**FINAL PROJECT REPORT**

**Project Title**: Mid-Columbia survey for sweet cherry viruses and vectors

**PI**: Jay W. Pscheidt, Extension Plant Pathology Specialist
**Organization**: Oregon State University
**Telephone**: 541-737-5539
**Email**: pscheidj@science.oregonstate.edu
**Address**: Department of Botany and Plant Pathology
**Address2**: 1089 Cordley Hall
**City/State/Zip**: Corvallis/Oregon/97331-2903

**Cooperators:** Lauri Lutes (OSU), Steve Castagnoli (OSU), Ashley Thompson (OSU), Drew Hubbard (GS Long), Jeff Heater (Columbia Gorge Fruit Growers Association), Inga Zasada (USDA-ARS)

**Other funding sources**

**Agency Name:** OSU Extension Service
**Amount Awarded:** $4,000
**Notes:** Annual discretionary statewide travel funds used to get to sampling sites.

**Agency Name:** USDA-ARS-HCRL
**Amount Awarded:** $4,000
**Notes:** Use of consumable supplies budget leveraged from USDA virus project.

**Total Project Funding:** $109,306

**WTFRC Funding: None** **OSCC Funding:** $109,306

**Budget History**

| **Item** | **(2017-2018)** | **(2018-2019)** |
|---|---|---|
| **Salaries (**GRA Stipend**)** | 23,776 | 24,242 |
| **Benefits (**Health Insurance**)** | 6,720 | 6,855 |
| **Wages** | | |
| **Benefits (**OPE**)** | 100 | 102 |
| **Equipment** | | |
| **Supplies** | | |
| **Travel** | | |
| **Miscellaneous (**OSU fees**)** | 18,520 | 21,000 |
| **Plot Fees** | | |
| **Total** | $49,106 | $52,200 |

## OBJECTIVES

**Objective 1**: Determine areas with sweet cherry trees expressing symptoms associated with Little Cherry Disease (caused by the pathogens *Little cherry viruses 1 & 2* and/or X-Disease phytoplasma) in Oregon's Mid-Columbia region.

**Objective 2:** Conduct regional Mid-Columbia survey for *Cherry leaf roll virus* and the *Cherry leaf roll virus* complex with *Prune dwarf virus* and *Prunus necrotic ringspot virus* on sweet cherry.

**Objective 3:** Survey Mid-Columbia sweet cherry orchards for nematode-transmitted viruses (such as *Cherry rasp leaf virus, Tobacco ringspot virus,* and *Tomato ringspot virus*) and their vectors.

**Objective 4:** Investigate spread and diversity of *Tomato ringspot virus* isolates from cherry in known-infected areas.

## SIGNIFICANT FINDINGS

- X-Disease found associated with little cherry symptoms in The Dalles, Dallesport (WA), and Mosier, but not Hood River valley.
- Several known *Cherry leaf roll virus*-infected trees removed in Oregon.
- Yet another orchard in The Dalles area was found with *Tomato ringspot virus* (ToRSV) infected trees.
- Nematode virus vector, *Xiphinema americanum*, was found in two commercial orchards in association with *Tomato ringspot virus* (ToRSV) infected trees. Visual symptoms were found reliably associated with ToRSV infected trees but a low percentage of samples without obvious symptoms also tested positive for ToRSV.

## RESULTS & DISCUSSION

**Objective 1**: Determine areas with sweet cherry trees expressing symptoms associated with Little Cherry Disease (caused by the pathogens *Little cherry viruses 1 & 2* and/or X-Disease phytoplasma) in Oregon's Mid-Columbia region.

In 2018 and 2019, 55 symptomatic and 6 asymptomatic sweet cherry leaf samples were collected for diagnostic testing associated with Little Cherry Disease and X-Disease with the help of local cooperators. Four symptomatic samples were collected from the Willamette Valley, while the rest were from the Mid-Columbia region. All samples from the Willamette Valley tested negative for the X-Disease phytoplasma. All but 3 of the other 52 symptomatic samples expressing little, unripened fruit tested positive for the X-Disease phytoplasma, *Candidatus* phytoplasma pruni, using a general phytoplasma real-time PCR assay (Table 1). One native *Prunus* sp. and six asymptomatic samples tested negative for the X-Disease phytoplasma. All samples tested negative for little cherry virus 2.

Table 1: 2018-2019 Little Cherry/X-Disease Survey Results

| | | | Number of positive samples | |
|---|---|---|---|---|
| Region | Year | # symptomatic /total | LChV2 qRT-PCR | Phytoplasma qPCR |
| Hood River Valley, OR | 2018 | 0/1 | 0 | 0 |
| | 2019 | 1/1 | 0 | 0 |
| Mosier, OR | 2018 | 5/5 | 0 | 5 |
| | 2019 | 2/2 | 0 | 2 |
| The Dalles, OR | 2018 | 20/24 | 0 | 20 |
| *3 Mile* | | 11/11 | 0 | 11 |
| *15 Mile* | | 0/1 | 0 | 0 |
| *Dufur* | | 0/1 | 0 | 0 |
| *Mill Creek* | | 9/12 | 0 | 9 |
| The Dalles, OR | 2019 | 23/23 | 0 | 23 |
| *3 Mile* | | 10/10 | 0 | 10 |
| *15 Mile* | | 0/0 | 0 | 0 |
| *Dry Hollow* | | 6/6 | 0 | 6 |
| *Dufur* | | 1/1 | 0 | 1 |
| *Mill Creek* | | 4/4 | 0 | 4 |
| *Unknown* | | 2/2 | 0 | 0 |
| Dallesport, WA | 2018 | 2/2 | 0 | 2 |
| Willamette Valley, OR | 2019 | 4/4 | 0 | 0 |
| **TOTAL** | | **57/62** | | |

These results indicate that the X-Disease phytoplasma is present throughout cherry production regions in The Dalles and Mosier, OR, as well as across the river in Dallesport, WA, but not in the Hood River Valley or the Willamette Valley, OR. Awareness of this disease and removal of infected trees should help control spread throughout the region.

**Objective 2:** Conduct regional Mid-Columbia survey for cherry leaf roll virus and the cherry leaf roll virus complex with prune dwarf virus and prunus necrotic ringspot virus on sweet cherry.

Past surveys revealed the presence of cherry leaf roll virus (CLRV) in The Dalles. One of the CLRV-infected orchards was removed and replanted in 2017. Another CLRV-infected orchard was removed in 2018. No new CLRV symptoms were reported in 2018 or 2019. Growers with the last few remaining trees known to have CLRV were encouraged to remove them.

**Objective 3:** Survey Mid-Columbia sweet cherry orchards for nematode-transmitted viruses (such as cherry rasp leaf virus, tobacco ringspot virus, and tomato ringspot virus) and their vectors.

Historically, the nematode-transmitted viruses, *Tobacco ringspot* and *Tomato ringspot,* have been known to occur on sweet cherry in Oregon. There is also historical evidence of another nematode-transmitted virus, *Cherry rasp leaf*, the causal agent of flat apple in Oregon's prominent production region, Hood River. In a statewide survey for sweet cherry viruses, *Tomato ringspot virus*, was found in Hood River and the Grande Ronde Valley. Due to this, a follow-up survey was conducting after bringing awareness of these diseases to the sweet cherry industry. No enation symptoms were

submitted for testing in the Mid-Columbia region in 2018. In 2019, a sample was submitted from an orchard in The Dalles and tested positive for *Tomato ringspot virus.*

**Objective 4:** Investigate spread and diversity of *Tomato ringspot virus* isolates from cherry in known-infected areas.

Understanding the distribution of viral symptoms, the virus itself and its nematode vector will help us determine how to manage this disease. Two infected orchards were selected for a more in-depth study of disease and pathogen distribution.

*Commercial Orchard #1 in The Dalles*

On May 21, 2018, a commercial cherry orchard in The Dalles was surveyed for symptoms associated with tomato ringspot virus (ToRSV). A total of 1,952 trees were surveyed for enations, dwarfed leaves, rosetting and dieback and rated on a 0-4 scale to indicate severity (0 = no ToRSV symptoms (rosetting, enations, 1 = Minimal ToRSV symptoms present, 2 = Moderate ToRSV symptoms present, 3 = Obvious ToRSV symptoms present, 4 = Tree stump with ToRSV symptoms on leaves). Tree locations were marked with an "X" if the tree had been removed partially (stump) or with an "M" if it was missing entirely. The orchard was primarily cv. 'Bing' on Mazzard rootstock with Van and Rainier pollinizers. Pollinizers were painted with different colors and used as reference points. Pollinator locations were indicated with a "Y" for yellow indicating cv. 'Van' and "R" for red indicating cv. 'Rainier'. A "+" symbol was used to note if suspected virus symptoms were observed that were not expected to be associated with ToRSV. Leaf samples were collected from a subsection of 400 trees (large rectangle, Figure 1) for testing by ToRSV ELISA. Due to missing trees, a total of 379 samples were tested. Of the samples tested, 92.9% of the samples expressing symptoms associated ToRSV tested positive for ToRSV, 6.6% tested positive when no visual symptoms were observed, and 0.5% tested negative when symptoms were observed, Figure 2.

Soil samples were collected on June 5, 2018, from a smaller subsection of 50 trees (5 rows by 10 trees in each row), as well as two small sections in areas where symptoms were not observed (medium and small rectangles in Figure 1) for identification of nematodes. Dagger nematodes were identified in 77.6% of the sites in the subsection of 50 trees, and in 25% of the samples from the regions where symptoms are not present on the outer edge of the orchard., Figure 3A. All dagger nematodes present in June were juveniles.

Soil samples were collected again on October 17, 2018 from the same regions. Dagger nematodes were identified in 88% of the subsection of 50 trees, and in 75% of the samples from the regions where symptoms were not present on the outer edges of the orchard, Figure 3B. The number of nematodes present in October was greater than in June. In June, there were an average of 11.16 (range 0-117) nematodes/250 g of soil, while there were 66.35 (range 0-313.5) nematodes/250 g of soil in October. Adult female dagger nematodes were found in the October survey and were archived for future studies relating to virus detection and nematode characterization.


Figure 1: Disease severity rating in commercial sweet cherry cv. 'Bing' orchard in The Dalles, OR; 0 = No 1 = ToRSV Symptoms (rosetting, enations), 2 = Minimal ToRSV symptoms present, 3 = Obvious ToRSV symptoms present, 4 = Tree stump with ToRSV symptoms on leaves, X = Dead stump (no leaves), M = Tree removed, Y = yellow marked pollinator, R = red marked pollinator, + = Suspected virus symptoms not associated with ToRSV, hashlines = *Xiphinema* sp. nematodes

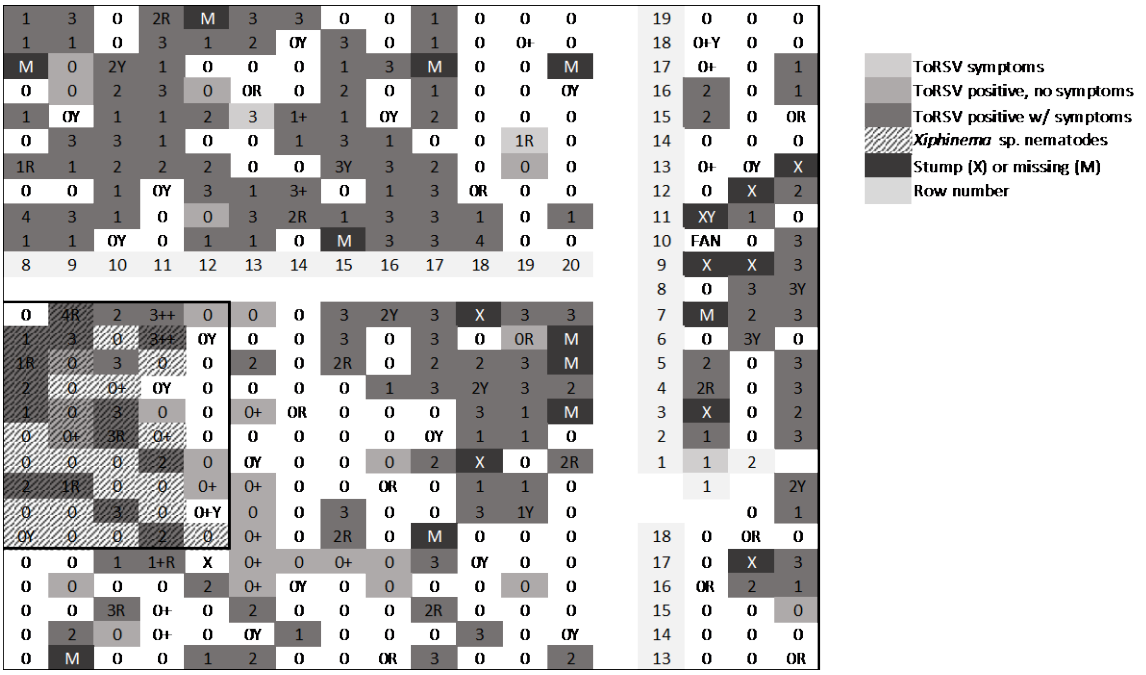


Figure 2 (a subsection of Figure 1 above): Correlation of symptom presence with diagnostic result for *Tomato ringspot virus* in commercial sweet cherry cv. 'Bing' orchard in The Dalles, OR

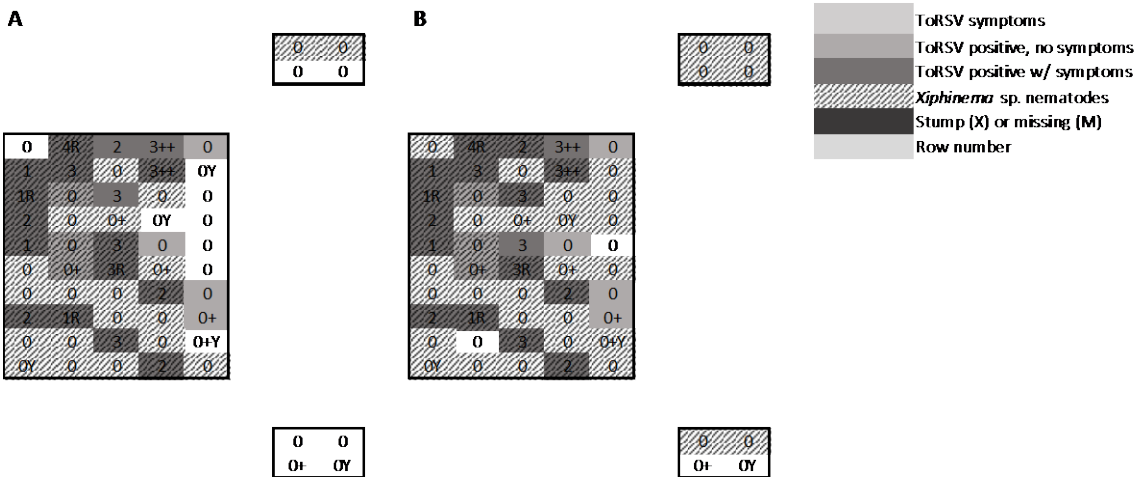


Figure 3 (a subsection of Figure 2 above): Dagger nematode presence in commercial sweet cherry cv. 'Bing' orchard in The Dalles, OR

*Commercial Orchard #2 in The Dalles*

On May 30, 2019, another commercial cherry orchard in The Dalles was surveyed for symptoms associated with tomato ringspot virus (ToRSV). A block of 455 trees (366 living) were surveyed for enations, dwarfed leaves, rosetting and dieback and rated on a 0-4 scale to indicate severity (0 = no ToRSV symptoms (rosetting, enations, 1 = Minimal ToRSV symptoms present, 2 = Moderate ToRSV symptoms present, 3 = Obvious ToRSV symptoms present, 4 = Tree stump with ToRSV symptoms on leaves). Tree locations were marked with an "X" if they had been removed partially (stump) or with an "M" if they were missing entirely. A "+" symbol was used to note if suspected virus symptoms were observed that were not expected to be associated with ToRSV, Figure 4. Due to missing trees, a total of 366 leaf samples were tested. Of the samples tested, 94.3% of the samples expressing symptoms associated ToRSV tested positive for ToRSV, 1.4% tested positive when no visual symptoms were observed, and 4.4% tested negative when symptoms were observed.

Soil samples were collected on June 6, 2019, from subsection of 45 trees for identification of nematodes in a section of the orchard that had symptomatic and asymptomatic trees. Four samples were also collected from two areas with asymptomatic plants. Dagger nematodes were identified in 33.3% of the sites in the subsection of 45 trees, and in 50% of the samples from the regions where symptoms are not present on the outer edge of the orchard. The average number of dagger nematodes found was 7.07 (range 0-71.5) nematodes/ 250 g of soil.

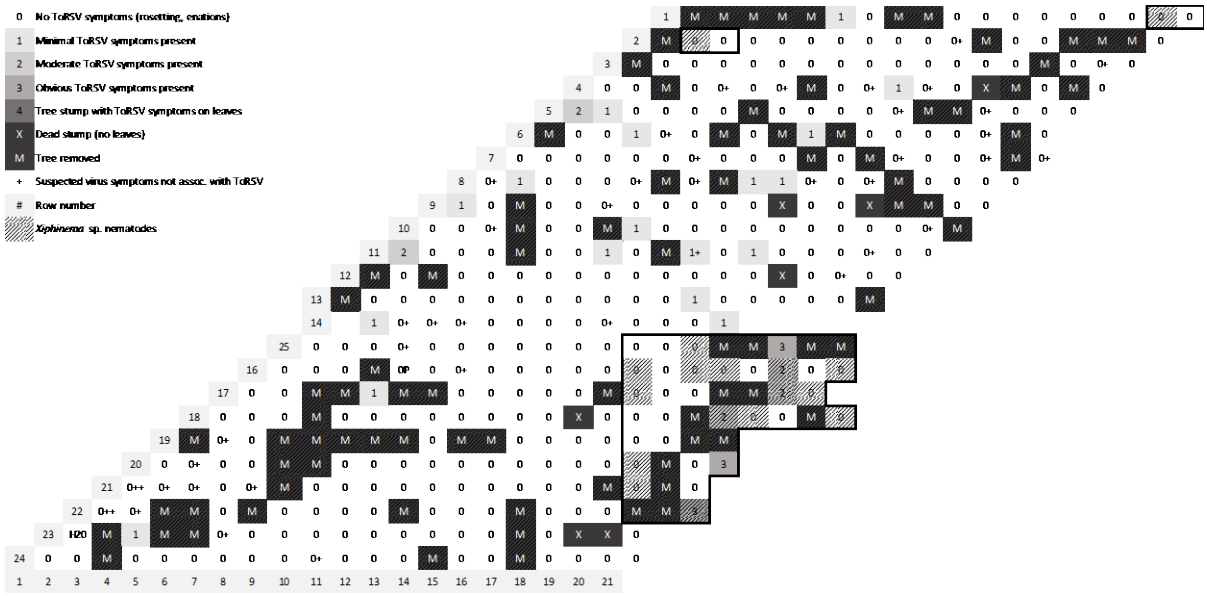


Figure 4: Disease severity rating in commercial sweet cherry orchard in The Dalles, OR; 0 = No 1 = ToRSV Symptoms (rosetting, enations), 2 = Minimal ToRSV symptoms present, 3 = Obvious ToRSV symptoms present, 4 = Tree stump with ToRSV symptoms on leaves, X = Dead stump (no leaves), M = Tree removed, + = Suspected virus symptoms not associated with ToRSV, hashlines = *Xiphinema* sp. nematodes

The two orchards surveyed for this study had tested positive for ToRSV; however, the distribution of the virus in the orchard and the correlation with the dagger nematode vector was unknown. The results suggest that there is no correlation between disease and nematode presence, as nematodes were found near trees with and without disease and did not seem to be aggregated in one area. From

this, we suggest to growers that if a nematode-transmitted disease is present in an orchard, it cannot be assumed that trees without symptoms are free of virus. If the virus and vector are present, the disease may be widespread in the orchard.

*Mid-Columbia Research and Extension Center*

Soil samples were collected on March 20, 2018, at a known *Tomato ringspot virus* (ToRSV)-infected research block at the Mid-Columbia Research and Extension Center. Samples were processed for the extraction and identification of dagger nematodes (*Xiphenema americanum*), the known vector of ToRSV. Matching the presence of nematodes in the orchard with locations of known ToRSV positive trees, allowed for the determination of eight areas where the virus and vector were present to test for the natural transmission of ToRSV to a variety of hosts. The following hosts were planted on April 13, 2018: apple (Scarlett Spur on MM106 rootstock), blueberry (Legacy), cherry (Lapins on Krymsk 6 rootstock), cherry (G6 rootstock), grape (self-rooted Chardonnay), peach (Loring on seedling rootstock) and raspberry (Meeker).

Bud, leaf, and/or root tissues were sampled from all hosts at planting and tested for the presence of ToRSV using ToRSV-specific ELISA. All samples tested negative for ToRSV (Table 2). Each tissue type (apple, blueberry, cherry, grape, peach and raspberry) was spiked with ToRSV-positive material in a 1:10, 1:100, 1:1000 serial dilution to account for possible inhibition of the test. None of the tissue types indicated the presence of inhibitors. Eight out of ten grape plants did not survive and were re-planted on June 4, 2018. Testing via ToRSV ELISA was performed again on root and leaf tissue to ensure plants were ToRSV negative (Table 2). Leaf samples were collected in September 2018 to test if transmission had occurred. None of the hosts tested positive, indicating no natural transmission had occurred.

On September 14, 2019, three cucumber seedlings at the cotyledon stage, as well as three cucumber seeds were planted in each plot positioned 8-10 inches around a sprinkler. Leaf and root samples were collected from each tree and small fruit host, cucumber, and weeds (plantains and dandelions) on October 4, 2019. Soil samples were collected from each of the eight plots to confirm presence of dagger nematodes. Results are pending. All foliar samples collected from the tree and small fruit hosts, cucumbers, and weeds tested negative for ToRSV (Table 2). Testing of root tissue is still in progress.

Table 2: Testing results for samples collected at Mid-Columbia Research and Extension Center

| | | 4/13/18 (Pre plant) | | 6/4/2018 (Re plant) | | 7/19/18 | | 9/17/18 | | 10/4/19 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Host | Tissue Type | n | # ToRSV + ELISA | n | # ToRSV + ELISA | n | # ToRSV + ELISA | n | # ToRSV + ELISA | n | # ToRSV + ELISA |
| Apple (Scarlett Spur on MM106) | bud | 9 | 0 | - | - | - | - | - | - | - | - |
| | leaf | - | - | - | - | - | - | 9 | 0 | 8 | 0 |
| | root | 9 | 0 | - | - | - | - | - | - | 8 | TBD[1] |
| Blueberry (Legacy) | leaf | 10 | 0 | - | - | - | - | 10 | 0 | 10 | 0 |
| | root | 10 | 0 | - | - | - | - | - | - | 10 | TBD |
| Cherry (G6) | bud | 10 | 0 | - | - | - | - | - | - | - | - |
| | leaf | - | - | - | - | - | - | 10 | 0 | 9 | 0 |
| | root | - | - | - | - | - | - | - | - | 9 | TBD |
| Cherry (Lapins) | bud | 6 | 0 | - | - | - | - | - | - | - | - |
| | leaf | - | - | - | - | - | - | 6 | 0 | 5 | 0 |
| | root | 6 | 0 | - | - | - | - | - | - | 5 | TBD |
| Grape (Chardonnary, self-rooted) | leaf | - | - | 8 | 0 | - | - | 10 | 0 | 10 | 0 |
| | root | 10 | 0 | 8 | 0 | - | - | - | - | 10 | TBD |
| Peach (Loring on seeding) | bud | 9 | 0 | - | - | - | - | - | - | - | - |
| | leaf | - | - | - | - | - | - | 9 | 0 | 8 | 0 |
| | root | 9 | 0 | - | - | - | - | - | - | 8 | TBD |
| Raspberry (Meeker) | leaf | 10 | 0 | - | - | - | - | 9 | 0 | 9 | 0 |
| | root | 10 | 0 | - | - | - | - | - | - | 9 | TBD |
| Cucumber (seedlings) | leaf | - | - | - | - | - | - | - | - | 7 | 0 |
| | root | - | - | - | - | - | - | - | - | 7 | TBD |
| Cucumber (seeds) | leaf | - | - | - | - | - | - | - | - | 3 | 0 |
| | root | - | - | - | - | - | - | - | - | 3 | TBD |
| Weeds (dandelion, plantain) | leaf | - | - | - | - | 1 | 0 | - | - | 8 | 0 |
| | root | - | - | - | - | 1 | 0 | - | - | 8 | TBD |

[1]TBD = To be determined

## Economic Importance

We hope that this survey has been a wakeup call for the Oregon sweet cherry industry to take action on important diseases that threaten cherry production. These diseases kill trees and/or yields and efforts should be made to find and remove infected trees. Many of the infected trees are from older orchards that may have already been scheduled for renovation. Removal of infected trees and treatment for soilborne nematode vectors will help protect newer orchards that keep the area vital and productive.

## REFERENCES


Lutes, L. A. and Pscheidt, J. W. 2019. Spread and host-specificity of *Tomato ringspot virus* on sweet cherry (*Prunus avium*) in Oregon. Phytopathology. (*Abstract accepted*)

Ito, T., and Suzaki, K. 2017. Universal detection of phytoplasmas and Xylella spp. by TaqMan singleplex and multiplex real-time PCR with dual priming oligonucleotides ed. Ruslan Kalendar. PLoS ONE. 12:e0185427.

## EXECUTIVE SUMMARY

**Project Title:** Mid-Columbia survey for sweet cherry viruses and vectors

**Key Words:** sweet cherry, virus, Little Cherry Disease, X-Disease, Tomato ringspot virus

A statewide diagnostic survey in 2016-2017 revealed the presence of important virus and phytoplasma induced diseases that threaten the Oregon sweet cherry industry. A focused survey for these diseases in the Mid-Columbia, the most prominent sweet cherry production in the state, as well as, the adjacent region in Washington, was conducted.

Little Cherry/X-Disease symptoms were found in this survey in The Dalles, Dallesport, and Mosier, but not in the Hood River Valley. Samples from these orchards tested positive for the X-Disease phytoplasma but negative for *Little cherry virus 2*. A few symptomatic samples were collected from the Willamette Valley, but each tested negative.

*Cherry leaf roll virus* (CLRV) was found in the previous survey, but no new reports of CLRV were made in 2018-2019; however, several known CLRV-infected orchards were removed. During this survey another orchard in The Dalles area was found with *Tomato ringspot virus* (ToRSV) infected trees. Despite an increased awareness of nematode-transmitted disease among the Oregon sweet cherry industry, only one new positive result was found.

The distribution of *Tomato ringspot virus* (ToRSV) was studied at two commercial orchards in The Dalles. The nematode vector was found in association with symptoms at both orchards. Visual symptoms of enations (gall-like formation on the underside of the leaf), as well as rosetting (bunching of leaves due to shortened internodes) were found reliably associated with ToRSV infected trees. A low percentage (1-6%) of samples without obvious symptoms also tested positive for ToRSV, highlighting the importance of virus testing.

An experiment to test if ToRSV could be transmitted to other hosts was conducted at the Mid-Columbia Research and Extension Center in Hood River by interplanting several tree and small fruit hosts in a known-infected orchard in areas where the dagger nematode vector was present. After an 18-month period, ToRSV did not naturally infect any of the interplanted hosts, including cherry trees used as controls.

We hope these surveys have been a wakeup call for the Oregon sweet cherry industry to take action on important diseases that threaten cherry production. These diseases kill trees and/or yields and efforts should be made to find and remove infected trees.