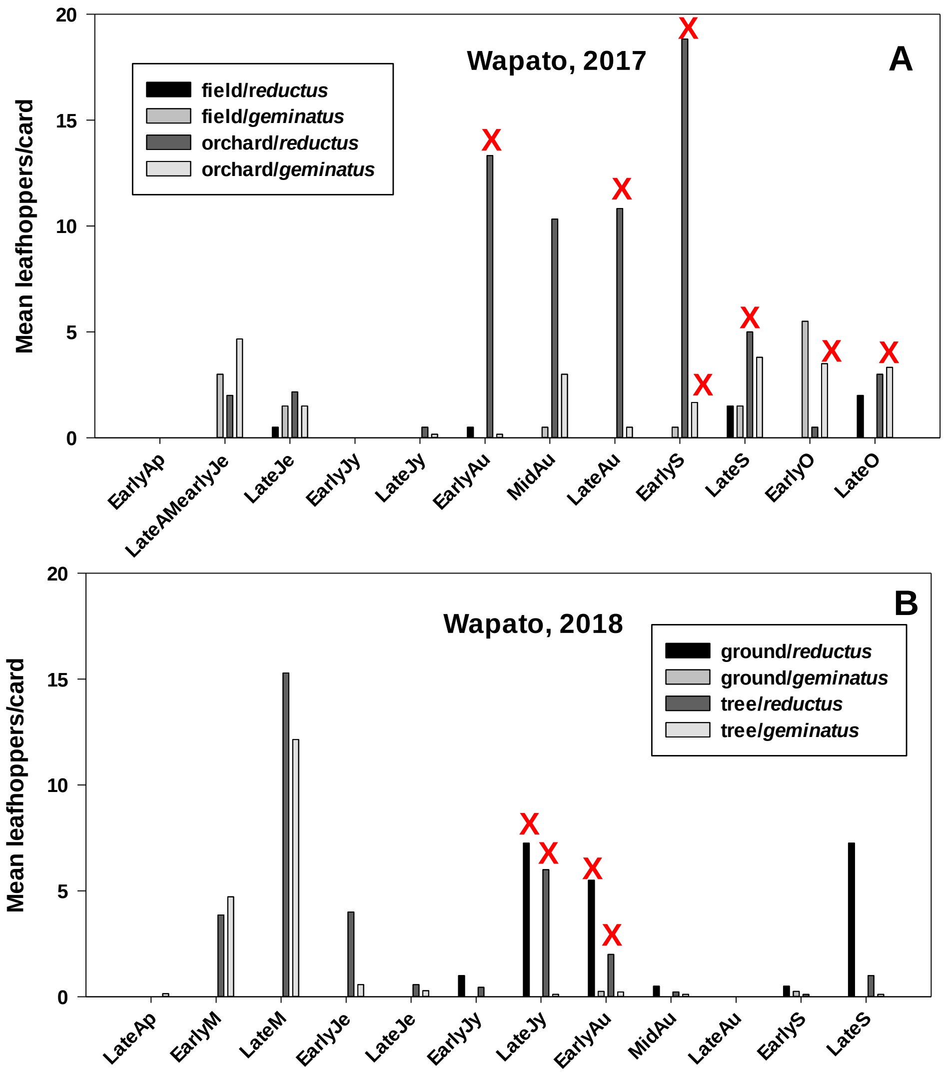

Figure 6. Seasonal abundance of and incidence of Western X (X over bar) in *Colladonus* spp. leafhoppers at the Wapato site, 2017-2018. Molecular analyses of late season 2018 samples are not complete.

Wenatchee sticky trap data in 2015 (collected in 2015 and analyzed during the grant period) and 2016 showed very low numbers of *Colladonus* leafhoppers throughout the growing seasons (data not shown). This was due to widespread spraying of cherry orchards in both years. One leafhopper sample from September 2016 was positive for WX.

Tables 2&3. Incidence of Western X in *Colladonus geminatus* (Table 2) and *C. reductus* (Table 3) collected in cherry orchard and extra-orchard habitats, 2016-2018. Extra-orchard habitats included alfalfa, sagebrush, *Purshia* sp., volunteer cherry, and herbaceous weeds.

Table 2. *Colladonus geminatus*						
	Cherry orchard			Extra-orchard habitat		
2016 sites	# samples	# positive	%	# samples	# positive	%
Mattawa1	100	4	4	71	7	10
Mattawa2	84	8	10	0	0	0
Mattawa3	29	0	0	20	3	15
Mattawa4	96	8	8	100	47	47
Granger	29	7	24	39	8	21
Benton City	3	1	33	7	1	14
2017 sites	# samples	# positive	%	# samples	# positive	%
Mattawa1	64	6	9	16	1	6
Mattawa2	--	--	--	20	1	5
Mattawa3	2	0	0	9	1	11
Mattawa4	11	1	9	9	0	0
Granger	10	3	30	13	1	8
Wapato	51	2	4	9	0	0
Selah	5	0	0	35	1	3
2018 sites	# samples	# positive	%	# samples	# positive	%
Wapato*	35	0	0	--	--	--

Table 3. *Colladonus reductus*						
	Cherry orchard			Extra-orchard habitat		
2016 sites	# samples	# positive	%	# samples	# positive	%
Mattawa1	104	15	14	10	0	0
Mattawa2	102	5	5	0	0	0
Mattawa3	23	0	0	13	1	8
Mattawa4	40	0	0	21	3	14
Granger	105	28	27	60	10	17
Benton City	2	1	50	31	3	10
2017 sites	# samples	# positive	%	# samples	# positive	%
Mattawa1	31	1	3	4	0	0
Mattawa2	--	--	--	16	0	0
Mattawa3	0	0	0	4	0	0
Mattawa4	4	0	0	1	0	0
Granger	36	7	19	39	3	8
Wapato	148	27	18	3	0	0
Selah	0	0	0	2	0	0
2018 sites	# samples	# positive	%	# samples	# positive	%
Wapato*	92	19	21	--	--	--

*Sample processing is not complete. Sticky card sampling continued into October 2018 but these samples have not yet been processed.

Objective 2. Conduct survey of host plants for leafhoppers and/or WX phytoplasma in affected orchards

We collected and identified 33 potential host plant species in our ecological survey. We attempted to maintain selected species in the greenhouse for host plant studies. Unfortunately, the numbers of leafhoppers required for these studies were not available. However, data from sticky trap and sweep net sampling in extra-orchard habitats provided host plant information.

Table 4. Plants identified in orchard and extra-orchard ecosystems that are potential host plants for *Colladonus* spp. leafhoppers and/or Western X phytoplasma.

Family	**Species**	**Common name**	**Orchard floor/border**	**Sageland**
Chenopodiaceae	*Chenopodium album*	Common lambsquarter	*	
Chenopodiaceae	*Atriplex spinosa*	Spiny hopsage		*
Chenopodiaceae	*Salsola tragus*	Russian thistle		*
Apiaceae	*Lomatium macrocarpum*	Large-fruit desert parsley		*
Apiaceae	Lomatium simplex	Great Basin desert parsley		*
Asteraceae	*Achillea millefolium*	Common yarrow	*	
Asteraceae	*Acroptilon repens*	Russian knapweed	*	
Asteraceae	*Arctium minus*	Common burdock	*	
Asteraceae	*Cyclachaena xanthifolia*	Burweed marsh elder	*	
Asteraceae	*Sonchus oleraceus*	Common sowthistle	*	
Brassicaceae	*Sisymbrium loeselii*	False London rocket	*	
Caryophyllaceae	*Arenaria serpyllifolia*	Thyme-leaf sandwort		*
Elaeagnaceae	*Elaeagnus angustifolia*	Russian olive	*	
Fabaceae	*Medicago lupulina*	Black medic	*	
Fabaceae	*Medicago sativa*	Alfalfa	*	
Fabaceae	*Melilotus officinalis*	Yellow sweet-clover	*	
Fabaceae	*Trifolium repens*	White clover	*	
Fabaceae	*Vicia villosa*	Hairy vetch	*	
Geraniaceae	*Geranium pusillum*	Small flower cranes-bill		*
Liliaceae	*Brodiaea douglasii*	Wild hyacinth	*	
Liliaceae	*Calochortus macrocarpus*	Sagebrush mariposa lily		*
Onagraceae	*Epilobium sp.*		*	
Onagraceae	*Oenothera villosa*	Common evening primrose	*	
Oxalidaceae	*Oxalis corniculata*	Creeping wood-sorrel	*	
Plantaginaceae	*Plantago major*	Common plantain	*	
Polygonaceae	*Persicaria maculosa*	Lady's thumb	*	
Polygonaceae	*Rumex crispus*	Curly dock	*	
Polygonaceaea	*Polygonum aviculare*	Prostrate knotweed	*	
Portulacaceae	*Portulaca oleracea*	Common purslane	*	
Rosaceae	*Amelanchier alnifolia*	Western serviceberry	*	
Rosaceae	*Prunus virginiana*	Chokecherry	*	
Rosaceae	*Purshia tridentata*	Antelope bush		*
Vitaceae	*Parthenocissus vitacea*	Virginia creeper	*	

Based on sticky trap and sweep samples, several species have been identified as probable host plants for *C. reductus* and *geminatus*. They are: alfalfa (*Medicago sativa*), clover spp. (*Trifolium* spp.), antelope bush (*Purshia tridentata*), and common mallow (*Malva neglecta*). If large stands or fields of these plants are found in orchard floor vegetation or next to orchard blocks, large populations of vector leafhoppers can build up, which creates a greater potential for WX transmission by leafhopper.

Objective 3. Examine the capability of selected leafhopper species to transmit WX phytoplasma. In 2016, we used several propagation methods (whip grafting, bottle grafting, sucker transplants) to establish an infected *Prunus* source of Western X but were unsuccessful. Acquisition and inoculation (transmission) studies were accomplished in 2017 and 2018. As we had no infected source of WX available to conduct the experiments in-house, we relied primarily on field-collected plant and leafhopper materials. We maintained clean, disease-free *C. reductus* and *C. geminatus* colonies in the laboratory on clean celery. Several approaches were taken to test the leafhoppers' potential to transmit Western X (Table 5). Either "clean" leafhoppers from the colonies or "dirty" leafhoppers from the field were used. In the no-choice trials, leafhoppers were confined to a small mesh bag over a clean plant shoot; this may have affected their natural flying and foraging behavior. In the "choice" experiments, leafhoppers could freely fly about in a dome-shaped mesh cage; this environment would be closer to a natural environment for them. One transmission was successful: *C. reductus* collected from an infected cherry block transmitted WX to a previously WX-free *Prunus tomentosa* tree. Recipient plants will continue to be tested for WX into the late fall/early winter.

Table 5. Western X acquisition and inoculation trials with the leafhoppers *Colladonus reductus* (n = 45) and *geminatus* (n = 30), 2017-2018.

Leafhopper vector source*	Acquisition source	Recipient plant	Choice or no-choice	No. trials	Transmissions observed?**
Colladonus sp. from colony	Cherry leaves from field	Celery seedlings	No-choice	20	none
Colladonus sp. from colony	Cherry leaves from field	*Prunus tomentosa*	No-choice	10	none
Colladonus sp. from field	From infected cherry trees	*P. tomentosa*	No-choice	6	1
Colladonus sp. from colony	Cherry leaves from field	*P. avium*	No-choice	12	none
Colladonus sp. from colony	Cherry leaves from field	Radish plants	No-choice	15	none
Colladonus sp. from colony	Cherry leaves from field	Radish seedlings	Choice of recipient	8	none
Colladonus sp. from field	From infected cherry trees	Celery seedlings	No-choice	4	none

*Summarized for brevity. Most studies involved either *C. reductus* or *geminatus* but not both.
**Trials are not complete. Additional testing will be done in fall and winter 2018-2019.

SIGNIFICANCE TO THE INDUSTRY AND POTENTIAL ECONOMIC BENEFITS

This information may be used by growers to improve the timing of both chemical and cultural leafhopper management practices, which in turn will improve management of little cherry disease caused by Western X phytoplasma. Our data show that there are two main time periods of WX-positive leafhopper activity in cherries—the spring and late summer into fall—although time periods may vary among orchards and year to year. To adopt a more targeted approach to leafhopper management, chemical controls could be implemented during those time periods, which should help to reduce costs of spraying for vector control.

EXECUTIVE SUMMARY

This project aimed to conduct surveys of leafhoppers and their potential host plants in Western X affected orchards and determine the incidence of Western X phytoplasma in populations of different leafhopper species. Western X is a pathogen of sweet cherries with infected trees exhibiting symptoms such as undersized and bitter-tasting cherries. Recommendations for preventing the spread of this disease include leafhopper vector control, leafhopper host plant control, and removal of trees. We also sought to determine the potential for *Colladonus reductus* and *C. geminatus* to transmit the Western X pathogen to cherries.

We conducted surveys of leafhoppers in affected orchards in 2016 to 2018, beginning in March/April and concluding in October. While some blocks were sampled over three years, sampling had to be discontinued in other blocks removed by the grower. Two leafhopper vector species, *C. geminatus* and *C. reductus*, were the most abundant species found on sticky cards in cherry tree foliage and in habitats outside of orchards in most sites.

Molecular diagnostic testing showed that for *C. geminatus*, 40/519 samples (8%) from within affected orchards and 71/348 samples (20%) from extra-orchard habitats, were positive for Western X phytoplasma. Likewise, for *C. reductus*, 103/687 samples (15%) from within affected orchards and 20/204 samples (10%) from extra-orchard habitats were positive for WX. Other infrequently collected potential vector species of leafhoppers that were tested for WX (number positive/sample size in parentheses) included: *Fieberiella florii* (1/12), *Osbornellus* sp. (1/23), *Scaphytopius* sp. (2/43), *Paraphlepsius* sp. (0/16), *Euscelidius variegatus* (1/35), *Macrosteles* sp. (0/15), *Circulifer tenellus* (0/22), and *Erythroneura* sp. (0/3).

From collections of weed and sagebrush plants and data from sticky card and sweep samples, we compiled a list of 33 potential host plant species for leafhoppers and/or Western X. A new project on native hosts of Western X phytoplasma began in the 2018 field season.

In late season 2017 and 2018, we conducted a total of 75 acquisition and/or inoculation studies with *C. geminatus* (n = 30) and *C. reductus* (n = 45). One transmission was successful: *C. reductus* collected from an infected cherry block transmitted WX to a previously WX-free *Prunus tomentosa* tree. We continue to check recipient plants during the fall into winter. While 1 success out of 45 *reductus* experiments may seem low, it only takes one leafhopper to introduce the disease to a block. Also, if leafhopper populations are high, as they were during peak periods in Mattawa1, Mattawa4, and Wapato blocks, this may account for the rapid spread of WX.

This information may be used by growers to improve the timing of both chemical and cultural leafhopper management practices, which in turn will improve management of little cherry disease caused by Western X phytoplasma. Our data show that there are two main time periods of WX-positive leafhopper activity in cherries—the spring and late summer into fall—although time periods may vary among orchards and year to year. To adopt a more targeted approach to leafhopper management, chemical controls could be implemented during those time periods, which should help to reduce costs of spraying for vector control.

To prevent the spread of Western X, management of leafhopper vectors is only one component of an integrated strategy. Growers should also consider other ways in which Western X is spread such as root grafting or planting trees that are not pathogen-free. Additional research is currently underway and ongoing at the Clean Plant Center Northwest to investigate other aspects of Western X pathology and transmission.