FINAL PROJECT REPORT

Project Title: Developing a management strategy for little cherry disease

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Other funding sources: None

Total funding: $56,083

Budget History:

Item	Year 1:	Year 2:	Year 3:
Salaries	143,464	13,056	
Benefits	5,655	5,484	
Wages			
Benefits			
Equipment			
Supplies	9,000	9,344	
Travel			
Plot Fees			
Miscellaneous			
Total	$28,199	$27,884	

OBJECTIVE:
The overall objective of this project is to **develop an industry-wide strategy to prevent the continued intrusion of little cherry disease into sweet cherry production regions**.

SIGNIFICANT FINDINGS:

- The resurgence of little cherry disease in Chelan and Grant Counties of Washington State is primarily associated with Little cherry virus 2.

- Smaller numbers of little cherry disease reports in Yakima County are associated with Little cherry virus 1 and the Western X phytoplasma.

- Grape mealybug was identified as a new and important vector that transmits Little cherry virus 2.

RESULTS AND DISCUSSION:

Little cherry disease is a serious economic disease of sweet cherry that has been present in Washington State since the 1940s but made a dramatic resurgence in 2010. Analysis in our laboratory demonstrated that this recent outbreak is associated primarily with Little cherry virus-2. Disease management is limited to destroying symptomatic trees, planting disease-free trees and controlling the insect vector.

The incidence of little cherry disease abruptly increased in many Washington commercial sweet cherry orchards during the 2011 and 2012 growing seasons. The result has been severe economic damage in affected orchards. Trees with little cherry disease produce small cherries with poor flavor development, and reduced sugar content making the fruit unmarketable, particularly with the current industry emphasis on premium fruit size and quality. However, some cultivars such as 'Bing' are fairly tolerant of little cherry disease. Once the trees become infected, they will exhibit the typical small fruit for one or two seasons; this is referred to as the shock phase. After this period, the fruit will return to near-normal size although the quality will still be sub-standard. The severity of little cherry disease expression varies depending on environmental conditions during the growing season, and colder than normal spring temperatures can induce the return to the shock phase of symptom expression in 'Bing'. It is believed that the cold spring of 2010 imitated the return of already infected trees to the "shock" phase of disease expression. As a result of this event, substantial amounts of fruit were sold at discounted prices or not packed at all. Additionally, many mature trees that are no longer commercially productive because of little cherry disease are being removed. The presence of infected orchards that serve as reservoirs of little cherry disease along with insect vectors creates a potential for extensive damage. Therefore, a sustainable integrated approach to control little cherry disease and its vector is needed, particularly for organic production.

Earlier studies in British Columbia identified apple mealybug (*Phenacoccus aceris* (Signoret)) as the primary vector associated with the previous little cherry disease outbreaks caused by little cherry virus-2. However, this insect species was not reported as a pest in Washington until the late 1990s and only a few apple mealybug populations have been recorded. The recent increase in the prevalence of little cherry virus-2 in the Pacific Northwest suggested that there may be changes in the vector population. Unlike populations of apple mealybug that have been declining, grape mealybug (*Pseudococcus maritimus* (Ehrhorn)) is becoming a common pest of both pome and stone fruits. The reduction in use of broad spectrum insecticides made possible by the use of spinosad bait (GF-120, Dow Agrosciences, Indianapolis, IN) for the key pest of cherries (western cherry fruit fly, *Rhagoletis indifferens* Curran) may have contributed to an increase in grape mealybug populations in sweet

cherry. The possible association between this insect and the spread of little cherry virus-2 was examined.

A natural infestation of mealybugs on myrobalan plum (*Prunus cerasifera*) trees was tentatively identified as grape mealybug based on general appearance. Slide mounts of several life stages confirmed the identity of grape mealybug and this colony was used for subsequent transmission experiments. Shoots from a sweet cherry tree inoculated with the North American LC5 strain of little cherry virus -2 was used as a virus source. In a growth chamber, crawlers were placed on shoots cut from the infected orchard tree and, after an acquisition period of seven days, approximately 50 insects were transferred to each potted 'Bing' cherry tree on Mazzard known to be free of little cherry disease. The young recipient potted trees (approximately 10 leaf stage) were growing in a second growth chamber at 23°C with a 16 hour light cycle. After one week, the trees were treated with a soil application of imidacloprid (Marathon 1% G; Olympic Horticultural Products, Mainland, PA) plus three times at one week intervals with horticultural oil to eliminate the mealybugs. This process was repeated on two separate groups of trees at different times during the growing season to yield a total of 20 young cherry trees that had been exposed to potentially viruliferous mealybugs. At 2 and 4 months after the transmission period; leaf tissue that developed after the transmission period was collected from each of the recipient trees for molecular testing. A similar test was done on a control plant that was not infested with mealybugs. After natural defoliation and a 3 month dormant period, emerging growth was again sampled and tested; this was 10 months after the initial transmission period. During each sampling, total RNAs were isolated from leaves and tested by reverse transcription polymerase chain reaction (RT-PCR) using two sets of primers in separate reactions. Identity of the RT-PCR amplicons was confirmed by sequencing.

Grape mealybug-mediated transmission of little cherry virus-2 to sweet cherry was confirmed by RT-PCR in 80% (16 of 20) of infected trees by molecular assays. Control samples, infested with no grape mealybugs, did not yield any bands. It is possible that the positive reaction in the RT-PCR was the result of virus carried by grape mealybug debris on the leaf surface but not transmitted. Hence, plants were allowed to continue to grow in the greenhouse and new growth was tested four months after inoculation to verify that the positive diagnostic reaction was the result of virus synthesis by the infected plant rather than surface contamination. To further negate this possibility, plants were allowed to go into dormancy at 6°C during winter and then transferred to a shade house to resume growth in the spring. When leaves collected from newly grown shoots were tested 10 months after the potential transmission period, identical results were obtained. The test results at different time points confirmed the establishment and spread of the virus in newly developed leaves of sweet cherry trees. The virus-specific amplicons from six independent trees were verified by sequencing. Both the partial replicase and coat protein gene sequences showed 99 to 100% sequence similarity among them and with corresponding regions of the LC5 strain of little cherry virus-2, confirming the identity of the virus. This work demonstrates that grape mealybug is an efficient vector of little cherry virus-2, one of the causal agents of little cherry disease. The confirmation of virus transmission by this common pest has great significance for sweet cherry producers in the Pacific Northwest.

This work demonstrates for the first time that grape mealybug is an efficient vector of little cherry virus-2. Grape mealybug has been recorded as a pest of tree fruits since the early 1970s, but pears were the primary host of concern. Recent versions of Washington's Crop Protection Guide do not include recommendations for grape mealybug control on cherry because its status as a pest is considered very minor. As a disease vector, its pest status is greatly elevated. This pest is most likely to be found in the tops of older trees, where spray penetration is poorest, and as such is considered difficult to control.

In 2012 alone, 72 cherry samples were submitted to our laboratory for virus testing in association with little cherry disease. More than two-thirds were infected with at least one virus-like agent. From these sample, 24 were selected and the coat protein determined. The nucleotide identity ranged from 83 to 100%. This information was used identify segments of the coat protein coding region that were conserved across all of the virus isolates and that could provide the reagents for recombinase polymerase assay, a cost effective diagnostic tool. The results are very promising and Washington State University is engaging in the next step of developing a diagnostic tool for little cherry virus-2.

EXECUTIVE SUMMARY:

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A recent outbreak of little cherry disease in Washington State is associated with little cherry virus-2. The cherry cultivars that predominate in the region are less sensitive to this virus, but cool spring weather can induce development of severe symptoms. Such a cool spring occurred in 2010. As a result many trees in many orchards exhibited recognizable little cherry disease symptoms for the first time. The incidence of symptomatic trees has been over 30% in some orchards. The incidence of diseased trees has continued to increase since that first event. The possible involvement of a virus vector population was considered. The previously known vector, apple mealybug, is present in very low numbers. However, population of a different mealybug species, the grape mealybug, is increasing in fruit growing areas. Our study demonstrated that grape mealybug is an efficient vector of little cherry virus-2 and that the presence of this insect may be contributing significantly to the spread of little cherry disease.

In an effort to correctly identify whether poor fruit yield is the result of physiological conditions or infection by little cherry virus-2, a new molecular technology is being adopted that will offer growers and consultants access to a relatively inexpensive method to detect this virus.