FINAL REPORT

WTFRC Project #: CH-01-14 **Organization Project #: 13C-3361-5291**

Project Title: Protecting Pacific Northwest cherry orchards from serious virus threats.

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Cooperators: Various growers

Project Objectives:

Develop a strategy to control the rapid decline of cherry trees associated with *Cherry leaf roll nepovirus.*

a) Determine transmission mechanisms for *Cherry leaf roll virus* in orchards of the Pacific Northwest and conditions affecting the disease development associated with this virus.
b) Initiate small-scale trial to determine symptoms of *Cherry leaf roll virus* on new and promising cherry cultivars.

Determine the biology of an emerging and very severe virus disease of cherries in the Columbia River Valley.

a) Determine sources of an emerging virus disease in Central Washington and the impact of these diseases on cherry varieties and rootstocks.
b) Develop diagnostic capabilities suitable for monitoring little cherry viruses in orchards of the northwest.

Significant findings:

Project Objective 1:

- A rapid decline of trees of sweet cherry trees is induced by *Cherry leafroll virus* in association with one or more of the *Ilarviruses* that are common in Pacific Northwest cherry orchards (i.e., *Plum American line pattern*, *Prunus necrotic ringspot* and *Prune dwarf ilarviruses*).
- Most trees exhibiting significant *Cherry leaf roll virus*-induced decline are 12 to 25 years old.
- No *Cherry leaf roll virus* is detected in registered mother trees in nurseries participating in the Washington certification program.
- *Cherry leaf roll virus* is pollen-borne. However, the exact mechanism by which this virus is naturally transmitted to cherry trees remains to be determined.
- A voluntary monitoring program was established to keep *Cherry leaf roll virus* out of commercial pollen sources.
- Identification of *Cherry leaf roll virus* in the Pacific Northwest, and recognition of the impact that it can have on sweet cherry production led to an industry-wide survey funded by the Commission.

Project Objective 2:

- *Little cherry virus-4* has been identified in sweet cherry trees of Washington and Pennsylvania. This was the first detection of this virus in the US. This is significant because the means by which *Little cherry virus-4* spreads in the orchard is unknown.
- Western X disease has re-emerged as a major disease of sweet cherry production. This follows a series of unusually mild and dry winters.

- We developed a laboratory test to detect trees infected with a new, rapidly spreading virus in the mid-Columbia Basin. Aggressive tree testing and removal has contained the virus.
- The development of more sensitive testing methods has revealed the presence of *Apple chlorotic leaf spot virus* associated with several instances of declining cherry trees.
- *Tobacco ringspot virus* was identified for the first time in cherry trees in Washington State. Correct identification resulted in tree removal.

Results and discussion:
Cherry production underwent an ambitious revitalization program during the early 1950's. To restore productivity of an ailing industry, trees affected with virus-like diseases were removed throughout the Northwest cherry growing districts. With the advent of certification programs in the early sixties, the incidence of virus diseases abated significantly. However, the emergence of several "new" viruses and the re-establishment of others have resulted in significant reductions in yield and profitability for affected cherry producers.

Objective 1: Our research revealed the presence of a virus that had not been detected in our orchards before. The symptoms of *Cherry leaf roll virus* by itself are not dramatic in the common cherry varieties grown in the Pacific Northwest. Virus infection does reduce tree vigor and yield, and the fruit is small and late ripening, but there are no intense disease symptoms. However, infected trees are weakened, and delayed fruit ripening with poor fruit size complicates orchard management.

There is another group of the viruses (the *Ilarviruses*) that are pollen-borne, and very common in all cherry production areas of the world. These viruses include *Prunus necrotic ringspot virus* and *Prune dwarf virus*. Because of the pollen-borne nature of these viruses, most orchard trees become infected with one or more of these viruses by the time the orchard is 15 years old. Since the trees are already mature and productive, the modest impact on production is largely tolerated. A third pollen-borne *Ilarvirus* is *Plum American line pattern virus*, but this is very rare in our production blocks. Our research has revealed that when one or more of these three viruses are present in a tree that is also infected with *Cherry leaf roll virus*, the mature and otherwise productive sweet cherry trees begin a period of rapid decline that ultimately results in their removal from the orchard.

The walnut isolate of *Cherry leaf roll virus* is known to be widespread in walnut in California where it causes significant financial losses, but our discovery of the cherry isolate of *Cherry leaf roll virus* was the first in this continent. Consequently, it was necessary to very quickly learn critical information so that growers could manage their orchards effectively.

A molecular-based laboratory test was developed that allowed us to confirm the identity of the virus as the cherry isolate of *Cherry leafroll virus* and to begin limited testing. These early results indicated that the isolates from several disparate locations in the Yakima Valley were identical. Antisera from laboratories around the world were acquired and evaluated for their ability to detect local strains of the virus. Antisera from the walnut isolates in California reacted only weakly with our local cherry strain and hence were of no value for routine testing. Test reagents for the elderberry strain reacted reasonably well with the cherry isolate. However, sensitivity and reliability of the serological ELISA was increase substantially when antisera specific for the cherry isolate was located from a European source and imported for our diagnostic purposes. Extensive virus testing was used to determine the distribution of *Cherry leaf roll virus* within infected trees, and to determine the most appropriate sampling methods for virus detection. This included determining the part of the season when virus detection is most reliable, the type of tissue that provides the most reliable results and the number of samples that must be collected from each tree for accurate detection. This critical information was then used as the basis for all subsequent tests, including the current industry-wide survey.

In Washington, 928 trees were sampled representing 48 separate production blocks of sweet cherry. [Note: this does not include the trees tested as part of the WTFRC-funded industry survey initiated in 2003.] Of more that 200 trees that tested positive for *Cherry leaf roll virus*, only five trees had been in the orchard for less than 7 years. Similarly, only a very small number of infected trees were more than 30 years old. Sites of *Cherry leaf roll virus* infections were in blocks throughout the lower Yakima River Valley and the lower Columbia basin ranging from Union Gap to Mesa.

Within the blocks that contained the virus, the incidence of *Cherry leaf roll virus* ranged from less than 1% to 32% of the trees infected. Through discussion with growers and fieldmen, it appears that the symptoms in the most severely affected blocks were first observed about 10 years ago.

It is difficult to develop a clear picture of the rate of spread of *Cherry leaf roll virus* since data is available for such a short period. As part of creating a baseline from which to monitor virus spread, large plots in three blocks were mapped in great detail. Every tree was tested for *Cherry leaf roll virus* plus three common *Ilarviruses*. Every tree in these blocks was re-tested in each of two subsequent years. Now there is clear evidence that *Cherry leaf roll virus* does spread naturally in the orchard. However, continued monitoring will be required to obtain an estimate of the rate of spread.

In addition, registered mother trees in the WSDA Certification program were tested for *Cherry leaf roll virus*, and all were free of this virus.

Cherry leaf roll virus belongs to a group of viruses that are predominantly transmitted by nematodes and/or by pollen. We identified the presence of a potential nematode vector, *Xiphinema rivesi*, in several of the affected orchards. However, three years of screenhouse and orchard trials did not reveal any transmission of the local strains of *Cherry leaf roll virus* by this nematode. In California, the walnut strain of *Cherry leaf roll virus* is pollen transmitted. Because there are significant differences in virus strains and pollen physiology (*i.e.* wind versus insect borne pollen), this assumption cannot be applied automatically to the situation in cherries. Our previous research confirmed that pollen from *Cherry leaf roll virus*-infected trees is ELISA-positive. Moreover, the virus in this form is infectious and is able to initiate infection when virus-laden pollen is mechanically rubbed onto experimental herbaceous hosts or when pollen from infected trees is applied to herbaceous plants in the presence of thrips. We are currently conducting research to resolve whether thrips can mediate the transmission of *Cherry leaf roll virus* from pollen to young cherry trees.

The mechanism by which the disease is spreading requires further study. However, the pattern seen in the distribution of infected trees has been very distinctive. An initial infection develops in an orchard, potentially several miles from any known source of infection or other cherry trees. Once established, disease radiates outward slowly from the initial infection site to adjacent trees. Initially, only one or two shoots of a newly infected tree will express symptoms, but eventually, all of the major scaffold limbs become involved.

Studies of this virus are still in their initial stages. However, from the early results of some grower efforts, it appears that tree removal is a viable strategy for stopping the spread of this virus. However, it has also become evident that such tree removal efforts must be aggressive, and infected trees completely removed at the first indications of symptoms. Of course, it is prudent to confirm the identity of the pathogen before removing the tree.

Objective 2: Through frequent contact with growers and fieldmen, we have become aware of several disease situations in the sweet cherry industry. We actively investigated these instances to determine the nature of the pathogen involved, and to provide information on its control, when available, to the grower.

Foveaviruses: In a Central Washington orchard, a virus disease emerged that induced severe die back of shoot tips and even major scaffold limbs, leaf loss, small fruit that is late ripening and dramatic foliar symptoms. The wood cylinder of the 'Montmorency' interstock was severely pitted.

Within 4 years, this disease spread through a significant portion of an otherwise productive 12-year old sweet cherry orchard and more than 170 mature trees were removed. This aggressive tree removal by the grower significantly slowed the spread of diseased trees, but continued monitoring is essential to determine the potential long-term impact of this virus. We developed a molecular laboratory test that is adequate for a limited number of samples, but more accessible diagnostic methods should be developed.

We partially characterized the genome of this virus and determined that it is part of the rusty mottle group of cherry viruses. That is, this virus is related to *Cherry rusty mottle virus*, *Cherry necrotic rusty mottle virus*, and *Sour cherry green ring mottle virus*. These viruses form a cluster of related but distinct viruses that infect cherry trees.

Western-X disease*:* In the 1950's, Western X disease was widely prevalent in the sweet cherry industry, to the degree that survival of an economically viable industry was threatened. This situation was one of the factors that precipitated a statewide effort to eliminate virus-infected trees and led to the establishment of the State certification program. This disease is caused by a form of bacteria (phytoplasma) that behaves, in many respects, like a virus. It is transmitted by any one of three species of leafhopper and over-winters in a number of weedy plants. A series of warm dry winters has led to a resurgence of this disease. Infected trees produce small, poorly colored cherries that do not ripen. There may be additional symptoms that are specific to different cherry varieties and rootstocks. It is difficult to control the transient populations of leafhopper that can first introduce Western X disease into an orchard, but their control in the orchard throughout the growing season plus identification and removal of infected trees are essential elements of a program to stop spread of this disease within the orchard.

Little cherry disease: Little cherry disease is causing significant crop losses in some orchards of the Pacific Northwest. The little cherry disease virus that is common here is *Little cherry virus-3*. This virus is spread by the apple mealybug and the spread of the virus can be significantly reduced or stopped by controlling this insect. However, during our studies, we revealed the presence of a very different virus that can be associated with this disease, *Little cherry virus-4*. The disease symptoms caused by these viruses are very similar, but the viruses themselves are different and actually belong to different virus genera. The spread of *Little cherry virus-4* can be very rapid as was seen in northern Germany in the 1990's. However, unlike *Little cherry virus-3*, the means by which this virus is spread is unknown so no specific control measure can be recommended.

We developed laboratory tests that identify and distinguish between the three pathogens that cause little cherry diseases: *Little cherry virus-3*, *Little cherry virus-4* and Western X phytoplasma. Although the tests are time consuming and expensive, they are available on a very limited basis and allowed us to determine that these three pathogens are currently in our industry causing substantial economic loss to individual growers.

Apple chlorotic leafspot virus: This virus occurs frequently in older apple trees where it causes little or no production difficulties in most varieties. Although it is also known to infect *Prunus* species, it was not thought to be very important in sweet cherry production. Our recent efforts have revealed the presence of *Apple chlorotic leafspot virus* in several cherry orchards exhibiting a variety of disease symptoms from severe ring spot and leaf necrosis to tree decline. We are improving our diagnostic capabilities to allow us to better detect and investigate the role of this virus in diseases of sweet cherry. The importance of this virus in sweet cherry production may have been seriously underestimated.

Clearly, viruses are not involved in every case of orchard decline. However, it is important to recognize when viruses induce or contribute to tree decline or reduced orchard income. It is also critical to know which virus(es) are involved in orchard decline in order to take appropriate measures to save the substantial capital investment represented by orchard trees.

Project budget:

The total funding received from the Washington Tree Fruit Research is summarized below:

Item	**3-Year Total (2001-03)**
Salaries	$34,182
Benefits	$11,021
Wages	---
Benefits	---
Equipment	---
Supplies	$21,409
Travel	---
Miscellaneous	---
TOTAL RECEIVED	$66,612

The National Research Support Project 5 made substantial in-kind contributions of virus-free research material, access to tree fruit virus collections, plus labor and expertise required for much of the virus testing and transmission studies. USDA-CSREES provided $31,668 for 1 year to expand the level of testing for *Cherry leaf roll virus* in the 3 western cherry producing States of Washington, Oregon and California. The Nursery License Research Fund administered by the Washington State Department of Agriculture has provided $14,145 for the first 2 years of a *Cherry leaf roll virus* transmission study. Washington State University provided significant levels of infrastructure and technical support through the duration of this project.