FINAL PROJECT REPORT **YEAR**: 4 of 4 (no cost extension)
WTFRC Project Number: CH14-110

Project Title: Developing a management strategy for little cherry disease

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Cooperators: Tim Smith–WSU Regional Extension Specialist, Grower cooperators

Total Project Request: Year 1: $63,479 **Year 2**: $65,020 **Year 3**: $62,743

Other funding sources

Agency Name: Stemilt Growers LLC
Amt. requested: $10,000
Notes: This funding is to support the development of field diagnostic kits for Little Cherry Virus 2.
Agency Name: WSDA Specialty Crop Block Grant – 'Managing Little Cherry Disease'
Amt. Funded: $199,820
Notes: WTFRC funding was used as match for this grant

Budget 1
Organization Name: WSU-TFREC **Contract Administrator:** C. Johnston/J. Cartwright
Telephone: 509-335-4564/ 509-663-8181 **Email address:**carriej@wsu.edu/joni.cartwright@wsu.edu

Item	2014	2015	2016	2017
Salaries[1]	26,738	27,808	26,499	
Benefits[2]	9,074	9,436	8,934	
Wages[3]	6,240	6,490	6,750	
Benefits[4]	605	630	655	
Equipment	0	0	0	
Supplies[5]	15,756	15,590	14,580	
Travel[6]	5,066	5,066	5,325	
Miscellaneous	0	0	0	
Plot Fees	0	0	0	
Total	63,479	**65,020**	62,743	

Footnotes: [1]Salaries are for post-doctoral scientists (for Beers, Eastwell) and faculty salaries (Gallardo) and research associate (Gallardo). [2]Benefits range from 27.47 to 41.85%. [3]Wages are for summer help (Beers). [4]Benefits for wages are 9.7%. [5]Supplies are PCR supplies (Eastwell); diagnostic kits (Beers), and grafted cherry trees/potting supplies (Beers). [6]Travel is for Motor Pool rental and gas (Beers) for travel to plots, and travel for focus group meetings (Gallardo).

Obj. 1. Determine mechanisms of Little Cherry Virus 2 (LChV2) transmission via insect vectors (apple and grape mealybug [AMB and GMB]). The goals of this objective are to gain new information about the vector-virus interaction; specifically, a minimum virus-acquisition feeding period for GMB and to quantify virus acquisition in the field via various life stages of both AMB and GMB.

Significant Findings:

- LChV2 infection is not always correlated with an active MB infestation. The initial infection via insect vectors may have occurred previously, but symptoms become evident only in subsequent years.
- Overall, mealybugs collected from LChV2-negative trees tested negative for LChV2 regardless of life stage, species, or proximity to LChV2-infected trees.
- After feeding for 24 hours, about 70% pf GMB nymphs acquired LChV2 from infected potted trees.

Results and Discussion:

Mechanisms of LChV2 transmission via insect vectors. In 2014-2015, 22 LChV2-infected orchards were visited, of which only 12 had active mealybug populations. Mealybug presence or absence was based on an extensive search during the visits, and the knowledge of the grower or consultant. We concluded that LChV2 infection is not always correlated with an active mealybug infestation. We also addressed LChV2 acquisition for various stages of AMB and GMB from infected trees. Mealybug eggs, mothers (an adult female in direct proximity with an egg mass and the presumed source of the eggs), small nymphs (0.5-1.5 mm), large nymphs (2-4 mm), and adults not associated with egg masses were collected from LChV2 positive and negative trees, in orchards with a history of LChV2 infection. When mealybugs from LChV2-positive trees were tested using RT-PCR, we found that 4 out of 10 eggs masses, 3 out of 7 females, 6 out of 9 small nymphs, 1 out of 3 large nymphs, and 3 out of 5 adults tested positive. All samples collected from LChV2 negative trees (2 ovisacs, 3 mothers, 12 small nymphs, 2 large nymphs, and 13 adult females) tested negative for LChV2 (Fig. 1). Both of these results were unexpected; first, that not all mealybugs feeding on LChV2-positive trees are positive, and secondly, that there was preliminary evidence of transovarial virus transmission. *Closteroviridae* (the family of viruses to which LChV2 belongs) are known to be semi-persistent, and not retained through molting, let alone from mother to offspring (transovarial transmission). More testing is warranted to determine if the LChV2-positive results found in egg samples is an actual infection or just superficial contamination from the positive mother.

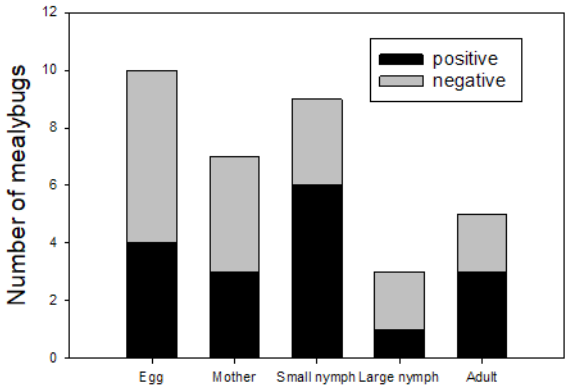

Fig 1. Number of mealybugs (eggs, mothers, small nymph (0.5-1.5 mm), large nymph (2-4 mm), and adult) collected from LChV2 positive trees that tested positive and negative for LChV2

Time needed for virus acquisition/transmission. Small potted 'Bing' trees on Mazzard rootstocks were maintained in the WSU-TFREC greenhouse. During January 2016, dormant budwood was collected from a 'Bing' tree in an orchard in Rock Island, WA, known to be positive for LChV2. Budwood was cleft-grafted onto the base of greenhouse plants in an attempt to infect them with LChV2. The trees were maintained in the greenhouse and leaf samples were tested in July 2016 to determine LChV2 infection status. Of the 10 greenhouse trees cleft budded with LChV2 infected plant material, only four were determined to be LChV2-positive when leaves were tested. The four

LChV2-positive trees were then infested with mealybug crawlers. After 24 hours of feeding, all 26 mealybug samples collected were nymphs measuring between 1-2 mm in length. Out of these samples, 18 (69.2%) were determined positive for LChV2 infection. After 6 days, 23 nymph and 4 adult samples were collected. Of the nymphs, 13 (56.5%) were determined positive for LChV2 infection, and of the adults, 2 (50%) were determined positive.

Obj. 2. Determine control methods for AMB and GMB in conventional and organic cherries. Vector control is a component of managing Little Cherry Disease (LCD), and relatively little was known about GMB on cherries, and no data existed for AMB control in Washington. In order to time insecticides for AMB, observations on phenology were recorded and used for application timing. Both field and greenhouse studies were conducted for control of the two vectors; absent a usable population of AMB on cherries, an infested apple block was used.

Significant Findings:

- Control strategies targeting AMB are most effective when sprayed at the delayed dormant (DD) timing targeting second instar crawlers, and organophosphates+oil provided good control.
- GMB field tests indicated promising results for systemic applications (Admire as a soil drench, and Ultor as a foliar spray). Admire, Aza-Direct and Centaur as systemics also suppressed GMB in greenhouse studies. Aza-Direct and M-Pede foliar sprays did not provide control.
- A parasitoid wasp, *Anagyrus schoenherri*, was collected from AMB and identified from the WSU TFREC Sunrise orchard. This is the first North American record for this species, which could be an important biocontrol agent for AMB.

Results and Discussion:

Phenology and parasitism. While AMB has a relatively low impact on apples (where it is not a vector), a high population on apple served to provide a better examination of phenology and better statistical separation of treatments. In 2014-2016, AMB was monitored weekly at WSU's Sunrise Orchards, in a conventional apple orchard with a high density of AMB. During the course of these observations, adult wasps were discovered near the mealybugs, and collected for identification by an expert in this group, Dr. Serguei Triapitsyn (UC Riverside). They were identified as *Anagyrus schoenherri* (Westwood 1837). This the first record of this species in North America[1] (Plate 1).

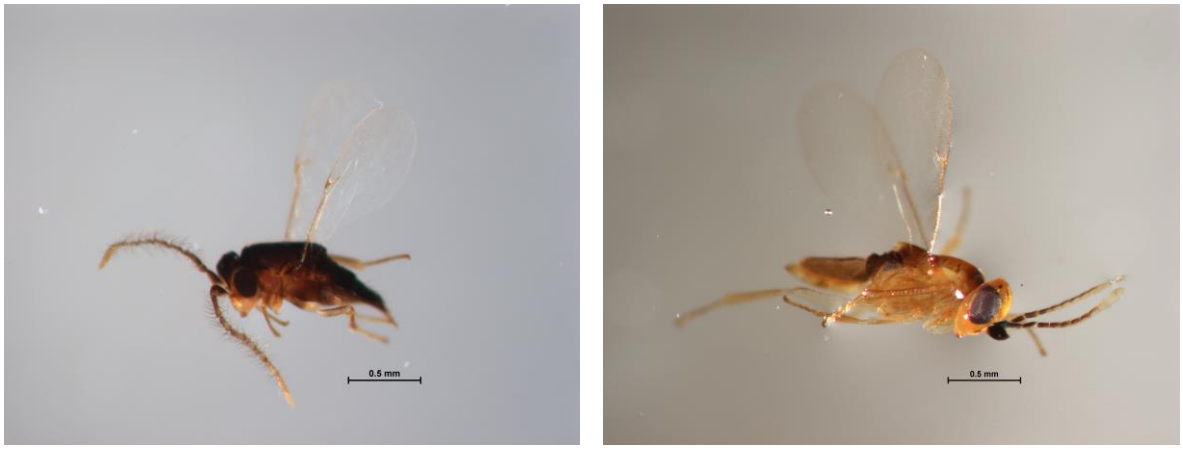

Plate 1. Male (left) and female (right) *Anagyrus schoenherri* (Westwood 1837), parasitoid wasps of apple mealybug.

[1] Bixby-Brosi, A. J., E. H. Beers, and S. V. Triapitsyn. 2017. Discovery of *Anagyrus schoenherri* (Westwood, 1837) (Hymenoptera: Encyrtidae) in the Nearctic Region, a parasitoid of the apple mealybug *Phenacoccus aceris* (Signoret, 1875) (Hemiptera: Pseudococcidae) in Washington, U.S.A., with notes on the host. Pan-Pac. Entomol. 93: 163-171.

The most complete record of host and parasitoid phenology is from 2015; however, this year was preceded by an unusually mild winter, thus any dates may be 2-3 weeks ahead of an average year. In late February, AMB were in overwintering shelters in the bark, although females had begun to emerge. Emergence was nearly complete by early March, and females began feeding at the base of buds. The emergence of the winged AMB males was later than that of the females, beginning in late March, and continuing into early April, when mating took place. Parasitized mealybugs (mummies) were first noted in early April, and adult wasps had emerged by mid-April, and were seeking mealybug hosts. By late April, female mealybugs had reached the adult stage and had started laying eggs. Their ovisacs were found on various parts of the tree, including crevices in the wood, under bark, leaves, shoots and spurs. Samples during the period indicated up to ≈80% of the mealybugs were parasitized. The parasitized mealybugs laid very few eggs. Parasitoid males emerged during mid-late May, and waited near ovisacs for female parasitoids to emerge. AMB eggs began to hatch in late May, but crawlers remained inside the ovisacs for some period before emerging to feed on the leaves. The phenology of the parasitoid relative to the host (Fig. 2) is based on 2016 data, a more typical year.

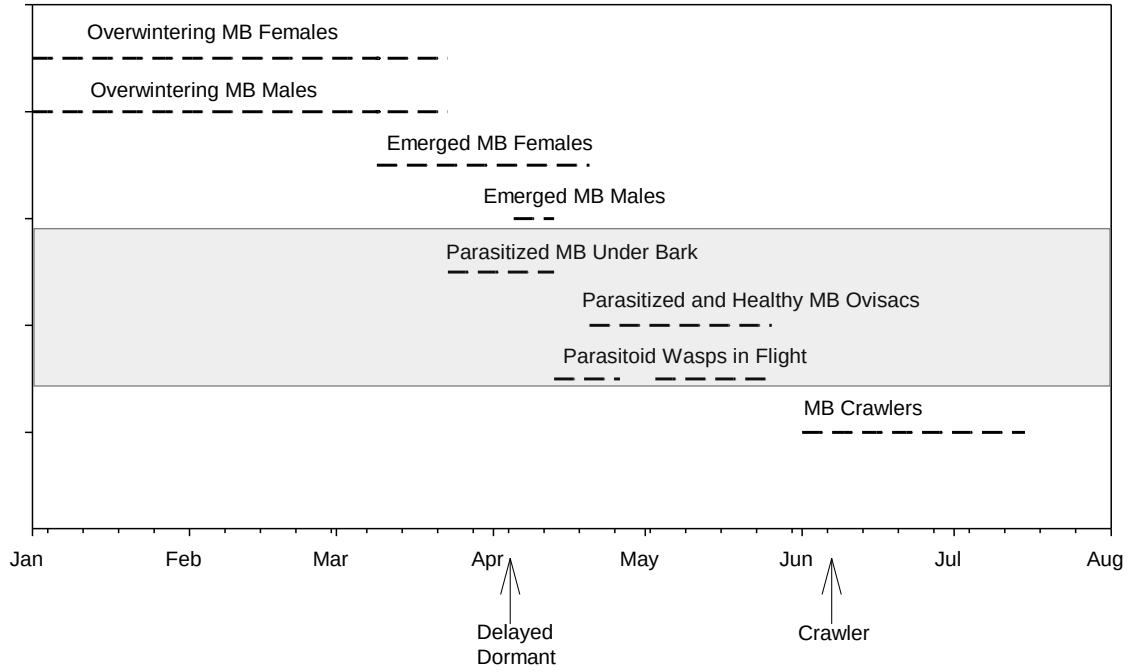

Fig. 2. Apple mealybug phenology observed in an infested apple orchard at WSU Sunrise Orchards in 2016. Parasitoid wasp, *A. schoenherri*, was observed within AMB mummies as well as in flight (shaded area).

In 2016, the first observation of parasitism occurred in nymphs under bark on 23 March. On 13 April, parasitoid wasps were seen flying, mating, and injecting eggs into mature female AMB. A few female mealybugs beginning to make ovisacs were collected from the field on 20 April, and when examined microscopically, parasitoid larvae were found inside. All collected ovisacs on 27 April and 3 May were also parasitized. Collections on 18 May revealed that most of the parasitoids had emerged, leaving only empty mummies behind. Parasitoid wasps then inject eggs into newly hatched crawlers.

Yellow sticky cards were used to monitor the flight of *A. schoenherri* adults. Yellow cards/traps were collected and replaced on a weekly or biweekly basis, starting on 21 April and ending on June 2. Numbers of *A. schoenherri* on sticky cards peaked between 3 May and 26 May, with over 100 individuals on each card. Very few *A. schoenherri* were captured during other times.

AMB Control. Field tests were conducted in the Sunrise apple block in 2014-2016, timing applications for either tree or insect phenology. Delayed dormant (DD) treatment targeted overwintering females, while mid-summer treatments targeted emerging crawlers. Petal fall (PF) treatments were used to test systemic compounds, and thus were based primarily on tree physiology. All tests were applied airblast to four single-tree replicates, with replicates based on pre-treatment counts, and post-treatment counts at intervals. The exception was the 2016 experiment, when AMB densities were very low, and only a single evaluation was done in early July.

The 2014 test indicated that the best treatments (as indicated by lowest post-treatment mean densities of nymphs) were Lorsban+oil at DD and Diazinon at crawler emergence (Fig. 3). In 2015, the Lorsan+oil treatment was similarly successful, although replicate variability did not allow statistical discrimination from the check. Centaur+oil and Diazinon+oil at DD, along with diazinon at crawler emergence also resulted in lower numbers of nymphs, but with the same lack of statistical separation (Fig. 4). In 2016, none of the treatments were statistically different than the check (Fig. 5); however, check populations had declined from 15 nymphs/leaf in 2014, to 1.5/leaf in 2015, and 0.15/leaf in 2016, making testing challenging.

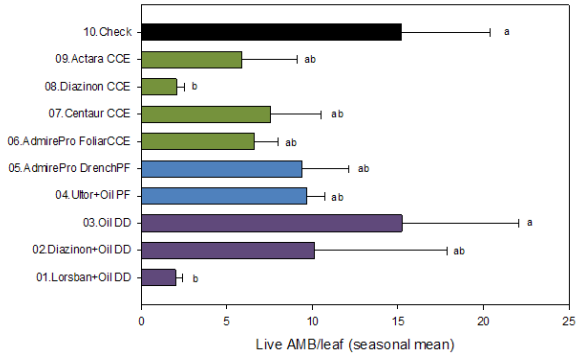

Fig. 3. Effects of compounds applied at delayed dormant (DD), petal fall (PF) and at crawler emergence (CE) on AMB numbers in an infested apple orchard at WSU Sunrise Orchards, 2014

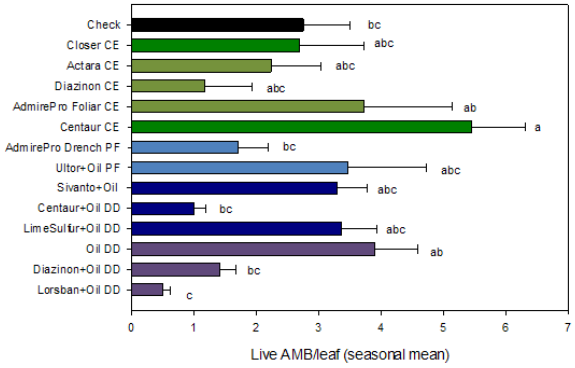

Fig. 4. Effects of compounds applied at delayed dormant (DD), petal fall (PF) and at crawler emergence (CE) on AMB numbers in an infested apple orchard at WSU Sunrise Orchards, 2015

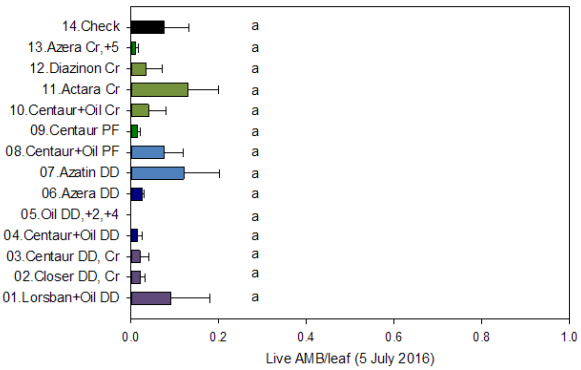

Fig. 5. Effects of compounds applied at delayed dormant (DD), petal fall (PF) and at crawler emergence (Cr) on AMB numbers in an infested apple orchard at WSU Sunrise Orchards, 2016

GMB Control. In 2015, an experiment was conducted on a GMB population in a commercial cherry orchard in East Wenatchee, WA. Treatments were applied airblast at 200 gpa at PF, 2 weeks after PF, or the crawler stage of the 1st generation in late June. The two systemic materials were applied 14 days after PF (Ultor as a canopy spray at 200 gpa), and Admire Pro as a soil drench (5 gal/tree). Treatments were assessed by counting the number of live GMB/cluster in mid-July. Due to the high spatial variability of this species, the treatment means were not statistically separable (Fig. 6). However, this test indicated that the two systemic treatments were promising, and warrant further investigation.

A second field experiment was conducted in 2016 to determine the effects of organic compounds on a heavy infestation of GMB in an organic plum orchard in Rock Island, WA. Pesticides were applied with a backpack sprayer to the point of drip on 14 July with label rates of Aza-direct and M-pede, and an untreated check (4 replicates/ treatment). Five days after treatment (19 July), labeled egg masses were removed from trees using forceps, and percentage of live and dead crawlers/eggs was determined using a microscope. The average percentage live crawlers, eggs, and total live (crawlers + eggs) was similar for all treatment groups (Fig. 7). These results suggest that organic compounds provided no control of GMB crawlers in this experiment. Eggs and crawlers in the nest are protected by waxy filamentous secretions of the ovisac, making them extremely difficult to reach with insecticides. In this experiment, egg masses were removed from trees for analysis when a percentage of eggs had hatched, and hatched crawlers were either crawling around in or leaving the egg mass. The fate of the mobile, unprotected, newly-hatch crawlers is unknown, since we only looked at crawlers within the nest. We may have had a different result if we were able to effectively sample these mobile crawlers. Predation and parasitism played a major role in reducing this GMB population, as we observed a number of syrphid predators and a parasitoid wasp in many egg masses.

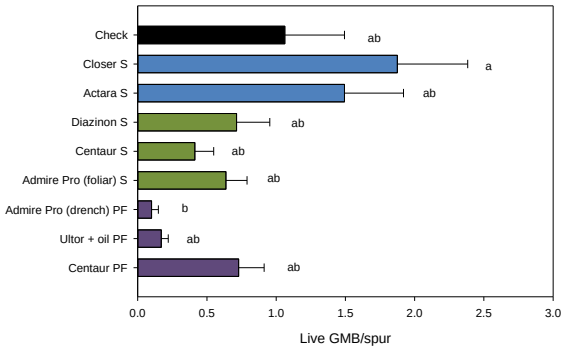

Fig. 6. Effects of compounds applied at petal fall (PF) and at crawler stage (S) on GMB numbers in commercial Bing cherry orchard, 2015

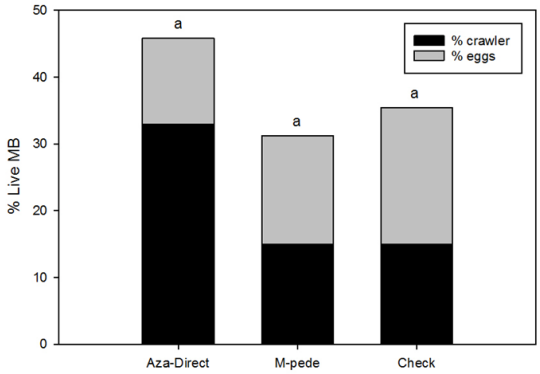

Fig. 7. Percent live GMB crawlers and viable eggs in egg masses treated with organic compounds, Rock Island, WA, 2016

A greenhouse experiment on potted trees was conducted to determine the efficacy of systemic materials for GMB applied either as a drench or a foliar spray. Potted cherry trees ('Bing'/Mazzard) were treated with Admire Pro and Aza-Direct (soil drench) or Ultor and Centaur (foliar) 7 days after mealybugs were transferred to the trees. Analyses were performed on the difference between the pre- and post-treatment counts. Nymph numbers were reduced to zero on Centaur-treated plants at 19 days post-treatment and nearly to zero on trees treated with Admire Pro and Aza-Direct at 28 days (Fig. 8a). Admire Pro, Aza-Direct, and Centaur reduced adult numbers to zero 19 days after application (Fig. 8b). Ultor did not significantly reduce GMB numbers for any life stage, however, it should be noted that an adjuvant (recommended for use with Ultor to increase systemic activity) was omitted from the treatment, and may have reduced efficacy.

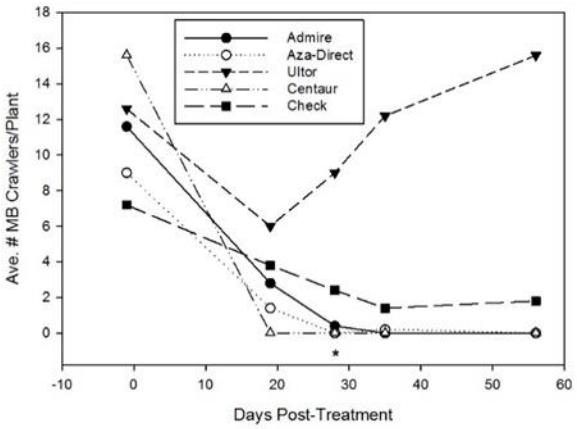

Fig. 8a. Effects of foliar- and drench-systemic compounds and an insect growth regulator on the average number of GMB crawlers/plant over time. *significant reduction in avg. crawlers/plant compared to check, 2016

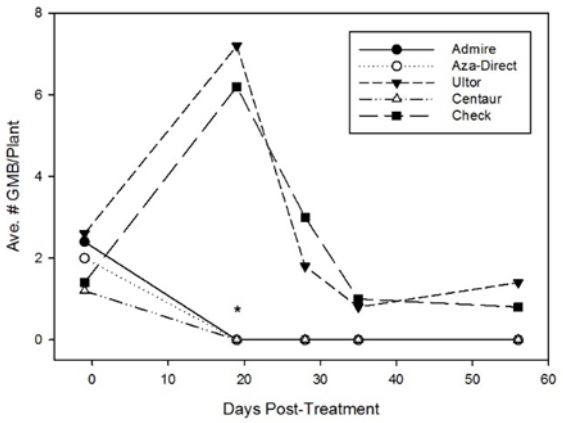

Fig. 8b. Effects of foliar- and drench-systemic compounds and an insect growth regulator on the average number of GMB adults/plant over time. *significant reduction in avg. adults/plant compared to check 2016

Obj. 3. Develop and deploy field diagnostic assays to detect LChV2 and differentiate it from other pathogens that induce similar symptoms (LChV1 and Western X phytoplasma [WX]).

Significant Findings:

- A new genetic variant of LChV2 was discovered in Washington orchards. This genetic variability contributes to reduced sensitivity of the assay systems.
- The commercial LChV2 kit (Reverse Transcription Recombase Polymerase Assay, or RT-RPA) for detecting LChV2 was modified and it now recognizes genetic variants of the virus that were not detected with previous kits.
- WX has been found to be an important pathogen associated with LCD in Grant and Chelan counties. It was previously associated with LCD in Yakima County.
- A reliable assay system was developed for WX based on the RPA format. This allows more precise identification of the pathogens associated with little cherry disease, a critical factor since that influences appropriate disease management decisions.
- The RPA kit for detecting WX phytoplasma was modified and it now recognizes isolates of the bacteria that were not detected in the previous season.

Results and Discussion:

Validation of LChV2 field kits: A diagnostic kit based on RT-RPA technology for LChV2 was made commercially available in the spring of 2014. However, kit performance was subpar for two fundamental reasons: 1) unexpected genetic variability of LChV2; and 2) limited experience with this assay system for the detection of LChV2. The RT-RPA kit was re-tooled using nucleotide sequence information obtained from unique genetic variants of LChV2. Using LChV2 infected trees maintained in the greenhouse of the Clean Plant Center Northwest; a prototype of the re-tooled kit successfully detected the unique LChV2 variant as well as common LChV2 strains. The redesigned test kit still discriminated between LChV2 and the other agents associated with little cherry disease including LChV1 and WX. Field data collected during the 2014 and 2015 growing season highlighted optimal sampling times and sample size, which were incorporated into revised protocols.

Development of RPA assay for WX and LChV1: An RPA assay system for WX phytoplasma was developed; however, three WX PCR positive samples during the 2016 growing season were not detected by the kit. Examination of nucleotide sequence from two regions of the WX genome did not reveal genetic variation of these three samples with other isolates of WX phytoplasma. Several

attempts to accommodate detection of these three WX PCR positive samples by the previous WX RPA assay system (i.e., changing primer and probe concentrations) were also not successful, prompting re-designing of the WX RPA assay system. A newer version is available that detects the three WX PCR positive samples. Crude leaf extracts from 23 WX PCR positive samples were then tested by the new version of WX RPA assay system; all 23 samples were positive. We examined the spatial variation in WX in infected trees, which is known to be unevenly distributed. Symptoms are most apparent when fruit are nearing harvest, and in late summer-early fall when leaves appear yellow to orange, referred to as 'bronzing'. Leaf samples from symptomatic and non-symptomatic branches from seven WX infected trees were tested by both PCR and RPA. All symptomatic branches tested positive for WX by both PCR and RPA but only one non-symptomatic branch was positive (Table 1). Overall, these results showcase the comparable sensitivity of RPA with PCR and further highlight the necessity of uniform sampling in order to get reliable detection of WX. The newer version of the WX RPA assay system needs to be tested extensively to ensure its reliability in detecting field isolates of the pathogen.

Table 1. Detection of WX phytoplasma by PCR and RPA from symptomatic and non-symptomatic branches of known WX infected trees.

WX positive tree	PCR		RPA	
	Symptomatic branch	**Non-symptomatic branch**	**Symptomatic branch**	**Non-symptomatic branch**
1	++	-	++	-
2	++	-	++	-
3	++	-	++	-
4	++	-	++	-
5	++	-	++	-
6	++	-	++	-
7a	++	-	++	-
7b	++	++	++	++
WX positive (purified DNA)	++	++	++	++
water	-	-	-	-

Legend: ++, strong positive reaction
+, weak positive reaction
-, negative reaction

We also examined temporal variability in sampling success, using different tissues. Both assay formats were unreliable in detecting WX during the earliest part of the season (mid-March: full bloom) but gave consistent positive detection a month after full bloom (starting on mid-Apr) (Table 2). Crude sap preparations of leaves from 29 samples gave consistent positive reactions in the WX PCR and RPA assays. Taken together, a reliable RPA assay for WX targeting the idpA region of the pathogen was developed that is suitable for use in crude sap extracts.

Table 2. Detection of WX phytoplasma by PCR and RPA in various tissues throughout the growing season.

WX tree	PCR						RPA					
	26-Mar	23-Apr	21-May	9-Jun	26-Jun	19-Aug	26-Mar	23-Apr	21-May	9-Jun	26-Jun	19-Aug
Tree #1:												
leaves	+	++	++	++	++	++	-	++	++	++	++	++
bark scraping	+	++	++	++	++	++	+	++	++	++	++	++
flower stem	+						+					
flower petal	-						-					
fruit stem		++	++	++	++			++	++	++	++	
green shoots				++	++					++	++	
Tree#2:												
leaves	-	++	++	++	++	++	-	+	++	++	++	++
bark scraping	+	++	++	++	++	++	-	+	++	++	++	++
flower stem	-						-					
flower petal	-						-					
fruit stem		++	++	++	++			++	++	++	++	
green shoots				++	++					++	++	
Tree #3:												
leaves	+	++	++	++	++	++	+	+	++	++	++	++
bark scraping	+	++	++	++	++	++	+	+	++	++	++	++
flower stem	+						+					
flower petal	-						-					
fruit stem		++	++	++	++			++	++	++	++	
green shoots				++	++					++	++	
Tree #4:												
leaves					++	++					++	++
bark scraping					++	++					++	++
flower stem												
flower petal												
fruit stem					++						++	
green shoots					++						++	
Tree #5:												
leaves					++	++					++	++
bark scraping					++	++					++	++
flower stem												
flower petal												
fruit stem					++						++	
green shoots					++						++	

Legend: ++, strong positive reaction; +, weak positive reaction; -, negative reaction; black shaded box, not applicable; gray shaded box, not tested.

Obj. 4. Assess the economic impact of LChV2 given its effects on crop yield, crop quality, and tree death.

Significant Findings:

- In hypothetical management scenarios, aggressive management of LCD (tree removal, monitoring/testing, additional mealybug sprays) led to better financial outcomes compared to no management when LChV2 is present. Assuming the management tactics slow the rate of disease spread, the orchard remained profitable throughout its 25 year life.
- Where no management options were used, profits were negative by year 12 (5% rate of spread) or 16-17 (3% rate of spread).
- In all scenarios, profits declined over time in an orchard with LCD in comparison to a non-infected orchard.

Methods:

We developed average production costs of 'Bing' and 'Sweetheart' cherries derived from interviewing producers and averaging the results. This provided the baseline production costs and returns for two common sweet cherry cultivars. This data was used as a base to project costs and returns if the orchard were affected by LCD, with different management practices and different rates of disease spread. Our figures were developed for a 10-acre block with a planting density of 272 trees/acre, and a productive life of 25 years. While the actual rate of disease spread is still unknown, we developed profit scenarios based on multiple hypothesized rates of spread. Our assumptions included 100% symptomatic expression and cullage in the year of infection (which likely overestimates impact). Returns for fresh-market fruit were estimated at $2.05/lb, while returns for culls were $0.20/lb throughout the life of the orchard.

The first management scenario (**Scenario 1**) entailed no LCD management by the grower. In this scenario, the grower is unaware that they have LCD in the orchard, and no mitigation or control measures are taken. The picking crew will not be warned about LCD and will pick all cherries in the trees. All of the fruit will be sent to the packinghouse, and all cherries from infected trees will be sorted as culls, and paid at the cull price. Three infection rates were projected, viz., 1%, 3%, and 5% of the remaining trees become infected each year.

In the second management scenario **(Scenario 2),** the grower is informed about LCD and takes all possible measures to control it, including monitoring for disease symptoms, testing symptomatic trees, removing positive trees, and spraying for the vector (mealybugs). Initially, management is based on visual symptoms, which are not available until the trees begin bearing fruit (year 3). Three infection rates are projected, viz., 0.5%, 1%, and 2% per year, *based on the assumption that the rate of spread is reduced by the management practices.* Infected trees are detected in June of a given year and removed in October of that year.

Results and Discussion:

Scenario 1, no management. Compared to the baseline for both Bing and Sweetheart cherries (Fig. 9), the total crop yield per acre is the same as the baseline but more cherries are sorted into culls throughout the productive life of the orchard, leading to a reduced profit. At all three infection rates considered (1%, 3%, and 5%) all profits are positive in year 4, and decline in years 4-25. At 1% infection rate profits do not become negative, at 3% profits become negative in year 16 for Bing and 17 for Sweetheart, at 5% rate of disease spread, profits become negative at year 12 for both Bing and Sweetheart.

Scenario 2, all possible management. Compared to the baseline, crop yield is lower because of infected tree removal. This leads to reductions in total returns, but is offset by lower harvest labor costs, warehouse packing charges and profits. At all three infection rates considered (0.5%, 1% and 2%) all profits are positive in year 4, and while they continue to decline slowly, they do not become negative in the life of the orchard.

Several points are important in these scenarios for management decisions, which become apparent if we compare the two scenarios at the same rate of disease spread (1%) at year 25 of the orchard life. Whether through fruit cullage (Scenario 1) or tree removal (Scenario 2), the fresh market production is only ca. 3% lower in Scenario 1 vs 2, and likewise the total return is not much affected. However, the much higher (46%) net return in Scenario 2 is due to reduced picking and packing costs where trees are removed when infected. The higher cost of monitoring, spraying, testing, and tree removal is trivial (ca. $500/yr) compared labor costs, providing an additional impetus for intensive management of this disease.

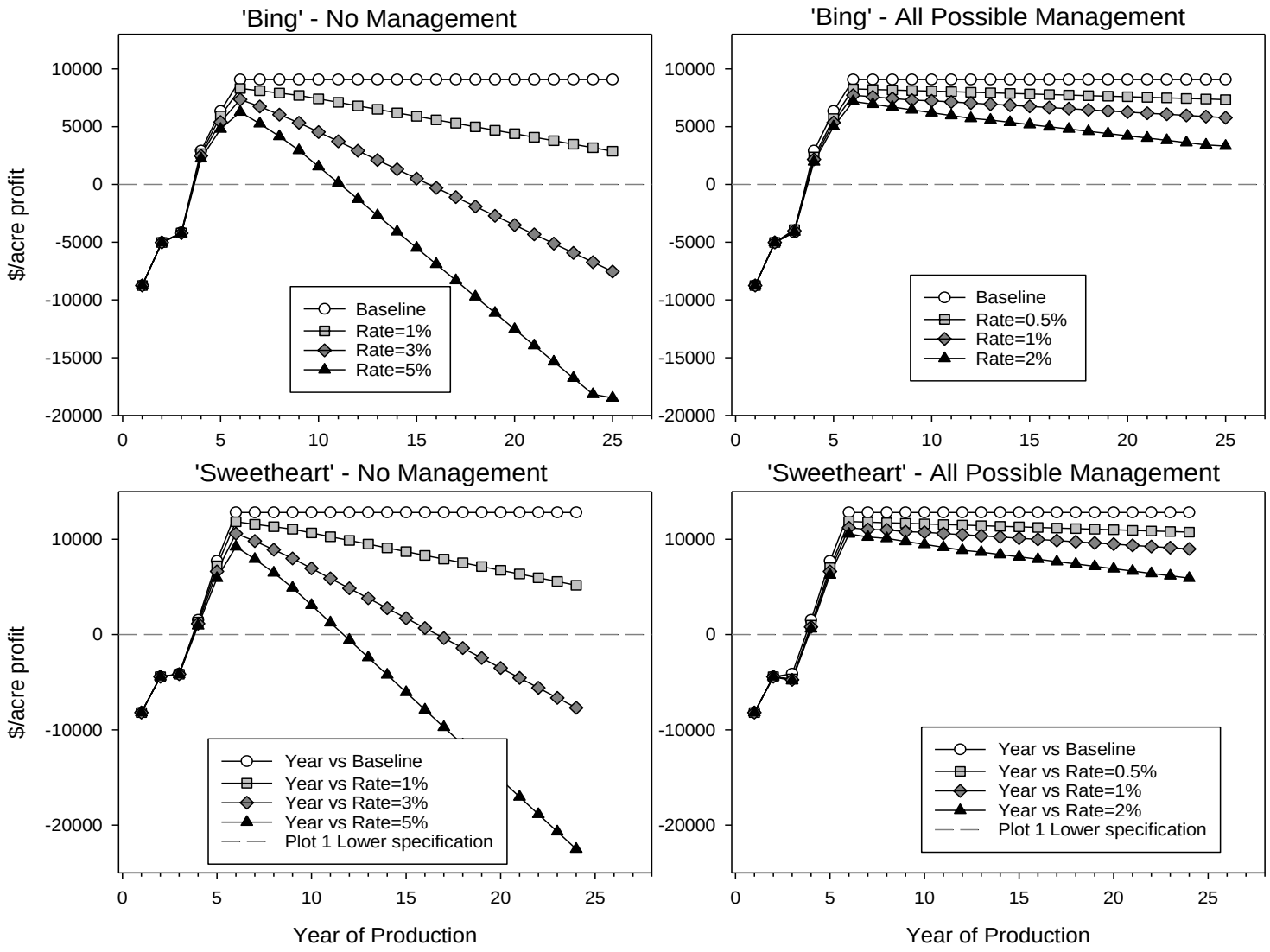

Fig 9. Comparison of estimated annual profits under different LCD scenarios for ‘Sweetheart’ cherries.

Executive Summary: This project addressed some fundamental questions concerning Little Cherry Disease (LCD) in the primary production districts of Eastern Washington. While this disease has a history going back decades, it has figured little in the management recommendations in Washington State. Unlike other viral diseases of cherries, the symptoms are more subtle and variable, and not fatal to the tree. Thus, when LCD was brought to the attention of the Washington industry in 2010, it was likely many years after the disease had already spread in some regions. It also occurred during a period of rapid expansion of the highly profitable sweet cherry industry in Washington, which increased 3.5-fold in acreage between 1985 and 2016.

Surprisingly little is known about the epidemiology of this disease, both at the organismal (individual tree) scale, or at the orchard or regional scale. It is reported to be vectored by mealybugs, scion grafting, and root grafting, but the literature on these modes of transmission is sparse. The mode of transmission has huge implications for disease control, but this aspect is largely unstudied, as is the economics of disease control. Lastly, the spatial and temporal variability and subtlety of the symptoms make accurate diagnosis based on visual symptoms unreliable, while the more reliable PCR diagnosis is an expensive alternative. The result is that decision-making for growers who may have LCD in their orchard is greatly complicated.

Regarding insect vectors, it was not until Washington researchers demonstrated that grape mealybug (GMB) was a competent vector that the full potential impact in our region was understood. Apple mealybug (AMB), the only known vector up until that time, was relatively rare in Washington, and restricted for the most part to apple blocks. Conversely, GMB was a ubiquitous and spreading pest in all tree fruits, including cherries. Our studies revealed several unexpected results: 1) that only half the orchards with LCD had an active mealybug infestation; that AMB was relatively rare in sweet cherry; that the infection status of GMB from LChV2 positive trees was only ca. 50%, and that all stages could be positive for LChV2. These results, while preliminary, are troubling for management decisions for LCD.

Insecticidal control of the vector is normally recommended in the case of vectored diseases, and LCD is no exception. The work in this project has focused on control of the less-known AMB, with additional evidence for control of the GMB. Unfortunately, the organophosphate insecticides remain among the more effective materials, which are likely to be withdrawn from the market at some point in the future.

The attempt to develop a rapid, user-friendly kit for molecular diagnosis of viral pathogens has met with mixed results. There is still a level of expertise necessary to use the kit effectively, and the cost is not dissimilar to traditional (and arguably more definitive) laboratory PCR methods. An additional complicating factor is the prevalence of Western X (WX) virus, which produces symptoms similar to that of LChV2. While the WX RPA methodology has been developed in the course of this project, it has not yet been commercialized.

The economics of LCD have been difficult to parse out, but the most interesting insight is that despite tree loss, intensive management has a better economic outcome. The increases in management costs are trivial compared to the reduced picking and packing costs associated with tree removal, which should help slow disease spread.

Lastly, the matching SCBG project has greatly informed the results of the current project. We have looked at the pattern of LCD in eight orchards, and in many cases confirmed the spatial relationship of infection, where new infections are within one to three trees of a current or previous infection. Unfortunately, the hypotheses for spread by root grafting and vectors produce much the same results, and the pattern does not help us differentiate between the two. Other insights are that observers (experienced fieldmen) usually correctly identified LCD trees, but they mis-identified non-LCD trees with a high frequency. An additional insight is that the spatial variability within an infected tree is significant, and that the expression of symptoms is highly correlated with PCR diagnosis, making sampling during the non-fruiting period a significant issue for this disease. As expected, the visual symptoms are highly correlated with a measurable loss in fruit size, color and quality from this aptly named disease.