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

Budget 1
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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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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).

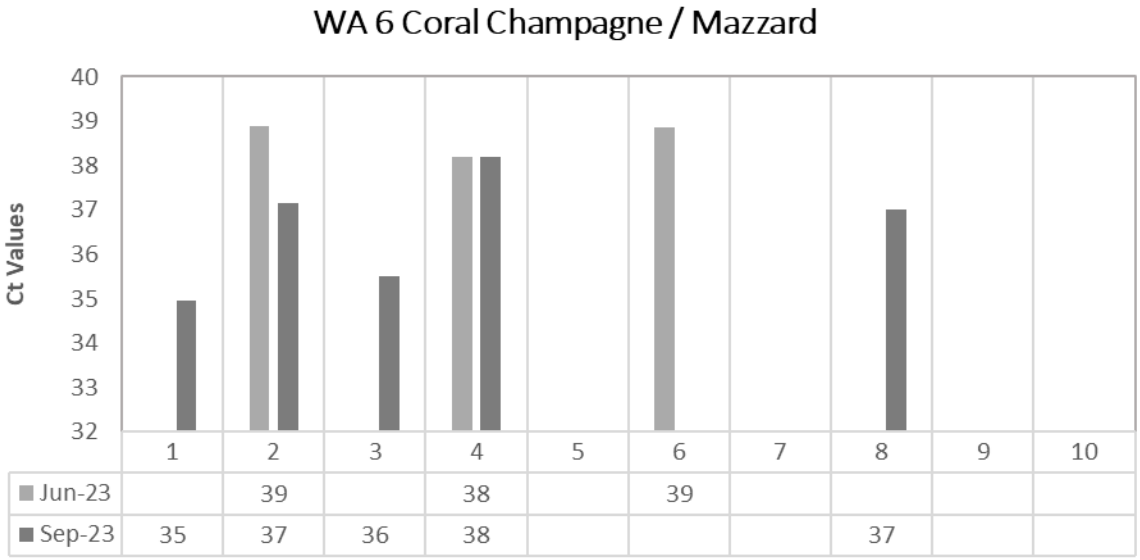

Figure 2. Ct values for XDP on individual trees at different sampling time.

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

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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/

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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