FINAL PROJECT REPORT

Project Title: Reducing the impact of virus diseases on quality cherry production.

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Numerous growers

Other funding sources

Agency Name: USDA-APHIS
Amount awarded: $42,300 as part of a much larger grant to support the efforts of the National Clean Plant Network at WSU-Prosser. This portion of the grant assisted in the maintenance of the orchard plantings.
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: $133,560

Budget History:

Item	2010	2011	2012
Salaries	22,537	22,150	23,460
Benefits	8,198	9,711	9,853
Wages	0	0	0
Benefits	0	0	0
Equipment	0	0	0
Supplies	12,000	12,661	12,990
Travel	0	0	0
Plot Fees	0	0	0
Miscellaneous	0	0	0
Total	$42,735	$44,522	$46,303

OBJECTIVES:

The **overall project objective** is to identify viruses that cause low quality and quantity fruit production, and to develop an understanding of virus biology that will ultimately lead to the **development of effective management strategies for growers**.

Goal 1: Determine the ability of rootstock and inter-stock selections to limit the spread of cherry leaf roll virus and related viruses.

Goal 2: Determine the means of long distance transmission for cherry leaf roll virus.

Goal 3: Document the responses of new cherry cultivars to viruses.

SIGNIFICANT FINDINGS:

- Cherry leaf roll virus has appeared in several additional cherry production regions of the state.
- The selection of rootstock and scion significantly alters the expression of disease symptoms in response to cherry leaf roll virus.
    - Rootstocks could become the cornerstone of a long range management program to limit the transmission of cherry leaf roll virus under certain orchard settings and in the nursery industry
- A new native plant species has been identified as a host for cherry rasp leaf virus and may explain the intense virus pressure in certain areas of the state. This realization could impact site selection and preparation for new cherry or apple plantings.
- The viruses that cause two cherry diseases present in Washington State and the west coast have been identified. This permitted development of diagnostic tests for the accurate identification of pathogens associated with disease in cherry orchards.
    - Availability of diagnostic tests is fundamental to future research on disease epidemiology in the region including the identification of vectors and alternate hosts of the disease agents.

RESULTS AND DISCUSSION:

Cherry leaf roll virus:
Infection of cherry trees in Washington State by cherry leaf roll virus was first identified in 1998. Although cherry leaf roll virus was previously reported in golden elderberry in Washington, this was the first reported instance of cherry leaf roll virus in cherry. The incidence of the cherry leaf roll virus varies dramatically across different regions of the state. There are two areas of extremely high virus pressure: one in the lower Columbia Valley and one in the Yakima Valley. Outside of these distinct areas, the disease pressure is significantly lower but not absent. The virus is now found with increasing frequency throughout all cherry production areas. The most appropriate grower response to infection will vary depending upon in which location the infection is located.

Our analysis of the virus coat protein sequence, and eventually of the entire virus genome, revealed that the cherry isolate is genetically different from the golden elderberry isolate, suggesting a biological barrier has prevented the virus moving between these hosts. Further analysis revealed that native blue elderberry, ornamental black elderberry and rhubarb plants in Washington also harbor cherry leaf roll virus, but again these are genetically distinct isolates of the virus. Thus, the virus isolates that infect cherry appear to be isolated from the virus isolates infecting other host plants. The source of new infections in isolated cherry blocks remains unclear. It has been observed that a single, new infection can occur in an established orchard miles distance from the nearest known source of cherry leaf roll virus. This strongly suggests that an aerial transmission route exists. Based on similarity to the walnut isolate of cherry leaf roll virus where pollen transmission has been

demonstrated, controlled pollination of 10,000 cherry blossoms with virus-infected pollen was performed. This process resulted in the development of cherry leaf roll virus-infected seed, embryos, seedlings and fruit stems. However, this failed to result in transmission of virus to a single tree exposed to infected pollen. This suggests that if this is a route for transmission, it is very inefficient. Our data has demonstrated that the major means by which the virus spreads within an orchard is through root grafting. The efficiency of this process is determined in part by root zone architecture. Taken together, these observations suggest that if transmission by root-grafting can be eliminated, the impact and spread in a single orchard after a new infection could be substantially reduced. This is supported by experience with several growers. When the first infection in an orchard is detected, and the infected tree promptly removed using techniques recommended by this program, cherry leaf roll virus is easily contained.

Rootstock selection was examined as a parameter in determining the impact of cherry leaf roll virus on cherry production. The appropriate rootstock selection, independent of other horticultural properties of the rootstock/scion combination, is informed by the pressure of cherry leaf roll virus in the vicinity. In one of the concentrated areas of cherry leaf roll infection, choosing a tolerant rootstock/scion combination may permit economic success. This is predicated on the assumption that reduced yield and fruit quality caused by infection can be offset but superior performance of the trees. In an area of low virus pressure, a rootstock/scion combination that declines quickly after infection would be an effective means of quickly identifying new infections and preventing secondary spread. To evaluate these possibilities, nine blocks of trees were planted in 2009. Each block consisted of ten virus-free 'Bing' budded onto a rootstock. In August, 2010, the trees were inoculated by T-budding with cherry leaf roll virus infected buds. In four trees of each block, the bud was placed below the graft union to simulate infection through the root system. In another four trees of each block, the virus-infected bud was grafted above the graft union to mimic infection entering the tree through an aerial route. Two trees of each block were reserved as un-inoculated control trees. Exceptions were with 'Mazzard' rootstock where only three trees were inoculated instead of four, and with 'Gisela 12' where the set size was doubled.

Within one growing season, there was evidence of tree stress and reduced growth of trees on some rootstocks. The greatest reductions in growth were observed where the 'Bing' scion was inoculated and growing on 'Colt', 'Gisela 6' or Citation rootstock with Zee-stem interstock. In the beginning of the second season of observations, all of the trees (4 out of 4) growing on 'Colt' rootstock where the scion was inoculated had died but none of the trees in which the 'Colt' rootstock was inoculated were exhibiting any signs of stress or significant reductions in tree growth (Table 1). This confirms that 'Colt' responds to cherry leaf roll virus with a hypersensitive reaction, thus causing a layer of dead cells to form between the infected 'Bing' and the rootstock, resulting in rapid death and decline of the scion. In the case of the rootstock inoculated 'Colt', the same hypersensitive reaction slows or prevents the movement of the virus from the bud into the rootstock and thus protects the tree from infection. This is the desirable response that would enable a virus management program to be developed based on this rootstock since infections are quickly eliminated in the orchard and secondary spread through root grafting is prevented. In many sites in Washington State, trees growing on 'Colt' rootstock are not very precocious. We attempted to circumvent this by using the Zee-stem interstock on 'Colt' rootstock. This hybrid interstem has been used to increase precocity and decrease tree size on other rootstocks. However, the graft union between the 'Colt' and Zee-stem were very brittle and may not be workable in commercial production.

The only other rootstock to exhibit a rapid response to cherry leaf roll virus infection was 'Gisela 6' rootstock. All of the infected scions collapsed by the end of the second growing season after inoculation. However, unlike 'Colt', trees growing on 'Gisela 6' rootstock where the rootstock was inoculated also collapsed. Thus, 'Gisela 6' does not provide direct protection against root graft

transmission of cherry leaf roll virus, but would still assist in virus control by quickly eliminating sources of inoculum.

Trees growing on the Zee-stem interstock/Citation rootstock combination exhibit severe sign of stress (premature leaf reddening in late summer) and reduced growth as measured by trunk cross sectional area (Table1). Thus, growing trees on this combination may be used as sentinel plants to indicate the movement of cherry leaf roll virus into an orchard, but would not offer any direct control.

The results of this rootstock trial point the way for potential management strategies that could be implemented during orchard planning and redevelopment. The data also highlight the impact of cherry leaf roll virus on tree growth. For example, on 'Mazzard' rootstock, the trunk cross sectional area of inoculated trees after two years is approximately one-half relative to un-inoculate controls. All of these experiments were conducted under conditions where cherry leaf roll virus is the only virus in the research trees. Observation over the past 12 years has made it very clear that cherry leaf roll virus in combination with other common viruses of cherry such as *Prunus* necrotic ringspot virus and prune dwarf virus results in much more severe symptom development and tree decline than with cherry leaf roll virus alone. Therefore, the modest reductions observed in the tree growth in this study would be expected to be much greater in the practice where mixed infections are common place.

A small potted tree experiment indicates that scion also plays a role in disease severity. As previously reported, 'Lapins' was the most tolerant scion when tree growth is considered. The growth of 'Tieton' and 'Skeena' were most severely affected by cherry leaf roll virus. A longer term and larger research trial will be necessary to evaluate the effect of cherry leaf roll virus on the quality and quantity of fruit.

Table 1. The trunk cross sectional area of trees were measured and averaged two years after inoculation with cherry leaf roll virus. The scion in each case is 'Bing'.

Rootstock	**Average trunk cross sectional area –in.^{2} (number of surviving trees)**		
	Non-inoculated	Scion inoculated	Rootstock inoculated
Mazzard	5.68 (n=2)	2.17 (n=2)	3.04 (n=3)
Gisela 5	1.90 (n=2)	2.10 (n=4)	2.40 (n=4)
Gisela 6	3.89 (n=2)	0[a]	2.10 (n=3)
Gisela 12	5.55 (n=3)	3.37 (n=6)	5.91 (n=4)
Z-stem/Citation	6.92 (n=2)	3.50 (n=4)	5.32 (n=4)
Colt	7.36 (n=2)	0[a]	5.91 (n=4)
Krymsk 5	7.08 (n=2)	5.05 (n=4)	6.14 (n=4)
Krymsk 6	2.59 (n=2)	3.54 (n=4)	3.44 (n=4)
a. The four trees in each of these treatments died during the second growing season.			

Cherry rasp leaf virus:
Cherry rasp leaf virus and the diseases caused by it have been reported in several cherry production regions west of the continental divide. The first reports in Washington State arose during the virus disease surveys of 1942. At that time, cherry rasp leaf disease was reported to be extensive in

orchards of the Yakima Valley. Through years of orchard replacement, the virus has become very uncommon in the Yakima Valley and is now found primarily in Chelan County where it persists in localized areas. The explanation for this transition has been unclear. In 2012, investigation of areas surrounding a cherry rasp leaf virus-infected cherry orchard led to the observation of elderberry shrubs with deformed leaves. Virus profile testing revealed that these shrubs are infected with cherry rasp leaf virus. This virus is nematode transmitted so the presence of the virus in the wild native vegetation would serve as a reservoir of the virus from which it can enter the orchard. Most viruses related to cherry rasp leaf virus are also seed transmitted. This remains to be confirmed in the case of cherry rasp leaf virus-infected elderberry, but the wide dispersal of infected seed in the area by birds may account for the high incidence of the virus in this area. Wild blue elderberry is common in the canyon bottoms of the Wenatchee cherry growing area. This represents the first report of this shrub as a host of cherry rasp leaf virus. The potential ingress of cherry rasp leaf virus from shrub land into cherry orchards should be considered during the selection of the orchard site and the preparation of the orchard and adjacent land for planting.

Cherry rusty mottle disease group of viruses:
Cherry rusty mottle is a graft-transmissible disease of sweet cherries first described in the early 1940s in Washington State. Because of the graft transmissible nature of the disease, a viral nature of the disease has long been assumed but not demonstrated. The 3'-terminus regions of virus-like RNA were amplified from trees affected with cherry twisted leaf (CTL), apricot ring pox (ARP), cherry necrotic rusty mottle (CNRM), cherry rusty mottle (CRM) and cherry green ring mottle (CGRM) diseases. Phylogenetic analysis of virus-like sequences from a total of 24 trees representing these diseases along with published sequences of Cherry green ring mottle virus (CGRMV) and Cherry necrotic rusty mottle virus (CNRMV) from other geographic regions revealed segregation into four major populations, each corresponding to one of the diseases (Figure 1). Virus sequences within each of these clades were designated as clade I: Cherry twisted leaf associated virus (CTLaV), clade II: CNRMV, clade III: Cherry rusty mottle associated virus (CRMaV), and clade IV: CGRMV. Segregation into these clades correlated with symptom expression on *Prunus avium* cultivars 'Bing' and 'Sam', and *Prunus serrulata* 'Kwanzan'. Examination of frequency distribution data derived from pairwise sequence comparisons and symptomatology suggests that CTLaV, CNRMV, CRMaV and CGRMV are separate virus species. This is the first report of specific association of virus and virus sequences with cherry rusty mottle and cherry twisted leaf diseases.

Primers were designed to permit detection of each of the proposed virus species individually. This has allowed accurate assessment of the virus profile of viruses in orchard trees. Data from the study revealed that mixed infections, that is multiple viruses present in a single tree, are very common (Table 2), and that the interaction of the viruses within a single tree can modify disease symptoms, thereby making a diagnosis based on visual symptoms alone difficult.

Table 2. Symptomatic source trees used in the study of the family *Betaflexiviridae*.. The viruses listed are in addition to the virus-like sequences used in sequence analysis in Figure 1.

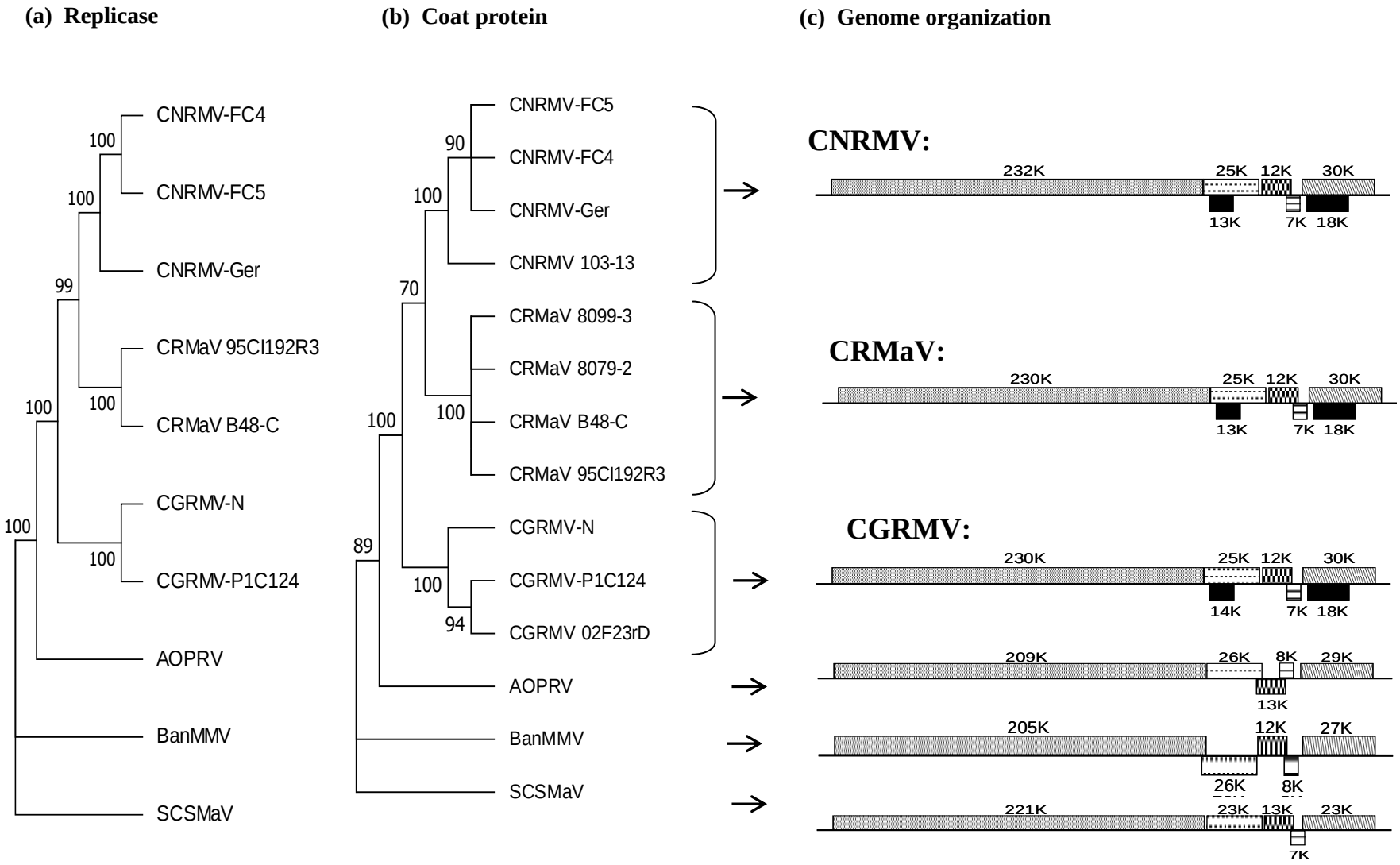
Source tree	Original collection site	Maintenance host	Other viruses detected[1,2]
Source trees maintained at the Clean Plant Center – Northwest, Washington State University			
B48-C	Benton Co., WA	*P. avium* 'Bing'	PDV
101-13	N/A[3]	*P. avium* 'Bing'	CMLV, CVA, PDV
103-13	N/A	*P. avium* 'Sam'	PDV
103-15	N/A	*P. avium* 'Bing'	ACLSV, CLMV
02F23rD	N/A	*P. avium* 'Bing'	PDV
04E17R1	The Dalles, OR	*P. avium* 'Bing'	ACLSV, PDV, PNRSV
04E36	Stockton, CA	*P. avium* 'Bing'	CVA, PDV, PNRSV
95CI189R2	Wenatchee, WA	*P. avium* 'Sam'	CVA, PDV, PNRSV
95CI190	Wenatchee, WA	*P. avium* 'Sam'	ACLSV, CVA, PDV
95CI191R3	Wenatchee, WA	*P. avium* 'Sam'	ACLSV, CVA, PDV, PNRSV
95CI192R3	Wenatchee, WA	*P. avium* 'Bing'	CVA, PDV, PNRSV
95CI205R1	Wenatchee, WA	*P. armeniaca*	None detected
98CI73R1	Moxee, WA	*P. mahaleb*	PDV
98CI194	Mattawa, WA	*P. avium* 'Bing'	CVA
99CI01	Granger, WA	*P. avium* 'Bing'	CVA, PDV, PNRSV
Symptomatic source trees from commercial orchards			
8079-1	Malaga, WA	*P. avium* 'Bing'	ACLSV, CLRV, CVA, PDV, PNRSV
8099-5	Prosser, WA	*P. avium* 'Bing'	CVA, PDV
8241-2	Wenatchee, WA	*P. avium* 'Bing'	CMLV, CVA PDV
8241-4	Wenatchee, WA	*P. avium* 'Bing'	ACLSV, CVA, PDV, PNRSV
8242-3	Wenatchee, WA	*P. avium* 'Bing'	CVA, LChV-2, PDV, PNRSV
8244-4	Malaga, WA	*P. avium* 'Bing'	CVA, PDV
8265	Granger, WA	*P. avium* 'Bing'	CMLV, PDV, PNRSV

1. ACLSV=Apple chlorotic leafspot trichovirus; CLRV=Cherry leaf roll nepovirus; CMLV=Cherry mottle leaf trichovirus; CVA=Cherry virus A capillovirus; LChV-2=Little cherry virus 2 ampelovirus; PDV= Prune dwarf ilarvirus; PNRSV=Prunus necrotic ringspot ilarvirus.

2. American plum line pattern ilarvirus; Cherry raspleaf cheravirus; Little cherry virus 1 unclassified Closteroviridae; Hop stunt pospiviroid; Peach latent mosaic avsunviroid, Xylella fastidiosa and phytoplasmas were not detected in these samples.

3. N/A indicates that information on the place of origin of samples is not available.

Figure 1. Sequence analysis of virus isolates from the rusty mottle group of diseases that affect cherry and their comparison to other members of the family *Betaflexiviridae.*



Project Title: Reducing the impact of virus diseases on quality cherry production.

EXECUTIVE SUMMARY:

Cherry leaf roll virus:
Disease caused by cherry leaf roll virus is appearing in additional cherry production regions of Washington State. The emphasis on premium cherry production increases the risk of cherry leaf roll virus-infected orchards being marginalized because the fruit does not meet current industry standards. Therefore, a management plan needs to be considered in planning new cherry plantings. The selection of scion and rootstock significantly alter disease expression. Moreover, the appropriate selection of rootstock could become the foundation of a cherry leaf roll virus disease management program. Some rootstocks such as 'Colt' and 'Gisela 6' respond very aggressively to infection by cherry leaf roll virus. This rapid response quickly eliminates the potential for secondary spread in the orchard. Alternatively, growth of trees on 'Gisela 5' rootstock does not seem to be impacted by infection. A longer term experiment will be needed to assess the impact on fruit production and in the presence of other viruses.

Cherry rasp leaf virus:
There has been a shift in the epidemiology of disease caused by cherry rasp leaf virus over the past 50 years. Our research revealed a new native plant species (blue elderberry) as a host for cherry rasp leaf virus. This may explain the intense virus pressure in certain areas of the state where elderberry grows in abundance. The potential for seed transmission increases this concern. Awareness of this newly reported host for the cherry rasp leaf virus could impact site selection and preparation for new cherry or apple plantings.

Cherry rusty mottle group of diseases:
The viruses that cause three diseases presence in Washington State and the west coast have been identified. Prior to this time the diseases were presumed to be caused by "virus-like agents" based on graft transmissibility. Our data led to development of virus-specific diagnostic tests for the accurate identification of pathogens associated with diseases in cherry orchards. Our study has shown that the presence of multiple viruses in a single tree can make disease diagnosis based on visual symptoms alone difficult and inaccurate. The virus-specific assays thus provide an accurate profile of the viruses present. This is important in knowing what cultural practices can and should be modified to minimize further spread of the disease. For future research, the availability of diagnostic tests is fundamental to studies of disease epidemiology in the region including the identification of vectors and alternate hosts of the disease agents.