FINAL PROJECT REPORT

Project Title: Cherry virus diagnostic survey of Oregon
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OTHER FUNDING SOURCES

Agency Name: Department of Botany and Plant Pathology – Teaching Assistantship
Amt. awarded: $25,828
Notes: Graduate student Lauri Lutes was awarded this teaching assistantship. Tuition was subtracted from this amount.

Agency Name: OSU Extension Service
Amt. awarded: $3,000
Notes: Annual discretionary statewide travel funds used to get to sampling sites.

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

TOTAL PROJECT FUNDING

Organization Name: Agricultural Research Foundation (Oregon State University)
Contract Administrator: Russ Karow
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Item	(2015-2016)	(2016-2017)*
Salaries (GTA Stipend**)**	5,715	23,318
Benefits (Health Insurance**)**	2,401	9,795
Wages		
Benefits (OPE**)**		68
Equipment		
Supplies		
Travel		
Miscellaneous (OSU fees**)**	323	1,445
Plot Fees		
Total**	8,439	34,626

*Anticipating 2% increase for 2016-2017 school year. **Anticipating tuition remission in the amount of $1,350 for the summer 2016 term. Anticipating tuition remission in the amount of $13,500 for the 2016-2017 school year.

ORIGINAL OBJECTIVES

Objective 1: Sample symptomatic (and healthy) cherry trees, pointed out by multiple growers, from each of the tree fruit production areas of Oregon.
Objective 2: Determine the most likely cause of these symptomatic cherry trees, virus or not!
Objective 3: Follow up the diagnosis with management recommendations to each grower.
Objective 4: Survey historical records for occurrence of cherry viruses in Oregon.
Objective 5: Summarize the survey information to report on the threat viruses may (or may not) pose to the Oregon cherry industry (and indirectly to the WA industry).

SIGNIFICANT FINDINGS

- First report of *Tomato ringspot virus* (ToRSV) in Hood River, The Dalles, and Grand Ronde Valley regions
- First report of *Cherry leaf roll virus* (CLRV) in Oregon (The Dalles)
- *Cherry leaf roll virus* (CLRV) found with *Prune dwarf virus* (PDV) and *Prunus necrotic ringspot virus* (PNRSV) on same host, which causes more rapid, severe decline
- Report of *Little cherry virus 2* in the The Dalles, OR
- Resurgence of X-Disease in The Dalles.
- Enations, rosetting, and little, immature fruit are indicative of the most severe viruses on sweet cherry in Oregon

RESULTS & DISCUSSION

Objective 1: Sample symptomatic (and healthy) cherry trees, pointed out by multiple growers, from each of the tree fruit production areas of Oregon.

Over a two-year period, 7 regions with commercial sweet cherry orchards in Oregon were sampled May through September. In 2016, sampling focused on The Dalles/Mosier, Hood River, the Willamette Valley, and the Umpqua Valley. Samples were collected from the Rogue Valley, the Grande Ronde Valley, and Milton-Freewater regions to round out the survey in 2017. Based on results from the first year and the importance of the sweet cherry industry in the region, additional samples were collected from Hood River and The Dalles in 2017.

By working with collaborators throughout Oregon, orchards with suspected virus problems were identified. Upon scouting the orchards, samples were collected from trees expressing virus-like symptoms, including: foliar chlorosis, mottling, enations (gall-like formations on the underside of the leaf), and rosetting (bunching of leaves due to shortened internodes), as well as trees with sections of little, immature fruit. For each tree sampled, 10-20 leaves were collected for analysis by virus-specific ELISA. Samples were screened with the following ELISA (Agdia, Inc., Elkhart, IN; DSMZ, Braunschweig, Germany; AC Diagnostics, Fayetteville, AR): *Cherry leaf roll virus* (CLRV), *Cherry rasp leaf virus* (CRLV), *Plum pox virus* (PPV), *Prune dwarf virus* (PDV), *Prunus necrotic ringspot virus* (PNRSV), *Tobacco ringspot virus* (TRSV), and *Tomato ringspot virus* (ToRSV). Isothermal AmplifyRP® (Agdia, Inc., Elkhart, IN) was used for detection of *Little cherry virus 2* (LChV2). These diagnoses allowed for regional identification of viruses throughout the state (Table 1).

Due to the potential of other *Prunus* species harboring viruses that could infect commercial orchards, 15 samples were collected from non-*P. avium* trees (*P. subhirtella, P. emarginata, P. serrulata*) in 2016 and 2 *P. emarginata* samples were collected in 2017. None of these samples tested positive for the viruses tested.

For each symptomatic sample a comparison sample was collected from an asymptomatic neighboring tree (typically 2-3 trees and/or rows removed) of the same cultivar and age. These comparison samples were used to identify inconsistencies between the visual symptoms observed and viruses present. Of the 192 samples tested, 29 symptomatic samples tested negative for the viruses tested and 29 asymptomatic samples tested positive for PDV or PNRSV. Of the 29 symptomatic samples that tested negative, 10 were not *Prunus avium*. The symptoms observed on the other 19 samples could be associated with herbicide damage or other abiotic factors.

As expected, pollen-transmitted viruses, PDV and PNRSV, were found in all regions including those not previously reported, including: the Umpqua Valley, which had no viruses reported on sweet cherry; as well as, Hood River, where PDV and PNRSV on sweet cherry had not been known. Due to the pollen-transmitted nature of these two viruses, it is not unexpected to find them throughout the state. When occurring as a single infection, these viruses express minimal foliar symptoms leading to insignificant yield loss that often goes undetected.

Cherry leaf roll virus (CLRV) was detected in The Dalles, OR, in summer 2016. Since this virus had never been previously reported in Oregon and its potential impact to kill trees, 24 more samples were collected in the area in September 2016 to get an idea of the localized prevalence of the virus. Eight of the sixteen symptomatic samples tested positive for CLRV. Two additional samples were collected in spring 2017 for reverse transcription polymerase chain reaction (RT-PCR) analysis and downstream sequencing. Sanger sequencing and BLASTn analysis revealed a 99% identity to CLRV isolate Olm1, an isolated obtained from naturally-infected *P. avium* cv. 'Bing' in North America (Eastwell 2012).

The enation-producing, nematode-transmitted virus, ToRSV, was also discovered in Hood River. Knowing that a nematode-vectored virus is present, growers can make more informed decisions regarding fumigation before replanting or establishing new orchards. Additionally, it is not uncommon for vineyards to be planted where former cherry orchards once stood. For this reason, it is important to know that ToRSV (and TRSV) are known pathogens of grape, both causing reduced fruit set and uneven ripening (Moyer et al. 2014). Therefore, fumigation or a fallow period prior to planting should occur to rid the soil of potential nematode vectors.

Observation of visual symptoms is insufficient for distinguishing between the *Little cherry viruses 1 &* 2 and the virus-like phytoplasma, X-Disease. For this reason, 14 samples (20 leaves and 10 fruit stems per sample) collected from The Dalles, OR, expressing Little Cherry Disease symptoms were analyzed by a general phytoplasma quantitative real-time PCR assay after 8 symptomatic samples tested negative with the LChV2 Amplify RP® assay. Nine symptomatic samples tested positive by General Phytoplasma real-time PCR (qPCR) and all 5 asymptomatic samples tested negative (Table 1). It should be noted that one sample pair was collected from the symptomatic and asymptomatic portions of a single tree, and produced a positive and negative result on the respective diseased and asymptomatic leaf tissue. This highlights the importance of collecting tissue from a diseased area of the tree, which most notably will be a branch or section with immature, insipid fruit on a tree that has produced otherwise healthy-looking fruit. Since these samples could be multiply infected, a nucleic acid (DNA and RNA) has been extracted from these samples for follow-up testing for *Little cherry viruses 1 & 2* using a more sensitive RT-PCR assay.

Table 1: Number of *Prunus* sp.[a] samples testing positive for virus and virus-like pathogens in each Oregon region surveyed in 2016-2017

			Number of positive samples								
Region[b]	**Year**	**# symptomatic /total**	**CLRV[c] ELISA**	**CRLV ELISA[d]**	**LChV2 RPA[e]**	**PDV ELISA**	**PNRSV ELISA**	**PPV ELISA**	**ToRSV ELISA**	**TRSV ELISA**	**Phytoplasma qPCR[e]**
WV	2016	11/16	0	0	0	2	6	0	0	0	ND
WV	2017[3]	1/2	0	0	ND	0	0	0	0	0	ND
HR	2016	6/13	0	0	ND	5	1	0	2	0	ND
	2017	4/7	0	ND[f]	ND	7	5	0	4	0	ND
TD	2016	23/42	8	0	0	18	7	0	1	0	ND
	2017	16/26	2	ND	ND	14	7	0	3	0	9
RV	2017	12/21	0	ND	ND	5	5	0	0	0	ND
UV	2016	9/14	0	0	ND	1	2	0	0	0	ND
MF	2017	17/28	0	ND	ND	21	7	0	0	0	ND
GR	2017	14/23	0	ND	ND	12	7	0	1	0	ND
TOTAL		**113/192**									

[a] In 2016, 15 samples were collected from non-*P. avium* trees (*P. subhirtella, P. emarginata, P. serrulata).* In 2017, two *P. emarginata* samples were collected. All other samples were from *P. avium* trees.

[b] WV = Willamette Valley, HR = Hood River, TD = The Dalles/Mosier, RV = Rogue Valley, UV = Umpqua Valley, MF = Milton-Freewater, GR = Grande Ronde

[c] CLRV = *Cherry leaf roll virus*, CRLV = *Cherry rasp leaf virus*, LChV2 = *Little cherry virus 2*, PDV = *Prune dwarf virus*, PNRSV = *Prunus necrotic ringspot virus*, PPV = *Plum pox virus*, ToRSV = *Tomato ringspot virus*, TRSV = *Tobacco ringspot virus*

[d] Samples were not screened for CRLV in 2017 due to the lack of reliable ELISA.

[e] Due to the cost associated with the LChV2 RPA and Phytoplasma real-time PCR (qPCR) assay, only samples expressing symptoms of Little Cherry Disease were tested.

[f] ND = not determined

Objective 2: Determine the most likely cause of these symptomatic cherry trees, virus or not!
Orchards were identified by regional cooperators and growers based on virus-suspected disease symptoms. Although all orchards visited had symptoms associated with viruses several were not due to virus. Diagnosis of these orchard problems included: lack of irrigation water, bacterial canker, gophers, crown gall and Phytophthora root rot. If a virus on a diseased sample was not found through initial ELISA screening and an abiotic diagnosis was not made, an alternative assay was used to identify the cause of disease.

One *P. avium* cv. 'Bing' sample with "pixelated", mosaic foliar symptoms collected from the Rogue Valley did not produce a positive result in the ELISA virus screening process. This sample was sent to Agdia, Inc. (Elkhart, IN) for further testing using the following group PCR assays (target viruses in parentheses): Ilarvirus *(American plumline pattern virus, Apple mosaic virus, Prune dwarf virus, Prunus necrotic ringspot virus*), Closterovirus (*Plum bark necrosis stem pitting-associated virus, Little cherry virus 1 & 2*), Potyvirus *(Plum pox virus),* Nepovirus (*Arabis mosaic virus, Cherry leafroll virus, Cherry rosette virus, Myrobalan latent ringspot virus, Tobacco ringspot virus, Tomato ringspot virus, Stocky prune virus*), Tombusvirus (*Tomato bushy stunt virus, Petunia asteroid mosaic virus, Carnation Italian ringspot virus*), Trichovirus (*Cherry mottle leaf virus*). A positive result was found using general Nepovirus primers, and downstream sequencing produced a 94% identity match to the putative virus *Prunus virus F*.

A weeping cherry sample collected in the Willamette Valley was found expressing chlorotic almost mosaic-like leaf symptoms on the newest growth. After initial screening with ELISA, no virus was found. This sample was sent to Agdia, Inc. (Elkhart, IN) for further testing using the same group PCR assays as listed above. This sample tested negative for all virus groups tested. Based on the extensive testing and peculiar expression of symptoms on the younger leaf tissue, which is not typical of viral symptoms, it was determined that the symptoms observed were not associated with a viral pathogen. Exact cause is still unknown.

Objective 3: Follow up the diagnosis with management recommendations to each grower.
A total of 113 symptomatic leaf samples were collected and analyzed in 2016-2017. After each diagnosis was made, the results were communicated with each grower or regional cooperator. Results were communicated via email, phone or in person.

Objective 4: Survey historical records for occurrence of cherry viruses in Oregon.
About 800 *Prunus* sp. records were assessed for the presence of specific viruses at the Oregon State University Plant Clinic (1956-2016). These records were submitted by county extension agents, growers, or homeowners for the purpose of disease diagnosis. From these records, several of the findings in the literature were corroborated and an unpublished record of *Tobacco ringspot virus* was identified in the Grande Ronde Valley (Table 2).

At the Oregon State University Herbarium, 373 *Prunus* sp. vouchers were inspected for obvious symptoms commonly associated with viruses, including: foliar mosaics, mottling, ringspots, and enations. One bitter cherry (*P. emarginata*) sample collected from the Umpqua Valley (Douglas County) in 1954 had a notable mosaic symptom not considered to be an artifact of the preservation process according to several herbarium curators. Bitter cherries at this location were revisited in 2016 and 2017, but similar symptoms were not observed at this site. A *P. avium* cv. 'Bing' sample collected in Medford, OR, in 2017 expressed similar symptoms and tested positive for *Prunus virus F*, as described under Objective 2.

Table 2: Presence of viruses and virus-like pathogens known to infect sweet cherry in Oregon

			Region							
Name of Pathogen	Present on sweet cherry in Oregon?	Oregon (Region Unknown)	Willamette Valley	Hood River	The Dalles/ Mosier	Umpqua Valley	Rogue Valley	Milton-Freewater	Grand Ronde Valley	Other
American plum-line pattern virus	Yes	+(flowering cherry)[9,10]								
Apple chlorotic leaf spot virus	Yes				+[8]					
Apple mosaic virus	No									
Arabis mosaic virus	No									
Cherry green ring mottle virus	Yes	+[2]	✦	+[8]						
Cherry leaf roll virus	Yes									
Walnut strain	Yes		+(walnut)[6]							
Olm1 strain	Yes				★					
Cherry mottle leaf virus	Yes	+[1,2]	✦	+[8]	✦				✦	
Cherry necrotic rusty mottle virus	Yes	+[1,2]	✦	+[8]	✦				✦	
Cherry rasp leaf	No			+(apple)[4]						
Cherry rusty mottle virus	Yes	+[2]	+[1]✦	+[8]	+[1,8]✦			✦	✦	
Cherry twisted leaf assoc. virus	Yes		✦		+[10]✦					
Cherry virus A	Yes		+[5]✦							
Hop stunt viroid	No		+(hop)[7]							
Little cherry virus-1	Yes		+[1]✦							
Little cherry virus-2	Yes				+[11]					
Peach latent mosaic viroid	No									
Plum bark necrosis stem pitting-associated virus	No									
Prune dwarf virus	Yes	+[1]	+[8]✦	+[8]★	+[8]✦	★	★	✦	★	
Prunus necrotic ringspot virus	Yes	+[1]	✦	★	+[8]✦	★	★	✦		✦(Coastal)
Rugose mosaic strain	Yes		✦		✦?				✦	
Prunus virus F	Yes						★			
Tobacco mosaic virus	Yes	+[10]								
Tobacco ringspot virus	Yes								✦	
Tomato ringspot virus	Yes			★					★	
Eola rasp leaf strain	Yes		+[3]✦							
X-Disease	Yes				+[1]★		+[12]	+[1]		+(Malheur)[1]
Not currently known in North America										
Cherry rosette virus	No									
Myrobalam latent ringspot virus	No									
Petunia asteroid mosaic virus	No									
Plum pox virus	No									
Stocky prune virus	No									

[1] MacSwan and Raymer 1959; [2] Hadidi, et al.; [3] Milbrath and Reynolds 1961; [4] Parish 1977; [5] Poudyal et al. 2015; [6] Miller et al. 1958; [7] Cindy Ocamb, personal communication; [8] Eastwell, personal communication; [9] Zeller and Milbrath 1942; [10] USDA-ARS 1976; [11] Drew Hubbard, personal communication [12] Sugar and Long, personal communication

✦ OSU Plant Clinic Record ★ Indicates finding from statewide survey

Objective 5: Summarize the survey information to report on the threat viruses may (or may not) pose to the Oregon cherry industry (and indirectly to the WA industry).

Based on the information gathered through historical records and the statewide sampling survey, viruses have been identified and rated based on their prevalence and potential impact (Table 3). Viruses were rated on a scale of 0 to 10 with 0 representing "no grower action needed" and 10 representing "action is imperative." This ranking scheme considered the ability of the virus to kill trees, significantly reduce yield, cause unmarketable fruit, and the mode of transmission.

We suspect that most growers would not be concerned about a virus with a rating of 5 or lower. Ratings of 6 or higher represent important viruses that will impact production and spread to other trees. Action can range from implementing an insecticide program to minimize vector spread to orchard removal, which could be followed by fumigation to manage other vectors.

Table 3: Grower action rating of sweet cherry cv. 'Bing' virus and virus-like pathogens of importance to Oregon

		Found on sweet cherry in Oregon?	
Name of Pathogen(s)	Action Rating (Pscheidt)[a]	Historically	This Survey
Cherry leaf roll virus* (plus PDV and/or PNRSV*)	7	-	+
Cherry mottle leaf virus	6-7	+	ND[b]
Cherry necrotic rusty mottle virus	7	+	ND
Little cherry virus 1	6-7	+	-
Little cherry virus 2	6-7	-	+
Plum pox virus	10	-	-
***Prunus necrotic ringspot virus* (rugose strain)**	6	+	+
Tomato ringspot virus	6	+	+
X-Disease	8	+	+

[a] 0 = no action, 10 = action is imperative
[b] ND = not determined

It may still take weeks to go from "I think it is a virus", collecting and sending in samples, to getting a report back on which viruses might be found. Key symptoms have been identified in association with more severe viruses that may be used to more rapidly initiate an action. These symptoms include enations and little, immature fruit on one or more branches of a tree.

Impact and economic benefits

The historical information from the OSU Plant Clinic and Herbarium contributed to our understanding of the occurrence sweet cherry viruses in Oregon. In the sampling survey, participating growers and regional cooperators were able to identify problems in their orchards and receive a diagnosis, even if the causal agent did not turn out to be viral. An article published by Good Fruit Grower provided an overview of the project objectives and findings after the first year (Dininny & Mullinax 2017).

Cherry leaf roll virus (CLRV) was found for the first time on sweet cherry in The Dalles, OR. Despite being a member of the genus Nepovirus, CLRV is not known to be transmitted by nematodes. The walnut strain of CLRV is known to be pollen-transmitted, but there is still much unknown about the transmission of this devastating virus on sweet cherry (Hadidi et al. 2011). CLRV causes a slow decline when occurring alone, but a synergistic effect occurs when a tree is multiply-infected with the PDV or PNRSV. In this case, a rapid decline will occur. With use of Google street imaging, a rapid decline over a 4-year period was observed. The diagnostic work from this survey prompted the removal of a CLRV-infected orchard with ~%7 trees in decline, to prevent further spread in The Dalles region. This information is being disseminated to the scientific community as a Disease Note in the journal *Plant Disease* (Lutes & Pscheidt 2017, *in press*).

Although there are dozens of viruses that infect sweet cherries, there are only a few that should elicit an immediate response. The grower action rating and identification of symptoms associated with the more severe viruses should allow growers to make more informed management decisions. For example, this survey confirmed that PDV and PNRSV are likely to be present in sweet cherry orchards throughout the state based on their pollen transmission method. These two viruses produce foliar symptoms (mottle, ringspots, "lacey" holes in leaves), but do not reduce yield significantly or kill trees as a single infection. However, foliar enations (gall-like formations on the underside of leaf) and rosetting (bunching of leaves due to reduced internodes) are associated with more severe viruses, including: CLRV and ToRSV. An action rating scale was introduced at the Mid-Columbia Cherry Day in February in The Dalles, Oregon, and published in the May 15th 2017 Issue of Good Fruit Grower (Pscheidt 2017).

As a result of this work, updates have been made to the widely accessible PNW Plant Disease Management Handbook (https://pnwhandbooks.org/plantdisease), including images of symptoms and management recommendations for cherry (*Prunus* spp.) diseases.

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EXECUTIVE SUMMARY

The Washington cherry industry has had to understand, detect, and manage several new viral diseases over the last 15-20 years. It was not known if these same viral diseases were a problem in the Oregon cherry industry. A thorough search of published literature, Oregon State University Plant Clinic and Herbarium records, and physical state-wide orchard sampling found many of the same important viral diseases.

Cherry leaf roll virus (CLRV) was found for the first time on sweet cherry in Oregon. This virus was found in just a couple of adjacent orchard blocks in The Dalles. Infected trees were in decline with poor growth, rosetting, leaf enations, and hardly any fruit. One of the blocks has been removed but continual monitoring of declining trees in this region is recommended.

Several nematode-transmitted viruses, including *Tomato ringspot virus* (ToRSV) and *Tobacco ringspot virus* (TRSV), were found in many areas (The Dalles, Hood River, the Grand Ronde Valley). This means that nematode sampling is strongly recommended prior to planting new cherry orchards, especially if they are not scheduled to be fumigated. This is not only relevant for replanting sweet cherry orchards, but also when transitioning to other susceptible crops such as grapes, peaches, plums, or apples.

Little Cherry Disease was also found in this survey. Little Cherry Disease is characterized by fruit that does not ripen, develop flavor, and/or brix by harvest. The disease can be caused by *Little cherry virus 1 & 2* and/or the X-Disease phytoplasma. We tracked an unpublished record from the Clean Plant Center Northwest (Prosser, WA) that confirmed the presence of *Little cherry virus 2* in The Dalles, OR. Several samples with symptoms of Little Cherry Disease from The Dalles tested positive for phytoplasma. This indicates there may be a resurgence of X-Disease in this area.

A few other production-limiting viral diseases were found in this survey including: the rugose strain of Prunus necrotic ringspot virus and an orchard in the Willamette Valley with Cherry rusty mottle disease.

For most growers and field representatives, the world of cherry viruses is a confusing bowl of alphabet soup. It can be confusing for plant pathologists, as well. To help simplify this world we came up with the grower action rating, a scale of 1 to 10, to indicate which viruses should get more attention than others. This helped reduce the possibility of dozens of viruses down to an important 7 that growers should be worried about. In addition, finding any of two different symptoms – enations or little cherries – should immediately be cause for concern. We think this will help growers take appropriate actions to limit the damage and spread of these diseases.