**FINAL PROJECT REPORT** **YEAR**: 3

**Project Title**: Orchard management practices for *little cherry virus 2*


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**Cooperators**: None.


**Total Project Request:** $171,172 Year 1: $57, 512 Year 2: $55,716 Year 3: $57,944

**Other funding sources**

*None.*

**Budget**

**Organization Name:** Washington State University **Contract Administrator:** Katy Roberts

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| Item | 2017 | 2018 | 2019 |
|---|---|---|---|
| **Salaries**[1] | $7,361 | $7,655 | $24,213 |
| **Benefits**[2] | $2,157 | $2,243 | $8,329 |
| **Wages** | $3,120 | $3,245 | 0 |
| **Benefits** | $324 | $337 | 0 |
| **Equipment** | 0 | 0 | 0 |
| **Supplies** | $44,550 | $42,236 | $25,000 |
| **Travel** | 0 | 0 | $0 |
| **Plot Fees** | 0 | 0 | $0 |
| **Miscellaneous** | 0 | 0 | $0 |
| **Total** | $57,512 | $55,716 | $57,542 |

**Footnotes:**

1. 0.15 FTE for year 1 and 2, and 0.5 FTE for year 3.
2. Benefits calculated at standard Washington State rates.

## OBJECTIVES

1) *Examine the effect of rootstocks on the concentration of LChV2.*

2) *Quantify the accumulation of LChV2 in different host tissues throughout the growing season.*

3) *Determine the population structure of LChV2 within Washington cherry production regions.*

## SIGNIFICANT FINDINGS

- In established infections, LChV2 can be detected in all tissue of the plant year round. However, the virus was most abundant in the fruit stem and woody tissue.
- The X-disease phytoplasma cannot be detected year round. Concentrations of the phytoplasma reach detectable levels at harvest and can be detected into the fall, however distribution is not uniform throughout the tree. Leaves are unreliable. Fruit stem and wood tissue appear to be the best tissues to test for the X-disease phytoplasma.
- LChV2 infects multiple rootstocks. Early in infection movement of the virus is basipetal, with highest concentrations in the roots and below the graft.
- Single-strand conformation polymorphism analysis confirmed that there are multiple isolates of LChV2 in Washington state.

## RESULTS AND DISCUSSION

LChV2 is one of the pathogens responsible for Little cherry disease (LCD), which produces small, misshapen fruit with poor taste. This project was undertaken to gain a better understanding of LChV2 biology with the goal of improving disease management. X-disease phytoplasma, which also causes little cherry disease, was included in the third year of the study as it has greatly increased in incidence in Washington along the Columbia River, and in the Wenatchee area.

To determine how LChV2 moves in different rootstocks, three rootstock varieties (Gisela 6, Gisela 12, and Krymsk 5) were chip grafted with wood from a tree infected with LChV2. At four, eight, twelve, and sixteen weeks, a subset of trees for each rootstock was dissected. Material was collected from fibrous roots, the primary root, wood scraping below the graft, wood scraping above the graft, pooled wood scrapings from branches, and a pooled leaf sample. This material was collected from four to five trees per rootstock at each time point. Quantitative PCR was performed to determine the concentration of LChV2 in each of these tissues. It was found the LChV2 moved basipetally, or downward towards the roots following initial infection, and was most concentrated below the graft and in the roots (Figure 1). Gisela 12 showed the most pronounced difference in LChV2 concentration in tissues below and above the graft. However, for all three rootstocks the virus did make it into the leaf tissue of the rootstock. Plans are in place to repeat this in Mazzard rootstocks.

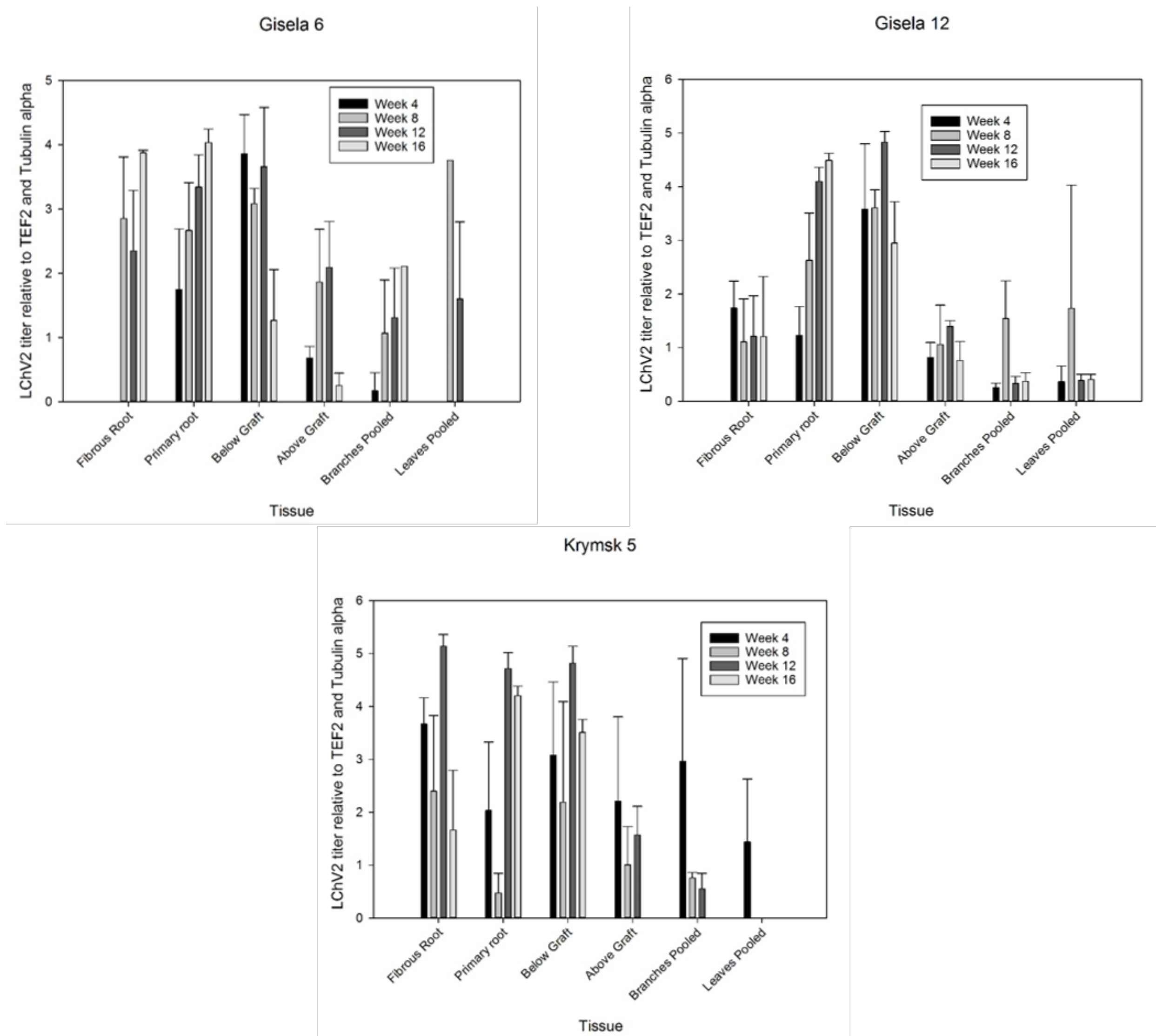


**Figure 1.** LChV2 titer across time and tissue type for Gisela 6, Gisela 12, and Krymsk 5 rootstocks.

A two year study was conducted examining titer and distribution of LChV2 in a Lambert cherry which had been infected for several years. Several tissue types were examined throughout the year (Table 1). RNA was extracted and qPCR analysis was performed to determine LChV2 titer. TEF2 and tubulin alpha were used as reference genes. LChV2 was distributed throughout the tree and was detected in all tissues throughout the year (Figure 2). However, LChV2 titer was greatest in the fruit stem and wood scraping. Although there were some differences between the two years, the overall pattern is similar. In both years, titer in leaf tissue was at its lowest in early May. Petiole and midrib concentrations were similar at each time point except in August of both years when midrib titer was higher. In the flower/fruit stem titer increased from April to May for both years. Titer in fruit increased closer to harvest although this increase was greater in the first year. Although in established infections LChV2 is distributed throughout the plant, it does appear that to improve the likelihood of detecting the virus when present, fruit stems and wood scrapings are the best material for analysis.

**Table 1.** Time points and tissues for titer and distribution of LChV2 (Tissues with highest concentration at that time point are in bold.

| Time | Tissue |
|---|---|
| December - January | Buds, **Wood Scraping**, Roots |
| March - Mid April | Buds, **Wood Scraping**, Roots |
| Mid to Late April | **Wood Scraping**, Roots, Flower Stem |
| Late April - Early May | Midrib, Petiole, **Wood Scraping**, Fruit, Fruit Stem |
| Late May - Early June | Midrib, Petiole, Wood Scraping, Roots, Fruit, **Fruit Stem** |
| Mid June - Early July | Buds, Midrib, Petiole, Wood Scraping, Roots, Fruit, **Fruit Stem** |
| Mid August | Buds, Midrib, Petiole, **Wood Scraping** |
| Mid October | Buds, Midrib, Petiole, **Wood Scraping** |

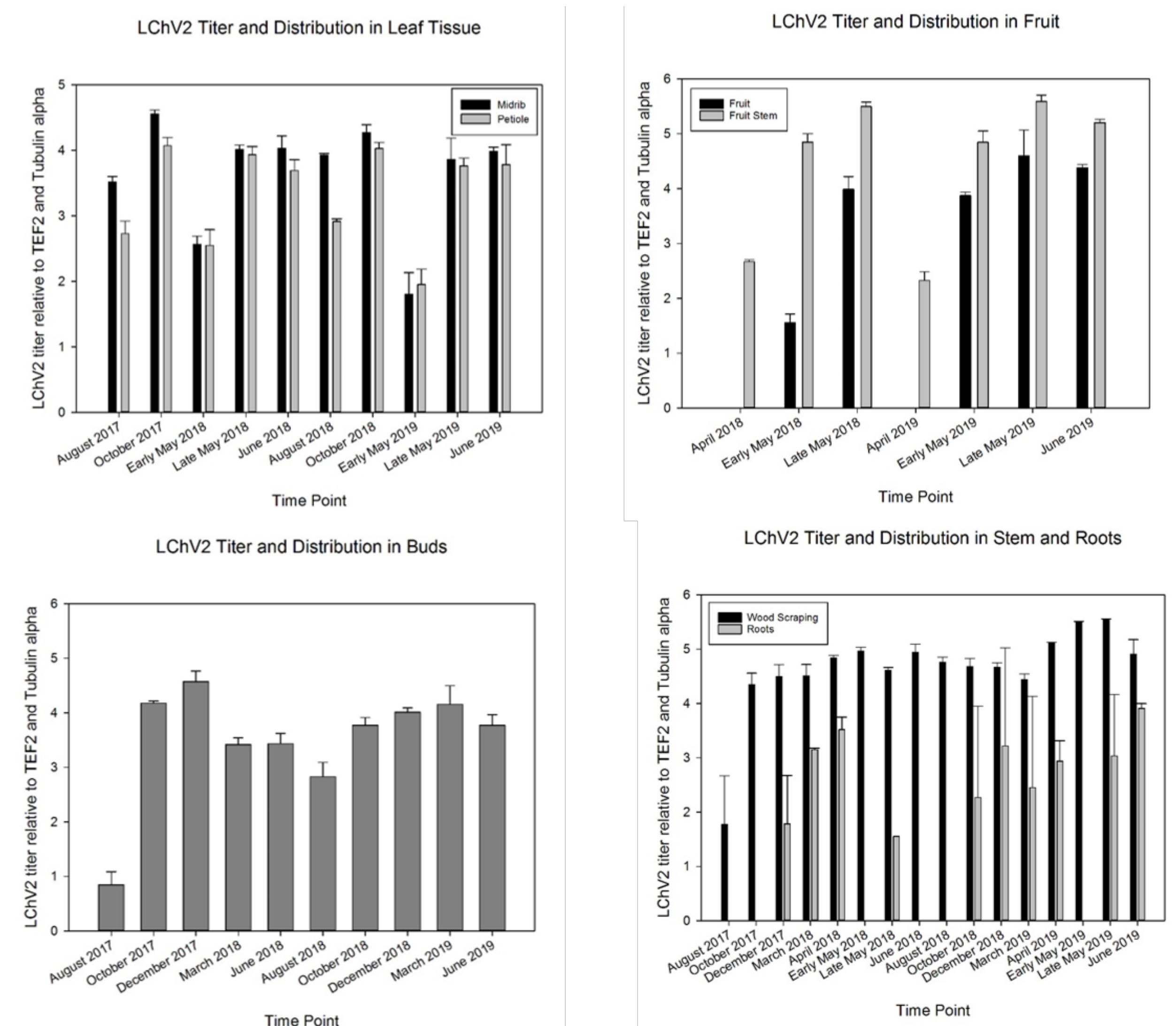


**Figure 2.** Titer and distribution of LChV2 in leaf, fruit, buds, stem and roots for year one and two.

The behavior of X-disease phytoplasma in cherry trees proved to be very different from LChV2. Samples were collected from trees that had tested positive in previous years. DNA was extracted from the

samples and qPCR was performed using ITS as a reference gene. Sampling for titer and distribution was begun in the spring, however it was not until shuck fall in May that the X-disease phytoplasma could be detected (Figure 3). Even then it could not be detected in leaves or fruit, only fruit stem and wood scrapings. Two weeks before harvest the pathogen could be detected in all four tissues, however detection in leaves was not consistent. Concentration in the fruit stem did increase between May and June. After harvest, buds were included in testing and these were positive in July and early September. Leaves, the tissue most commonly screened for the X-disease phytoplasma, are not reliable and were not positive until close to harvest. It is unknown what role, if any the winter weather had in delaying detection of the X-disease phytoplasma. Another year of data collection is planned, however this will be included in a new proposal. In addition, leaf, fruit stem, and fruit tissue was collected from several infected cultivars at harvest. Titer and distribution will also be examined in these samples.

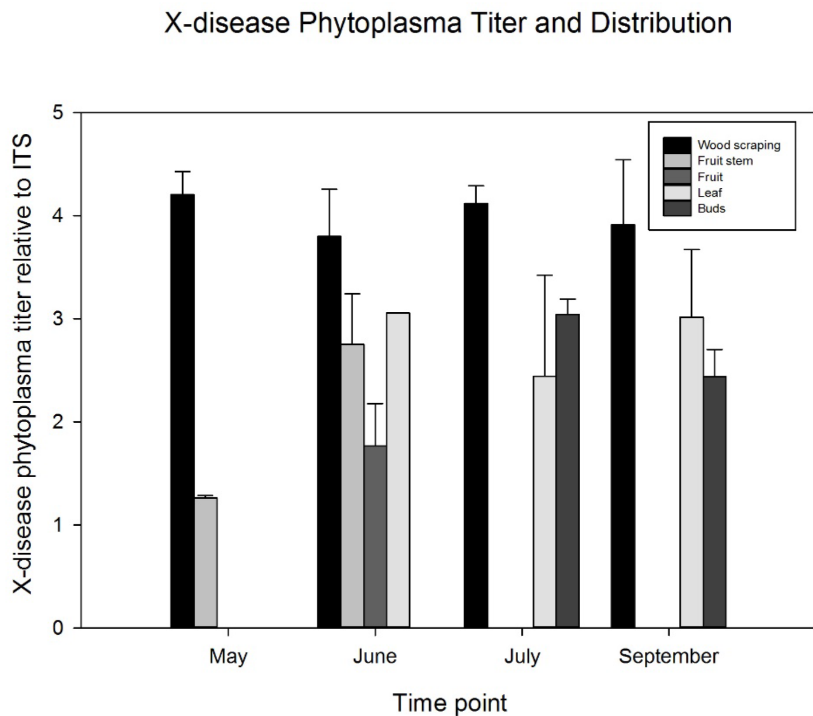


**Figure 3.** Titer and distribution of the X-disease phytoplasma.

Lastly, an examination of LChV2 samples from Washington State was conducted to identify unique isolates of the virus. This was done with the goal of increasing the amount of sequence data available for this virus so a more accurate qPCR assay can be developed. Positive samples from across the state were subjected to single strand conformation polymorphism analysis in which different sequences generate different banding patterns (Figure 4). While it was intended that the different variants identified be sequenced, the rapid escalation of the LCD epidemic in 2019 delayed this process into winter. Furthermore sampling during the 2019 season identified plants that were displaying classic LCD symptoms yet were negative for both LChV2 and X-disease phytoplasma. While it is unlikely that these are infected by an LChV2 isolate missed by extant tests and is more probably the X-disease phytoplasma, these samples will be sequenced over the winter to determine the causal agent. Together, this data will allow for a better assessment of the existing assays and development of an improved assays if necessary.

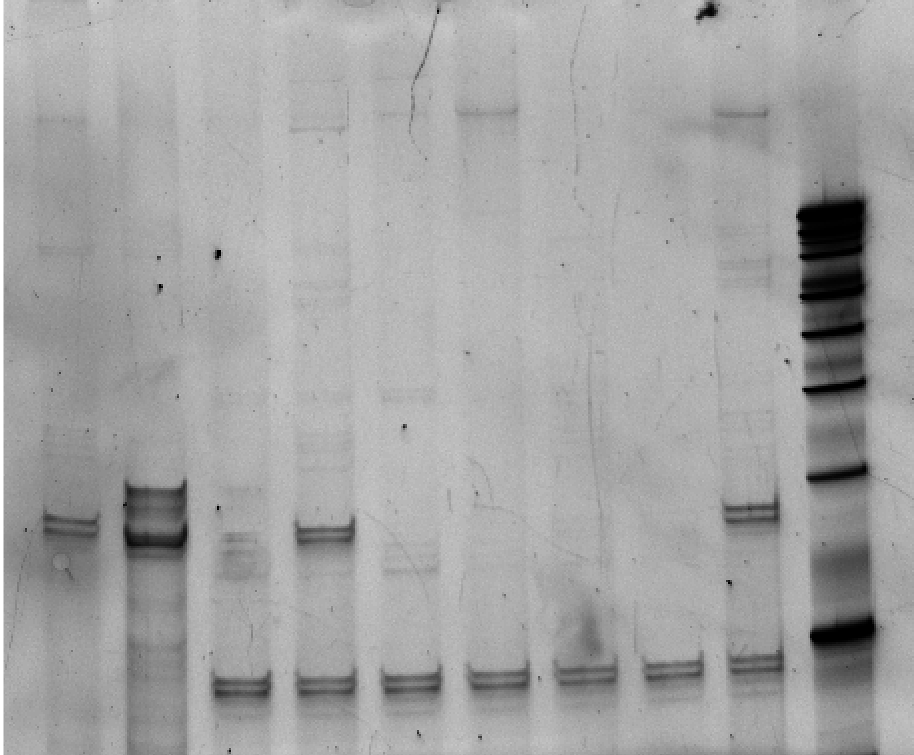

**Figure 4.** SSCP analysis on LChV2 samples. For each sample, the same region of LChV2 was amplified, however the banding patterns indicate that some of these samples have different sequences.

Collectively these data will lead to improved orchard management for control of LChV2. The rootstock analysis shows that, for these three rootstocks at least, once LChV2 is introduced, it can move throughout the plant even though it favors basipetal, or downward movement, towards the roots. The data for Mazzards will need to be included, but it is unlikely that rootstock choice affects susceptibility to LChV2. The titer and distribution study revealed that, in established infections, the virus is distributed throughout the tree and can be detected year round. SSCP analysis demonstrated that different isolates of LChV2 are present in the state and sequencing will determine how much these isolates differ. These data will inform decisions regarding LChV2 detection and should lead to improved detection of the virus. Early detection is an important part in managing this disease.

While growers should remain vigilant with regards to LChV2, the X-disease phytoplasma has in the last couple of years become the predominant cause of little cherry disease in Washington State. Testing of grower samples by the Clean Plant Center Northwest showed a 14% incidence of LChV2 and a 24% incidence of the X-disease phytoplasma. This year those numbers changed to 1% and 63%, respectively. LChV2 has declined while the X-disease phytoplasma has sharply increased. Additionally, the X-disease phytoplasma has been detected as far north as Wenatchee where LChV2 has traditionally been the predominant of the two pathogens. To address this, in the last year we examined titer and distribution of the X-disease phytoplasma. The phytoplasma is far more difficult to detect than the virus. Prior to harvest it is very difficult to detect in any tissue. Tissue distribution is not even and leaves in particular, which are the tissue most often used in testing, are unreliable. The lack of sequence data for this pathogen also makes it difficult to know if any assay will detect all strains. Future work will focus more heavily on the X-disease phytoplasma than LChV2 as the X-disease phytoplasma is the predominant pathogen in the state.

## EXECUTIVE SUMMARY

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**Keywords:** Little cherry disease, Little cherry virus 2 (LChV2), X-disease phytoplasma


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## FUTURE DIRECTIONS

The data presented here will result in improved detection and management of LChV2. Knowing how the virus is distributed allows for better sampling. Having sequence data from multiple isolates in the state will allow for the development of an improved qPCR assay if needed. Identification of trees with the virus, either through symptoms on the fruit or detection of the virus by qPCR, and removal of those infected trees is key to controlling and reducing the spread of LChV2.

While growers should remain vigilant with regards to LChV2, this pathogen is no longer the primary agent of little cherry disease. X-disease phytoplasma increased significantly in the state in the summer of 2019 and has spread north, being detected as far north as Wenatchee. Based on samples brought into the Clean Plant Center Northwest in 2018 and 2019, LChV2 incidence has dropped from 14% to 1% and X-disease phytoplasma incidence has increased from 24% to 63%. This one year increase is alarming. To address X-disease phytoplasa in terms of management, we began to look at titer and distribution of the phytoplasma. Unlike LChV2, which can be detected year round, the X-disease phytoplasma cannot be reliably detected in infected trees until summer. At harvest is the best time to begin sampling and sampling should be complete by the end of September. Leaves, the tissue most frequently sampled, were not reliable. Future projects will shift their focus mostly to the X-disease phytoplasma as this is the most problematic of the two pathogens. Currently, knowledge about this pathogen is very limited. Existing control options are not slowing down the spread. It is necessary to not only improve detection, but to understand the biology of this pathogen so either tolerant cultivars can be developed or improved control strategies can be employed.