Northwest Cherry Research Review

Red Lion, Pasco

Thursday, 11/9/2017

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FINAL PROJECT REPORT

Project Title: Finding the Achilles' heel of a new virus infecting stone fruits

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Cooperators: N/A

Other funding sources

Agency Name: USDA-APHIS Center for Plant Health Science and Technology
Amt. requested: $50,013 was received in FFY 2014 to determine the incidence of the new luteovirus-like virus in the foundation program of the CPCNW.
Notes: WSU is including this information on other funding available for the support of similar research undertaken by the faculty member (K. Eastwell). These resources are listed to identify other support granted for this research and are not included as a commitment of cost-share by the institution

Total Project Funding: $83,178

Budget History:

Item	2015	2016	2017
Salaries	$8,143	$8,469	$8,808
Benefits	$3,339	$3,472	$3,611
Wages	$0	$0	$0
Benefits	$0	$0	$0
Equipment	$0	$0	$0
Supplies	$15,935	$15,689	$15,712
Travel	$0	$0	$0
Plot Fees	$0	$0	$0
Miscellaneous	$0	$0	$0
Total	$27,417	$27,630	$28,131

Objectives

This project had four major objectives, looking at different aspects of the viruses' biology in an attempt to determine whether these two viruses were a significant threat to the cherry industry in the PNW, and if so identify areas where control measures could be applied.

Obj. 1. *Determine if aphids are vectors of the newly discovered virus, and which aphid species in particular can transmit the virus to adjacent trees.*

Obj. 2. *Identify the relevant members of the host range that may be a reservoir of the virus in the fruit producing region of the cherry industry.*

Obj. 3. *Observe the development of symptoms on cherry cultivars that are critical to the cherry industry*

Obj. 4. *Develop a robust assay system for the detection of this virus.*

Significant Findings

Obj. 1. This objective was completed.

Obj. 2. This objective was completed

Obj. 3. This objective was completed.

Obj. 4. This objective was 75% completed.

- Both viruses have relatively limited distribution in Washington orchards: *Nectarine stem pitting-associated virus* was detected in 1.8% of the trees tested (8 out of 450), whereas *Prunus virus F* was detected in 11.7% (51 out of 450).

- Both viruses show low titer and scattered distribution in sweet cherry trees, suggesting that they are poorly adapted this species. Similarly, neither virus was observed to produce disease symptoms on sweet cherry.

- Neither virus was found to infect bitter cherry (*Prunus emarginata*) chokecherry (*P. virginiana*) suggesting that these two species are not reservoirs for further infection.

- Neither virus was found to be transmitted by the green peach (*Myzus persicae*) or black cherry (*M. cerasi*) aphids in this study, in part due to low virus titer and distribution.

- Real-time PCR assays were developed for these two viruses, allowing sensitive and accurate detection.

RESULTS & DISCUSSION

This project focused on examining the biological impact and risk of spread of two newly reported viruses. The first, *Nectarine stem pitting-associated virus* (NSPaV), is luteovirus first observed in imported nectarine cultivars in California in 2013 (Bag et al. 2015), that was, as the name suggests, associated with stem pitting disease symptoms. The second virus, *Prunus virus F* (PrVF) was originally detected in a sweet cherry tree in a commercial orchard in Grant county, Washington State, that showed leaf spot symptoms associated with cherry necrotic rusty mottle disease (Villamor et al. 2017). Deep sequencing of this tree indicated the presence of *Cherry necrotic rusty mottle virus*, as well as *Apple chlorotic leaf spot virus*, *Cherry virus A*, and *Prune dwarf virus*. In addition to these known viruses, a novel fabavirus was identified, which was named, in the absence of an associated disease symptom, *Prunus virus F* (Villamor et al. 2017).

Given that the cherry industry is at present dealing with the little cherry disease complex, the emergence of additional pathogens is unwelcome, therefore this project was proposed to carry out a brief risk assessment for NSPaV and PrVF. Four objectives were studied, three examining the biology of these two viruses: host range, symptom expression, and vector transmission, and a final objective to develop diagnostic assays for future detection and diagnosis.

Host range

When these two viruses were found to be present in Washington state, samples were collected to determine their incidence in five sweet cherry producing counties (Table 1). NSPaV occurred only in eight out of 450 samples tested, and only in Grant county. PrVF, on the other hand, was detected 51 out of the 450 samples, from orchards in Yakima, Grant and Chelan counties.

Table 1. Occurrence of NSPaV and PrVF within cherry production regions of Washington State.

County	NSPaV positive samples	PrVF positive samples	Total samples
Benton	0	0	75
Douglas	0	0	25
Grant	8	21	63
Chelan	0	13	175
Yakima	0	17	112
Total	**8 (1.8%)**	**51 (11.3%)**	**450**

Given the incidence of both viruses in major cherry producing regions of the state, a two-fold approach was taken. Previous research had shown that NSPaV was graft transmissible to *P. avium* and to the indicator species *P. tormentosa* (Villamor et al. 2016), while PrVF was graft transmissible to *P. avium*, and the interspecific hybrid rootstock Krymsk-6 (*P. cerasus x (P. cerasus x P. maackii*). In addition, PrVF was detected on Gisela rootstocks with parentage consisting of combinations of either *P. cerasus, P. canescens*, or *P. avium*. Given these observations, a greenhouse trial was established to see whether either virus could readily infect Bing cherry on two common rootstocks, Mazzard and Gisela-6. These trees were subsequently inoculated with either NSPaV or PrVF as a

single infection, or with both NSPaV and PrVF in a double infection in April 2016. Eighteen months later the plants were sampled and sectioned for virus presence and symptom expression. Sequential sections of the taproot, the stem of the rootstock below the graft union, the stem of the scion above the graft union, and terminal shoot and leaf tissue were collected and screened by virus-specific real-time PCR for virus presence (Table 2). We found that NSPaV had a very patchy distribution in both scion host combinations, with no systemic (found throughout the plant) infection observed. PrVF distribution was more consistent, although it was rare to find a systemic infection in either host.

Table 2. Distribution of NSPaV and PrVF in Bing on Mazzard and Gisela-6 rootstocks as determined by real-time PCR.

	Bing on Mazzard				Bing on Gisela-6			
Virus	**Scion Shoot**	**Scion Stem**	**Rootstock Stem**	**Rootstock Taproot**	**Scion Shoot**	**Scion Stem**	**Rootstock Stem**	**Rootstock Taproot**
NSPaV	0/3	0/1	1/3	1/3	0/5	0/5	1/5	1/5
PrVF	1/3	1/3	1/3	1/3	2/5	4/5	3/5	2/5
NSPaV w/PrVF	0/3	0/3	0/3	1/3	1/5	1/5	0/5	0/5
PrVF w/NSPaV	1/3	1/3	1/3	0/3	4/5	1/5	1/5	2/5

Both viruses accumulated to a very low titer in either scion-rootstock combination (Figure 1). This, combined with the scattered distribution and generalized root-biased tropism suggests that that they are poorly adapted to commercial *Prunus* spp., and are unable to establish a full systemic infection.

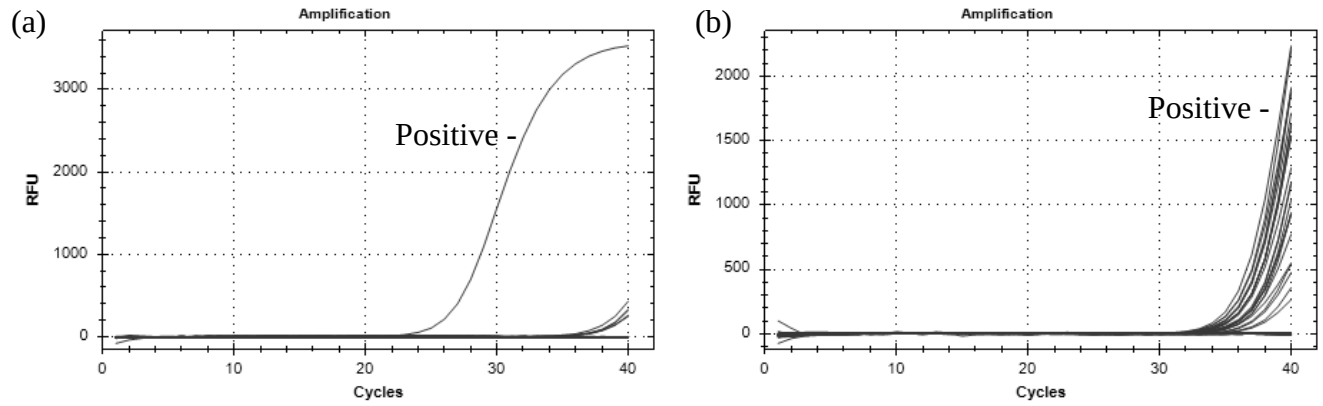

Figure 1. Titer of (a) NSPaV, and (b) PrVF in Bing scion material on either Mazzard or Gisela-6 rootstock as determined by real-time PCR. The positive control sample is marked.

In addition to examining the ability of these two viruses to infect commercial cherry, we also performed and experiment to see whether they could infect common, wild cherry species. *P. emarginata* (bitter cherry) and *P. virginiana* (choke cherry) were graft-inoculated with bark patches from NSPaV (in the 2015 and 2016 growing seasons) or PrVF (2016 only) infected sources, and tested for virus presence. No virus was found in either host during the 2015/2016 seasons by endpoint PCR, nor was either virus detected using the newly developed real-time PCR assays during the 2017

season. These data would suggest that both viruses have a limited host range within the *Prunus* genus, and that the likelihood of spread into, or from, wild cherries is remote.

Symptom expression

One of the major questions posed in this study was whether these two recently discovered viruses, NSPaV and PrVF, are potentially pathogenic to cherry. No foliar or trunk symptoms were observed on infected field plants that could definitely be associated with either of these two viruses; the trees did exhibit overall poor growth and slightly reduced fruit size, which may be attributed to infection by another virus or pathogen, or to poor orchard management. In order to confirm that neither virus affected commercially significant cherry cultivars, we established a greenhouse trial with Bing scions on Mazzard or Gisela-6 rootstock as described earlier. As can be seen in Table 3, no distinctive symptoms that could be attributed to infection by either pathogen were observed, nor were there any abnormalities to distinguish them from the uninoculated negative controls.

Table 3. Symptoms observed in Bing on two different rootstocks after infection with NSPaV and/or PrVF.

Host	Symptoms Observed	Inoculum		
		NSPaV	PrVF	NSPaV + PrVF
Bing on Mazzard	Foliar	0/3	0/3	0/3
	Stem Pitting	0/3	0/3	0/3
Bing on Gisela-6	Foliar	0/5	0/5	0/5
	Stem Pitting	0/5	0/5	0/5

These data would suggest that neither virus is a significant pathogen of cherry. However, we advise caution as this was not an exhaustive study of a) all potential scion and rootstock combinations, b) was conducted under greenhouse conditions, and c) did not examine potential interaction with other more prevalent viral pathogens of cherry, such as *Prune dwarf virus* or *Little cherry virus 2*. On the other hand, as discussed earlier, both viruses appear to be poorly adapted to cherry cultivars, and thus have difficulty establishing a systemic infection. Maladaptation is important as it reduces the likelihood of disease symptoms occurring in isolation; this may however change should interaction with a co-infecting virus occur.

Vector transmission

The third aspect of these two viruses examined was their vector transmissibility. Both belong to genera, *Luteovirus* and *Fabavirus* respectively, whose members have been shown to be aphid transmissible; as vector type is usually a common factor linking related viruses, aphid were used in this study. Insect transmission of both virus was attempted using black cherry (*Myzus cerasi*) or green peach (*M. persicae*) aphids. Neither species was able to transmit NSPaV to either sweet cherry (*P. avium*) or peach (*P. persica*) seedlings, despite using both 24hr and 72hr virus acquisition periods.

Similarly, both species failed to transmit PrVF to sweet cherry seedlings; peach plants were not used for PrVF transmission as so far it has only been detected in sweet cherry. For PrVF we used a short acquisition period as fabaviruses are transmitted a non-persistent manner in which long feeding times can reduce the efficiency of virus transmission. Screening of a small sample of green peach aphids after the acquisition period by PCR was negative, suggesting no virus was acquired. These data may indicate neither species of aphid is appropriate vector for NSPaV or PrVF, or more simply, that these two viruses are not vectored by aphids. However, the titer of these two viruses in cherry, as reported earlier, suggests another possibility. While endpoint PCR was performed prior to transmission to ensure that the plants were virus positive, subsequent real-time PCR results suggest that the titer of each was simply too low for the aphids to successfully acquire enough virus to transmit. From the Ct values we may estimate that the average tissue section has between 1-10 virus copies present, whereas most successfully aphid transmitted viruses maintain titers of 100-1000 fold higher. Based on this result we conclude that these two viruses are unlikely to be transmitted to neighboring trees or orchards at a significant frequency.

Detection

Finally, during the course of this study two additional genomes each for NSPaV and PrVF were generated via high-throughput sequencing. These sequences, combined with publically available genomes allowed us to build an alignment to examine genetic diversity within these two viruses, and identify regions of the genome suitable for assay design. Also taken into consideration was the extremely low titer of both of these viruses in cherry, the decision was taken to design real-time PCR assays, as this platform provides greater speed and sensitivity than endpoint PCR.

Taqman-based real-time assays were designed against the GP3 fusion protein (bases 3104-3194) of the NSPaV genome, and against the RNA1 polyprotein (bases 3140-3266) of PrVF; PrVF RNA2 showed considerable divergence between isolates and was not considered for assay design. Both real-time PCR assays were optimized for reaction time, annealing/extension temperature, and magnesium concentration against known positives held in the CPCNW collection. Unfortunately due to the paucity of extant NSPaV, and to a lesser extent, PrVF isolates, the two assays have not been completely validated; this will be completed prior to publication. The assays were found to be highly sensitive, detecting trace amounts of their respective target viruses in infected tissue during the host range and cherry virus symptom (Figure 1) experiments described earlier.

Summary

This study, on the biology and detection of two recently discovered viruses infecting stone fruits, *Nectarine stem pitting-associated virus*, and *Prunus virus F*, revealed that neither virus is widespread in the Washington cherry industry, and unlike the Western X phytoplasma for example, do not infect wild cherry relatives and thus appears to lack reservoir species. While both viruses could infect commercial cherry varieties, they appear to have significant difficulty doing so, exhibited through scattered distribution in the plant and low virus titer. These factors likely contributed to a lack of successful aphid transmission in this study, and suggest that its ability to spread may be limited. Similarly, neither virus was observed to produce visible symptoms on Bing on two different rootstocks, indicating that they do not, unlike *Little cherry virus 2*, present a significant threat to the Washington cherry industry, with the caveat that this was not an exhaustive study on all possible scion-rootstock combinations or stresses (environment, coinfection with other viruses, etc) that could induce disease. Finally, through this study we were able to produce a Taqman-based real-time PCR assay for each virus, allowing sensitive and accurate detection and continued monitoring.

Literature Cited

Bag, S., Al Rwahnih, M., Li, A., Gonzalez, A., Rowhani, A., Uyemoto, J. K., & Sudarshana, M. R. (2015). Detection of a new luteovirus in imported nectarine trees: a case study to propose adoption of metagenomics in post-entry quarantine. *Phytopathology*, *105*(6), 840-846.

Villamor, D. E. V., Mekuria, T. A., Pillai, S. S., & Eastwell, K. C. (2016). High-throughput sequencing identifies novel viruses in nectarine: insights to the etiology of stem-pitting disease. *Phytopathology*, *106*(5), 519-527.

Villamor, D. E. V., Pillai, S. S., & Eastwell, K. C. (2017). High throughput sequencing reveals a novel fabavirus infecting sweet cherry. *Archives of virology*, *162*(3), 811-816.

EXECUTIVE SUMMARY

This project focused on examining the biological impact and risk of spread of two newly reported viruses infecting stone fruit, *Nectarine stem pitting-associated virus* (NSPaV), and *Prunus virus F* (PrVF). Four objectives were proposed, three examining the biology of these two viruses: host range, symptom expression, and vector transmission, and a final objective to develop diagnostic assays for future detection and diagnosis.

We found that while both viruses are present in Washington state, neither is widespread in commercial cherry orchards, with incidences of 1.8 and 11% for NSPaV and PrVF respectively, and is limited to select counties. While both viruses can infect sweet cherry species, virus titer is low and both exhibit a scattered distribution in plants. Interestingly, neither virus was able to infect the wild cherry relatives *Prunus emarginata* (bitter cherry) and *P. virginiana* (choke cherry), suggesting that these are not reservoir species, reducing the risk of spread. Stunting and reduced fruit size in the field could not be conclusively associated with these two viruses and no symptoms were observed on Bing cherry on either Mazzard or Gisela-6 rootstock in greenhouse experiments, indicating that these not likely significant pathogens of cherry, though should continue to be monitored. Aphid transmissibility of these two viruses was not observed experimentally using two different species, the black cherry (*Myzus cerasi*) and green peach (*M. persicae*) aphids. This is likely due to low virus titer in the source plants, but may also indicate that another species is the vector. Finally, through this study we were able to produce a Taqman-based real-time PCR assay for each virus, allowing sensitive and accurate detection and continued monitoring.

In summary, based on this data neither virus appears to be an emerging threat to the cherry industry, with no identified reservoir species, and a limited ability to spread. Using the tools developed in this project, continued monitoring of the incidence of these two viruses is encouraged, as is scouting and visual inspection to confirm that no symptoms that can be associated with these viruses are emerging. There are two areas that deserve further investigation however, to see whether either of these viruses interacts with economically significant pathogens of cherry, such as *Little cherry virus 2* or *Prunus necrotic ringspot virus*, and to further examine their ability to cause disease of a wider range of scion and rootstock combinations, and under different environmental conditions.

FINAL PROJECT PROPOSAL

Project Title: Sources of primary cherry powdery mildew inoculum - revisited

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Cooperators: Ms. Neusa Guerra[3] (WSU-IAREC, Prosser), Washington and Oregon State Growers

[1] Research lead on fruit quality aspects and identification of volatiles of objectives 2
[2] Research lead on modeling component of objective 2
[3] Technical assistant

Total Project Request: **Year 1:** 24,872 **Year 2:** 25,040 ~~**Year 3:** 31,219~~

Other funding sources
None

Budget 1
Organization Name: WSU-IAREC Contract Administrator: Lisa Bruce
Telephone: 509-786-2226 **Email address:** lisa-bruce@wsu.edu

Item	**2016**	**2017**	~~**2018**~~
Salaries			
Benefits			
Wages[4]	4800	4800	~~4800~~
Benefits	480	480	~~480~~
Equipment			
Supplies[5]	6000	6000	~~6000~~
Travel[6]	2500	2500	~~2500~~
Miscellaneous			
Plot Fees			
Total	13780	13780	~~13780~~

Despite an extensive two-year study, we found no evidence of alternative sources (to chasmothecia) of primary inoculum for the initiation of powdery mildew epidemics. The untimely passing of Dr. Yan Wang also precludes accomplishment of objective 2. Therefore, 2018 funding will not be requested.

Budget 2
Organization Name: **OSU-MCAREC** **Contract Administrator:** Russ Karow
Telephone: 541-737-4066 **Email address:** Russell.Karow@oregonstate.edu

Item	2016	2017	~~2018~~
Salaries[1]	4,584	4,722	~~4864~~
Benefits[2]	1008	1038	~~1069~~
Wages			
Benefits			
Equipment			
Supplies[3]	5,000	5,000	~~5,000~~
Travel[4]	500	500	~~500~~
Miscellaneous			
Plot Fees			
Total	11,092	11,260	~~11,433~~

Footnotes:
[1]300hr for a Biological Science Tech. at $15.28/hr. 3% increase is factored into Year 2 and 3.
[2]OPE: 22% of the wage, with a 3% annual increase.
[3]Supplies: renting and maintaining cold rooms; GC/MS supplies including gases (helium, nitrogen, hydrogen, air, and standard gases), gas tank rental, and chemicals; shipping fees.
[4]Travel to field

Budget 3
Organization Name: WSU-AgWeatherNet **Contract Administrator:** Lisa Bruce
Telephone: 509-786-2226 **Email address:** lisa-bruce@wsu.edu

Item	2016	2017	~~2018~~
Salaries[1]			~~4,620~~
Benefits			~~1,386~~
Wages			
Benefits			
Equipment			
Supplies			
Travel			
Miscellaneous			
Plot Fees			
Total			~~6,006~~

Footnotes:
[1]Dr.Melba Salazar

Significant findings

- The stem cavity (stem-fruit attachment zone) is the primary infection court for the fungus. Fruit infections are initiated in this area. If the cavity is unavailable for the fungus (in our study it was sealed with Vaseline), fruit did not become infected, even if disease pressure was high (100% disease incidence in the tested orchard). Mycelium radiates away from the stem cavity towards the cheeks and down to the stylar end. (Figure 2, Table 1)

    - If the stem cavity is sealed early during the growing season, fruit development is impaired. However, knowing how fruit infection starts has major implications for future disease management strategies.

- Attachment of conidia to the leaf is a quick process (about 2h). With the exception of Sweetheart. Here, conidia attached nearly instantly (Figure 1).

    - The importance of leaf properties should be investigated. For example, it is known that hydrophobicity of the host substrate plays a major role in conidial attachment and germination success; younger leaves are more hydrophobic than older leaves. This may explain why young leaves are much more susceptible to infection than older leaves (ontogenic resistance). Observational evidence from work in the breeding program also showed that some "odd" looking cherry leaves were not (or less) infected by the fungus.

- Overwintering of powdery mildew as mycelium on cherry seeds is unlikely. Overwintering of powdery mildew as chasmothecia followed by ascospore release in the spring is the only known means of initiating an epidemic (Grove 1998).

    - Survival rate of cherry pits after overwintering on the orchard floor is low (less than 2%, on average). With the onset of winter (December), no conidial viability could be detected on cherry pits. No powdery mildew infection was observed on seedlings, even if the seed came from a previously infected cherry.

Objectives

1. Characterize the role of cherry fruit in fungal life and disease cycle

2. Identify cherry volatiles and characterize their impact on spore germination

Due to the tragic passing of Dr. Yan Wang this objective has been cancelled and it has been decided to end the study in 2017 (and not 2018).

3. Monitor flag shoot like development and post-harvest fruit management in commercial orchards in WA and OR

Objective 1

Conidia adhesion assays. Conidial adhesion (attachment) is the first interaction between fruit and pathogen. Upon contact with a host surface, fungal conidia secrete a liquid exudate that tightly binds the conidia to the host. This process is time and substrate dependent and needed to protect the fungal conidia from being removed by wind and rain. The time required for conidial adhesion to leaf surfaces was assessed on Bing, Black Republican, Early Robin and Sweetheart. Briefly, circular leaf disks were cut from a clean leaf and gently dipped onto an actively sporulating powdery mildew culture. After various time periods (0 to 8 min, 15, 45, 60, 75, 90, 105, 120, 135, 150, 180, 201, and 240 min) two inoculated leaf disks were removed and gently immersed into 1ml of water. The number of conidia transferred from the leaf disk in the water was quantified. The leaf disk was allowed to air-dry and adhesive tape was used for sampling of conidia from leaf disk surfaces for subsequent microscopic analyses. The proportion of conidia found in the water was compared to the proportion of conidia found on the leaf disk for each time point. In theory, once the spore has successfully attached to the leaf surface, it should not be easily removed by water. Hence, the proportion of conidia found in the water should decrease and the proportion of conidia found on the leaf disk should increase over time. This general trend can be observed for all but Sweetheart (Figure 1). In all trials, the proportion of conidia attached to Sweetheart leaves was greater than the proportion of conidia flushed into the water starting the moment conidia were brought into contact with the leaf surface. In the other cultivars, conidia were first detected on leaf surfaces after 1 min (Black Republican and Early Robin) and 5 min (Bing). The proportion of conidia retained on the leaf increased over the 2h time period. It has been shown that with increasing leaf age the hydrophobicity of the leaf decreases significantly, which could be a factor for the increased susceptibility of young leaves to powdery mildew infection (Bringe, Schumacher et al. 2006). All leaf disks used in this study were the same age to account for hydrophobicity. Still, attachment of powdery mildew conidia to Sweetheart leaf disks was very rapid and it would be interesting to investigate the leaf properties of this cultivar and compare it to other cultivars. Observational evidence from work conducted in the breeding program points toward an effect of leaf properties and ability of the fungus to cause disease.

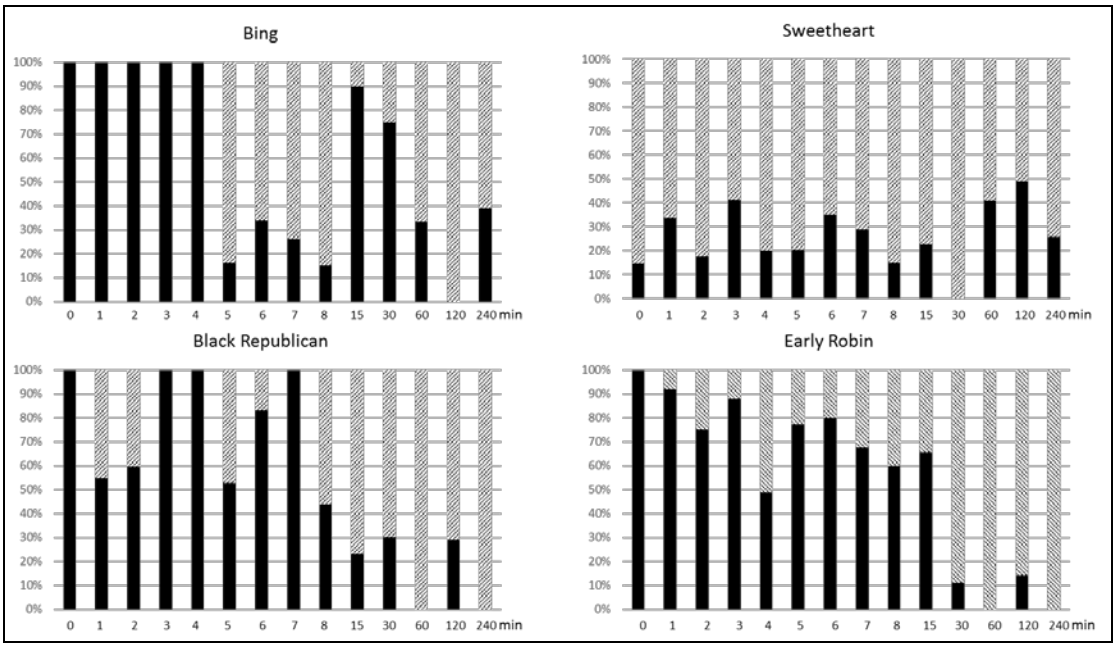
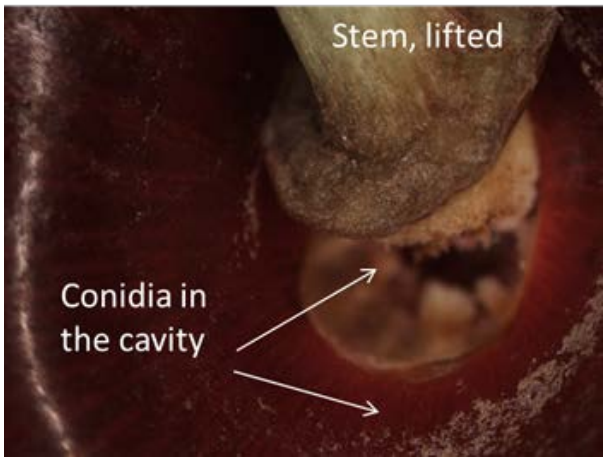


Figure 1. Proportion of conidia flushed off into the water (black bar) versus retained on the leaf (stripped bar) after various time periods (0 min to 2h).

Stem-fruit attachment area. Previous observations pointed toward the stem-fruit attachment area (from here on referred to as stem cavity) as the preferred powdery mildew infection court. The experiment was designed to establish the importance of this specific region during initiation of fruit infection. Sets of cherry clusters were uncovered and the fruit bowl was sealed off with Vaseline. Fruits were left uncovered and exposed to natural, airborne conidia. Negative control clusters remained covered all season. Cherries were evaluated for powdery mildew incidence and severity at harvest using a 0-3 severity scale (Calabro 2007). The results of this study were spectacular. Vaseline treated fruit (as well as the always covered negative control) remained disease free during both study years. This clearly shows that the primary infection court for the fungus is the stem cavity and that infection do not usually start on the cheeks or the stylar end. A possible explanation could be an easier access point for the fungus to penetrate the fruit epidermis. The cavity also provides a natural hideout for conidia, which can usually be seen (with the microscope) if the stem is lifted from the cherry. However, fruit development was significantly impaired in fruit receiving Vaseline treatment on May-21 and somewhat impaired in fruit treated on June 4. Only fruit treated on June 19 developed without impairment. Disease management strategies should take this into account.



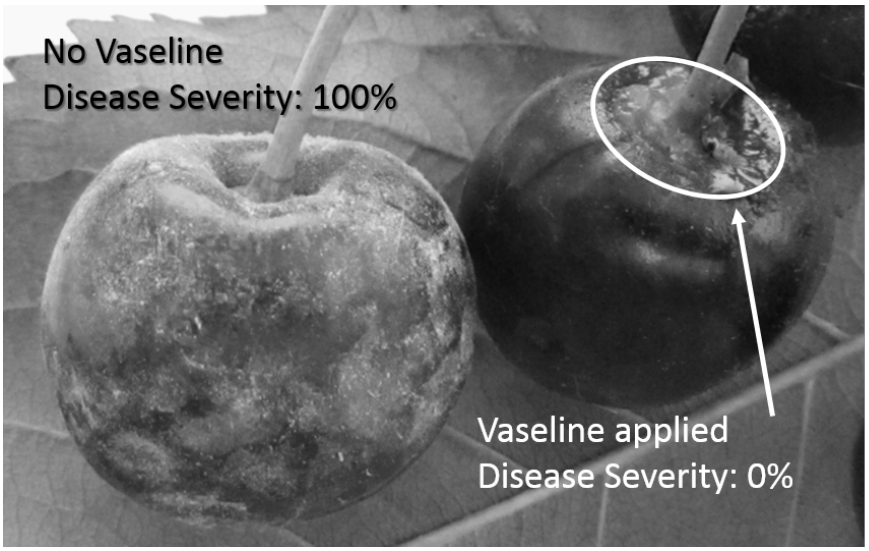


Figure 2. Two Sweetheart cherries from the same cluster. The cherry on the left was left untreated; the cherry on the right had Vaseline applied to the stem cavity on June 19. Onset of fruit infection was June 26 – 28 (2017).

Table 1 Powdery mildew (PM) development on cherries treated with Vaseline

		Severity[2] scale				July 25, 2017	
		0	1	2	3		
Bing	**Date^**	**0**	**1-33%**[2]	**34-66%**	**>67%**	**PM % Incidence**[3]	**Avg PM Severity**[4]
Experiment 1[1]	21-May	8	0	0	0	0	0
	4-Jun	12	0	0	0	0	0
	19-Jun	16	0	0	0	0	0
	UTC[5]	6	9	10	25	88	2.1
Experiment 2	21-May	12	0	0	0	0	0
	4-Jun	16	0	0	0	0	0
	19-Jun	12	0	0	0	0	0
	UTC	0	2	22	26	100	2.5
Experiment 3	21-May	8	0	0	0	0	0
	4-Jun	20	0	0	0	0	0
	19-Jun	11	0	0	0	0	0
	UTC	0	0	8	42	100	2.8
Sweetheart	**Date^**	**0**	**1-33%**[2]	**34-66%**	**>67%**	**PM % Incidence**[3]	**Avg PM Severity**[4]
Experiment 1	21-May	15	0	0	0	0	0
	4-Jun	12	0	0	0	0	0
	19-Jun	9	0	0	0	0	0
	UTC	6	2	12	26	87	2.3
Experiment 2	21-May	9	0	0	0	0	0
	4-Jun	16	0	0	0	0	0
	19-Jun	12	0	0	0	0	0
	UTC	0	0	10	40	100	2.8
Experiment 3	21-May	8	0	0	0	0	0
	4-Jun	16	0	0	0	0	0
	19-Jun	12	0	0	0	0	0
	UTC	0	4	8	38	100	2.7

^ Day Vaseline was applied to the stem-fruit area

[1] Single tree experiments. One tree per experiment.

[2] % fruit surface area affected by powdery mildew
[3] Proportion of fruit diseased.

[4] Severity was averaged based on the following formula: $\bar{x} = \frac{\sum_{i=1}^{5} C_i \cdot N}{\sum_{i=1}^{5} N_i}$ where: $\bar{x}$ = weighted average of infection severity, C_i = infection severity [1, 2, 3], N_i = number of samples sorted into severity of infection
[5] UTC = Untreated control. Never bagged. Natural disease occurrence.

Shoot assays. After harvest, infected cherries (cvs 'Bing' and 'Sweetheart') were sorted into four categories (0 = no infection,1 = 1-33% fruit surface area colonized; 2 = 34-66% fruit surface area colonized; 3 = >66% fruit surface area colonized). Cherries free of powdery mildew were used as a negative control. To simulate overwintering, 100 cherries per category will be buried just below the soil surface in the experimental cherry orchard at WSU-Prosser. Cherries harvested from commercial orchards (The Dalles, OR) were also left in the respective orchard for the winter. Germinated seedlings were recovered the following spring, individually potted and grown in isolation in a greenhouse. Percentage of seedlings developing flag shoots were recorded. Not a single seedling grown from overwintering pits developed powdery mildew. It has to be mentioned that the survival rate (measured as germination rates) of cherry pits in the orchard was below 10% at WSU and below 5% in The Dalles. This study did not give any indication that powdery mildew survives as mycelium on cherry pits.

Fungal activity was measured on overwintering cherry seeds. Briefly, 3x 20 seeds from decaying, previously infected fruit were obtained from the orchard floor (WSU) in the middle of October, November and December. Seeds were washed in sterile water and subjected to the PMA viability assay. The assay was able to detect minute amounts of the fungus in October and November with viability of conidia below 2%. No fungal viability was detected in December.

Objective 3
The possibility of powdery mildew surviving on cherry pits in the orchard was monitored in commercial orchards in Oregon and Washington. Surveys started with the onset of vegetative tree growth and ended when irrigation was turned on. First irrigation is linked to ascospore dissemination, which is the common route of powdery mildew overwintering. Briefly, nine transects (10 trees x 10 trees each) were evaluated for on-ground shoots and overwintering pits were collected from the orchard floor surrounding one random tree in the transect. The number of seeds was recorded and seeds were evaluated for fitness. Empty seeds were discarded and healthy seeds were germinated. Seedlings were grown in isolation in a greenhouse. Occurrence of powdery mildew on seedlings was noted. **No early season flag shoots were detected** in any participating orchard or in seedlings grown in the greenhouse. All orchard floors had a vigorous occurrence of root shoots (98-99%) compared to true seedlings (1 to 2%). The low occurrence of seedlings is also explained by the low viability (2%) of seeds found on the orchard floors after the winter. Powdery mildew started growing on root shoots. However, the source of the powdery mildew on root shoots could not be determined and most likely was related to ascospore release from overwintering chasmothecia (given shoot proximity to bark fissures).

Management strategies ranged from frequent or moderate chemical root shoot removal ('burn-back') to general mowing. Overall, the orchards with frequent root shoot removal had the least amount of powdery mildew disease. Mowing seemed less effective since root shoots located close to the base of the trees were not removed. In all cases, orchard floor management stopped shortly after harvest. Root shoots growing during this period frequently (in 8 out of 9 orchards) were infected with

powdery mildew. This infection spread to the trees likely resulting in the production of chasmothecia (‘Next Year’s mildew’).

Taken all of these observations into account, survival of powdery mildew on seeds and the successful growth of both the seed and the fungus in the spring may be an event with a very low incidence and likely no commercial importance.

Executive Summary:

What we know so far about the infection process of cherry fruit:
In general, fungi explore their habitat with rapidly-growing, sparsely-branched hyphae, then, when some of those hyphae find a nutrient resource, the extension rate declines, rate of branching increases, and the mycelium captures and exploits the resource, from which it subsequently send out a new generation of exploratory hyphae and/or populations of conidia are produced. It is important to recognize that fungal growth is dynamic. One part of a mycelium may be growing as a rapidly extending, sparsely branched exploratory sector, another part may be a highly branched and interconnected network exploiting a nutrient resource, while a third region maybe actively involved in formation of conidia (mode of dispersal). Conidia (spores) are produced at greater quantities when nutrient become limited.

On cherries, conidia accumulate on cherry fruit surfaces throughout the cherry growing season. Once powdery mildew shows up on leaves*, powdery mildew can be detected on the fruit. The early fruit infection is invisible but characterized by a steady increase in fungal mass throughout the cherry growing season. At this point, the fungus is still resting and not actively growing. The increase of fungal mass on cherries is likely due to increasing foliar infections and airborne conidia concentrations that steadily land on fruit. The onset of visible fruit infection is sudden and not related to fruit maturity. Once the fruit infection has begun, it can be found on all cultivars. In order for a cherry to become infected, the conidia have to be located in the stem cavity area. Those conidia will germinate and produce hyphae to explore and feed at an exponential rate and in a pattern that produces the characteristic morphology of the mycelium. Mycelium radiates away from the stem cavity towards the cheeks and down to the stylar end, and not in reverse order. This is also contrary to leaf infection. Here, several powdery mildew colonies may appear on different locations and/or upper or lower leaf surface, grow which eventually leads to a fusion of these colonies. Also, while leaf infections start with a few infected leaves which constantly re-infect new leaves, cherry fruit seem to become (visibly) infected simultaneously. The exact trigger for fruit infection to start is not known, but could be related to a change of fruit volatiles or be a density-dependent process. Even though abundant fungal mycelium is produced on cherry fruit, it is likely not associated with overwintering (asexual form of overwintering). The main route of overwintering remain chasmothecia (sexually produced overwintering structures) which harbor ascospores that become released in the spring (Grove and Boal 1991). The start of first irrigation, wetness and temperature are main factors in such releases, which have been described by Grove (1998). Managing the production of chasmothecia post-harvest is still essential to contain next season's epidemic. However, since such productions occur post-harvest, powdery mildew management has usually ceased in the orchard.

** For reference: In 2015 and 2016, foliar powdery mildew started 30 days after full bloom (dafb) and 14dafb in 2017*

Literature cited
Bringe, K., C. F. Schumacher, M. Schmitz-Eiberger, U. Steiner and E.-C. Oerke (2006). "Ontogenetic variation in chemical and physical characteristics of adaxial apple leaf surfaces." Phytochemistry **67**(2): 161-170.

Calabro, J. M. (2007). Biology of sweet cherry powdery mildew, Oregon State University.
Grove, G. G. (1998). "Meteorological factors affecting airborne conidia concentrations and the latent period of Podosphaera clandestina on sweet cherry." Plant disease **82**(7): 741-746.

Grove, G. G. and R. J. Boal (1991). "Overwinter survival of Podosphaera clandestina in eastern Washington." Phytopathology **81**(4): 385-391.

FINAL PROJECT REPORT

Project Title: PM viability during postharvest handling of cherry fruit

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Cooperators: Fred Scarlett (Northwest Fruit Exporters), Dave Martin (Stemilt Growers LLC), David Anderson (Northwest Fruit Exporters), Mike Willett (Northwest Horticultural Council), Neusa Guerra (WSU-IAREC), Zirkle Fruit in Prosser

Total Project Request: **Year 1:** $ 62,507 **Year 2:** $ 57,987

Other funding sources: None

Budget 1
Organization Name: WSU-IAREC **Contract Administrator:** Hallie Faulk
Telephone: 509-786-2226 **Email address:** prosser.grants@wsu.edu

Item	2015	2016	2017
Salaries[1]	$ 34,620	$ 36,005	No-cost extension
Benefits	$ 14,887	$ 15,482	
Wages			
Benefits			
Equipment[2]	$ 5000		
Supplies[3]	$ 6500	$ 5000	
Travel[4]	$ 1000	$1000	
Miscellaneous [5]	$ 500	$ 500	
Plot Fees			
Total	$ 62,507	$ 57,987	

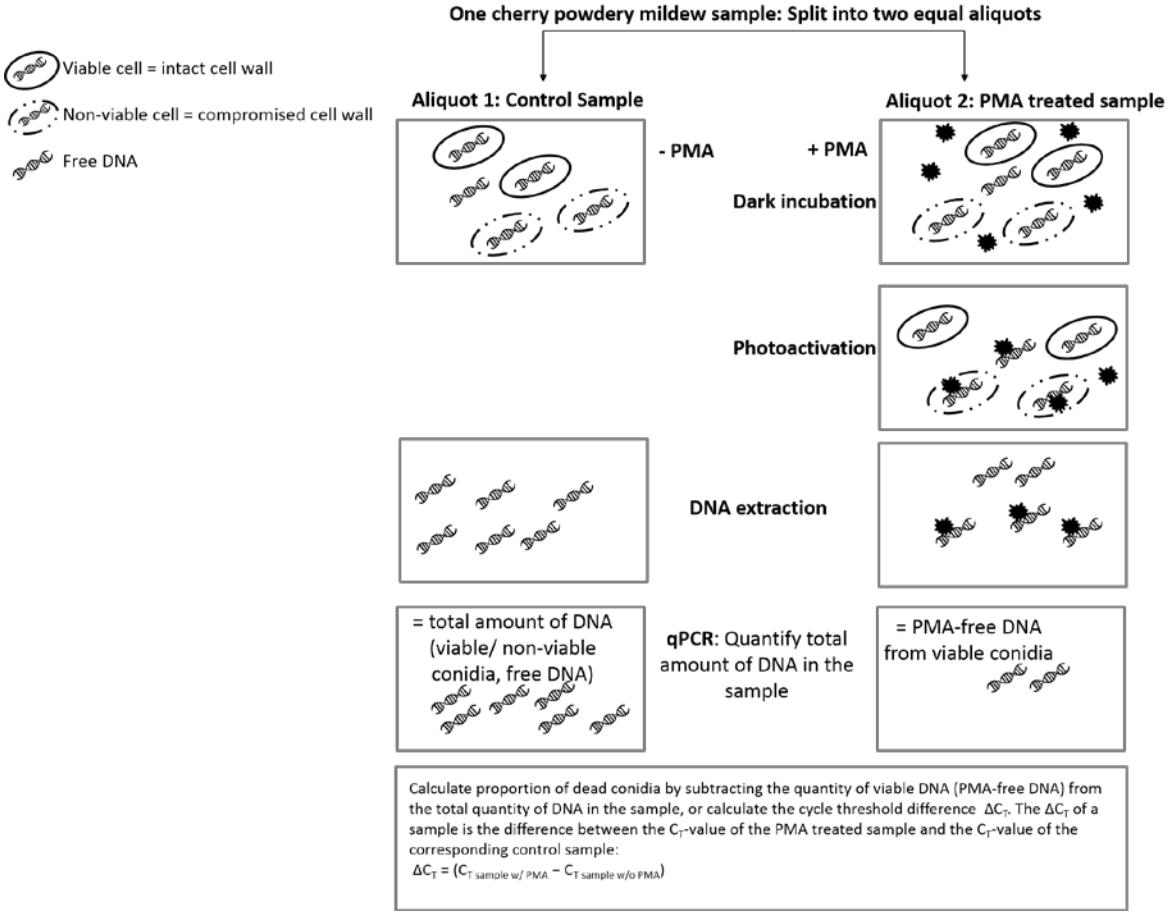
Footnotes:
[1]Associate in research
[2]PMA-Lite™ LED photolysis device, orbital plate shaker, multichannel precision pipettes)
[3]Reagents and material (anhydrous glycerol, DNA extraction kits, qPCR related and general lab supplies, Nitex cloth)
[4]industry wide travel to collect cherry fruit during various post-harvest handling stages
[5] shipping cost of cherry fruit during Washington State off-season to allow extended season research

Objectives

1. Development and validation of a robust viability assay using propidium monoazide (PMA) in conjunction with quantitative PCR to distinguish between viable and non-viable inoculum (conidia) of *Podosphaera clandestina*, the causal agent of cherry powdery mildew.

Recap: Propidium monoazide qPCR (PMA-qPCR) provides an indication of viability based on membrane integrity and the detection of nucleic acids. It can therefore be used for the detection of intact viable cells within a sample. Briefly, the sample which will be analysed is pre-treated with a nucleic acid binding dye (PMA that is considered to be membrane impermeable. During the treatment process the dye is upon photoactivation able to bind to extracellular (free) DNA or DNA from cells with compromised cell walls/membranes. This effectively removes the bound DNA from the sample as it cannot be amplified during the subsequent qPCR. This assay has therefore successfully been used to differentiate between intact and dead cells.

Several methodological parameters may affect PMA efficiency. Among them, 1) the concentration of the dye and 2) the length of photoactivation and of dark incubation appear to be key parameters. The purpose of this objective was to evaluate the efficacy and accuracy of the PMA qPCR procedure to determine conidia viability of cherry powdery mildew (PM). The general PMA qPCR workflow:



2. Quantify and monitor inoculum viability and identifying latent periods on sweet cherry fruit during fruit development and following customary post-harvest handling conditions.

Recap: The occurrence of powdery mildew (*Podosphaera clandestina*) on mature fruit severely limits export opportunities for growers and marketing entities. To date, we have not much insight on powdery mildew viability on harvested fruit, before and after methyl bromide fumigation prior to export, etc. This objective measured powdery mildew viability post-harvest (after methyl bromide fumigation) using the aforementioned PMA viability method.

Significant Findings

- Even if invisible to the eye, the amount of conidia (conidia) found on developing cherry fruit increases significantly week by week as soon as powdery mildew shows up on the leaves (Figure 1). The percentage of conidia that are viable on apparently healthy fruit is small (less than 10%, on average) but increases significantly at the onset of visible infection (up to 60% in 2016). Controlling the invisible increase of conidia mass on developing fruit with preventative fungicides seems imperative.

- Fumigation leads to a significant decrease of conidia viability. However, killing efficacy nearly never reaches 100%. Surviving conidia are capable of producing new infections on susceptible leaves (fresh leaves and fumigated leaves).

- Viability of conidia on fumigated cherries decreased to 0% after 3 days post fumigation (Table 7).

    - ***HOWEVER: The same trend was found for mildew survival on pruned branches. Therefore, it cannot be stated that the loss of viability was due to a delayed effect of the fumigation treatment.***

- Fumigated leaves support powdery mildew growth from both fumigated and fresh powdery mildew colonies. This shows that fumigation leaves a small percentage of viable conidia that can establish new infections if susceptible host tissue is present. Host susceptibility is not reduced by methyl bromide gas.

- Hydrocooling reduced the amount of powdery mildew conidia on fruit surfaces by an average of 56% (range from 44 to 68%). The greatest decrease of viability was achieved through a combination of cold storage and hydrocooling followed by fumigation (Table 6).

Results and Discussion for Objective 1

PMA™ = phenanthridium, 3-amino-8azido-5[3-(diethylmethylammonio)propyl]-6-phenyl dichloride. PMA solution (20mM in H_2O) can be purchased from the manufacturer (Biotium Inc., Hayward, CA). PMA can be diluted in sterile distilled H_2O to produce 2mM and 10mM stock solutions. Stock solutions should be aliquoted and stored in brown (light impermissible) microcentrifuge tubes at -20˚C until needed.

Validation of protocol to distinguish between viable and non-viable *P. clandestina* conidia. The discrimination between viable (live = conidia with an intact cell wall) and non-viable (dead = conidia

with damaged cell walls) conidia is one key element of the here described viability qPCR methodology. The proper discrimination has to be validated for the organism of interest. Therefore, a *P. clandestina* conidia suspension was freshly prepared and kept at room temperature under constant agitation on a standard laboratory magnetic stir plate. Immediately, aliquots (400µl) were pipetted into sterile 1.5ml light transparent Eppendorf tubes. Tubes were labelled 'Dead-PMA', 'Dead-Control', 'Live-PMA' and 'Live-Control'. *P. clandestina* conidia in tubes with the prefix 'dead' were killed by heat treatment for 2h at 85˚C in a standard laboratory water bath. Loss of viability was also examined by inoculating susceptible sweet cherry leaf disks (cv. Bing) with the heat-killed *P. clandestina* conidia suspensions. Leaf disks were incubated alongside a positive (+ viable conidia) and negative (+ sterile water) control for 21 days at 20°C in a diurnal growth chamber.
All tubes with the suffix 'PMA' were subjected to PMA treatment while tubes with the suffix 'Control' were left untreated. DNA extraction followed by qPCR was performed to calculate cycle threshold (C_T) values. The results of the dead and live conidia examination can be found in Table 1. The change measured in the viable population ("Dead-PMA" minus "Live-Control") is approximately 99.9% indicating that the heating protocol killed all conidia. These results are concordant with results from the leaf disk assay in which no growth had been observed from the heat killed conidia suspension. In order to demonstrate total recovery of conidia and DNA in the test, comparison of "Dead-Control" and "Live-Control" treatments were evaluated. The difference in C_T was small (mean = 1.0), indicating good recovery of all of the conidia/DNA. Finally, the comparison of the "Live-PMA" minus "Live-Control" indicates that a small part of the initial population of conidia were dead before heat treatment. Since *P. clandestina* is an obligate biotroph it can only be cultured on its host. By the time fungal growth is sufficient to carry out experiments (about 21 days), a mixture of old, possibly dead, and young, very viable conidia have formed on the leaves. The results of the "Live PMA" (which reflects the DNA from the intact/viable conidia) minus "Live Control" (which reflects the DNA from all conidia in the suspension) show this natural development. Overall, results indicate that the protocol is very well suited to differentiate between viable and non-viable conidia of *P. clandestina*.

Table 1. Mean cycle threshold (C_T) differences and standard deviation (STD) for viable (live) and heat killed (dead) *Podosphaera clandestina* conidia. Assay conditions: 10 min dark incubation, 20 min photoactivation, 20µl final PMA concentration

	Cycle threshold difference (ΔC_T)		
	Dead PMA - Live Control	*Dead Control - Live Control*	*Live PMA - Live Control*
Experiment 1	8.2	-0.6	3.1
Experiment 2	14.5	3.6	4.6
Experiment 3	10.7	0.04	6.1
Experiment 4	16.1	0.9	3.1
Mean	**12.4**	**1.0**	**4.2**
STD	3.6	1.9	1.5

Final PMA concentration: 20µM (e.g. add 4µl of the 2mM PMA stock solution to 400µl conidia suspension); **Dark Incubation interval:** 10 minutes (room temperature), **Photoactivation interval:** 20 minutes exposure to blue light emitting diodes (LED)

General protocol to evaluate sweet cherry powdery mildew viability on cherry fruit:
Step 1: Fruit sampling and washes. Thirty to forty cherries (per replicate) can be submerged in 500ml sterile water (1 L Pyrex bottles) containing 0.001% Tween20, a mild non-ionic surfactant and a dispersant to help distributing conidia evenly in the liquid. Bottles should be immediately placed on

a rotary shaker at 300rpm for 5 to 10 minutes to rinse the fungus of the fruit surface. The resulting wash solution will contain conidia from the sample. Immediate sample processing is recommended.

Step 2: Filtration, PMA treatment, and DNA extraction. Each cherry wash solution is filtered through a 12-micron polycarbonate membrane using vacuum assisted filtration. Filter size (12 micron) is small enough to retain powdery mildew conidia contained within the wash solution and big enough to separate out bacterial and smaller fungal conidia, which will pass through the filter. The filter is removed aseptically with the help of sterile forceps, rolled loosely with the conidia containing side facing inward and placed into an 5mL Eppendorf tube containing 2mL of 0.001% Tween water. The tube is vortex vigorously for 2 min (e.g. on a horizontal vortex) to dislodge conidia from the filter membrane. Remove filter and discard. Vortex tube again and split the liquid into two 1.5mL clear (light permissible) centrifuge tubes (800µl per tube). Label tubes. The first tube will serve as the control sample to measure the total amount of DNA in the sample (see: general PMA qPCR workflow). Place this tube in the freezer until DNA extraction. The second tube will be treated with PMA to determine how many conidia in the sample are viable. Add 8µl of the 2mM PMA stock solution to 800µl conidia suspension under low light to achieve a 20mM final PMA concentration. Gently shake the tube and place in a dark box (or drawer) for a 10 min dark incubation period. Mix by inverting the tube halfway through the incubation period. After the incubation, gently mix the tube again and expose the conidia suspension to blue light emitting diodes (LED) using the PMA-Lite™ LED Photolysis Device (Biotium Inc., Hayward, CA) to activate and permanently cross-link the PMA dye to all accessible DNA. As a direct consequence of the irreversible crosslinkage, the targeted DNA cannot be amplified during the subsequent qPCR step. DNA extractions can be performed using any routine protocol (e.g. the MoBio UltraClean Microbial DNA isolation kit from MoBio Laboratory Inc., Carlsbad, CA) following the manufactures' protocol.

Step 3: qPCR assay.

A primer pair specific for *P. clandestina* and optimized for the use with qPCR was developed previously by Calabro (2007). The forward primer sequence: 5'CTCCACCCGTGTGAACTGA. The reverse primer sequence: 5'GAGGTCATCCAAAATATATGTGT. Quantitative real-time PCR (qPCR) assays can be performed, e.g., on a LightCycler 480 system (Roche, Indianapolis, IN) using 96-well plates in a total reaction volume of 20 µl. Each reaction consists of: 10 µl of SYBR Green mastermix (e.g. PerfeCTa® SYBR® Green FastMix® from Quanta Biosciences, Beverly, MA), 1µl of each primer at 400 nM final concentration, 3µl of PCR grade water and 5µl of template DNA. Each well of the 96-well plate is loaded manually with 20µl of the reaction mix and sealed with an adhesive cover before running the qPCR assay. A positive control with a known concentration of purified *P. clandestina* DNA and a no template control (NTC) are added to each assay for quality control. The qPCR assay is carried out according to the following protocol: Pre-incubation at 95°C for 10 min; 45 PCR cycles at 95°C for 10 s, 58°C (primer specific annealing temperature) for 20 s, 72°C for 30 s with fluorescent data collection; and a cooling period of 10 sec at 40°C. Melting curve analysis of the PCR products is conducted following each assay to confirm that the fluorescence signal originated from specific PCR products and not from primer-dimers or other artefacts. DNA samples, including unknowns, positive and NTC, are tested in duplicates. Results are recorded only if no signal was detected from the negative control. The instrument software can be used to calculate the threshold cycle (C_T) values using the second derivative method.

Standards are included in each qPCR assay in order to calculate the DNA concentration of the unknown samples. Purity and DNA concentration of the standards can be determined with a NanoDrop spectrophotometer (NanoDrop products Wilmington, DE). Five standards are prepared using a 10-fold dilution series. DNA from each dilution (tested in triplicates) serves as a template to construct a standard curve. The threshold cycle (C_T) values, linear regression coefficient (R^2), line equation and PCR efficiency (E) are calculated with the instrument software. The efficacy of the qPCR should be at least 90%.

Data analysis. At the end of each qPCR run, the amount of DNA per sample and the C_T value for each sample will be calculated by the instrument. C_T values are inversely proportional to the amount of DNA in the sample. Typically, C_T values below 29 cycles show abundant DNA, and C_T values above 38 cycles indicate minimal amounts. To evaluate the effect of PMA treatment on a sample, the cycle threshold difference (ΔC_T) is calculated. The ΔC_T of a sample is the difference between the C_T value of the PMA treated sample and the C_T value of the corresponding control sample: ΔC_T = (C_T sample w/ PMA − C_T sample w/o PMA). In general, a ΔC_T of about 3.6 is equivalent to a 10-fold difference in the concentration of the amplified organism.

Results and Discussion for Objective 2

Invisible conidia on developing fruit.
In 2017, clusters of developing fruit (cv Sweetheart) were removed weekly to assess natural disease pressure (Figure 1). During the duration of the sampling, cherry fruit were not visibly infected with powdery mildew. Hence, the natural disease pressure described in Figure 1 reflects the invisible increase of conidia (expressed as the amount of fungal DNA found on the cherries) up until the visible onset of PM signs in the orchard (June 26 - June 28). With the start of the foliar epidemic (May 24) in the orchard, the amount of conidia found on fruit increased significantly week after week. This increase was only halted during June 14 and June 21, where the amount of conidia found on fruit was significantly similar to each other. The ΔC_T difference between week 0 (May 17) and week 6 (June 28) is 6.0 (26 – 20 = 6.0) which equals an approximate 1000-fold increase in powdery mildew mass. This indicates that there were 1000 times more conidia on fruit on June 28 compared to May 17 (Figure 1). During the invisible infection time, powdery mildew is not active on fruit; viability is less than 10%. Hence, the increase of conidial quantities observed on developing fruit resulted from a continuous deposition of airborne conidia produced on infected leaves. However, most conidia deposited on the fruit do not remain viable. Viability of conidia increases significantly with the onset of visible fruit infection (fungus starts to grow and sporulate).

This confirms our findings from 2016 where the increase of powdery mildew conidia viability on fruit was measured. There was a 168-fold increase in sporulation (expressed as the amount of DNA quantified by qPCR) between June 18 and July 1, 2016 (Figure 2). Only 10% of conidia found on fruit were viable on June 18. This number increased to 62% by the beginning of July (Figure 2). In general, viability on developing cherry fruit was 10% or less on asymptomatic fruit. Once fruit becomes infected, conidia viability increases. Since powdery mildew colonies are dynamic and composed of mycelium and conidia (differing in age) conidia viability never reaches 100%. In times of rapid growth (visible ongoing infections) conidia viability is also greatest. However, even during latent periods (invisible infections), a small percentage of conidia always remain viable (between 2 and 10%). Controlling the increase in conidia through preventative fungicide sprays or manipulation of the orchard environment seems imperative. Spray coverage is key, conidia deposited on fungicide treated surfaces do not attach, and consequently, quantities are reduced.

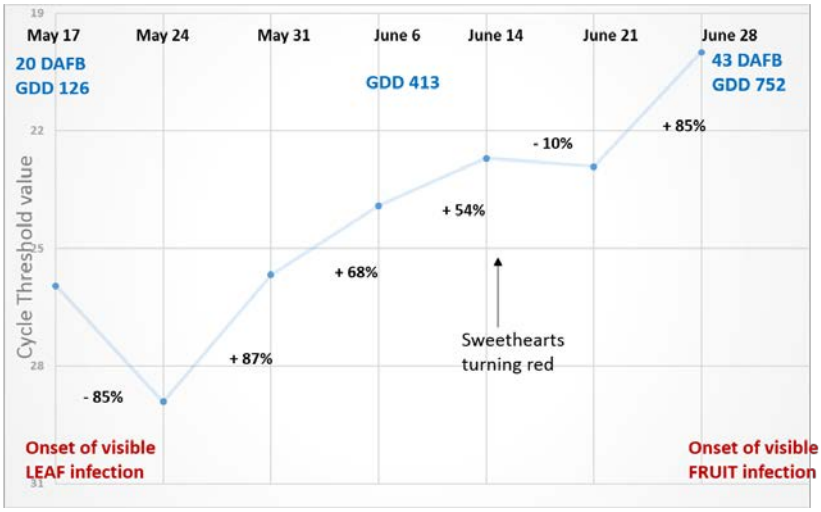


Figure 1. 2017: 6-week increase of conidia on developing Sweethearts at WSU-Prosser. Fruit were asymptomatic for powdery mildew (healthy looking) until June 28. Cycle threshold (C_T) values reflect the average quantity of DNA from *P. clandestina* conidia obtained from 60 cherries (15 cherries per rep, 4 reps). Lower C_T values indicate high amounts of DNA, while higher C_T values mean lower amounts of DNA. The values displayed next to the graph indicate % increase (or decrease) compared to the previous week. The biofix to calculate Growing Degree Days (GDD) was January 1st. DAFB refers to Days after Full Bloom.

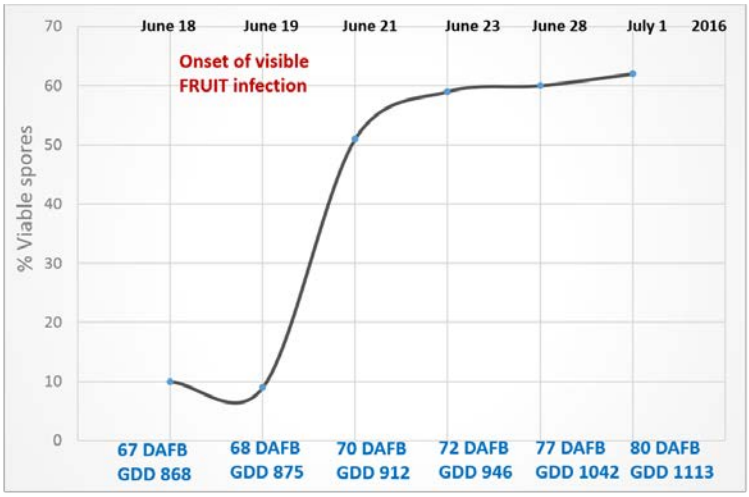


Figure 2. 2-week increase of powdery mildew conidia viability on Sweethearts in 2016. Cherries were grown at WSU (Prosser, Roza station, block D51). The onset of visible infection started between June 19 and June 21, 2016 (GDD 868-875). The onset of foliar infection started May 11 (GDD 399). Biofix for GDD is January 1st.

RESULTS related to Methyl Bromide Fumigation

Hydrocooling and conidial viability. The average time span for cherries to undergo commercial hydrocooling is about 3 minutes. This study investigated the effect of the chlorine found in the hydrocooling water on powdery mildew conidia viability. The results showed that a 24h exposure of powdery mildew conidia to hydrocooling water had no significant fungicidal effect. No decrease in viability was noted. However, hydrocooling is a very forceful event during which the fruit is in contact with the chilled water. We replicated the 3 min hydrocooling process in the lab by simply submerging the fruit in chilled hydrocooling water, moving them gently. As a result of this pre-fumigation fruit wash, quantities of conidia on sweet cherry fruit surfaces was reduced by an average of 56%.

Conidia survival after methyl bromide (MB) fumigation

Fumigation chamber protocol (for fruit intended to ship to Australia):
Chamber Temperature: 47°F (to less than 51°F)
Dosage of MB: 4.5 pounds of MB per 1000 cubic feet or 72 grams/ M^3
Cubic foot volume of chamber: 29,366

In 2016, the effect of fumigation on conidial survival was studied. Fruit were harvested at the experimental orchard (WSU, Roza, block D51) the day of the fumigation and transported to Zirkle Fruit in the late afternoon. Fruit were contained in a perforated, open box or in a single layer on a perforated plastic tray. Fumigation was conducted overnight. The next morning fumigated cherries were retrieved, bagged immediately to prevent cross-contamination and transported to the lab. Fumigated cherries were exclusively handled in a laminar flow hood (positive air flow). Samples were processed the same day. There was significant variation in the reduction of conidia viability observed in bulk processed cherries (cherries in a box). Viability ranged from 81.8% to 98.8% (Table 2 and Table 5). When analyzed layer by layer, cherries sampled from the middle layer (layer 3) had significantly less dead conidia after fumigation (Table 2). In the same study, removing the stem from the cherry prior to fumigation had no significant effect. However, there was a trend that stem removal increased the efficacy of fumigation which is likely due to a sheltering effect of the stem to the conidia. Fumigation was less successful on cv 'Sweetheart' compared to cv. 'Bing' cherries (mean reduction of 87.5 versus 99.3% with stems attached and 86.7 versus 99.6% with stems removed) (Table 2).

Table 2 Efficacy of methyl bromide in reducing powdery mildew viability on cherry fruit fumigated in bulk (box) or single layers (tray)

			Run[a] 1	***Run 2***	***Mean***
Trial	**Cultivar**	**Position in box**	**% dead conidia after fumigation**		
Box - Trial 1	Bing	Random	96.4	97.2	96.8
Box - Trial 2	Bing	Top	96.8	98.0	97.4
		Layer 1	98.5	99.9	99.7
		Layer 2	96.4	89.3	94.0
		Layer 3	92.7	50.5*	81.0
		Layer 4	96.8	99.3	98.5
		Bottom	97.5	99.8	99.3
		Mean	**96.5**	**89.5**	**98.0**
			Run1	***Run 2***	***Mean***
Trial	**Cultivar**	**Stem^**	**% dead conidia after fumigation**		
Tray- Trial 1	Bing	+	94.4	93.6	94.0
Tray- Trial 2	Bing	+	99.6	99.4	99.5
Tray- Trial 3	Sweetheart	+	87.9	87.1	87.5
		Mean	**97.8**	**97.2**	**97.5**
Tray- Trial 2	Bing	-	99.7	99.5	99.6
Tray- Trial 3	Sweetheart	-	87.1	86.2	86.7
		Mean	**94.4**	**93.8**	**94.1**

[a] Each sample was tested in duplicates in two independent qPCR analyses (Run 1 and Run 2). The reported value is the average of three replicates (30 cherries each) per sample (per tray or per layer in the box).
^ The stem was either removed (-) or left attached (+) to the fruit before fumigation.
* Value is statistically different from the others

As can be seen in Table 3, the fungicidal effect of methyl bromide on single cherries varies. For some cherries, none (0%) or few (0.1%) of conidia survived the gas treatment while survival rates on other cherries reached 30%. Since cherries from Experiment 1 and 2 were randomly chosen from a box, higher survival rates may be explained by the position of the cherry in the box as could be seen in Table 2. The same variability was seen for cherries fumigated in bulk (box) where survival ranged from 1% to 16.7% and for cherries fumigated in a single layer on a tray where survival ranged from 3.6 to 12.9%.

Results in Table 5 show the effect of cold storage and hydrocooling followed by fumigation on conidia survival. **The sole process of submerging cherries in hydrocoolant water for 3 min before fumigation reduced the average conidia number by 56%.** Both cold storage and hydrocooling reduced conidia viability even without fumigation. The combination of cold storage, hydrocooling and fumigation resulted in the largest amount of dead conidia (99.8 to 100%) in cherries fumigated on a tray (Table 4) and 92.2 to 99.4% in cherries fumigated in a box (Table 5).

Table 3 Fungicidal effect of methyl bromide viability measured on single sweet cherries

	Experiment 1 ^	***Experiment 2***^	***Experiment 3***~
Workflow: Orchard →Fumigation	**Outcome: % dead conidia after fumigation**		
Tray, single layer[a]	96.4	90.6	87.1
Box, multiple layers (bulk) [a]	93.6	99.0	83.3
Cherry 1	**70.3***	98.9	100 [b]
Cherry 2	97.7	99.9	99.9
Cherry 3	92.3	98.3	99.9
Cherry 4	99.8	**72.2***	99.7
Cherry 5	99.3	99.1	95.9
Cherry 6	72.2	99.6	89.3a
Cherry 7	99.3	99.4	91.7
Cherry 8	97.2	99.6	99.1
Cherry 9	99.1	99.6	99.6
Cherry 10	100.0	99.8	n/a
Cherry 11	99.8	99.9	n/a
Cherry 12	99.9	87.9	n/a
Cherry 13	99.9	99.4	n/a
Cherry 14	97.0	97.8	n/a
Cherry 15	99.9	99.9	n/a
Cherry 16	99.5	87.9	n/a
Mean (single cherries)	**95.2**	**96.2**	**97.2**
STD (single cherries)	9.6	7.5	4.1

[a] Mean of three replicates (tray) or five replicates (box). Each replicate contained 30 cherries.

^ Single cherries were obtained randomly from a bulk sample (fumigated in a box)

~ Single cherries were fumigated in an open clamshell container, n/a – not available

* Value is statistically different from the others

In 2016, we observed that conidia harvested from fumigated cherries were not able to establish infections on fresh, susceptible leaves even though conidia viability was never reduced to 0%. One

possible explanation could be that the effect of the gas has a delayed effect on membrane integrity. A similar effect has been observed for radiated conidia (e.g. UV radiation) during which the conidia dies but the cell membrane stays intact for a period of time before the cell disintegrates. In 2017, the focus of the fumigation study was to confirm or refute this effect on fumigated conidia. To this end, cherries were fumigated and subsamples were processed for up to 3 days post fumigation starting immediately after fumigation (like in the previous year) and every 24h thereafter. The results are shown in Table 6: Fumigation significantly reduced conidia viability (96.2 to 99.8% conidia dead after fumigation). Viability slowly declined within the following 48h and reached 0% after 72h (3dp fumigation) in all three experiments. However, if methyl bromide fumigation indeed kills all conidia and the % viable conidia detected by the PMA assay are dead conidia with an intact cell membrane but no cell function, then these fumigated conidia should not be able to infect susceptible leaves (as was observed in 2016). To this end, branches with vigorously growing powdery mildew colonies were cut from orchard trees, kept in water and fumigated. Fumigated powdery mildew inoculum was used in leaf disk assays using a) fresh, susceptible leaves, and b) fumigated, susceptible leaves. As a comparison, non-fumigated powdery mildew conidia were used to inoculate a) fresh, susceptible leaves, and b) fumigated susceptible leaves. The four combinations tested were: Fumigated Inoculum/Fumigated Leaves = double negative; Fumigated Inoculum/ Fresh Leaves; Fresh Inoculum/Fumigated Leaves; Fresh Inoculum/ Fresh Leaves = positive control. Powdery mildew grew in all leaf disk assays regardless whether the fungus or leaves were fumigated. ***It can be concluded that fumigation does not kill all the conidia but leaves a small percentage of survivors that are capable of initiating disease if presented with a susceptible host. Also, Fumigation does not affect leaf susceptibility***. A useful reminder: A 99.9% reduction would reduce 1,000,000 conidia to 1,000 conidia. A 99.99% reduction would reduce 1,000,000 down to 1 conidia. A single conidium is enough to initiate an area wide epidemic.

Table 4 Comparative fungicidal effect of stem cold storage and hydrocooling followed by methyl bromide fumigation on powdery mildew viability

Workflow: *Orchard*	*→ Pre-Treatment*			*→ Fumigation*	***Outcome:*** ***How many conidia are still alive?***		
Harvest cherries	**Stem**[a]	**Cold**[b]	**Hydrocooled**[c]	**Fumigated**[d]	**Experiment 1**	**Experiment 2**	**Experiment 3**
					% dead conidia before fumigation		
Control[e]	+	No	No	No	64.1	53.6	78.5
Control	+	**Yes**	No	No			99.9
Control	+	No	**Yes**	No	91.2	82.2	
Control	+	**Yes**	**Yes**	No			99.5
					% dead conidia before fumigation		
Fumigated	+	No	No	**Yes**	90.6	87.1	
Fumigated	-	No	No	**Yes**	99.4	86.2	
Fumigated	+	**Yes**	No	**Yes**	40.0		98.8
Fumigated	-	**Yes**	No	**Yes**	43.8		94.0
Fumigated	+	No	**Yes**	**Yes**		98.2	99.9
Fumigated	-	No	**Yes**	**Yes**		94.0	99.8
Fumigated	+	**Yes**	**Yes**	**Yes**		99.9	99.9
Fumigated	-	**Yes**	**Yes**	**Yes**		100.0	99.8

[a] The stem was either removed (-) or left attached (+) to the fruit before fumigation.
[b] Cherries were stored in a cold room (4°C, 39°F) for 4 to 6 hours before fumigation.
[c] Cherries were submerged in chilled hydrocooler water (39°F) for 3 min before fumigation.
[d] Cherries were fumigated in a single layer on a plastic tray.
[e] Control was harvested, treated and evaluated. Reflects viability of conidia on orchard fruit before fumigation.

Table 5. Efficacy of methyl bromide (alone or in combination with cold storage and hydrocooling) in reducing powdery mildew viability on sweet cherries fumigated in bulk (perforated, open box)

	Experiment 1 *No Pre-treatment*[b]	***Experiment 2*** *No Pre-treatment*[b]	***Experiment 3*** *No Pre-treatment*[b]	***Experiment 4*** *+ Cold & Hydrocooling*[c]	***Experiment 5*** *+ Cold & Hydrocooling*[c]
	% dead conidia before fumigation				
Control[a]	90.1	64.1	50.5	78.5	72.2
Position in box	**% dead conidia after fumigation**				
Top layer	98.0	77	56.4	77.0	100.0
Middle layer	99.1	74	79.8	99.9	99.6
Bottom layer	99.3	94.4	79.8	99.6	98.7
Mean[d]	**98.8**	**81.8**	**72.0**	**92.2**	**99.4**
STD[d]	**0.7**	**11.0**	**13.5**	**13.1**	**0.7**

[a] Control was harvested, treated and evaluated. Reflects viability of conidia on orchard fruit before fumigation.
[b] Cherries went straight from the orchard to fumigation
[c] Cherries were stored in cold storage (39°F) for 6 hours and submerged in chilled (37°F) hydrocoolant water for 3 minutes before fumigation.
[d] Mean of 3 reps with 30 cherries each per layer; combined Mean of 270 cherries per box; STD = Standard Deviation

Table 6. Time-course development of conidial viability after methyl bromide fumigation

	Experiment 1		***Experiment 2***		***Experiment 3***	
	ΔC_T[a]	% dead[a]	ΔC_T[a]	% dead[a]	ΔC_T[a]	% dead[a]
Control, pre-fumigation^	1.5	**61.7**	2.0	**72.2**	1.0	**47.2**
0 dp fumigation*	8.7	**99.6**	5.1	**96.2**	9.6	**99.8**
1dp fumigation	11.4	**99.9**	6.9	**98.8**	11.5	**99.9**
2dp fumigation	9.3	**99.7**	7.9	**99.4**	8.8	**99.6**
3dp fumigation	12.5	**100.0**	11.9	**100.0**	12.5	**100.0**

[a] average of 8 replicates with 30 fruit per replicate. $\Delta C_T = (C_{T\ \text{sample w/ PMA}} - C_{T\ \text{sample w/o PMA}})$. In general, a ΔC_T of about 3.6 is equivalent to a 10-fold difference in the concentration of the amplified organism.

^ Percentage of dead powdery mildew conidia found on a freshly picked and visibly contaminated orchard sample (4 reps, 30 fruit each) before fumigation.

* Fumigated samples were picked up at Zirkle Fruit at the end of the fumigation cycle and processed immediately (0dp), or stored at 4°C for 24h (1dp), 48h (2dp) or 76h (3dp) before processing.

Executive Summary:

Conventional methods to identify Podosphaera clandestina, the causal agent of sweet cherry powdery mildew, are based on cultural and morphological characteristics (phenotype), and quantification is mainly based on spore counting or dilution plating. These methodologies are time-consuming, require individual expertise in fungal taxonomy and are not able to differentiate between live and dead conidia. However, the question whether the fungus is dead or alive bears great importance for cherry powdery mildew research. Propidium monoazide qPCR (PMA-qPCR) provides an indication of viability based on membrane integrity and the detection of nucleic acids. It can therefore be used for the detection of intact viable cells within a sample. In this study the PMA-qPCR methodology was optimized, validated and applied to quantify viable cells of *P. clandestina* in naturally infected samples, after various post-harvest handling (cold storage, hydrocooling) of the fruit and after methyl bromide fumigation.

Conidia can be found on fruit with the onset of foliar powdery mildew. Even if invisible to the eye, the amount of conidia found on developing cherries increases significantly week by week. The fungus is inactive during this invisible time of infection and the increase of conidial numbers on fruit is likely due to a constant deposition of conidia originating from infected leaves. Even if foliar infection is well controlled, or not visible in the orchard, airborne propagules can be carried by the wind from nearby, infected orchards. Or infections can be hiding on the orchard floor (unmanaged root suckers) or high up in the upper canopy (if spray regimes do not cover the upper canopy as well as the lower canopy). The fungus has many ways of coming into an orchard. The fungal inactivity on fruit during the invisible infection period is also shown by the small percentage of viable conidia (less than 10%, on average). Conidial viability on fruit increases significantly when infection switch from invisible to visible. Visibility indicates that the fungus is actively growing. Powdery mildew control has to start at the invisible time of infection. Spray coverage is key.

Fumigation leads to a significant decrease of conidia viability. However, killing efficacy nearly never reached 100% and left a small percentage of survivors that were capable of initiating disease if presented with a susceptible host. The greatest decrease of viability was achieved through a combination of cold storage and hydrocooling followed by fumigation. A three-minute immersion in chilled, hydrocoolant water reduced the amount of powdery mildew conidia on fruit surfaces by an average of 56%. The hydrocoolant water itself had no significant fungicidal effect. Methyl bromide did not affect leaf susceptibility; the fungus was able to grow on leaves exposed to the gas for the length of a fumigation cycle. However, conidial viability decreases with time leaving all conidia dead after 72h (post fumigation). The same pattern of conidial die off was seen in pruned (infected) branches left on the orchard floor. Therefore, it cannot be concluded that the decrease in viability was caused by the fumigation but seems to be the natural course of conidial die off on a non-viable host.

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.

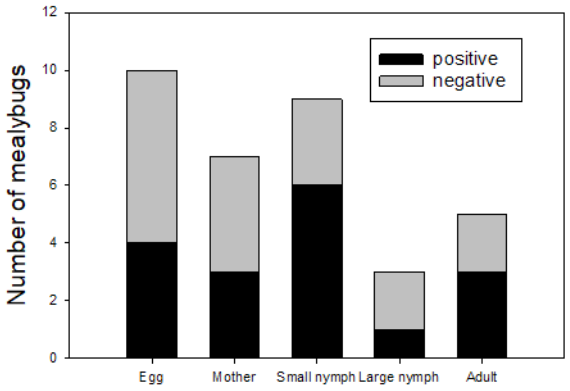


Fig 1. Number of mealybugs (eggs, mothers, small nymph (0.5-1.5 mm), large nymph (2-4 mm), and adult) collected from LChV2 positive trees that tested positive and negative for LChV2

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).

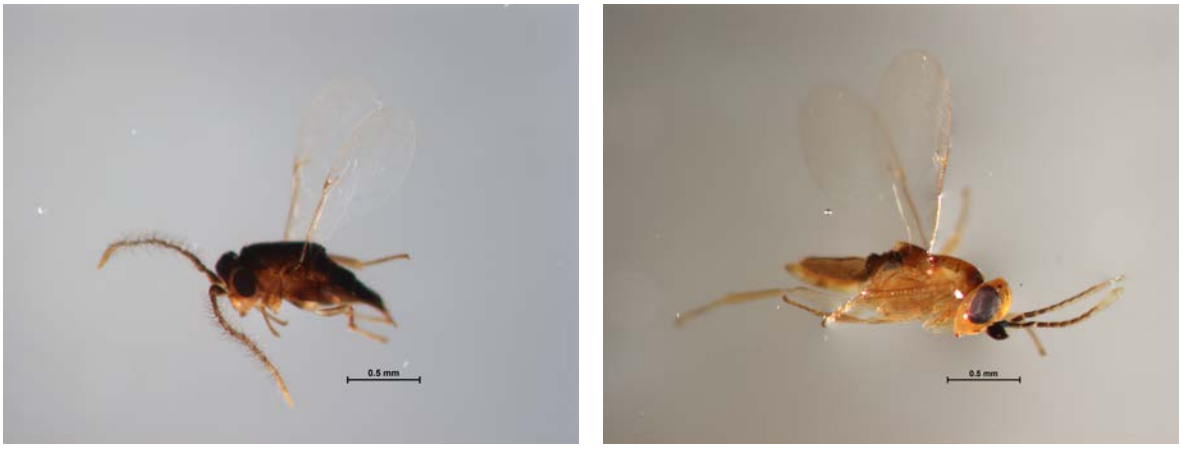


Plate 1. Male (left) and female (right) *Anagyrus schoenherri* (Westwood 1837), parasitoid wasps of apple mealybug.

[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.

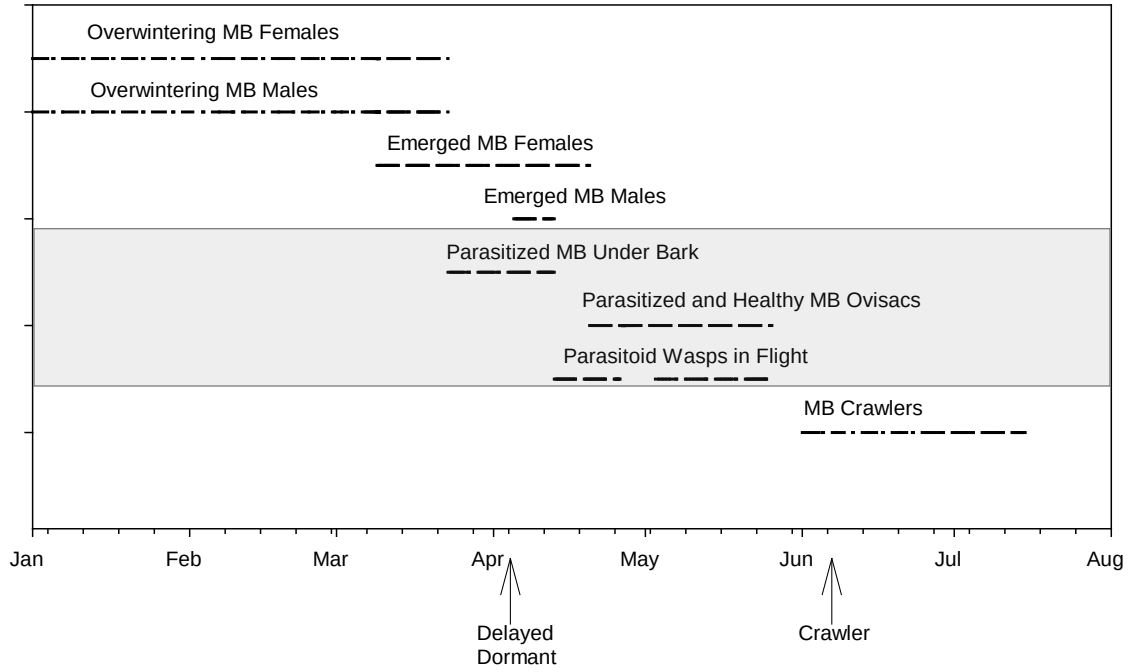


Fig. 2. Apple mealybug phenology observed in an infested apple orchard at WSU Sunrise Orchards in 2016. Parasitoid wasp, *A. schoenherri*, was observed within AMB mummies as well as in flight (shaded area).

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.

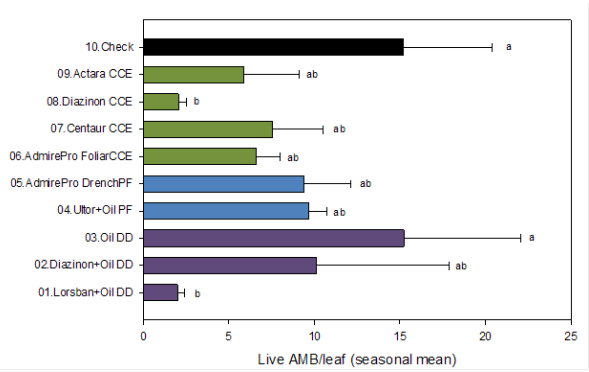


Fig. 3. Effects of compounds applied at delayed dormant (DD), petal fall (PF) and at crawler emergence (CE) on AMB numbers in an infested apple orchard at WSU Sunrise Orchards, 2014

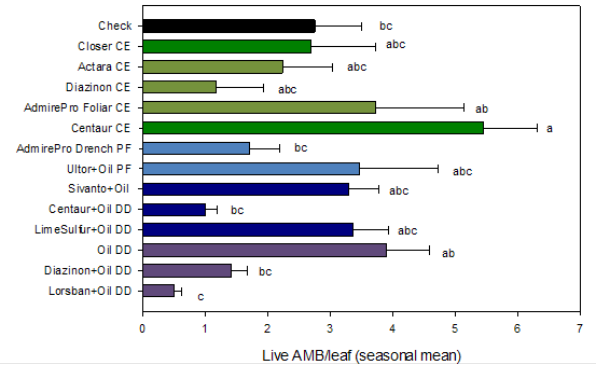


Fig. 4. Effects of compounds applied at delayed dormant (DD), petal fall (PF) and at crawler emergence (CE) on AMB numbers in an infested apple orchard at WSU Sunrise Orchards, 2015

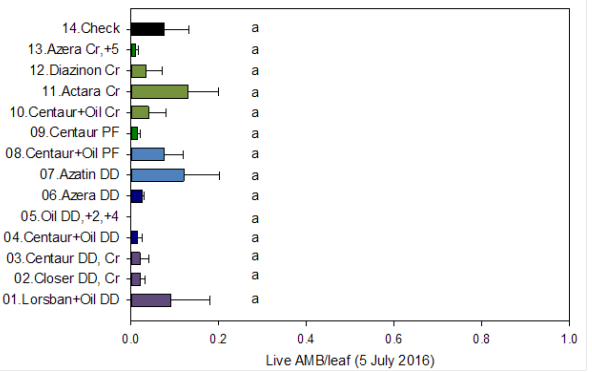


Fig. 5. Effects of compounds applied at delayed dormant (DD), petal fall (PF) and at crawler emergence (Cr) on AMB numbers in an infested apple orchard at WSU Sunrise Orchards, 2016

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.

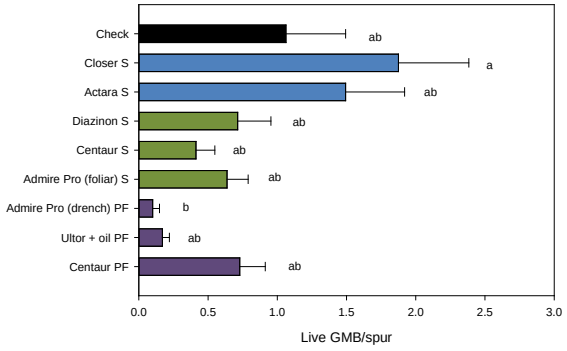


Fig. 6. Effects of compounds applied at petal fall (PF) and at crawler stage (S) on GMB numbers in commercial Bing cherry orchard, 2015

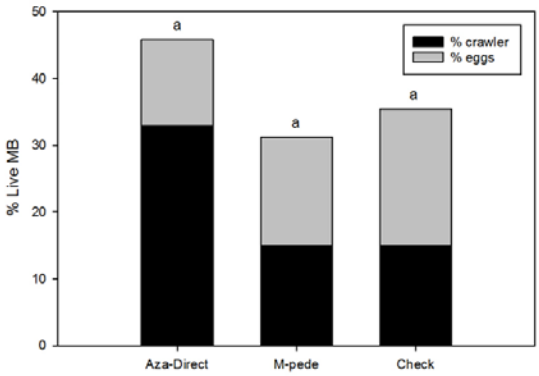


Fig. 7. Percent live GMB crawlers and viable eggs in egg masses treated with organic compounds, Rock Island, WA, 2016

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.

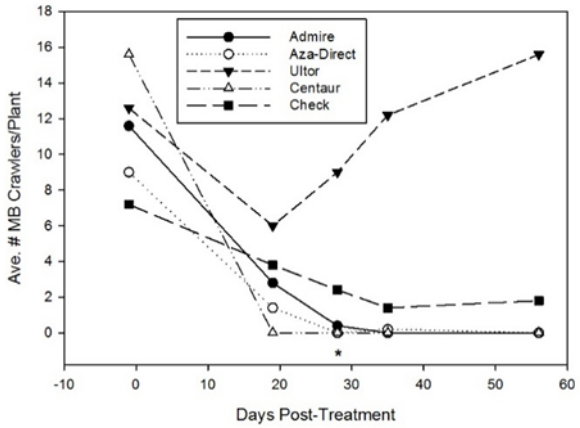


Fig. 8a. Effects of foliar- and drench-systemic compounds and an insect growth regulator on the average number of GMB crawlers/plant over time. *significant reduction in avg. crawlers/plant compared to check, 2016

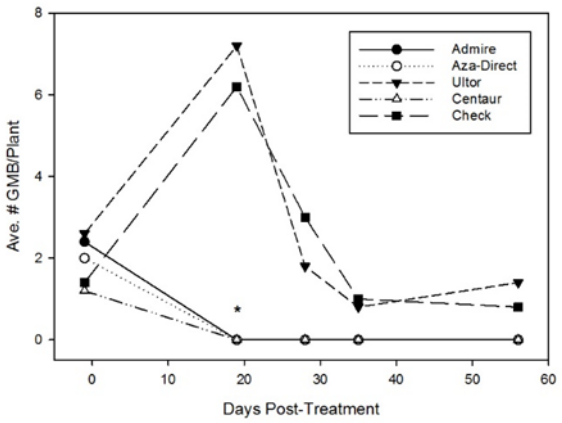


Fig. 8b. Effects of foliar- and drench-systemic compounds and an insect growth regulator on the average number of GMB adults/plant over time. *significant reduction in avg. adults/plant compared to check 2016

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.

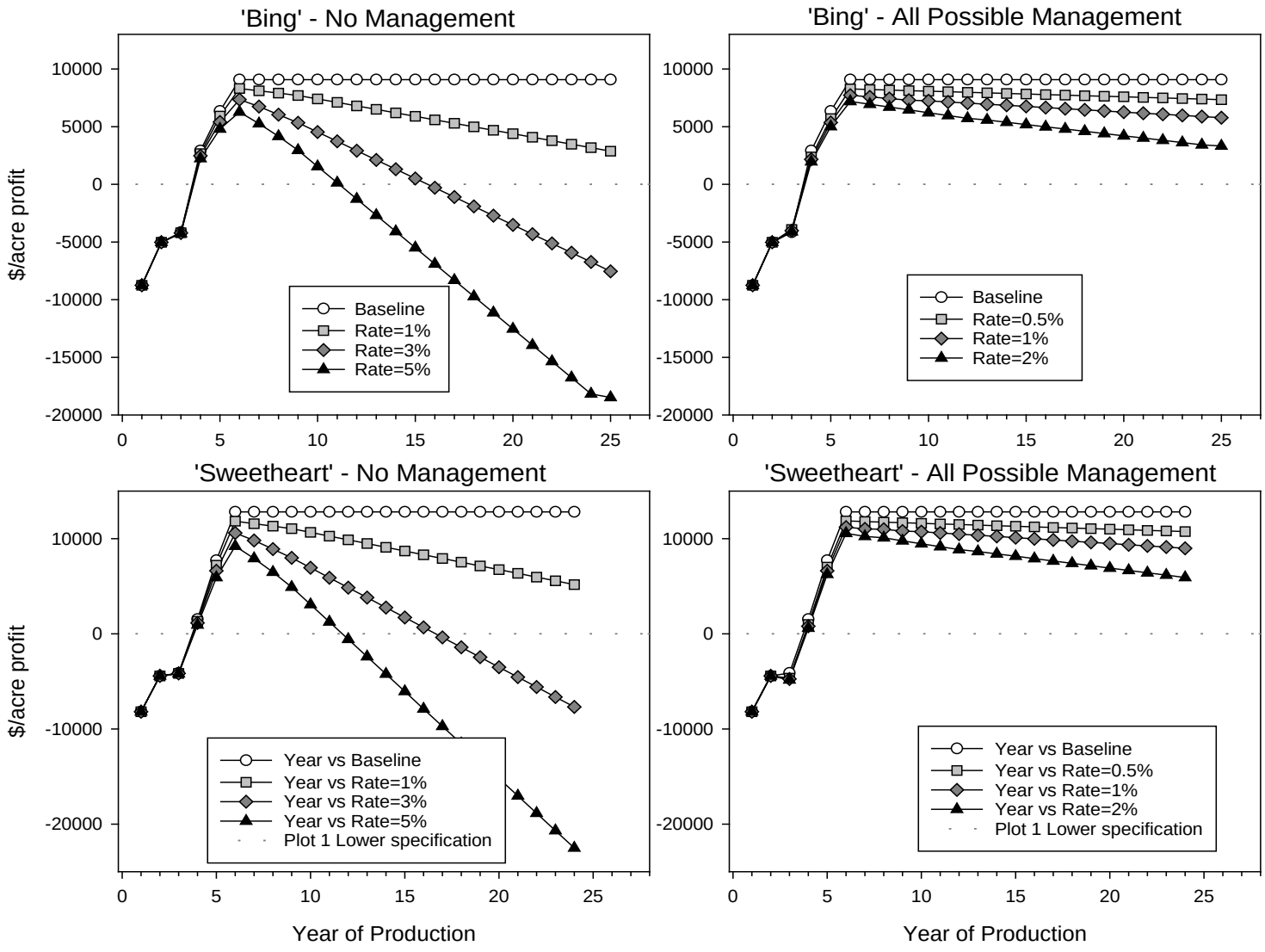


Fig 9. Comparison of estimated annual profits under different LCD scenarios for ‘Sweetheart’ cherries.

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.

WSDA SPECIALTY CROP BLOCK GRANT PROGRAM
FINAL PERFORMANCE REPORT

Grant Agreement Number: K1511

Report Submitted By: Elizabeth Beers

Note: This document is required to be written in the third person.

Project Summary

1. Provide a background for the initial purpose of your project. Include the specific issue, problem, or need that was addressed by the project.

Little cherry disease (LCD) is a serious virus-caused disease of sweet cherry, which has been present at low levels in Washington (WA) State since the 1940s, but became increasingly evident as a state-wide problem during 2011-2013. The disease is incurable, and initially results in unpicked limbs, then tree or orchard removal. Trees with LCD produce cherries of small size and poor flavor, which make the fruit unmarketable. Apple mealybug is the documented vector of a causal pathogen of LCD, little cherry virus 2 (LChV2). This mealybug was recently recorded for the first time on sweet cherry in WA. In 2012, the grape mealybug, a well-established pest of sweet cherry in WA, was shown for the first time to also be a capable vector of LChV2. The absence of recommendations for controlling these virus vectors in WA sweet cherry makes management of LChV2 difficult. Mealybugs are notoriously difficult to kill because they are covered with waxy filaments, and spend most of their life cycle protected in crevices in bark or leaf axils. These life history characteristics make them extremely difficult to reach with foliar insecticides. The first instar (crawlers), the stage most vulnerable to insecticides, emerge over a prolonged time period making multiple applications necessary. This project seeks to address these issues by evaluating chemical control options for LCD vectors. Insecticide options include soil drench and foliar applied systemic insecticides (all registered on cherry) and an insect growth regulator for control of various mealybug life stages.

Correctly diagnosing LCD is challenging because symptoms may take years to appear and can vary depending on the weather. Molecular assays, currently the only tools available to reliably detect LCD in plant material, are too expensive to be cost effective for large-scale testing ($80/tree), therefore, growers typically only test symptomatic trees or a subset of symptomatic trees. Management decisions based on the results of these assays range from the removal of only symptomatic trees to the removal of an entire orchard. The first scenario leaves potentially infected, non-symptomatic trees nearby to serve as a disease reservoir, while the second scenario may be unnecessarily removing healthy trees resulting in a replanted orchard and the associated economic loss. More information regarding the presence and movement of LCD through orchards is necessary to developing a comprehensive management plan. This project also investigated the likelihood of healthy trees becoming infected based on the proximity to previously infected trees or orchard areas and the probability of non-symptomatic trees serving as virus reservoirs.

2. Establish the motivation for your project by presenting the importance and timeliness of the project.

Undetected LChV2-infected trees allowed to remain in an orchard will serve as a disease reservoir and enable spread of LCD to nearby trees or orchards over time. This disease imposes an economic penalty for the cherry grower when small, poorly colored fruit are

either left unpicked or sorted out in the warehouse. Therefore, reducing the spread of LCD by controlling mealybugs, the known vectors of LChV2, and establishing sampling strategies that will optimize finding newly infected trees is important and timely.

3. If the project built on a previously funded SCBGP project, describe how this project complimented and enhanced the previously completed work.
This project was not built on a previously funded SCBGP project.

Project Approach

4. Briefly summarize the activities performed and tasks achieved during the project period. Whenever possible, describe the work accomplished in both quantitative and qualitative terms. Include the significant results, accomplishments, conclusions and recommendations. Describe favorable and unusual developments.

1. Establish colonies of apple and grape mealybug for lab bioassays to test chemical controls. Grape mealybug (GMB) colonies were maintained in vented plastic containers on sprouted potatoes in a growth room (83 °F, 14:10 (L:D), and 34% relative humidity). An apple mealybug (AMB) colony was initiated, but was eventually killed by a parasitoid that was brought in from the field via parasitized females. The emerged parasitoid wasps were identified as *Anagyrus schoenherri* (Westwood, 1837). This identification is the first Nearctic find of this European species. A manuscript entitled, "Discovery of *Anagyrus schoenherri* (Westwood, 1837) (Hymenoptera: Encyrtidae) in the Nearctic region, a parasitoid of the apple mealybug *Phenacoccus aceris* (Signoret, 1875) in Washington State, with notes on the host," is in review for publication in *Pan-Pacific Entomologist*.

2. Complete lab bioassays using various stages of apple and grape mealybug. A greenhouse experiment was conducted on 1-yr-old, potted 'Bing' trees on Mazzard rootstock to determine the effects of foliar and soil-applied systemic insecticides on GMB. Mealybugs from colonies were transferred to greenhouse trees and given 7 days to become established, and then treated with Admire Pro and Aza-Direct as soil drenches. Ultor, a foliar-systemic compound and Centaur, an insect growth regular, were both applied to the point of drip with a backpack sprayer, using an equivalent insecticide concentration of 100 gpa. Counts of GMB crawlers (0.1-0.5 mm in length), nymphs (0.6-2 mm in length), and adults (>2 mm in length) were made 1 day pre-treatment, and 18, 27, 35, and 56 days after treatments were applied. The average number of crawlers, nymphs, adults, and total GMB was calculated for each treatment for each sampling date. Statistical analysis was done by using the difference between the pre- and the post-treatment means for a given date. Data were analyzed using the Statistical Analysis System (SAS 2016). PROC MIXED was used to conduct an analysis of variance, and treatment means were separated using a pairwise comparison of the least-squares means. Average crawler and total mealybug differences were similar for all treatments and all dates (data not shown). Nymph numbers were reduced to zero on Centaur-treated plants at 18 days post-treatment (Figure 1) and close to zero on trees treated with Admire Pro and Aza-Direct at 27 days. Admire Pro, Aza-Direct, and Centaur reduced adult numbers to zero 18 days after application. Ultor did not significantly reduce GMB numbers for any life stage; however the addition of an adjuvant (recommended on the label), might have increased the effectiveness of this product. These results are published in *Arthropod Management Tests*.

3. Locate 8 (4/year) sweet cherry orchards containing variable levels of LCD infected trees. Four sweet cherry orchards with a history of LChV2 infection were located for use in 2015 field studies. Orchard A and C were 'Bing', Orchard B was 'Rainier', and Orchard D was a 'Lapin' orchard. In 2016, four additional sweet cherry orchards, with a history of LChV2 infection were located for field studies. Orchard E and G were 'Rainier' cherries, Orchard F was 'Skeena', and Orchard H was a half 'Chelan' and half 'Bing'. Growers/orchard owners consented to the use of trees (±250/orchard) for leaf and fruit samples, access for fieldmen (for diagnosing visual symptoms), and researcher visits.

4. Collect plant material for molecular assays from 250 trees in 8 (4/year) LCD orchards. Plant material was collected from Orchards A, B, C, and D during 2015 and E, F, G, H in 2016. Different tissues were collected depending on time of season. Sampling periods were spread out over the year to manage the PCR workflow, given the 3-month maximum storage period for samples. Dormant season sampling (January and February) required 8 inches of dormant bud wood collected from each of the tree's leaders (minimum of five bud wood samples per tree). Harvest/post-harvest season sampling (June to September) required 2 leaves from each of 5 leaders, or a total of 10 leaves per tree. Because of the large size of the orchards, it was not possible to sample every tree; therefore 2 categories of trees were sampled: 1) adjacent trees – trees that were directly adjacent to or 15 trees (within or across rows) away from trees that were removed due to previous LChV2 infection and 2) suspect trees – trees showing visual disease symptoms, but outside of the "adjacent tree" area. In 2015, "random" trees were sampled as well; however, no LChV2-positive trees were identified using this method, and it was dropped from the 2016 sampling procedure. Samples (wood or leaf tissue) were then analyzed using PCR.

5. Perform molecular analysis (RNA extraction and PCR) on plant material samples (1,000/year) from LCD orchards to determine virus infection (Jan-May 2015 and 2016). Reverse transcription polymerase chain reaction (RT-PCR) was used to determine LChV2 infection in collected plant material. RNeasy Plant Mini Kits were used to extract and purify RNA from plant material. Molecular analysis of dormant bud wood required extracting phloem from samples using a sterile razor blade, while leaf tissue was processed without alteration. 'SuperScript III One-step RT-PCR System with Platinum Taq DNA Polymerase' was used in combination with known primers specific to LChV2 (developed by Ken Eastwell's lab, WSU, Prosser, WA), for the reverse transcription. PCR products were identified using gel electrophoresis, with known positive and negative controls in each gel.

Molecular analysis for LChV2 identification was completed for all samples for both years. Surprisingly, many trees showing symptoms of LCD had negative PCR results for LChV2; those symptomatic trees were resampled for Western X (WX) phytoplasma, another causal pathogen of LCD.

6. Walk through LCD orchards and categorize trees as symptomatic or non-symptomatic based upon visual symptoms (1-2 weeks before harvest, June-August 2015 and 2016). Trees were individually labeled in a 500-700 tree block, in and around the LCD sample area in each orchard. During the week before harvest, when LCD symptoms are most obvious, a group of fieldmen (experienced in identifying LCD) walked through the block. Based on visual symptoms, fieldmen identified the trees as symptomatic or non-symptomatic. Depending on availability, 3-8 fieldmen walked though individual orchards. All participants walked through an orchard on the same day to avoid differences in symptom appearance that can occur over time. Walkthroughs were performed on two separate dates in orchard H for the two cultivars which ripened at different times. Many of the trees determined by fieldmen

to have visual symptoms of LCD were negative for LChV2 via PCR. Up to 10 symptomatic trees infection in Orchard B, F, E, and H that tested negative for LChV2 were resampled and tested for WX.

7. Collect a 2-lb clamshell of cherries from each tree in a subset of 20 trees (10 symptomatic and 10 non-symptomatic) in each LCD orchard (1-3 days before harvest, June-August 2015 and 2016). A 2-lb clamshell of cherries was collected from a subset of trees including, up to10 positive and 10 negative trees (depending on availability). In most cases, the trees were chosen and the fruit was picked before knowing the LChV2-infection status, thus only visual symptoms could be used as a clue to infection. Therefore, some fruit presumed to be collected from a positive tree was actually from a negative tree, and sample sizes were unequal for positive and negative. Of the four orchards sampled in 2015, collections were made from 6 to 10 positive trees. In 2016, very few trees were LChV2-positve in the chosen orchards, and therefore, collections from positive trees were made in only 2 of the 4 orchards.

8. Perform fruit quality assessments on cherry samples (cherry size, color, and firmness) directly after they are collected (June-August 2015 and 2016). Fruit size and quality (size, color, brix, firmness, acidity) were measured 1-3 days before the start of commercial harvest to determine if LChV2 infection caused changes in fruit quality. These measurements were completed by Stemilt Growers R&D labs. Firmness was measured non-destructively using a fruit firmness tester (FirmTech 2, BioWorks, Wamego, KS). In addition to firmness, this device also calculated fruit diameter and row size (defined as the number of cherries that will fit in a 10.5 inch (276 mm) container (Webster and Looney, 1996)). Brix (% soluble solids) was measured with a refractometer (Atago PAL-1, Kirkland, WA). Titratable acidity (expressed as % malic acid) and pH was measured with an autotitrator (888 Titrando, Metrohm USA LLC, Riverview, FL). Fruit color was measured in 2016. Hue angle was measured with a color meter (Minolta Chroma Meter CR 300), and visual rating of cherry redness (red cultivars only) was conducted with the CTIFL color chart on a scale of 0 (light) to 6 (dark).

Firmness was significantly lower in LCD-positive cherries in 2 orchards, higher in 2 orchards, and not significantly different in 2 orchards (Table 1). Fruit size, however, was consistently smaller and soluble solids consistently lower in LCD-positive fruit from all 6 orchards. In 3 of 5 orchards, pH was higher in LCD-positive fruit, but % malic acid was unchanged. In the 2016 'Bing' sample, the CTIFL color rating was lower in LCD-positive fruit; hue angle was significantly different in both 2016 orchards (Bings were less red, Rainiers were more green) in LCD-positive fruit.

9. Sample a 50-tree subset in LCD orchards for mealybug species, presence, and abundance (Post-harvest, July-September 2015 and 2016). Mealybug sampling was conducted in the study orchards on LCD-positive and LCD-negative trees using a ladder. No mealybugs were found during the course of the sampling.

10. **Walk through LCD orchards and categorize trees as symptomatic or non-symptomatic based on reddish leaf coloration (late summer-early fall 2015 and 2016).** Based on 2015's observations that there were no obvious color differences between known positive and negative trees, this assessment was discontinued in 2016.

11. Statistical analysis and modeling of data collected in LCD orchards (September-December 2015 and 2016). Using data collected during 2015 and 2016, Dr. Marc Evans

(WSU, Program in Statistics) performed a directional spatial analysis to determine evidence of a spatial relationship between infected trees within the same row and across rows. All data were analyzed using the Statistical Analysis System (SAS 2016). To determine if there was a spatial pattern among the PCR tested LChV2-positive or -negative trees, a spatial analysis of disease infection using PROC GLIMMIX was performed. The orchard trees were coded by position in the orchard with X = row position and Y = column position and coded for LChV2 infection as positive (1) or negative (0). These X and Y positions were used to develop the correlation structure among trees that were LChV2-positive or -negative.

Two of the three 2015 orchards showed a significant spread pattern within rows, but not between rows. The third orchard did not display a significant spread pattern in either direction; however, statistics showed that it was more likely to spread within than between. For 2015 orchards, new LChV2 infections were located on trees within a 1- to 3-tree radius of trees previously removed from the orchard, due to historic LChV2 infections. In one orchard three LChV2-positve trees were identified in a completely different location of the orchard, where LChV2 had not been previously detected. The trees were detected based on visual symptoms and were later confirmed LChV2 positive with PCR.

Of the 2016 orchards, only 2 could be analyzed for spatial patterns due to lack of sufficient LChV2-infected trees. In one orchard, where 4 additional LChV2 trees were located within a 1-tree radius of previous infections, statistics showed that the disease was more likely to spread within rows than between. In another orchard 13 of the 14 additional LChV2 infected trees were all located within a 1- to 3-tree radius of previous infection. Analysis revealed the disease was likely to spread within and between rows in this orchard.

In order to determine if visual symptoms are good indicators of LCD infection, an analysis comparing visual symptoms identified by fieldmen to actual LCD infection, as determined by PCR, was also completed. PROC FREQ was used to produce a one-way frequency table to determine the percent of LChV2 infected trees, verified by PCR, determined to be positive by at least one fieldman, the percent of LChV2-positive trees correctly classified by fieldmen (true positive), and percent of LChV2-negative trees classified as positive by fieldmen (false positive); 1 = determined positive (PCR and/or fieldman) and 0 = determined negative (PCR and/or fieldman). The proportion of fieldmen who indicated trees were positive for LChV2-positive trees (correct decision) and LChV2-negative trees (incorrect decision or false positive) was determined by using PROC MEANS. A Chi-square test was used to determine if the percentage of positive guesses for positive trees (true positive) and negative guessed for negative trees (true negative), differs from random chance (significantly exceeds 50%).

In all but two orchards, all LChV2-positive trees were identified by at least one fieldman. Trees in those two orchards (total of 3) did not show the typical visual symptoms of LChV2 infection, and were therefore overlooked by all participants. Fieldmen (at least one) incorrectly identifying negative trees as positive (false positive) ranged from 9 to 96% of trees in orchards.

When looking at the frequency of fieldman guesses, the number of correct guesses for positive trees (true positive) compared to the number incorrect guesses (false negative) significantly exceeded 50% or differed from random chance in all but two orchards. One of those orchards contained a number of trees that appeared to show symptoms, but were LChV2-negative. In this case the number of positive guesses on LChV2-positive trees didn't differ much from the number of positive guesses on LChV2-negative trees. The other orchard had only one LChV2-positve tree, and was only identified by one participant, while

other LChV2-negative trees in the orchard were identified as positive by most or all participants as being positive.

The number of true negative guesses compared to false positive guesses differed from random chance in all orchards. This indicates that fieldmen were overall good and unified at determining LChV2-negative trees.

12. Provide research updates to producers and consultants during winter meetings (November-February, 2015-16 and 2016-17). Completed outreach activities during October 2015-February 2017:

1) Washington State Tree Fruit Association Annual Meeting; Yakima, WA; December 8, 2015
2) G.S. Long Grower Meeting; Chelan, WA; December 16, 2015
3) Orchard Pest & Disease Management Conference; Portland, OR; January 14, 2016
4) Cherry Institute, Yakima, WA; January 15, 2016
5) Stone Fruit Day, Wenatchee, WA; January 19, 2016
6) Northwest Wholesale Grower Meeting; Oroville, WA; January 26, 2016
7) Developing a management strategy for little cherry disease, WA State Annual Tree Fruit Association Meeting. Wenatchee, WA, December 2016
8) Managing the vectors of little cherry disease. Orchard Pest and Disease Management Conference, Portland, OR, January 2017
9) Detecting and managing little cherry disease, Wenatchee Stone Fruit Day. Wenatchee, WA, January 2017
10) Developing a management strategy for little cherry disease, Bluebird Annual Meeting. Wenatchee, WA, January 2017
11) Viruses: Little Cherry/ Western X & More, Organic Pest and Disease Management Fruit School, Wenatchee, WA, February 2017

13. Prepare reports. Reports were prepared as requested for the granting agency, as well as for the agency providing matching funds (Washington Tree Fruit Research Commission).

5. Describe the significant contributions and roles of project partners.

The primary project partners were the Washington State Tree Fruit Research Commission and Stemilt Growers LLC. Stemilt Growers LLC contributed by performing field work and completing fruit quality assessments. The Washington State Tree Fruit Research Commission provided the matching funds from a 3-year grant (2014-2016).

6. If the overall scope of the project benefitted commodities other than specialty crops, indicate how project staff ensured that funds were used to solely enhance the competitiveness of specialty crops.

Only specialty crops were benefited.

Goals and Outcomes Achieved

7. Describe the activities that were completed in order to achieve the performance goals and Expected Measurable Outcomes for the project.

One goal of this project was to provide educational and informative extension presentations to cherry industry groups that incorporate the newest findings associated with LCD management. In January of 2016, a survey of 60 attendees at an outreach talk indicated high levels of concern with LChV2. Before the talk, 68% could identify the best time to assess visual symptoms, and after the talk this improved to 90%. Before the talk, 88% knew the correct sampling strategy, which improved to 97% after the talk. After hearing the information provided, 78% plan to change their current management strategy for LChV2.

Another outreach presentation in January 2017 focused on correct identification of LCD via visual symptoms versus PCR test and LCD management strategies. The audience (124 participants) consisted of over 90% cherry growers or managers, who were mostly all aware of LCD and its effects, and at least 25% were actively managing it in an orchard they owned or managed. At the end of the presentation the group was asked to look at a series of 10 images of fruit and based on visual symptoms, determine the LCD status (positive or negative) of the tree containing these cherries. Infection status (4 negative, 6 positive) of the trees was determined via PCR prior to this presentation. When the infection status was negative, 60% to 82% of the participants guessed correctly. When the infection status was positive, 53% to 100% of the participants guessed correctly. These results emphasized to the participants that while visual symptoms are important, they can be easily mistaken, and a PCR test done by a certified laboratory is essential.

Additionally, an informative web-page developed in conjunction with Washington State University Extension http://treefruit.wsu.edu/crop-protection/disease-management/little-cherry-disease/. The web-page provides current and updated information on LCD identification and management.

8. If any Expected Measurable Outcomes were long term, summarize the progress that has been made towards their achievement.

Recommendations for mealybug control on sweet cherry will be incorporated in the next version of the WSU Crop Protection Guide.

Information regarding the spatial distribution of LCD and the economic thresholds for orchard/tree removal is being summarized, and will be published in scientific journals and made available on the WSU website. A manuscript on the economic thresholds for orchard removal is in advanced stages of preparation.

9. Provide a comparison of the activities and goals established for the project with the actual accomplishments.

Goal #1: As a result of this project, cherry growers and/or their IPM consultants will have the necessary IPM information to protect their sweet cherry crop from mealybugs/LCD vectors. A bioassay comparing two drench systemic insecticides (Admire Pro and Aza-Direct), one foliar systemic insecticide (Ultor), and one insect growth regulator (Centaur) was completed on GMB-infested greenhouse trees. Admire Pro, Aza-Direct, and Centaur reduced nymph and adult numbers to almost zero between 18 and 27 days post-treatment application (Figure 1). Since LCD has become a state-wide problem, many growers and managers have used Centaur as a mealybug control method. Previously, very little empirical evidence of the Centaur's efficacy existed. A field experiment, as part of a sister project, showed a reduction in AMB crawler numbers when Centaur was applied at the delayed dormant timing. The active ingredient in Admire Pro (imidacloprid) and in Aza-Direct (azadirachtin) can reduce

mealybug numbers in grape vines via soil drench application (Lo and Walker 2011, Balikai 1999). Based on these results and previous evidence, all three of these compounds show promise as mealybug controls in sweet cherry orchards. Due to problems with parasitism in AMB colonies, greenhouse bioassays with this species could not be performed.

Goal #2: Sample data from trees in LCD orchards will be used to develop measurable indicators to predict the probability of non-symptomatic trees serving as reservoirs of LCD, and to guide in making management decisions to remove whole orchards or just a subset of infected trees. In 2015 measurable indicators such as small fruit, reduced sugar content (determined by tasting fruit), and lighter color proved to be good indicators of LChV2 infection. Fruit quality analysis on LCD-positive and -negative cherries in 2015 and 2016 showed consistently smaller fruit size and lower and soluble solids in LCD-positive fruit from all orchards. In the 2016 'Bing' sample, the CTIFL color rating was lower in LCD-positive fruit and hue angle was significantly different in both 2016 orchards (Bings were less red, Rainiers were more green) in LCD-positive fruit.

Based on 2015 data, it appears that many 'newly' infected trees are located within a 3-tree radius of prior infections, and that monitoring and sampling efforts to locate LChV2 infected trees should be prioritized in this zone. An additional search (using visual symptoms) will be necessary to locate other patches of infected trees. The results from 2016 orchards seem to stray from 2015 conclusions, with less contiguous LChV2 infections. A large number of trees showed visual symptoms of LCD, but molecular analysis results were negative for LChV2. Many symptomatic trees were tested for WX (an unexpected addition to the project), however, only a small number were positive. There are a number of additional viral infections or the combination of one or more viruses (cherry rasp leaf, cherry decline, cherry mottle leaf, etc.) that were not tested for in these orchards, and may have been the cause of visual symptoms resembling LCD.

10. Clearly convey the achievement of your Expected Measurable Outcomes by describing the baseline data that was gathered and the achievement (or progress toward achievement) of your set targets.

The vector management recommendations are ready for incorporation into the Crop Protection Guide, and have been widely presented at grower meetings. The use of sample requests as an indicator of LCD spread within the state is likely a poor indicator. An increase in sample requests is more likely a result of increased awareness of LCD, which will lead to containment of the disease through vector control and tree removal.

Beneficiaries

11. Describe those who have benefited from the completion of your project and its accomplishments.

The sweet cherry growers of Washington State have benefited from the information gathered by this project. Sweet cherry growers in other parts of the United States and the world, which may contend with LChV2 infection, can apply what has been learned in Washington to their own region.

12. Clearly state the quantitative data that concerns the beneficiaries of the project and/or that describes the economic impact of the project.

The economic analysis from the matching project from the Washington Tree Fruit Research Commission indicated that the break-even point for profitability of a cherry orchard is a 10% loss in production either through tree removal or unpicked/culled fruit. This can be a guide for growers for orchard removal/replant due to LCD.

Lessons Learned

13. Offer insights into the lessons learned by project staff as a result of completing this project. Include the positive and negative results and conclusions of the project.

When considering measurable indicators of LChV2, it is clear based on the eight orchards examined, mealybug presence is not as important as was initially thought. All orchards had a history of mealybug infection; however, during the examination time, no mealybugs were found in any orchard, but LChV2 was found in all but one orchard. Mealybugs can be an indicator of infection, but they don't have to be in the orchard for an active infection to be present. Leaf color in autumn has also been associated with LChV2 infection; however, no clear connection was found between fall color and positive infection.

The pattern of newly infected trees tended to mostly be related to the proximity of previous infections, about a 3-tree radius. In some cases new infections were not close to previous infections, but were discovered based on visual symptoms. To slow the spread of this disease, or to eliminate it from the orchard requires careful scouting close to previous infection, but also throughout the orchard. It is also possible that symptomless infections remain undetected in areas of the orchard that were not tested, however this is impossible to determine without prohibitively expensive PCR sampling.

14. Describe any unexpected outcomes or results that were an effect of implementing your project.

The most unexpected outcome was the sole orchard that had trees with visual fruit symptoms throughout, but was negative for LChV2. In the remaining orchards, visual symptoms were fairly reliable indicators of infection. This underscores the need for testing to verify the status of symptomatic trees.

15. If activities, goals or Expected Measurable Outcomes were not achieved, identify and share your lessons learned to help others expedite problem-solving.

Activities #1 and #2 were only partially completed, in that a GMB colony was established and tested, but the AMB colony failed due to parasitism. However, several years of field tests on AMB in the matching WTFRC project provided adequate information for making control recommendations.

Additional Information

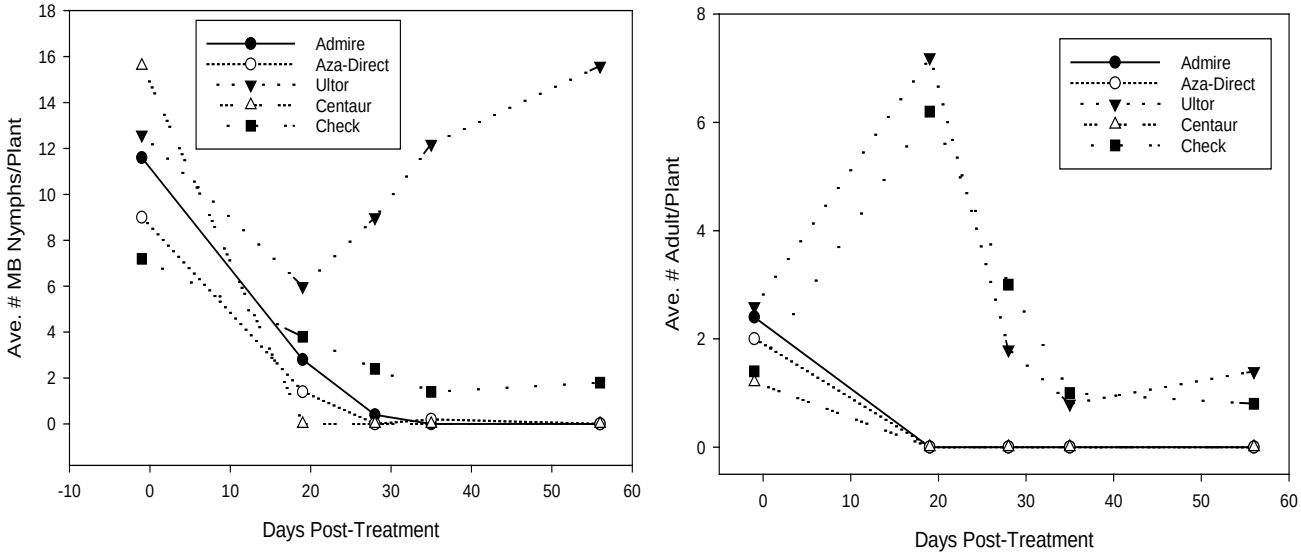
16. Provide the total level of cash or in-kind matching donations utilized for your project. Describe the amounts, sources and ways in which the donations were utilized.

This project was matched (cash) with a three-year grant from the Washington Tree Fruit Research Commission in the amount of $151,242 (see attached letter of matching pledge).

This was used primarily for salaries. Stemilt Growers LLC provided in-kind donations of ca. $80,000 in field work and fruit quality analysis for Activities #7 and #8.

17. Provide any additional information available (i.e. publications, websites, photographs) that was not applicable to any of the prior sections.

Figure 1. Potted tree bioassay of insecticides for control of grape mealybug



Treatments were applied on 6 May, 2016: Admire Pro and Aza-Direct = Soil drench; Ultor and Centaur = Foliar application

Table 1. Fruit quality differences between LCD-positive and LCD-negative fruits, 2015-2016

Year	Cultivar	LCD infection	n	Firmness	Row size	Soluble solids (%)	pH	% Malic acid	CTIFL color	Hue angle
2015	Bing	01.Positive	14	325.02 b	12.17 a	13.51 b	.	.	.	.
		02.Negative	4	349.97 a	10.22 b	19.83 a	.	.	.	.
2015	Rainier	01.Positive	5	325.39 a	10.45 a	18.22 b	3.95 a	0.8040 a	.	.
		02.Negative	14	304.44 a	9.44 b	21.82 a	4.00 a	0.8259 a	.	.
2015	Bing	01.Positive	9	298.95 a	11.87 a	18.44 b	4.85 a	1.1788 a	.	.
		02.Negative	10	280.47 b	9.91 b	21.92 a	4.30 b	1.3236 a	.	.
2015	Lapins	01.Positive	10	378.62 a	12.11 a	12.82 b	4.10 a	0.9198 a	.	.
		02.Negative	10	310.48 b	9.28 b	17.11 a	3.94 b	0.8860 a	.	.
2016	Rainier	01.Positive	3	304.00 a	11.27 a	11.53 b	3.75 a	0.8000 a	.	93.59 a
		02.Negative	10	281.12 a	9.15 b	19.80 a	3.81 a	0.8060 a	.	39.35 b
2016	Bing	01.Positive	10	366.31 b	11.67 a	14.42 b	3.77 a	1.0338 b	2.56 b	24.07 a
		02.Negative	10	432.96 a	9.58 b	22.24 a	3.67 b	1.1399 a	4.35 a	15.77 b

FINAL PROJECT REPORT

Project Title: Streamlining the Pacific Northwest sweet cherry breeding program

PI:	Cameron Peace	**Co-PI (2):**	Bernardita Sallato
Organization:	WSU-Horticulture	**Organization**:	WSU-Horticulture
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City/State/Zip:	Pullman/WA/99164	**City/State/Zip**:	Prosser/WA/99350

Cooperators: WSU Cherry Breeding Program Advisory Committee (BPAC), OSU-MCAREC

Total Project Request: **Year 1:** $150,000

Budget 1
Organization Name: Washington State University **Contract Administrator:** Carrie Johnston
Telephone: (509) 335 4564 **Email address:** carriej@wsu.edu

Item	2017
Salaries	27,000
Benefits	8,964
Wages	29,370
Benefits	6,168
Fruit lab supplies & maintenance	2,367
Field supplies & maintenance	32,350
Analytical services	10,000
Travel	4,031
Plot fee	4,750
Total	125,000

Budget 2
Organization Name: OSU-MCAREC **Contract Administrator:** Russell Karow
Telephone: (541) 737 3228 **Email address:** Russell.Karow@oregonstate.edu

Item	2017
Salaries	4,357
Benefits	3,006
Wages	13,000
Benefits	333
Equipment	
Fees and Supplies	4,304
Travel	
Miscellaneous	
Total	25,000

OBJECTIVES

Overall goal

Create a robust foundation for Pacific Northwest sweet cherry breeding within an objective, resource-driven, protocol-based framework that quantitatively targets industry priorities.

Specific objectives

1) Establish and deploy a robust **horticultural management** system that efficiently raises and maintains **healthy plant materials** at all breeding stages

2) Establish and deploy a robust **performance evaluation** system that effectively targets the **Early and Late Mahogany market classes**

SIGNIFICANT FINDINGS

- Much streamlining of the breeding orchard and activities was achieved, and evaluation of breeding germplasm identified and confirmed the most promising material.

- The breeding orchard's physical footprint was significantly reduced, with removal of 10 acres (45% of total), ~3500 Phase 1 trees (50% of total), 11 Phase 2 selections no longer to be considered, and two acres (30%) of parent blocks – totaling an estimated $36,000 in annual maintenance and evaluation costs.

- The breeding orchard condition was improved and maintained by applying best horticultural management practices and diligent observation throughout the season.

- Virus identification led to removal of two advanced selections and prompted evaluation of alternative strategies to efficiently detect and deal with virus presence in breeding trees

- Several promising Phase 2 selections stood out in 2017 evaluations: R1, R3, R17, R19, and especially R29. R29 was large, firm, sweet, and had good storability, but low acidity.

- In Phase 1, 13% of ~700 fruiting seedlings were considered worthwhile for lab-based fruit quality evaluation (77% mahogany-type and 23% blush-type). Two promising seedling in previous years continued to stand out, and should receive extra attention in 2018.

- The PNW sweet cherry breeding program is in good shape for a new permanent breeder

RESULTS & DISCUSSION

Objective 1: Establish and deploy a robust **horticultural management** system that efficiently raises and maintains **healthy plant materials** at all breeding stages

1a: Reduce physical footprint
The physical footprint across Phase 2, Phase 1, and Parent blocks was significantly decreased, capping previously escalating costs and dilution of attention. In total, 10 acres of trees were removed

in 2017 (8 acres of Phase 1 F block and 2 acres of parent blocks), 3560 Phase 1 trees were removed (~3000 of F block and ~560 in C block), and 11 selections in Phase 2 were removed from ongoing consideration. These reductions eliminate $36,000 from ongoing orchard maintenance costs and evaluation, and provide greater efficiencies in allocation of breeding attention. Details for each level of breeding germplasm are described below.

- Phase 2: The WSU team with BPAC advice discarded nine advanced selections (out of a total of 24 going into 2017) considered not promising enough in fruit quality and/or productivity or that exhibited fatal flaws, according to established PNW industry needs and priorities. Trees of these nine discarded selections (R2, R4, R7B, R8, R9B, R13, R14, R15, and R25, where "B" specifies blush-type) were not physically removed from the block, to avoid spacing disturbance and to re-utilize clean existing trees as rootstocks for future advanced material. Two further advanced selections had to be removed due to virus (PDV) presence (R18B at Prosser and R24 at Prosser and Pasco).

- Phase 1: The complete F block (8 acres, 3176 seedlings) was removed in March, removing 45% in acreage and 50% in tree number of the Phase 1 physical footprint. This provides a reduction of $32,000 in what would have been additional orchard maintenance costs. In the remaining C block of seedlings, we identified unwanted trees (redundant, weak, virus-infected, consistent poor fruit quality, fatal flaws, etc.) using 2015 and 2016 phenotypic data and health assessments. These unwanted trees were removed between May and August. This thinning of approximately every second tree in the first nine rows of C block removed a total of 560 trees, which was 28% of Phase 1 seedlings in C block, and resulted in improved health and evaluation efficiency of remaining trees.

- Parents: Two inefficient blocks of trees being maintained in past years as parents (totaling 2 acres, 30% of acreage of parent trees) will be removed by the end of the year. This planned removal was the result of identification of redundant trees concentrated in the two blocks, with very few non-redundant potential parents within. Parents that were considered valuable to retain within these to-be-removed blocks were tested for viruses. Two out of 13 parents tested were positive to PDV and were discarded (BB and HH). Clean material was collected and propagated into virus-free rootstock (Gisela 6). The propagation procedure and clean rootstocks were generously provided by Scott Harper, Director of the Clean Plant Center, and propagated trees are currently being maintained at the PNW Clean Plant Center in Prosser.

1b: Renew and protect the parent block

In 2017, we did not propagate a select set of parents for future crossings due to a lack of virus-free rootstocks available this year and the fact that the hiring of a cherry breeder was approved for early 2018. The decisions of whether and how to establish a protected parent block are therefore left to the new breeder. Instead, valuable information is being obtained on the available parents to support the breeders' decisions: virus status and genetic contributions to the next generation according to DNA profile. Maps of parent block trees were also updated.

- Virus status: Tissue samples from one tree of each replicated parent in the main parent block (B53) were collected and are currently being tested efficiently for PDV and PNRSV viruses using the grafting technique of bud chips into a *Prunus tomentosa* indicator. The procedure and resources were generously provided by Dr. Lauri Guerra, WSDA plant pathologist. Results are expected by the end of this year. This procedure is expected to reduce greatly the number of samples to be subsequently evaluated for other viruses.

- DNA profiles: Leaf samples were obtained for each parent (as well as all current P2 and P1.5 selections, which are also potential parents) for whole-genome DNA profiling supported by the RosBREED project. For this DNA profiling, an expanded genome-scanning tool was developed in the RosBREED project in 2017, with the first data on cherry breeding individuals expected to arrive in November. (The resulting descriptions of genetic potential will be described as "genomic predictions" as well as visualized as "haplotype mosaics" that show ancestry and valuable genetic factors across the chromosomes of each individual, to be available for the new breeder.)

1c: Horticultural management

Horticultural practices were constantly improved and supervised during 2017. Some of the incorporated practices during 2017 were:

- Pruning, training, and tree thinning: Because of observations of excessive shading and blind wood, trees in the first nine rows of C-block Phase 1 seedlings were pruned in early spring to modify the current training system. Also, about half the trees in these rows were removed (as described above in *Reduced physical footprint: Phase 1*). Light penetration into the block was greatly improved. Trees have been lowered in size which improved efficiency of orchard activities such as sprays and harvest. Following the heavy pruning in spring, trees were summer-pruned to improve architecture and encourage fruiting wood for subsequent seasons (Figure 1).

- Irrigation: Using the irrigation scheduler software developed by WSU and with guidance from Troy Peters, WSU specialist, we modified the irrigation programing according to soil type, irrigation system, and water-withholding capacity of each area. Continued irrigation monitoring led to removal of an additional drip line in a shallow row of the Roza Phase 2 block, improving tree health. The irrigation system in C block was also modified from spaghetti tube irrigation to sprinklers to allow establishment of a cover crop next season and improve root lateral growth.

- Nutrient management: Standard soil and foliage analyses were developed for nutrient diagnostics in the breeding orchard. The subsequent fertilization program was based on tree demand and soil supply. The biggest challenge has been to manage soil and root growth variability across C block of Phase 1 seedlings.

- Disease management: Standard pest management practices were conducted by the Roza orchard crew starting on March 30. Only the P2 selection block received treatment to control powdery mildew (PM). Other blocks were not controlled for PM because evaluation of PM resistance/susceptibility is required for Phase 1 seedlings and was also being evaluated in 2017 in a parallel project in the genetic stock C53 block and parents. Dr. Claudia Probst, WSU pathologist, reported the first signs of PM in the second week of June. The high pressure observed during the 2017 season enabled an efficient and accurate evaluation of foliar susceptibility and some evaluation of fruit susceptibility in Phase 1. To reduce the detrimental pressure on vegetative growth and fruit quality for next year, we used a fall control spray. Aaron Avila, G.S. Long, generously provided product and advice for this fall application.

- Virus control and monitoring: In collaboration with members of the Clean Plant Center and WSDA experts, blocks were monitored starting in April for identification of virus-related symptoms. In Phase 2 blocks, we evaluated all trees of all selections and standard cultivars for PNRSV and PDV. Two selections showed virus symptoms and tested positive to PDV: R18B and R24B. These selections were immediately removed. To avoid such wasteful elimination of Phase 2 selections and to maintain orchard health, for future years we propose routine, random virus-testing of 20% of seedlings.

Figure 1. Pruning, training, and tree thinning in Phase 1. Top: Seedlings before (left) and after (right) spring pruning. Bottom: summer pruning in C52 block.

- Propagation: Two previously advanced selections, propagated by Willow Drive Nursery, were planted in the Phase 2 block at Roza (B48) in April: R29 and R45. For other material, a different propagation system was used. To ensure we efficiently retain good performers and potential parents, we trialed the method of grafting into existing established trees that are otherwise discarded. Two sets of the most promising Phase 1 F-block seedlings from 2015 and 2016 evaluations, as described in last year's report, were propagated in this manner. The first set (new R46–R51 selections: 3x Early Mahogany, 3x Late Mahogany) were grafted onto trees of previously discarded selections in the B48 Phase 2 block. The second set (potential new parents) were grafted onto some scaffolds of other parent trees on the edge of the genetic stock block (C53). The grafting service was generously donated to the breeding program by Mike Argo Grafting. The method showed great success in terms of tree growth and propagation efficiency. We will monitor effects on precocity and fruit quality to inform a cost:benefit consideration of this propagation method as a standard practice.

Because of strong pressure applied for PM, foliar PM-resistant seedlings were readily distinguished from susceptible ones (Figure 2). Some seedlings susceptible to fruit PM were also able to be detected, although absence of fruit infection did not mean resistance because fruit incidence was much lower across the block than foliar incidence.

Figure 2. Seedlings new shoot with evident foliar PM resistance CR11T64 (left) and a neighboring tree susceptible to PM, CR11T63 (right).

Activity 2: Establish and deploy a robust **performance evaluation** system that effectively targets the **Early and Late Mahogany market classes**

2a: Performance evaluation
A streamlined protocol was used for performance evaluations efficiently targeting industry priority traits. Activities were synchronized across all locations, including full bloom timing observation, green fruit thinning, netting, and fruit quality evaluation at harvest and post-storage. In-field Phase 1and Phase 2 evaluations were conducted at a minimum of twice per week throughout the harvest season. At least once per week we walked the blocks with BPAC members and other industry visitors. Special emphasis was placed on flavor and overall appreciation of the fruit of selections and promising seedlings, for which was incorporated a weekly evaluation by BPAC members and collaborators.

Phase 2
Overview
Several promising Phase 2 selections stood out in 2017 evaluations: R1, R3, R17, R19, and especially R29. R19 is Early Mahogany, four days after Chelan and 10 days before Bing in both 2016 and 2017, while the other four selections are Mid-season Mahogany from approximately one week before to four days after Bing timing. R19 and R29 were evaluated for the first time in Phase 2 this year and only in Pasco. R29 was the most exceptional selection (large, firm, sweet, and good storability, but low acidity).

A total of 12 selections were evaluated in Phase 2 in 2017. These selections consisted of one Early Mahogany (EM – R19), two Early Blush (EB – R16B, R28B), four Mid-season Mahogany (MM – R1, R3, R17, R29), one mid-to-late Mahogany depending on location (MM/LM – R6), and four Late Blush (LB – R5B, R10B, R11B, R12B). The number of selections evaluated at each Phase 2 trial location (Prosser, Pasco, and Hood River) was reduced by at least 40% compared to 2016. Nine selections were evaluated at Prosser, five at Pasco, and four at Hood River, along with the standard cultivars of Chelan (Prosser, Pasco), Early Robin (all locations), Bing (Prosser, Pasco), Rainier (Prosser, Hood River), and Sweetheart (all locations).

Phenology

Starting the season, phenological development from bud break to green fruit was recorded for every advanced selection at the three locations. Bloom time in Pasco occurred April 13 to 25. Maximal temperatures during bloom were between 55 and 65 º F and the accumulated degree days (base 50 ºF) were between 24 and 38. Similar observations were made at Prosser a week later, where bloom was spread from April 17 to May 2, maximal temperatures were 55–65º F, and accumulated degree days were 24–44. In Hood River, the bloom time was April 20–28, with temperatures of 42–47 º F. These cooler conditions during bloom at Hood River compared to 2016 permitted an extended bloom period that overlapped among selections. At all sites, the standard cultivar Chelan was the first to bloom, followed a couple of days later by Early Robin, Rainier, R1, R3, and R11B. Five days after Chelan were Bing, Selah, Sweetheart, and the selections R16B and R6. The later selections to bloom were R10B a couple of days after Bing and R5B and R12B 4–5 days after Bing. In Pasco, we were able to evaluate for the first time the selections R19, R21B, R24B, R28B, and R29. The selection R28B bloomed at the same time as Chelan and selections R10B, R21B, and R24B three days later. The mahogany R29 had its full bloom at the same time as Bing and R19 was the last to reach full bloom, 4 days later.

Four weeks after bloom, trees were thinned to 30 fruit per foot of fruiting wood and all selections were rated for crop load levels, doubles, and other observed defects. The selections R3, R6, and R10B had high crops, equivalent to Sweetheart, Bing, and Chelan, so they all needed intense thinning. The selections R1 and R17 were the opposite, with low crops and no need to thin, equivalent to Early Robin. This season, only Early Robin and Sweetheart (and no selections) showed some doubles, but in both cases it was below 5%.

At the onset of harvest, we recognized a lack of objective indicators to guide the assessment of an adequate harvest date for each selection. In contrast with standard cultivars for which we have expectations of fruit size, sweetness, acidity, and color, such parameters for advanced selections (and seedlings) have not yet been established and this could lead to mistakes in harvest timing. To mitigate this uncertainty, we performed multiple harvest times and used the resulting fruit quality data to attempt to characterize each selection for fruit maturity. This information will serve as a guide for future accurate evaluations and more accurate allocation to market class.

At the Roza orchard in Prosser we recorded only one rain event of 0.11 inches within 14 days of harvest. However, we did not observe cracking issues in any current Phase 2 selections. Nevertheless, the already-discarded selection R4 had > 90% cracking (and had 100% cracking on 2016).

Performance

All fruit of Phase 2 selections were evaluated for fruit size, firmness, soluble solids content (SSC), titratable acidity (TA), and general sensory flavor, with target trait thresholds of row 10 size, 10 g weight, and 300g/mm firmness for early and late-season selections, and better than 9.0 row and more than 11 g weight for mid-season selections. For those selections with enough fruit, additional evaluations were performed for pedicel-fruit retention force (PFRF) and post-storage performance (after three weeks of regular-atmosphere cold storage: firmness, SSC, TA, luster, pitting, shrivel, and stem browning).

Selections R1, R3, R17, and R29 had better performance in several aspects of harvest fruit quality and post-storage condition than the standard Bing in Prosser and Pasco (Tables 1–4), although R17 was only grown in Prosser and R29 was only evaluated in Pasco. The selections R1 and R3 were reclassified from Early Mahogany to Mid-season Mahogany because at Prosser they were harvested on the same day as Bing.

Table 1. Harvest performance of Phase 2 selections at Prosser in 2017 (averaged over five trees). Values in shaded boxes are better than standards. Values in parentheses are well below those of standards and below thresholds.

Selection or standard cultivar	Market class	Harvest date	Harvest date vs. Bing (days)	Fruit Wt. (g)	Fruit row size	Fruit width (mm)	Fruit firmness (g/mm)	Skin color (1-7)	Juice SSC (ºBrix)	Juice TA (%)
Chelan	EM	20-Jun	-7	7.7	11.2	24.3	303	6.0	20.3	0.92
Early Robin	EM	21-Jun	-6	11.5	9.6	28.6	401	B	20.3	0.57
R16B	EB	22-Jun	-5	10.6	10.0	27.3	313	B	19.6	0.89
Bing	MM	27-Jun		8.2	10.7	25.6	261	5.2	23.6	0.96
R3	MM	27-Jun	0	9.5	10.2	26.8	315	5.9	21.7	0.74
R1	MM	27-Jun	0	11.4	9.6	28.6	298	5.3	24.1	1.13
R17	MM	27-Jun	0	9.7	10.2	26.8	315	5.1	22.8	0.89
Rainier	LB	27-Jun	0	7.7	10.7	25.7	260	B	25.1	0.73
R5B	LB	3-Jul	+6	8.3	11.2	24.2	281	B	(19.2)	1.03
R10B	LB	7-Jul	+10	12.4	9.8	28.1	(226)	B	25.7	0.77
R11B	LB	7-Jul	+10	(6.7)	11.0	24.7	300	B	23.4	1.14
R12B	LB	7-Jul	+10	9.8	10.4	26.5	251	B	24.6	0.92
Sweetheart	LM	7-Jul	+10	8.5	11.0	24.7	288	5.3	25.9	0.92
R6	LM*	12-Jul	+15	12.0	9.7	28.3	260	5.0	23.2	1.22

* In 2017, R6 was LM in Prosser but MM in Hood River; it has also varied in past years

Selections R19, R28B, and R29 were evaluated for the first time in Phase 2 and only in Pasco (Table 2). Selection R24B also had its first crop but had to be discarded due to virus presence, as mentioned earlier. Selection R19 had better performance than its standard Chelan. Selection R28B was only as good as its standard Early Robin. Selection R29 showed particularly promising characteristics compared to its standard Bing, with R29 having substantially larger and firmer fruit. Selection R29 has a sweet flavor and an attractive appearance (shiny luster and good color development) at harvest and post-storage. However, it has low acidity compared to Bing. In 2018 we expect enough crop for R29 in Pasco for a full harvest and storage evaluation. Additional trees of this selection were replanted in 2017 in the Phase 2 block at Roza, from which we expect enough of a crop for a partial evaluation in 2019 and 2020 and for a full harvest evaluation in 2021.

Selections R5B, R6, R10B, R11B, R12B, and R16B did not perform better than the standards in fruit size and/or firmness wherever they were grown, and were often worse (Tables 1–4).

Table 2. Harvest performance of Phase 2 selections at Pasco in 2017 (averaged over five trees). Values in shaded boxes are better than standards. Values in parentheses are well below those of standards and below thresholds.

Selection or standard cultivar	Market class	Harvest date	Harvest date vs. Bing (days)	Fruit Wt. (g)	Fruit row size	Fruit width (mm)	Fruit firmness (g/mm)	Skin color (1-7)	Juice SSC (ºBrix)	Juice TA (%)
Chelan	EM	12-Jun	-14	8.0	11.0	25	297	3.67	15.9	0.74
R19	EM	16-Jun	-10	10.2	9.5	29	437	4.64	25.7	1.10
Early Robin	EB	19-Jun	-7	10.9	9.7	28	322	B	16.9	0.51
R28B	EB	19-Jun	-7	11.0	9.6	29	357	B	18.4	0.85
Bing	MM	26-Jun		9.4	10.3	27	250	5.97	24.0	1.05
R3	MM	19-Jun	-7	12.5	9.1	30	336	5.26	20.6	0.82
R1	MM	26-Jun	0	13.0	9.1	30	315	5.22	23.3	1.11
R29	MM	30-Jun	+4	14.8	8.7	32	321	4.65	19.9	0.51

Table 3. Harvest performance of Phase 2 selections at Hood River in 2017 (averaged over five trees). Values in shaded boxes are better than standards. Values in parentheses are well below those of standards and below thresholds.

Selection or standard cultivar	Market class	Harvest date	Harvest date vs. Bing (days)	Fruit Wt. (g)	Fruit row size	Fruit width (mm)	Fruit firmness (g/mm)	Skin color (1-7)	Juice SSC (ºBrix)	Juice TA (%)
Early Robin	EB	7-Jul	-4	10.6	9.4	29	341	B	16.8	0.62
R16B	EB	7-Jul	-4	11.3	9.3	30	344	B	18.1	0.62
Rainier	LB	7-Jul	-4	10.4	9.4	29	280	B	17.7	0.60
R12B	LB	19-Jul	8	11.2	9.4	29	277	B	20.5	0.56
R5B	LB	28-Jul	17	10.7	9.5	29	263	B	18.1	0.68
Bing	MM	11-Jul		9.4	10.3	27	250	5.97	24.0	1.05
R6	MM*	11-Jul	0	11.5	9.2	30	334	4.70	(18.4)	0.89
Sweetheart	LM	28-Jul	17	9.6	9.8	28	305	4.47	18.3	0.79

* In 2017, R6 was LM in Prosser but MM in Hood River; it has also varied in past years

Table 4. Post-storage performance of Phase 2 selections at Prosser and Pasco in 2017 (averaged over five trees). Values in shaded boxes are better than standards. Values in parentheses are well below those of standards and below thresholds.

Selection or standard cultivar	Trial location	Market class	Firmness (g/mm)	SSC (ºBrix)	TA %	Luster (1-3)	Natural pitting (%)	Induced pitting (%)	Shrivel (%)	Stem browning (1-4)
Chelan	Prosser	EM	321	20.5	0.73	2	10	5	13	2
Early Robin	Prosser	EB	342	21.3	0.51	3	6	5	5	2
R16B	Prosser	EB	302	21.2	0.72	3	10	9		1
Bing	Prosser	MM	286	25.0	0.71	3	12	25	7	4
R3	Prosser	MM	311	23.5	(0.49)	3	10	5	10	2
R1	Prosser	MM	344	25.3	0.91	2	10	25	20	2
R17	Prosser	MM	337	23.8	0.70	3	9	9		2
Rainier	Prosser	LB	273	26.7	0.53	3	12	50		2
R5B	Prosser	LB	(228)	20.5	0.73	3	38	26		2
R10B	Prosser	LB	(238)	25.3	0.62	2	14	50	5	2
R11B	Prosser	LB	313	23.6	0.99	3	14	22		3
R12B	Prosser	LB	258	24.4	0.69	3	22	50		3
Sweetheart	Prosser	LM	346	25.9	0.78	3	11	20		3
R6	Prosser	LM	272	23.7	0.96	3	26	39		1
Chelan	Pasco	EM	294	16.4	0.59	2	8	5	10	2
R19	Pasco	EM	383	24.9	0.89	3	30		5	2
Early Robin	Pasco	EB	342	21.3	0.51	3	6	5	5	2
R28B	Pasco	EB	334	20.1	0.68	3	0			3
Bing	Pasco	MM	286	25.0	0.71	3	12	25	7	4
R3	Pasco	MM	307	(19.6)	0.60	3	10	5	23	2
R1	Pasco	MM	323	21.9	1.03	2	20	25	20	4
R29	Pasco	MM	356	21.5	(0.43)	3	10	15	10	4

Phase 1

Few exceptional Phase 1 seedlings stood out in 2017. Fruit size was difficult to evaluate this season as it was generally low including for the standard cultivars. Those rare seedling that did have particularly large fruit were too soft. Traits evaluated in the field were fruit size then firmness then flavor, and a visual estimation of whether the proportion of visual defects was too high. Of a total of ~700 fruiting seedlings, almost 100 (13%) were considered worthwhile for lab-based fruit quality evaluation, of which 77% were mahogany-type and 23% blush-type. The same selection thresholds as Phase 2 were used, by sensory evaluation in the field and with both instrumental measures in the lab. Seedlings with fruit averages meeting the first two essential trait thresholds of fruit size and firmness were evaluated for further traits of SSC, TA, PFRF, and post-storage performance. 2017 was the first year that fruit were evaluated for storage potential in Phase 1. During the weekly BPAC visit, fruit of the most promising Phase 1 seedlings in cold storage was available for inspection and sensory evaluation.

Despite the most attention given to the Early and Late Mahogany market classes, most of the promising seedlings evaluated in 2017 were Mid-season Mahogany, several of which exhibited better

fruit quality than Bing, and two Late Blush seedlings type equivalent to Early Robin (Table 5). Seedling CR01T78 was previously noted for its promising performance in 2016 and 2014 and CR05T59 was also promising in 2016. Storage evaluation of these seedlings was considered insufficient in 2017 and so they should be closely monitored in 2018.

Table 5. Phase 1 promising seedlings from 2017 evaluation. Values in shaded boxes exceed thresholds. Values in parentheses are well below thresholds.

Selection or standard cultivar	Market class	Harvest date	Harvest date vs. Bing (days)	Fruit weight (g)	Fruit row size	Fruit width (mm)	Fruit firmness (g/mm)	Skin color (1-7)	Juice SSC (ºBrix)	Juice TA (%)
Mahogany types										
Chelan	EM	20-Jun	-13	9.4	11.1	24.5	335	5.14	19.2	1.06
CR03T035	MM	27-Jun	-6	9.7	9.9	28.0	403	5.90	21.5	
Bing	MM	3-Jul	0	(8.4)	(11.0)	24.7	(238)	5.80	23.8	1.02
CR01T078*	MM	3-Jul	0	11.2	9.7	28.4	376	5.26	23.7	1.02
Sweetheart	LM	25-Jul	+22	(7.1)	(11.9)	22.6	299	4.36	20.8	0.84
Blush types										
Early Robin	EB	27-Jun	-6	10.9	9.6	28.7	353	blush	21.4	0.54
Rainier	LB	3-Jul	0	10.1	10.2	26.9	271	blush	25.4	0.93
CR05T059*	LB	3-Jul	0	11.1	9.6	28.8	317	blush	21.0	1.20
CR15T046	LB	3-Jul	0	10.7	9.8	28.1	304	blush	23.3	

* Selections considered promising in previous years

Further Breeding Program Advisory Committee engagement

A BPAC meeting was held on May 11, 2017, and another will be held on November 8. Prior to these meetings, summarized data, recommendations by Peace and Sallato, information on orchard status and planned interventions, and breeding operation streamlining initiatives and outcomes were distributed for discussion in the meetings. Meeting minutes, a pre-season update, an early-season update, and a post-season update were also shared by email. BPAC members advised on various decisions in horticultural management and performance evaluation, including critical decisions about which selections to retain for evaluation in the 2017 season and which ones to discard from further consideration.

EXECUTIVE SUMMARY

Breeding-based genetic solutions provide long-term economic sustainability. The Pacific Northwest Sweet Cherry Breeding Program was re-established in 2004 to develop superior new cultivars for the Washington and Oregon industries. Extensive breeding resources, including a diverse germplasm base, laboratory and cold storage facilities, evaluation protocols and equipment, and expertise have been established. A continuum of genetically improved plant material now exists in the breeding orchard, from parents to seedlings to selections. The overall goal for 2017 was to ensure a robust foundation for the new permanent breeder. Specific objectives, and major outcomes achieved for each, are described below.

1) Establish and deploy a robust **horticultural management** system that efficiently raises and maintains healthy plant materials at all breeding stages

    - The breeding orchard condition was improved and maintained by applying best horticultural management practices and diligent observation throughout the season.

    - The breeding orchard's physical footprint was significantly reduced, with removal of 10 acres (45% of total), ~3500 Phase 1 trees (50% of total), 11 Phase 2 selections no longer to be considered, and two acres (30%) of parent blocks – totaling an estimated $36,000 in annual maintenance and evaluation costs.

    - Virus identification led to removal of two advanced selections and prompted evaluation of alternative strategies to efficiently detect and deal with virus presence in breeding trees

2) Establish and deploy a robust **performance evaluation** system that effectively targets the Early and Late Mahogany market classes

    - Several promising Phase 2 selections stood out in 2017 evaluations: R1, R3, R17, R19, and especially R29. R29 was large, firm, sweet, and had good storability, but low acidity.

    - In Phase 1, 13% of ~700 fruiting seedlings were considered worthwhile for lab-based fruit quality evaluation (77% mahogany-type and 23% blush-type). Two promising seedling in previous years continued to stand out, and should receive extra attention in 2018.

The Pacific Northwest sweet cherry breeding program is in good shape for a new permanent breeder from 2018 onward.

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FINAL PROJECT REPORT

Project Title: Cherry virus diagnostic survey of Oregon
PI: Jay W. Pscheidt, Extension Plant Pathology Specialist
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Cooperators: Lynn Long (OSU, Wasco Co. Extension); Steve Castagnoli (OSU, Hood River Co. Extension); Sue Root (Oregon Cherry Growers); Brooke Edmunds (OSU, Regional Extension Agent); Clive Kiser (Umatilla Co. Extension Agent); Steve Renquist (Douglas Co. Extension Agent); Rick Hilton and Achala KC (Southern Oregon Research and Extension Center, SOREC); Russ and Mary West (3H Ranch); Darrin Walenta (Union Co. Research & Extension Center); Andrea Galloway (Julibee Farms); Dipak Poudyal and Shannon Lane, Jeffery Grant (ODA); Jeff Heater (The Dalles Fruit Co.)

OTHER FUNDING SOURCES
Agency Name: Department of Botany and Plant Pathology – Teaching Assistantship
Amt. awarded: $25,828
Notes: Graduate student Lauri Lutes was awarded this teaching assistantship. Tuition was subtracted from this amount.

Agency Name: OSU Extension Service
Amt. awarded: $3,000
Notes: Annual discretionary statewide travel funds used to get to sampling sites.

Agency Name: USDA-ARS-HCRL
Amt. awarded: $4,000
Notes: Use of consumable supplies budget leveraged from USDA virus project.

TOTAL PROJECT FUNDING
Organization Name: Agricultural Research Foundation (Oregon State University)
Contract Administrator: Russ Karow
Telephone: (541) 737-4066 **Email address:** Russell.Karow@oregonstate.edu

Item	(2015-2016)	(2016-2017)*
Salaries (GTA Stipend**)**	5,715	23,318
Benefits (Health Insurance**)**	2,401	9,795
Wages		
Benefits (OPE**)**		68
Equipment		
Supplies		
Travel		
Miscellaneous (OSU fees**)**	323	1,445
Plot Fees		
Total**	8,439	34,626

*Anticipating 2% increase for 2016-2017 school year. **Anticipating tuition remission in the amount of $1,350 for the summer 2016 term. Anticipating tuition remission in the amount of $13,500 for the 2016-2017 school year.

ORIGINAL OBJECTIVES

Objective 1: Sample symptomatic (and healthy) cherry trees, pointed out by multiple growers, from each of the tree fruit production areas of Oregon.
Objective 2: Determine the most likely cause of these symptomatic cherry trees, virus or not!
Objective 3: Follow up the diagnosis with management recommendations to each grower.
Objective 4: Survey historical records for occurrence of cherry viruses in Oregon.
Objective 5: Summarize the survey information to report on the threat viruses may (or may not) pose to the Oregon cherry industry (and indirectly to the WA industry).

SIGNIFICANT FINDINGS

- First report of *Tomato ringspot virus* (ToRSV) in Hood River, The Dalles, and Grand Ronde Valley regions
- First report of *Cherry leaf roll virus* (CLRV) in Oregon (The Dalles)
- *Cherry leaf roll virus* (CLRV) found with *Prune dwarf virus* (PDV) and *Prunus necrotic ringspot virus* (PNRSV) on same host, which causes more rapid, severe decline
- Report of *Little cherry virus 2* in the The Dalles, OR
- Resurgence of X-Disease in The Dalles.
- Enations, rosetting, and little, immature fruit are indicative of the most severe viruses on sweet cherry in Oregon

RESULTS & DISCUSSION

Objective 1: Sample symptomatic (and healthy) cherry trees, pointed out by multiple growers, from each of the tree fruit production areas of Oregon.

Over a two-year period, 7 regions with commercial sweet cherry orchards in Oregon were sampled May through September. In 2016, sampling focused on The Dalles/Mosier, Hood River, the Willamette Valley, and the Umpqua Valley. Samples were collected from the Rogue Valley, the Grande Ronde Valley, and Milton-Freewater regions to round out the survey in 2017. Based on results from the first year and the importance of the sweet cherry industry in the region, additional samples were collected from Hood River and The Dalles in 2017.

By working with collaborators throughout Oregon, orchards with suspected virus problems were identified. Upon scouting the orchards, samples were collected from trees expressing virus-like symptoms, including: foliar chlorosis, mottling, enations (gall-like formations on the underside of the leaf), and rosetting (bunching of leaves due to shortened internodes), as well as trees with sections of little, immature fruit. For each tree sampled, 10-20 leaves were collected for analysis by virus-specific ELISA. Samples were screened with the following ELISA (Agdia, Inc., Elkhart, IN; DSMZ, Braunschweig, Germany; AC Diagnostics, Fayetteville, AR): *Cherry leaf roll virus* (CLRV), *Cherry rasp leaf virus* (CRLV), *Plum pox virus* (PPV), *Prune dwarf virus* (PDV), *Prunus necrotic ringspot virus* (PNRSV), *Tobacco ringspot virus* (TRSV), and *Tomato ringspot virus* (ToRSV). Isothermal AmplifyRP® (Agdia, Inc., Elkhart, IN) was used for detection of *Little cherry virus 2* (LChV2). These diagnoses allowed for regional identification of viruses throughout the state (Table 1).

Due to the potential of other *Prunus* species harboring viruses that could infect commercial orchards, 15 samples were collected from non-*P. avium* trees (*P. subhirtella, P. emarginata, P. serrulata*) in 2016 and 2 *P. emarginata* samples were collected in 2017. None of these samples tested positive for the viruses tested.

For each symptomatic sample a comparison sample was collected from an asymptomatic neighboring tree (typically 2-3 trees and/or rows removed) of the same cultivar and age. These comparison samples were used to identify inconsistencies between the visual symptoms observed and viruses present. Of the 192 samples tested, 29 symptomatic samples tested negative for the viruses tested and 29 asymptomatic samples tested positive for PDV or PNRSV. Of the 29 symptomatic samples that tested negative, 10 were not *Prunus avium*. The symptoms observed on the other 19 samples could be associated with herbicide damage or other abiotic factors.

As expected, pollen-transmitted viruses, PDV and PNRSV, were found in all regions including those not previously reported, including: the Umpqua Valley, which had no viruses reported on sweet cherry; as well as, Hood River, where PDV and PNRSV on sweet cherry had not been known. Due to the pollen-transmitted nature of these two viruses, it is not unexpected to find them throughout the state. When occurring as a single infection, these viruses express minimal foliar symptoms leading to insignificant yield loss that often goes undetected.

Cherry leaf roll virus (CLRV) was detected in The Dalles, OR, in summer 2016. Since this virus had never been previously reported in Oregon and its potential impact to kill trees, 24 more samples were collected in the area in September 2016 to get an idea of the localized prevalence of the virus. Eight of the sixteen symptomatic samples tested positive for CLRV. Two additional samples were collected in spring 2017 for reverse transcription polymerase chain reaction (RT-PCR) analysis and downstream sequencing. Sanger sequencing and BLASTn analysis revealed a 99% identity to CLRV isolate Olm1, an isolated obtained from naturally-infected *P. avium* cv. 'Bing' in North America (Eastwell 2012).

The enation-producing, nematode-transmitted virus, ToRSV, was also discovered in Hood River. Knowing that a nematode-vectored virus is present, growers can make more informed decisions regarding fumigation before replanting or establishing new orchards. Additionally, it is not uncommon for vineyards to be planted where former cherry orchards once stood. For this reason, it is important to know that ToRSV (and TRSV) are known pathogens of grape, both causing reduced fruit set and uneven ripening (Moyer et al. 2014). Therefore, fumigation or a fallow period prior to planting should occur to rid the soil of potential nematode vectors.

Observation of visual symptoms is insufficient for distinguishing between the *Little cherry viruses 1 &* 2 and the virus-like phytoplasma, X-Disease. For this reason, 14 samples (20 leaves and 10 fruit stems per sample) collected from The Dalles, OR, expressing Little Cherry Disease symptoms were analyzed by a general phytoplasma quantitative real-time PCR assay after 8 symptomatic samples tested negative with the LChV2 Amplify RP® assay. Nine symptomatic samples tested positive by General Phytoplasma real-time PCR (qPCR) and all 5 asymptomatic samples tested negative (Table 1). It should be noted that one sample pair was collected from the symptomatic and asymptomatic portions of a single tree, and produced a positive and negative result on the respective diseased and asymptomatic leaf tissue. This highlights the importance of collecting tissue from a diseased area of the tree, which most notably will be a branch or section with immature, insipid fruit on a tree that has produced otherwise healthy-looking fruit. Since these samples could be multiply infected, a nucleic acid (DNA and RNA) has been extracted from these samples for follow-up testing for *Little cherry viruses 1 & 2* using a more sensitive RT-PCR assay.

Table 1: Number of *Prunus* sp.[a] samples testing positive for virus and virus-like pathogens in each Oregon region surveyed in 2016-2017

			Number of positive samples								
Region[b]	**Year**	**# symptomatic /total**	**CLRV[c] ELISA**	**CRLV ELISA[d]**	**LChV2 RPA[e]**	**PDV ELISA**	**PNRSV ELISA**	**PPV ELISA**	**ToRSV ELISA**	**TRSV ELISA**	**Phytoplasma qPCR[e]**
WV	2016	11/16	0	0	0	2	6	0	0	0	ND
WV	2017[3]	1/2	0	0	ND	0	0	0	0	0	ND
HR	2016	6/13	0	0	ND	5	1	0	2	0	ND
	2017	4/7	0	ND[f]	ND	7	5	0	4	0	ND
TD	2016	23/42	8	0	0	18	7	0	1	0	ND
	2017	16/26	2	ND	ND	14	7	0	3	0	9
RV	2017	12/21	0	ND	ND	5	5	0	0	0	ND
UV	2016	9/14	0	0	ND	1	2	0	0	0	ND
MF	2017	17/28	0	ND	ND	21	7	0	0	0	ND
GR	2017	14/23	0	ND	ND	12	7	0	1	0	ND
TOTAL		**113/192**									

[a] In 2016, 15 samples were collected from non-*P. avium* trees (*P. subhirtella, P. emarginata, P. serrulata).* In 2017, two *P. emarginata* samples were collected. All other samples were from *P. avium* trees.

[b] WV = Willamette Valley, HR = Hood River, TD = The Dalles/Mosier, RV = Rogue Valley, UV = Umpqua Valley, MF = Milton-Freewater, GR = Grande Ronde

[c] CLRV = *Cherry leaf roll virus*, CRLV = *Cherry rasp leaf virus*, LChV2 = *Little cherry virus 2*, PDV = *Prune dwarf virus*, PNRSV = *Prunus necrotic ringspot virus*, PPV = *Plum pox virus*, ToRSV = *Tomato ringspot virus*, TRSV = *Tobacco ringspot virus*

[d] Samples were not screened for CRLV in 2017 due to the lack of reliable ELISA.

[e] Due to the cost associated with the LChV2 RPA and Phytoplasma real-time PCR (qPCR) assay, only samples expressing symptoms of Little Cherry Disease were tested.

[f] ND = not determined

Objective 2: Determine the most likely cause of these symptomatic cherry trees, virus or not!
Orchards were identified by regional cooperators and growers based on virus-suspected disease symptoms. Although all orchards visited had symptoms associated with viruses several were not due to virus. Diagnosis of these orchard problems included: lack of irrigation water, bacterial canker, gophers, crown gall and Phytophthora root rot. If a virus on a diseased sample was not found through initial ELISA screening and an abiotic diagnosis was not made, an alternative assay was used to identify the cause of disease.

One *P. avium* cv. 'Bing' sample with "pixelated", mosaic foliar symptoms collected from the Rogue Valley did not produce a positive result in the ELISA virus screening process. This sample was sent to Agdia, Inc. (Elkhart, IN) for further testing using the following group PCR assays (target viruses in parentheses): Ilarvirus *(American plumline pattern virus, Apple mosaic virus, Prune dwarf virus, Prunus necrotic ringspot virus*), Closterovirus (*Plum bark necrosis stem pitting-associated virus, Little cherry virus 1 & 2*), Potyvirus *(Plum pox virus)*, Nepovirus (*Arabis mosaic virus, Cherry leafroll virus, Cherry rosette virus, Myrobalan latent ringspot virus, Tobacco ringspot virus, Tomato ringspot virus, Stocky prune virus*), Tombusvirus (*Tomato bushy stunt virus, Petunia asteroid mosaic virus, Carnation Italian ringspot virus*), Trichovirus (*Cherry mottle leaf virus*). A positive result was found using general Nepovirus primers, and downstream sequencing produced a 94% identity match to the putative virus *Prunus virus F.*

A weeping cherry sample collected in the Willamette Valley was found expressing chlorotic almost mosaic-like leaf symptoms on the newest growth. After initial screening with ELISA, no virus was found. This sample was sent to Agdia, Inc. (Elkhart, IN) for further testing using the same group PCR assays as listed above. This sample tested negative for all virus groups tested. Based on the extensive testing and peculiar expression of symptoms on the younger leaf tissue, which is not typical of viral symptoms, it was determined that the symptoms observed were not associated with a viral pathogen. Exact cause is still unknown.

Objective 3: Follow up the diagnosis with management recommendations to each grower.
A total of 113 symptomatic leaf samples were collected and analyzed in 2016-2017. After each diagnosis was made, the results were communicated with each grower or regional cooperator. Results were communicated via email, phone or in person.

Objective 4: Survey historical records for occurrence of cherry viruses in Oregon.
About 800 *Prunus* sp. records were assessed for the presence of specific viruses at the Oregon State University Plant Clinic (1956-2016). These records were submitted by county extension agents, growers, or homeowners for the purpose of disease diagnosis. From these records, several of the findings in the literature were corroborated and an unpublished record of *Tobacco ringspot virus* was identified in the Grande Ronde Valley (Table 2).

At the Oregon State University Herbarium, 373 *Prunus* sp. vouchers were inspected for obvious symptoms commonly associated with viruses, including: foliar mosaics, mottling, ringspots, and enations. One bitter cherry (*P. emarginata*) sample collected from the Umpqua Valley (Douglas County) in 1954 had a notable mosaic symptom not considered to be an artifact of the preservation process according to several herbarium curators. Bitter cherries at this location were revisited in 2016 and 2017, but similar symptoms were not observed at this site. A *P. avium* cv. 'Bing' sample collected in Medford, OR, in 2017 expressed similar symptoms and tested positive for *Prunus virus F,* as described under Objective 2.

Table 2: Presence of viruses and virus-like pathogens known to infect sweet cherry in Oregon

			Region							
Name of Pathogen	Present on sweet cherry in Oregon?	Oregon (Region Unknown)	Willamette Valley	Hood River	The Dalles/ Mosier	Umpqua Valley	Rogue Valley	Milton-Freewater	Grand Ronde Valley	Other
American plum-line pattern virus	Yes	+(flowering cherry)[9,10]								
Apple chlorotic leaf spot virus	Yes				+[8]					
Apple mosaic virus	No									
Arabis mosaic virus	No									
Cherry green ring mottle virus	Yes	+[2]	✦	+[8]						
Cherry leaf roll virus	Yes									
Walnut strain	Yes		+(walnut)[6]							
Olm1 strain	Yes				★					
Cherry mottle leaf virus	Yes	+[1,2]	✦	+[8]	✦				✦	
Cherry necrotic rusty mottle virus	Yes	+[1,2]	✦	+[8]	✦				✦	
Cherry rasp leaf	No			+(apple)[4]						
Cherry rusty mottle virus	Yes	+[2]	+[1]✦	+[8]	+[1,8]✦			✦	✦	
Cherry twisted leaf assoc. virus	Yes		✦		+[10]✦					
Cherry virus A	Yes		+[5]✦							
Hop stunt viroid	No		(hop)[7]							
Little cherry virus-1	Yes		+[1]✦							
Little cherry virus-2	Yes				+[11]					
Peach latent mosaic viroid	No									
Plum bark necrosis stem pitting-associated virus	No									
Prune dwarf virus	Yes	+[1]	+[8]✦	–[2]★	+[8]✦	★	★	✦	★	
Prunus necrotic ringspot virus	Yes	+[1]	✦	★	+[8]✦	★	★	✦		✦(Coastal)
Rugose mosaic strain	Yes		✦		✦?				✦	
Prunus virus F	Yes						★			
Tobacco mosaic virus	Yes	–[10]								
Tobacco ringspot virus	Yes								✦	
Tomato ringspot virus	Yes			★					★	
Eola rasp leaf strain	Yes		+[3]✦							
X-Disease	Yes				+[1]★		+[12]	+[1]		+(Malheur)[1]
Not currently known in North America										
Cherry rosette virus	No									
Myrobalam latent ringspot virus	No									
Petunia asteroid mosaic virus	No									
Plum pox virus	No									
Stocky prune virus	No									

[1] MacSwan and Raymer 1959; [2] Hadidi, et al.; [3] Milbrath and Reynolds 1961; [4] Parish 1977; [5] Poudyal et al. 2015; [6] Miller et al. 1958; [7] Cindy Ocamb, personal communication; [8] Eastwell, personal communication; [9] Zeller and Milbrath 1942; [10] USDA-ARS 1976; [11] Drew Hubbard, personal communication [12] Sugar and Long, personal communication

✦ OSU Plant Clinic Record ★ Indicates finding from statewide survey

Objective 5: Summarize the survey information to report on the threat viruses may (or may not) pose to the Oregon cherry industry (and indirectly to the WA industry).

Based on the information gathered through historical records and the statewide sampling survey, viruses have been identified and rated based on their prevalence and potential impact (Table 3). Viruses were rated on a scale of 0 to 10 with 0 representing "no grower action needed" and 10 representing "action is imperative." This ranking scheme considered the ability of the virus to kill trees, significantly reduce yield, cause unmarketable fruit, and the mode of transmission.

We suspect that most growers would not be concerned about a virus with a rating of 5 or lower. Ratings of 6 or higher represent important viruses that will impact production and spread to other trees. Action can range from implementing an insecticide program to minimize vector spread to orchard removal, which could be followed by fumigation to manage other vectors.

Table 3: Grower action rating of sweet cherry cv. 'Bing' virus and virus-like pathogens of importance to Oregon

		Found on sweet cherry in Oregon?	
Name of Pathogen(s)	Action Rating (Pscheidt)[a]	Historically	This Survey
Cherry leaf roll virus **(plus PDV and/or PNRSV)**	7	-	+
Cherry mottle leaf virus	6-7	+	ND[b]
Cherry necrotic rusty mottle virus	7	+	ND
Little cherry virus 1	6-7	+	-
Little cherry virus 2	6-7	-	+
Plum pox virus	10	-	-
Prunus necrotic ringspot virus **(rugose strain)**	6	+	+
Tomato ringspot virus	6	+	+
X-Disease	8	+	+

[a] 0 = no action, 10 = action is imperative
[b] ND = not determined

It may still take weeks to go from "I think it is a virus", collecting and sending in samples, to getting a report back on which viruses might be found. Key symptoms have been identified in association with more severe viruses that may be used to more rapidly initiate an action. These symptoms include enations and little, immature fruit on one or more branches of a tree.

Impact and economic benefits

The historical information from the OSU Plant Clinic and Herbarium contributed to our understanding of the occurrence sweet cherry viruses in Oregon. In the sampling survey, participating growers and regional cooperators were able to identify problems in their orchards and receive a diagnosis, even if the causal agent did not turn out to be viral. An article published by Good Fruit Grower provided an overview of the project objectives and findings after the first year (Dininny & Mullinax 2017).

Cherry leaf roll virus (CLRV) was found for the first time on sweet cherry in The Dalles, OR. Despite being a member of the genus Nepovirus, CLRV is not known to be transmitted by nematodes. The walnut strain of CLRV is known to be pollen-transmitted, but there is still much unknown about the transmission of this devastating virus on sweet cherry (Hadidi et al. 2011). CLRV causes a slow decline when occurring alone, but a synergistic effect occurs when a tree is multiply-infected with the PDV or PNRSV. In this case, a rapid decline will occur. With use of Google street imaging, a rapid decline over a 4-year period was observed. The diagnostic work from this survey prompted the removal of a CLRV-infected orchard with ~%7 trees in decline, to prevent further spread in The Dalles region. This information is being disseminated to the scientific community as a Disease Note in the journal *Plant Disease* (Lutes & Pscheidt 2017, *in press*).

Although there are dozens of viruses that infect sweet cherries, there are only a few that should elicit an immediate response. The grower action rating and identification of symptoms associated with the more severe viruses should allow growers to make more informed management decisions. For example, this survey confirmed that PDV and PNRSV are likely to be present in sweet cherry orchards throughout the state based on their pollen transmission method. These two viruses produce foliar symptoms (mottle, ringspots, "lacey" holes in leaves), but do not reduce yield significantly or kill trees as a single infection. However, foliar enations (gall-like formations on the underside of leaf) and rosetting (bunching of leaves due to reduced internodes) are associated with more severe viruses, including: CLRV and ToRSV. An action rating scale was introduced at the Mid-Columbia Cherry Day in February in The Dalles, Oregon, and published in the May 15th 2017 Issue of Good Fruit Grower (Pscheidt 2017).

As a result of this work, updates have been made to the widely accessible PNW Plant Disease Management Handbook (https://pnwhandbooks.org/plantdisease), including images of symptoms and management recommendations for cherry (*Prunus* spp.) diseases.

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EXECUTIVE SUMMARY

The Washington cherry industry has had to understand, detect, and manage several new viral diseases over the last 15-20 years. It was not known if these same viral diseases were a problem in the Oregon cherry industry. A thorough search of published literature, Oregon State University Plant Clinic and Herbarium records, and physical state-wide orchard sampling found many of the same important viral diseases.

Cherry leaf roll virus (CLRV) was found for the first time on sweet cherry in Oregon. This virus was found in just a couple of adjacent orchard blocks in The Dalles. Infected trees were in decline with poor growth, rosetting, leaf enations, and hardly any fruit. One of the blocks has been removed but continual monitoring of declining trees in this region is recommended.

Several nematode-transmitted viruses, including *Tomato ringspot virus* (ToRSV) and *Tobacco ringspot virus* (TRSV), were found in many areas (The Dalles, Hood River, the Grand Ronde Valley). This means that nematode sampling is strongly recommended prior to planting new cherry orchards, especially if they are not scheduled to be fumigated. This is not only relevant for replanting sweet cherry orchards, but also when transitioning to other susceptible crops such as grapes, peaches, plums, or apples.

Little Cherry Disease was also found in this survey. Little Cherry Disease is characterized by fruit that does not ripen, develop flavor, and/or brix by harvest. The disease can be caused by *Little cherry virus 1 & 2* and/or the X-Disease phytoplasma. We tracked an unpublished record from the Clean Plant Center Northwest (Prosser, WA) that confirmed the presence of *Little cherry virus 2* in The Dalles, OR. Several samples with symptoms of Little Cherry Disease from The Dalles tested positive for phytoplasma. This indicates there may be a resurgence of X-Disease in this area.

A few other production-limiting viral diseases were found in this survey including: the rugose strain of Prunus necrotic ringspot virus and an orchard in the Willamette Valley with Cherry rusty mottle disease.

For most growers and field representatives, the world of cherry viruses is a confusing bowl of alphabet soup. It can be confusing for plant pathologists, as well. To help simplify this world we came up with the grower action rating, a scale of 1 to 10, to indicate which viruses should get more attention than others. This helped reduce the possibility of dozens of viruses down to an important 7 that growers should be worried about. In addition, finding any of two different symptoms – enations or little cherries – should immediately be cause for concern. We think this will help growers take appropriate actions to limit the damage and spread of these diseases.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: CH-16-104

Project Title: ABC of sweet cherry powdery mildew: adaption, behavior and control

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Total Project Request: **Year 1:** $81,321 **Year 2:** $82,187 **Year 3: $84,435**

Other funding sources: None

Budget 1
Organization Name: WSU-IAREC **Contract Administrator:** Katy Roberts
Telephone: 509-335-2885 **Email address:** arcgrants@wsu.edu

Item	2016	2017	2018
Salaries[1]	36,504	37,964	**39,483**
Benefits[1]	17,522	18,223	**18,952**
Wages			
Benefits			
Equipment			
Supplies[2]	25,000	25,000	**25,000**
Travel[3]	1000	1000	**1000**
Miscellaneous[4]	1295*		
Plot Fees			
Total	81,321	82,187	**$84,435**

Footnotes:
[1]Associate in Research
[2]Molecular supplies (DNA extraction, sequencing costs, PCR and qPCR related chemicals, primer development), Nitex mesh for *in vivo* studies, general supplies for greenhouse and laboratory (petri dishes, agar), fungicides
[3]Sampling trips through Washington and Oregon State
[4]Geneious Software license, international shipping of DNA samples

Objectives

1. **Adaptation:**
   a. Isolate and characterize cherry powdery mildew populations in commercial orchards in WA and OR
   b. DNA based identification of the causal agent of cherry powdery mildew and multigene phylogenetic reconstruction of the evolutionary relationships among global cherry mildew entities
2. **Behavior:**
   a. Compare virulence structures of identified clades/ subgroups
   b. Identify niche (host tissue) preferences
   c. Identify reproductive strategies and, if heterothallic, mating type frequencies
3. **Control:**
   a. Evaluate response of powdery mildew spores to fungicide sprays before fruit infection is established (visible) using viability qPCR
   b. Identify critical spray periods in which fungicidal protection is most needed to suppress onset or minimize severity of fruit infection

Significant Findings

- 93 fungal genotypes have been collected in Washington State and Oregon
    - The internal transcribed spacer (ITS) region suggests clonality of the genotypes (no clades or subgroups). A multi-locus analyses should refute or confirm this observation.
- Conidia survival 24h after sulfur treatment in a commercial orchard was 0.3% (99.7% dead conidia) (Table 1)
- Conidial survival on cherry fruit decreased to 0% after fungicide treatment (Table 2)
- Conidia survival decreased daily on the foliage of pruned branches left on the orchard floor reaching 0% (all dead) after 3 days.
    - ***HOWEVER: This leaves the fungus 2 days to get airborne and re-infect nearby foliage!***
- Fungal isolates from seven cherry growing regions were tested against sensitivity towards Quintec (FRAC 13), Fontelis (FRAC 7), Luna Sensation (FRAC 7 & 11) and Vivando (FRAC U8). No resistance was detected (Table 3).

Methods, Results & Discussion

Fungal isolate collection

Powdery mildew isolates were collected in commercial orchards in Oregon: Hood River, The Dalles, and Corvallis; and Washington: Brewster, Chelan, Dallesport, Mattawa, Pasco, Prosser, Quincy, Wenatchee, White Salmon, and Zillah. Multiple isolates were obtained from each region and grown as single conidia to obtain a clean genotype. DNA extractions were performed using the MoBio UltraClean Microbial DNA isolation kit (MoBio Laboratory Inc., Carlsbad, CA) following the

manufactures' protocol. Overall, we obtained 38 genotypes from Oregon and 55 genotypes from Washington. Fungal genotypes are stored in the freezer.

Species specific primers for multiple genes and the mating-type genes are still being developed using a primer software and previous publication of genes in related fungal species. Due to the regional limitations of the cherry powdery mildew, there are not many published or developed primers for *P. clandestina*. A first round of amplifications were conducted using the internal transcribed spacer (ITS) region. ITS is a universal genetic region used in fungal taxonomy to identify fungal species. However, its resolution in distinguishing between genotypes of the same fungal species is limited. Therefore we sequenced the complete ITS (ITS1/2) region to achieve better resolution and compared this region to related powdery mildew species (apple and rose powdery mildew). We also successfully developed primers to sequence a partial region of the β -tubulin II (TUB2) gene.

ITS sequence alignments resulted in 100% sequence identity suggesting that *P. clandestina* populations from all sweet cherry growing regions are clonal. However, this assumption is based on just one locus. The sexual stage of *P. clandestina* is very common in Washington and Oregon (chasmothecia) and presents the main route of overwintering for the fungus. Considering the constant mating, a clonal population structure seems highly unlikely. It is more likely that ITS was the wrong genetic locus to differentiate between these groups. After discussion with Swamy Prashant, a molecular plant pathologist at WSU in Prosser, we are pursuing a more complicated route of identifying population structures. More complicated because the genetic regions of interest have not been studied yet in most powdery mildew.

Conidia survival on leaves after sulfur treatment and on pruned branches left on the orchard floor. On May 11, powdery mildew was collected in a commercial orchard that had an early powdery mildew epidemic. This epidemic preceded the onset of foliar powdery mildew in the experimental orchard at WSU. The initial infection occurred on water sprouts originating in the tree crotch. One day before samples were collected, the trees received a standard sulfur application. Powdery mildew samples were transferred to the lab; conidia were washed off the leaves and treated with PMA to determine viability. The mean reduction in fungal viability after application of sulfur was 99.7%. Unfortunately, we could not include a negative control since the entire orchard had been treated with sulfur and mildew had not yet appeared in the WSU orchard yet.

Table 1 Effect of sulfur treatment on PM conidia viability

	PMA	***Mean C_T***	***ΔC_T (% dead conidia)***	***Mean ΔC_T (% dead conidia)***
Sample* 1	no	28.1	**3.5 (89%)**	**8.8 (99.7%)**
Sample 1	yes	31.6		
Sample 2	no	20.9	**14.1 (99.99%)**	
Sample 2	yes	35.0		

*Each sample consisted of 8 pruned branches. Ten leaves were removed randomly and processed to determine conidia viability.

Pruned branches were left on the orchard floor to follow PM survival on its dying host. Conidia were completely undetectable with the PMA assay after 3 days (0% survival). However, for two days conidial activity was still measurable on the branches, leaving the potential to get airborne and establish new infections on susceptible host tissue and the orchard.

Powdery mildew growth on fungicide treated cherries (cv. Bing)

Developing fruit clusters were covered with a 8 micron nylon mesh (Nitex) bag at fruit set to avoid natural infection of the cherries by *P. clandestina*. Clusters of cherries were uncovered on June 15, 2017 and inoculated with a liquid powdery mildew suspension (25,000 conidia/mL). Cherries were recovered with the nylon mesh bag and the fungus was allowed to develop for three days. After 72h, clusters were uncovered again and sprayed with either Quintec (FRAC 13), Fontelis (FRAC 7), Luna Sensation (FRAC 7 & 11), or water (untreated control). The maximum allowable rate per application was used and spray coverage was100%. On July 15, 2017, no fungal growth (disease incidence) was observed on the fungicide treated cherries. Disease incidence on the untreated control ranged from 65 to 90% (Table 2). Results from the viability qPCR showed that no viable conidia were detected in the fungicide treated cherries on July 15. This experiment showed that fungicide applications halter the onset of powdery mildew even though viable conidia are present (but infection is still invisible) on the fruit. The fungicide application led to an abortion of conidia (100% dead after 4 weeks).

Table 2. Powdery mildew Disease Incidence (%) of fungicide treated Bing cherries with active conidia attached to them

	Test[a] 1	***Test 2***	***Test 3***
	% Disease Incidence[b] on July 15, 2017		
Quintec	**0**	**0**	**0**
Fontelis	**0**	**0**	**0**
Luna Sensation	**0**	**0**	**0**
UTC[c]	**65**	**90**	**85**

[a] Each test was conducted on three random trees (cv. Bing) at WSU (Roza, block D51)
[b] Disease Incidence = proportion of diseased cherries [(Number of diseased cherries / total number of cherries)*100]
[c] UTC = untreated control; cherries received no fungicide application. Mean of three clusters of cherries per tree.

Fungicide resistance assays using fungal isolates from different growing regions

Powdery mildew isolates were collected from commercial orchards in Cashmere, Mattawa, Pasco, Prosser, Stemilt Hill, The Dalles (OR), and Wenatchee after harvest. *Podosphaera clandestina* colonies were maintained on cherry seedlings in the greenhouse and on detached leaves (cultivars Bing and Sweetheart) in a growth chamber (22˚C, 14h photoperiod). Detached leaves were kept on autoclaved cotton ovals saturated with 5% filter sterilized sucrose solution in petri dishes. To obtain clean fungal cultures, a single leaf with distinctive colonies was selected and used to transfer single mycelial chains to susceptible (surface sterilized) cherry leaves. Petri dishes containing the inoculated leaves were sealed with parafilm to keep humidity levels high. Colonies were transferred to fresh leaves every 3 weeks.

Note: In some orchards a steep gradient between powdery mildew infection of the lower and upper canopy was found, with some only showing (severe) infection in the upper canopy. If this is observed, spray regimes should be re-evaluated to ensure coverage in the upper canopy is maximized.

Briefly, susceptible leaves (sweet cherry cultivar 'Bing') were harvested from trees grown in a disease-free greenhouse. After surface sterilization (5 minutes in 10% bleach water v/v followed by a triple rinse with sterile di water) 15 mm leaf disks were excised with a cork borer. Leaf disks were dipped in a full strength or half strength fungicide solution, air-dried, and transferred with their abaxial side up to 24-well tissue culture plates containing 500 µl of agar (1% w/v) per well. Leaf disks not treated with fungicides served as a positive control. A standardized conidial suspension (10,000 conidia/mL) was prepared fresh from infected leaves. Each leaf disk was inoculated with a 20ul drop of the conidial suspension (200 conidia per drop). Leaf disks inoculated with water served as a negative control. Plates were covered with a lid, sealed with parafilm to avoid humidity loss and incubated in a diurnal growth chamber (14h photoperiod, 71°F) for 21 days. Fungal growth on untreated (UTC) leaf disks was measured and compared to the growth of the fungus on fungicide treated leaf disks. Each UTC was inoculated with the isolate from the respective growing area. The following fungicides were tested: Quintec (FrAC 13), Fontelis (FRAC 7), Luna Sensation (FRAC 7 & 11) and Vivando (FRAC U8). Results are presented in Table 3: No fungicide resistance against the four tested fungicides was seen for the isolates tested in this study.

Table 3: Fungicide resistance leaf disk assays

Trade name	**Active Ingredient**	**FRAC Group**	**Applied at[a]**	***The Dalles*** **OR**	***Cash-mere**** **WA**	***Stemilt Hill*** **WA**	***Mattawa*** **WA**	***Pasco*** **WA**	***Prosser*** **WA**	***Wenatchee*** **WA**
Fontelis	Penthiopyrad	7	Max	-[c]	-	-	-	-	-	-
Fontelis	Penthiopyrad	7	Half	-	-	-	-	-	-	-
Luna Sensation	Fluopyram & Trifloxystrobin	7&11	Max	-	-	-	-	-	-	-
Luna Sensation	Fluopyram & Trifloxystrobin	7&11	Half	-	-	-	-	-	-	-
Quintec	Quinoxyfen	13	Max	-	-	-	-	-	-	-
Quintec	Quinoxyfen	13	Half	-	-	-	-	-	-	-
Vivando	Metrafenone	U8	Max	-	-	-	-	-	-	-
Vivando	Metrafenone	U8	Half	-	-	-	-	-	-	-
UTC[b]	**water**	none		+	+	+	+	+	+	+

[a]Allowable rate per application; Max = maximum, full strength; Half = 50% of maximum
[b]UTC = untreated control; isolates from the respective area grown on untreated leaf disks
[c] Absence of fungal growth is indicated by a minus sign (-); presence of fungal growth is indicated with a plus sign (+)
* 2 locations in Cashmere were sampled. Both had the same results.

CONTINUING REPORT **PROPOSED DURATION:** Year 2 of 3

Project Title: Enhanced strategies to reduce postharvest splitting of cherries

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Cooperators: TIC Gums, Van Doren Sales, Inc., Chelan Fruit, Stemilt Growers LLC and Allan Bros. Inc., Shield Bags and Printing Company, Washington Fruit & Produce Co. and others.

Budget: **Year 1**: $31,407 **Year 2**: $33,185 **Year 3**: **$34,753**

Budget 1
Organization Name: Washington State University **Contract Administrator:** Katy Roberts
Telephone: 509-335-2885 **Email address:** arcgrants@wsu.edu

Item	2016	2017	**2018**
Salaries	$14,092	$14,656	**$15,242**
Benefits	$1,235	$1,285	**$1,337**
Wages	$9,055	$9,417	**$9,794**
Benefits	$1,275	$1,327	**$1,380**
Equipment			
Supplies	$5,000	$5,000	**$5,000**
Travel	$750	$1,500	**$2,000**
Plot Fees			
Miscellaneous			
Total	$31,407	$33,185	**$34,753**

Footnotes: Budget is requested to cover salaries and wages for the students working on the project. Money is also requested for purchasing laboratory supplies and small equipment for the experiments. Travel funds are requested to visit our co-operators for project work, specifically for the plant trials.

1. OBJECTIVES:
The original objectives proposed were:

1. Develop an understanding of the mechanism by which gum acacia helps reduce cherry splits.
2. Enhance the film forming ability of gum acacia by other low cost friendly edible coatings and modifiers.
3. Optimize the level of embedded desiccant in the packaging to help reduce cherry splitting.
4. Conduct post-packing cooling studies with enhanced coatings to help reduce stem browning.
5. Evaluate the consumer acceptance of the cherries coated with the optimized edible coatings.

Work in second year (2017) was accomplished on the following aspects:

- Selected the best coatings that worked to reduce cracking and stem browning in the year 2016 trials.
- Studied the interaction effect of the select coatings from the 2016 study with packing liners loaded with different concentration of desiccant.
- Coatings: Gum Acacia Senegal (Grade 1), Gum Acacia Seyal (Grade 2) with modifiers agar and sodium alginate used in the lab trials with sweet cherry *var*. Tieton.
- Conducted trials on packing line with Gum Acacia Senegal (Grade 1) for 5 different liners with the sweet cherry *var*. Skeena. Also, tested above mentioned coatings with just one type of liner (obtained from packing facility) to compare the difference within the coating treatment.
- Liners used: desiccant loading @ 0%, 2%, 4% and 6% concentrations.
- Performed sensory analysis on the coated cherries in the 4th week of storage, *var*. Skeena, to see if consumers can detect any difference in the coated and uncoated cherries.

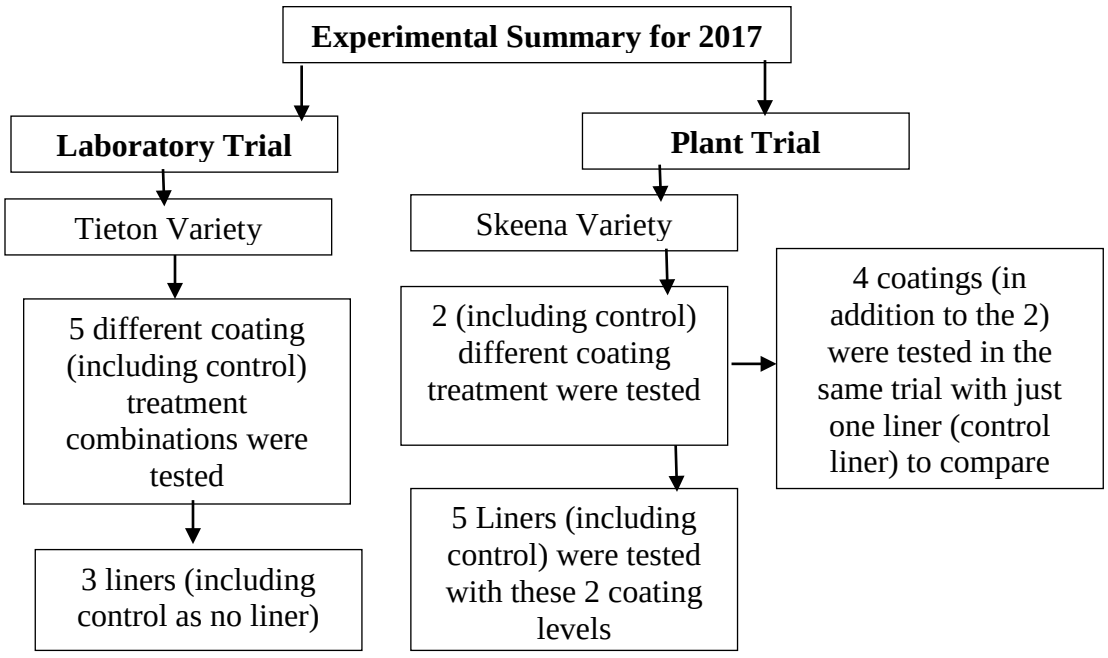


Figure 1. Flow chart showing the summary of the experiments conducted in 2017. All treated cherry samples were subjected to storage studies for 5 weeks.

2. SIGNIFICANT FINDINGS:

Overall findings:

- Gum Acacia Senegal and Gum Acacia Seyal with 2% loaded desiccant liner had lower values for cracking and Gum Acacia Seyal (90%) and Agar (10%) with 2% loaded desiccant liner reduced stem browning at the end of the storage study, in the lab trials.
- Gum Acacia Senegal with 0% loaded desiccant liner had lower values for cracking, Gum Acacia Senegal with 6% loaded desiccant liner was observed to be beneficial to reduce the stem browning, in the plant trials.
- Packaging with the desiccant aided in reducing the moisture in the packages, which in turn helped to reduce the cracking.
- Reducing the moisture on the Cherries before packing is very critical in controlling the post-harvest cracking.
- The plant trial samples showed molding after week 4. This was not normal and after careful review it was found that the samples had high moisture in the packages.
- The new white mesh belts did not prove to be effective with the air knives in removing the excess moisture/coating solution from the cherry surface.
- Based on this, we came to conclusion that the air knife treatment need be implemented carefully, to ensure that the excess moisture is removed from the cherries before packing.
- Sensory evaluation of the product coated and uncoated, after 4th week of storage, did not show any significant differences in the consumer acceptance, in overall quality as well as individual attributes.
- Sensory testing was done on the *var*. Skeena, from the plant trials.
- Final test needs to be conducted in 2018 (during the final year of the project) to confirm all the findings and make the final recommendations to the industry.

Following are the significant findings categorized for different Cherry varieties:

2.1 Tieton (Lab Trials) 5 levels of coatings and 3 levels of liners

- "Gum Acacia Senegal 100%" (0.5%) with 2% loaded desiccant liner and "Gum Acacia Seyal 100%" (0.5%) with 2% loaded desiccant liner were observed to reduce the surface cracking at the end of storage period.
- "Gum Acacia Seyal 90% & Agar 10%" (0.3%) with 2% loaded desiccant liner was effective in reducing the stem browning and retained the green color till end of storage period than other coating-liner combinations.
- "Gum Acacia Seyal 100%" (0.5%) with 0% loaded desiccant liner had the least weight loss values.
- "Gum Acacia Seyal 100%" (0.5%) with 4% loaded desiccant liner was effective for maintaining the fruit pH;
- "Gum Acacia Seyal 90% & Agar 10%" (0.3%) with 0% loaded desiccant liner had lower values for soluble solids and thus helped in retaining the increase in sugar concentration of the fruit.
- "Gum Acacia Seyal 100%" (0.5%) with 4% loaded desiccant liner maintained the acidity of the fruit well.

2.2.1 Skeena (Plant Trials) 2 levels of coatings and 5 levels of liners

- "Gum Acacia Senegal 100%" (0.5%) with 0% loaded desiccant liner was effectively delaying the cracking of fruit over time.
- "Gum Acacia Senegal 100%" (0.5%) with 6% loaded desiccant liner kept the stem from turning brown over the storage time.
- "Gum Acacia Senegal 100%" (0.5%) with 6% loaded desiccant had higher values for firmness, keeping turbidity intact.
- "Gum Acacia Senegal 100%" (0.5%) with 4% loaded desiccant liner had a lower pH value and higher acidity in terms of malic acid content.

2.2.2 Skeena (Plant Trials) 4 levels of coatings with single control (no desiccant) liner

- The data from these samples did not show any differences between all the treatments till the end of 3 weeks of testing.
- The 4th week was not tested.
- Following this, during the 5th week, we saw all the bags (for all treatments) had significant mold growth. Due to this mold growth, we could not evaluate the quality of the cherries.
- Significant results were not obtained from this set of experiments.
- Upon close observation of all the experimental details including the plant trial line set-up, we discovered that the major difference this year was the belts on the packing line.
- This year, the packing line had the "white mesh belts" (Figure 2b). This belt has very small pores, which tend to have higher surface tension and thus does not drain all the water that is stripped off from the cherries by the air knife.
- Based on this our recommendation is not to use these types of belts under the air knife.

2.3 Sensory Analysis

No difference was observed in the in overall acceptance, aroma, sweetness, sourness and flavor of the cherries, for all the treatments. The treatments included, i) Control (no coating); ii) Gum Acacia Senegal (100%) at 0.5% concentration; iii) Gum Acacia Senegal (95%) & Sodium Alginate (5%) at

0.5% and iv) Gum Acacia Senegal (90%) & Agar (10%) at 0.3% concentration, treatments for Skeena cherries at week 4 of storage.

3. METHODS:

3.1 Materials:

Sweet cherries packed in carton boxes (*var.* Tieton) were procured from a packing house in Naches, WA. The cherries were stored in the pilot plant at Washington State University (WSU), Pullman. A total of 4 coatings, plus the control (5 treatments) were tested on these cherries. A total of 2 liners including control (no liner) were included in the study. This trial has been referred to as "Lab Trial", throughout this report.

A plant trial for *var.* Skeena, was also conducted with the help of a cooperator in Naches, WA. Two studies were conducted in this trial. First comprised of 2 coatings with a control (no coating) treatment and 5 liner types with varying desiccant levels. Second, 4 different coatings including control (no coating) with a single liner. The air knife treatment, to remove the excess coating from the surface was applied for all treatments in the plant. These cherries were then packed in carton boxes and brought to the pilot plant facility at WSU where they were stored in refrigerated storage (42 ^{0}F & 75% RH). This trial has been referred to as "Plant Trial" throughout this report.

3.2 Coating Application:

3.2.1 Lab Trial

For the cherries coated at the laboratory, dipping method was used. Cherries were dipped in buckets/container filled with the solution for 1 minute, ensuring that all the cherries were properly immersed in the solution. The excess coating was drained off with the help of a strainer and air dried at room temperature for 45 min. Following this, the cherries were stored in the walk-in refrigerator for the storage studies, in the commercial bags and boxes. This study was conducted over a period of 5 weeks where the quality parameters were tested every week.

3.2.2 Plant Trial

The waterfall method of coatings was used during the plant trials followed by the removal of excess coating with air knives. The system consisted of two air knives along with a blower, mounted on the conveyor belts in the plant. The coated cherries were air dried just before packing. The coated and air-dried cherries were packed in standard bags and boxes. Following this, the cherries were transported to WSU and stored in the walk-in refrigerator for the storage studies. This study was carried for 5 weeks, the quality parameters tested on week1, week 2, week 3 and week 5.

3.3 Coatings Used:

Tables 1 and 2, show the details of the coatings and liners used in Lab trials respectively

Table 1. Details of the coatings used in the lab trials with *var.* Tieton

Sr. no.	Code	Coating	Solution Conc. %
1	**ASD**	Gum Acacia Senegal 100%	0.5
2	**ASF**	Gum Acacia Seyal 100%	
3	**NASF**	Gum Acacia Seyal 95% & Sodium Alginate 5%	
4	**AGSF**	Gum Acacia Seyal 90% & Agar 10%	0.3
5	**CRT**	No Coating	

Footnote: Coatings selected based on effectiveness in reducing cracking and stem browning in 2016 study.

Table 2. Liners used in Lab trial

Sr. no.	Code	Liner with % desiccant loading
1	**A**	No Liner
2	**B**	2
3	**C**	0

Tables 3 & 4 show the details of coatings and liners used in the plant trials with *var*. Skeena

Table 3. Details of the coatings used in the plant trials with Skeena variety

Sr. no.	Code	Coating	Solution Conc. %
1	**ASD**	Gum Acacia Senegal 100%	0.5
2	**NASD**	Gum Acacia Senegal 95% & Sodium Alginate 5%	
3	**AGSD**	Gum Acacia Senegal 90% & Agar 10%	0.3
4	**CRT**	No Coating	

Foot note: ASD and CRT were tested with liners A, B, C, D & E. While all the coatings (1 through 4) were tested with just liner E.

Table 4. Liners used in Plant trial

Sr. no.	Code	Liner with % desiccant loading
1	**A**	4
2	**B**	2
3	**C**	0
4	**D**	6
5	**E**	Obtained from packing facility

3.4 Fruit Quality Testing Parameters

Cherries were analyzed for seven quality parameters each week through the entire storage period of respective trials, as described in the original proposal. Quality parameters determined were Weight loss, Cracking, Firmness, Pedicel browning, pH, Total Soluble Solids (TSS) (^{o}Bx), Titratable Acidity (% malic acid).

3.5 Sensory Analysis

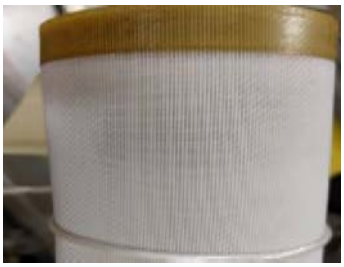
The sensory analysis of untrained panel (n=120) was carried out for the Skeena variety cherries with the coatings listed in section *2.3.* The fruit selected for the sensory analysis belonged to the Liner Type E.

4. RESULTS & DISCUSSION

- Gum Acacia Seyal performed well with the packing liner loaded with 2% desiccant in terms of reducing cracking.
- The effect of the combination; modifiers with Gum Acacia Seyal, cannot be concluded firmly, to have worked well while studying the interaction effect with packing liner with varying embedded desiccant.
- Gum Acacia Senegal which had proven to have achieved the aim of reducing cracking and stem browning in previous year's study did perform well. Although its effectiveness was observed in a combination of liner types.
- In the plant trial, modifiers; Agar and Sodium Alginate, with Gum Acacia Senegal were tested to check if any synergistic effect to improve the fruit quality. Gum Acacia Senegal alone provided better comparative results.

- Some variability in the data in the results of plant trial may have arisen due to the change in the conveyor belt system from year 2016 trial which resulted in inefficient drying of the sample before it was packed into bags.
- This led to mold growth in all the treatment bags, leading us to unreliable data from this particular plant trials.
- Although this confirms that, for efficient removal of the excess moisture from the coated cherries, it is important to have the proper drain belt, at least under the air knives.
- Figure 1, shows the types of the belts used in 2016 and 2017. The belts used in 2016 trials, were better drain belts than the ones used in the year 2017.
- Thus, for the final trials in the year 2018, we will make sure to specifically use the belts that drain better.

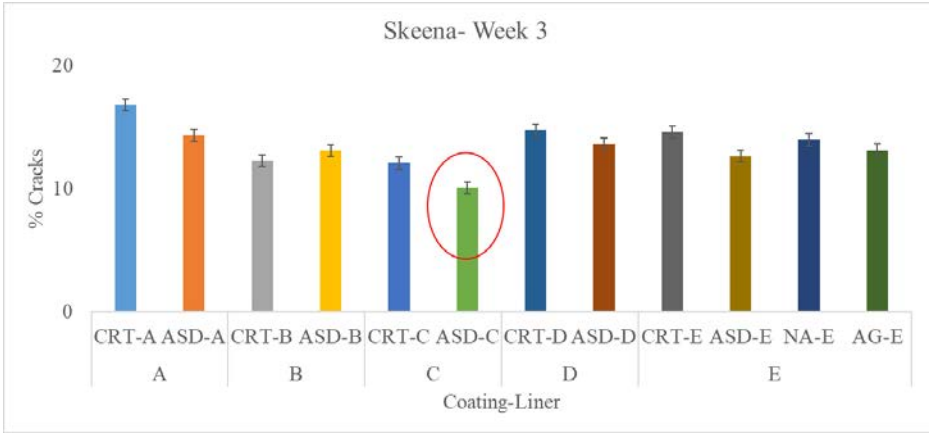
(a) Belt used in 2016



(b) Belt used in 2017

Figure 2. Belts used under the airknives in the plant trials.

Figure 3, shows the results on the % cracking in the cherries, from the plant trials and the end of week 3.



A significant reduction in cracking was observed at week 3 with Gum Acacia Senegal with Liner embedded 0% desiccant.

Figure 3. Percent cracking in cherries with ASD and Control at various liner levels at Week 3 for the plant trials with Skeena variety. ASD - Gum Acacia Senegal (100%) at 0.5% concentration. Liner C – 0% desiccant loading.

Sensory Analysis Summary

- From the preliminary sensory analysis of the cherries with four different treatments, no differences were observed in the acceptance of aroma, sweetness, sourness, cherry flavor and overall acceptance.
- Consumers could not detect the difference for the above parameters among “Gum Acacia Senegal 100%” (0.5%), “Gum Acacia Senegal 95% & Sodium alginate 5%” (0.5%), “Gum Acacia Senegal 90% & Agar 10%” (0.3%) and the control with no coating.

5. IMPACTS TO THE CHERRY GROWERS

- By reducing the cracking and the pedicle browning, there will be extended shelf life for the Cherries to be sold in the market by retaining the fruit quality.
- This will potentially help increase the income to the Cherry growers.

6. NEXT YEARS PROPOSED WORK

- Final plant trials to be conducted to evaluate the effectiveness of all the major findings.
- Conduct sensory trials for the 4^{th} and 5^{th} weeks of storage.
- Make final recommendations to the industry.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3

Project Title: Ensuring long-distance ocean shipping arrival quality of PNW cherries

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Email: dongyu@oregonstate.edu

Cooperators: Jinhe Bai, David Gibeaut, Johnny Gebbers, Yingli Li, Shaoying Zhang, Steve Castagnoli

Total Project Request: **Year 1:** $45,542 **Year 2:** $46,794 **Year 3: $48,086**

Budget:
Organization Name: OSU-MCAREC **Contract Administrator:** Russ Karow
Telephone: 541-737-4066 **Email address:** Russell.Karow@oregonstate.edu

Item	2016	2017	2018
Salaries	29,407[1]	30,289	31,198
Benefits	5043[2]	5245	5455
Wages	4,584[3]	4,722	4,864
Benefits	1,008[4]	1,038	1,069
Equipment			
Supplies	5,000[5]	5,000	5,000
Travel	500[6]	500	500
Miscellaneous			
Total	45,542	46,794	**48,086**

Footnotes:
[1]Postdoctoral Research Associate: 2/3 FTE. 3% increase is factored into Year 2 and 3.
[2]OPE: 2/3 FTE at 17.15%. 4% increase is factored into Year 2 and 3.
[3]Wages: 300hr for a Biological Science Tech. at $15.28/hr. 3% increase is factored into Year 2 and 3.
[4]OPE: 22% of the wage, with a 3% annual increase.
[5]Supplies: fruit, fruit quality and nutrient analyses, fruit volatile compound analyses, GC and GC/MS supplies (helium, nitrogen, hydrogen, standard gases), gas tank rental, chemicals, and MCAREC cold room and land use fees.
[6]Travel to grower fields and packinghouses

OBJECTIVES

Flavor deterioration is a major arrival issue for long-distant ocean shipping (3-5 weeks) of PNW sweet cherries. The goal of this project is to understand the mechanism of cherry flavor deterioration and develop commercially feasible protocols to maintain postharvest quality of PNW sweet cherry cultivars.

1. Understand the mechanisms of flavor deterioration (year 1 and 2).
    a. Bland flavor
    b. Bitter taste
    c. Internal browning (IB)
    d. Anaerobic aroma
2. Identify pre- and postharvest factors affecting flavor deterioration and internal browning (year 1-3).
3. Determine reliable predictors for cherries with long postharvest flavor life (year 1-3).

SIGNIFICANT FINDINGS

Mechanisms of flavor deterioration

1. Bland flavor is closely correlated with the loss of malic acid (titratable acidity, TA), but it is not correlated with soluble solids content (SSC).
2. The development of bland flavor in 'Skeena' mainly results from decreases in aroma volatile compounds (mainly aldehydes and alcohols).

Identify pre- and postharvest factors affecting flavor deterioration and shipping quality

1. Among the major cultivars ('Bing', 'Skeena', 'Lapins', 'Sweetheart', and 'Regina') produced in the PNW, 'Sweetheart' and 'Skeena' retained variety specific flavor during storage longer than 'Bing', 'Lapins', and 'Regina'.
2. A pre-harvest simulated rain event did not affect 'Lapins' and 'Skeena' fruit quality, bitter taste or the rate of flavor deterioration in 2017.
3. Calcium levels in cherries affected flavor deterioration and bitter taste development. The fruit with higher calcium concentration were more resistant to softening, loss of TA, and flavor deterioration
4. Late harvest fruit had lower TA and flavor sensory scores along with increased bitter taste and internal browning after 5 weeks of storage at 32 °F.
5. Compared to fruit stored at 32 and 34 °F, fruit stored at 36 °F had lower fruit firmness and TA along with higher stem browning and decay after 4-5 weeks storage/shipping.

Determine reliable predictors for cherries with long postharvest flavor life

1. In 'Lapins' fruit, postharvest flavor life was positively correlated with fruit tissue content of Ca, P, Mg, and K.
2. Fruit with higher N and lower Ca and P was more likely to develop bland flavor and bitter taste.

METHODS

1. Understand mechanisms of flavor deterioration of cherry after 3-5 weeks of cold storage.

a. Changes of carbohydrates: SSC, TA
b. Changes of volatile aroma compounds: GC, GC-MS (mass spectrometry)
c. Dynamics of TAC, individual antioxidants, MDA, TP, PPO, POD, O-quinones
d. Correlation of fruit physiology and biochemistry with sensory trained panel evaluations

2. Identify pre- and postharvest factors affecting flavor deterioration.

a. Cultivar
b. Heat: pre- and post-heating (>95 °F)
c. Rain: before and after rain (simulated)
d. Fruit nutrition: pre-harvest Ca application
e. Harvest maturity: based on Ctifl color scores (4, 5, 6, 7)

f. Commercial MAP liners: low, medium, and high gas permeability
g. With and without post-packing forced-air cooling
h. Storage/shipping temperature: 32, 34, 36°F

3. Determine predictors for cherries with long postharvest flavor life.

a. Fruit were collected from orchards with distinct flavor deterioration during shipping.
b. Measure fruit quality parameters: SSC, TA, TAC, individual antioxidants
c. Measure fruit nutrient content: (N, P, K, Ca, Mg, S, B, Fe, Zn)
d. Correlate flavor life with these quality attributes.

RESULTS

1. Mechanisms of flavor deterioration of sweet cherries after 5 weeks of cold storage

a. Changes of carbohydrates: SSC, TA

In 2016 and 2017, 'Sweetheart' and 'Skeena' had higher TA at harvest compared to the other cultivars. 'Sweetheart' and 'Skeena' TA degraded at a faster rate in storage. However, SSC did not change significantly between harvest and after 5 weeks of storage (Fig. 1). After 5 weeks of storage, bland flavor was closely related to the absolute levels of TA, as fruit with 0.6% malic acid was perceived to have much more bland flavor than fruit with 0.8% malic acid. The sugar/acid ratio was highly correlated with bland flavor (r = 0.871) and may be a good indicator of potential for bland flavor development.

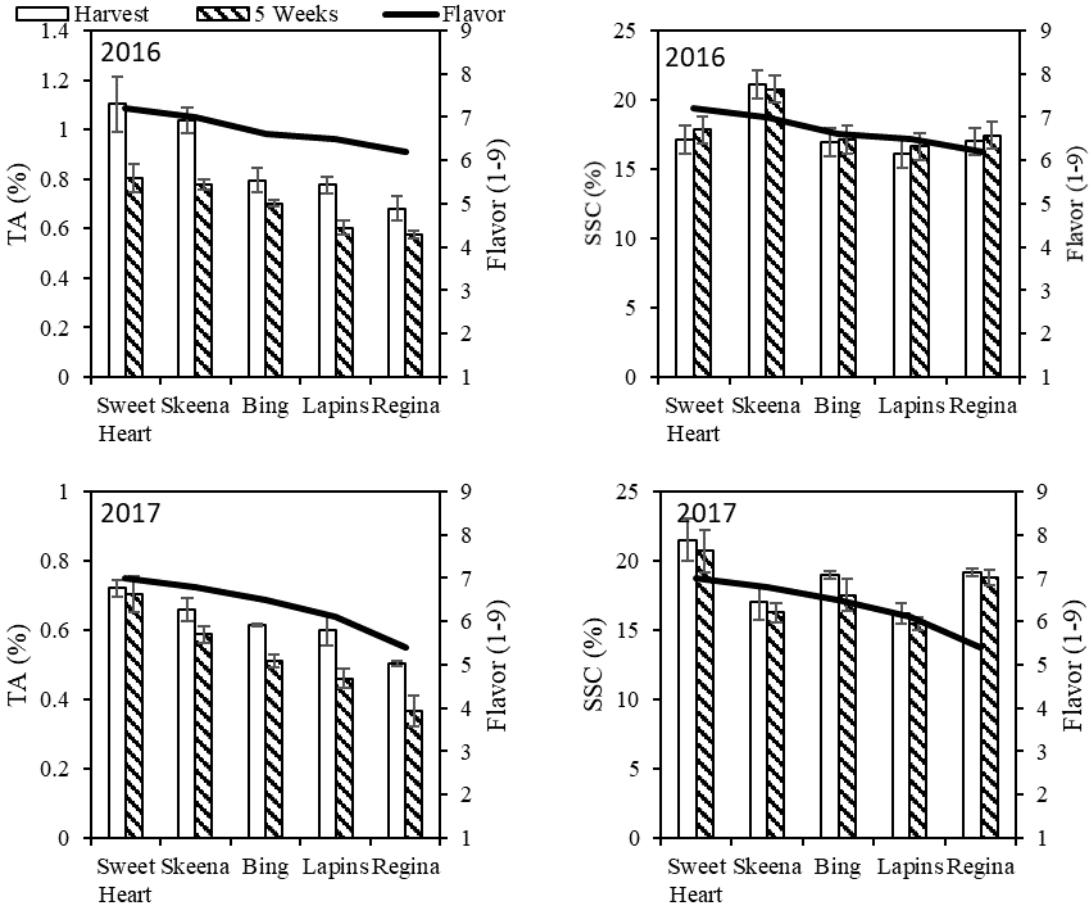

Fig. 1 TA, SSC, and flavor scores of 'Sweetheart', 'Skeena', 'Bing', 'Lapins', and 'Regina' cherries during 5 weeks of storage at 32 °F in 2016 and 2017.

***b. Changes of volatile aroma compounds*: GC-MS (mass spectrometry)**

Tests conducted with 'Skeena' fruit sourced from five orchards indicated that aroma volatile compounds (including acetaldehyde, pentanol, (Z, Z)-2,4-hexadiene, (E)-2-penten-1-ol, (E)-2-hexen-1-ol, D-limonene, and benzyl alcohol) decreased after 5 weeks of storage, while benzaldehyde increased significantly ($p < 0.05$) compared to levels at harvest (Table 1).

A principal component analysis (PCA) was performed using 19 aroma volatiles. 51.68% of the total variation was accounted for by two principal components (PC1 and PC2, Fig. 2). 'Skeena' sourced from five orchards at harvest (solid circles) and after 5 weeks of storage (open circles) separated into two clusters. One cluster (dashed line and solid circles) was positively correlated with PC1 and was characterized by higher levels of acetaldehyde, pentanol, (Z, Z)-2,4-hexadiene, (E)-2-hexen-1-ol, 3-methyl-3-buten-1-ol, (E)-2-penten-1-ol, 2-methyl-2-buten-1-ol, hecanol, dodecane, and benzyl butyrate, while the other cluster (solid line and open circles) showed reductions in those aroma volatile compounds.

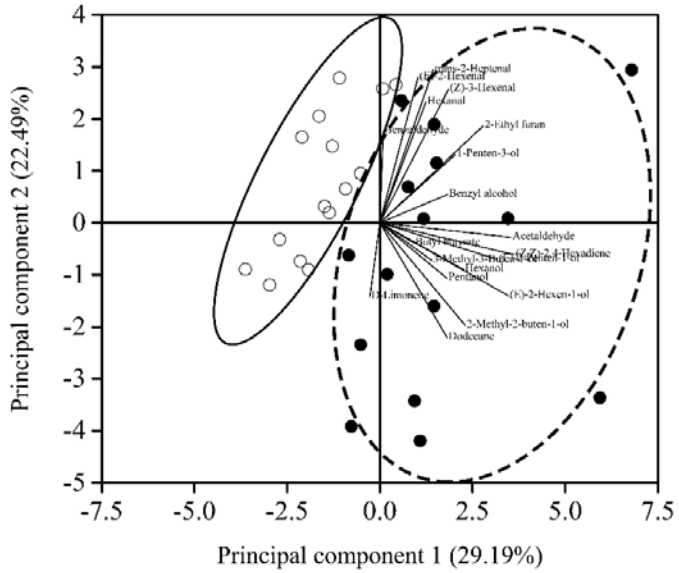

Fig. 2 Principal component analysis (PCA) of 19 aroma volatiles compounds in 'Skeena' cherries from 5 orchards at harvest (● solid circles) and after 5 weeks of storage (○ open circles).

c. Dynamics of TAC, individual antioxidants, MDA, TP, PPO, POD, O-quinones

Data are not presented because similar results with year-1.

d. Correlation of fruit physiology and biochemistry with sensory panel evaluations

Data are still being analyzed.

2. Identify pre- and postharvest factors affecting flavor deterioration

a. Cultivar Data are not presented because the results were shown in Result 1.a.

b. Heat Data will be presented in the final report.

c. Simulated Rain event:

Three trees of both 'Lapins' and 'Skeena' were sprayed with 8 gallons of tap water over 25 minutes (ambient air temperature ~87 °F; water temperature ~69 °F) using a hand pressure sprayer when fruit was close to commercial harvest maturity. Fruit were harvested before simulated rain, then harvested again after 8 h. No significant differences ($p > 0.05$) in fruit firmness, size, SSC, TA, flavor sensory, or bitter taste were observed between fruit treated or not treated with simulated rain (Table 2). Next season, we will conduct additional tests of simulated rain on fruit flavor deterioration one week before commercial maturity using deionized water.

Table. 1. Aroma volatile compounds identified in 'Skeena' cherries from 5 orchards at harvest and after 5 weeks storage at 32°F.

Retention Time (s)	Compounds	Orchard 1		Orchard 2		Orchard 3		Orchard 4		Orchard 5	
		H	5 w	H	5 w	H	5 w	H	5 w	H	5 w
3.80	Acetaldehyde	0.73±0.14	0.65±0.12	1.05±0.44	0.45±0.20	1.06±0.38	0.56±0.10	1.02±0.27	0.69±0.07	0.83±0.19	0.60±0.14
4.85	Pentanol	0.43±0.07	0.38±0.03	0.54±0.27	0.26±0.06	0.49±0.05	0.30±0.07	0.47±0.03	0.30±0.04	0.41±0.19	0.22±0.05
8.12	(Z,Z)-2,4-Hexadiene	1.33±0.44	0.76±0.16	2.06±0.47	1.03±0.70	1.86±0.40	0.96±0.32	1.81±0.83	1.13±0.38	1.30±0.77	0.86±0.29
10.10	1-Penten-3-ol	0.41±0.09	0.48±0.06	0.85±0.48	0.54±0.35	0.69±0.21	0.67±0.08	0.83±0.19	0.90±0.35	1.05±0.67	0.90±0.34
10.72	2-Ethyl furan	0.43±0.07	0.47±0.07	0.73±0.36	0.45±0.03	0.88±0.39	0.72±0.13	1.42±0.39	0.85±0.03	0.88±0.27	0.97±0.07
11.94	3-Methyl-3-Buten-1-ol	1.93±1.15	2.49±0.12	3.20±0.53	2.28±0.40	2.20±1.05	2.73±0.54	2.22±0.62	2.99±0.22	1.34±0.17	1.82±0.87
13.62	(E)-2-Penten-1-ol	0.25±0.03	Nd	0.35±0.16	Nd	0.25±0.01	Nd	0.58±0.10	Nd	0.31±0.00	Nd
13.76	2-Methyl-2-buten-1-ol	2.81±0.48	2.02±0.38	3.52±1.41	1.47±0.82	1.65±0.14	2.13±0.29	2.13±0.93	1.32±0.42	1.27±0.67	1.30±0.03
14.84	(Z)-3-Hexenal	2.74±1.11	2.16±0.86	2.06±0.69	2.41±0.63	3.20±0.50	4.19±1.16	3.51±1.57	2.77±1.06	3.94±0.68	3.22±0.38
14.94	Hexanal	44.38±9.99	46.97±12.35	39.47±12.56	40.94±10.19	53.52±7.57	55.17±9.83	66.45±7.46	47.45±2.45	56.58±12.45	57.44±16.21
17.06	trans-2-Heptenal	3.75±1.38	3.19±1.10	2.58±0.7	3.73±0.97	4.95±0.59	6.81±1.13	6.16±2.45	4.18±1.53	6.43±1.02	5.07±0.14
17.51	(E)-2-Hexenal	132.9±29.43	114.92±7.51	108.56±13.16	127.68±14.80	161.76±13.15	192.67±25.39	171.61±21.51	135.52±7.14	187.64±24.37	151.62±4.77
17.82	(E)-2-Hexen-1-ol	71.07±3.07	40.35±4.44	100.92±20.60	48.76±9.17	91.95±4.69	48.71±1.52	83.54±17.58	47.43±9.47	55.95±6.08	37.66±5.10
17.89	Hexanol	21.90±2.73	18.93±6.36	37.99±10.33	22.88±6.92	18.17±4.17	23.64±5.55	26.79±7.77	23.16±6.99	22.53±4.98	17.33±1.90
23.09	Benzaldehyde	4.72±1.48	7.47±0.85	7.25±2.78	10.84±5.15	8.51±2.47	16.21±4.83	18.83±4.10	29.21±9.51	6.22±1.48	14.11±3.94
25.52	D-Limonene	20.76±14.03	0.78±0.34	9.55±1.48	5.20±4.97	5.76±1.95	1.86±1.46	5.87±2.62	1.27±0.59	5.57±4.23	1.37±0.55
26.58	Benzyl alcohol	4.33±0.35	2.75±0.47	5.20±3.32	2.97±1.66	7.11±5.58	4.27±0.50	13.88±6.43	9.76±3.37	2.11±0.72	1.43±0.40
32.15	Dodecane	0.81±0.36	Nd	0.53±0.28	Nd	0.54±0.22	Nd	Nd	Nd	Nd	Nd
39.00	Butyl butyrate	7.22±0.26	6.74±1.16	6.93±2.58	5.01±1.59	4.69±0.87	5.98±0.80	7.21±1.84	5.80±1.25	6.91±2.18	5.25±0.61

Nd: not detectable; H: at harvest; 5 w: after 5 weeks of storage. Yellow color shows the significant decrease of flavor compounds, while red color shows the significant increase after 5 week of storage. Data were presented by total ion 10^7.

Table 2. Fruit quality and flavor deterioration of 'Lapins' and 'Skeena' cherries treated with simulated rain during 5 weeks of storage at 32 °F.

Cultivar	Storage time	Treatment	FF (g mm^{-1})	Fruit size (mm)	TA (%)	SSC (%)	Bitter Taste (%)	Flavor
'Lapins'	Harvest	Before rain	331±9b	27.4±1a	0.53±0.02a	16.1±1.1a		
		After rain						
	3 w	Before rain	336±20b	27.1±2a	0.50±0.01b	16.6±0.6a		
		After rain	333±18b	27.1±1a	0.50±0.02b	16.4±1.5a		
	5 w	Before rain	363±32a	27.5±1a	0.46±0.03c	16.0±1.3a	3.97±0.15a	6.1±0.2a
		After rain	360±15a	27.4±1a	0.45±0.06c	16.3±1.2a	3.87±0.15a	6.1±0.2a
'Skeena'	Harvest	Before rain	354±7a	30.3±0.8a	0.55±0.03a	17.0±1.2b		
		After rain						
	3 w	Before rain	347±30a	29.6±1.0a	0.51±0.03b	18.4±0.8a		
		After rain	346±26a	29.5±0.7a	0.50±0.03b	16.2±1.1b		
	5 w	Before rain	359±22a	29.8±0.8a	0.49±0.02b	16.3±0.7b	3.10±0.20a	6.8±0.3a
		After rain	353±31a	29.5±0.2a	0.48±0.02b	16.3±0.8b	3.17±0.21a	7.1±0.2a

Data within columns with different letters are significantly different by Fisher's Pretected LSD test at *P* = 0.05.

d. Fruit nutrient content:

According to our previous results (2014-2016, improving shipping quality of cherry by preharvest Ca and NaCl spray), the optimum $Ca(NO_3)_2$ spray rate (0.1-0.15%), timing (start pit-hardening), and frequency (6 times at weekly intervals from pit-hardening to harvest) were found to increase fruit tissue Ca content. In 2017, application of $Ca(NO_3)_2$ at the rate of 0.15% (1.24 lb/acre, 100 gal/acre) was applied 6 times at weekly interval from pit-hardening to harvest. These pre-harvest Ca applications significantly increased fruit tissue Ca content ($p < 0.05$) of 'Skeena', 'Lapins' and 'Regina' cherries at harvest (Fig. 3). After 5 weeks of storage, Ca-treated fruit maintained higher fruit firmness, TA, and flavor sensory score along with lower bitter taste. SSC did not change in 'Skeena' and 'Regina' or decreased in 'Lapins'.

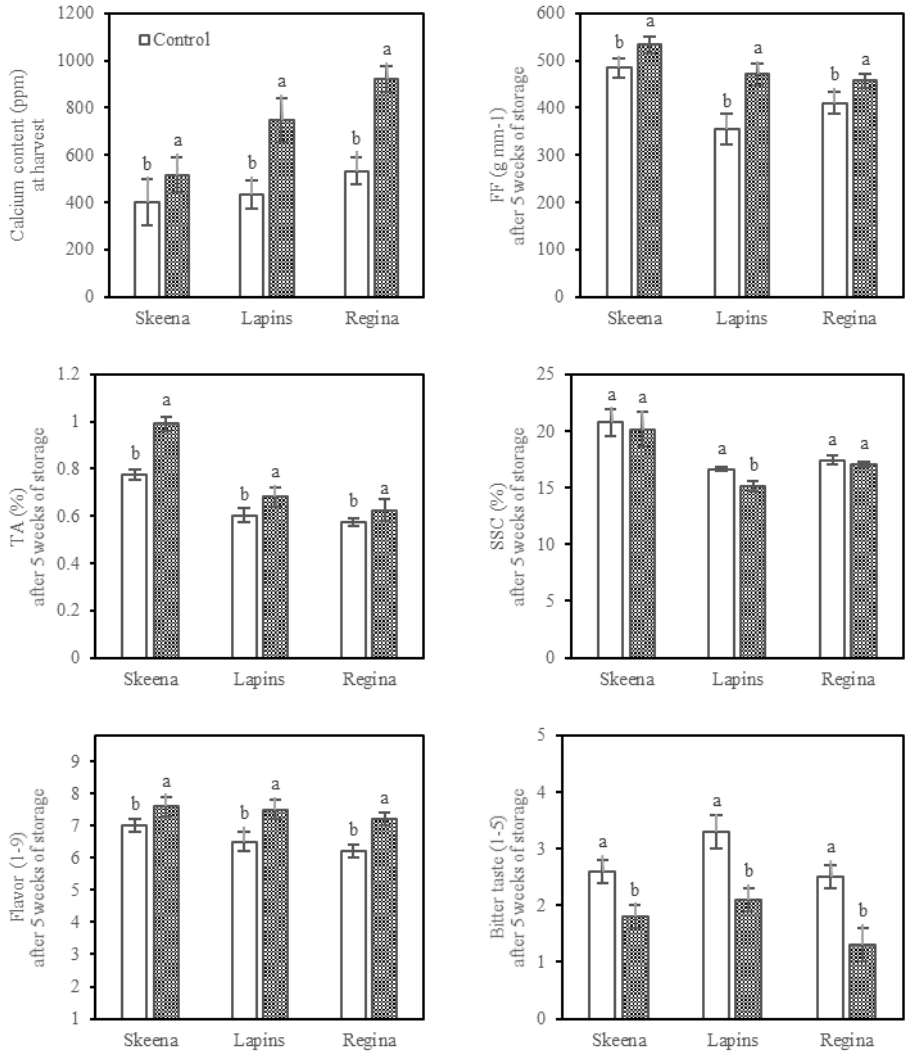

Fig. 3 Effects of pre-harvest spraying $Ca(NO_3)_2$ on Ca content, fruit quality, flavor sensory, and bitter taste of 'Skeena', 'Lapins', and 'Regina' after 5 weeks at 32°F.

e. Harvest maturity:

'Bing' fruit were harvested when average skin color had Ctifl color score of 4, 5, or 6. Similar to the 2016 results for 'Lapins' and 'Regina', the late harvest fruit had lower TA and flavor sensory score along with higher bitter taste and internal browning after 5 weeks of storage at 32 °F.

Late harvested fruit were more susceptible to flavor deterioration, softening, pitting, pedicel browning, and fruit skin luster loss. Data will be presented in the final report.

f. Commercial MAP liners: Data were reported in year-1.
g. Post-packing forced-air cooling: Data were reported in year-1.
h. Storage/shipping temperature

'Lapins' and 'Regina' cherries were harvested at commercial maturity. Fruit were stored at 32, 34 and 36 °F. Compared to fruit stored at 32 and 34 °F, fruit stored at 36 °F had lower fruit firmness and TA along with higher stem browning and decay after 4-5 weeks storage/shipping. Compared to 32°F, fruit stored at 36°F had inferior flavor after 5 weeks storage/shipping. Data will be presented in the final report.

3. Determine reliable predictors for cherries with long postharvest flavor life

In 2016, 'Skeena' fruit from 9 orchards were sampled for quality evaluation and fruit nutrition measurement. In 2017, 'Lapins' cherries from 9 orchards (2 orchards in OR and 7 orchards in WA) were sampled for quality evaluation and fruit mineral content (Table 3). PCA results indicated that flavor intensity was highly positively correlated with Ca and P after 5 weeks at 32 °F. K and Mg were slightly positively correlated with flavor intensity. However, higher N with lower Ca and P accelerated flavor deterioration (bland flavor and bitter taste) (Fig. 4).

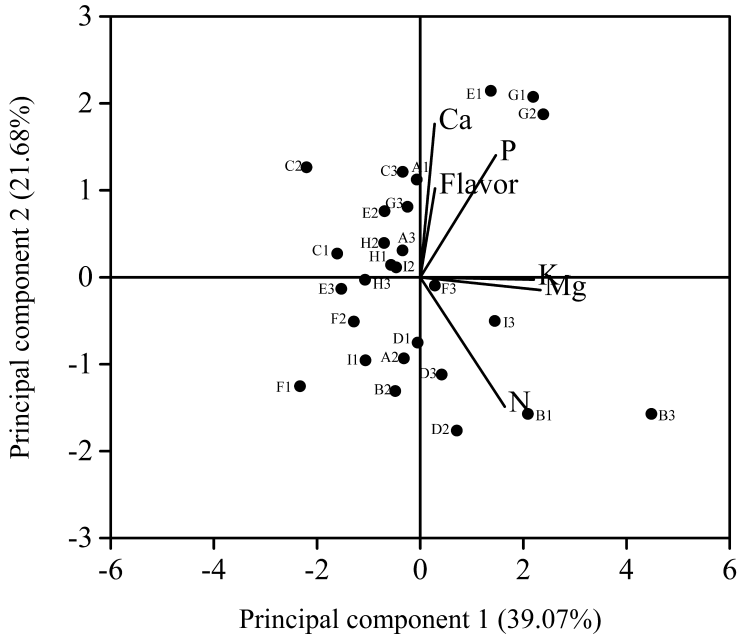

Fig. 4. The relationship of flavor after storage/shipping with fruit nutrient content in 'Lapins' cherries from 9 orchards.

WTFRC INTERNAL PROGRAM: FINAL PROJECT REPORT

Project Title: 2017 Cherry Pesticide Residue Study

PI:	Tory Schmidt	**Co-PI (2):**	Steve Thun
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City:	Wenatchee	**City:**	Sherwood
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Cooperators: Rick Jordan, Gerardo Garcia, Santos Lopez, Doug Stockwell, Doyle Smith

Other funding sources:

Most pesticides were donated by their registrants

Total Project Funding (in last __1__ years):

Budget History:

Item	2017:
Salaries	3000
Benefits	600
Wages	1200
Benefits	400
Equipment	
Supplies	1000
Travel	500
Shipping	300
Plot Fees	
Miscellaneous	
Analytical Lab Fees	2000
Total	**9000**

2017 WTFRC CHERRY PESTICIDE RESIDUE STUDY

For the seventh consecutive year, the WA Tree Fruit Research Commission conducted a study of residues of commonly used pesticides on cherry fruit at harvest. Digital versions of this report and similar studies on apple and cherry are available at www.treefruitresearch.com. For current information on maximum residues levels (MRLs) and other regulatory issues, please consult the Northwest Horticultural Council at http://nwhort.org/export-manual.

Airblast spray application

TRIAL DETAILS

- Mature 'Bing'/Mazzard multiple leader open vase trees on 10' x 20' spacing near Orondo, WA
- 14 insecticides/acaricides & 6 fungicides applied at or near maximum rates and minimum pre-harvest and re-treatment intervals; a foliar fertilizer containing potash and phosphite applied early in season at rates & timings consistent with industry use patterns
- Applications made by Rears PakBlast PTO-driven airblast sprayer with 16 oz Regulaid in 200 gal water/acre
- 0.61" cumulative total rainfall recorded on 8 separate days during study: heaviest rain events were approximately 0.2" which fell on May 11 & 13 (42 and 40 days before harvest)
- Samples submitted overnight to Pacific Agricultural Labs (Sherwood, OR) for chemical analysis

RESULTS & DISCUSSION

As before, this study simulates a *worst case scenario* for residues of legally applied pesticides using aggressive rates, timings, and spray intervals. Most materials were applied twice as allowed by product labels, whether or not commercial use patterns would do the same. With that approach, all residues complied with domestic tolerances but **most exceeded some key foreign tolerances,** whether from published MRLs or national default values:

Insectides/acaricides: Centaur, Bexar, Agri-Mek 0.15SEC, Mustang MAX, Assail 70WP, Baythroid XL, Delegate WG, Danitol 2.4EC, Perm-Up 3.2EC, Carbaryl 4L, Onager

Fungicides: TopGuard, Orbit, Topsin 4.5FL

Fertilizer: 19% potash + 33% phosphite foliar fertilizer

MRLs change frequently and cherry producers should routinely monitor the most current information (http://nwhort.org/export-manual) to facilitate compliance with dynamic foreign standards. While fruit from this study were not rinsed prior to analysis, similar studies in 2011 and 2012 found no clear evidence of consistent residue reduction from commercial hydrocooler cycles.

Cherries with residues at harvest

For the second consecutive year, we included an early-mid season program of potash/phosphite fertilizer applied 3 times at 14 day intervals starting around shuck fall; these programs are used by some Northwest cherry growers for nutritional value and to promote overall tree health. While the US EPA does not regulate residues of foliar fertilizers, phosphite residues are regulated by the European Union (EU) as part of its residue definition for fosetyl-Al (Aliette), which is not registered for use on bearing cherry trees in the US. The EU has set a tolerance for fosetyl-Al residues which includes phosphonic acid and all of its salts at 2 ppm; as in 2016, our 2017 samples did not carry any measurable traces of actual fosetyl-Al, but did contain levels of phosphite well in excess of the EU standard. Growers hoping to ship cherries to Europe should avoid use of any phosphite products unless the fosetyl-Al MRL is redefined or significantly relaxed.

Results of this lone unreplicated trial are shared for informational purposes only and should not be construed as endorsements of any product, reflections of their efficacy against any arthropod or fungal pest, or a guarantee of similar results regarding residues for any user. Cherry growers should consult with extension team members, crop advisors, and warehouses to develop responsible pest control programs.

Measured residue levels vs. MRLs for pesticides applied with 16 oz Regulaid in 200 gal water/acre. 'Bing'/Mazzard, Orondo, WA. WTFRC 2017.

Common name	Trade name	Application rate	Application timing(s)	Measured residue	US tolerance[1]	Lowest export tolerance[2]
		per acre	*days before harvest*	*ppm*	*ppm*	*ppm*
phosphite	33% phosphite fertilizer	64 oz	43, 28, 14	**31**	na	2 (EU)*
buprofezin	Centaur	34.5 oz	28, 14	**1.2**	1.9	1 (Kor)
tolfenpyrad	Bexar	27 oz	28, 14	**0.97**	2	0.01 (Tai)
abamectin	Agri-Mek 0.15SEC	20 oz	21	**0.018**	0.09	0.01 (EU)
zeta-cypermethrin	Mustang MAX	4 oz	21, 14	**0.23**	1	0.1 (Can)
acetamiprid	Assail 70WP	3.4 oz	21, 7	**0.93**	1.2	0.2 (Kor)
beta-cyfluthrin	Baythroid XL	2.8 oz	21, 7	**0.083**	0.3	0.01 (Tai)
spirotetramat	Ultor	14 oz	21, 7	0.041	4.5	3 (many)
spinosad	Entrust	2.5 oz	14, 7	0.15	0.2	0.2 (many)
spinetoram	Delegate WG	7 oz	14, 7	**0.16**	0.3	0.05 (EU)
flutriafol	TopGuard	14 oz	14, 7	**0.50**	1.5	0.01 (Jap)
metrafenone	Vivando	15.4 oz	14, 7	<0.01	2	0.01 (EU)
fenpropathrin	Danitol 2.4EC	21.3 oz	14, 3	**2.8**	5	0.01 (EU)
permethrin	Perm-Up 3.2EC	8 oz	14, 3	**1.2**	4	0.05 (EU)
carbaryl	Carbaryl 4L	96 oz	10, 3	**10**	10	0.01 (EU)
cyantraniliprole	Exirel	20.5 oz	10, 3	0.54	6	6 (many)
propiconazole	Orbit	4 oz	10, 1	**0.53**	4	0.01 (EU)
thiophanate-methyl	Topsin 4.5FL	30 oz	10, 1	**0.684**	20	0.3 (EU)
hexythiazox	Onager	24 oz	7	**0.45**	1	0.1 (Kor)
fluxapyroxad	Merivon	6.7 oz	7, 1	0.59	3	2 (Tai)
pyraclostrobin	Merivon	6.7 oz	7, 1	0.79	2.5	1 (HKG,Tai)

[1] 18 July 2017. http://nwhort.org/export-manual/comparisonmrls/cherry-mrls , https://www.globalmrl.com

[2] Major export markets for Pacific Northwest cherries; 18 July 2017; tolerances may be based on published MRLs or default values. http://nwhort.org/export-manual/comparisonmrls/cherry-mrls , https://www.globalmrl.com

* EU tolerance for fosetyl-Al defined as the sum total of residue levels of fosetyl-Al, phosphonic acid and all of its salts (including phosphite)

** Reported thiophanate-methyl values reflect the sum total of thiophanate-methyl and carbendazim residue levels

For more information, contact Tory Schmidt (509) 669-3903 or email tory@treefruitresearch.com

WASHINGTON TREE FRUIT RESEARCH COMMISSION

CONTINUING PROJECT REPORT **PROPOSED DURATION**: 3 Years
ONE-YEAR DELAY IN INITIATION

Project Title: Advancing precision pollination systems for yield security

PI: Matthew Whiting
Organization: WSU-IAREC
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City/State/Zip: Prosser WA 99350

Cooperators: Connor Dykes, Hayden Farms, WA Fruit & Produce, Russ LeSage, Wolverton Orchards; Harold Schell, Firman Pollen Co., OnTarget Spray Systems, Cameron Peace

Total Project Request: **Year 1: 74,566** Year 2: 77,609 Year 3: 80,777

Other funding sources
Firman Pollen Company will provide pollen for this project at no cost – estimated at $5,000; On Target Spray Systems is providing a technician (estimated 140 hrs/year for this project) and a sprayer (retail value of $20,000) for this research.

Budget 1
Organization Name: Washington State University **Contract Administrator:** Katy Roberts
Telephone: (509) 335-2885 **Email address:** katy.roberts@wsu.edu

Item	2017	2018	2019	2020
Salaries	$40,856		$42,491	$44,191
Benefits	$5,482		$5,760	$6,053
Wages	$12,480		$12,980	13,500
Benefits	$1,248		$1,298	$1,350
Equipment	$0		$0	$0
Supplies	$8,000		$8,320	$8,653
Travel	$6,500		$6,760	$7,030
Miscellaneous				
Plot Fees				
Total	$74,566	**0**	$77,609	$80,777

Footnotes: Salaries for graduate research assistant, wages for hourly assistance @$12/hr; supplies for insect netting, supplies for limb and tree cages, lab consumables for suspension development and fruit quality testing; travel for intra-state travel to plots and international to Chile for 10 days each year

Justification:

The ability to consistently set a target crop is fundamental to orchard productivity and profitability. Yet perennially growers face challenges due to pollenizers (e.g., insufficient density, ineffective distribution, irregular bloom overlap, spread of pollen-borne disease, etc.) and pollinators (e.g., increasing cost, inconsistent hive performance, colony collapse disorder, etc.). Climate change effects and increasingly variable spring weather conditions will further complicate each of these issues. In short, fruit set is unpredictable and variable among cultivars and years. We propose to develop a precision pollination system for sweet cherry using previously collected pollen applied in a suspension via electrostatic sprayer. Our recent studies have shown promise for this system – we have preliminary yet promising results on the ability to pollinate (and, more importantly, fertilize) apple, pear, and sweet cherry flowers with a liquid pollen suspension. This project will evaluate the key components of a precision pollination system including pollen rate, application timing, application technology (i.e., electrostatic vs. airblast systems), and the effect of the suspension media on fruit set. Each year, select complementary trials will be conducted on common cultivars in Chile to hasten discovery and system evaluation. Pollen suspension media will be further investigated to extend pollen viability and improve the solubility of pollen. Additionally, in select trials, seed genotyping will be carried out to evaluate the efficacy of the pollination system. If successful, this research will eliminate concerns over the ancient pollinizer + pollinator model for pollination, and improve yield security and profitability for sweet cherry growers.

Objectives:

Our long-term goal is to improve yield security and yield by developing and deploying a reliable precision pollination system that can 1) supplement current grower pollination practices or, 2) replace the current (ancient) system of planting pollenizers and renting pollinators. We will continue to work with grower collaborators, Firman Pollen Company, and On Target Spray Systems to be sure that research progress is easily translatable to commercial-scale solutions.

1. Refine pollen rate and application timing to improve efficiency of precision pollination systems
2. Optimize pollen suspension constituents to preserve pollen viability and improve solubility
3. Investigate pollen production systems
4. Use commission funded work to strengthen regional and national research proposals

Update:

We require a 1-year delay on this project because, given the timing of the funding decisions, we could not recruit a new graduate student for this spring. We did however make progress toward objectives 1 and 3, as outlined below, and Katie Taylor, a new M.S. student has started her program Fall 2017.

Objective 1. We setup two trials in ‘Early Robin’ orchards to investigate the effects of supplemental pollination treatments on fruit set and fruit quality. In a block of Y-trellised ‘Early Robin’ north of Pasco we compared several supplemental pollen treatments with untreated control. Each replicate was a block of 5 trees and applications of liquid pollen suspension were made with an electrostatic sprayer from On Target Spray Systems. Dusting applications were made by the grower with several passes at 15 g pollen/acre each pass. Fruit set was unusually high this year, and treatment effects were subtle (Table 1). The highest fruit set was from 60 g of pollen/acre. There was no rate response to supplemental pollen. This likely reflects the excellent year the region experienced for fruit set –

many growers reported record high yields. Excellent weather (i.e., mild temperatures, light or no wind, lack of rain) during bloom across most of the region led to high rates of pollination and fruit set. In these situations, supplemental pollen is not likely to improve fruit set because pollination is not limiting. Overall, fruit set was about 24% in this orchard – extraordinarily high for 'Early Robin'. Our previous studies have typically found 'Early Robin' fruit set to be less than 10%.

Table 1. Effect of supplemental pollination treatments on fruit set in 'Early Robin' sweet cherry limbs n=10.

Treatment	Fruit set (% available flowers) +/- SEM
Untreated control	20.7 +/- 2.9
Supplemental pollen dusting (3@15g/acre)	27.5 +/- 4.6
Liquid pollen suspension 1@15 g/acre	23.8 +/- 2.8
Liquid pollen suspension 1@30 g/acre	19.0 +/-3.1
Liquid pollen suspension 1@60 g/acre	30.1 +/-5.5
Liquid pollen suspension 1@120 g/acre	21.8 +/- 1.7
Suspension media alone	24.5 +/- 2.4

Interestingly, we documented tremendous variability in fruit set among our replicate limbs, across all treatments. This ranged from about 2% to 66%, with an average of 24%. We intend to better understand this variability by studying key factors in subsequent years.

In a second 'Early Robin' block near Buena, we compared supplemental pollen applications with programs designed to extend ovule longevity (ReTain® and Harvista®) and untreated control. A single application of 30 g of pollen/acre in liquid suspension was made to blocks treated with ReTain or Harvista. Fruit set overall was also high, at about 24% across all treatments. The improved fruit set from anti-ethylene bioregulators confirms our previous research that suggested rapid ovule senescence limits 'Early Robin' productivity, rather than pollination.

Table 2. Effect of supplemental pollination (30g/acre) with or without anti-ethylene bioregulators.

Treatment	Fruit set (% available flowers) +/- SEM
Untreated control	14.0 +/- 4.5
Liquid pollen suspension 1@30g/acre	17.1 +/- 3.1
AVG	24.8 +/- 3.5
AVG + liquid pollen suspension	27.7 +/- 3.3
Harvista 1x	29.9 +/- 2.6
Harvista 2x	25.4 +/- 2.9
Harvista 1x + liquid pollen suspension	23.5 +/- 3.3
Harvista 2x + liquid pollen suspension	26.5 +/- 2.1

*thanks to Hanrahan orchard and TFRC interns for data collection

Objective 3. We collected pollen from 10 cultivars at the WSU-Roza farm and have samples ready for analysis of viability. In addition, we worked with Firman Pollen Co. in the establishment of a new orchard for pollen production. Pruning and training decisions were discussed with the goal of maximizing pollen yield per acre.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: CH-17-101

Project Title: Predicting flower bud hardiness of commercial sweet cherry cultivars

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Co-PI(3): David Gibeaut
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Co-PI(4): Todd Einhorn
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City/State/Zip: East Lansing/MI/48824

Cooperators: Sean Hill, Lynn Mills, C & M Orchards at Prosser, WA

Total Project Request: **Year 1:** $83,802 **Year 2: $86,094** **Year 3:** $94,938

Other funding sources
None

Budget 1
Organization Name: Washington State University **Contract Administrator:** Katy Roberts
Telephone: (509) 335-2885 **Email address:** arcgrants@wsu.edu

Item	2017	**2018**	2019
Salaries	13,029	**13,550**	14,092
Benefits	4,833	**5,026**	5,227
Wages	13,440	**13,978**	14,537
Benefits			
Equipment			6,000
Supplies	1,500	**500**	
Travel	1,000	**1,040**	1,082
Miscellaneous			
Plot Fees			
Total	33,802	**34,094**	40,938

Footnotes:

Budget 2
Organization Name: OSU-MCAREC **Contract Administrator:** L.J. Koong
Telephone: 541 737-4866 **Email address:** l.j.koong@oregonstate.edu

Item	2016	**2017**	2018
Salaries	31,000	**32,240**	33,530
Benefits	17,695	**18,403**	19,139
Wages			
Benefits			
Equipment			
Supplies	1,305	**1,357**	1,331
Travel			
Plot Fees			
Miscellaneous			
Total	50,000	**52,000**	54,000

Footnotes: approximate values

OBJECTIVES

1. To standardize and employ procedures for cold hardiness determination for sweet cherry for different locations and sweet cherry cultivars.
2. To develop a precise model to predict sweet cherry bud cold hardiness for different cultivars as a function of local weather conditions using public weather data collected in Washington and Oregon.
3. Initiate a validation process of the model involving a network of user-collaborators (beta testers) and conduct field damage surveys of any potentially harmful freeze events.
4. To implement the model as a decision support tool on the AgWeatherNet portal and local systems in Oregon for use by local sweet cherry growers and orchard managers in Washington and Oregon.

SIGNIFICANT FINDINGS

- Multi-spur sampling technique adopted
- DTA analysis -1.8 °F per hour considered a fast rate but more realistic than -7.2 °F per hour
- Freeze Tolerance in Endo and Ecodormancy (FTEED) was rewritten in Excel for increased precision and efficiency
- DTA analysis revealed no LTEs on Sept 29th, 2017 from The Dalles
- DTA analysis revealed LTEs at 10 °F on Oct 6th, 2017 from the Dalles signaling the onset of acclimation to freezing
- Predictive modelling of Freeze Tolerance in Endo and Ecodormancy (FTEED) was published for the year 2016-17 on website: blogs.oregonstate.edu/gdavs
- FTEED was given to Sean Hill for coding and presentation on AgWeatherNet
- DTA data is currently being collected in WA to compare rates of freezing for Bing, Chelan and Sweet heart.
- Preliminary models have been develop for Bing, Chelan and Sweet heart using the data previously collected in WA during the past seasons of 2012-2013, 2013-2014, 2014-2015.

RESULTS & DISCUSSION

Objective 1. To standardize and employ procedures for cold hardiness determination for sweet cherry for different locations and sweet cherry cultivars.

This activity is in progress; partial data has been collected in both OR and WA, analysis of the data will be conducted and data collection will continue during fall, and early winter of 2017 and throughout 2018-2019 seasons.

Procedures.

Field assessments are initiated by the removal of one fruiting-spur from 30 trees and transporting them to the lab under near ambient temperature. Buds are removed from the spurs and 15 buds are wrapped in foil then placed in the DTA apparatus. After the DTA procedure, the buds are removed, weighed, dried and reweighed. In this manner, the calculated relative water content can be correlated to the onset of dormancy break.

Differential thermal analysis (DTA) methods can employ different rates of freezing; however, the low temperature exotherm (LTE) temperatures of pistil primordia will be affected. LTE temperatures were about 4.8 °F lower when a slow freezing rate of -1.8 °F per hour was used compared to a fast rate of -7.2 °F per hour. The slower rate of -1.8 °F per hour, although fast in nature, was considered a more realistic rate to use in further experiments. Data acquired previously at -7.2 °F per hour will be adjusted by comparison to simultaneous slow and fast freezing rates in Oregon (OR).

In WA we started measuring the hardiness of the buds with DTA, using a rate of 7.2°F/h (equivalent to 4°C/hour) and 1.8°F/h (equivalent to 1 °C/hour) with the purpose of compare the results and adjust if necessary with the data obtained in OR in order to standardize the procedure for the hardiness determination.

Objective 2. To develop a precise model to predict sweet cherry bud cold hardiness for different cultivars as a function of local weather conditions using public weather data collected in Washington and Oregon.

This objective will continue during early spring of 2018-2019 and 2019-2020 seasons. There are preliminary data collected and analyzed in OR and WA. We are working on two approaches for the benefit of the industry. A rate based model was developed in Oregon and presented (FTEED, blogs.oregonstate/gdavs). Analysis for the upcoming season will emphasize an assessment of the acclimation to freeze tolerance during the period between late summer and the acquisition of dormancy. Preliminary analysis will be presented at the review meeting in Nov 2017 for Freeze Tolerance in Endo- and Ecodormancy an approach developed in OR, and we will continue supporting the development of a model for the complete season including dormant and non-dormant flower buds, with the purpose to better satisfy the needs of the growers and industry.

In WA, seasonal preliminary models have been develop for Chelan, Bing and Sweet heart using the data previously collected (in previous projects supported by the TFRC) during the seasons of 2012-2013, 2013-2014, 2014-2015. In this moment statistical analysis is been doing and will continue for sensitivity analysis for different cultivars and dates in both locations.

Procedures: Because the process of freeze acclimation/deacclimation is temperature dependent, modelling this behavior can be done by a time vs rate model. Rate values, one for freezing and another for thawing temperatures, have been determined in the literature and we have observed similar results. Time values are the accumulation of time spent either in the frozen or thawed state, and the result of time multiplied by either the acclimation or deacclimation (frozen or thawed) rate predicts the lethal temperature. FTEED was designed to predict what the lethal temperature would be if the orchard experienced a freezing rate of -1.8 °F per hour during the next day. Included in FTEED are frost starting dates, threshold temperature values, and acclimation/deacclimation rates that diminish to zero as thresholds are approached. FTEED was published on line for the 2016-17, and will be published again for the 2017-18 season.

On October 6, 2017 acclimation to freezing was indicated by the appearance of LTEs in DTA of Sweetheart in The Dalles. Because the appearance of LTEs typically occurs before the first freeze date that triggers the FTEED program, further temperature modelling covering the duration between late summer and first freeze, will be required. Well-known chilling requirement models of dormancy acquisition will be adapted to sweet cherry.

Dormancy break and the progression toward bloom is seasonally variable, as was dramatically apparent the last two seasons. DTA analysis that is the foundation of the FTEED model during dormancy no longer works after bud phenology reaches first-swelling. However, bud phenology becomes a very good predictor of cold hardiness. Therefore, a prediction of bud phenology is a prediction of cold hardiness. Cultivar differences in cold hardiness are explained by the difference in degree days required to achieve a given phenology. An extensive growing-degree model prediction of cherry development (GDAVS) from dormancy to maturation will be combined with FTEED as part of the overall prediction of cold hardiness.

Objective 3. Initiate a validation process of the model involving a network of user-collaborators (beta testers) and conduct field damage surveys of any potentially harmful freeze events.

We will work closely with WSU Extension and industry representatives as beta testers, including those organizations currently providing freeze chamber data to Washington stone fruit producers. We will incorporate industry feedback to improve the tool and decision aid to the benefit of the local cherry growers, orchard managers and industry.

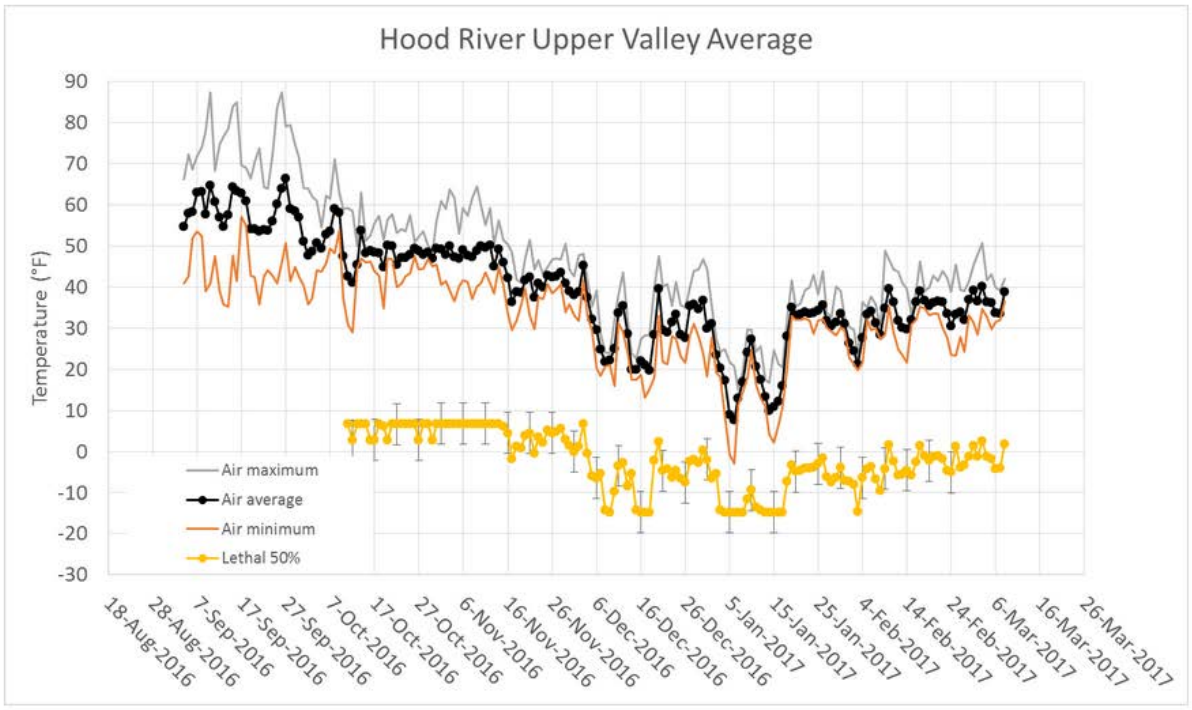
Objective 4. To implement the model as a decision support tool on the AgWeatherNet portal and local systems in Oregon for use by local sweet cherry growers and orchard managers in Washington and Oregon.

An information delivery system and media tool will be fully developed in collaboration with the growers and the industry, to present the models on the web as a Decision Aid Tool. Preliminary web site has been expanded for cherry cold hardiness with information of models developed from previous projects in OR (Fig 1) and WA (Fig 2), its inclusion and improvement into the AgWeatherNet Portal will be a continuous activity for the following years of the project.

Results of the model, Freeze Tolerance in Endo and Ecodormancy (FTEED) were presented with weekly updates for the 2016-17 season from 56 weather stations across Washington and the Columbia Gorge (blogs/Oregonstate/gdavs). A graphical presentation of the average temperature and predicted lethal temperature was presented (Fig 1). Temperature and lethal temperature values for the individual weather stations were also tabulated on this site. Subsequently, the FTEED model was rewritten for efficiency, temporal precision and flexibility as a research tool. The rewrite will also enable more frequent updates from OR for the 2017-18 season. The model was given to Sean Hill for coding and presentation on AgWeatherNet. When ready, predictions of lethal temperatures will be updated in 15 minute intervals and available to growers.

Preliminary seasonal models developed for Chelan, Sweet heart and Bing using data from previous seasons were included in the AgWeatherNet for Beta-testing (Fig 2).

Overall, the PI and Co-PI's are making great progress in completing proposed activities in a timely manner.



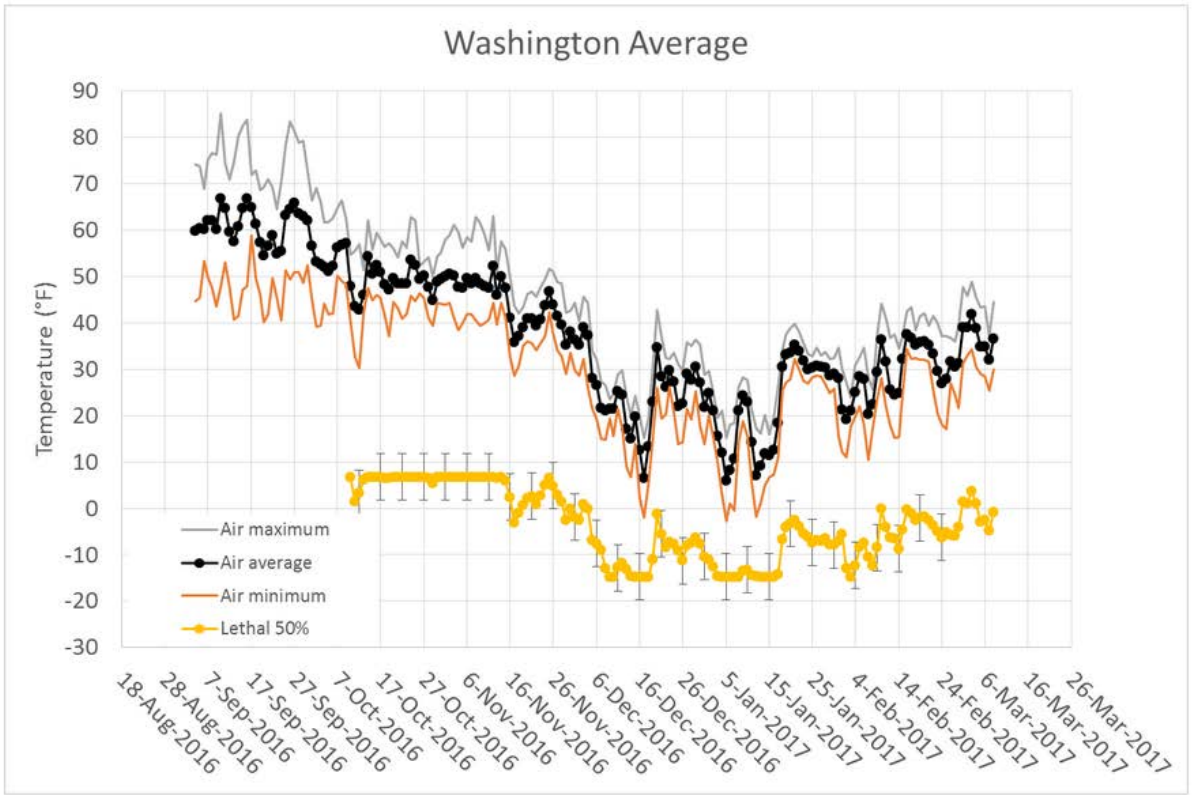


Fig 1. Freeze Tolerance in Endo- and Ecodormancy (FTEED) of Sweet Cherry Floral Buds. (Preliminary model developed in OR).

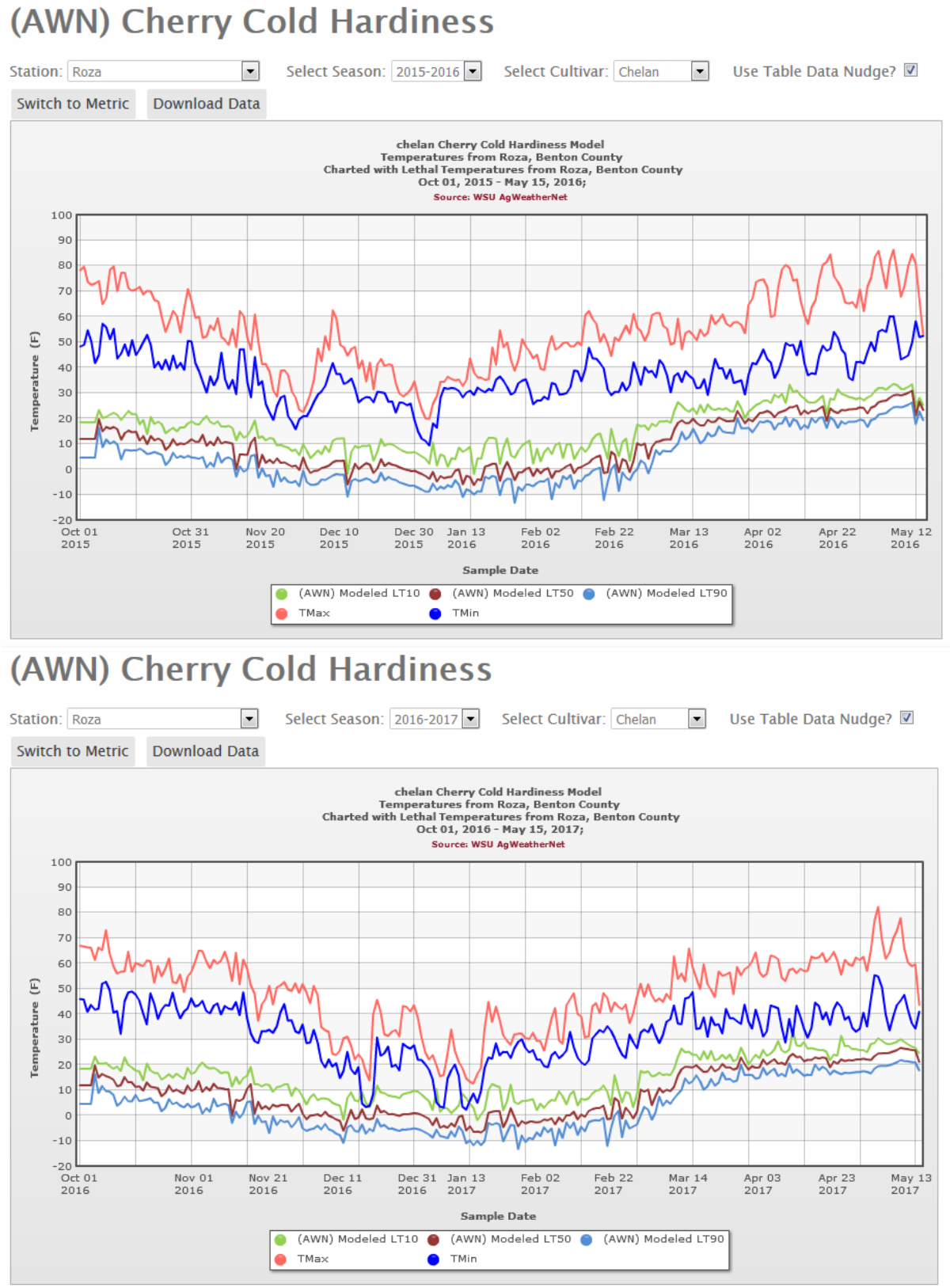


Fig 2. Flower bud hardiness of Sweet Cherry (Prel. model developed with data collected in WA).

CONTINUING PROJECT REPORT **YEAR:** 1 of 2

Project Title: Preservation and retention of green stems

PI:	David Gibeaut	Todd Einhorn
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City/State/Zip:	Hood River OR 97031	East Lansing MI 48824

Cooperators: Stacy Cooper, Brad Fowler

Total Project Request: Year 1: 15,708 Year 2: **15,708**

Other funding sources: none

Budget 1
Organization Name: OSU-MCAREC **Contract Administrator:** L.J. Koong
Telephone: 541 737-4866 **Email address:** l.j.koong@oregonstate.edu

Item	2017	2018
Salaries	10,000	10,000
Benefits	5,708	5,708
Wages		
Benefits		
Equipment		
Supplies		
Travel		
Miscellaneous		
Plot Fees		
Total	15,708	**15,708**

OBJECTIVES

Green stems are a good indicator of freshness for the consumer and stems that are retained on the fruit have the longest postharvest life. Deficiencies in the lignification of the five major vascular bundles that supply and connect the developing ovule and flesh lead not only to losses at harvest and postharvest but also defects at the stem bowl connection which provide an opening for moisture loss or absorption and pathogen attack. Reinforcement of these bundles and those throughout the stem with silicate solutions could improve stem retention and stem quality.

SIGNIFICANT FINDINGS

- A single application at shuck fall of Si compounds modestly increased the stem pull-force and size of sweet cherry at harvest
- Fruit retention on the tree was high for all treatments
- Stem pull-force in silicate treated fruit maintained higher stem-pull force than controls
- Stem color in silicate treated fruit was less brown after 2-4 weeks storage

METHODS

Four orchards, two with Lapins and two with Skeena were used. Six to seven trees near shuck fall were selected for each of four treatments. Treatment solutions (table 1) were prepared in 14 L buffered water and applied to whole trees with a backpack sprayer. Near commercial harvest, 30 spurs from each tree (30-spur method) were collected for the first assessment that included a count of fruit and fruitless stems per spur as an estimate of fruit retention on the tree. For postharvest assessment, a similar amount of spur-fruit were collected, combined among treatment trees, and stored at 1.8 °F in clamshells wrapped in plastic.

Stem-pull force was assessed with fruit at room temperature using a push/pull force gauge (DS2-11, IMADA). Fruit were weighed individually. Stem color was assessed with a chroma meter (CR 410, Konica Minolta). Fruit firmness, diameter, total acidity and soluble solids were assessed with standard procedures.

Sodium and potassium solutions were prepared to contain similar amounts of Si; however, the Ca solution is actually a suspension and the Si content was approximately 50 greater than in the soluble Si solutions (Table 1.).

Table 1. Silicate solutions

Product	Silicate	stock (Si)	dilution	g Si per L	g or mL per 14 L	mL 250ppm KPO4 buffer per 14L
Sodium metasilicate pentahydrate	$Na_2SiO_3 \cdot 5H_2O$	212.14 g/mol	1.7 mMolar	0.0477	5.0489 g	1.4 mL
Armor SI	$K_2O \cdot SiO_2$	10%	1 mL/L	0.046743	14 mL	2.8 mL
Mainstay	$2CaO \cdot SiO_2$	22%	5 mL/L	2.337	70 ml	5 mL

RESULTS & DISCUSSION

The two late harvest orchards, Lapins or Skeena were far lower in stem-pull force (Fig. 1) and exhibited no significant difference between treatments for stem-pull force, fruit firmness, total acidity and soluble solids (not shown); however, fruit weight was significantly greater in the late harvest orchards (Fig 1.). Among all treatments and orchards, no clear differences in fruit firmness, total acidity and soluble solids showed were observed (not shown). Fruit color was also unaffected (not shown).

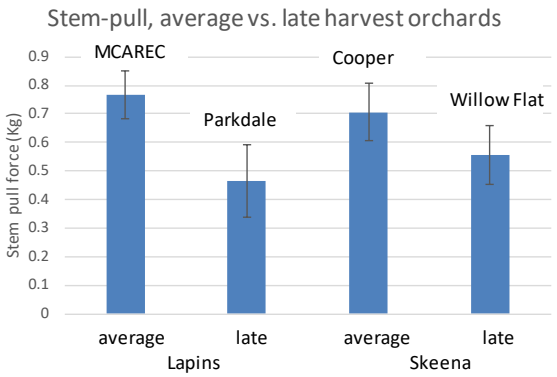
A quality aspect of sweet cherry that is sometimes overlooked is the retention of fruit on the tree. Our assessment using a 30-spur method allows a count of fruitless stems. We hypothesized Si could ameliorate losses from the tree before or during harvest. Significant fruit losses however, typically occur in stressed conditions. The 2017 growing season was mild and we observed minimal losses, less than2%, and no differences between treatments (not shown).

Stem-pull force at harvest was modestly higher in silicate treatments compared to controls in the two average harvest timing orchards (Fig. 2). Interestingly, fruit weight was similarly affected (Fig. 3).

Stem-pull force after 1 to 3 weeks storage indicated that silicate treatments maintained higher values during storage (Fig. 4).

Stem browning after 2 and 4 weeks storage was assessed for Lapins at MCAREC (Fig. 5). Silicate treatments showed less browning (lower values) than controls.

Silicate supplementation shows promise for improving sweet cherry quality. Effects from a single application near shuck fall were modest but indicated an improved vasculature was formed. It is conceivable that improved vasculature could promote fruit size and other quality attributes. Frequent supplementation may also be beneficial. Emphasis for next year will be placed on spray timing and frequency.



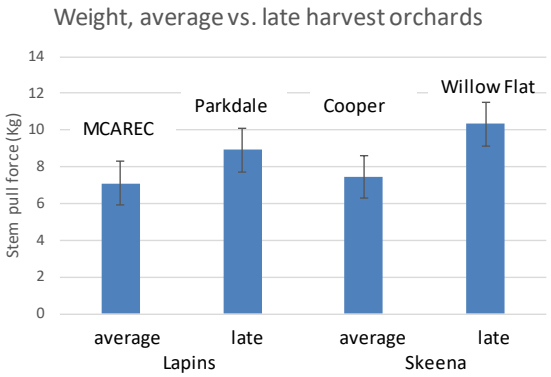
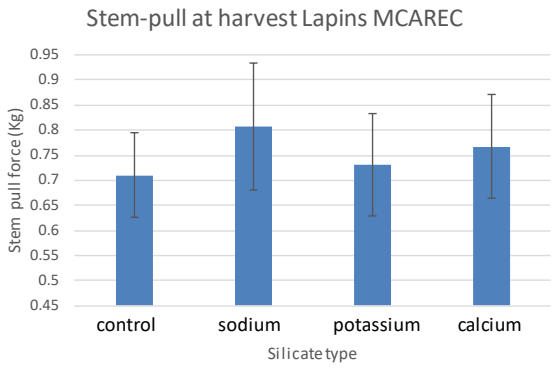


Figure 1. Lapins at MCAREC and Skeena at Cooper Barn were harvested at average timing from bloom; whereas, Lapins at Parkdale and Skeena at Willow Flat were harvested 7-10 days later. Stem pull-force was assessed with a push/pull force gauge (DS2-11, IMADA). Values are the average and standard deviation of 6-7 trees. Each tree value was the average of fruit from 30 spurs.



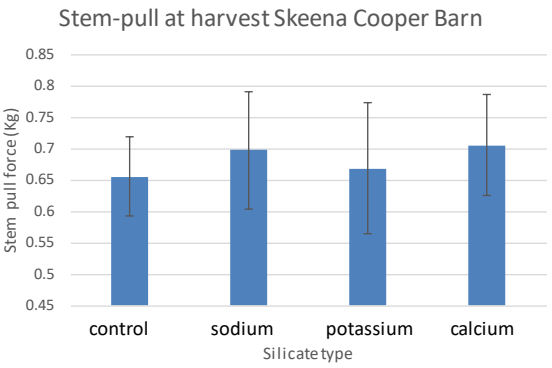
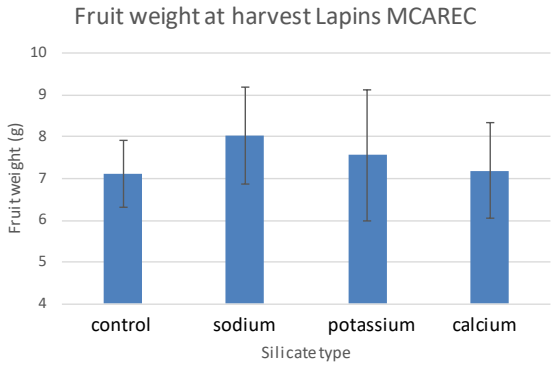


Figure 2. Stem pull-force was assessed with a push/pull force gauge (DS2-11, IMADA). Values are the average and standard deviation of 6-7 trees. Each tree value was the average of fruit from 30 spurs.



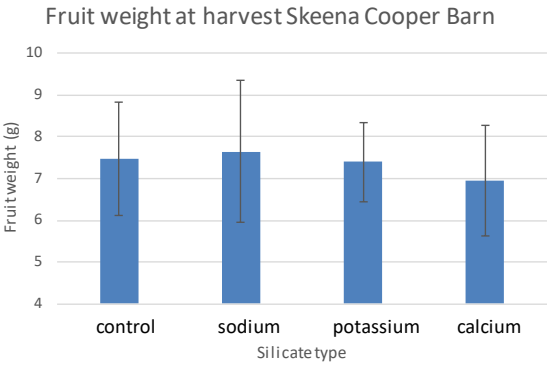


Figure 3. Individual fruit weights were obtained after the stem-pull force assessment. Values are the average and standard deviation of 6-7 trees. Each tree value was the average of fruit from 30 spurs.

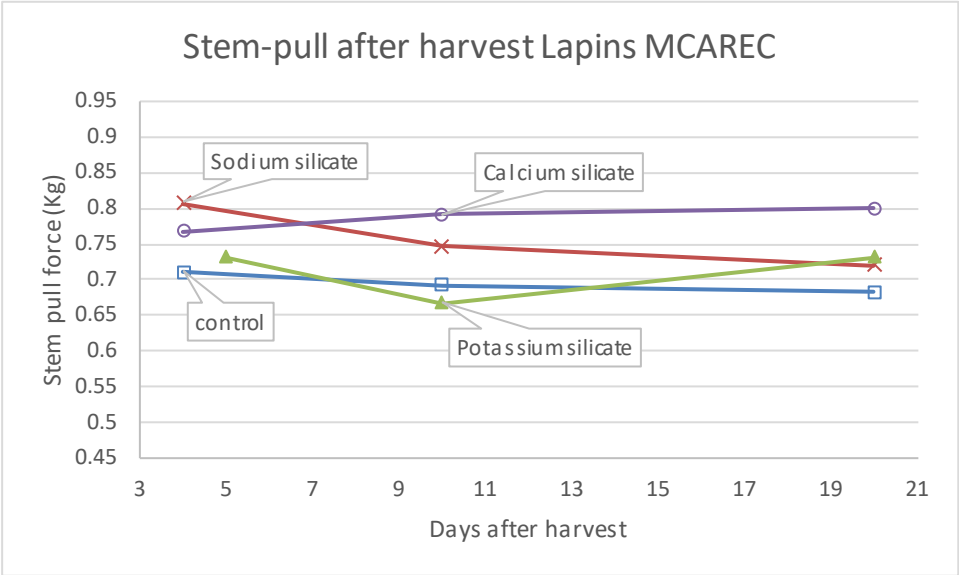


Figure 4. Stem pull-force was assessed with a push/pull force gauge (DS2-11, IMADA). Values are the average and standard deviation of 6-7 trees. Each tree value was the average of fruit from 30 spurs.

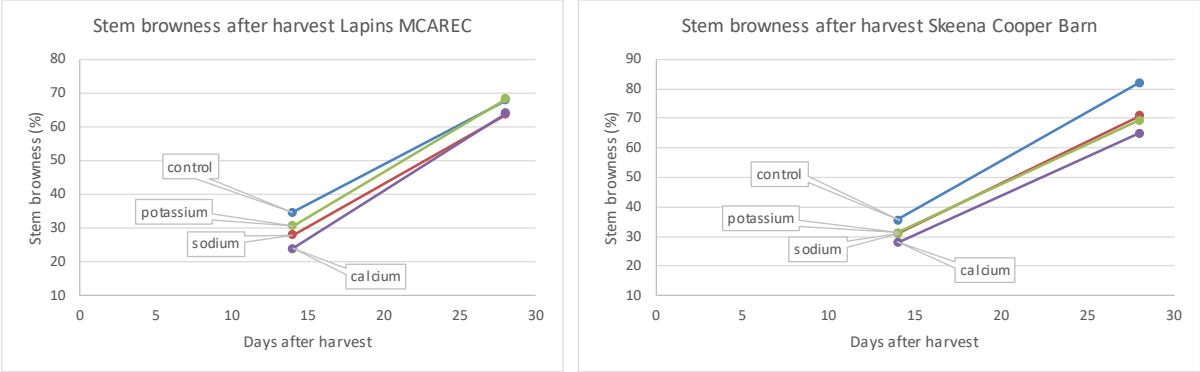


Figure 5. Browning of stems after harvest was assessed with a chroma-meter (CR 410, Konica Minolta). Greater values indicate that more surface area of the stems are brown.

CONTINUING PROJECT REPORT **YEAR:** 1 OF 2

Project Title: Model reporting

PI:	David Gibeaut	Todd Einhorn
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Address 2:		
City/State/Zip:	Hood River OR 97031	East Lansing MI 48824

Cooperators: to be determined

Total Project Request: **Year 1:** 31,416 **Year 2: 31,416**

Other funding sources
none

Budget 1
Organization Name: OSU-MCAREC **Contract Administrator:** L.J. Koong
Telephone: 541 737-4866 **Email address:** l.j.koong@oregonstate.edu

Item	2017	2018
Salaries	20,000	20,000
Benefits	11,416	11,416
Wages		
Benefits		
Equipment		
Supplies		
Travel		
Miscellaneous		
Plot Fees		
Total	31,416	**31,416**

OBJECTIVES

Two temperature based models of sweet cherry development were created to provide a decision aide support for growers, packing houses and marketers. The model, Freeze Tolerance in Endo and Ecodormancy (FTEED), is a predictive model of dormant bud lethal temperatures. A predictive model of bud hardiness in winter is required for decision support because real-time analysis requires two or three days--after the information was desired. The model, Gibeaut Einhorn Growing Diurnal Variable simulation (GDAVS), is a growing degree model of development that has been created using extensive phenology data from dormancy to maturation. The GDAVS model in combination with previously determined bud hardiness/phenology estimates (Fig. 1) can be used for bud hardiness predictions after the break of dormancy because bud hardiness is closely tied to phenology—a prediction of phenology is also a prediction of bud hardiness (Fig. 2). GDAVS also provides cultivar and location specific estimates of the seasonal progression of tree and fruit development which are useful for orchard management and crop predictions.

Reporting of both models publically on my website (blogs.oregonstate.edu/gdavs) will provide time sensitive information to the industry.

SIGNIFICANT FINDINGS

- Predictive modelling of Freeze Tolerance in Endo and Ecodormancy (FTEED) was published for the year 2016-17 on website: blogs.oregonstate.edu/gdavs
- FTEED was rewritten to provide predictions at 15 minute intervals
- FTEED was also made more flexible so that other crops can be easily analyzed by simply altering the temperature and rate settings specific to the crop
- Growing Degree Day model of cherry development from dormancy to maturation (GDAVS) was expanded to include other models such as the common Single-sine for comparison
- GDAVS was applied to Lynn Long's cultivar trial data comprising 40 cultivars and years spanning 2005 to 2017

METHODS

Models were constructed in Excel spreadsheets. The website editor is WordPress.

Dormancy to bloom phenology data was taken using the 30-spur method for Bing, Regina, Skeena and Sweetheart at MCAREC.

Temperature data were from AgriMet, IFPnet and recently from Columbia Gorge Fruit Growers Weather provided by the Rainwise Net.

Data will be added manually to prepared spreadsheets that contain site specific calculations. Once updated and calculated, tabulated and graphic reports will be posted

RESULTS & DISCUSSION

Constructing models in Excel is desirable for research because it has easily managed design flexibility and portability across platforms. Portability will become useful when mainframe computing is implemented. Blog posting is also useful at this time because it allows me to change and update information relatively easily as I develop the models.

Results of the model the FTEED model were presented with weekly updates for the 2016-17 season from 56 weather stations across Washington and the Columbia Gorge (blogs/Oregonstate/gdavs). A graphical presentation of the average temperature and predicted lethal temperature was presented.

Temperature and lethal temperature values for the individual weather stations were also tabulated on this site.

The IFPnet weather stations are located in orchards in the Columbia Gorge; whereas, AgriMet stations are not. Users should be aware of any temperature offset values they may presently use when evaluating the model results. No dangerous cold events in winter were predicted in the Columbia Gorge; however, some sites including MCAREC experienced a late-October light frost that may have caused some damage. While assessing phenology at MCAREC I observed a small percentage of pistil damage in some border trees.

The GDAVS required extensive reworking to increase calculation efficiency and to provide a more general design of phenology input data. The gains in efficiency allowed the addition of other growing degree model calculations for comparison such as the commonly employed Single-sine method. These comparison are crucial to the evaluation. This more robust and flexible design can be applied to other crops as well.

We have begun to collect and analyze temperature data for the 2017-18 season. Reporting of both models on the website, blogs.oregonstate.edu/gdavs, are forthcoming.

I recently added to GDAVS the bloom and maturation data provided by Lynn Long OSU Extension, for the cultivar trials at OSU's Cemetery Block in The Dalles OR. These data will be made available when evaluation is complete.

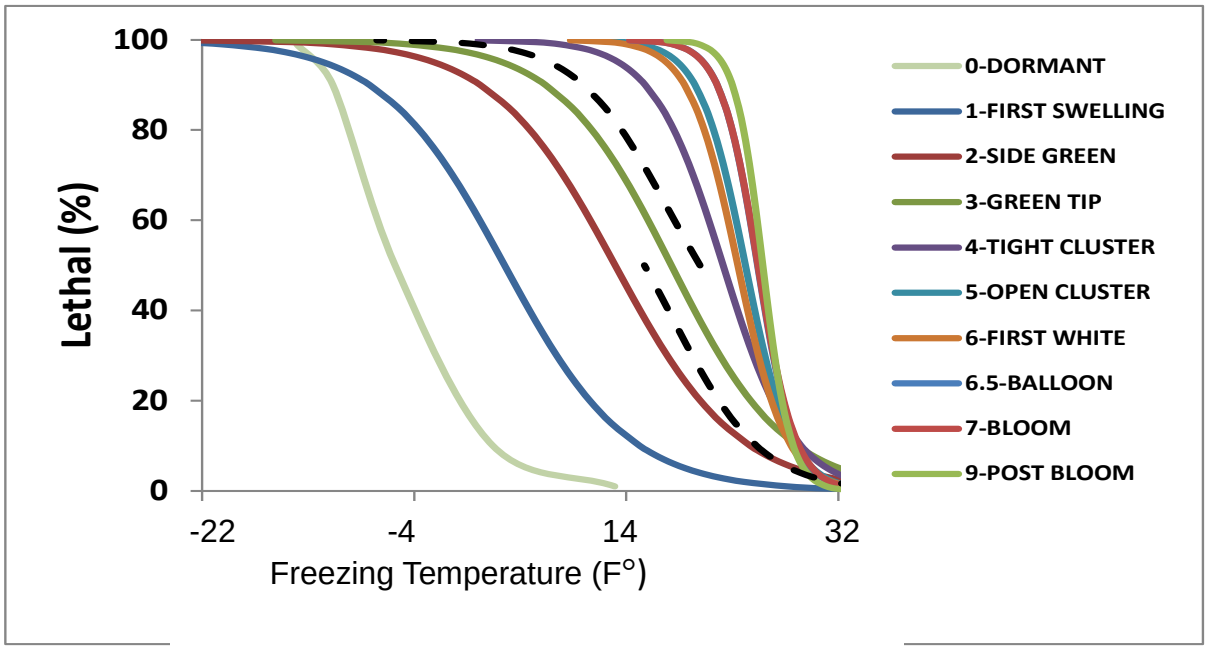

Figure 1.Bud hardiness is predictable by assessing the phenology stage.

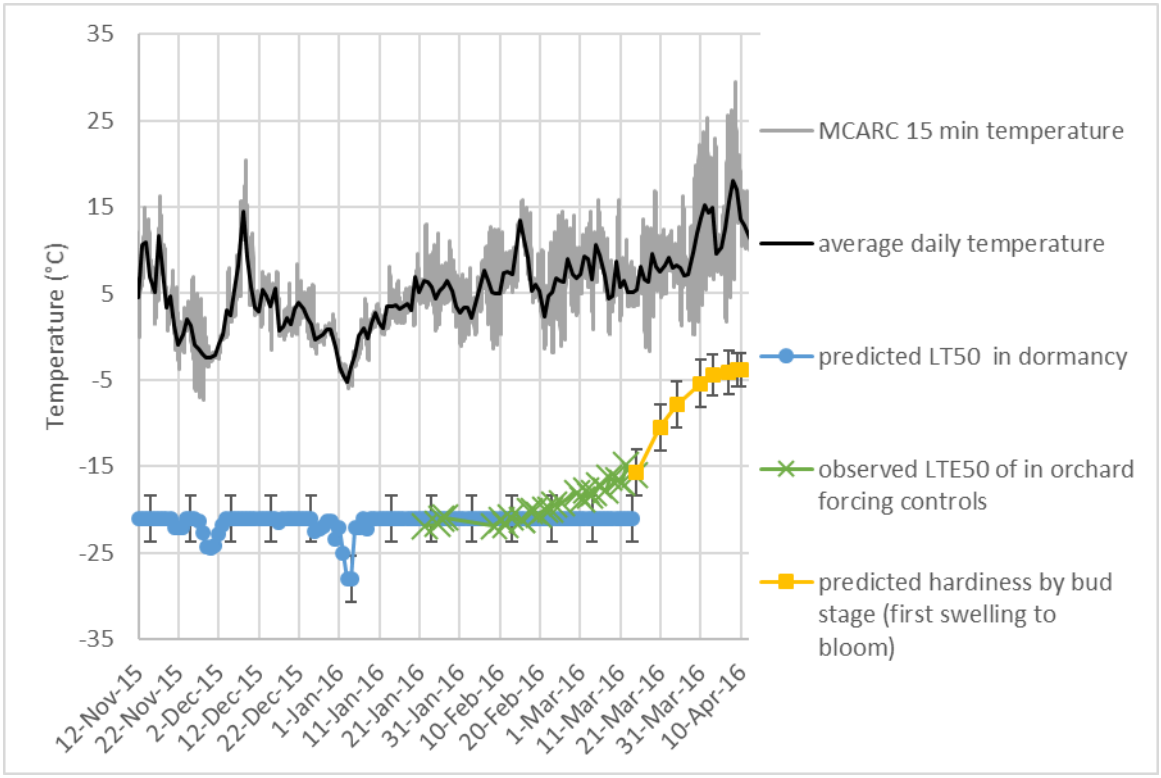

Figure 2. An example of the model results for Regina at MCAREC with experimentally determined values (see Einhorn final report 2016) of critical temperatures.

FINAL PROJECT REPORT

Project Title: Printing of color-maturation profiles for PNW dark-red sweet cherries

PI:	David Gibeaut	Todd Einhorn
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Address 2:		
City/State/Zip:	Hood River OR 97031	East Lansing MI 48824

Cooperators: Rob Blakey WSU Tree Fruit Extension; Northwest Cherries Inc

Total Project Request: **Year 1:** 6,142

Other funding sources
none

Budget 1
Organization Name: OSU-MCAREC **Contract Administrator:** L.J. Koong
Telephone: 541 737-4866 **Email address:** l.j.koong@oregonstate.edu

Item	2017
Salaries	2,000
Benefits	1,142
Wages	
Benefits	
Equipment	
Supplies	3,000
Travel	
Miscellaneous	
Plot Fees	
Total	6,142

OBJECTIVES
The colors of dark-red sweet cherry cultivars in the PNW are related to growth and maturation. Therefore, an accurate assessment of color will also be an assessment of how much additional fruit growth can be expected. A printed, field usable product of the color-maturation profile could be useful as a decision aide support.

SIGNIFICANT FINDINGS
- Fruit color from blush to mahogany
- 2-sided plastic cards
- Sizing holes
- Developmental indices of diameter and weight
- Color index numbered to be similar to the CTIFL color chart
- Colors named
- Row size holes with metric measurement
- Inch and millimeter rulers
- Cherry color size prediction spreadsheet

METHODS
Color-maturation profiles of dark-red sweet cherry were determined for cultivars: Chelan, Bing, Lapins, Skeena and Regina located throughout the Columbia Gorge (WTFRC grant "New programs to increase fruit size and improve harvest quality"). This data was collected twice weekly for the duration between green and mature cherry fruit. Light and size calibrated images provided the average color and size used for the color-maturation profiles and printing. Paper, heavy cardstock and laminated products were printed with good color reproduction but the paper based media were too fragile to withstand field work. Attempts at adding texture to the card by mottling the colors was unsatisfactory; therefore, monochrome colors were selected for printing A company, Plastic Printers Inc., were identified as a good candidate that could provide durable plastic prints that were die-cut for accurate hole sizing. Files for printing were prepared as PowerPoint.xps files.

RESULTS & DISCUSSION
Three versions were printed on plastic: a color test card (Fig. 1); a sizing card (Fig. 2); and a pocket ruler card (Fig. 3). Color reproduction in the test card was good; therefore a hole sizing card was devised. Some growers also wished to have a small pocket size color card without the sizing holes which we printed as well.

After the initial printing of 500 copies of version 1, extra money was provided by Northwest Cherries Inc.to defray half the cost of printing 2000 copies of versions 1 and 2. On advisement, two color names were also changed for versions 2 and 3: Cherry 2 was changed to Rose 2, and Berry 4 was changed to Crimson 4.

To help users understand how to use the card as a size prediction tool, a "How-to" video and size prediction calculator was by produced by Rob Blakey, WSU Tree Fruit. (treefruit.wsu.edu/videos/how-to-postharvest-pnw-dark-sweet-cherry-development-chart-index/)

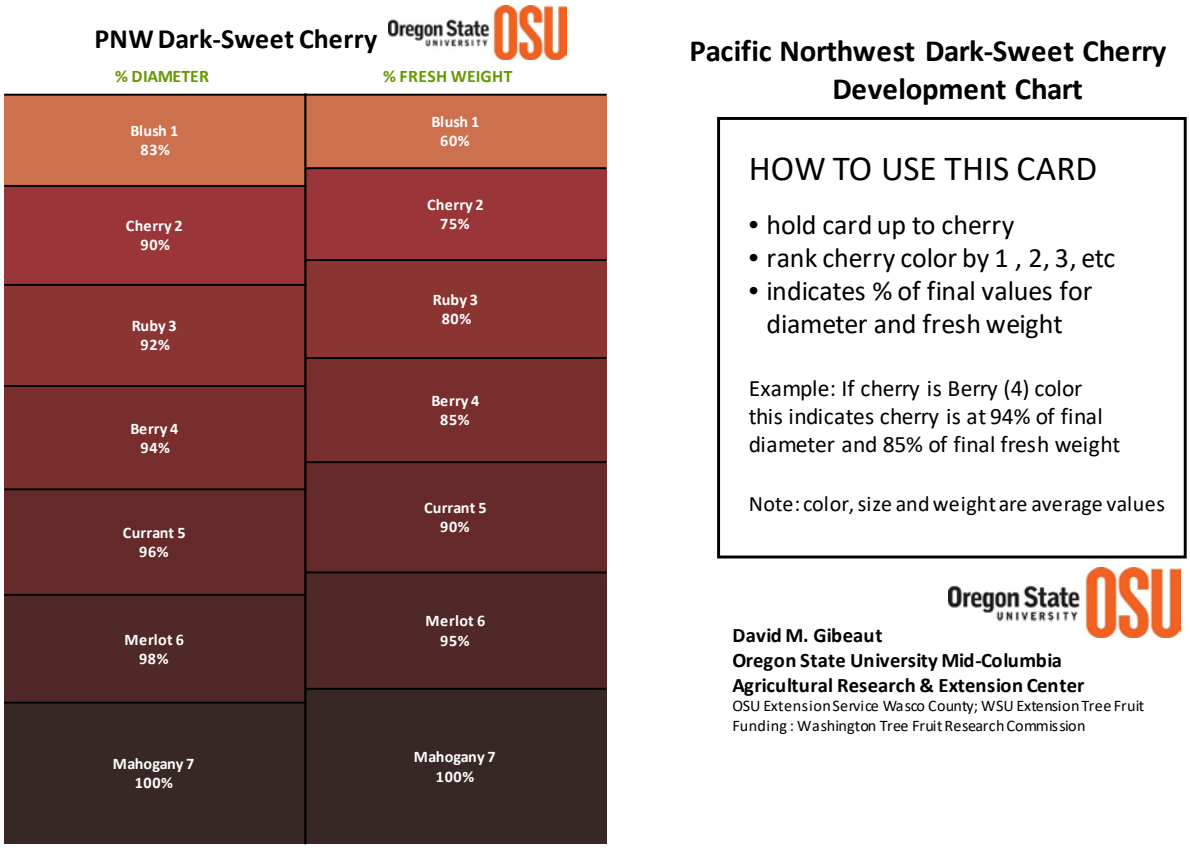

Figure 1. Version 1, Feb 2017. Color and developmental index card.

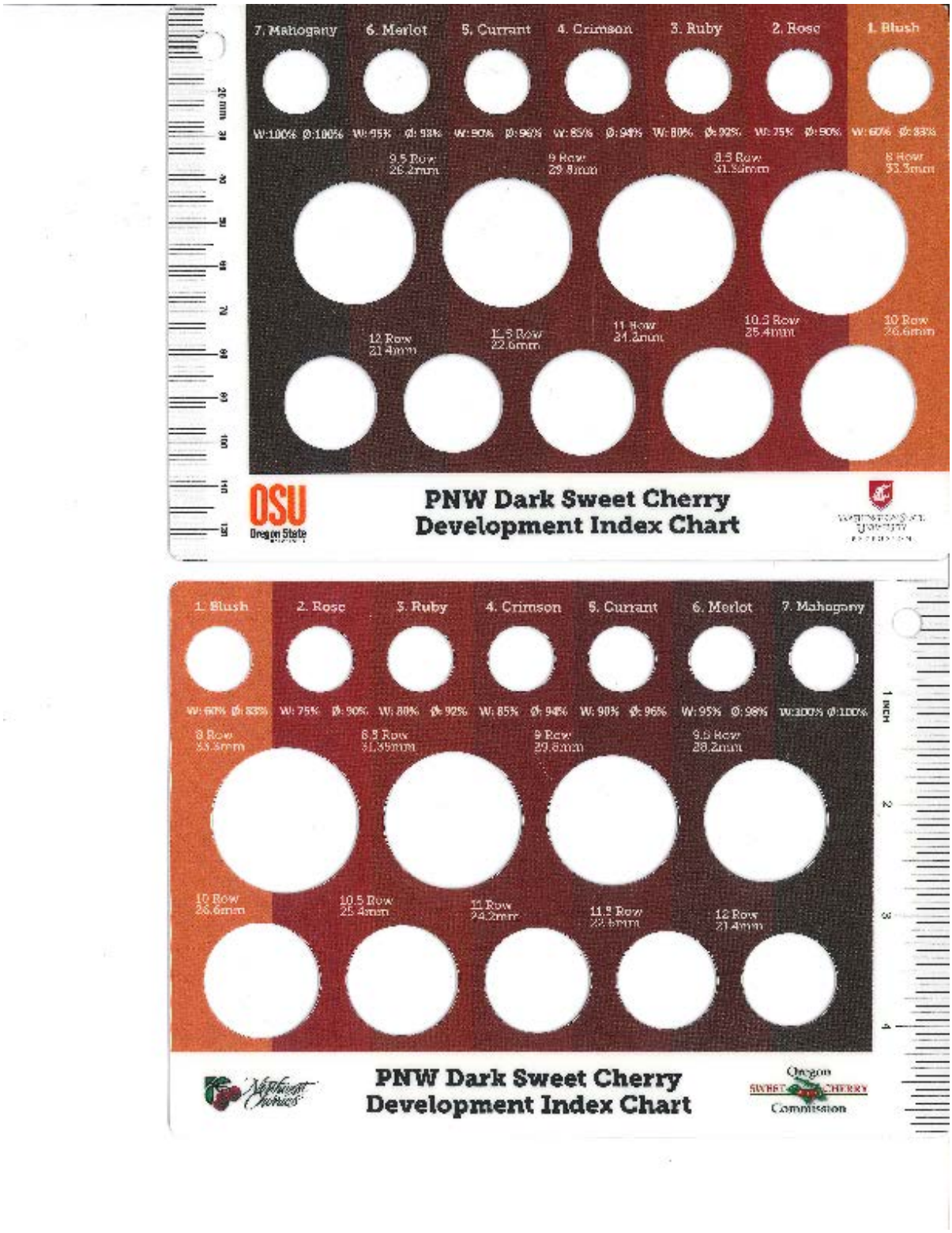

Figure 2. Version April 2, 2017. Sizing card.

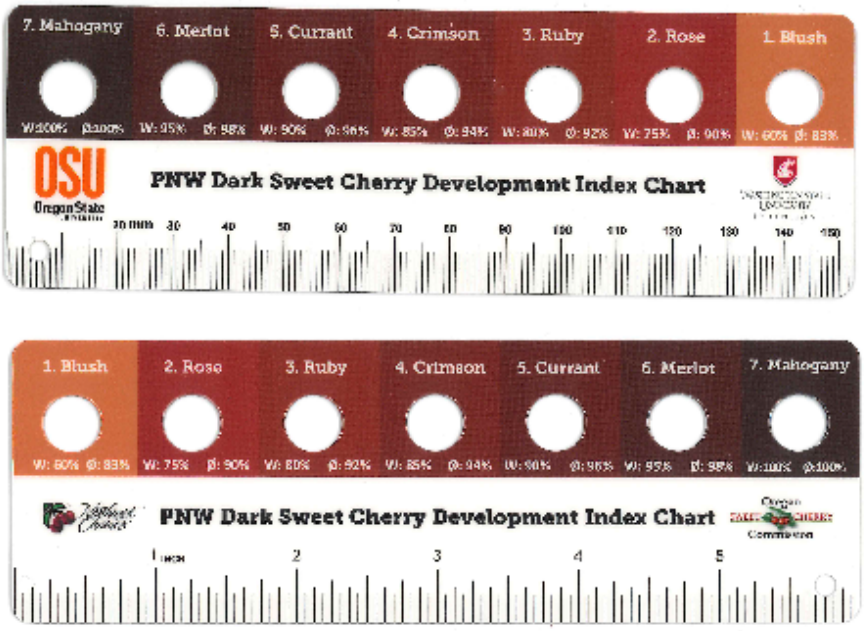

Figure 3. Version 3, April 2017. Pocket Ruler.

EXECUTIVE SUMMARY

The majority of the budget was used to cover printing costs. The second and third versions were printed and distributed at the 2016 Cherry Pre-harvest Tour organized by Lynn Long OSU Extension. Northwest Cherries Inc. provided funds for printing additional cards and Rob Blakey helped distribute them in WA.

CONTINUING PROJECT REPORT **YEAR**: Year 1 of 3

Project Title: MSU Cherry Rootstocks: Pre-commercialization

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Cooperators: Tim Dahle, Scott McDougall, Dan Plath, Aran Urlacher, Dale Goldy, Denny Hayden, Ian Chandler (see Table 2)

Total Project Request: **Year 1:** $81,012 **Year 2: $82,532** **Year 3:** $84,963

Other funding sources - None

Budget 1 – Amy Iezzoni
Organization Name: Michigan State University **Contract Administrator:** Greta McKinney
Telephone: (517) 353-0391 **Email address:** mckin134@anr.msu.edu

Item	2017	2018	2019
Salaries (technician)[a]	$ 5,500	$ 5,775	$ 6,064
Benefits[b]	2,335	2,492	2,635
Wages			
Benefits			
Equipment			
Supplies[c]	600	600	600
Travel[d]	4,500	4,500	4,500
Trees & shipping[e]	11,721		
Plot Fees			
Total	$ 24,656	**$ 13,367**	13,799

Footnotes:
[a]Technician will analyze and prepare summary tables and figures of the plot data and conduct the DNA diagnostics.
[b]Benefits calculated at 42.46%,43.15% and 43.46% for 2017, 2018 and 2019, respectively.
[c]Laboratory supplies for the DNA diagnostics.
[d]Travel for A. Iezzoni to visit the test plots, liner nurseries and finished tree nurseries.
[e] The cost of the trees and tree shipping for the 2017 plantings for Obj. 1 and 2.

Budget 2 – Lynn Long
Organization Name: Oregon State University **Contract Administrator:** L.J. (Kalvin) Koong
Telephone: **Email address:** l.j.koong@oregonstate.edu

Item	2017	2018	2019
Salaries		$ 8,400	$ 8,400
Benefits		$ 2,772	$ 2,722
Wages	$ 5,100	$ 5,600	$ 6,400
Benefits	510	560	640
Equipment			
Supplies	200	200	200
Travel	40	40	40
Plot Fees	660	660	660
Miscellaneous			
Total	$ 6,510	**$ 18,232**	$ 19,112

Footnotes:
[1]Previous 2018 and 2019 totals were $7,060 and $ 7,940. The increase is due to the reduction in salary support by OSU and Wasco County due to Lynn Long's retirement.

Budget 3 – Bernardita Sallato[1]
Organization Name: Washington State Univ. **Contract Administrator:** Katy Roberts
Telephone: (509) 335-2885 **Email address:** katy.roberts@wsu.edu

Item	2017	2018	2019
Salaries	$ 2,990	$ 15,450	$ 16,068
Benefits	2,270	7,547	7,849
Wages	25,472	14,400	14,976
Benefits	17,489	10,498	10,918
Equipment			
Supplies			
Travel	1,625	3,038	2,241
Plot Fees			
Miscellaneous			
Total	$ 49,846	**$ 50,933**	$ 52,052

Footnotes:
[1]Budget for 2017 was to the WTFRC with Tom Auvil as Co-PI. Due to changes at the WTFRC, for 2018, and moving forward, this project was transferred to B. Sallato. The assistance of Jim McFerson that was instrumental in accomplishing the 2017 planting activities is gratefully acknowledged.

OBJECTIVES:

1. Compare the performance of the MSU cherry rootstocks to currently available sweet cherry rootstocks using intensive cherry production systems.
2. Track the MSU rootstock performance in trials with PNW grower cooperators that are experimenting with a wider range of scions and orchard systems.
3. Collaborate with cooperating nurseries and the Clean Plant Center Northwest-Fruit Trees to ensure MSU cherry rootstocks are available as virus certified and genetically verified.

SIGNIFICANT FINDINGS:

- For the 2015 plantings (Obj. 1), trees on the MSU rootstocks were significantly smaller than trees on the control rootstocks [Gisela 5 (Gi5), Gi6, Krymsk 5 (K5) and K6] measured as TCSA.
- For the 2015 plantings (Obj. 1), in general, across scion varieties and locations, the highest tree and per acre yields were obtained with Gi5 and Clinton and the lowest yields were obtained with K5 and K6.
- For the 2015 plantings (Obj. 1), the rootstock effect for fruit size varied among locations. In part, this could be due to the different environmental conditions and training systems, including differential abilities to tolerate the high heat at the Mattawa plot.
- For the 2015 plantings (Obj.1), in general, fruit maturity date was accelerated on the MSU rootstocks followed by Gi5 and Gi6 and then the Krymsk rootstocks using mean color and brix as a measure of maturity.
- Project team members visited grower plots (Obj. 2) to monitor performance and provide cultural practice recommendations. Collectively these plots encompass seven scions and four training systems. These plantings will not only provide critical comparisons of the new rootstocks, but they will also illustrate how these new rootstocks perform given difference scions, orchard systems and environments.
- All five MSU cherry rootstocks had been virus certified by the CPCNW-FT; however, this virus certification did not include screening for CVA. Therefore, this year, all five MSU rootstocks were screened for CVA and found to be negative.
- The MSU rootstocks were trademarked under the name Corette™ to facilitate experimental plantings and are commercially available at several nurseries.
- The Iezzoni lab provided DNA diagnostic support as needed at no cost to the collaborating nurseries to assure rootstock trueness-to-type at various stages of liner and finished tree production.

METHODS:

1. Compare the performance of the MSU cherry rootstocks to currently available sweet cherry rootstocks using intensive cherry production systems.

2015 plantings (Table 1): The three replicated rootstock trials that were planted in 2015 will continue to be pruned and trained based on the training system used for each plot (Table 1). For all three sites, trees will be harvested to obtain individual tree yields. The fruit from the Mattawa and East Wenatchee plots will be transported to the Sallato laboratory in Prosser for fruit quality evaluations. The Oregon fruit will be transported to the OSU cherry laboratory located at the Extension office in Wasco County. Evaluations will be done with a goal of 100 fruit per 5-tree replicate for the following traits: bulk fruit weight and cracking. Next, a sample of 50 fruit will be evaluated for skin color, row size, fruit size in mm, and firmness. Brix and titratable acidity will be measured from a bulked sample of 25 fruit. After harvest, the tree trunk circumference will be measured 10 cm above the graft line and used for calculations of yield efficiency. Per acre yields will be calculated using tree spacings based on projections of rootstock canopy size (see Tables 3 and 4, footnote 2).

Table 1. Summary of rootstock plantings made in spring 2015 at three locations: The Dalles (TD), Ore., Mattawa (MA) & East Wenatchee (EW), Wash.

Scion cultivars	Regina, Early Robin, Sweetheart
MSU rootstocks	Cass, Clare, Clinton and Lake[1]
Control rootstocks	Gi5, Gi6, Krymsk 5 (Sweetheart), Krymsk 6 (Regina, Early Robin)
Pollinators	Chelan (Early Robin), Sam (Regina)
Replication	20 trees per each scion/rootstock combination (four 5 tree replications)
Training system: TD	Sweetheart and Early Robin trees were headed to establish a bush system. Regina trees were trained to a steep leader system.
Training system: MA	Two narrow rows on a 4 wire angle canopy trellis
Training system: EW	Super Slender Axe, 2 very narrow rows on 4 wire angle canopy trellis[2]
Within row spacing: TD	8 ft
Within row spacing: MA[3]	3 ft (Gi6), 2.5 ft (K5, K6, Clinton), 2ft (Cass, Lake, Clare)
Within row spacing: EW[3]	4 ft (Gi6, K5, K6, Clinton), 2 ft (Cass, Lake, Clare)

[1]'Regina'/Cass was not included at East Wenatchee due to insufficient tree numbers.

[2]Wires 2.3 (0.7m) apart vertically

[3]Spacings are under each trellis arm; therefore, spacings down the row would be divided by ½.

2017 plantings: The objective of these trials is to evaluate the fifth cherry rootstock, Crawford, in comparison with the most similar rootstocks, Clinton and Gi5. Crawford was selected for advancement a year later because of good performance at the WSU-Prosser plot with 'Bing' scion. The scions are 'Regina' and 'Sweetheart' with 20 trees per combination per location. The combination 'Regina'/Cass was added as this combination was under-represented in the 2015 plantings. In 2017, two of the plots were planted next to the 2015 plantings [The Dalles (hosted by Tim Dahle) and East Wenatchee (hosted by McDougall & Sons)] while the Mattawa plot was hosted by Zirkle due to lack of space next to the current Mattawa plot. Due to the rootstocks' precocity and the SSA training system, at the Mattawa and East Wenatchee sites, in the spring, the top portion of the leader where laterals have not yet been induced, will be subjected to a round of light trunk girdling at green bud stage to induce lateral. This will be followed by four applications at weekly intervals of 6Ba + Ga $_{4+7}$ (Promalin, Typey, Perlan) at maximum labeled rates. Data to be collected in 2018 will include plant survival, trunk cross sectional area, and suckering. In 2019, fruit data will be taken as above for the 2015 plantings.

2. Track the MSU rootstock performance of trials with PNW grower cooperators that experiment with a wider range of scions and orchard systems.

The 2016 and 2017 plantings of the MSU rootstocks were arranged based upon requests from individual growers (Table 2). In three cases, the producers received the liners directly from three propagators (Duarte Nursery, Protree Nursery and North American Plants). Replication size was capped at 200 trees per combination due to the experimental status of the MSU rootstocks. The project team will visit the plots to monitor performance and provide cultural practice recommendations as requested.

3. Collaborate with cooperating nurseries and the Clean Plant Center Northwest-Fruit Trees to ensure MSU cherry rootstocks are available as virus certified and genetically verified.

All five MSU cherry rootstocks (Cass, Clare, Lake, Crawford, and Clinton) have been virus certified by the CPCNW-FT and stock plants are being maintained for any future distributions. These rootstocks, trademarked under the name Corette™, are commercially available from several nurseries. The relative ease of liner and finished tree production at these nurseries will continue to be assessed

through visits of Iezzoni to these nurseries. The Iezzoni lab will provide DNA diagnostic support as needed at no cost to the collaborating nurseries to assure rootstock trueness-to-type at various stages of liner and finished tree production.

Table 2. PNW trials planted in 2016 (*) and in 2017 testing the MSU cherry rootstocks.

Location	Scion(s)	No. of MSU Rootstocks (~ rep. size [1])	Producer
The Dalles, OR[2]	Ebony Pearl, Burgundy Pearl	All 5 (40)	Omeg Orchards
The Dalles, OR	Coral	All 5 (45)	Dahle Orchards
Dallesport, WA	Bing, Chelan	All 5 (30)	Orchard View
Mattawa, WA[3]	Benton	All 5 (200)	Zirkle Fruit
Mattawa, WA*	Skeena	4[4] (150)	Stemilt
Mattawa, WA	Coral	All 5 (20)	Wash. Fruit & Produce
Pasco, WA	Coral	All 5 (20)	Hayden Farms

[1]The number of trees for each rootstock/scion combination.
[2]The rootstock liners were planted in the orchard in spring 2016 and budded fall 2016.
[3] The rootstock liners were budded in August 2016 and planted in place in spring 2017.
[4] Crawford was not included.

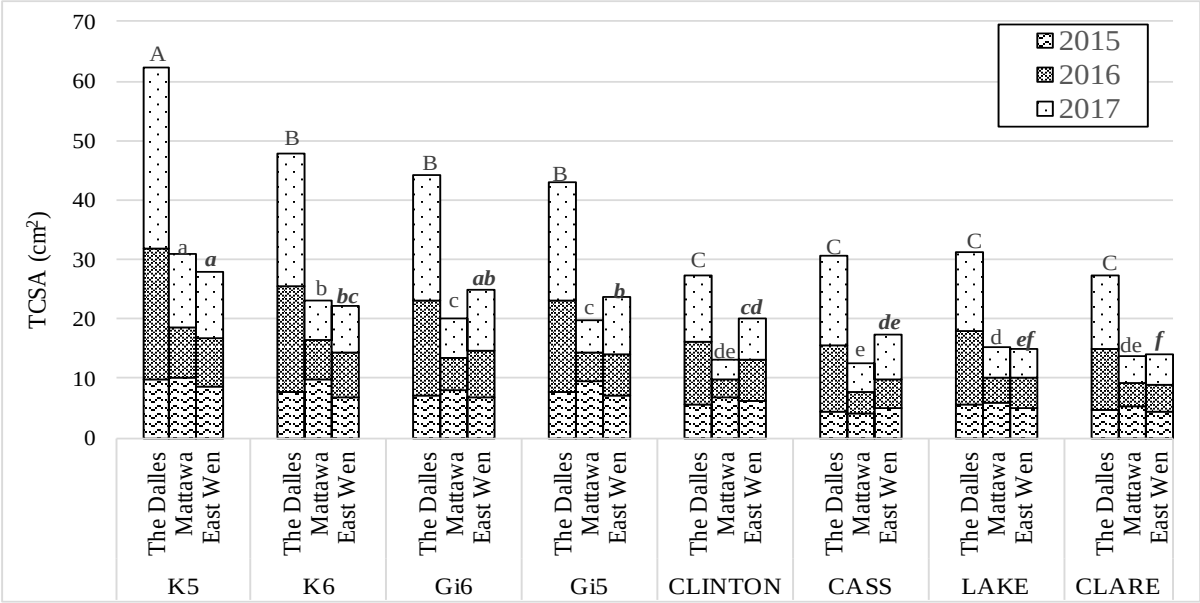
RESULTS & DISCUSSION:
Obj. 1. 2015 plantings: For the 2015 plantings, trees on the MSU rootstocks were significantly smaller than trees on the controls rootstocks [Krymsk 5 (K5) and K6, Gisela 5 (Gi5), Gi6] measured as TCSA (Fig. 1). Fruit for all three scion cultivars ('Regina', 'Sweetheart' and 'Early Robin') were harvested from the plots in E. Wenatchee and The Dalles, however only 'Regina' was harvested from Mattawa, as the two other cultivars at this site had severely reduced crop loads due to spring freeze damage. In The Dalles, where 'Regina' was trained to steep leader system, the mean tree yields for the rootstocks were not significantly different (Table 3). For 'Early Robin' at The Dalles and trained to a KGB, the yields on Clinton were highest compared to the other three MSU rootstocks while for 'Sweetheart', also trained to a KBG, the highest yields were on Gi5. For the SSA plot at Wenatchee, yields across varieties tended to be significantly higher with Clinton or Gi5 and lowest for K5/K6 (Table 4). At Wenatchee, compared to the other rootstocks, Cass and Clare trees had significant floral spur thinning done to encourage branching and this may have contributed to the lower yields. For the Mattawa SSA planting, 'Regina' trees on Clinton also had the highest tree yields. When the different tree densities and TCSA were taken into consideration (Table 4), the yields per acre and yield efficiencies were more uniform across the dwarfing rootstocks.

The rootstock effect for fruit size varied among locations (Tables 3 and 4). For The Dalles, there were no significant rootstock effects for 'Regina', however, for 'Early Robin' and 'Sweetheart' fruit size means were smaller on Cass. At Wenatchee, mean fruit size for all three scions tended to be significantly smaller on Lake and K6 or K5. However, at Mattawa, mean fruit size for 'Regina' on Cass and Lake were not significantly smaller than that on the other rootstocks, and instead fruit size tended to be larger than that on the other rootstocks. Lake, Cass and Clare all appeared to have less heat stress at the Mattawa site, whereas there was relatively less heat stress at the two other sites.

In general fruit maturity date was accelerated on the MSU rootstocks followed by Gi5, Gi6 and then the Krymsk rootstocks for the Washington plantings using mean color and brix as a measure of maturity (Table 5). The one exception was for red over-color rating at Wenatchee where 'Early Robin' fruit from trees on Clare and Lake had similar color ratings as trees on K6. The differences in maturity at time of harvest are likely the causes for the differences in firmness. For example 'Regina' fruit from the SSA plots grown on K6 had a higher mean firmness and less red color than fruit grown

on trees on the other rootstocks (Table 5). There were no significant differences for fruit color or firmness based on rootstock for steep leader 'Regina' trees at The Dalles.

Fig. 1. Mean comparison of trunk cross-sectional area (TCSA; cm^2) for trees on 4 MSU rootstocks, K5, K6, Gi6, and Gi5 and planted in 2015. Means are summed over three scions ('Early Robin', 'Regina', and 'Sweetheart') and presented for three locations (The Dalles, OR, Mattawa and East Wenatchee, WA). Boxes represent growth over one season.[1,2]



[1]Means that are significantly different for 2017 TCSA ($P < 0.05$) within location are denoted by different letters [Comparisons for The Dalles = capital letters, Mattawa = small letters, Wenatchee = small italic letters).
[2]Strong branching near the ground due to the KGB training system used at The Dalles contributed to the larger trunk sizes compared to the SSA plots in Wash.

Table 3. Individual tree yields for 'Early Robin, 'Regina' and 'Sweetheart' grown on four MSU rootstocks, Gi5, Gi6, Krymsk 5 and Krymsk 6 for trees planted in 2015 in The Dalles, OR. Harvest dates: 'Early Robin' – June 17, 'Regina' – July 8, and 'Sweetheart' – July 19, 2017. [1,2]

Rootstock selection	Early Robin			Regina			Sweetheart		
	Tree yield (lb)	Tons per acre	Row size[3]	Tree yield (lb)	Tons per acre	Row size[3]	Tree yield (lb)	Tons per acre	Row size[3]
Gi5	0.55 ab[4]	0.23 ab	9.4 abc	6.29 a	1.96 a	9.7 a	2.65 a	0.82 a	9.2 a
Gi6	0.73 ab	0.14 b	9.3 ab	5.18 a	1.35 a	9.8 a	0.66 b	0.17 b	9.3 ab
K5/K6[5]	0.67 ab	0.18 ab	9.5 bc	3.09 a	0.80 a	9.9 a	0.37 b	0.09 b	9.5 ab
CASS	0.42 b	0.16 ab	9.6 c	3.97 a	1.54 a	9.9 a	0.29 b	0.12 b	9.7 b
CLARE	0.51 b	0.20 ab	9.3 ab	6.11 a	2.38 a	9.7 a	0.28 b	0.11 b	9.6 ab
CLINTON	0.91 a	0.28 a	9.2 a	4.30 a	1.34 a	9.8 a	0.83 b	0.26 b	9.2 ab
LAKE	0.50 b	0.19 ab	9.3 abc	3.14 a	1.23 a	9.8 a	0.50 b	0.20 b	9.6 ab

[1]Training systems: 'Regina' – Steep Leader and 'Early Robin' and 'Sweetheart' – Kym Green Bush.
[2]Yield per acre was calculated as average yield per tree × number of trees per acre with 519 trees/acre (6 ft × 14 ft) for K6 and Gi6; 622 trees/acre (5 ft × 14 ft) Gi5 and Clinton; and 778 trees/acre (4 ft × 14 ft) for Clare, Cass, and Lake. Ton=US short ton=2000 lbs
[3]Row size data was generated using a BioWorks FirmTech machine
[4]Means that are significantly different ($P < 0.05$) are denoted by different letters.
[5]'Sweetheart' is on Krymsk 5 (K5) and 'Early Robin' and 'Regina' are on Krymsk 6 (K6)

Table 4. Individual tree yields, yield efficiency, yield per acre, fruit weight and row size for 'Early Robin', 'Regina' and 'Sweetheart' grown on four MSU rootstocks, Gi5, Gi6, K5, and K6 and trained using a Super Slender Axe (SSA) from trees planted in 2015 at East Wenatchee and Mattawa, WA[1].

Scion	Location	Rootstock selection	Tree yield (lb)	Yield efficiency (kg/cm^2)	Yield per acre (tons/acre)[2]	Fruit weight (g)[3]	Row size (row card)[3]
Early Robin	Wenatchee	Gi5	2.41 a[4]	0.046 a	2.19 a	11.4 a	9.6 b
		Gi6	1.54 bc	0.033 ab	1.40 ab	11.5 a	9.5 ab
		K6	0.75 d	0.016 b	0.68 b	9.7 c	10.1 c
		CASS	1.01 cd	0.031 ab	1.83 a	11.2 ab	9.4 a
		CLARE	1.20 cd	0.047 a	2.18 a	11.5 a	9.4 a
		CLINTON	2.17 ab	0.054 a	1.96 a	11.9 a	9.5 ab
		LAKE	1.23 cd	0.039 ab	2.23 a	10.4 bc	10.0 c
Regina	Wenatchee[5]	Gi5	4.00 a	0.088 ab	3.63 ab	8.0 ab	11.0 b
		Gi6	2.95 a	0.056 abc	2.67 b	8.4 ab	10.7 a
		K6	0.77 b	0.016 c	0.70 c	7.6 b	11.0 b
		CLARE	2.60 a	0.115 a	4.72 a	7.9 ab	10.9 b
		CLINTON	3.37 a	0.098 a	3.05 b	8.6 a	10.7 a
		LAKE	1.18 b	0.051 bc	2.14 b	7.7 b	11.0 b
	Mattawa[6]	Gi5	2.14 bc	0.090 abc	3.11 abc	9.1 a	10.7 c
		K6	0.27 c	0.004 c	0.39 c	8.8 a	10.6 c
		CASS	1.76 bc	0.089 bc	3.19 ab	9.1 a	10.5 bc
		CLARE	2.26 b	0.101 ab	4.09 ab	9.3 a	10.4 b
		CLINTON	3.27 a	0.138 a	4.74 a	9.8 a	10.6 c
		LAKE	1.69 bc	0.070 bc	3.06 bc	9.2 a	10.1 a
Sweetheart	Wenatchee	Gi5	4.98 a	0.100 a	4.52 a	6.8 abc	11.3 b
		Gi6	3.14 b	0.054 b	2.85 b	6.9 abc	11.3 b
		K5	0.56 d	0.009 c	0.51 c	6.7 bc	11.2 ab
		CASS	1.96 c	0.053 b	3.55 ab	7.0 ab	11.1 ab
		CLARE	1.90 c	0.060 b	3.45 ab	7.2 ab	11.0 a
		CLINTON	4.01 ab	0.078 ab	3.63 ab	7.4 a	11.1 a
		LAKE	1.90 c	0.051 b	3.44 ab	6.4 c	11.5 c

[1]Fruit were harvested on the following dates: 'Early Robin' East Wenatchee, – June 27; 'Regina' East Wenatchee, – July 11 and Mattawa – July 6; and 'Sweetheart' East Wenatchee – July 19, 2017. Spring freeze damaged resulted in the crop loss of 'Early Robin' and 'Sweetheart' at Mattawa.

[2]Yields per acre were calculated as average yield per tree × number of trees per acre. Trees/ac for Wenatchee: 1,815 trees/acre (2 ft ×12 ft) for K6, Gi5, Gi6, and Clinton and 3,630 trees/acre (1 ft × 12 ft) for Clare, Cass, and Lake. Trees/acre for Mattawa: 2,904 trees/ha (1.25 × 12 foot spacing) for K6, Gi5, and Clinton; 2,420 trees/acre (1.5 ft × 12 ft) for Gi6; and 3,630 trees/acre (1 ft × 12 ft) for Clare, Cass, and Lake. Spacings are down the row; therefore, spacings under each trellis arm would be doubled. Ton = US short ton = 2000 lbs

[3]Fruit weight and row size were measured from 100 and 50 fruit per replicate, respectively.

[4]Means that are significantly different ($P < 0.05$) are denoted by different letters.

[5]Due to insufficient trees 'Regina'/Cass was not included in this planting.

[6]Data was not available for Gi6 as the 'Regina' trees delivered from the nursery turned out to be an unknown variety.

Table 5. Mean fruit firmness (g/mm) and fruit skin color for 'Regina' grown on three MSU rootstocks, Gi5, Gi6, and Krymsk 6 from trees planted in 2015 in East Wenatchee and Mattawa, WA and trained to the SSA and The Dalles trained to a steep leader. Fruit were harvested on July 11, 6, and 8, 2017, respectively. Firmness data was generated using a BioWorks FirmTech machine. Color data is according to the sweet cherry CTIFL color card.

Rootstock selection	E. Wen CTIFL Color Rating	E. Wen Firmness (g/mm)	Mattawa CTIFL Color Rating	Mattawa Firmness (g/mm)	The Dalles CTIFL Color Rating	The Dalles Firmness (g/mm)
Gi5	4.5 b	296 c[1]	4.9 c	315 b	5.9 a	309 a
Gi6	4.6 b	310 c	NA	NA	6.0 a	317 a
K6	4.3 b	412 a	4.3 c	350 a	6.1 a	301 a
CASS	-	-	5.3 ab	226 d	5.8 a	292 a
CLARE	5.3 a	354 b	4.9 c	307 bc	6.1 a	273 a
CLINTON	5.3 a	343 b	5.1 bc	311 b	6.4 a	280 a
LAKE	5.2 a	352 b	5.5 a	291 c	6.1 a	271 a

[1]Means that are significantly different ($P < 0.05$) are denoted by different letters.

Obj 1. 2017 plantings: These three plots were planted at the E. Wenatchee, Mattawa, and The Dalles locations. The first two plots were trained to an SSA and The Dalles plot was trained to start a KGB system.

Obj 2. Project team members visited the plots (Table 2) to monitor performance and provide cultural practice recommendations as requested. The 'Skeena', 'Coral', and 'Benton' plantings established in Wash. were managed to initiate an SSA system, while the 'Bing'/'Chelan' planting was managed to initiate a modified SSA/TSA system. All plantings are high density with fixed trellis. In Oregon, the 'Coral' planting was trained to a KGB system and the 'Ebony Pearl'/ 'Burgundy Pearl' planting is being trained to a UFO. Collectively these plantings will not only provide critical comparisons of the new rootstocks, but they will also illustrate how these new rootstocks perform given difference scions, high density orchard systems, and environments.

Obj. 3. All five MSU cherry rootstocks (Cass, Clare, Lake, Crawford and Clinton) have been virus certified by the CPCNW-FT; however, this virus certification did not include screening for CVA. Therefore, this year all five MSU rootstocks were screened for CVA and found to be negative. Stock plants are being maintained for any future distributions. Patents were applied for these rootstocks in October 2016 and they were trademarked under the name Corette™ to facilitate experimental plantings. The rootstocks are available from several nurseries. The relative ease of liner and finished tree production at these nurseries will continue to be assessed through visits of A. Iezzoni to these nurseries. The Iezzoni lab provided DNA diagnostic support as needed at no cost to the collaborating nurseries to assure rootstock trueness-to-type at various stages of liner and finished tree production.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number: CH-17-108

Project Title: Screening for fruit powdery mildew resistance in the breeding program

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Cooperators: Gary Grove, Neusa Guerra, new permanent WSU cherry breeder

Total Project Request: **Year 1:** 17,348 **Year 2: 40,475**

Other funding sources:

Agency Name: WTFRC/OSCC
Amt. awarded: $150,000 (2017)
Notes: "Streamlining the Pacific Northwest Sweet Cherry Breeding Program." PI: Peace. Co-PI: Sallato.

Agency Name: USDA-NIFA Specialty Crop Research Initiative
Amount awarded: $10.0 M (Sep 2014 – Aug 2019)
Notes: "RosBREED: Combining disease resistance with horticultural quality in new rosaceous cultivars" PI: Iezzoni. Co-PIs include Peace.

Agency Name: WTFRC/OSCC
Amt. requested: $150,000 (2018)
Notes: "Sweet cherry breeding: identifying genetically superior selections." PI: Peace. Co-PIs: Sallato, Blakey.

Organization Name: WSU Pullman **Contract Administrator:** Katy Roberts
Telephone: 509 335 2885 **Email address:** arcgrants@wsu.edu

Item	2017	2018
Salaries [a]	9,001	**12,454**
Benefits	3,872	**5,323**
Wages [b]		**8,000**
Benefits		**800**
Equipment		
Supplies [c]		**7,096**
Travel [d]		**2,327**
Miscellaneous		
Plot Fees [e]	4.475	**4,475**
Total	17,348	**40,475**

[a] 0.25 FTE for an associate in research; 1 month salary and benefits for genetic screening technician in the WSU Tree Fruit Genotyping La
[b] Time slip field workers
[c] Bags for artificial inoculation of fruit; DNA test development consumables
[d] Prosser-Pullman travel for meetings among PIs
[e] Plot fees and maintenance of block C53

OBJECTIVES

Goal: Develop a reliable, efficient assay for revealing genetic potential for fruit powdery mildew (PM) resistance that can be routinely used in the PNW sweet cherry breeding program.

Specific objectives:

1. Determine genetic potential for fruit powdery mildew resistance/tolerance in the PNW sweet cherry breeding program by evaluating a large, representative germplasm set using natural and artificial inoculation
2. Convert large-effect genetic factors discovered for fruit powdery mildew resistance/susceptibility into a diagnostic DNA test for routine breeding use

SIGNIFICANT FINDINGS

- Fruit PM resistance = Foliar PM resistance, genetically
- A successful season was achieved of discriminating resistance from susceptibility for fruit and foliar PM in the PNW sweet cherry breeding program; infection pressure was high.
- The detached leaf disk assay was very effective and, given the broader results obtained, could be suitable as a phenotypic screen for fruit as well as foliar resistance/susceptibility.
- Fruit and foliar PM resistance were each observed to be under strong genetic control. Many individuals were identified that appear to be fully resistant to fruit PM infection, and were descended from particular parents.
- Complete absence of fruit and foliar PM infection was associated with a dominant allele inherited from 'Moreau'/PMR-1 and Mildew-Immune Mazzards. The two sources are probably the same as they provide the same effect alone or in combination, and have been used extensively in local crosses for at least the last 10 years.
- Other genetic contributors to resistance and/or suppression of infection spread might exist in the examined breeding material. Another season of observation is required with an even higher spread of fruit infection across the block.
- The experience of this season establishes a strong basis for next season's validation of sources and carriers of resistance in breeding material and establishment of a DNA test method to eliminate fruit PM-susceptible plants efficiently and routinely.

METHODS

Two-year plan: **2017** – Use preliminary fruit infection protocols (field and in vitro) for collection of a first season of data. Then optimization of protocols from 2017 experiences. Begin genetic dissection of fruit resistance. **2018** – Use optimized evaluation protocols in the 2018 fruiting season on a small but efficient set of breeding program trees to validate 2017 results. Develop DNA test using the robust infection data collected over the two seasons.

Germplasm used and tree management to encourage PM pathogen growth: All evaluated trees were growing at WSU-IAREC's Roza Unit. Most experiments were conducted on the genetic stock trees ("Genetic Stocks") of the RosBREED block (C53) that represents the diversity of the breeding program in a series of families from many parents. These individuals were DNA-profiled at high resolution in the RosBREED project. Each individual is usually present as two tree replicates, usually growing side-by-side. These 335 distinct genetic stock individuals (almost 600 trees total) are maintained in eight rows with about 84 trees per row. A subset of genetic stock trees ("Segregating Families", 160 trees, 88 individuals) were chosen from among families descended from suspected resistance sources 'Moreau' (including 'Chelan', PMR-1, BB, CC, etc.) and/or Mildew-Immune

Mazzards (MIM 17 and MIM 23). These families were known to be segregating (i.e., varying distinctly among individuals) for alleles at a chromosome 5 region previously reported to be associated with foliar PM resistance. Positive controls for fruit PM infection were also evaluated: 11 cultivars (Attika, Benton, Kristin, Lambert, Rainier, Sandra Rose, Santina, Selah, Skeena, Sweetheart, and Vista) growing in the breeding program's mother block B53.

Orchard management was conducted according to WSU standards, with a few alterations to suit this year's experiment to promote powdery mildew development. This year there were no fungicide sprays or pruning, irrigation intervals were longer to raise humidity, and additional fertilizer was applied to promote terminal bud growth. Additionally, powdery mildew spores, obtained from an adjacent high-disease-pressure orchard of 'Bing' and 'Sweetheart' trees, were evenly dispersed with a mist sprayer on May 17, 24, and 31.

Foliar PM evaluation in the orchard: To gather detailed data on how early in the season individual trees became infected and to follow the progression of powdery mildew development across block C53, all Genetic Stock trees were screened for the presence of foliar powdery mildew in the lower canopy (June 6 and 28). The final evaluation was conducted on July 25, in which *foliar PM incidence* (total number of leaves infected) and *foliar PM severity* (% leaf surface area infected) was rated for both the lower and upper canopy (five shoots per height, five leaves per shoot) of each tree. Foliar disease severity was assessed by direct estimation; each leaf was assigned a severity value (ranging from 0 to 100%). (In contrast, fruit disease was evaluated with an ordinal severity scale, which is explained below). All trees rated as "zero-mildew" were revisited for a fourth time on August 4 and visually checked for signs of PM infection.

Foliar PM evaluation in the lab: The detached leaf disk assay, performed only on the Segregating Families, is a previously optimized lab-based assay. In brief, the first emergent or fully expanded leaves from a terminal shoot were collected from each tree, transported to the lab, and surface-sterilized (10% bleach for 5 minutes followed by a triple rinse with sterile distilled water). Circular disks (12 mm in diameter) were cut from leaves using a disinfected cork borer. Leaf disks were then transferred with their lower side up to 24-well tissue culture plates containing 500 µl of agar (1% w/v) per well. An assay consisted of four leaf disks from four independent leaves per tree. Susceptible leaf disks from cultivar 'Bing' were included as a positive control (inoculated) and negative control (not inoculated) in each assay. Each leaf disk (test samples and positive control) was then inoculated with a 20-µl drop of standardized spore solution (20,000 spores per mL). The solution was allowed to remain on the leaf disk for 5 minutes to allow spores to settle and make contact with the leaf surface. After 5 minutes, the liquid portion of the inoculum was removed with a sterile cotton swap because powdery mildew spores do not favor free water to germinate. Plates were sealed with parafilm to avoid humidity loss and incubated for 21 days at 20°C (14 h light period) in a plant growth chamber. A leaf disk was rated positive if a visual infection was observed at the end of the incubation period. A leaf disk was rated negative if no visual infection was apparent and no fungal structures were visible under the stereoscope. The proportion (%) surface area infected was visually estimated for each positive leaf disk sample and the positive control. To ensure these visual ratings were accurate, total DNA was extracted from the infected leaf disks for each genotype and subjected to a quantitative PCR assay that determines presence of the pathogen's DNA. The amount of DNA of each sample was compared to that of the positive control. Because visual ratings were soon found to be accurate, the quantitative PCR back-up test was only conducted for the first 50 trees tested. All assays were performed and evaluated by Ms. Neusa Guerra, the senior research technician in the cherry powdery mildew program. Ms. Guerra conducted this study with no prior knowledge of the resistance status of the trees – thus it was a blind evaluation.

Fruit PM evaluation: Fruit collection from Genetic Stock trees started on July 7, one week after the onset of fruit infection on 'Bing' and 'Sweetheart' in the adjacent high-disease-pressure orchard, and

weekly thereafter (July 12 and 19) until the end of July. At each evaluation, 20 to 50 fruit were harvested at random, transported to the laboratory, and evaluated for *fruit PM incidence* and *fruit PM severity*. Color development at time of picking was also noted. On July 19, infected fruit from eleven cultivars and five P1 seedlings were also evaluated. An ordinal rating scale of fruit infection was used. Each observed fruit was assigned to one of the following six classes: **0**: No infection; **1**: 1% fruit surface area infected; **2**: 1.1 – 10% fruit area infected; **3**: 10.1-30% fruit surface area infected; **4**: 30.1-50% fruit surface are infected; **5**: 50.1-100% fruit surface area infected (Figure 1). Disease incidence was calculated as the proportion of fruit with at least 1% infection (i.e., classes 1-5 out of classes 0-5). Disease severity (weighted average for each tree) was calculated as the weighted average of infected fruit across classes 1-5.

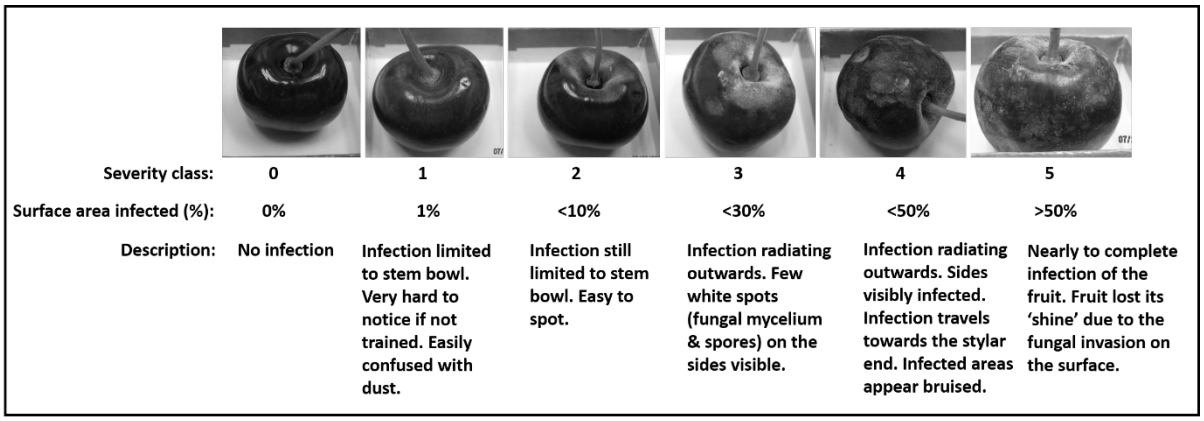

Figure 1. Rating scale for fruit powdery mildew evaluation.

Preliminary genetic dissection of resistance/susceptibility: For foliar PM, trees showing any infection, regardless of quantitative degree, were designated as "susceptible". Trees showing no infection in the field and lab assays were designated as "resistant". This binary designation was possible because of high foliar infection pressure across the block allowed few "escapes". For lab-assayed trees, cases of one replicate tree being recorded as susceptible while the other was resistant were interpreted as being mislabeling of trees or samples. Cases of replicate trees that didn't receive lab assays but were mismatched in their orchard observations were interpreted as being escapes.

For fruit PM, trees with any infection were designated as "susceptible", while trees with no infection could be resistant or could be susceptible escapes. The hypothesis that fruit resistance is under strong genetic control was tested as Genetic model 1 (expect either: replicate trees of the same individual will always have the same fruit PM phenotype, which assumes no escapes and no tree or sample mislabeling; or replicate trees will share the same fruit PM phenotype significantly more often than would occur randomly given the overall frequency of fruit infection for trees in the block, which assumes some tree or sample mislabeling is possible).

Further genetic models were tested after consolidating data across replicate trees to arrive at foliar and fruit PM resistance/susceptibility designations for each distinct germplasm individual. These phenotypic designations were compared to each individual's DNA profile, particularly focusing on a region on chromosome 5, *Pmr1*, where the genetic factor underlying the resistance of the Toyama selection PMR-1 (parent of selections and breeding parents AA, BB, CC, etc.) was previously reported to be located. Because several families included two PM-resistant parents (e.g., BB x MIM 23, EE x MIM 17, PMR-1 x 'Moreau'), totaling 50 individuals, the *Pmr1* resistance factor was inherited in seedlings of such families as 2 copies, 1 copy, or zero copies (1:2:1 Mendelian segregation). Five hypotheses were tested of genetic models for major factors underlying the genetic

control of fruit and foliar PM resistance in our available breeding germplasm. Genetic model 2 was to confirm that the resistance allele from 'Moreau'/PMR-1 is dominant for foliar resistance (expect individuals with one or two copies will be resistant to foliar PM while those with zero copies will be susceptible). Genetic model 3 was to check if the foliar resistance allele from the MIM parents is positionally and effectively the same as the 'Moreau'/PMR-1 source (expect all individuals in families with both MIM and 'Moreau'/PMR-1 parents that have zero copies of the resistance allele will be susceptible to foliar PM and all others with 1-2 copies of the resistance allele, regardless of source, will be resistant to foliar PM). Genetic model 4 checked if the fruit and foliar resistance allele are the same (expect all individuals susceptible to fruit PM will also be susceptible to foliar PM). Genetic model 5 was in contrast to model 4, to check if resistance alleles for fruit and foliar resistance are at different genomic locations and thus inherited independently (expect individuals susceptible to fruit PM will be either susceptible or resistant to foliar PM according to the overall observed frequency of those foliar infection phenotypes).

RESULTS & DISCUSSION

Summary: Resistant and susceptible breeding individuals were successfully discriminated for both fruit and foliar PM infection. Although the typically difficult-to-induce fruit infection was indeed difficult in the 2017 season, enough disease spread on fruit and leaves was achieved through the experimental block that preliminary conclusions could be made on the genetic control of fruit PM infection. These conclusions are that (1) the main genetic factor giving fruit resistance is the same as that giving foliar resistance, (2) the foliar resistance source from PMR-1 (inherited from 'Moreau', which 'Chelan' also inherited) is the same as that from the small-fruited Mildew-Immune Mazzards, (3) this resistance allele is dominant, such that plants only need to inherit one copy to be resistant to both fruit and foliar PM, and (4) seedlings and selections with genetic resistance to fruit-and-foliar PM infection are common in the PNW sweet cherry breeding program – because of extensive use of sources carrying the resistance allele for at least the last 10 years of crossing. Evaluations next season will build on experience gained this season in adequately spreading PM infection and efficiently evaluating degree of resistance. These evaluations are required to confirm the above findings on the genetics of PM resistance in sweet cherry.

Foliar PM infection, in-orchard evaluation: The first occurrence of foliar PM infection in the C53 block of genetic stocks was observed on May 31, sixteen days after the onset of disease in the adjacent high-disease pressure D51 block. Based on four independent times of foliar disease evaluation in the orchard, 135 trees representing 74 unique individuals were rated as negative (zero-mildew), while 438 trees were rated as positive for disease (25 of which had 100% incidence) and represented a block-wide infection rate of 76%. Among the positive trees, foliar disease *incidence* ranged from 2 to 100% (average 41%) of leaves infected. In two cases among the Genetic Stocks, one tree of the pair of supposedly identical genotypes had no infection observed while the other tree had low incidence and severity (rated as positive for disease susceptibility). These mismatches are assumed for now to be cases of tree mislabeling during propagation, and indicate a need to test all trees of this block for trueness-to-type. Not being true-to-type might account for a few other cases of both trees of a pair showing resistance/susceptibility opposite to that predicted for their DNA profile.

Among the positive trees for incidence, foliar disease *severity* ranged from 0.02% to 54.2% (average 5.2%) of leaf area infected by the fungus (Table 1). For several trees, infection was visible on leaves as isolated single colonies, covering about 2% of the leaf surface area, which had established but did not continue to grow during the season. Our interpretation is that these trees have an underlying resistance to powdery mildew but the fungus has developed a way to break this resistance and, over time, would be able to actively infect such trees. We found 16 pairs of trees that showed this pattern, none of which carried the major resistance genetic factor described later.

Table 1. Foliar PM severity among the breeding program-representative trees of the RosBREED genetic stock block, recorded at the Roza research orchard on July 25, 2017.

Trees	Foliar PM severity (proportion of leaf area infected by the fungus)							
	0%	**1%**	**-10%**	**-20%**	**-30%**	**-40%**	**-50%**	**>50%**
Number	135	143	223	54	10	4	3	1
Proportion	24%	25%	39%	9%	2%	1%	1%	0.2%

Foliar PM infection, in-lab evaluation: The detached leaf disk assay provided clear results for 156 trees (88 individuals) on whether their leaves were able to support pathogen growth. Quantitative PCR results detecting pathogen DNA presence confirmed the leaf disk results. The detached leaf disk assay also almost always matched information available from DNA profiling (described later), and was therefore confirmed to be robust for future phenotyping. Such phenotyping could be in the context of a research project or for routine screening of material in the breeding program in situations where such a phenotypic screen is preferred over a genotypic screen (DNA test of seedlings or trees).

Fruit PM infection, in-orchard evaluation: 265 trees showed no fruit infection – many of these probably represent escapes. Fruit infection was observed for 113 trees (30%), with fruit disease *incidence* ranging from 3 to 100% (average 29%, median 22%). Fruit disease *severity* was generally low in trees that had some infection; most infected fruit had less than 10% of their surface affected (Table 2). Higher levels of fruit incidence and severity were observed on the south side of the orchard which faces the high-disease-pressure D51 'Bing'/'Sweetheart' mildew block. Because this was the first year in which C53 did not receive mildew protection, disease pressure was generally lower than in the always-unprotected D51. For 2018 it is advised to keep C53 unprotected to increase infection in the north part of the block. For most of the cultivars used as positive controls, at least half the fruit on the trees were infected. However, the least infection at only 25% of fruit was observed for 'Lambert'. Severity ratings were between class 2 and 3 (i.e., 10 to 30% of fruit surface area infected). There were only 10 trees among the Genetic Stocks with a similar high infection severity.

Table 2. Fruit PM severity of infected fruit among the breeding program-representative trees of the RosBREED genetic stock block, recorded at the Roza research orchard in July, 2017. Note that fruit in the 0 severity class do not factor into the weighted average.

Trees	Fruit PM severity (weighted average of 1-5 severity classes)					
	0	**1**	**2**	**3**	**4**	**5**
Number	265	45	58	9	1	0
Proportion	70%	12%	15%	2%	0.3%	0%

Genetic dissection of fruit and foliar PM resistance: Results from 2017 were consistent with fruit and foliar PM resistance being the same genetic mechanism. Other major findings were that fruit and foliar PM resistance is under strong genetic control, the PMR-1 source of fruit and foliar resistance is probably the same as that from the Mildew-Immune Mazzards (MIMs), and resistance is contributed by a dominant allele from all of these sources.

A strong signal of genetic resistance to foliar PM was observed and was inherited from suspected sources. Of the 331 orchard-phenotyped individuals in the Genetic Stock block, 78 (23%) were designated as resistant to foliar PM and 254 (77%) as susceptible. Mislabeling of trees or samples was identified in 3 cases (3% of lab-assayed individuals). Escapes were identified in 15 cases

(8% of pairs of individuals orchard-phenotyped). Therefore, the total error rate of the dataset for foliar PM was estimated to be around 10%. All resistant individuals were in families descended from 'Moreau'/PMR-1 or MIMs except for six cases out of 78, which was within the phenotyping error rate and so might represent escapes or mislabeling (but they will be examined closely next season to see if they instead represent one or more new genetic sources of resistance).

A strong signal of genetic resistance to fruit PM was observed. Of the 228 individuals in the Genetic Stock block orchard-phenotyped for fruit PM, 145 (64%) had no recorded fruit infection (=resistant or escapes) and the other 83 (36%) were designated as susceptible to fruit PM. Escapes were identified in 32 cases (8% of pairs of individuals phenotyped only in the orchard). Out of 134 individuals for which replicate trees were phenotyped for fruit PM, 32 (24%) did not match between the replicates, which was interpreted as the escape rate for fruit PM. Combined with tree mislabeling errors, the total error rate of the dataset for fruit PM was estimated to be around 25%. Given this error rate, only part (b) of Genetic model 1 could be tested. Pairs of replicate trees for each individual were much more likely to have the same fruit PM phenotype than to be mismatched; Chi-square analysis determined that the observations were not consistent with random infection ($p<0.001$).

The *Pmr1* resistance allele for foliar PM in 'Moreau'/PMR-1 was confirmed to be dominant and resistance in MIMs was determined to also be the *Pmr1* allele. Testing of Genetic models 2 and 3 found that individuals with 1-2 copies of the resistance allele from either source were almost always phenotypically resistant to foliar PM, while individuals with zero copies of the resistance allele were almost always susceptible. Therefore, the MIM and Moreau resistance alleles are located at the same genomic region (*Pmr1*), are both dominant in their effect of imparting foliar PM resistance, and can be considered as equivalent. (The few discrepancies are assumed for now to represent tree mislabeling or data-handling errors but will be closely examined in 2018.)

The resistance allele for fruit PM was determined to be the same as the *Pmr1* foliar resistance allele, and regardless of germplasm source. Of the 82 individuals susceptible to fruit PM, 80 were also susceptible to foliar PM (Table 3). Thus, Genetic model 4 was supported. (The two discrepant individuals are assumed for now to represent errors in data-handling but will be carefully examined in 2018.) Genetic model 5, independence of foliar and fruit resistance, was not supported (Chi-square analysis: observations not consistent with random infection, $p<0.001$). If the two were independent, 63 of the 82 fruit-susceptible individuals would be expected to be susceptible and 19 resistant.

Table 3. Association of fruit PM resistance with foliar PM resistance.

Foliar PM phenotype	Number of individuals observed with fruit PM phenotype		
	Resistant/Escape	Susceptible	All
Resistant	28	2	30
Susceptible	116	80	196
All	144	82	226

Other resistance sources: Beyond the *Pmr1* genetic resistance factor, other sources of resistance might exist in the material tested. Some cases of zero-mildew (both fruit and foliar) were observed in trees not carrying a *Pmr1* resistance allele. 'Regina' was a parent of some of these trees (three cases) as was 'Venus' (two cases). Some families had no fruit incidence and low foliar incidence, for which 'Regina' and 'Venus' were commonly the parents. With the current dataset, distinction is difficult between susceptible trees that escaped fruit infection and true resistance; likewise it is difficult to identify genetic factors underlying degrees of fruit infection (as opposed to the clear-cut resistant vs. susceptible for presence vs. absence of the *Pmr1* resistance allele). Such dissection will require the second season of evaluation in 2018, with a focus on particular families, inclusion of a wide susceptibility range of cultivars and breeding parents, and increased fruit infection across the block.

CONTINUING PROJECT REPORT **YEAR**: 3 of 3 (3rd year report)

Project Title: Managing acclimation, hardiness and bacterial canker of sweet cherry

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Cooperators: Grower: Stacey Cooper (The Dalles trial), Brad Fowler (Hood River trials)

Total Project Request: **Year 1:** $43,657 **Year 2:** $43,820 **Year 3:** $44,503

Other funding sources
None

Budget 1: Hubbard
Organization Name: OSU-MCAREC **Contract Administrator: L.J. Koong**
Telephone: 541 737-4866 **Email address: l.j.koong@oregonstate.edu**

Item	2015	2016	2017	2018
Salaries[1]	19,750	20,343	20,953	
Benefits[2]	10,107	10,177	10,250	
Wages				
Benefits				
Equipment				
Supplies[3]	8,500	8,000	8,000	
Travel[4]	1,800	1,800	1,800	
Miscellaneous [5]	3,300	3,300	3,300	
Plot Fees[6]	200	200	200	
Total	43,657	43,820	44,503	0

Footnotes: [1]Salary is for graduate student (D. Hubbard) at 0.25FTE and postdoc at 0.2FTE. A 3% increase is factored into years 2 and 3; [2] Benefits are based on a graduate student static cost and the actuals of a postdoc rate; [3]supplies include lab consumables, nursery stock & supplies and several chest freezers and rates for microscopy lab use at OSU-Corvallis; [4]travel is for # trips to Corvallis at 0.565 cents per mile and travel to research plots in The Dalles; [5]shipping and nutrient analysis (factor $25/ship date for shipping fees and $12/sample x # of samples per date); [6]greenhouse space at 0.21 cents/sqft/mo and cold room space at 0.94 cents/sqft/mo

Objectives:

1. Examine the role of acclimation and induced early winter damage on infection by *Pseudomonas syringe* pv *syringe* (*Pss*) and subsequent bacterial canker formation.

2. Determine the location of epiphytic populations & infection points of *Pss* on sweet cherry tissues using microscopy techniques.

3. Evaluate commercial & experimental plant growth regulators for their ability to induce defoliation and increased cold hardiness.

4. Evaluate the effects of defoliating compounds on nutrient remobilization and tissue content during dormancy and early spring development.

Significant Findings:

Objective 1

- Regrowth of plant tissue subjected to varying freezing temperatures showed only a slight reduction in overall growth in inoculated treatments for both the natural and artificially acclimated plant tissue.
- Inoculation with incubation 24 hours prior to freezing does not appear to be an efficient method of delivery for disease development.

Objective 3

- All defoliation treatments were efficient at abscising leaves, though only on a single sampling date was an increase in acclimation observed.
- A reduced rate of ABA applied multiple times gave compelling evidence of both enhancing remobilization of nutrients and defoliating trees several weeks ahead of the control.

Objective 4

- Leaves showed significant Nitrogen remobilization from one of the treatments, while the rest had little to no time for sufficient reuptake
- Zinc and Boron showed rapid remobilization in all treatments, with the exception of lime sulfur which burned the leaves far too quickly.

Objective 1

2015: Gisela 6 rootstocks were received from North American Plants (NAP) in McMinnville, OR in late August. Plants were roughly 9 inches tall. These plants were segregated by the following acclimation treatments: 1) Naturally acclimated under ambient, outdoor conditions 2) Non-acclimated in a greenhouse (75^{o}F daytime and 60^{o}F nighttime) and 3) artificially induced to acclimate by exposing plants to low night time temperatures within a cold storage unit and moved outdoors during the day. After sufficient cooling was achieved, plants were again divided equally into inoculated & non-inoculated treatments. Inoculations were carried out prior to exposure to freezing. A suspension (3.1×10^{8}) of a local *Pss* isolate was applied to run-off and bagged immediately to maintain high humidity and held at ambient temperature (68^{o}F) for 24 hrs. Tissue washes conducted on plants after incubation showed an average recoverable *Pss* population of 1.4×10^{7}. Based on direct measurements, we identified sub-freezing temperatures that generated an increasing level of tissue injury until the kill points were reached for each of the three acclimation treatments (Table 1).

Table 1. Test temperatures to achieve similar freeze damage for each of three different acclimation levels (°C)

	Acclimation level		
	Non-Acclimated	Artificially Acclimated	Naturally Acclimated
UTC	---	---	---
Temp 1	-2°	-4°	-4°
Temp 2	-6°	-8°	-8°
Temp 3	-8°	-15°	-13°
Temp 4	-12°	-17°	-15°

Freeze runs with rootstocks began daily on 1 November with 2 reps of each of the 30 treatments per day. To accommodate all treatment x replicate combinations required 4 days of freezing. We segregated the inoculated and non-inoculated populations between two identical programmable freeze chambers in order to minimize transfer of bacteria between treatments. The temperature was reduced at a rate of 1°C per hour to better simulate natural freeze events. These plants were removed from the chambers after a minimum of 1 hr exposure to the designated temperatures. Once these plants were removed, they were held in isolated growth chambers at 60°F until the 4 days of freezer runs were complete. These plants were then held in a walk-in cooler at 34°F for one week before temperatures were reduced to 32°F for the remainder of the winter. Plants were removed from the walk-in on 15-April and allowed to break bud and grow in a controlled climate greenhouse for the 2016 season. Plants were measured upon removal and then again when growth had terminated in September 2016.

Growth data showed a slight reduction in growth of inoculated compared to non-inoculated in both the natural & artificially acclimated tissues, but puzzlingly, not in the non-acclimated treatment (Figure 1). However, these data do not account for buds that did not break and grow shoots in the spring due to tissue death (which was markedly more pronounced as temperatures decreased). These data, therefore, will be re-analyzed to capture this effect. Additionally, disease symptoms did not appear to develop over the 2016 growing season, which requires modification of our inoculation protocol for fall 2016/winter 2017. We intended on conducing additional freeze runs during the month of November, but due to equipment malfunction, heat was lost in the greenhouse and all non-acclimated tissue was lost due to exposure to multiple days of low temperatures (i.e., acclimation).

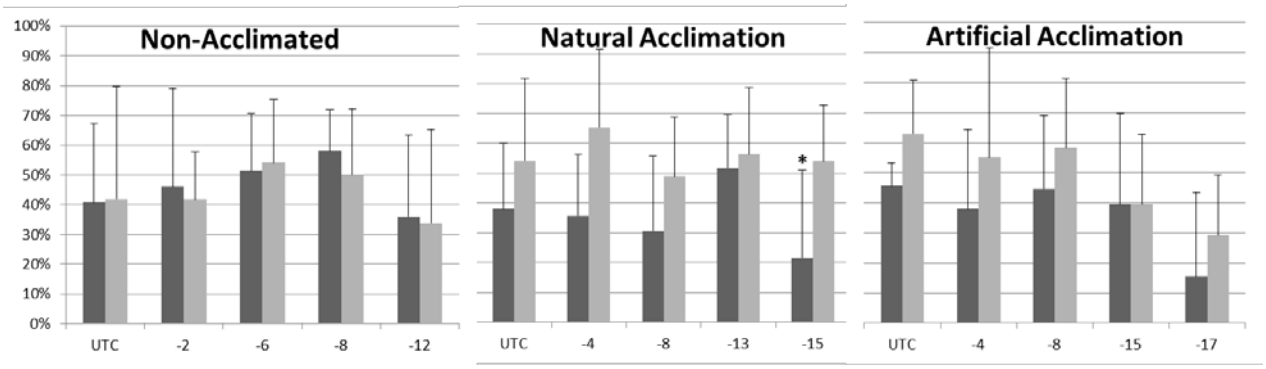

Figure 1. Percentage of relative regrowth of Gisela 6 rootstock of 3 levels of acclimation after being subjected to differing injurious temperatures. Dark bars represent inoculated treatments, while light bars are non-inoculated. Lines are top of bars are ± one standard deviation. * represents significance (P = 0.05)

2016: Mazzard rootstocks, rather than Gisela, from NAP in early September. These plants have not completed their growth for the season, and are presently being hardened off. Several artificial freeze tests are planned for this season to evaluate the role of acclimation and non-acclimation on freeze injury with and without inoculation. Modifications to the inoculation procedure will also be tested this year. The freeze procedure will remain in place with additional temperature mapping of the freeze chamber to account of variability within the unit. Heating equipment in the Greenhouse has

been restored to working order and will be monitored to ensure that the environment remains controlled. This experiment was repeated 4 times over the course of 3 weeks.

Plants were frozen at temperatures noted prior, then inoculated following a 24hr incubation period, bagged for humidity in a growth chamber at 50°F. These were held on a day/cycle for 96hrs, then placed into a cold storage room at 32°F. During late winter, the defroster unit malfunctioned for some period of time (sending the cold room between 32°F and 65°F several times a day), desiccating the tissue to a point of mortality. Once this was discovered, plants were promptly removed and placed into the greenhouse to regrow, but the drought injury was severe enough, no meaningful data could be recovered.

2017: For fall 2017 experiments, Gisela rootstocks were acquired from NAP in August due to availability. These plants are currently being regrown after a severe powdery mildew outbreak immediately following arrival at MCAREC defoliated many of them. Once growth has terminated, they will be graded and placed into either a natural acclimation state (a covered, unheated structure), a growth chamber to be acclimated systematically or left in the greenhouse at an ambient temperature of ~65°F. Due to the loss of the 2016 season, the same experiments will be replicated again 3-4 times in order to correctly capture the relationship between acclimation, freeze injury and *Pss* infection

Objective 2

Due to the lack of disease development in plants from objective 1, tissue immersed in fixative was not assessed via SEM as locations of damage were unknown. This year, tissue, once hardened off, will be subjected to artificial freeze assays without inoculum and inspected with a light microscope to better understand locations & signs of damage. Once these locales are identified, tissue from these areas will be fixed and saved for SEM inspection over the winter of 2017. From what little disease symptoms did develop on 2015 tissue, it was far too general to isolate specific areas for microscopy. Similar results were seen in 2016, leading us to abandon this objective. Leftover funding will be returned to OSCC following the completion of existing objectives.

Objective 3

2015: In a grower collaborator orchard, treatments of elemental (lime sulfur) or commercial & experimental plant growth regulators (ABA and ACC) were evaluated for their ability to induce early defoliation and cold hardiness. Defoliation efficiency was examined objectively as the percentage of leaves to senesce and abscise (4 shoots per rep). ABA and lime sulfur applications were made on 7 October followed by ACC applications on 21 October. All PGR treatments included 0.1% Simulaid. Overall, all treatments significantly sped up the process of defoliation, whether it be by chemically burning leaves (Lime Sulfur) or seemingly, by increasing the rate of natural abscission (Table 2).

Table 2. Defoliation efficiency of chemical compounds over 6 weeks beginning 14 days post application in 2015

		Evaluation Date						
Treatments	Rate	10/20	10/27	11/3	11/12	11/15	11/23	12/1
UTC	---	4% a[#]	7% b	8% c	10% d	37% c	56% c	100% a
ABA	500ppm	22% a	41% a	52% b	59% c	74% b	85% b	100% a
ABA	1000ppm	23% a	64% a	71% ab	75% b	84% ab	96% ab	100% a
ABA then ACC	500ppm 500ppm	19% a	48% a	77% ab	87% ab	97% a	98% a	100% a
ABA then ACC	1000ppm 1000ppm	20% a	53% a	99% a	100% a	100% a	100% a	100% a
ACC	500ppm	8% a	10% a	78% ab	93% ab	100% a	100% a	100% a
Lime Sulfur	10% (v/v)	21% a	79% a	85% a	88% ab	92% a	92% a	100% a

Means within a column followed by the same letter do not differ significantly (*P*=0.05) based on significant difference

Flower buds of the aforementioned treatments were evaluated for their hardiness by differential thermal analysis (DTA). DTA detects freeze events (i.e., exotherms) that signify flower death. Buds were evaluated biweekly beginning prior to applications, at which time no exotherms were detectable (implying that flowers were not acclimated). Exotherms were observed 3 weeks after the initial applications. An increase in the number of exotherms was seen for all treatments with subsequent sampling dates (Table 3). Despite numerical differences in the percentage of kill points observed among treatments, high variation led to insignificant differences among treatments. The first frost event of the fall occurred 4 November.

Table 3. Percentage of recoverable flower exotherm peaks on a series of evaluation dates

		Evaluation Date			
Treatments	Rate	10/28/15	11/11/15	11/25/15	1/8/16
UTC	---	10.86%	11.51%	86.18%	100.00%
ABA	500ppm	18.21%	20.92%	89.67%	100.00%
ABA	1000ppm	37.50%	17.43%	88.16%	100.00%
ABA then ACC	500ppm 500ppm	29.89%	36.68%	94.02%	100.00%
ABA then ACC	1000ppm 1000ppm	39.02%	39.63%	100.00%	100.00%
ACC	500ppm	26.69%	55.83%	92.64%	100.00%
Lime Sulfur	10% (v/v)	57.41%	42.90%	84.57%	100.00%

2016: Beginning on 6 October, initial samples were taken with applications of ABA & Lime Sulfur subsequently following. Treatments were altered for 2016, including multiple applications of lowered rates of ABA to address the short-lived nature of the molecule *in vivo*. Shoots were marked and counted similar to 2015 (Table 4). Contrary to 2015's trial, floral peaks could be found on the initial DTA assays. This occurrence of peaks so much earlier than 2015 did not allow the testing of floral buds to confirm they are expiring in the mass ice nucleation mentioned prior. Following similar protocols as mentioned in 2015, spur samples were taken weekly and analyzed via DTA. Detection of peaks is represented similar to last year with some modifications of treatments (Table 4). Similar results to prior years regarding the high variation of detectable peaks and a relatively stable LT50 of buds across sampling dates, insignificant differences were found across treatments. Possible explanations for the stable LT50's could be due to a constant temperature between transportation and subsequent processing of buds prior to being placed in the freezer units.

Table 4. Defoliation efficiency of chemical compounds over 4 weeks beginning 12 days post application in 2016

		Evaluation Date								
Treatments	Rate	10/18	10/21	10/25	10/28	11/4	11/11	11/15	11/18	11/21
UTC	---	2% a[#]	3% a	3% a	4% a	6% a	18% a	47% a	71% a	96% a
ABA (x3)	500ppm	9% a	21% ab	28% ab	44% bc	55% b	87% b	97% b	100% b	100% a
ABA (x3)	250ppm	10% a	32% ab	45% b	63% c	73% bc	94% b	97% b	100% b	100% a
ABA then ACC	500ppm 1000ppm	5% a	51% a	75% c	85% d	86% b	91% b	98% b	98% b	96% a
ABA then ACC	1000ppm 1000ppm	20% a	67% b	73% c	89% d	92% b	98% b	100% b	100% b	100% a
ACC	1000ppm	5% a	8% a	23% a	57% b	68% bc	90% b	99% b	100% b	98% a
Lime Sulfur	10% (v/v)	50% b	58% c	71% c	81% d	82% b	88% b	93% b	94% b	97% a

Means within a column followed by the same letter do not differ significantly (P=0.05) based on significant difference

Table 4. Percentage of recoverable flower exotherm peaks on a series of evaluation dates

		Evaluation Date					
Treatments	Rate	10/06/16	10/14/16	10/20/16	10/27/16	11/03/16	11/18/16
UTC	---	48.15%	22.78%	100.00%	100.00%	94.44%	100.00%
ABA (x3)	500ppm	29.44%	54.07%	98.89%	100.00%	100.00%	100.00%
ABA (x3)	250ppm	27.41%	42.78%	100.00%	100.00%	91.11%	100.00%
ABA then ACC	500ppm 1000ppm	12.78%	50.00%	88.33%	100.00%	100.00%	100.00%
ABA then ACC	1000ppm 1000ppm	43.33%	51.11%	100.00%	96.11%	100.00%	100.00%
ACC	1000ppm	37.22%	30.00%	94.44%	100.00%	85.00%	100.00%
Lime Sulfur	10% (v/v)	43.33%	52.22%	87.22%	89.44%	87.78%	100.00%

2017: Following promising results from the nutrient remobilization and defoliation data of 2016, treatments were altered for the current year. Treatments have also been replicated in 2 more cherry blocks and in a potted tree experiment, all 3 of which are in the Hood River Valley, in addition to the mature 'sweetheart' block used in The Dalles, OR. Two commercial 'sweetheart' cherry blocks roughly 4 miles SE of Odell, OR were chosen based on late harvest dates and age of blocks, one is young, but of bearing age and the other is still immature and vegetative. The young potted 'Bing' trees are also being used. Treatments on the three new locations are Multiple applications of 250ppm ABA, 1000ppm ACC and a solution of 10% Lime Sulfur. The 2 commercial blocks will be evaluated for defoliation rate and potential winter damage from freeze events next spring. The young potted trees will also receive these treatments in addition to 2 chemical treatments for *Pss* before and after inoculation occurs in late October.

Initial sampling made 28 September in the Dalles trial and no peaks were present. The following sampling date, 5 October, did have peaks present. The following day, buds from the same orchard were run again with the experiment ending shortly before peaks had been seen the day prior. These buds were dissected and visually inspected for ovary mortality and it was found ~95% of these were dead following the mass ice nucleation seen, answering the question of where the "invisible peaks" were in prior experiments.

Objective 4

2015: Tissues were dissected into leaf, bud & spur as sampling occurred. These tissues have been sent to the lab for analysis following the decision to continue with the project, but due to a long queue, results have not been received.

2016: As stated above, all tissue was dissected into separate parts and being dried in ovens to eliminate excess water. The tissue from 2016 were submitted and returned and subsequently analyzed. Remobilization charts were built to understand the rate of remobilization.. Nitrogen appears to be the most limiting mineral for remobilization as it appears to be a slow process (Figures 1). Although the actual percentage removed from the leaves appears to be large, the actual nitrogen found in bud & spur tissue appears to be similar, except for the lime sulfur treatment which, again, burned the foliage far too quickly to leave much viable tissue intact. The Zinc and Boron appeared to remobilize quickly once signaled from PGR treatments.

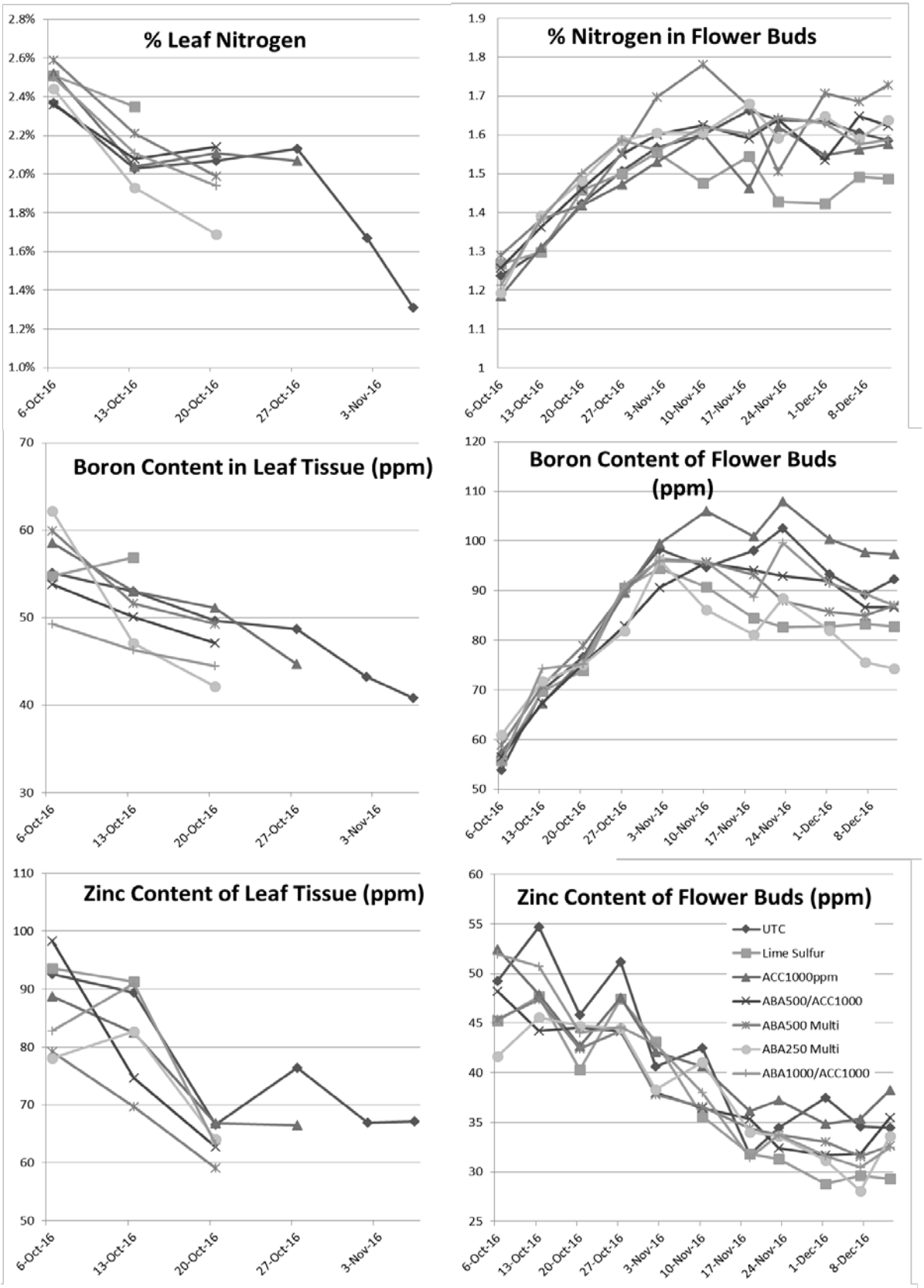

Figure 1. Nutrient levels in various 'Sweetheart' Sweet Cherry tissues from Fall 2016 (from left to right). Percent leaf Nitrogen. Percent Nitrogen content in flower buds. Leaf Boron content in leaf tissue in parts per million (ppm). Boron content (ppm) in flower buds. Leaf Zlnc content (ppm) in leaf tissue. Zinc content (ppm) in flower buds. Treatment key in bottom left figure

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: CH-16-102

Project Title: Integrated pest management of spotted wing drosophila in sweet cherry

PI: Elizabeth H. Beers
Organization: WSU-TFREC
Telephone: 509-663-8181 ext. 234
Email: ebeers@wsu.edu
Address: WSU-TFREC
Address 2: 1100 N. Western Ave.
City/State/Zip: Wenatchee, WA 98801

Total Project Request: Year 1: $85,424 Year 2: $57,932 **Year 3: $60,064**

Other funding sources

Agency Name: **WSDA SCBG**
Amt. awarded: $237,908 (September 30, 2013 - September 29, 2016)
Notes: Research Intern and time slip is paid from this grant through Sept. 29, 2016; hence the WA request for 2016 is limited to 6 months for these two budget items.
Previous WTFRC SWD project for Beers was used as match for SCBG.

WTFRC Collaborative Expenses: None

Budget 1
Organization Name: WSU-TFREC **Contract Administrator:** Katy Roberts/J. Cartwright
Telephone: 509-335-2885/ 509-663-8181 **Email:** arcgrants@wsu.edu/joni.cartwright@wsu.edu

Item	2016	**2017**	**2018**
Salaries[1]	16,042	32,085	**33,368**
Benefits[2]	6,192	12,385	**12,880**
Wages[3]	4,922	8,364	**8,699**
Benefits[4]	118	448	**467**
Equipment			
Supplies[5]	5,000	1,000	**1,000**
Travel[6]	1,150	1,150	**1,150**
Miscellaneous			
Plot Fees[7]	2,500	2,500	**2,500**
Total	$35,924	$57,932	**$60,064**

Footnotes: [1]Salaries 0.60 FTE Research Intern, [2]Benefits, Research Intern 38.6%; [3]Wages, student (summer) @$20.51/hr, 20 hrs/week x 12 weeks; Research Assistant $12/hr x 20 hr/week x 13 weeks; [4] Benefits student 2.4%, Research Assistant 10%; [5] Five whole tree cages @$800/cage (2016 only), SWD rearing supplies, traps and lures; [6]Travel to plots, $0.54/mile x 100 miles/year; [7]Plot fees. $1,000/acre x 2.5 acres for Sunrise 4 and Columbia View 14.

Budget 2 (PW Shearer)
Organization Name: OSU MCAREC **Contract Administrator:** Russ Karow
Telephone: 541-737-4066 **Email address:** Russell.Karow@oregonstate.edu

Item	2016	2017	**2018**
Salaries[1]			
Benefits[2]			
Wages	31,320		
Benefits	10,187		
Equipment			
Supplies[3]	3,411		
Travel[4]	1,582		
Plot Fees	3,000		
Miscellaneous			
Total	49,500		

Footnotes:
[1]Two Temp BSRT1, 6 mo ea, $15/hr, 3% raise each year
[2]Benefits: $850/mo
[3]Traps, lures, sampling equipment, insect rearing
[4]In state travel to research sites, $0.575/mile

Objectives:

1. Test chemical control products to determine their ability to prevent infestation (years 1-3).
2. Test chemical control products to determine ability to kill early stages of SWD in fruit (years 1-3)
3. Test provisional spray thresholds to determine initial and subsequent spray timing in commercial orchards (years 2-3).

Significant Findings:

- Entrust provided long-term (up to 21 days) of mortality of SWD, and good fruit protection relative to Cormoran
- Entrust mixed with an attracticide caused similar levels of mortality as GF-120, although activity was slower. Residual activity was greatly reduced relative to GF-120 or Entrust applied with an airblast sprayer.
- Ecotrol reduced ovipositions by 2.6-fold relative to the check, and methyl benzoate+oil reduced ovipositions by 5.3-fold.
- The new formulation of Suzukii Trap bait did not capture more SWD than the old formulation at low densities, and was less selective. Both Dros'Attract and Suzukii Trap baits are more selective for SWD (versus other Drosophila) than the Scentry lure, but SWD capture is lower.

Method - Laboratory Bioassays:
The addition of an attracticide to spinsoad (Entrust SC) was tested in two laboratory bioassays for enhancing the speed of kill of female SWD. The attracticide mixture was compared to GF-120, a pre-mix of an attractant plus a toxicant. The test compounds were mixed with the equivalent rate of spinosad (Entrust) as that in GF-120. The bioassay was conducted in 1-qt plastic containers lined with untreated cherry leaves. The leaves were stapled to the inside of the arena, and 5 droplets of the attracticide plus toxicant, attracticide alone (to determine if the attracticide itself was toxic), and GF-120 were pipetted onto the leaves (Plate 1). The arenas were provisioned with *Drosophila* medium to ensure fly survival in the checks. After adding the droplets to the arena, 10 seven-day-old female SWD were transferred to the arena. Mortality was assessed at 6, 24 and 48 hours after treatment (HAT).

Plate 1. SWD bioassay arena with untreated cherry leaves, 2017

Results: In both bioassays, mortality was low at 6 HAT, but improved at 24 and 48 HAT for all of the treatments including a toxicant (Figs. 1, 2). In the preliminary bioassay, the GF-120 treatment has 100% mortality at 24 h, but the attracticide+Entrust did not reach that level until 48 h (Fig. 1). The attracticide alone was never significantly different than the check. This preliminary test indicated that this approach may be feasible. The second bioassay compared two formulations of the attracticide to GF-120 (Fig. 2). The two formulations performed similarly, but both had slower kill at 24 h than GF-120. After 48 h, mortality levels were similar in the three treatments. These tests provide additional

evidence that an attracticide may provide a useful level of suppression, but will not suffice for stand-alone control. The benefit of having an attracticide separate from the toxicant is that materials may be selected for faster kill, or rotated for resistance management purposes.

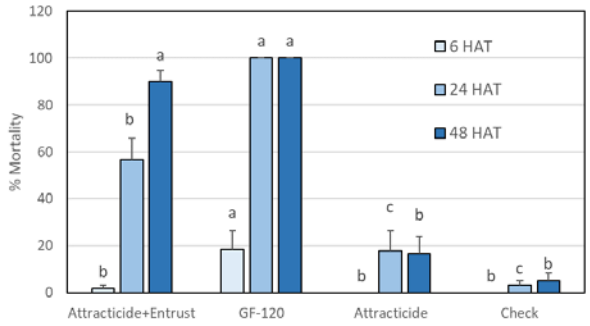

Fig. 1. SWD attracticide bioassay, single attracticide, 2017

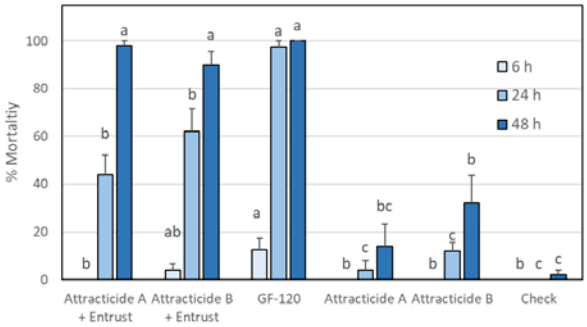

Fig. 2. SWD attracticide bioassay, two formulations, 2017

Method - Field-aged reside bioassay of attracticides: The laboratory bioassay of attracticides was followed up with a test of residues under field conditions, to determine how long they are effective. The bioassay method was similar to the one described above, except that the droplets were applied to cherry leaves in an orchard, and brought in at intervals after treatment (0, 1, 3, 7, and 14 days after treatment or DAT) to determine length of residual activity. In additon, untreated cherry fruits were added to the arenas as an oviposition substrate to determine if the attracticides could provide fruit protection. The attracticide treatments were compared to Entrust applied airblast and an untreated check.

The cherry leaves with aged droplets were retuned to the laboratory at the specified post-treatment interval, and stapled to the arenas as described above. The stems of untreated cherries (5 per arena) were suspended from the lid of the arena to allow females access to all surfaces for oviposition (Plate 2). Flies were transferred to the arenas, and allowed to feed and oviposit. The lid with suspended fruit was removed after 16 h, and replaced with a plain lid. The oviposition punctures on the fruit were counted and recorded, and the lid transferred to a plain container to allow larval development and adult emergence. These were held at 22 °C (72 °F) for 16 days, and the resulting male or female SWD counted and recorded. In the treated arena, fly mortality was recorded after 6, 24 and 48 h (the first evaluation was done with cherries still present, the latter two after their removal).

Plate 2. SWD bioassay arena with untreated cherries suspended by their stems, 2017

Results: As in the lab-only bioassay, mortality was very low after 6 h, and improved at the 24 and 48 h evaluations (Table 1). Unfortunately, this allowed sufficent time for the females to oviposit in the cherries. In most evaluations, the ovipostion rate and adult emergence in the attracticide treatments was not significantly different than the check. However, the GF-120 and Entrust treatments consistently provided higher levels of mortality for an extended period of time.

The artificial spatial scale of the arenas forces the flies into close contact with the fruit for the duration of the bioassay, whereas in field situations their would be movement in and out of the

canopy, with peaks of activity at dawn and dusk. This may allow more exposure to a toxicant before seeking an oviposition site, thus the attack rate is likely overestimated in this bioassay.

Table 1. SWD field-aged residue study of attracticides, 2017

DAT	Trt	n	% Mortality (6 h)	% Mortality (24 h)	% Mortality (48 h)	Oviposition s/ 5 fruits	Adults emerged	Adults/ oviposition
0	1.Attracticide A+Entrust	6	1.67 a	8.33 c	55.00 b	8.83 a	5.83 a	0.89 a
0	2.Attracticide B+Entrust	6	1.67 a	40.00 b	85.00 a	14.00 a	5.67 a	0.54 a
0	3.GF-120	6	8.33 a	86.67 a	100.00 a	5.17 a	1.17 a	0.09 a
0	4.Spinosad	6	0.00 a	70.00 a	100.00 a	10.17 a	3.83 a	0.52 a
0	5.Check	6	0.00 a	10.00 c	26.67 c	21.33 a	5.00 a	0.25 a
1	1.Attracticide A+Entrust	6	0.00 a	3.33 c	6.67 c	35.17 a	12.83 a	0.46 a
1	2.Attracticide B+Entrust	6	1.67 a	28.33 b	73.33 b	21.00 a	13.33 a	0.66 a
1	3.GF-120	6	1.67 a	81.67 a	100.00 a	32.50 a	9.33 a	0.37 a
1	4.Spinosad	6	0.00 a	91.67 a	100.00 a	17.17 a	9.17 a	0.64 a
1	5.Check	6	0.00 a	0.00 c	0.00 c	41.50 a	19.83 a	0.51 a
3	1.Attracticide A+Entrust	6	0	1.67 b	55.00 b	38.67 a	10.50 a	0.24 b
3	2.Attracticide B+Entrust	6	0	11.67 b	58.33 b	38.33 a	15.83 a	0.46 ab
3	3.GF-120	6	0	21.67 b	86.67 a	20.67 a	10.83 a	0.49 ab
3	4.Spinosad	6	0	90.00 a	100.00 a	13.17 a	8.33 a	0.65 a
3	5.Check	6	0	8.33 b	16.67 c	42.83 a	17.17 a	0.36 ab
7	1.Attracticide A+Entrust	6	0	1.67 c	3.33 c	39.50 a	18.33 a	0.53 b
7	2.Attracticide B+Entrust	6	0	0.00 c	25.00 b	20.33 ab	15.17 a	0.81 ab
7	3.GF-120	6	0	35.00 b	96.67 a	21.00 ab	14.33 a	0.64 b
7	4.Spinosad	6	0	61.67 a	95.00 a	25.83 ab	9.67 a	0.54 b
7	5.Check	6	0	3.33 c	13.33 bc	5.17 b	4.67 a	1.27 a
14	1.Attracticide A+Entrust	6	0	18.33 a	23.33 a	51.00 ab	21.17 ab	0.46 a
14	2.Attracticide B+Entrust	6	0	8.33 a	30.00 a	77.33 a	24.17 a	0.36 a
14	3.GF-120	6	0	15.00 a	40.00 a	20.67 b	8.17 b	0.55 a
14	4.Spinosad	6	0	31.67 a	53.33 a	22.33 b	10.67 ab	0.61 a
14	5.Check	6	0	10.00 a	13.33 a	40.17 ab	15.83 ab	0.37 a

MIXED for CRD, lsmeans adj=Tukey, no trans

Method - Field-aged reside bioassay of conventional insecticides: A bioassay of two conventional insecticides was conducted to determine the length of residual control. Two rates of Cormoran (a mixture of novaluron and acetmiprid) were compared to Entrust and an untreated check. Treatments were applied with an airblast sprayer to a research cherry block (SRO4, 'Sweetheart') and the residues challenged at intervals with a laboratory colony of SWD. The arena and procedures were the same used for the attracticide field-aged bioassay.

Results: Entrust caused consistently high levels of mortality through 21 DAT. The two rates of Cormoran varied between about 40-80% mortality during the same period (Fig. 3). Because one of the components is an insect growth regulator (IGR), other modes of action may enhance activity over mortality alone. Oviposition deterrence (Fig. 4) and prevention of egg or larval development (as evidenced by adult emergence, Fig. 5) are two possible modes of action. However, there were only two instances (1 and 14 DAT) where ovipositions in the Cormoran treatments were significantly lower than the check, and only one instance (14 DAT) where the number of emerged flies was reduced relative to the check.

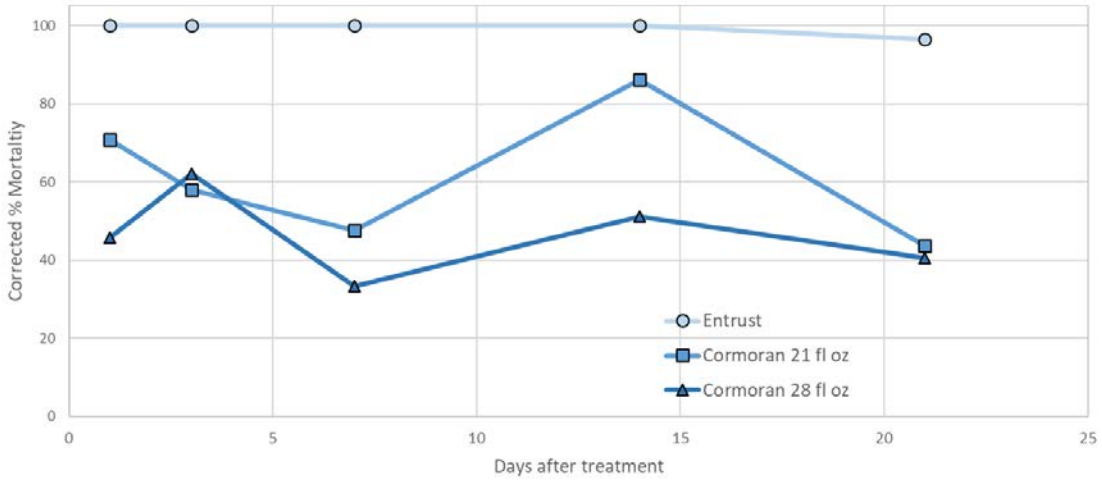

Fig. 3. Mortality caused by field-aged residues on cherry leaves and fruit, 2017

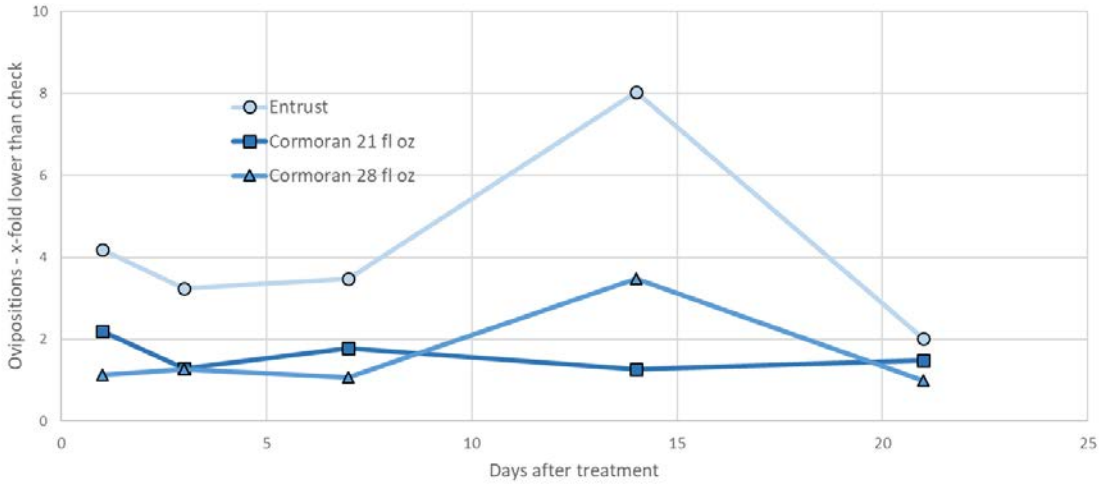

Fig. 4. Oviposition deterrence caused by field-aged residues on cherry leaves and fruit, 2017

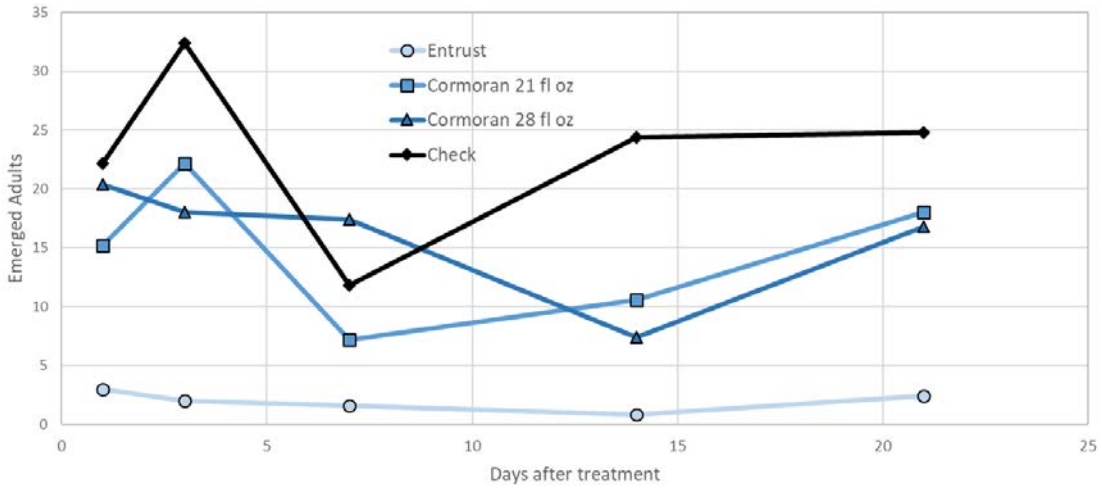

Fig. 5. Adult emergence by field-aged residues on cherry leaves and fruit, 2017

Method - Oviposition deterrence: A series of bioassays was conducted to determine if various types of compounds (mineral and essential oil based) would deter oviposition by SWD. The same bioassay arena described above was used, lined with untreated leaves. The fruit were sprayed with a laboratory atomizer, rotating the fruit in four cardinal directions to obtain thorough coverage. Ten female SWD from a laboratory colony were transferred to the arena and allowed to oviposit for 24 h. The fruit were removed, and ovipositions recorded.

Results: The first bioassay tested two essential oils and two pesticides derived from essential oils. While all treatment means of ovipositions were numerically lower than the check, only Ecotrol 0.5% was significantly lower (Fig. 6), resulting in 2.6 fewer ovipositions compared to the check. The second bioassay tested horticultural mineral oil (HMO) and Cinnerate, whose active ingredient is cinnamon oil. None of the treatments reduced ovipostions relative to the check (data not shown), despite previous work that demonstrated deterrency of HMO. The third bioassay tested laboratory grade essential oils (methyl benzoate, a fruit odor, and allyl sulphide, a garlic odor) and compared them to Stylet oil and a supreme-type 440 oil. Methyl benzoate+oil (used as an emulsifier) resulted in 5.3-fold fewer ovipositions than the check (Fig. 7).

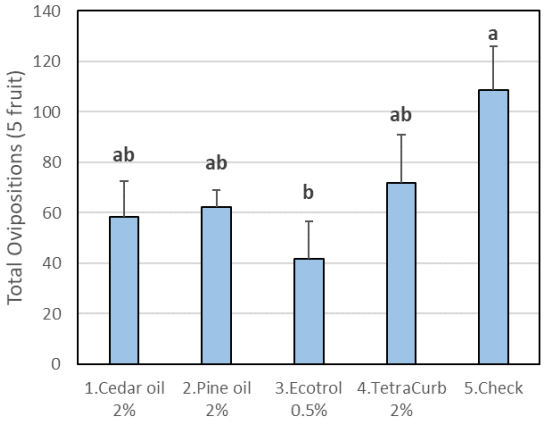

Fig. 6. SWD oviposition deterrence by oil based compounds, 2017

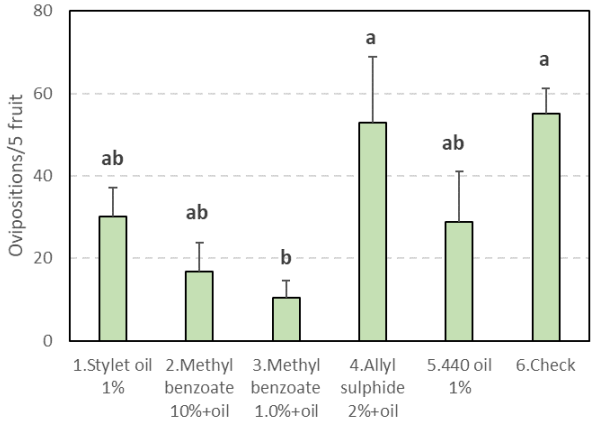

Fig. 7. SWD oviposition deterrence by oil based compounds, 2017

Method - Optimizing monitoring tools: Commercial SWD lures were tested during the 2017 season. Several liquid lures available from European manufacturers were tested (Suzukii Trap, Dros'Attract), and compared to the Scentry pouch lure and the older apple cider vinegar (ACV) standard.

Because of the low densities early in the season, the test did not commence until August. Traps were deployed in a commercial cherry orchard near Orondo, WA, in 10 replicate rows spaced 9 rows apart. Treatments within a row were spaced 8 trees (48 ft) apart, leaving 2-5 buffer trees at the row ends. Traps were suspended 3.3-4.9 feet above the ground, sheltered from direct sun by the tree canopy. The trap used to deploy all treatments was a 32-oz clear plastic jar with a red screw-on lid (a.k.a., the PBJ trap). A 2-inch piece of red duct tape (3M, St. Paul, MN) encircled the trap's circumference, with three 1.26 in diameter holes in the sides. The holes were covered with plastic screening (1/8 inch gutter mesh) to exclude larger arthropods (Plate 3). Trap contents were collected weekly for a 4-week period (22 Aug to 12 Sep). The drowning fluid or liquid bait replaced and treatments re-randomized within replicates on each collection date. Trap contents were brought back to the lab, and examined using magnification. SWD (male and female) and other *Drosophila* were counted and recorded.

Plate 3. PBJ trap used for SWD, 2017

Results: As in previous tests, the Scentry lure captured the highest number of SWD during the 4-week period, which was significantly higher than the ACV standard (Table 2). The new formulation of Suzukii trap did not improve captures in relation to the old formulation. Both the new and old formulations of Suzukii Trap and Dros'Attract trap bait were not significantly different than the check. The sex ratio was highly skewed to females during this period, but to a greater extent in the ACV and Scentry lures than the new Suzukii Trap and Dros'Attract lures. Other drosophila and % SWD more favorable in the older formulation of Suzukii Trap and Dros'Attract, and least favorable in the ACV and Scentry traps.

Table 2. SWD lure test, 2017

Treatment	n	Total SWD (Avg)	% Female SWD	Other Drosophila	% SWD (of all Drosophila)
1.ACV	10	0.03 b	100.00 ab	1364.50 b	0.00 b
2.Scentry	10	2.45 a	98.57 a	4740.00 a	0.06 b
3.Suzukii old	10	1.00 ab	81.39 ab	555.50 d	1.81 ab
4.Suzukii new	10	0.38 b	66.67 b	801.20 c	0.18 b
5.DrosAttract	10	0.65 ab	47.92 b	130.18 e	1.16 a

CONTINUING PROJECT REPORT **YEAR**: 3 of 3 (4th year report)
WTFRC Project Number: CH-14-106

Project Title: Insecticide resistance of Spotted Wing Drosophila in sweet cherry

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Total Project Request: **Year 1**: $32,058 **Year 2**: $93,397 Year 3: $83,899

Other funding sources: None

WTFRC Collaborative Expenses: None

Budget 1
Organization Name: WSU TFREC **Contract Administrator:** Joni Cartwright; Katy Roberts
Telephone: 509-663-8181 x221; 509-335-2885**Email:** joni.cartwright@wsu.edu; arcgrants@wsu.edu

Item	2014	2015	2016 **(revised)**
Salaries[1]	0	0	10,422
Benefits[2]	0	0	4,022
Wages[3]	7,800	8,112	8,400
Benefits[4]	757	787	843
Equipment	0	0	0
Supplies[5]	1,500	1,500	1,500
Travel[6]	2,966	2,966	4,000
Plot Fees	0	0	0
Miscellaneous	0	0	0
Total	$13,023	$13,365	$29,187

Footnotes (year 3 revised budget only):
[1]Salaries: Research Intern, 0.20 FTE
[2]Benefits on salaries: 38.6%
[3]Wages $14/hr, 40 hrs/week, 15 weeks/year;
[4]Benefits on wages: 10%.
[5]Supplies: traps, drosophila rearing supplies, baits and lures, office supplies/electronics
[6]Travel to research sites, motor pool rental, mileage, gas (2 months): $1600; travel to sites in WA and OR (lodging, per diem): $2400.

Budget 2 (Van Steenwyk)
Organization Name: University of California Berkeley Contract Administrator: Lynne Hollyer
Telephone: 510-642-5758 **Email address:** Lhollyer@berkeley.edu

Item	2014	2015	2016
Salaries	0	13,180	13,575
Benefits	0	5,878	6,462
Supplies	1,008	388	585
Travel	3,892	6,672	8,340
Total	$4,900	$26,118	$28,962

Footnotes:
Salary: Laboratory Research Assistant II at $2,636 per month for 5 months
Benefits: FY 15 = 44.6% and FY 16 = 47.6%
Supplies: Lab supplies for assay and rearing.
Travel: FY 14 = 35 trip for 200 miles/trip at 0.556/mi, FY 15 = 40 trips for 300 miles/trip at 0.556/mi. and FY 16 = 40 trips for 375 miles/trip at 0.556/mi.

Budget 3 (Zalom/Chiu)
Organization Name: University of California Davis **Contract Administrator:** Guyla Yoak
Telephone: (530) 752-3794 **Email address:** gfyoak@ucdavis.edu

Item	2014	2015	2016
Salaries	0	12,872	13,514
Benefits	0	84	88
Supplies	5,000	6,408	6,230
Miscellaneous	0	5,636	5,918
Total	$5,000	$25,000	$25,750

Footnotes:
Salary and Benefits: Graduate Student Researcher
Supplies: Lab supplies for molecular assays including DNA/RNA extraction, PCR, and DNA sequencing
Miscellaneous: Fees for Graduate Student Researcher

Budget 4
Organization Name: OSU MCAREC **Contract Administrator:** L.J. Koong
Telephone: 541-737-4066 **Email address:** l.j.koong@oregonstate.edu

Item	2014	2015	**2016 (revised)**
Salaries	--	10,485	0
Benefits	--	6,763	0
Wages	7,280	7498	0
Benefits	605	623	0
Equipment	--	--	--
Supplies	1,000	1,545	0
Travel	250	2,000	0
Miscellaneous	--	--	--
Plot Fees	--	--	--
Total	$9,135	$28,914	0

Footnotes:
Salary: Faculty Research Assistant 3 mo. Yr 2, 3, Benefits 28.24%+$1,267.51/mo. 3% increase/yr.
Wages: Summer assistant, 3 mo, $14/hr. Benefits 8.31%. 3% increase/yr.
Supplies: Lab supplies for assay and rearing. 3% increase/yr.
Travel to field. 0.556/mi. 3% increase/yr.

Objectives:
1. Design and test traps to capture live SWD adults for insecticide resistance studies (yr 1)
2. Develop discriminating doses of insecticides to test susceptibility of SWD populations (yr 1)
3. Complete development of primers for genetic analyses of SWD alleles that confer resistance (yr 1)
4. Screen SWD from multiple districts in CA, OR and WA for insecticide susceptibility (yr 2-3)
5. Correlate results from discriminating-dose and genetic studies (yr 2-3)

SIGNIFICANT FINDINGS

- Several styles of traps and techniques are effective for capture of live SWD.
- Low numbers of founding females are likely due to low densities in the orchards sampled.
- There were several instances of surviving females in the diagnostic dose screenings, even when re-tested. This may be indicative of the early stages of resistance, or too low a diagnostic dose.
- Two populations with possible resistance showed ca 10-fold reduction in a cytochrome P450 gene expression.

Note: a no-cost extension was requested to collect more SWD populations in the fall of 2017; rearing and bioassays on these populations will be completed by January of 2018.

Methods

Obj. 1. Design and test traps to capture live SWD adults for insecticide resistance studies (yr 1)

In the first year of the study, methods will be developed to collect adult SWD populations from orchards. This methodology will be utilized to capture adults for use in discriminating dose and target site and metabolic resistance screening in years 2 and 3. Current traps employ a liquid bait which also served to kill and retain the flies, and is thus not suitable for live capture. Several possible approaches suggest themselves, including 1) using a liquid bait, but utilize a screen to prevent flies from drowning in the fluid; such a trap will include measures to aid fly retention and survival (food, water, and shade); 2) using a dry lure in a similar type of trap.

Obj. 2. Develop discriminating doses of insecticides to test susceptibility of SWD populations (yr 1)

Baseline susceptibility information using a probit bioassay will be generated for candidate insecticides using an SWD population collected in OR in 2009, just after the detection of SWD. Insecticides screened will include Malathion, Sevin, Delegate, Entrust and Warrior. For each insecticide, a minimum of five concentrations will be evaluated which will provide responses between 25 and 95% mortality in addition to two doses that yield 100% mortality. Water will be used as a control. For each concentration there will be a minimum of 40 adult female SWD. Flies will be treated using a Potter Spray Tower, and mortality will be assessed 24 h post-treatment. The probit bioassays will be analyzed using PoloPlus program, and the diagnostic dose calculated as 2x the LC_{99}.

Obj. 3. Complete development of primers for genetic analyses of SWD alleles that confer resistance (yr 1)

In order to monitor the presence and frequency of mutations that confer target site resistance in *D. suzukii*, populations will be collected from different regions of the Western U.S. PCR-based assays and primers will be developed to amplify genomic regions that are associated with development of resistance. Research in this proposal will focus on: (i) *ace*, which encodes acetylcholinesterase and is a target for organophosphates and carbamates; (ii) *nAC-hR Dα6*, which encodes a subunit of the nicotinic acetylcholine receptor and is proposed as a target for spinosad/spinosyns; and (iii) *para*, which encodes a voltage-gated sodium channel that is a target for pyrethroids. The *D. suzukii* genome has recently been sequenced and annotated a high quality reference gene set, which will greatly facilitate primer design. Genomic DNA will be isolated from individual flies that are collected from the field populations and stored in 95% EtOH. PCR using primer sets that amplify

regions covering potential target site mutations will be performed using Accuprime Taq DNA polymerase (Life Technologies, Grand Island, NY) for high fidelity. Resulting PCR products will be purified using PCR purification kits (Qiagen, Valencia, CA) and subsequently submitted for DNA sequencing at the UC Davis Sequencing Core Facility. Results will be analyzed using sequence alignment packages, e.g., CLC sequence workbench, to determine the presence and allele frequency of nucleotide polymorphisms that might confer insecticide resistance.

4. Screen SWD from multiple districts in CA, OR and WA for insecticide susceptibility (yr 2-3)
At least 100 adult female SWD (and associated males) will be collected from each orchard screened (Table 1) using traps or sweep nets. These females will be used to produce cohorts of F_1 progeny for use in the diagnostic dose screening. At least 100 females (5-12 days old) from each population will be exposed to the diagnostic dose of each of the five insecticides. The females will be transferred to Petri dishes, sprayed in groups in a Potter Spray Tower and evaluated for mortality after 24 h. If there are any survivors in the diagnostic dose assay, it will be repeated. If there are still survivors in the repeat bioassay, a full probit line will be calculated using the methods in Obj. 2. Flies from each population screened will be sent to the Chiu lab for allele frequency tests (see Obj. 3)

Table 1. Cherry production districts within CA, OR and WA where populations of SWD will be collected and assayed for susceptibility to various insecticides

CA	OR	WA
N. San Joaquin	The Dalles	Okanogan Cty
Coastal	Hood River	Chelan/Douglas
	Willamette Valley	Col. Basin
	Milton-Freewater	Tri-Cities

Shaded regions are those from which populations have been collected and screened.

5. Correlate results from discriminating-dose and genetic studies (yr 2-3)
We will correlate our genomic data with insecticide bioassays performed on the corresponding fly strains in comparison to the genomic baseline SWD strain.

Results and Discussion.

Obj. 1. Design and test traps to capture live SWD adults for insecticide resistance studies (yr 1)
A number of trap designs were tested for live capture of SWD. Trap design focused on 1) attracting flies into the trap body; 2) preventing escape; and 3) keeping flies alive until retrieved. Some of the custom-fabricated prototypes were large, and labor-intensive/expensive to produce. The primary difficulty, however, was simply low SWD densities in the orchard. To overcome this, a larger number of traps (up to 100/block) was deployed, and flies were collected over a 1-3 day period. A second technique was found to be a practical means of collecting flies, viz., sweep netting beneath the trees. Where populations were adequate, sufficient flies could be collected in a few hours. The majority of the colonies were started with more than the target number of founding females (100), but a few fell short of this mark (Table 2).

Table 2. SWD population information for diagnostic dose screening

Year	state	Region	Orchard	Mgmt.	Coll. date	No. founding females
2014	WA	Orondo	AU	Conv	10/4/2014	199
2014	WA	Brewster	GL	Conv	11/4/2014	95
2014	WA	Royal City	RZ	Conv	11/10/2014	237
2014	WA	Malaga	SN	Conv	9/16/2014	138
2014	WA	Stemilt Hill	SH	Org	10/27/2014	113
2014	WA	Rock Island	SC	Conv	10/2/2014	106
2015	WA	Orondo	CC	Conv	7/14/2015	164
2015	WA	Orondo	CO	Org	8/17/2015	145
2015	WA	Malaga	SE	Org	7/20/2015	140
2015	WA	Rock Island	SC	Conv	7/28/2015	69
2015	OR	Hood River	MC	Conv	7/31/2015	135
2015	OR	Dallesport	DP	Conv	9/4/2015	125
2015	CA	Brentwood	BW	Org		11
2015	CA	Tracy	TC	Conv		9
2016	OR	Dayton	ST	Org	7/14/2016	94
2016	WA	Brewster	HA	Conv	9/2/2016	68
2016	WA	Prosser	OB	Conv	9/9/2016	561
2016	CA	Brentwood	BW	Org		30
2016	CA	Tracy	TC	Conv		18

Obj. 2. Develop discriminating doses of insecticides to test susceptibility of SWD populations (yr 1)

Discriminating doses (2x the LC_{99}) were developed for five pesticides: (Delegate (94.35 mg AI/liter), Entrust (221.24), Sevin (41,272), Malathion (523.58), and Warrior II (109.18), using a standard probit bioassay (Fig. 1). The reference colony used was named 'OSU', which was collected from a blueberry field in the Willamette valley in 2009, shortly after the first detection of SWD in the Pacific Northwest. This colony has been in continuous culture from 2009 until probit bioassays were conducted in 2014-2015.

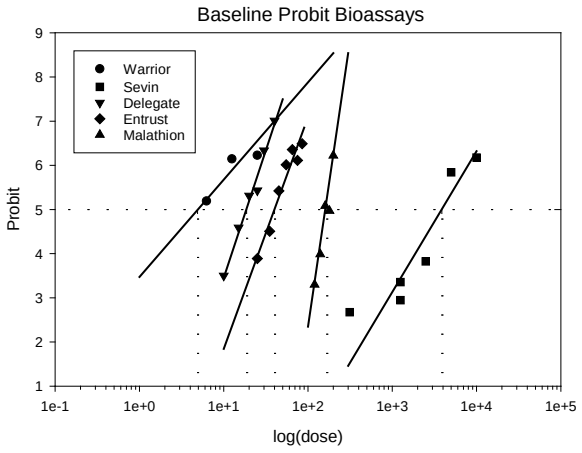

Fig. 1. Baseline probit bioassays for five candidate SWD pesticides

Obj. 3. Complete development of primers for genetic analyses of SWD alleles that confer resistance (yr1)

A total of 12 populations of SWD have been sequenced to date. We have focused the identification of differentially expressed genes (DEG) to those that are known to be involved in conferring metabolic insecticide resistance, e.g., metabolic detoxification (glutathione-S-transferase [GST], cytochrome

P450, and esterase) and reduced cuticle penetrance. Results of these analyses are presented in Objective 5. Bioinformatic analysis is in progress to yield single nucleotide variants (SNVs) in protein coding regions that can confer target-site resistance.

Obj. 4. Screen SWD from multiple districts in CA, OR and WA for insecticide susceptibility (yr 2-3)

To date, 19 populations have been screened against the candidate pesticides (Table 3). For the Washington populations, there were no survivors in the 2014 screenings. Unlike the 2015-16 data, these populations had been in culture 4-6 months versus the 4-7 weeks for the later collections. While the 2014 population screening did not conform to the protocol (collection close to harvest, screening of F_1 females), they represent an initial proof of concept for the diagnostic dose procedure. In 2015, however, there were 3 instances of survivorship in the initial screenings; only one population (CY/Org – Delegate) also had a survivor in the repeat screening. A full probit line was run on this population, and while the LC_{50} was slightly lower than the original OSU line (12.6 vs 18.7), the LC_{99} was slightly higher (59.4 vs 47.2).

Table 3. Percentage mortality in diagnostic dose screening of five candidate SWD insecticides

State	Year	Orchard	Regime	Delegate 5.04 oz	Entrust 11.82 fl oz	Malathion 6.99 fl oz	Sevin 34.4 qt	Warrior 5.61 fl oz
WA	2014	AU	Conv	100	100	100	100	100
		GL	Conv	100	100	100	100	100
		RZ	Conv	100	100	100	100	100
		SN	Conv	100	100	100	100	100
		SH	Org	100	100	100	100	100
		WB	Conv	100	100	100	100	100
	2015	CY	Conv	100	100	100	100	100
		SC	Conv	100	100	97	100	96
		SC rep	Conv			100		100
		CY	Org	89	100	100	100	100
		CY rep	Org	99				
		SN	Org	100	100	100	100	100
		DP	Conv	100	100	100	100	100
	2016	HA	Conv	100	100			
		OB	Conv	100	100	100	100	100
CA	2015	BW	Org	100	100	100	100	100
		TC	Conv	91	97	90	100	100
	2016	BW	Org	100	98			
		TC	Conv					
		GL	Conv	100	100	100		
		GN			100			
OR	2015	HR	Conv	100	100	100	100	100
	2016	ST	Org	100	100	100	100	100

Cells highlighted in yellow had 1 or more survivors. The designation ‘rep’ indicated a screening that was repeated due to survivors.

Obj.5. Correlate results from discriminating-dose and genetic studies (yr 2-3)
The goal of this objective is to correlate our genomic data with insecticide bioassays performed on the corresponding fly strains in comparison to the genomic baseline SWD strain, Specifically, we will focus on (1) gene expression changes indicative of metabolic upregulation of detoxification enzymes or genes known to be involved in reducing cuticle penetrance of insecticides; as well as (2) single nucleotide variants (SNVs) in protein coding regions that can potentially confer target-site resistance. Whereas the bioinformatic analysis for SNVs is in progress and is expected to be completed by the end of 2016, we have completed the differential gene expression analysis to identify genes that are up- and down-regulated in the various populations of SWD as compared to the SWD genome strain (Tables 4, 5).

Table 4. Number of Up- and Down-regulated genes in SWD populations as compared to the genome strain.

Strain	Collection Location	Collection Date	Up-regulated	Down-regulated
BT	Brentwood, CA	7/1/2015	867	1374
TC	Tracy, CA	9/10/2015	690	1696
CY	Bray's Landing, WA	7/14/2015	743	1331
CYO	Bray's Landing, WA	8/17/2015	767	1754
SN	Malaga, WA	7/20/2015	776	1726
SC	Rock Island, WA	7/28/2015	544	1552
DPt	Dallesport, WA	9/3/2015	1392	2264
HR	Hood River, OR	7/30/2015	1063	2008

Table 5. Differential expressions of selected metabolic detoxification genes. Values are $\log_2$(fold_change) compared to the SWD Genome Strain, and only shown if they are significant, i.e., value of +1 = 2-fold increase.

Strain	Cyp12a4	Cyp12b2	Cyp12c1	Cyp12d1-d	Cyp18a1	Cyp28c1	Cyp28d1	Cyp301a1	Cyp304a1
BT								0.54	
CY	-0.94		-0.60	-0.74			-0.78		2.76
CYO	-0.55	-0.96	-0.57			-1.03	-0.79		2.25
DP	-0.65	-1.12	-0.77	-0.89	-0.60	-1.38	-1.21		
HR	-0.45	-0.91	-0.58	-0.51		-1.11	-0.68		
SN						-1.59	-0.76		2.37
SC		-0.94						1.08	2.00
TC	-0.46	-0.85	-0.73			-1.28	-0.55		2.78

Strain	Cyp305a1	Cyp308a1	Cyp309a2	Cyp311a1	Cyp312a1	Cyp4ac1	Cyp4ad1	Cyp4d1	Cyp4d14
BT	-0.60		-0.80	-0.64				-0.75	2.10
CY			-0.89	-1.50		-0.73		-0.70	1.45
CYO	-0.63		-0.95	-1.50		-0.91		-0.59	1.74
DP	-1.08		-1.48	-1.65		-1.21		-0.85	1.38
HR	-0.96	2.17	-0.94	-1.47		-0.93		-0.57	1.94
SN		1.64					1.16	-0.75	1.65
SC			-0.45	-1.26	-3.33	-0.75		-0.96	1.61
TC			-0.43	-1.09	-3.34	-0.56			1.81

Strain	Cyp4d20	Cyp4d8	Cyp4g15	Cyp4p1	Cyp4p2	Cyp4s3	Cyp6a13	Cyp6a14	Cyp6a20
BT	-0.66	-1.82	0.77			-0.50		0.58	
CY	-1.39	-2.05		-0.56					
CYO	-0.97	-1.28		-0.49		-0.95	-0.68		
DP	-0.88	-2.21		-0.85	-0.57	-1.54	-0.80		-0.62
HR	-0.66	-2.04	0.72	-0.64		-1.71			
SN	-1.33	-1.17	0.64			-0.71			
SC	-0.81		1.01			-0.75			
TC	-1.23	-1.95	0.61			-0.53			

Strain	Cyp6a22	Cyp6a23	Cyp6d4	Cyp6d5	Cyp6w1	Cyp9b2	Cyp9c1	Cyp9h1	Est-6
BT	-1.12		1.12		0.72	-0.61			-0.55
CY	-0.94		1.09				-1.17		
CYO			0.75	0.67					-0.79
DP	-0.55	-0.57	0.69			-0.77	-1.27		-1.00
HR			0.76			-0.87		-1.09	-0.80
SN	-0.64		0.73	0.72					-0.54
SC	-0.64		0.78		0.59	-0.65			
TC	-0.74		0.83						-0.92

Strain	Est-Q	GstZ2	α-Est1	α-Est2	α-Est3	α-Est8
BT	-0.93					
CY	-1.57					
CYO	-1.44					
DP	-2.13	-0.78	-0.85	-1.44	-0.71	-0.77
HR	-1.33	-0.82			-0.43	
SN	-2.45					
SC	-1.00					
TC	-1.46					

Among all the metabolic detoxification genes, there is only one that shows changes in gene expression that occur in slightly resistant/tolerant populations, as shown in our bioassays. Cyp312a1 is a cytochrome P450 gene whose expression level is reduced by roughly 10-fold in the Spanish Castle and Tracy populations. We performed the same analysis for genes that are involved in regulating cuticle penetrance of insecticides to identify any correlation between SWD populations that are more tolerant to insecticides, and identified 3 genes that are differentially expressed (CCAP-R, Cam, l(3)mbn) (Table not shown).

Additional bioinformatics analysis on this data set is in progress to identify single site mutations (SNVs) that are known to confer insecticide resistance. It would be particularly interesting if we find SNVs in insecticide target genes in the three SWD populations that appear to be slightly resistant/tolerant to insecticides in our bioassays.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3

Project Title: Non-toxic RNAi-based biopesticide to control spotted wing drosophila

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Cooperator: Drs. Dr. Seung-Joon Ahn and Jacob Corcoran – Postdoctoral associate, USDA-ARS Horticultural Crops Research Unit, Corvallis, OR.

Total Project Request: $140,400 Year 1: $43,880 Year 2: **$48,260** Year 3: $48,260

Other funding sources

Agency Name: OBC, WBC, ORBC, WRRC, Oregon ARF

Amt. awarded: Total $94,900 (2015-2017)

Budget
Organization Name: Agricultural Research Foundation
Contract Administrator: Dr. Russ Karow
Telephone: 541-737-4066 **Email address:** Russell.karow@oregonstate.edu

Item	2017 (1st yr)	**2018 (2nd yr)**	2019 (3rd yr)
Salaries[1]	$25,000	$25,750	$25,750
Benefits[2]	$4,380	$4,510	$4,510
Wages[3]	$6,000	$12,000	$12,000
Equipment	$0	$0	$0
Materials & Supplies[4]	$8,000	$5,500	$5,500
Travel[5]	$500	$500	$500
Plot Fees	$0	$0	$0
Total	$43,880	**$48,260**	$48,260

Footnotes: USDA ARS in-house fund supports for equipment, facilities and supplies for RNAi project. [1]Salaries & [2]Benefit, 0.5 FTE Postdoc; [3]Wage, student assistant, $12/h x 20/w@4x6m & OPE $480; [4]Materialsand Supplies Molecular biology materials & supplies, RNAi materials & supplies (relatively expensive), and Insect rearing materials and supplies; [5]Travel, PI and/or postdoc to attend a cherry commission & grower meetings each year.

OBJECTIVES

The goal of this research objectives is the development of a novel environmentally-friendly control that is non-toxic insecticide and non-transgenic strategy to control SWD as well as other potential cherry pests. Although RNAi technology is a new insight and promising tool for insect pest management, there are still technical huddles remaining to successfully develop a next generation pesticide. RNAi approach to pest management consider three major challenges: 1) selection and identification of suitable target genes and/or physiological system with high level of gene silencing, 2) cost effective RNAi material production, and 3) development of a suitable delivery method into target pest. A large-scale production of RNAi in vitro using kits is too expensive, and not a practical approach for growers (#2). Therefore, there is required a mass production system to synthesis dsRNA through a microbial-based process such as a bacterial-based dsRNA production provides more practical application. To solve this problem, we have established a mass production system using a microbial-induced dsRNA production to increase the feasibility of RNAi application for SWD control (see previous studies and preliminary data 2). To control SWD the strategy of our RNAi approach is non-planted incorporated delivery method such as spray and/or bait-station application (#3).

In the present proposal, therefore we focus on the screening and identification of suitable RNAi target(s) from SWD (#1). A feasible approach for RNAi target gene screening is to search previous targets or systems observed already from same or similar insect groups. Another cost-effective approach is to search RNAi targets from known functional gene analysis and RNA mechanism studies from model insects to increase likelihood of success from the initial stage. Therefore, the screening strategy to identify appropriate RNAi target genes is particularly important. A feasible approach for RNAi target gene screening is to search previous targets or systems observed already from same or similar insect groups.

Our approach for RNAi target gene screening is based on our current RNAi research and previous RNAi results. We recently started the screening of RNAi candidates from SWD, and currently evaluate their impacts through various bioassay. In this proposal we continue to screen more target genes selected from SWD, and evaluate and identify suitable RNAi targets. To achieve this goal the following specific objectives need to be accomplished in this project:

1. Cloning and identify potential RNAi target genes from SWD (Yr. 1) – *completed*
2. Construct, design and biosynthesis dsRNAs for target genes (Yrs. 1 & 2) – partially completed and *continued*
3. Screen for efficacy using bioassay to measure RNAi impacts on SWD (Yrs. 2 & 3)
   3-1. Inject dsRNA into adult flies and monitor RNAi impacts (Yrs. 2& 3)
   3-2. Feed dsRNA to larvae and adults, and evaluate RNAi impacts (Yr. 3)

SIGNIFICANT FINDINGS (Year 1)

- Identified and sequenced > 30 genes from SWD for RNAi targets
- Constructed, and designed dsRNA (double-stranded RNA, RNAi material) for all targets
- Established SWD specific nano-injection system for the initial screening
- Screened 13 potential RNAi targets from SWD

METHODS

Our approach for RNAi target gene screening is based on our current RNAi research and previous RNAi results. We recently started the screening of RNAi candidates from SWD, and currently evaluate their impacts through various bioassay. In this proposal we continue to screen more target genes selected from SWD, and evaluate and identify suitable RNAi targets.

Obj. 1. Cloning and identify potential RNAi target genes from SWD (Yr. 1)
All selected genes will be directly identified from SWD adult or developmental stages. We will apply a PCR-based strategy and routine molecular cloning procedures to amplify target genes. Based on *D. melanogaster*'s genome sequences, we will employ a BLAST search with the published *D. suzukii* genome (http://spottedwingflybase.oregonstate.edu).

Housekeeping genes: Most of those genes have been targeted for RNAi-based approach to control pests. Selected target genes (17 targets) are: Actin genes, β-actin and actin 5C; β-coatmer proteins; cytochrome P450; regulatory particle non-ATPase 2 & regulatory particle triple-A ATPase 6; vacuolar H+ ATPase (V-ATPase), two subunits; vacuolar-sorting protein (snf7); protein transport proteins sec23 &6. Additional interesting genes, chitin synthase – insect chitin synthesis, frizzled and frizzled 2 for wing development will be added.

Neuropeptide hormones: A variety of peptide families have been identified and tested to find biological functions from insects that suggested a great target for pest management. Selected target genes (3 targets) are: Pyrokinin-2 (PK-2 = *Hug in*) and CAPA; diuretic hormones (DHs); CRF-like and calcitonin-like hormones; sex peptide (SP); eclosion hormone (EH) and ecdysis triggering hormone (ETH); allatostatins (ASTs); adipokinetic hormone (AKH). The other possible candidate NPs are: NP-F, kinins, myotropin, corazonin and ion transport peptides.

G-protein-coupled receptors (GPCRs): GPCRs have great potential for RNAi targets. Based on *D. melanogaster*'s GPCRs selected SWD's GPCRs for RNAi targets (12 targets) are: GPCRs for pyrokinin (PK1-R and PK2-R), AKH-R, ETH-R, AST-Rs, CAPA-R corazonin-R, dopamine receptors, DH-Rs, SP-R, vitellogenin R and chemosensory receptors.

Obj. 2. Construct, design and biosynthesis dsRNAs for target genes (Yrs. 1-2)
Specific primers and/or a degenerate primer set designed with 5'-T7 promoter appended will be designed to amplify partial lengths between 200- 400 nucleotides of each target gene because long dsRNAs are much more effective than ingested small RNAis (siRNAs). Once confirmed, the sequence of DNA fragment will serve as the template for dsRNA synthesis using a dsRNA synthesis kit (New England Biolabs). The negative dsRNA control (dsGFP) will be constructed by the same method described above for SWD.

Obj. 3: Screen for efficacy using bioassay to measure RNAi impacts on SWD (Yrs. 2-3)
3-1. Injection dsRNA into SWD: Synthetic SWD dsRNAs (0.1 - 1 µg) dissolved in RNase free water will be injected into adult stages of SWD using a Nanoliter 2010TM injector (WPI Instrument). Dr. Choi has experience with RNAi micro-injection into small insects such as ants and flies. Recently, PI's lab has established a novel and convenient microinjection system that is able to injection for SWD adults without anesthetization under a stereo-microscope. After injection of ~20 flies per treatment, phenotypic changes will be monitored for a week. Dr. Lee's laboratory has a convenient system to monitor longevity and fecundity of flies, and several bioassay options for monitoring flight or activity level. Once we evaluate RNAi impacts, we will decide on the next feeding assays because dsRNA injection directly into insect hemocoel is the best way for RNAi delivery.

3-2. Feeding dsRNA to SWD: For adult feeding assays, various dsRNA concentrations determined from the injection experiment will be mixed in a dry bread yeast. The mixed yeast with specific dsRNA will be sprayed on the surface of the artificial diet in a petri-dish to allow adult flies to feed in the cage. After feeding, flies will be monitored for phenotypic changes, and verified for gene silencing as described above. For larval feeding assays, the same concentration of dsRNAs will be added in the diet at cooled to below 60 ºC and mixed immediately. The first instars (20 individuals) will be transferred onto the diet with the dsRNA and monitored for larval mortality and developmental rates.

RESULTS & DISCUSSION

For optimal impact of dsRNA delivered to target cells through feeding, RNAi target genes should focus on non-cell-autonomous RNAi mechanisms including environmental and systemic RNAi if dsRNA can be internalized into the target cells through feeding. We have selected over thirty (> 30) RNAi targets based on the previous studies for insect RNAi targets and biological functions. These target genes include essential housekeeping genes that are required for the maintenance of basic cellular functions, neuropeptide (NP) hormones and GPCRs for SWD life stages.

Obj. 1. Cloning and identify potential RNAi target genes from SWD (Yr. 1): We selected and identified 32 SWD genes for potential RNAi targets (Table 1). These genes were designed to construct dsRNA and synthesized 11 dsRNAs (Fig. 1) through the RNAi screening process (Fig. 2).

Table 1. SWD RNAi candidates from three different groups and GFP, and nucleotide lengths of dsRNAs.

RNAi candidates	DNA template for RNAi synthesis	Gene family
SWD ID1	296 nucleotides	Neurohormone
SWD ID2	195 nucleotides	Neurohormone
SWD ID3	399 nucleotides	Hormone receptor
SWD ID4	244 nucleotides	Housekeeping
SWD ID5	253 nucleotides	Housekeeping
SWD ID6	255 nucleotides	Housekeeping
SWD ID7	253 nucleotides	Housekeeping
SWD ID8	250 nucleotides	Housekeeping
SWD ID9	251 nucleotides	Housekeeping
SWD ID10	254 nucleotides	Housekeeping
SWD ID11	254 nucleotides	Housekeeping
SWD ID12	250 nucleotides	Neurohormone
SWD ID13	299 nucleotides	Hormone receptor
SWD ID14	377 nucleotides	Housekeeping
SWD ID15	374 nucleotides	Housekeeping
SWD ID16	325 nucleotides	Hormone receptor
SWD ID17	299 nucleotides	Hormone receptor
SWD ID18	315 nucleotides	Hormone receptor
SWD ID19	325 nucleotides	Hormone receptor
SWD ID20	261 nucleotides	Hormone receptor
SWD ID21	362 nucleotides	Hormone receptor
SWD ID22	240 nucleotides	Hormone receptor
SWD ID23	360 nucleotides	Hormone receptor
SWD ID24	363 nucleotides	Housekeeping
SWD ID25	308 nucleotides	Housekeeping
SWD ID26	200 nucleotides	Housekeeping
SWD ID27	378 nucleotides	Housekeeping
SWD ID28	200 nucleotides	Housekeeping
SWD ID29	200 nucleotides	Housekeeping
SWD ID30	240 nucleotides	Housekeeping
SWD ID31	250 nucleotides	Chemosensory
SWD ID32	240 nucleotides	Chemosensory
GFP	350 nucleotides	unrelated gene as a control

We found some genes identified in this study were very different from those sequences published on the SWD genome data, indicating errors in the annotated genome or an incomplete SWD genome that needs to be confirmed for these target genes. Therefore, each target has been amplified and confirmed the sequence for the real SWD gene. The length of dsRNA for SWD ID2 was designed as a short fragment because the size corresponds roughly to full sequence (~200 base pairs). SWD ID3 is a receptor for a neuropeptide hormone functioning in egg development in the female SWD. In this report specific gene names and sequences are not disclosed until these targets are published for the patent and/or peer-reviewed journals.

Eight housekeeping genes as constitutive genes are expressed in all cell types at a level that does not fluctuate with the cell cycle. Functional examples of housekeeping genes for RNAi targets are related in the muscle physiology, detoxification, ATP metabolism, protein sorting and transporting, and cell membrane structure in cells. These genes have been selected for RNAi candidates to develop RNAi-based control for insect pests.

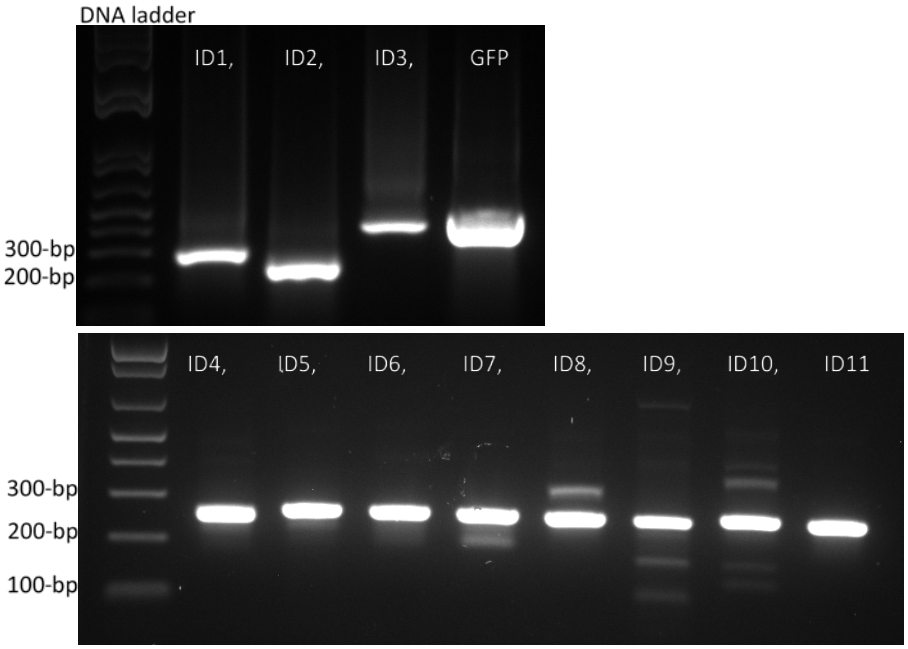

Figure 1. Photos of examples of dsRNAs synthesized from SWD ID1-3 & GFP (upper) and SWD ID4 -11 (lower) corresponded to DNA templates in Table 1.

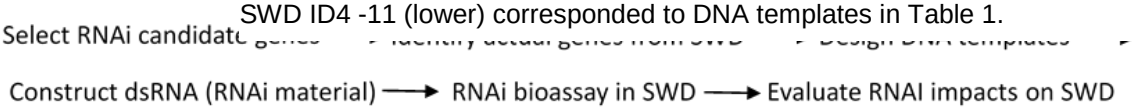

Figure 2. Outline of the screening process of RNAi targets to SWD

2. Microinjection tool: Recently, Dr. Choi has successfully established a SWD specific microinjection system using a Nanoliter 2010^{TM} injector fitted with custom-pulled borosilicate needles, and a homemade vacuumed tube to hold fly alive (Fig. 2). The system and skill is particularly important to inject a nano-litter volume (50 nL = 0.02 uL) into small insects such as SWD without or a minimum damage physically to the fly. After injected a shame or water only we found almost SWD adults (> 90%) were not physically affected and survived for two weeks monitored. Although dsRNA injection into SWD individually is not a practical approach, but it is the best and fastest method to

screen RNAi impact on pheno-and genotypic effects in the initial step because dsRNA injected into hemocoel (= blood vessel) will be directly delivered in the target cells.

3. Initial screening of 11 potential RNAi targets: During the past years the 1st screening with 11 RNAi candidates was investigated with over 2,000 nano-injections to SWD adult flies. We found effective phenotypic impacts, mainly mortality, from some of the RNAi injection into SWD flies (Fig. 4).

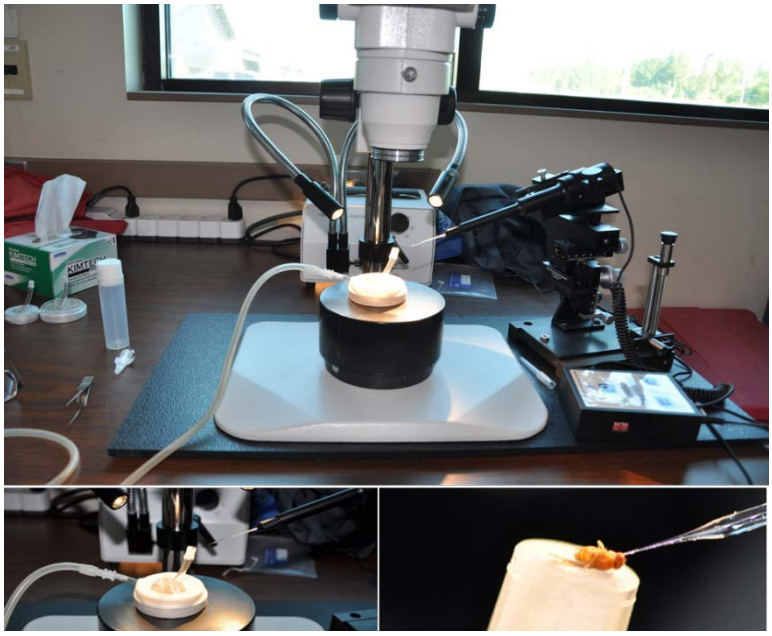

Figure 3. Photos of microinjection system (upper) equipped with a stereomicroscope, micromanipulator and a vacuumed tubed plate (lower left), and SWD adult injected by a capillary glass needle (lower right).

4. Continuing the project
Our long term goal is to develop biologically-based control method for SWD. If the funding is continued, following objectives will be completed for next year.

Specific Objectives – **Year 2**

1. Continue to identify target genes from SWD, and design dsRNA sequences of these genes and green fluorescence protein (GFP) as a control gene. Inject RNAi into adult flies and monitor RNAi impacts (*i.e.* fecundity or mortality) on SWD (Yr.2 &3).
2. Continue to screen for efficacy using bioassay to measure RNAi impacts on SWD - Inject dsRNA into adult flies and monitor RNAi impacts (Yrs. 2& 3).

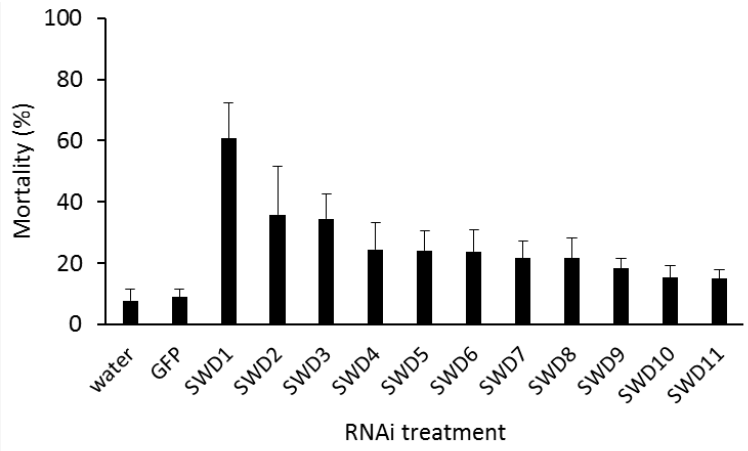

Figure 4. Mortality of SWD adult flies after injected dsRNA (1µg/fly) within 48h. Twenty flies were used with 3 replications at least for each RNAi target.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: CH-17-103

Project Title: Orchard management practices for *little cherry virus 2*

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Co-PI (2): Dr. Alice Wright
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Cooperators: None.

Total Project Request: $171,172 Year 1: $57, 512 **Year 2: $55,716** Year 3: $57,944

Other funding sources
None

Budget
Organization Name: Washington State University **Contract Administrator:** Katy Roberts
Telephone: (509) 335-2885 **Email address:** arcgrants@wsu.edu

Item	2017	**2018**	2019
Salaries	$7,361	**$7,655**	$7,961
Benefits	$2,157	**$2,243**	$2,333
Wages	$3,120	**$3,245**	$3,375
Benefits	$324	**$337**	$350
Equipment			
Supplies	$44,550	**$42,236**	$43,925
Travel			
Plot Fees			
Miscellaneous			
Total	$57,512	**$55,716**	$57,944

Footnotes:
1. 0.15 full time equivalents of a Post-Doctoral Research Associate.
2. Benefits calculated at standard Washington State rates.
3. Personnel for summer help
4. Supplies include:

Acquisition and retention of trees over three years	$12,993
Land preparation and irrigation installation	$3,000
Virus testing	$111,718
Mealybug colony establishment and maintenance	$3,000

OBJECTIVES

1) *Examine the effect of rootstocks on the concentration and mealybug transmissibility of LChV-2*.
Mazzard, Krymsk 5, Krymsk 6, Gisela 6, and Gisela 12 rootstocks, inoculated with LChV-2 isolate LC-5 will be screened for virus presence in April 2018. Canindex, Bing, Chelan, and Sweetheart scions will be grafted on to all plants in June-July, and monitoring of virus concentration will follow, as described in the methods, from September 2018. Mealybug transmission experiments will occur in spring of 2019 once the virus has had sufficient time to accumulate and infect the scion tissues. The timeline for this objective was delayed due to material availability and staffing changes at the CPCNW.

2) Quantify the accumulation of LChV-2 in different host tissues throughout the growing season.
In 2018, monitoring of virus distribution and titer will continue throughout the year as described in the methods section. From July-August 2018, sufficient data will have been collected to compare inter-year variation in titer in the same tree, providing a more comprehensive picture of the movement of LChV-2 through its host.

3) *Determine the population structure of LChV-2 within Washington cherry production regions*.
The population structure of LChV-2 will be examined using samples from the 2017 and 2018 growing seasons; the use of 2015-2016 samples was discarded due to the age of the extractions, and risk of sample degradation. Data collection will begin in November-December 2017 and continue throughout the following year. In a departure from the originally proposed methods, single-stranded conformation polymorphism (SSCP) will be used as the primary method for searching for distinct variants; isolates identified by SSCP will undergo direct (Sanger) sequencing, and, should a new genotype be discovered, high-throughput sequencing to reconstruct the genome will be performed.

SIGNIFICANT FINDINGS

- The titer of LChV-2 differs significantly between tissues, with bark tissue and buds being particularly low in summer. This suggests that summer budwood is a poor tissue for diagnostic screening.

METHODS

1. *Examine the effect of rootstocks on the concentration and mealybug transmissibility of LChV-2.*
In this study, the rootstocks Mazzard, Krymsk 5, Krymsk 6, Gisela 6 and Gisela 12 will be bark patch inoculated with LChV-2 isolate LC5, planted during the first year in an experimental field plot and maintained for the duration of the experiment. In the following year, the indicator cultivar 'Canindex1' and three commercial cultivars 'Bing', 'Sweetheart' and 'Chelan' will be grafted, in replicates of five, onto the rootstocks and the concentration of LChV-2 will be measured by quantitative RT-PCR at five different time points (between 1=March-April, 2=June-July, 3=August-September; 4=October-November; 5=December-January) throughout the growing season. Leaf symptom expression will also be recorded. To account for any occurrence of uneven virus distribution within the tree, sampling units will be collected uniformly throughout the tree canopy (tissue of different ages will be sampled from major scaffold limbs of the tree) and will consist of five petioles and leaves for sampling points 1-3, while phloem scrapings from dormant wood will be used for sampling points 4 and 5.

An additional set of plants will be established in the greenhouse during the first year, with treatments identical to the rootstock-virus concentration trial. These plants will be used for virus acquisition by crawlers of either apple (*Phenacoccus aceris*) or grape (*Pseudococcus maritimus*) mealybugs. The concentration of LChV-2 on these shoots will be measured by quantitative RT-PCR prior to mealybug feeding. After virus acquisition period of 7 days, 25 crawlers will be transferred to each

caged 'Canindex1' sweet cherry tree (five trees per scion-rootstock combination) free of LChV-2. The inoculated trees will be maintained in the greenhouse and treated with insecticide after one week to eliminate the mealybugs. Leaves will be collected 90 days after the inoculation period and tested for LChV-2 by RT-PCR. Additionally, the concentration of LChV-2 in each mealybug (for a total of 5 mealybugs) after virus acquisition feeding will also be measured by quantitative RT-PCR.

2. *Quantify the accumulation of LChV-2 in different host tissues throughout the growing season.*
For this part of the study, trees with known and established LChV-2 infections that are maintained in CPCNW field block will be used. Appropriate tissues will be collected throughout the growing season, for two years and the concentration of LChV-2 measured using quantitative RT-PCR. The tissue types to be examined will be as follows: [1] buds, [2] wood scraping and [3] roots during winter (between December and January); [4] flower/vegetative buds, [5] woodscraping and [6] roots (early white bud stage between late March and mid-April); [7] flower stem and [8] wood scraping (full bloom stage between mid and late April); [9] leaf petiole, [10] leaf midrib, [11] fruit stem, and [12] wood scraping (fruit set stage between late April and early May); [13] leaf petiole, [14] leaf midrib, [15] fruit stem, and [16] green shoot scraping (green fruit stage between late May and early June); [17] leaf petiole, [18] leaf midrib, [19] fruit stem, [20] green shoot scraping, [21] buds and [22] roots (mature fruit/harvest stage between mid-June and early-July); [23] leaf petiole, [24] leaf midrib, [25] green shoot/wood scraping, and [26] buds (late summer, mid-August); and [27] leaf petiole, [28] leaf midrib, [29] wood scraping, [30] buds and [31] roots (early fall, mid-October).

3. *Determine the population structure of LChV-2 within Washington cherry production regions.*
The population structure of LChV-2 within Washington cherry production regions will be determined using LChV-2 positive samples from the 2017 and 2018 growing seasons collected from different growing regions within Washington. Samples will be screened by single stranded conformation polymorphism (SSCP) analysis of three different coding regions of LChV-2 genome, with variants indicated by this method being identified by direct sequencing, and genotypic groupings of LChV-2 population across Washington cherry production regions deduced. Additionally, in coordination with Washington cherry growers, symptomatic trees (small, triangular, high shoulder fruits possibly infected with LChV-2) that have tested negative for LChV-2 and Western X phytoplasma in the upcoming 2018 growing season will be analyzed further by high throughput sequencing to identify a possible new genotypic variant of LChV-2; if a new genotypic variant is found, the current RT-PCR detection method will be re-optimized to accommodate detection of a newer variant of the virus. Representative samples from 2017-2018 including LChV-2 isolates maintained at CPCNW will be screened for the presence of the new genotypic variant of the virus.

RESULTS & DISCUSSION

1. *Examine the effect of rootstocks on the concentration and mealybug transmissibility of LChV-2.*
Rootstocks for this objective have been established in the field and greenhouse. In September they were grafted with stem tissue containing the LC5 isolate of LChV-2. These plants will be monitored for the presence of LChV-2 as described in the methods. In the summer we will proceed with grafting.

2. *Quantify the accumulation of LChV-2 in different host tissues throughout the growing season.*
A quantitative RT-PCR assay has been developed to detect LChV-2. Primers were developed for seven candidate reference cherry genes and, of these, three were selected for further optimization. Temperature, magnesium concentration, and primer concentration were optimized for the reference genes as well as the primers for LChV-2. Two of the three reference genes appeared stable; these are translation elongation factor 2 and tubulin alpha. These two reference genes will continue to be used throughout the study.

Due to personnel changes, only one time-point for assaying LChV-2 concentration in different tissues has been completed. This is the mid-August time point in which petiole, midrib, bud and wood-scraping tissues were sampled (Figure 1). The quantitative RT-PCR assay revealed that LChV-2 concentration was highest in the midrib and petiole and lowest in the bud and wood scraping tissues. This difference in concentration was observed in all three replicates, indicating that for screening purposes in late summer, petiole and midribs are better tissues to screen than budwood. Sampling will continue as described in the methods and the concentration of LChV-2 in theses tissues will be determined by this quantitative RT-PCR assay.

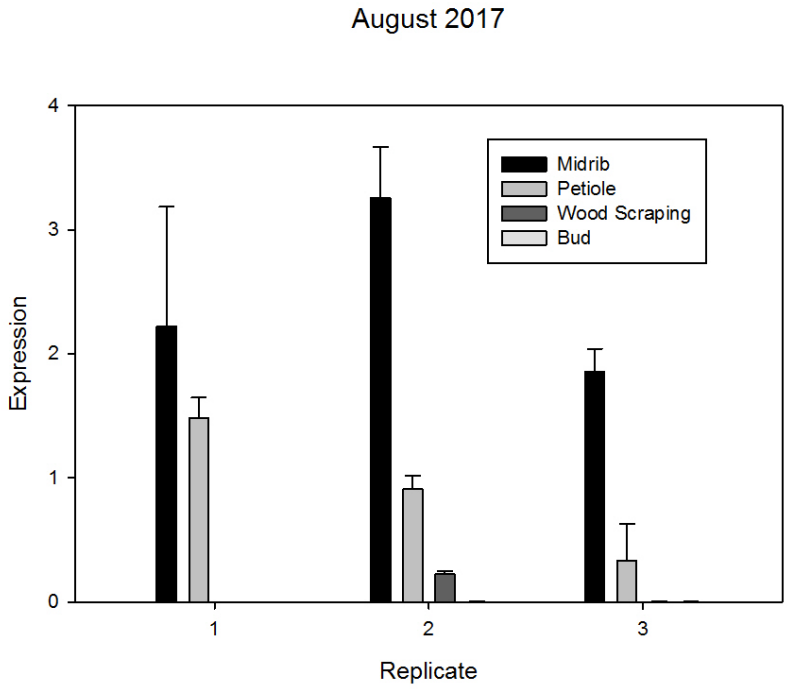

Figure 1. Quantification of LChV-2 concentration in midrib, petiole, wood scraping and bud tissues in August of 2017.

3. *Determine the population structure of LChV-2 within Washington cherry production regions.*
SSCP will be used to assess population structure for LChV-2. This technique uses gel electrophoresis to detect the presence of sequence differences between samples. So far primers have been designed for the helicase, RNA dependent RNA polymerase (RdRP), p39, and p55-p6. These primers have been tested against the LC5 isolate of LChV-2 (Figure 2). These primers will be screened against additional isolates and from that screening a subset of the primers will be selected for SSCP. Unique isolates identified by SSCP will be sequenced to assess sequence variability and high throughput sequencing will be performed if needed.

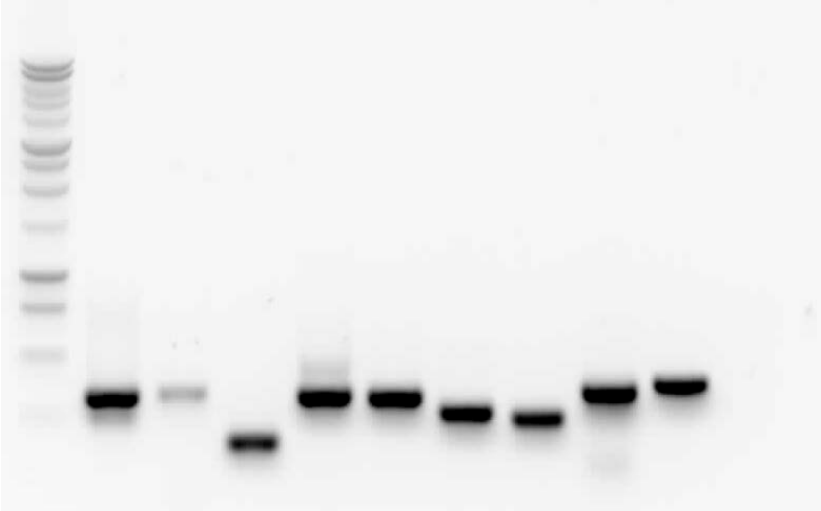

Figure 2. Testing of SSCP primers for amplification of segments of the LChV2 genome. The first lane is the ladder and the next nine are segments amplified by SSCP primers.

In summary, this project aims to establish whether there are conditions, be they host- or virus-specific, that are conducive to the spread of *Little cherry virus 2*, and to the onset of little cherry disease in Washington orchards. This year we have laid the groundwork necessary for the project to continue, establishing long-term experiments and building tools to help us gather and assess the data. The data from this project will help growers make informed choices in terms of planting and management practices.

CONTINUING PROJECT REPORT **YEAR: 2 of 3**
WTFRC Project Number: CH-16-105

Project Title: The hunt for leafhopper vectors of Western X in Washington cherries

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Cooperators: Washington cherry growers, Stemilt Growers

Total Project Request: **Year 1**: $33,740 **Year 2**: $34,603 **Year 3**: **$35,499**

Other funding sources
None

Budget 1
Organization Name: Washington State University **Contract Administrator:** Katy Roberts
Telephone: 509-335-2885 **Email address:** arcgrants@wsu.edu

Item	2016	2017	2018
Salaries[1]	$15,601	$16,226	**$16,875**
Benefits[2]	$5,939	$6,177	**$6,424**
Wages			
Benefits			
Equipment			
Supplies[3]	$12,200	$12,200	**$12,200**
Travel			
Miscellaneous			
Plot Fees			
Total	$33,740	$34,603	**$35,499**

Footnotes:
1. 0.25 and 0.10 FTE of Research Associates Ferguson and Wright, respectively.
2. Benefits calculated at standard Washington State rates.
3. Supplies include partial funding of:
Fuel to travel to research sites Leafhopper colony establishment and maintenance
Field sampling supplies Acquisition/retention of inoculated plants over 2 years
Sample extraction and PCR assays

OBJECTIVES

1. *Conduct survey of leafhoppers in Western X affected orchards.*
   The 2017 leafhopper survey in cherry-producing regions yielded informative data in the counties sampled (Grant and Yakima). We plan to conduct a similar study in 2018, beginning in March and concluding in October. Because of personnel changes and time limitations, we did not collect in Chelan County in 2017, although we plan to coordinate limited sampling in Wenatchee and Malaga during the 2018 season. One of the Yakima County sites that was new for 2017 removed their troubled Bing blocks soon after harvest. We have located additional sites in Yakima County to sample in 2018. We plan to continue extensive molecular testing of leafhoppers to determine which species have the potential to transmit Western X phytoplasma.
2. *Conduct survey of host plants for leafhoppers and/or WX phytoplasma in affected orchards.*
   During the growing season beginning in May, potential host plants for leafhoppers will be collected from field sites and propagated in the greenhouse. Data from sticky card sampling in ecosystems outside of the orchard will also provide information on potential host plants.
3. *Examine the capability of selected leafhopper species to transmit WX phytoplasma.*
   A colony of *Colladonus reductus* was established in late season 2016 and a colony of *C. geminatus* was established in late season 2017. As these colonies continue to thrive well on celery, we will be able to continue conducting transmission studies in the early and late 2018 season.

SIGNIFICANT FINDINGS

- **Two leafhopper vector species, *Colladonus geminatus* and *C. reductus*, were the most abundant species found on sticky cards in cherry tree foliage and in habitats outside of orchards in most sites.**
- **Data from sticky cards placed in extra-orchard habitats provided new information on alternative habitat/host plants, other potential leafhopper vector species (*Fieberella* sp., *Scaphytopius* sp.), and seasonal movement of *Colladonus* species.**
- **From the molecular diagnostic testing results, 18 of 388 *C. geminatus* and 18 of 440 *C. reductus* DNA samples from late 2016 and 2017, were positive for WX. We noted that these WX-positive samples were primarily found in the orchard during the latter part of the season, e.g., late August 2017, late August to early October 2016.**
- **A laboratory colony of *C. geminatus* was established on celery seedlings. The *C. reductus* colony that was initiated in 2016 continued to expand. We plan to use both species of leafhoppers for transmission studies in the early and late 2018 season.**
- **Twenty transmission experiments were initiated in August of 2017 with *C. reductus*, using infected cherry shoots from the field and recipient celery seedlings. Analysis of these transmissions is ongoing.**

METHODS

1. *Conduct survey of leafhoppers in Western X affected orchards.*
In 2018, we will conduct leafhopper sampling from March to October in Western X affected cherry orchards to determine species composition and peak activity periods. Yellow sticky cards (4" X 10") will be placed in the tree canopy to catch leafhoppers in cherry foliage, and sweep nets will be used to sample the orchard floor vegetation. Sticky cards will be placed at a height of 5-6 feet. We will use the same orchard sites we used this past summer, except for the Selah site, where the troubled Bing blocks were removed. We will add at least one new site in Yakima County. We plan to sample additional alternative habitat areas near orchards. We plan to sample for leafhoppers in Grant and

Yakima Counties. Provided that we have adequate time and technical assistance, sampling for leafhoppers is also planned in Chelan County cherry blocks.

Leafhoppers will be identified, counted, and preserved in ethanol until analysis. Leafhoppers separated by species and DNA will be extractioned. DNA samples will be tested for WX using a PCR assay.

2. *Conduct survey of alternate host plants for leafhoppers and/or WX phytoplasma in affected orchards.*
At the same sampling sites described in Objective 1 Methods, we will continue to characterize the plant species on the orchard floor and in neighboring ecosystems from spring to fall. We will attempt to propagate select weedy or woody host species in the greenhouse to determine their suitability as hosts for leafhopper and/or WX phytoplasma. We are especially interested in propagating *Purshia tridentata* (antelope bush), which is listed as a leafhopper host in past research studies.

3. *Examine the capability of selected leafhopper species to transmit WX phytoplasma.*
During the third year, we will continue to develop, propagate, and maintain infective sources of WX phytoplasma in greenhouse culture. An infective source is needed to conduct the transmission experiments. We will continue to maintain the *C. reductus* and *C. geminatus* colonies on celery. Assessment of vector competency of these species using clean laboratory-reared leafhoppers will involve at least one of these approaches: (1) acquisition of WX from infected field trees, (2) transmission from infected woody host to clean herbaceous recipient (celery), (3) transmission from infected herbaceous host to clean herbaceous recipient, and (4) transmission from infected cherry or peach host to clean recipients (cherry or peach seedlings). Potentially infected celery, cherry, and peach plants will be maintained in the greenhouse and observed for symptom development; leaf samples will be collected when appropriate to test for the presence of WX.

RESULTS & DISCUSSION

1. *Conduct survey of leafhoppers in Western X affected orchards.*
The 2017 leafhopper survey in cherry-producing regions yielded informative data in Yakima and Grant County orchard sites. We sampled at four sites in Mattawa, one in Selah, one in Granger, and one in Wapato. One of the sites in Mattawa was outside of the orchard that was removed in 2016. For the Selah site that was added in 2017, the troubled Bing blocks were removed soon after harvest; we continued to sample in other cherry blocks and extra-orchard habitats at that site.

We focused primarily on the two most abundant species, *Colladonus reductus* and *C. geminatus* but did record data on other potential vector leafhopper species. Data from sticky cards and sweep net samples yielded information on the leafhoppers present in the foliage of the cherry trees as well as the foliage of plant species in extra-orchard habitats (alfalfa, sagebrush, antelope bush, clover, etc.). While *C geminatus* is known to be a vector of WX in Washington State (Wolfe et al. 1951), little is known of the vector status of *C. reductus*. We found cherry leafhopper, *Fieberiella florii,* which is a significant vector of WX in California sweet cherries (Purcell et al. 1987), in the Granger block. Due to lack of personnel, sites were sampled infrequently during the spring. For all sites, we are still collecting and analyzing samples through late October 2017.

Samples from late season 2016 (Figs. 1, 2, 4, and 5) revealed that *C. reductus* numbers peaked in October in all four Mattawa sites (in sageland at two sites and in orchard at the other two sites), Granger (in alfalfa), and Benton City (roadside weeds) sites. *Colladonus geminatus* peaked in October at all four Mattawa sites, primarily in the neighboring sageland, but not in Granger or Benton City sites.

In the Granger block (Yakima County), *C. reductus* and *geminatus* remained more abundant in the alfalfa field across the road than in the cherry foliage throughout most of the 2017 season (Figs. 1B and 2B). *C. geminatus* showed a peak of activity in alfalfa in May/June, and *C. reductus* peaked in late July.

In the Wapato block (Yakima County), which was a new sampling site for 2017, both *Colladonus* species were found, with *C. reductus* being the dominant species (Figs. 3A and B). While *C. geminatus* peaked in the spring, *C. reductus* showed a larger increase in numbers during August in the orchard. Numbers of both leafhopper species likely would have continued to increase into September and October, but the orchard was treated with insecticide on September12th.

All of the Mattawa (Grant County) blocks had sageland within 200 feet of the edge of the orchard. Due to space limitations, only the Mattawa1 block data are presented in this report. In that block the incidence of *C. geminatus* was greater than *C. reductus* in the sageland during the spring, but by July numbers of both species were similar and more numerous in the orchard (Figs. 4B and 5B).

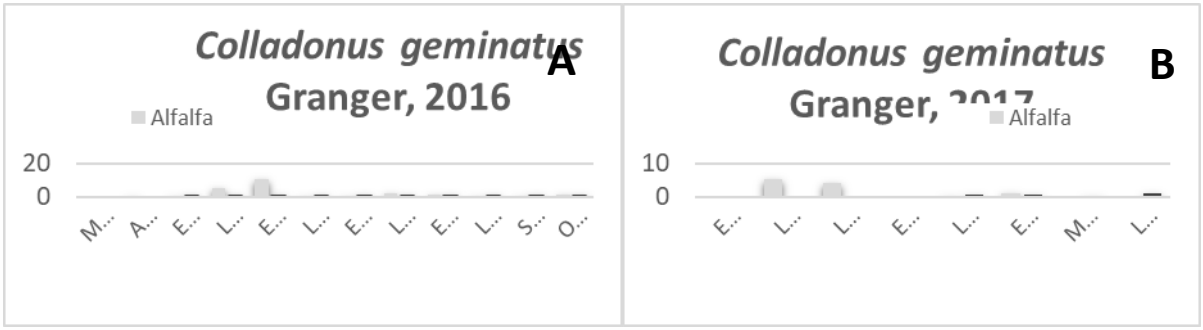

Fig. 1. *Colladonus geminatus* abundance (mean/card) in orchard and extra-orchard habitat (alfalfa field), Granger, Yakima County, WA, 2016-2017 (A and B).

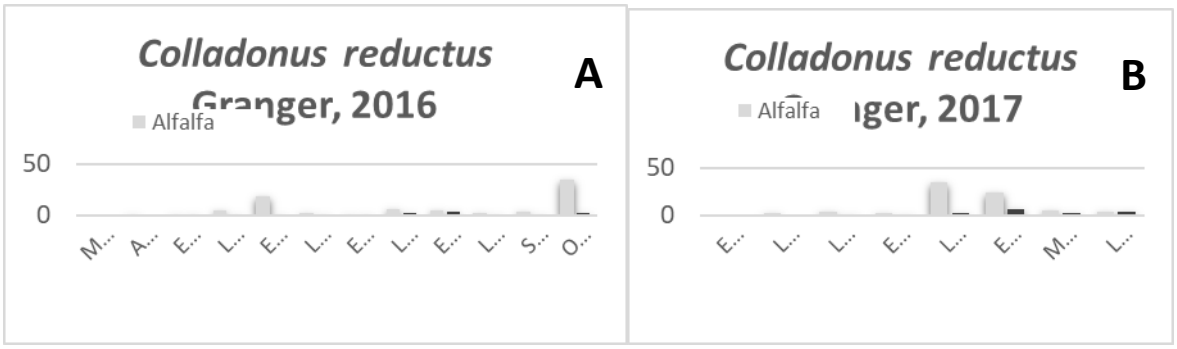

Fig. 2. *Colladonus reductus* abundance (mean/card) in orchard and extra-orchard habitat (alfalfa field), Granger, Yakima County, WA, 2016-2017 (A and B).

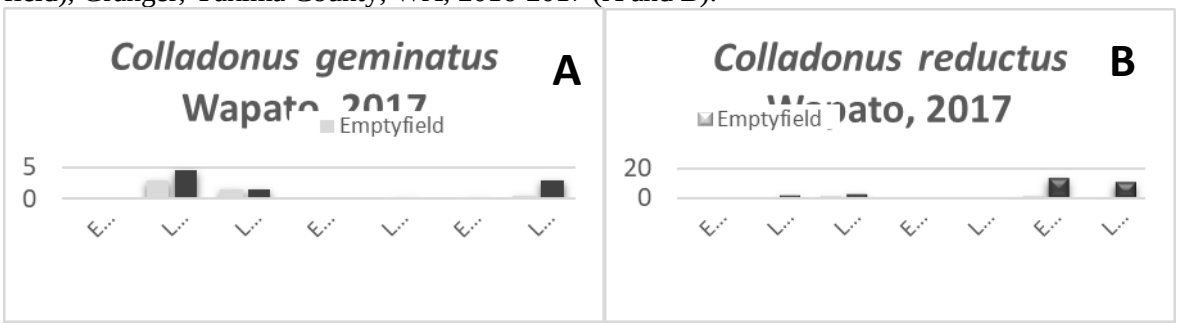

Fig. 3. *Colladonus geminatus* (A) and *reductus* (B) abundance (mean/card) in orchard block and extra-orchard habitat (empty field) in Wapato, Yakima County, WA, 2017.

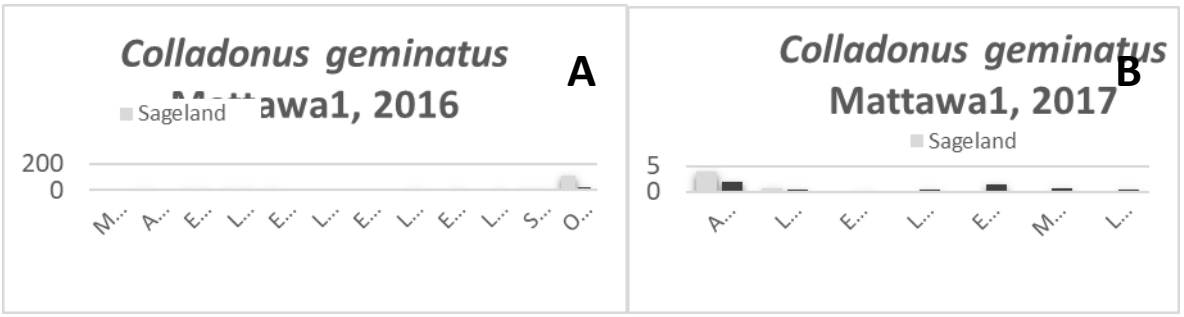

Fig. 4. *Colladonus geminatus* abundance (mean/card) in orchard and extra-orchard habitat (sageland) in northern Mattawa, Grant County, WA, 2016-2017 (A and B).

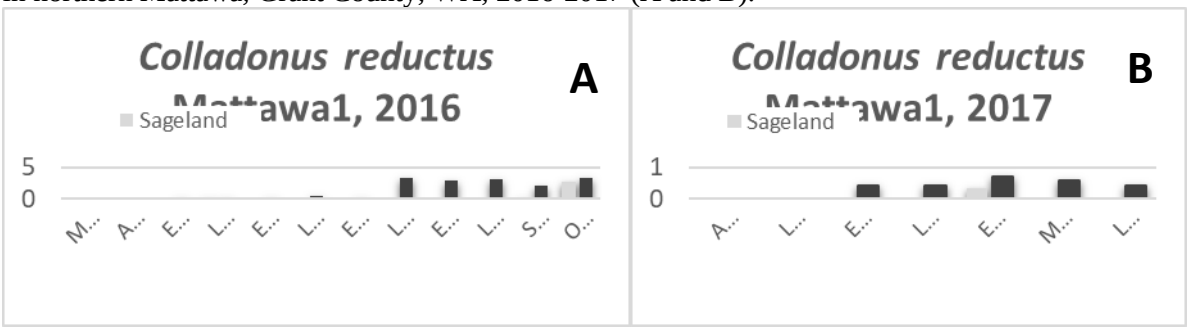

Fig. 5. *Colladonus reductus* abundance in orchard and extra-orchard habitat (sageland) in northern Mattawa, Grant County, WA, 2016-2017 (A and B).

From the molecular diagnostic testing results, 18 of 388 *C. geminatus* DNA samples and 18 of 440 *C. reductus* samples from late 2016 and 2017 through August were positive for WX. Table 1 provides a summary of molecular diagnostic testing results for 2016 and 2017 so far. The upsurge of positive orchard leafhopper samples in late 2016 season may be explained by the simultaneous increase in WX titer in the cherry trees at that time of the year (Suslow and Purcell 1982; Uyemoto and Luhn 2006**). During late season, leafhopper abundance was comparatively low in the extra-orchard habitats, although WX-positive specimens continued to be found. The drop in the number of positive leafhopper samples by September and October 2016 is likely an artifact due to the removal of two of the blocks that had widespread infections of WX. In 2017, *C. geminatus* positive for WX were found at five of the seven sites in late August (Selah, Granger, Mattawa1, 2, and 4). *C. reductus* samples positive for WX were found primarily at the Wapato site with others found in Granger and Mattawa sites.**

Table 1. Incidence of Western X in leafhoppers collected in cherry orchard and extra-orchard habitats, 2016 and 2017. Extra-orchard habitats included alfalfa, sagebrush, antelope bush, volunteer cherry, and roadside herbaceous weeds.

	Cherry orchard			Extra-orchard habitat		
Colladonus geminatus						
2016	# samples	# positive	%	# samples	# positive	%
Late April	0	--	--	28	22	79
Early May	22	5	23	59	37	63
Late May	34	1	4	21	1	5
Early June	6	0	0	8	0	0
Late June	2	0	0	4	0	0
Early July	5	1	20	1	0	0
Late July	8	1	13	5	2	40
Early Aug	13	5	38	2	1	50
Late Aug	6	1	17	6	1	17
September	109	4	4	11	1	9
October	102	0	0	57	0	0
2017						
April/May/June	13	0	0	17	0	0
Late June	7	0	0	21	0	0
Early July	1	0	0	2	0	0
Late July	6	0	0	3	0	0
Early August	6	0	0	7	0	0
Late August*	11	2	19	3	1	33
Colladonus reductus						
2016						
Late April	0	--	--	1	1	100
Early May	9	3	33	9	7	78
Late May	15	0	0	9	0	0
Early June	5	0	0	11	0	0
Late June	6	0	0	11	0	0
Early July	7	2	29	4	0	0
Late July	38	16	42	6	4	67
Early Aug	33	19	58	4	4	100
Late Aug	43	4	9	6	0	0
September	94	2	2	15	0	0
October	105	1	1	45	1	2
2017						
April/May/June	18	0	0	8	0	0
Early July	4	0	0	7	0	0
Late July	10	0	0	8	0	0
Early August	21	4	19	10	0	0
Late August*	39	6	15	3	0	0

*Sample processing is not complete. Sticky card sampling continued into October 2017 but these samples have not yet been processed.

2. *Conduct survey of host plants for leafhoppers and/or WX phytoplasma in affected orchards.*
We continued to collect potential host plant specimens and have maintained selected species (choke cherry, serviceberry, purshia) in the greenhouse for host plant studies. Numerous antelope bush (*Purshia* sp.) plants were purchased late in the season, as they were not available earlier, to be used in 2018 studies. We will initiate a leafhopper colony on *Purshia* this winter. Data from sticky cards and from observations during sweep net sampling provided new information on other potential host plants for leafhoppers and/or Western X. Several weed specimens were assayed for the presence of WX but were found to be negative. A broader-scoped study needs to be accomplished in order to elucidate the range of host plants other than sweet cherry for leafhopper vectors.

3. *Examine the capability of selected leafhopper species to transmit WX phytoplasma.*
Twenty transmission experiments were initiated in August 2017. Young shoots and leaves were collected from infected cherry trees and brought back to the lab. Up to 12 *C. reductus* were collected from the colony and placed onto infected shoots, allowing them to feed for 2-7 days of acquisition time. Then, leafhoppers were placed onto a clean celery plant. For the leafhopper to be infectious, a latent period of about a month must pass. We observed the leafhoppers feeding and reproducing on the recipient celery plants. Two months after initiation of experiment, the celery plants were/will be tested for the presence of WX. So far, no recipient celery plants have tested positive for WX. Analysis is ongoing.

A laboratory colony of *C. reductus* had been established on celery seedlings in late 2016; likewise a colony of *C. geminatus* was established on celery in late 2017. We plan to use these leafhoppers for transmission studies in 2018.

Practical application: This information may be used by growers to improve the timing of both chemical and cultural leafhopper management practices, which in turn will improve management of Little cherry disease. Our data show that there are two main time periods of WX-positive leafhopper activity in cherries, although time periods may vary a bit among orchards and year to year. To adopt a more targeted approach to leafhopper management, chemical controls could be implemented during those time periods.

Citations:

Purcell, A.H., Uyemoto, J.K., Van Steenwyk, R.A., Schreader, W.R., Suslow, K.G., and Kirkpatrick, B. 1987. Buckskin disease of cherry. Calif. Agric. 41: 26-27.

Suslow, K.G. and Purcell, A.H. 1982. Seasonal transmission of X-disease agent from cherry by leafhopper *Colladonus montanus*. Plant Disease 66: 28-30.

Uyemoto, J.K. and Luhn, C.F. 2006. In-season variations in transmission of cherry X-phytoplasma and implication in certification programs. J. Plant Pathol. 88: 317-320.

Wolfe, H.R., Anthon, E.W., Kaloostian, G.H., and Jones, L.S. 1951. Leafhopper transmission of western X-disease. J. Econ. Entomol. 44: 616-619.