FINAL PROJECT REPORT

Project Title: Managing virus diseases detrimental to cherry production

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Other funding sources

Agency Name: California Cherry Advisory Board
Amount awarded: $5,000 in 2008 and $10,000 in 2009

Agency Name: ANLA/HRI
Amount awarded: $132,000 (project ended Sept 30, 2008)
Notes: Objectives of the ANLA/HRI project partially overlapped with the characterization of the rusty mottle group of cherry viruses.

Total Project Funding:$100,680 from the WTFRC plus $15,000 from the CCAB

Budget History:

Item	**Year 1:** 2007	**Year 2:** 2008	**Year 3:** 2009
Salaries	$ 5,618	$10,722	$13,498
Wages	$ 3,275	$ 1,776	$ 4,706
Benefits	$ 2,212	$ 4,076	$ 4,576
Equipment	$ 0	$ 0	$ 0
Supplies	$31,635	$ 5,261	$ 9,016
Travel	$ 0	$ 40	$ 0
Miscellaneous			
Total Expended	$42,740	$21,875	$31,796[1]
Total WTFRC Funded	$36,938	$33,823	**$29,919**

1. Expenditures as of October 9, 2009. Remaining project expenses for salaries and benefits will be derived from the balance of WFTRC and CCAB funds.

OBJECTIVES:

Viruses cause lost production over the life of an infected tree; recurring annual losses are cumulative and have a significant negative impact on the overall economic viability of farm operations. Despite past progress, some viruses that affect cherry production continue to challenge efforts to minimize their negative impact on profitability. Those that continue to be problematic from an orchard management perspective include the viruses associated with little cherry disease, cherry leafroll, cherry raspleaf and the rusty mottle group of diseases. Specific objectives of this project include:

1. To develop alternative methods of managing virus diseases with particular reference to those where root-grafting and/or nematode transmission play significant roles in disease epidemiology.
2. To develop laboratory tests that increase grower accessibility to rapid virus diagnosis. The ability to correctly identify the underlying cause of poor fruit production is required for appropriate corrective measures to be implemented.

SIGNIFICANT FINDINGS:

- Root grafting is the major means by which cherry leafroll virus spreads within an orchard.
- Rootstock selection offers the potential of minimizing transmission of viruses by root grafting.
- Pollen transmission of cherry leafroll virus is suspected, but if it does occur, it is very inefficient.
- Viruses of the rusty mottle group can be dispersed through propagation and planting of symptomless carriers. Symptom expression is cultivar dependent and infected trees can be juxtaposed with other sweet cherry cultivars that express severe symptoms with resulting crop loss.
- Isolates of Little cherry virus 1 are genetically diverse; conserved regions of the genome were identified that permitted development of a reliable molecular assay.
- Little cherry virus 1 is present in western cherry producing regions.
- Broad spectrum molecular assays assist in detecting deleterious viruses where they are the cause of poor production in cherry orchards.

RESULTS AND DISCUSSION:

Objective 1: Development of alternative methods of managing viruses.

Cherry leafroll virus (CLRV) poses a serious risk to sweet cherry production in the Pacific Northwest. Our program performed many assays by request that demonstrated that the geographic distribution of known CLRV infections has expanded to new production regions of Washington State. As an aid to determining factors that affect the spread of CLRV, six plots in commercial blocks in the Yakima Valley were tested annually for CLRV over a period of ten years. Two distinct patterns of virus spread were observed. Dispersion from an infected tree to trees immediately adjacent to the original infection site was relatively fast. This rate of virus movement appears to be dependent on orchard architecture and is particularly rapid in sprinkler irrigated and older orchards. There was also a much less frequent transmission to new sites areas previously free of disease. These new foci of infection occurred within or outside of the original cherry block. The existence of these two scenarios points to the existence of two modes by which CLRV is transmitted from one tree to another.

Our research on the epidemiology of CLRV demonstrated that transmission through root grafts is an important route for tree-to-tree spread within an orchard. Consequently, genetically diverse rootstocks are being evaluated for their ability to provide field resistance to CLRV that could minimize or even eliminate this significant route of infection. A small on-farm trial was established in 2000 to test the influence of rootstock on this mechanism of virus transmission and was completed in 2008. Twenty trees of 'Bing' on 'Colt' (*P. avium* × *P. pseudocerasus*) rootstock and 20 trees of 'Bing' on 'Mazzard' (*P. avium*) rootstock were planted in plots in three separate orchards. Each year they were monitored for CLRV. It was suggested that pollen-borne virus may play a role in CLRV transmission, so any flower buds that developed during the first four years of this study were removed from the subject trees. During the first 7 years of testing, five of the 20 trees on 'Mazzard' rootstock

became infected, some within the second growing season. None of the trees on 'Colt' rootstock became infected with CLRV. During this same period, many replacement trees planted by growers on 'Mazzard' rootstock at these locations also become infected. This limited trial suggested that 'Colt' offers protection from the root grafting transmission of CLRV. In parallel studies conducted by others, 'Colt' rootstock has been shown to be effective in controlling the spread of *Tomato ringspot virus* (ToRSV), a nematode transmitted virus, in cherry trees. 'Colt' reacts to ToRSV with a hypersensitive reaction and thus prevented movement of virus away from the site of nematode inoculation. Both CLRV and ToRSV are members of the same genus of viruses, but there is no evidence that a nematode vector of CLRV exists in North America. ToRSV does, however, provide an example where rootstock selection can be used effectively to control virus transmission.

In the final year of this on-farm trial, one of the trees on 'Colt' became infected with CLRV. The tree declined rapidly and by mid-summer, the canopy was collapsing. Further examination revealed the development of necrotic tissue at the graft union. CLRV was detected by serological (ELISA) (Table 1) and molecular (RT-PCR) (data not shown) methods in two of the four leaders. None of the suckers emerging from the rootstock below the graft union contained detectable CLRV. This distribution of virus suggests that infection of the young tree had occurred through an aerial route. The appearance of necrotic tissue (dark discoloration) at the graft union suggests that 'Colt' rootstock responded to CLRV infection by development of a hypersensitive reaction leading to death of plant tissue adjacent to the infected 'Bing' scion. Development of necrotic tissue at the graft union restricts movement of nutrients and water to the scion leading to decline of the scion. This same relationship was subsequently observed in a mature orchard planted on 'Colt' rootstock where natural infection by CLRV had occurred. A zone of necrotic tissue developed at the graft union and the scions quickly declined. This dramatic response of 'Colt' rootstock leads to loss of the infected tree, and mimics the pattern of CLRV infection of walnut trees where pollen transmission of the virus leads to "black line" disease and death of walnut trees planted on northern California black walnut or Paradox rootstock. However, from a disease management perspective, the rapid decline of sweet cherry scions on 'Colt' rootstock quickly eliminates sources of virus-laden pollen from the orchard that would otherwise sustain the continued spread of the disease. Since CLRV has a distinct negative impact on fruit production and quality, the rapid decline and removal of an infected tree significantly minimizes the long term economic impact of CLRV infection in the orchard.

Table 1. Portions of a tree ('Bing' scion on 'Colt' rootstock) were tested by ELISA to determine the distribution of cherry leafroll virus after natural infection.

Tree position	ELISA Absorbance values (interpretation)	
South-east leaders	0.319 (+)	0.315 (+)
North-west leaders	0.002 (-)	0.053 (-)
Lower branch from main trunk	0.319 (+)	0.367 (+)
Suckers from 'Colt' rootstock	0.001 (-)	0.001 (-)

The above results indicate that 'Colt' rootstock offers important disease management options to mitigate the spread of CLRV: the rootstock prevents systemic movement of the virus from root grafts, and quickly eliminates sources of virus-infected pollen that would support secondary spread of infection to other trees. While 'Colt' rootstock confers good horticultural properties in sandy and/or rocky soils, trees on 'Colt' rootstock produce excessive vegetative growth and lack precocity when planted in rich deep soils (Perry *et al*., 1997). Therefore, other rootstocks and rootstock/interstock combinations are being evaluated for their potential to offer similar protection from the ingress of CLRV, but offering greater desirable horticultural characteristics in a wider array of settings. To investigate the potential of rootstocks to provide field resistance to soil-borne viruses, 132 trees on

rootstocks and rootstock/interstock combinations were propagated and planted. Rootstocks include 'Colt', 'Krymsk 5', 'Krymsk 7', 'Gisela 5', 'Gisela 6', and 'Gisela 12'. Zee-stem interstocks are reported to offer size control and precocity to cherry trees, so 'Zee-stem' interstocks on 'Citation 'and 'Myrobalan 29C' rootstock were included in this study. All rootstocks and rootstock/interstock combinations were grafted with a virus-free clone of 'Bing'. Finished trees of 'Zee-stem' interstocks on 'Colt' rootstock were also prepared, but the graft union of this combination was particularly fragile and impractical for further consideration during this trial. Finished trees were established in the orchard and graft-inoculated in June 2009 with 'Bing' infected with CLRV. The source of CLRV inoculum was tested by ELISA to ensure freedom from other common viruses. The inoculating chips were grafted onto trees of each combination either directly onto the rootstock or onto the scion. At the end of the first growing season after inoculation, the most overtly visible reaction to CLRV was observed where infected buds were placed directly on 'Krymsk 5' (*Prunus fruticosa* × *P. lannesiana*) rootstock. A severe hypersensitive reaction characterized by prolific gumming around the inoculation site is evident. This rootstock is also known to be sensitive to the ilarviruses *Prunus necrotic ringspot virus* (PNRSV) and *Prune dwarf virus* (PDV). 'Krymsk 7' (*Prunus lannesiana*) has not produced any reaction to direct budding of CLRV onto the rootstock, nor is this rootstock known to be sensitive to PNRSV and PDV. 'Krymsk 6' (*Prunus cerasus (Lyubskaya)* × *Cerapadus Michyunin* (*P. cerasus* × *P. maackii*)) is sensitive to infection by the ilarviruses but has a different genetic background so the response to CLRV cannot be predicted; 'Krymsk 6' was not included in this first trial. Of the Gisela series used in this study, initial observations suggest that 'Gisela 12' (*Prunus canescens* × *Prunus cerasus*) is responding adversely to inoculation by CLRV. There is no evidence of a hypersensitive reaction at the site where the infected chip is grafted directly to the rootstock. However, when the scion is inoculated, there is a proliferation of suckers from the rootstock which is suggestive of an adverse reaction at the graft union. 'Gisela 12' rootstock is not sensitive to the two ilarviruses PNRSV or PDV. Final interpretation of these grafting experiments cannot be made until trees are sacrificed and the graft union examined for the appearance of abnormalities; this will occur after subsequent growing seasons. The preliminary observation of a hypersensitive reaction from 'Colt', 'Krymsk 5' and potentially 'Gisela 12' suggests that there are multiple sources of genetic resistance to CLRV.

Serological tests (ELISA) by our program confirmed that pollen derived from cherry trees infected with CLRV carries a large amount of virus particles, and mechanical inoculations confirmed that the virus associated with the pollen is infectious. In an effort to obtain a measure of the risk of pollen transmission, clusters of flower buds were surrounded by organza cages before the blossoms opened. The organza cages prevent the introduction of pollen from other sources and limit movement of Western flower thrips and other insects. In control cages where no pollen was introduced, there was no fruit set. This demonstrated that the organza cages successfully exclude sources of compatible pollen. In May 2006, 800 blossoms of a virus-free tree were pollinated with CLRV-infected pollen. As previously reported, at the time of shuck fall, 50% of the pedicels tested contained CLRV detectable by RT-PCR. In this and all subsequent pollen trials, the branches and spurs exposed to virus-laden pollen are tagged with tree marking paint to ensure they are not removed during routine orchard pruning operations. In spring 2007, leaves adjacent to each of the spurs that had been pollinated with infected pollen were collected and tested for CLRV by RT-PCR. No samples yielded positive results. In 2008, adjacent leaves were again tested by ELISA and all were negative.

In 2007, pollination experiments were repeated on a different set of trees and 800 flowers of 'Van' were hand pollinated with 'Bing' pollen collected from CLRV-infected trees. In June of 2007, fruit was harvested from each cage and the pedicels extracted and tested by RT-PCR. Overall, 20% of the pedicels from cages into which CLRV-infected pollen was introduced yielded positive results by RT-PCR. In contrast to the 2006 experiment, most fruit was carried to maturity. When leaves adjacent to spurs of fruit formed in 2007 were sampled in spring 2008, no samples yielded positive results. This was consistent with results from the pollination experiments begun the previous season. Thus, in

each of two years, although CLRV is present in the pedicels of fruit after blossoms are pollinated with virus-infected pollen, the virus has not replicated to detectable levels in the adjacent vegetative tissue.

In 2009, caged blossoms of 'Van' trees were pollinated with pollen from CLRV-infected 'Bing' pollen; a total of 8,679 blossoms were exposed to the virus infected pollen. Trees from all experiments will continue to be monitored for the presence of CLRV.

To validate methods used for surveys and pollen transmission experiments, it is important to estimate the rate at which the virus is able to replicate to detectable levels and move through susceptible cultivars. Previous observations of naturally infected trees suggested that based on visual assessment of symptoms and supported by ELISA data, it may take 3 to 4 years for CLRV to become fully detectable throughout the tree. To verify, each of two major scaffold limbs of four trees were T-grafted with a single bark patch from an infected 'Bing' tree in August 2006. In spring 2007, CLRV could be detected only in the shoots immediately adjacent to the buds. In spring 2008, leaves from the base of each scaffold limb were assayed by ELISA for CLRV (Table 2). After one full growing season, CLRV had not moved and replicated to a level detectable by ELISA in all parts of the tree.

Table 2. One-year old shoots of two major scaffold limbs of each tree were inoculated with cherry leafroll virus-infected bark patches in the autumn of 2006. In spring 2008, leaves at the base of each scaffold limb were assayed by ELISA for cherry leafroll virus.

Tree designation	# scaffold limbs positive / # scaffold limbs tested
R-6	1/4
S-6	4/5
T-6	2/5
U-6	5/5
Total scaffold limbs with detectable virus	12/19

The amount of inoculum introduced by bark patch inoculations is much greater than that potentially introduced through pollination. This suggests that additional periods of observation are warranted to determine if CLRV had been successfully transmitted from infected pollen during the experiments initiated in 2006 thru 2009. In studies of CLRV pollen transmission in Europe, trees were monitored for four years after pollination in order to assess the rate of pollen transmission in other perennial species.

Impact and economic benefits: CLRV, like so many other viruses that infect sweet cherry, reduce the size and quality of fruit, and hence their marketability. This program identified this virus in the western US and alerted the industry to its presence so that measures could be implemented to reduce further encroachment by the virus and the diseases that it causes. Greater grower awareness has resulted in the identification and elimination of many infected and unproductive trees, thus reducing sources of inoculum that would foster further spread. Knowledge gained from our project has impacted industry-wide operations in an effort to control this virus. The nursery certification program implemented more stringent standards to reduce the entry of the virus into plantings through Washington State Certified cherry trees. One county has introduced and sustained control measures. Additionally, awareness of the potential aerial transmission of this virus led some pollen companies to engage the WSU ELISA Testing Service Center in a program to ensure that their products are free of CLRV. Root grafting appears to be the major route by which CLRV spreads to adjacent trees. It has been demonstrated that minimizing transmission via this process through rootstock selection dramatically slows the spread of the virus within infested orchards. Further studies are required to identify horticulturally beneficial rootstocks that bestow this same ability to reduce virus transmission via root grafting.

Objective 2. Development of laboratory tests to increase accessibility to rapid virus diagnosis.

Many of the viruses that significantly diminish fruit size and quality are members of the genus *Foveavirus*; these viruses are frequently referred to as the rusty mottle group of viruses and are encountered in many cherry production areas. Several are likely native to wild *Prunus* species of western North America. At least some of these diseases spread naturally via an aerial transmission route, but vectors of the diseases are not known. Green ring mottle virus and cherry necrotic rusty mottle virus are the best characterized members of this group. Green ring mottle virus does not induce recognizable symptoms on sweet cherry, but does on sour cherry and ornamental flowering cherry trees whereas the remaining viruses of the rusty mottle group have been defined by the range of symptoms that are induced on select sweet cherry cultivars. Diseases caused by putative members of this group include cherry rusty mottle, cherry necrotic rusty mottle, cherry twisted leaf, cherry stem pitting and Montmorency stem pitting. The diversity of viruses and the varied responses of different cultivars renders diagnosis based on visual observations very difficult. Moreover, symptoms of infection often resemble those of adverse physiological conditions, chemical injury, bacterial or fungal infections. The ability to confirm the presence or absence of foveaviruses and their identity would greatly aid growers in properly ascertaining underlying causes of poor tree productivity and associated symptoms before initiating a response to declining trees. During the course of this project, techniques developed in our laboratory were used to obtain sequence information from approximately 13% of the genome from each of 26 foveavirus isolates associated with distinct disease symptoms in cherry, plus seven isolates of green ring mottle virus that are symptomless in sweet cherry. This process confirmed the ability of "universal" foveavirus primers to reliably screen for members of this virus genus, and further analyses of the data provided a strong footing on which further studies are based. Further refinement is necessary to allow the assay system to distinguish between green ring mottle virus, which is symptomless in sweet cherry, and other foveaviruses that cause disease. Expanding the database of sequences representing regional isolates improves the ability to develop assays specific for pathogenic virus strains. In an effort to increase accessibility and affordability of diagnosis to growers, a serological assay was also sought. We characterized the genes that encode the structural proteins of the foveaviruses found in cherry and applied this information to develop polyclonal antibodies for use in ELISA. The resulting serological assay has the desirable characteristic of not detecting green ring mottle virus, while still detecting a large number of pathogenic viruses of the rusty mottle group. Approximately 50% of the pathogenic viruses in this group are recognized by the assay so further enhancement is needed. Success with the antibodies produced in this manner provides great optimism for the potential to provide future refinement in robust serological assays for the foveaviruses of cherry. Additionally, trials are underway to adapt ELISA procedural parameters so additional strains of the viruses are detected by these new antibodies.

Another molecular assay that detects foveaviruses (Foissac *et al*., 2005) has received increasing acceptance internationally. This is a polymerase chain reaction-based system that uses "TriFoCap" primers, and is capable of detecting members of multiple virus genera including trichoviruses, foveaviruses and capilloviruses. Thus, this single test would detect a wide range of viruses of concern to the fruit tree industry. Fortunately, there has been excellent agreement between the new assay formats ("TriFoCap" and "universal" foveavirus molecular assays) and the traditional greenhouse indexing. Unfortunately, the broader scope of viruses recognized by the "TriFoCap" primers can complicate real world interpretation. Our "TriFoCap" assay detected two previously unreported viruses in *Prunus* spp. samples. Although samples with these viruses were positive by woody indexing in the greenhouse, they were negative by the "universal" foveavirus assay. Sequence analysis indicated that the two new viruses are closely related to, but distinct from known foveaviruses and appear to be closely related to viruses that infect citrus and remain unclassified members of the *Flexiviridae* family. These observations highlight the advantages of non-specific

molecular assays for virus detection with the necessity of using tests with narrow specificities to define the pathogenic agent for the particular orchard disease of concern to a grower. Efforts are underway to refine the "TriFoCap" assay such that more information can be obtained about the nature of the viruses contained in the sample under analysis. This would add the desired specificity to the broad spectrum "TriFoCap" assay.

Detection methods for viruses associated with little cherry disease were also addressed. This disease is now known to be associated with two related viruses that are distinct and belong to different genera within the family *Closteroviridae*. Serological assays for Little cherry virus 2 (LChV2) are not routinely available, but molecular assays for LChV2 were developed many years ago (Eastwell & Bernardy, 2001). The development of reliable detection methods for Little cherry virus 1 (LChV1) have been evasive. Through this project, we revealed the diverse nature of the genomes of North American isolates of LChV1 relative to Eurasian isolates (Figure 2). With this knowledge, the first reliable molecular assays were developed (Bajet *et al*., 2008). Again, serological assays remain a very desirable objective for future development. The critical need for reliable detection of LChV1 has become much more evident in recent years. Coordinated efforts between this program and similar programs in Canada and Germany have confirmed that current biological methods for detection are unreliable. Furthermore, it has become evident that the host range of LChV1extends beyond sweet cherry. Several other *Prunus* spp. (peach, almond and plum) are now known to be symptomless carriers of the virus (Matic *et al.*, 2009). The latency of the virus in several fruit tree hosts and the difficulty of detection combine to create the potential for this virus to continue to make inroads into major cherry production regions. The mechanism(s) by which LChV1 is transmitted in the field, other than through the use of infected propagation material, remains unknown.

Orchards with poor fruit production were inspected for signs of little cherry disease (small, light colored fruit and late ripening). A small number of representative samples were collected from these orchards and tested by the above methods for the presence of the viruses known to be associated with

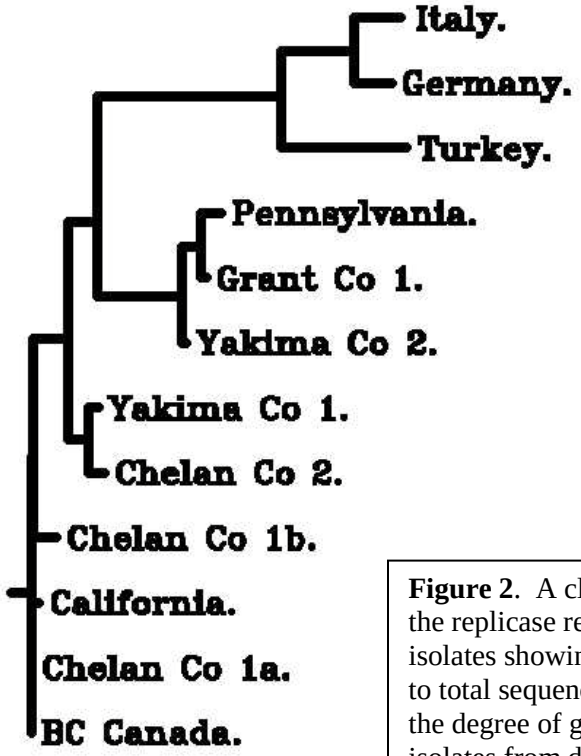

Figure 2. A cladogram of sequences from the replicase region of *Little cherry virus 1* isolates showing branch lengths in proportion to total sequence difference. This illustrates the degree of genetic variability of virus isolates from different geographic locations.

little cherry disease. From these data (Table 3), it is evident that LChV1 has become established in western North America. Because the symptoms expressed by infected trees are less severe than those

Table 3. Incidence of the viruses associated with little cherry disease in orchards with poor production.

Orchard designation and county	Trees with		
	LChV-1 only	LChV-2 only	Both LChV-1 + LChV-2
Yakima Co., orchard 1	4/4	0/4	0/4
Yakima Co. , orchard 2	3/13	6/13	0/13
Chelan Co., orchard 1	2/10	3/10	1/10
Chelan Co., orchard 2	0/3	0/3	1/3
Grant Co., orchard 1	10/18	0/18	0/18
Total Positives/Total Assayed	**19/48**	**9/48**	**2/48**

symptoms induced by LChV2, there is a greater tendency to assume that poor tree performance is the result of horticultural practices. The data generated in this project increases our ability to discern the underlying cause of the poor yields and to address that cause appropriately.

Impact and economic benefits: All of the virus diseases studied in this project are present in cherry production areas of western North America. The diseases associated with these viruses often resemble physiological conditions or symptoms induced by other pathogens. Therefore, it is critical that the grower has the tools to discriminate between potential underlying causes of poor cherry production and tree growth. The incorrect diagnosis would result in ineffective and frequently costly investments in remedial treatments with little or no relief from poor production.

The viruses from the rusty mottle group of viruses and those associated with little cherry disease directly impact fruit quality to different degrees. Trees infected with one or more of these viruses can display an extremely diverse array of symptoms that, in many cases, can only be distinguished from symptoms caused by other pathogens or agricultural practices with great difficulty. By increasing awareness of these viruses in the grower community and by providing the diagnostic tools for them, we hope to increase the ability with which diseased trees are identified. Viruses diseases do not respect property boundaries so these concerns are an industry issue. As the frequency of on-farm propagation increases, so does the need to ensure that these trees are free of deleterious viruses.

References cited in this report:

Bajet NB, Unruh TR, Druffel KL, Eastwell KC. 2008. Occurrence of two little cherry viruses in sweet cherries in Washington State. Plant Disease 92:234-238.

Eastwell KC, Bernardy MG. 2001. Partial characterization of a closterovirus associated with apple mealybug-transmitted little cherry disease in North America. Phytopathology 91:268-273.

Foissac X, Svanella-Dumas L, Gentit P, Dulucq M-J, Marais A, Candresse T. 2005. Polyvalent degenerate oligonucleotides reverse transcription-polymerase chain reaction: A polyvalent detection and characterization tool for trichoviruses, capilloviruses, and foveaviruses. Phytopathology 95:617-625.

Matic S, Minafra A, Sanchez-Navarro J, Pallas V, Myrta A, Martelli, GP. 2009. 'Kwanzan stunting' syndrome: detection and molecular characterization of an Italian isolate of Little cherry virus 1. Virus Research 143:61-67.

EXECUTIVE SUMMARY:

Cherry leafroll virus continues to encroach into cherry production regions of the Northwest. Grower access to diagnostic tests at WSU-Prosser for identifying infected trees is helping to reduce potential sources of inoculum. Root grafting is a major route of virus transmission and the judicious selection of appropriate rootstocks may be very helpful in minimizing the damage inflicted by cherry leafroll virus to our industry. At least one rootstock has been identified that responds to cherry leafroll virus with a hypersensitive reaction; this may be the foundation of one strategy to reduce the spread of disease. Pollen of infected trees is a rich source of infectious virus particles, and it has been assumed that pollen is at the center of long distance spread of cherry leafroll virus. Our data suggest that the virus does enter fruiting structures from infected pollen, but this translates into a new tree infection relatively infrequently, if ever. This lack of frequency makes the possibility of scientifically monitoring the migration of virus past the abscission layer between the pedicel and the fruiting spur impractical. As a consequence, studies should now be aimed at alternate means of aerial transmission.

The rusty mottle group of viruses causes several serious diseases in Pacific Northwest cherry orchards. The group consists of a complex array of virus genotypes that induce many symptoms that differ in appearance and severity. Viruses in this group appear to express symptoms that are very dependent on the specific variety that is infected, with symptoms ranging from none to severe. This enables the viruses to be distributed in varieties that act as symptomless carriers. Once planted in juxtaposition to other varieties, the viruses then infect sensitive cultivars with the possibility of causing severe crop loss. These viruses appear to spread naturally in orchard settings, but it is not clearly understood how members of this group of viruses are transmitted other than by propagation. Research has validated broad spectrum tests that can reliably detect viruses of this group. However, further refinement is needed in order to discriminate between those viruses that may be symptomless from those that can cause significant reduction in tree productivity. The first attempt to produce antibodies that can be used as the basis for a serological assay for these diverse viruses was very encouraging. Using the reagents developed in this study, a single ELISA will detect approximately one-half of the virus isolates that cause disease in the western states. Modifications to this initial process should be implemented to expand the range of viruses that can be detected. At the very least, future work to improve serological assays could produce complementary assays to expand the range of virus isolates detected.

Increased knowledge of Little cherry virus 1 is leading to greater concern about its role in cherry production. The genetic variability of this virus created great difficulties in developing accurate diagnostic methods. We identified well conserved portions of the genome that permitted the development of molecular assays that will detect all known strains. As our ability to detect and confirm the presence of the virus increased, it became apparent that the virus is already entrenched in major sweet cherry production areas. Concomitant with this observation, other programs have identified several additional *Prunus* species as symptomless carriers of Little cherry virus 1. Therefore, the potential exists for this virus to increase in its importance in the cherry industry. The means by which this virus is transmitted other than through the use of infected propagation material is unknown.