Northwest Cherry Research Review

Hood River Best Western, Hood River, OR

Tuesday, 11/13/18

Time	Page	Presenter	Project Title	Yrs
8:00		Willett	Welcome	
			Final project reports	
8:15	1	Gibeaut	Preservation and retention of green stems	17-18
8:30	11	Peace	Screening for fruit powdery mildew resistance in the breeding program	17-18
8:45	17	Boyer	Testing an oviposition deterrent for *Drosophila suzukii* in cherry orchards	18
9:00	25	Boyer	Environmental predictors of *D. suzukii* abundance in cherry orchards	18-19
9:15	36	Dong	Ensuring long-distance ocean shipping arrival quality of PNW cherries	16-18
9:30	47	Ferguson	The hunt for leafhopper vectors of Western X in Washington cherries	16-18
9:45	59	Gibeaut	Model reporting	17-18
10:00			Break	
10:15	64	Buchman	*D. suzukii* transgenic, population replacement, eradication, suppression	18
10:30	76	McCord	Sweet cherry breeding: identifying genetically superior selections	18
10:45	84	Hubbard	Managing acclimation, hardiness and bacterial canker	15-17
11:00	95	Ganjyal	Strategies to reduce postharvest splitting of cherries	16-18
	107	Beers	Insecticide resistance of SWD in sweet cherry: ***Written report only***	14-16
Group #			Continuing/short project reports 2:45 - 4:45	
N/A	119	Beers	Integrated pest management of spotted wing drosophila in sweet cherry: ***Written report only***	16-18
1	127	Choi	Non-nutritive sugar-based control strategy for spotted wing drosophila	18-19
1	132	Abrieux	Electronic sensors to capture spatiotemporal population density of SWD: ***No-cost extension***	18
1	139	Wright	How do Western X phytoplasma and LChV-2 cause little cherry disease?	18-20
1	144	Harper	Native hosts of the Western X phytoplasma	18-19
1	148	Wright	Orchard management practices for *Little Cherry Virus 2*	17-19
1	154	Choi	Non-toxic RNAi-based insecticide to control SWD	17-19
2	161	Pscheidt/ Lutes	Mid-Columbia survey for sweet cherry viruses and vectors	18-19
2	168	Whiting	Advancing precision pollination systems for yield security: ***1-year delay in project initiation****	17-20
2	175	Salazar	Predicting flower bud hardiness of commercial sweet cherry cultivars	17-19
2	183	Iezzoni	MSU sweet cherry rootstocks: pre-commercialization	17-19
2	191	Swamy	ABC of sweet cherry powdery mildew: adaptation, behavior and control: ***No-cost extension***	16-18
2	199	Schmidt	Cherry MRL	18-20

FINAL PROJECT REPORT

Project Title: Preservation and retention of green stems

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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 silicate 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

Year 2017

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 tmes than in the soluble Si solutions (Table 1.).

Methods

Year 2018

Si solutions (Table 1) were prepared and applied similarly to 2017 except CropSil was used in place of Mainstay. CropSil is highly soluble source of Si; whereas, Mainstay is a suspension combined with Ca. Up to three applications were done weekly beginning 1-week after bloom in contrast to the single application at 20 days from bloom in 2017 (Table 2).

Specific gravity was assessed by buoyancy. A basket was hung from the under-hook of a balance and the basket completely submerged in water. The balance was tared then dry cherries were place on top of the balance for the first weight. The cherries were then transferred to the basket, submerged, and weighed again. Specific gravity was determined as first weight / (first weight- second weight) at 20 °C.

New plantings (3-5 years) of Bing/K5 and Rainier/K6 as well as the same older (18yrs) Lapins as 2017 were used.

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
CropSil	proprietary	?	1 mL/L	?	14 mL	6.3 mL

Table 2. Phenology and timing of Si sprays at MCAREC

	Lapins		Bing	Rainier	Skeena
	2017	2018	2018		
Bloom 80%	April 23	April 16	April 14	April 16	April 20
First spray	+ 20	+14	+16	+14	+10
Second spray		+21	+22	+21	+17
Third spray		+28	+30	+28	+24
maturity	+82 days	+76 days	+ 71 days	+69 days	+ 76 days

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).

In 2018 specific gravity was assessed as an indicator of fruit development and quality. Specific gravity of fruit is directly related to soluble solids, and may be a superior indicator of fruit development than size alone. The 'lag or resting" phase of growth was clearly evident and coincided well with the pit hardening phase between 30 to 45 days from bloom. Irrigation was started late at MCAREC which affected the mature Lapins block more severely than the young planting of Bing and Rainier. The increase in specific gravity that coincides with final swelling was delayed in Lapins and overall, specific gravity was lower in these tress (fig.6).

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). In 2018 loss from the tree of mature fruit was again minimal.

Stem-pull force at harvest was modestly higher in silicate treatments compared to controls in the two average harvest timing orchards in 2017 (Fig. 2). Interestingly, fruit weight was similarly affected

(Fig. 3). In 2018 no significant difference between Si treatments were observed in stem-pull force (fig. 7) nor in fruit size (fig 8); however, each Si treatment and cultivar showed improvement in fruit quality as assessed by specific gravity (fig. 9).

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.

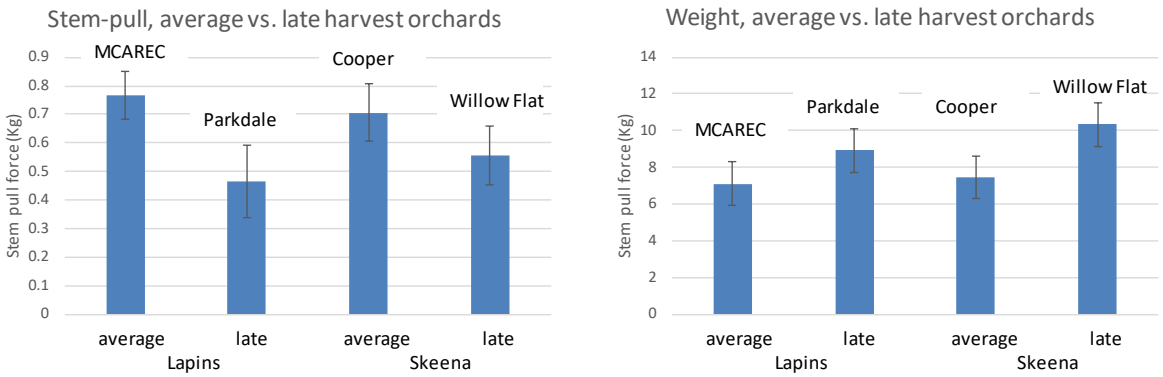

Figure 1. 2017. Lapins at MCAREC and Skeena at Cooper Barn were harvested at average timing from bloom; whereas, Lapins at Parkdale and Skeena at Willow Flat were harvested 7-10 days later. Stem pull-force was assessed with a push/pull force gauge (DS2-11, IMADA). Values are the average and standard deviation of 6-7 trees. Each tree value was the average of fruit from 30 spurs.

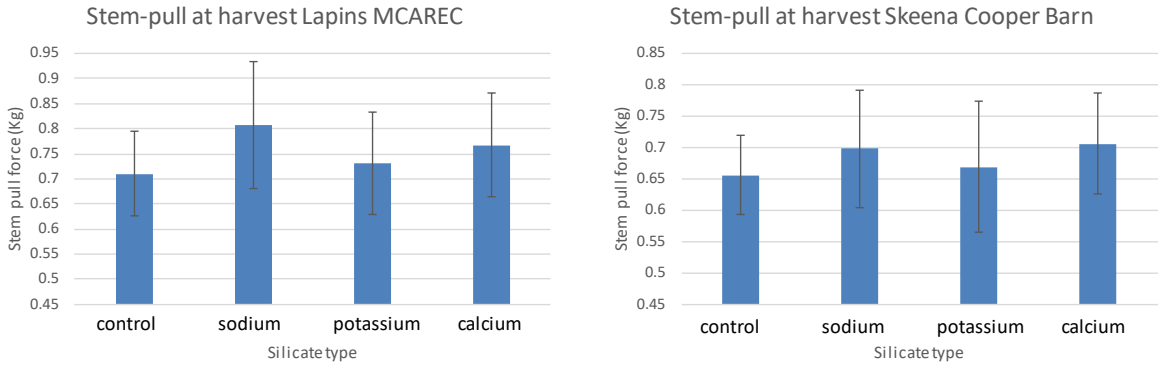

Figure 2. 2017. Stem pull-force was assessed with a push/pull force gauge (DS2-11, IMADA). Values are the average and standard deviation of 6-7 trees. Each tree value was the average of fruit from 30 spurs.

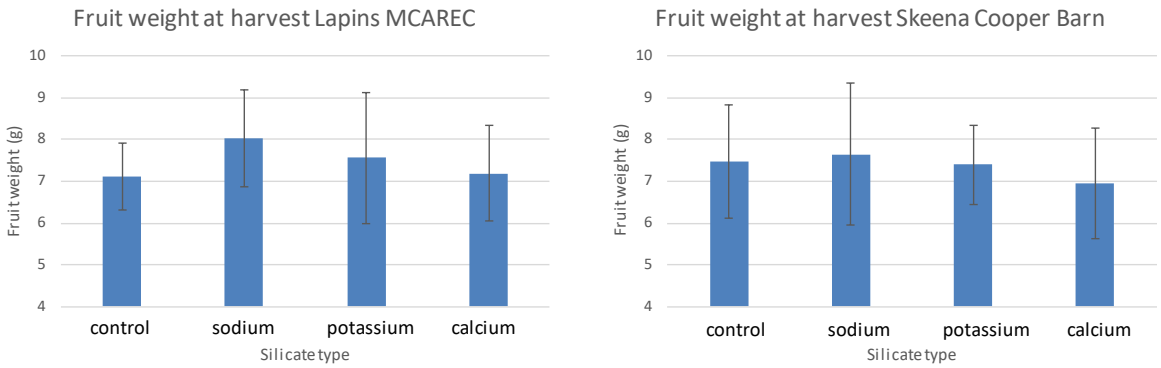

Figure 3. 2017. Individual fruit weights were obtained after the stem-pull force assessment. Values are the average and standard deviation of 6-7 trees. Each tree value was the average of fruit from 30 spurs.

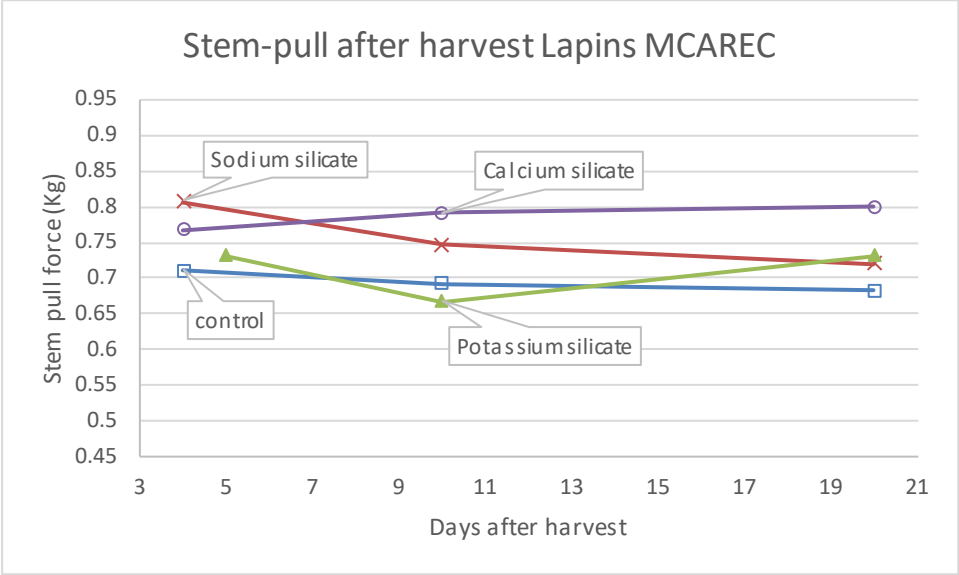

Figure 4. 2017. Stem pull-force was assessed with a push/pull force gauge (DS2-11, IMADA). Values are the average and standard deviation of 6-7 trees. Each tree value was the average of fruit from 30 spurs.

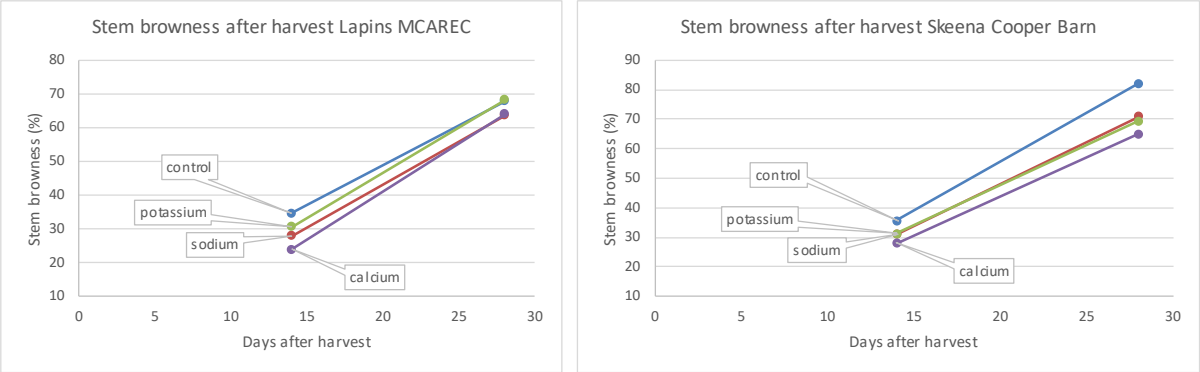

Figure 5. 2017. Browning of stems after harvest was assessed with a chroma-meter (CR 410, Konica Minolta). Greater values indicate that more surface area of the stems are brown.

2018

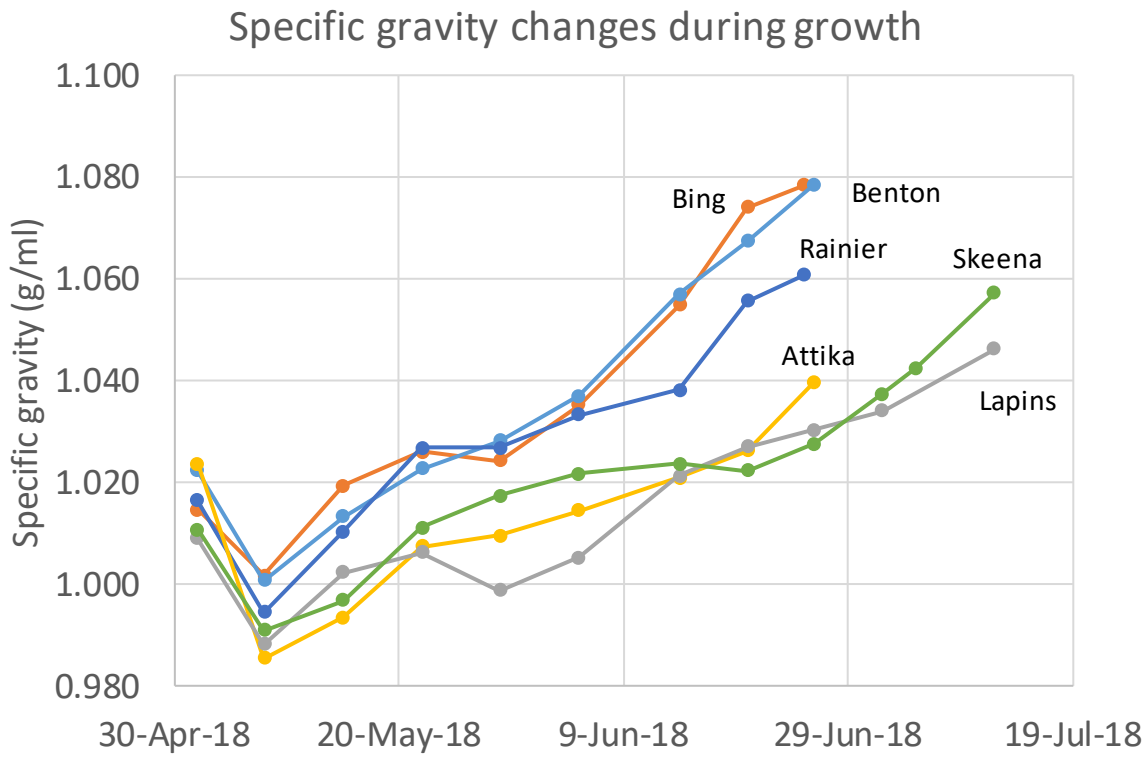

Figure 6. 2018. Specific gravity changes during growth reveal the effects of unintended water stress on Lapins where final swelling was delayed and reduced in magnitude. These trees were not sprayed with Si.

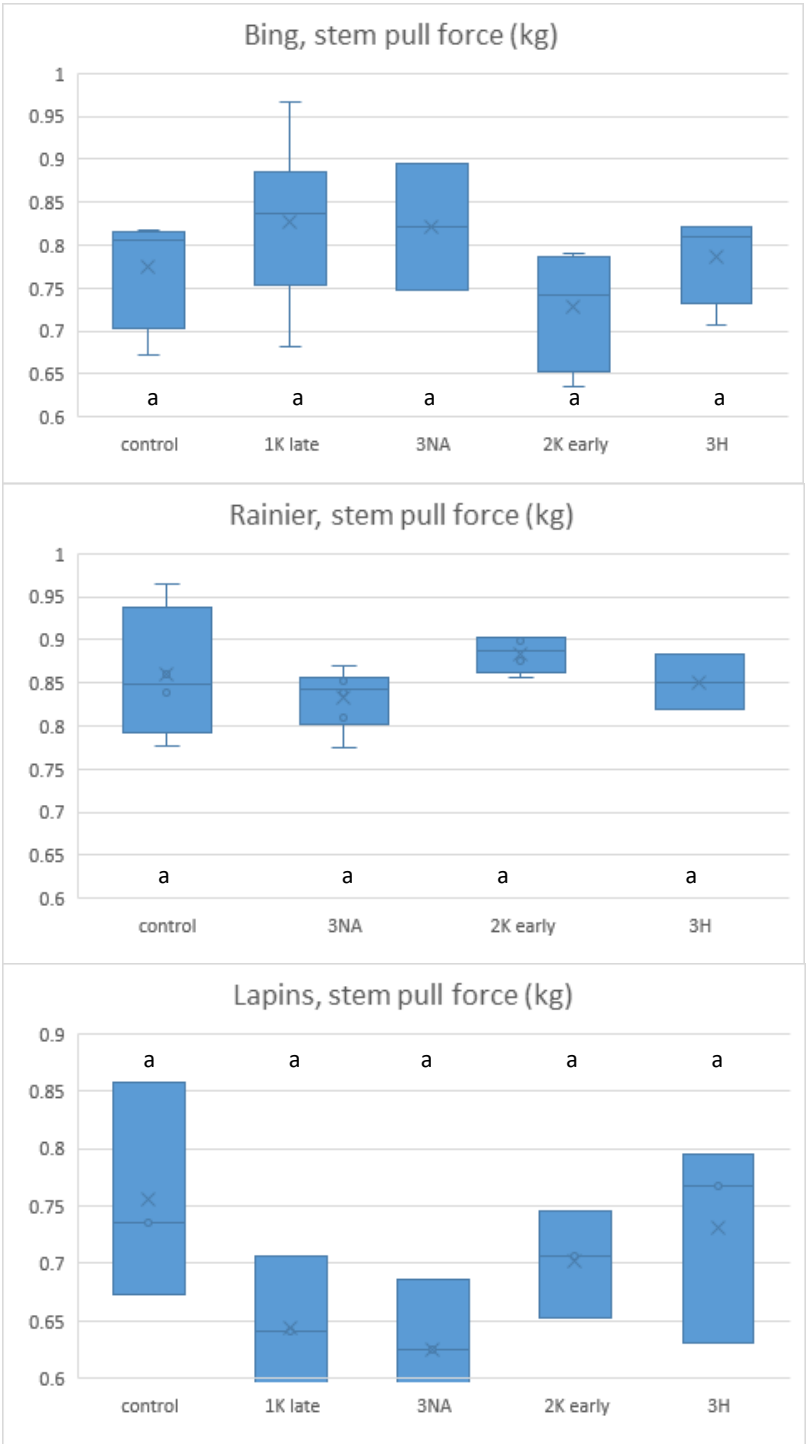

Figure 7. 2018. Stem-pull force showed no significant differences (LSD 95%) between Si treatments. Treatments were as follows; 1K late = potassium form at 21 days post-bloom, 3NA = sodium form at 7, 14 and 21 days post-bloom. 2K = potassium form at 14 and 21 days post-bloom. 3H = humic acid form at 7, 14 and 21 days post-bloom.

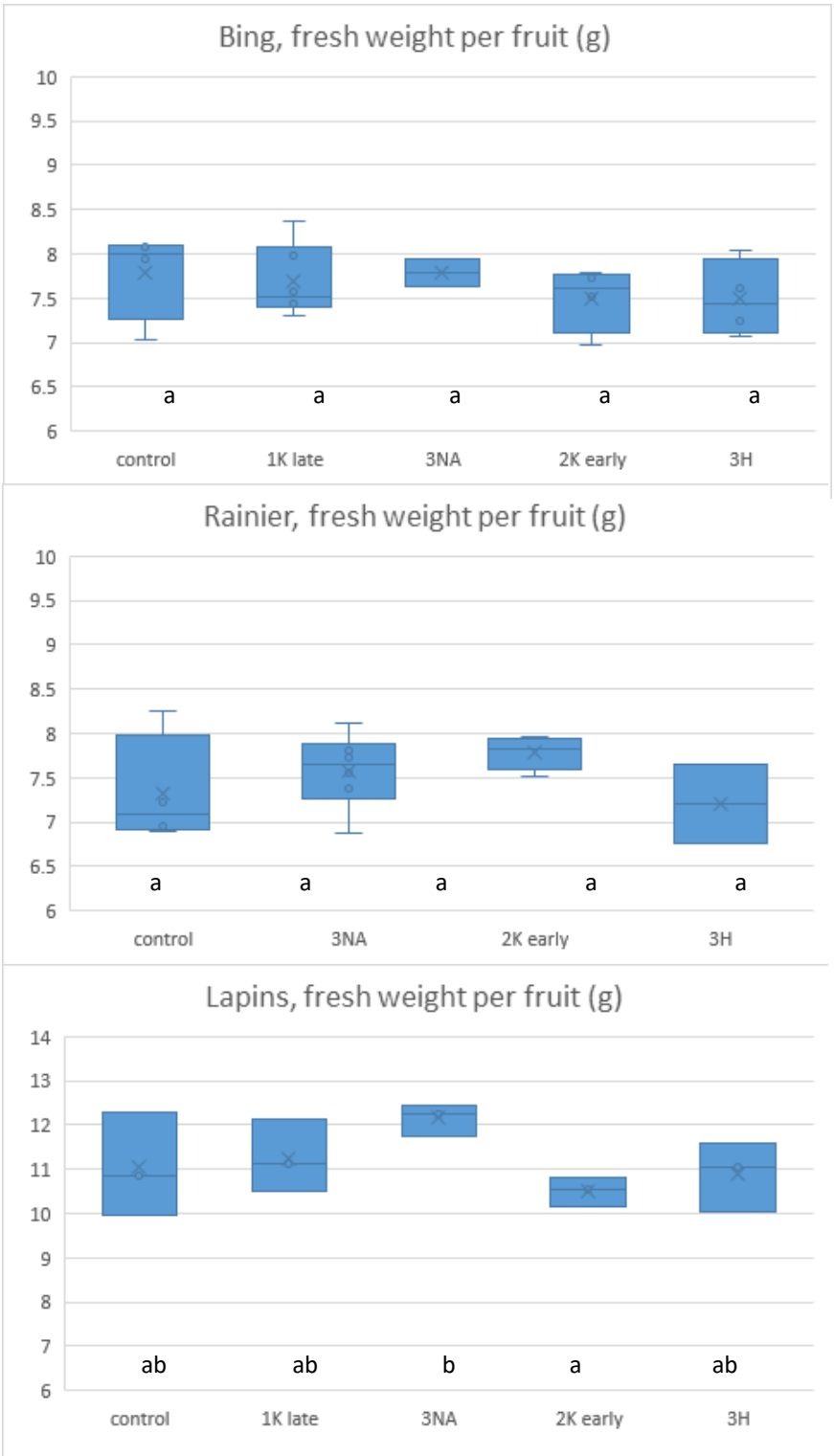

Figure 8. Fruit weight showed no significant differences (LSD 95%) between Si treatments. Treatments were as follows; 1K late = potassium form at 21 days post-bloom, 3NA = sodium form at 7, 14 and 21 days post-bloom. 2K = potassium form at 14 and 21 days post-bloom. 3H = humic acid form at 7, 14 and 21 days post-bloom.

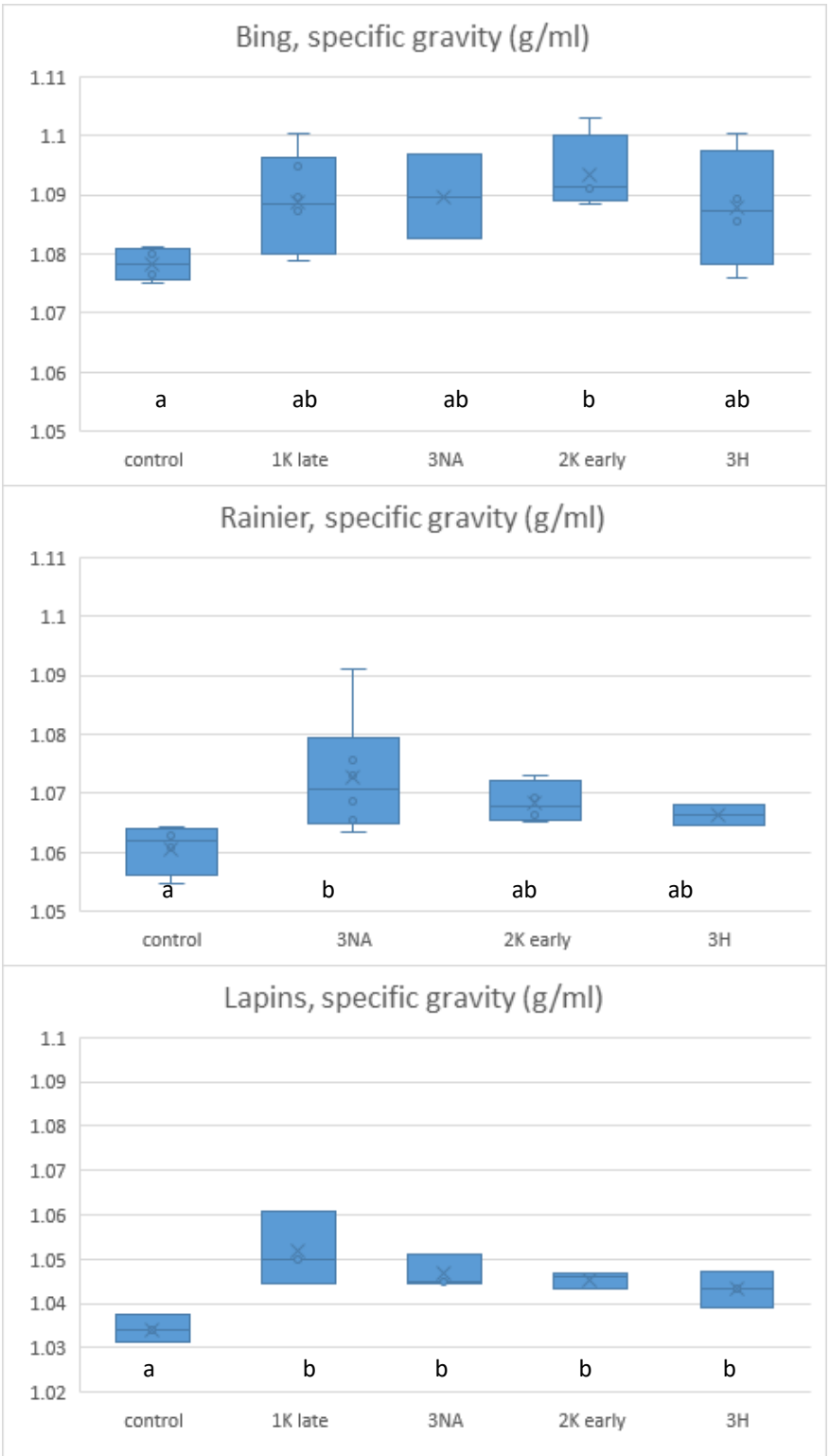

Figure 9. Fruit specific gravity showed some increase (LSD 95%) when Si was applied. Treatments were as follows; 1K late = potassium form at 21 days post-bloom, 3NA = sodium form at 7, 14 and 21 days post-bloom. 2K = potassium form at 14 and 21 days post-bloom. 3H = humic acid form at 7, 14 and 21 days post-bloom.

EXECUTIVE SUMMARY

The effect of Si treatment on stem retention gave mixed results. Only a modest increase was observed 2017 and no positive effect on stem-pull force was observed in 2018.

Post-harvest applications of Si reduced stem browning; however, use of Si solutions in packing-house rinses may create a disposal problem of the alkaline residue.

Silicate supplementation shows promise for improving sweet cherry quality. Effects from a single application near shuck fall (about 14 days after bloom) were modest but indicated an improved vasculature was formed. It is conceivable that improved vasculature could promote fruit size and other quality attributes.

Fruit quality in 2018 was assessed by the specific gravity of bulk samples of fruit (50-300 fruit). Specific gravity is directly related to soluble solids, especially near harvest but may also be used to monitor early fruit development and thereby identify plant stress conditions.

Fruit quality may be improved with Si applications as indicated by a small increase in fruit weight 2017 and by increased specific gravity in 2018.

FINAL PROJECT REPORT

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

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Cooperators: Gary Grove, Neusa Guerra, and Per McCord (WSU IAREC), Alexandra Johnson (graduate student, WSU Pullman)

Other funding sources

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

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

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" PIs: Iezzoni & Peace. Co-PIs include McCord.

Total Project Funding: $57,823

Budget History:

Item	Year 1: 2017	Year 2: 2018
WTFRC expenses		
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
Plot Fees [e]	4,475	4,475
Miscellaneous		
Total	17,348	40,475

[a] 0.25 FTE for an associate in research Prosser (2017 & 2018); 1 month salary and benefits for genetic screening technician in the WSU Tree Fruit Genotyping Lab (2018)

[b] Time slip field workers (2018)

[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

RECAP ORIGINAL OBJECTIVES

Overall 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
- One major factor of genetic resistance ("Pmr1") was determined to be widespread in our breeding program, inherited from PMR-1, 'Moreau', and Mildew-Immune Mazzards
- Complete absence of fruit and foliar PM infection was associated with a dominant allele inherited from any of the above-mentioned sources
- The Pmr1 status (lacking or carrying one or two copies of this genetic factor) was established for selections and parents in the breeding program
- Two effective screening methods are now available for identifying PM resistance vs. susceptibility in the breeding program:
  - Detached leaf disk assay
  - DNA test
- Other genetic factors for resistance and/or suppression of infection severity appear to exist in the breeding program

RESULTS & DISCUSSION

Over two seasons, resistant and susceptible breeding individuals were successfully discriminated for both fruit and foliar PM infection. In the orchard in 2017 and 2018, a high degree of disease spread on fruit and leaves was achieved throughout the experimental block. In the pathology lab in 2017, the detached leaf disk assay was found to be as effective as reliable quantitative PCR for pathogen presence/absence (indicating susceptibility/resistance). The clear discrimination across genetically variable germplasm with known pedigree structure allowed strong conclusions to be made about the genetic control of fruit PM infection:

(1) The main genetic factor giving fruit resistance is the same as that giving foliar resistance (Figure 1).
(2) The foliar resistance genetic factor "Pmr1" from the Toyama selection PMR-1 (that it inherited from 'Moreau', as did 'Chelan') is the same factor 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.
(4) Seedlings with genetic resistance to fruit-and-foliar PM infection are common throughout the PNW sweet cherry breeding program because of extensive use of parents with the resistance allele for the past decade.

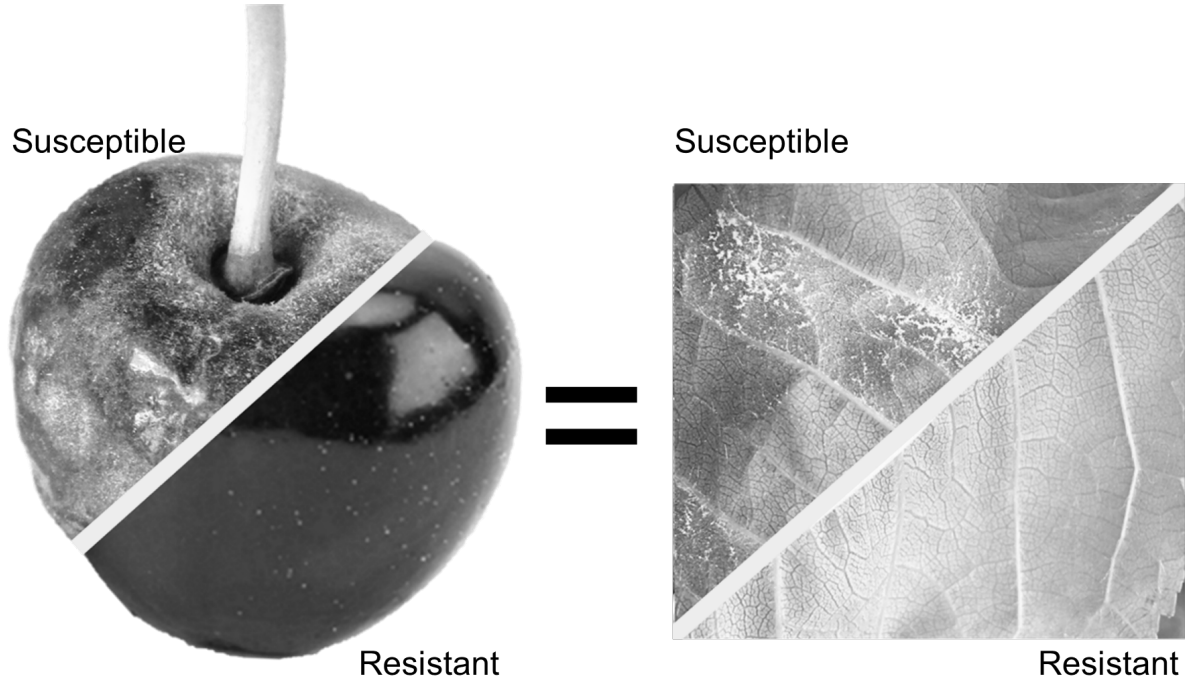

Figure 1: The main discovery of the project. Genetically, resistance vs. susceptibility to powdery mildew of fruit is the same as for leaves. The genetic factor (allele) responsible for resistance is called "Pmr1", which several breeding parents have. Trees having Pmr1 in their genetic makeup are resistant to both fruit and foliar PM. Trees without Pmr1, which includes most cultivars of the PNW industry, are susceptible to PM.

From 2018's field evaluation of the breeding program's parent block (B53), across which the pathogen was allowed to spread, all parents having Pmr1 in their genetic makeup had no PM infection symptoms – zero mildew for all replicate trees examined. Of the ~40 parents without Pmr1, only four had zero mildew for all of their replicate trees – which included 'Regina' and 'Hedelfinger'.

Other sources of resistance might exist in the material tested. In the genetically variable families of the evaluated RosBREED block (C53), some cases of zero-mildew (both fruit and foliar) were observed in trees of offspring not carrying a Pmr1 resistance allele. Mirroring observations in the B53 parent block, 'Regina' was a parent of several of these offspring, as was 'Venus' (whose mother is 'Hedelfinger'). Some families had offspring trees with no fruit incidence and low foliar incidence, for which 'Regina' and 'Venus' were also commonly the parents. However, the 2018 season's orchard evaluations of fruit infection did not include multiple days of assessment through the season and had several discrepancies between tree replicates of the same genotype and with the 2017 season's orchard observations. These issues undermined our ability to effectively dissect the genetics of quantitative resistance beyond Pmr1 that the 2017 results had pointed to.

From whole-genome DNA profiling (results of the RosBREED project), ‘Regina’, ‘Venus’, several other parent cultivars*, and at least a third of selections of the breeding program were determined to carry an allele at the *Pmr1* chromosomal region that looks similar to that of Mildew-Immune Mazzards – so we call this the “Pmr1-like” allele. Two seasons of observations found that most offspring with the Pmr1-like allele became PM-infected. However, in 2018 about one in every four ‘Regina’ offspring with the Pmr1-like allele were not infected (6 of 22) whereas all ‘Regina’ offspring (26) without that allele became infected. So, the Pmr1-like allele might be a “leaky” version of normal Pmr1 that sometimes provides the possibility for zero or low mildew infection but depends on environmental conditions or depends on alleles of other genomic regions present in some offspring. Or, the resistance (or lower susceptibility) of ‘Regina’ and offspring might be entirely due to genetic factors independent of Pmr1.

* Other cultivars we determined from DNA profiling to have a Pmr1-like allele include: ‘Venus’, ‘Schneiders’ (the mother of ‘Regina’), ‘Hedelfinger’ (the mother of ‘Venus’), ‘Sato Nishiki’, ‘Early Burlat’, and ‘Cristobalina’.

The clear, “single-gene” resistance provided by Pmr1 leads to the question of whether it can be overcome by the pathogen. If not, a powerful breeding strategy would be to eventually have all new cultivars carry this allele. If it can be overcome, it would still be useful to continue to enrich the breeding genepool with this allele. Another question revolves around the genetic control of the alternative PM resistance (or reduced susceptibility, tolerance, or leaky resistance) that a few cultivars such as ‘Regina’ have. If those further genetic factors could be identified, having a DNA test for them and knowing which parents, seedlings, and selections carry them would facilitate additional breeding strategies to combat this high-priority disease problem for the PNW cherry industry.

Two assays are now available for routinely discriminating resistance vs. susceptibility for both fruit and foliar PM resistance in cherry breeding germplasm: a DNA test and the detached leaf assay. These two efficient assays were confirmed to be effective for both fruit and foliar PM resistance, and both assays can be used on seedlings through to mature trees without exposing the whole plants to the pathogen. The DNA test can be integrated into routine operations of greenhouse-stage DNA testing of seedlings. The DNA test can also be used to confidently plan crosses that result in a high proportion of seedlings resistant to fruit and foliar PM, and to ascertain which selections have genetic resistance.

EXECUTIVE SUMMARY

This two-year project aimed to develop a reliable, efficient assay for revealing genetic potential for fruit powdery mildew (PM) resistance for routine used in the PNW sweet cherry breeding program. The aim was achieved by evaluating a large, representative germplasm set containing genetic variability for PM resistance. Two assays are now available for routinely discriminating resistance vs. susceptibility for both fruit and foliar PM resistance in cherry breeding germplasm: a DNA test and the detached leaf assay.

Over two seasons, resistant and susceptible breeding individuals were successfully discriminated for both fruit and foliar PM infection. In the orchard, a high degree of disease spread on fruit and leaves was achieved throughout the experimental block. In the pathology lab, the detached leaf disk assay was as effective as reliable quantitative PCR for pathogen presence/absence. The clear discrimination across genetically variable germplasm with known pedigree structure allowed strong conclusions to be made about the genetic control of fruit PM infection:

(1) The main genetic factor giving fruit resistance is the same as that giving foliar resistance.
(2) The foliar resistance genetic factor "Pmr1" from the Toyama selection PMR-1 (that it inherited from 'Moreau', as did 'Chelan') is the same factor 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.
(4) Seedlings and selections with genetic resistance to fruit-and-foliar PM infection are common throughout the PNW sweet cherry breeding program because of extensive use of parents with the resistance allele in the past decade.

Field observations of the 2018 season confirmed those of 2017 for the strong effect of Pmr1. However, the second season's orchard PM evaluations of the genetically variable RosBREED germplasm set were not fine-scaled enough to dissect degrees of resistance beyond Pmr1, i.e., that of 'Regina', 'Venus', and some other cultivars.

The clear, "single-gene" resistance provided by Pmr1 leads to the question of whether it can be overcome by the pathogen. If not, a powerful breeding strategy would be to eventually have all new cultivars carry this allele. If it can be overcome, it would still be useful to continue to enrich the breeding genepool with this allele. Another question revolves around the genetic control of the alternative PM resistance (or reduced susceptibility) that a few cultivars such as 'Regina' have. If those further genetic factors could be identified, having a DNA test for them and knowing which parents, seedlings, and parents carry them would facilitate additional breeding strategies to combat this high-priority problem for the PNW cherry industry.

FINAL PROJECT REPORT

Project Title: Testing an oviposition deterrent for *Drosophila suzukii* in cherry orchards

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Cooperators: Steve Castagnoli (Director, Mid-Columbia Agricultural Research and Extension Center), local cherry growers.

Budget: $12,500 **Year 1 (2018):** $12,500

Other funding sources
None

Budget 1
Organization Name: OSU-MCAREC **Contract Administrator:** R.S Karow
Telephone: (541) 737 4066 **Email address:** Russell.Karow@oregonstate.edu

Category	Details	Amount Requested
Salary:	Technician ($15/hour, 400 hours)	$6,000
Benefits:	OPE ($647 per term)	$720
Travel:	Domestic (in state)	$2,000
Supplies:	Insect monitoring and rearing supplies PVC pipes and netting for field cages Reagents for preparing gum	$2,850
Services:	Plot fee at MCAREC (0.3 acres)	$930
Total Requested		$12,500

Budget narrative:
Technician assistance will be used for data collection, field trial setup and sample maintenance. Benefits are 12% of salary, as per university guidelines. Domestic travel will pay for travel to field trial sites. Supplies are for netting materials, insect rearing colonies, reagents, and spray backpack and nozzles.

ORIGINAL OBJECTIVES:

1. To test the oviposition deterrent effects of a solid and liquid gum matrix for *Drosophila suzukii* (SWD) in cherry trees.
2. To test the potential for solid gum baits as efficient monitoring tools for *Drosophila suzukii* (SWD) oviposition.

SIGNIFICANT FINDINGS

- Liquid gum baits hold promise for decreasing SWD oviposition in cherry at a small scale, in bagged cherry clusters.
- At a larger scale (whole-tree enclosures), liquid gum baits were not effective at reducing cherry infestation.
- Due to the limited testing period, it was inconclusive whether gum bait stations can be used as an oviposition monitoring tool in cherry orchards.

RESULTS AND DISCUSSION

Oviposition deterrence

We tested liquid gum baits in small cherry clusters and whole tree enclosures in a young unsprayed cherry block (cv. Lapin) on 25 Jun 2018, and 5 Jul 2018.

For branch enclosures, we selected a cluster of 10-15 cherries, and covered it with a mesh bag. We selected 10 clusters to treat with gum, and 10 clusters as an untreated control with flies. Five mature males and five mature females were released inside each mesh bag between 6pm and 8pm inside each mesh bag. Flies were provided with a foam plug soaked in sugar water as a food source (Fig. 1). We placed a cloth rectangle with 5mL of liquid gum inside 10 treated mesh bags. As a laboratory control, we placed 10 cherries, 5 males, 5 females, and a cotton ball with sugar water inside a pint vented container, where flies could freely oviposit. After 72 h, we collected the cherries and incubated them for further 3 days in laboratory conditions. After this period, we extracted larvae from each cherry using a salt flotation method (Figs. 2, 3), and we counted 1) number of cherries with (any) larvae in each branch cluster, and 2) total number of larvae extracted from each branch cluster.

Figure 1. Cherry branch clusters to test gum.

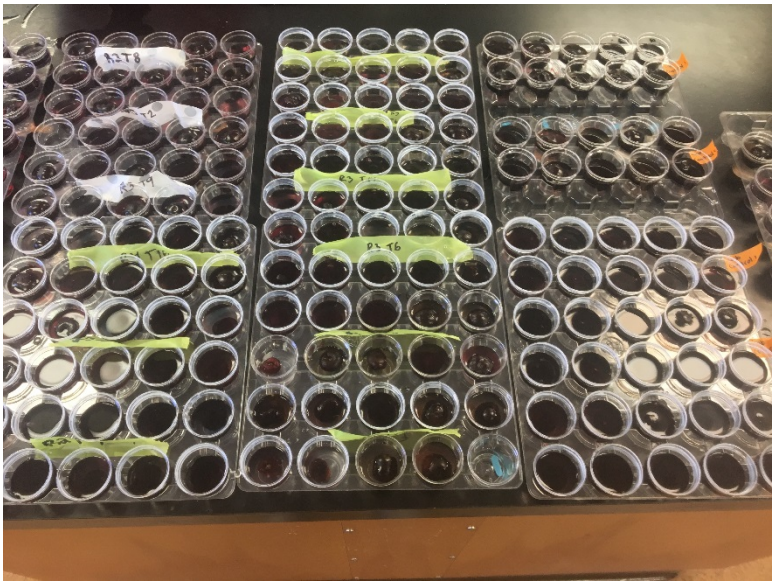

Figure 2. Salt flotation method to extract SWD larvae from individual cherries.

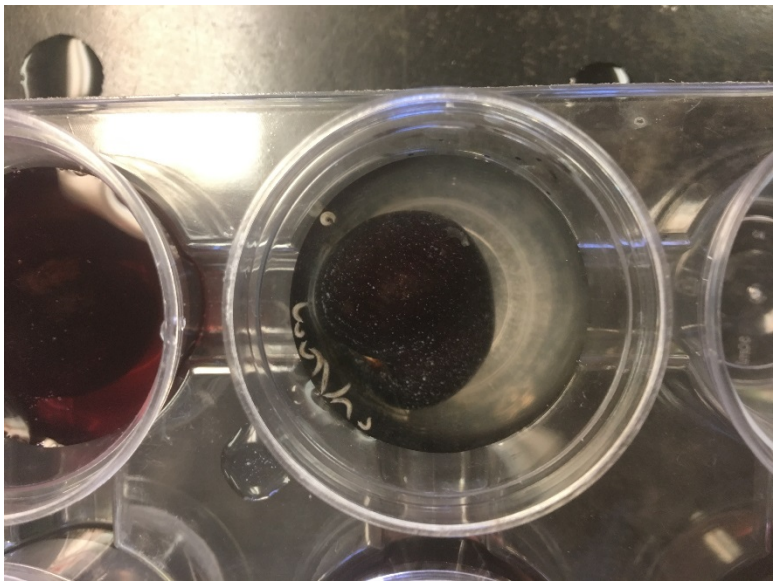

Figure 3. SWD larvae extracted from a single cherry.

There was a tendency for gum-treated clusters to have fewer infested cherries compared to untreated clusters (df = 1, 43, F = 12.67, $p < 0.01$; difference between gum-treated and untreated clusters $p = 0.055$). On the first date tested, there was on average a 33% reduction on number of infested cherries, on the second date tested, there was a 44% reduction on number of infested cherries (Table 1, Fig. 4).

Table 1. Number of infested cherries (mean ± SD) in a cluster of 10-15 cherries with and without a liquid gum application

Treatment	25 Jun 2018	5 Jul 2018
Gum	1 ± 1.3	2.4 ± 1.5
Untreated	1.5 ± 1.8	4.3 ± 2.2
Lab control	9.8 ± 0.4	7 ± 1.2

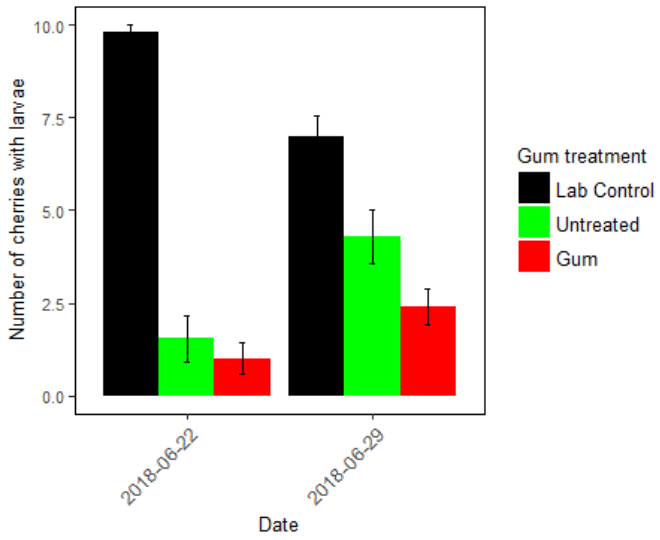

Figure 4. Number of infested cherries (mean ± SE) in each cluster treated and untreated with gum.

There was a non-significant tendency for gum-treated clusters to have fewer total larvae compared to untreated clusters (df = 1, 43, F = 10.16, $p < 0.01$; difference between gum-treated and untreated clusters $p = 0.065$). On the first date tested, there was on average a 40% reduction on number of total larvae, on the second date tested, there was a 58% reduction on number of total larvae (Table 2, Fig. 5).

Table 2. Total SWD larvae (mean ± SD) extracted from a cluster of 10-15 cherries with and without a liquid gum application

Treatment	25 Jun 2018	5 Jul 2018
Gum	1.3 ± 1.9	4.4 ± 4.1
Untreated	2.2 ± 2.7	10.5 ± 9.7
Lab control	45.0 ± 16.7	19.2 ± 17.2

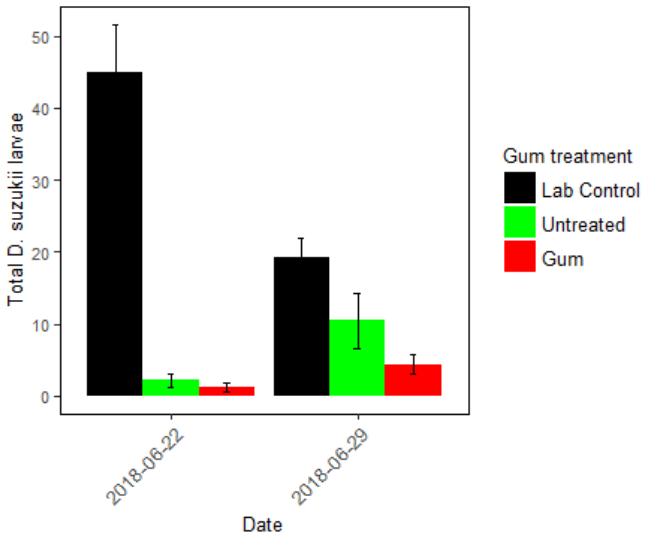

Figure 5. Number of extracted SWD (mean ± SE) in each cluster treated and untreated with gum.

For whole-tree enclosures, we covered 20 trees with a mesh cage (6ft x 6ft x10ft; Fig. 6). Inside each cage, 50 mature males and 50 mature females were released between 6pm and 8pm. 10 gum-treated trees had a cloth rectangle soaked in 100mL of liquid gum attached to a tree branch in the middle of the canopy (Fig. 7), and 10 untreated trees did not have any gum. After 72 h, we collected 20 cherries from the top of the canopy, 20 from the middle, and 20 from the bottom. Cherries were incubated for 3 more days in laboratory conditions. After this period, SWD larvae were extracted from the cherries using a salt flotation method.

Figure 6. Whole tree enclosures to test liquid gum.

Figure 7. Cloth rectangle soaked in 100mL of liquid gum, and attached to a branch in the middle of the tree canopy.

There was no effect of gum treatment in the total number of larvae extracted from the cherries ($df = 1, 93$, $F = 0.05$, $p = 0.81$). There was a location effect, and fewer larvae were extracted from the top of the canopy compared to the middle and bottom ($df = 2, 93$, $F = 22.08$, $p < 0.01$).

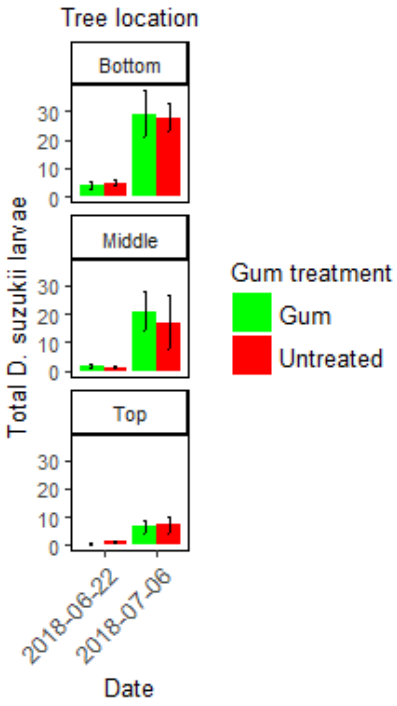

Figure 8. Total SWD larvae extracted from 20 cherries in the top, middle and bottom canopy in trees treated and untreated with liquid gum.

In general, these results showed that in small scales gum can be effective in reducing number on cherries infested, and total number of larvae. But when applied in large scale to a whole tree, gum was not effective in reducing infestation. This could be due to the total volume of gum applied needed to be effective; in this study we applied 100 mL of gum in each whole-tree enclosure, and it is possible that a larger amount is required. The fact that more larvae were collected from the middle and bottom canopy suggests that, with a limited quantity of product available, application efforts should be focused in this area. Another issue was that after 3 days of application, the gum was already dry and powdery, and possibly less attractive to SWD females. Other ongoing trials are currently exploring ways to maintain gum moisture (for example, by utilizing current irrigation systems). This gum formulation continues to be tested and improved, and more field trials are need to determine its effectiveness.

Monitoring

We tested solid gum as a monitoring tool for early oviposition in cherry orchards. An oviposition monitoring station consisted of 100mL of solid gum bait deposited in a petri dish, and placed on a tree branch underneath a commercial SWD Scentry lure. We placed two monitoring stations on the edges of a 0.3 acre cherry block (cv. Lapin) during May 2018. Due to the limited availability of gum, this experiment could not be replicated in other orchards, or tested for a longer period. Every week, the gum bait was replaced with fresh gum. Each gum bait was inspected under the microscope for the presence of SWD eggs. We additionally collected 10 cherries from 18 trees every week, and tested for SWD infestation using the salt flotation method described above.

During the oviposition monitoring period, no SWD eggs were found in the gum bait, and none of the cherries collected from the trees were infested with SWD. These results are inconclusive, and could be due to many factors: 1) SWD females were not attracted to the gum baits to oviposit, or 2) the SWD population in this orchard during the trialed period was too low to detect any oviposition. Future trials should expand the monitoring period throughout the growing season.

EXECUTIVE SUMMARY

Project Title: Testing an oviposition deterrent for *Drosophila suzukii* in cherry orchards.

As the quest for creating environmentally-friendly ways to control *Drosophila suzukii* (SWD) infestations in cherries continues, we here tested a food-grade product (gum) developed by our co-investigators. In preliminary laboratory trials, this gum substrate was shown to be very attractive to female SWD. When presented in choice experiments with other fruit, female SWD preferred to oviposit in the gum substrate, and consequently and reduced the number of eggs laid in multiple fruit. The effect of this gum in cherries at a field scale had not yet been tested.

We tested liquid gum in bagged cherry clusters in tree branches in the field, and whole-tree enclosures. When SWD were bagged in cherry clusters with 5 mL of gum, there was a tendency to have fewer infested cherries, and fewer SWD larvae. The number of infested cherries enclosed with liquid gum was reduced by 33-44%, compared to untreated control (with no gum). Likewise, the total number of SWD larvae extracted from cherry clusters enclosed with liquid gum was reduced by 40-58%.

In whole tree enclosures, we did not find an effect of exposure to gum on SWD infestations. Cherries collected from trees with gum had a similar number or SWD larvae compared to trees with no gum. SWD infestation was higher in the bottom and middle canopy, compared to the top canopy. In whole-tree enclosures, we applied 100mL of gum, and it is possible that this amount is not enough to attract SWD females to oviposit. Future studies should focus on testing variable amounts of gum in whole trees, refine optimal application rates, and the best locations to apply within the canopy.

In addition to oviposition deterrent field trials, we also tested solid gum as a potential oviposition monitoring tool in the field. We placed exposed petri dishes with solid gum and a commercial SWD lure on the edges of an unsprayed cherry orchard during the early season. Every week, we retrieved the gum and inspected it for the presence of SWD eggs. We did not find any eggs in the gum, but it is possible that we only monitored during a short period during the early season, when overall SWD populations are low in the field. Future studies should explore whether these gum lures can be used to monitor field oviposition throughout the cherry growing season.

FINAL PROJECT REPORT

Project Title: Environmental predictors of *Drosophila suzukii* abundance in cherry orchards

PI: Dalila Rendon, Postdoctoral research associate.
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Collaborators: Gabriella Boyer, MCAREC
Steve Castagnoli, Director, MCAREC.
Orchard owners in Hood River and Wasco counties
U.S Forest Service, Mt. Hood national forest.

Total Project Request: Year 1 (2018): $20,305 **Year 2 (2019):** No funds requested

Other funding sources: Genomic work for identification of small-nucleotide polymorphisms to characterize subpopulations of SWD will be done in Dr. Chiu's laboratory in UC Davis, and this work is covered by a current NIFA-SCRI grant.

Budget 1

Organization Name: OSU-MCAREC **Contract Administrator**: L.J. Koong
Telephone: (541)7374866 **Email address:** l.j.koong@oregonstate.edu

Item	2018	2019
Wages[1]	$14,400	
Benefits[2]	$1,400	
Supplies[3]	$2,365	
Travel[4]	$2,140	
Total	$20,305	$0

[1]Wages: 800hr for a Biological Science Tech. at $18.00/hr (20 hrs per week for 40 weeks, 0.5 FTE), 3% salary increase for year 2.
[2]Benefits: 10% of the wage.
[3]Supplies: Traps, lures, containers, insect cages, colony bottles, fly diet. HOBO temperature dataloggers and reader, software license for analysis of ecological data (CANOCO). License renewals and replacement for lost/broken dataloggers for year 2.
[4]Travel: Weekly travel to orchards and forest sites (~100 miles) for 40 weeks at $0.535 per mile

No funds will be requested from WTFRC for this project for 2019

ORIGINAL OBJECTIVES

1) To determine environmental predictors of SWD occurrence and fecundity such as altitude, monthly temperatures, host plant phenology, presence of pollinator refuges, and level of urbanization in cherry orchards and unmanaged areas.
2) To provide an improved risk assessment tool to improve the efficacy/accuracy of scheduling and frequency of insecticide applications in cherry orchards based on annual environmental and site variability.

The primary objective of this proposal was to determine the dispersal capabilities of SWD in no-agricultural areas where the pest is known to thrive. This projects falls within the research priority topic "SWD: Predicting location and intensity of infestations". We conducted this work in the Mt Hood National Forest, and rural/urban areas of Hood River and Wasco counties.

This study also aimed to help identify single nucleotide polymorphism (SNP) markers that can be used to develop genotyping assays to examine dispersal between geographical sites. SNP genotyping should be able to shed light on the origin of these flies early in the growing season to answer the following questions: Did they migrate from warmer low-elevation areas? Or are they overwintering populations from the previous season?

Together, an analysis of environmental variables and the identification of SNP markers will help pinpoint wild sink-and-source sites, i.e., spots where SWD can overwinter and then migrate from agricultural areas to wild unmanaged areas, and determine when SWD is most likely to be found in these areas.

SIGNIFICANT FINDINGS

- We found that environmental predictors of SWD abundance vary depending on winter temperatures. In years with cold winters, degree days (DD) accumulated at the end of the spring equinox (June 20), along with average maximum temperature during winter, and accumulated precipitation during winter were the strongest predictors of total SWD collected in June. In years with mild winters, site setting (urban/agricultural/forest) and winter precipitation were the best (but still weak) predictors of SWD abundance.
- Tracking winter temperatures (for example, number of days with minimum temperatures below 23F) are an important tool for making early spray management decisions. However, there is still a lot of unexplained variability in the data, and environmental predictors do not provide clear thresholds.
- Live traps were not effective as monitoring tools. In general we found more abundance of SWD in forest sites compared to orchards, but it is not clear whether elevation or host fruit had an effect.
- SWD was reared from wild hosts such as huckleberries and thimbleberries.
- Future molecular work can help disentangle whether forest and orchard populations are isolated, or migrate between sites.

RESULTS AND DISCUSSION

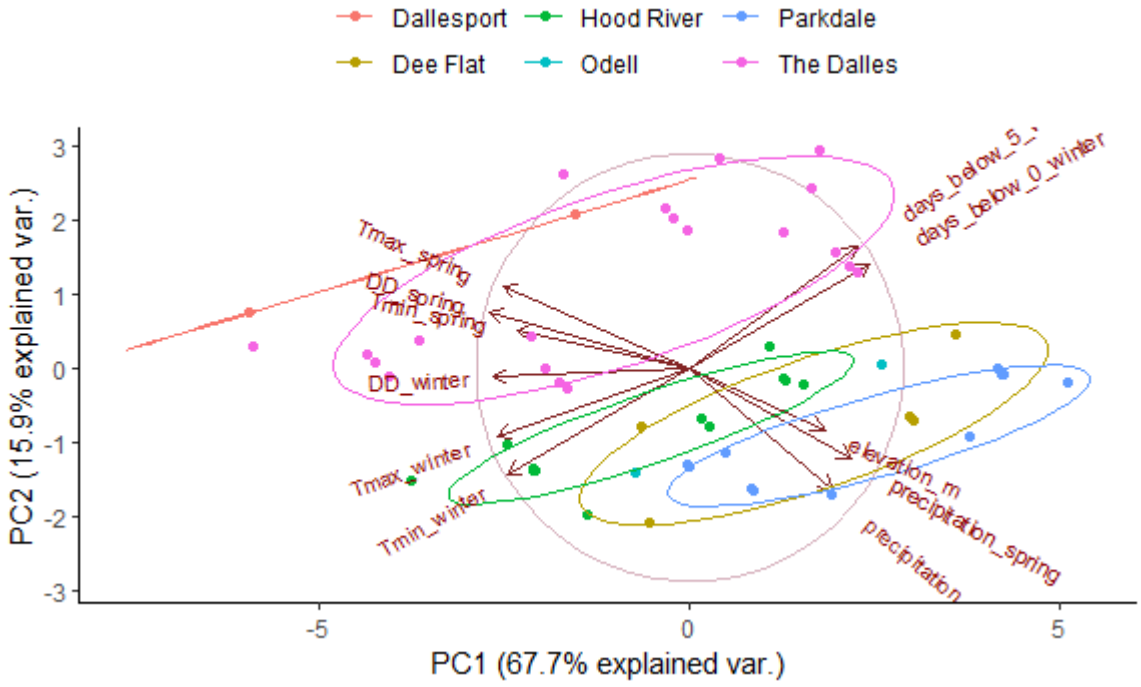
1) Which environmental factors best predict the total number of SWD trapped in various areas in Hood River and Wasco counties during 2017 and 2018?

To answer this question, we used the data from the trap network deployed by OSU extension in 2017 and 2018 in Hood River and Wasco counties. As an assessment of risk infestation, the response variable selected was total number of SWD collected in each trap during June of each year.
For each trap, we selected the nearest weather station from the U.S pest website (http://uspest.org/cgi-bin/ddmodel.us?sta=E9560&mdt=ins&spp=swd&cal=S1&tlow=50&thi=88&stm=1&std=1&styr=18&enm=9&end=20&cel=0&fcast=1&spyr=0&shd=1&mkt=0&mkg=1&ipc=1&evnts=3)
From each weather station, we extracted the following information:

- Average minimum temperature during winter solstice
- Average maximum temperature during winter solstice
- Average minimum temperature during spring equinox
- Average maximum temperature during spring equinox
- Total number of days with temperatures below 23F (-5°C) during winter solstice
- Total number of days with temperatures below 32F (0°C) during winter solstice
- Degree-days (DD) accumulated at the end of winter solstice (on March 20, using lower threshold of 50F, 10°C)
- Degree-days (DD) accumulated at the end of spring equinox (on June 20, using lower threshold of 50F, 10°C)
- Total precipitation during winter solstice
- Total precipitation during spring equinox
- Elevation

To reduce the number of environmental variables that might be correlated, we performed a principal component analysis (PCA). This tells us how each variable is associated with each other, visualized by location (Hood River / Odell / Parkdale / The Dalles / Dallesport / Mosier).

Figure 1. Biplot of principal component analysis of environmental variables.



In this case, the first two components explain 83% of the variation (PC1 + PC2, Fig. 1). Variables that point in similar (or directly opposite directions) are highly correlated, and therefore redundant. For instance, Tmax_winter (average maximum temperature during winter solstice) is positively correlated with Tmin_winter (average minimum temperature during winter solstice), and inversely correlated with number of days below freezing, and number of days below 23F. In case of highly correlated variables, it is more adequate to choose ones that have higher loadings on the first principal component.

Table 1. Loadings of each variable on principal components.

	Tmin_winter	**Tmax_winter**	**Tmin_spring**	**Tmax_spring**
PC1	-0.3146984	-0.3296766	-0.2972291	-0.3205501
PC2	-0.3806404	-0.2416034	0.133411	0.2904705
	days_below_5_winter	**days_below_0_winter**	**DD_winter**	**DD_spring**
PC1	0.2881748	0.3088001	-0.3361714	-0.3434887
PC2	0.4302847	0.3631645	-0.031987	0.1935672
	precipitation_winter	**precipitation_spring**	**elevation_m**	
PC1	0.2467444	0.2771615	0.2325543	
PC2	-0.432349	-0.3223878	-0.2195105	

After determining the loadings of each variable in the PCA, we selected a smaller number of variables to build a general linear model (GLM) that best predicted abundance of SWD collected during the month of June. In this case, we selected the environmental variables "average maximum temperature during winter solstice", "precipitation during winter solstice" and "degree-days accumulated during spring equinox".

In addition to the weather data extracted from the stations, we also selected the following categorical variables from each trap site:

- Fruit host (cherry / blackberry / peach)
- Setting (agricultural / urban / forest)
- Management (managed / unmanaged)
- Lure type (Apple cider vinegar ACV / ACV+ Trece commercial lure).

Using the Akaike criterion information (AIC) on environmental and categorical variables, we built the model that best described the abundance of SWD collected in June by dropping variables that did not contribute to data fit. Data was analyzed separately for 2018 and 2017. Due to the high number of traps without SWD in 2017, we used a zero-inflated Poisson model, and for 2018, we used a general linear model.

For 2017, the best model was:
Total SWD June = 0.037(DD spring) + 0.009(precipitation winter) – 2.76(Tmax_winter) – 5.87.
The strongest predictor in this model was DD spring ($p = 0.04$)

For 2018, the best model was:
Total SWD June = 8.79(setting forest) + 0.85(setting urban) + 0.009(precipitation_winter) +3.10
However, all these variables were weak predictors and there is a lot of unexplained variability.

Why were the models so different in 2017 and 2018?

Let's have a look at the relationship between the predictors and SWD (Figure 2). There is a clear separation between 2017 and 2018 data in variables such as mean minimum and maximum temperatures during winter (2017 having lower values, Fig. 2a, b), and number of days below 23F in winter (more days in 2017, Fig. 2e). This suggests that, in years with cold winters, the best predictor of SWD abundance was degree days (DD) accumulated during the spring equinox (until June 21, Fig. 2h). But in years with milder winters, the accumulation of DD will not be a reliable predictor of SWD abundance, and instead, the spatial setting (urban/ forest / agricultural, Fig 2n) and the precipitation during winter (Fig. 2i) can better predict where SWD will be more abundant.

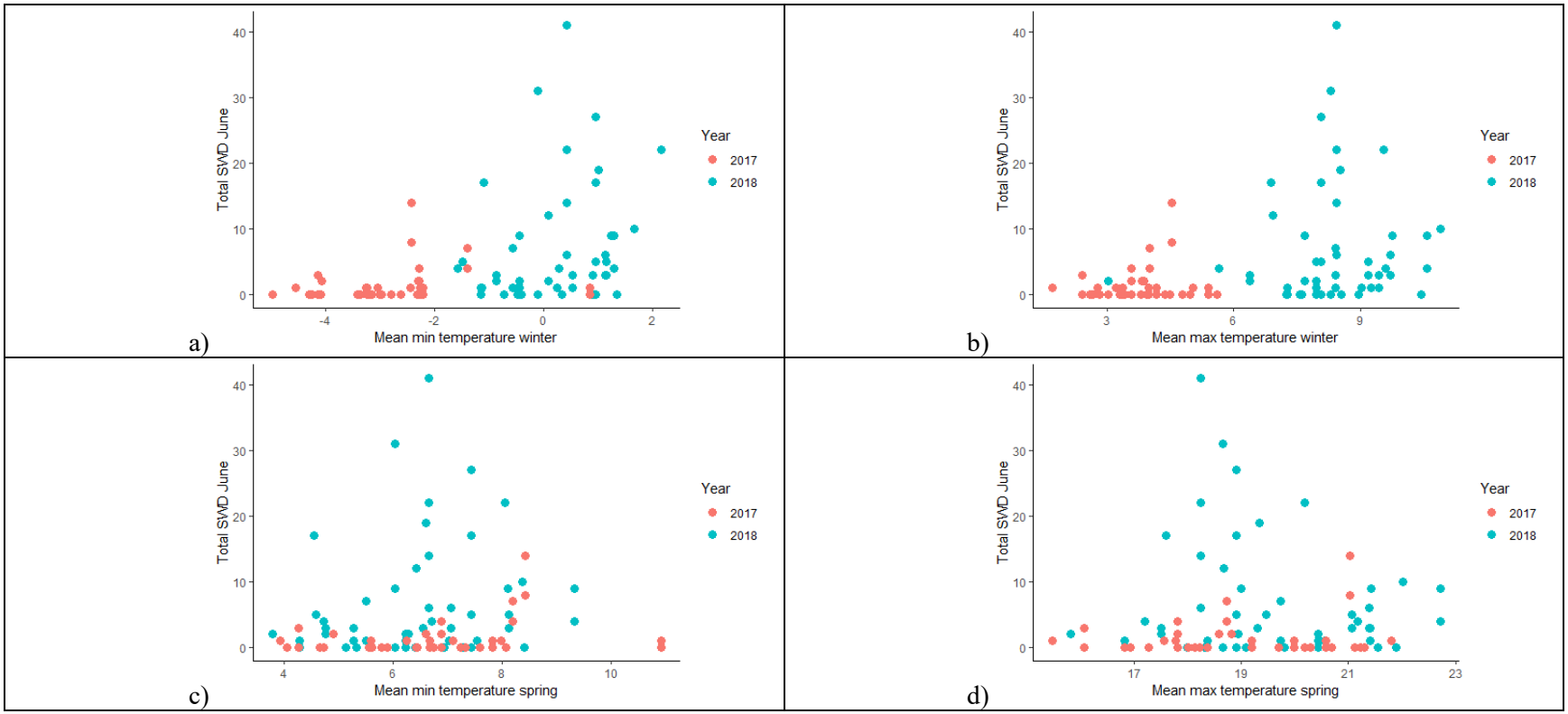
A predictor that is easy to track in terms of quantifying how cold a winter is as it progresses is number of days below 23F. There was a clear cut difference between both years; across the region in 2017 there were at least 12 days below 23F in winter, while in 2018, there were some sites with zero days below 23F in winter. As winter progresses, the low temperatures can be tracked in local weather stations, and once there are more than ~10 days with temperatures below 23F, then DD accumulated in spring will be more important in predicting SWD abundance.

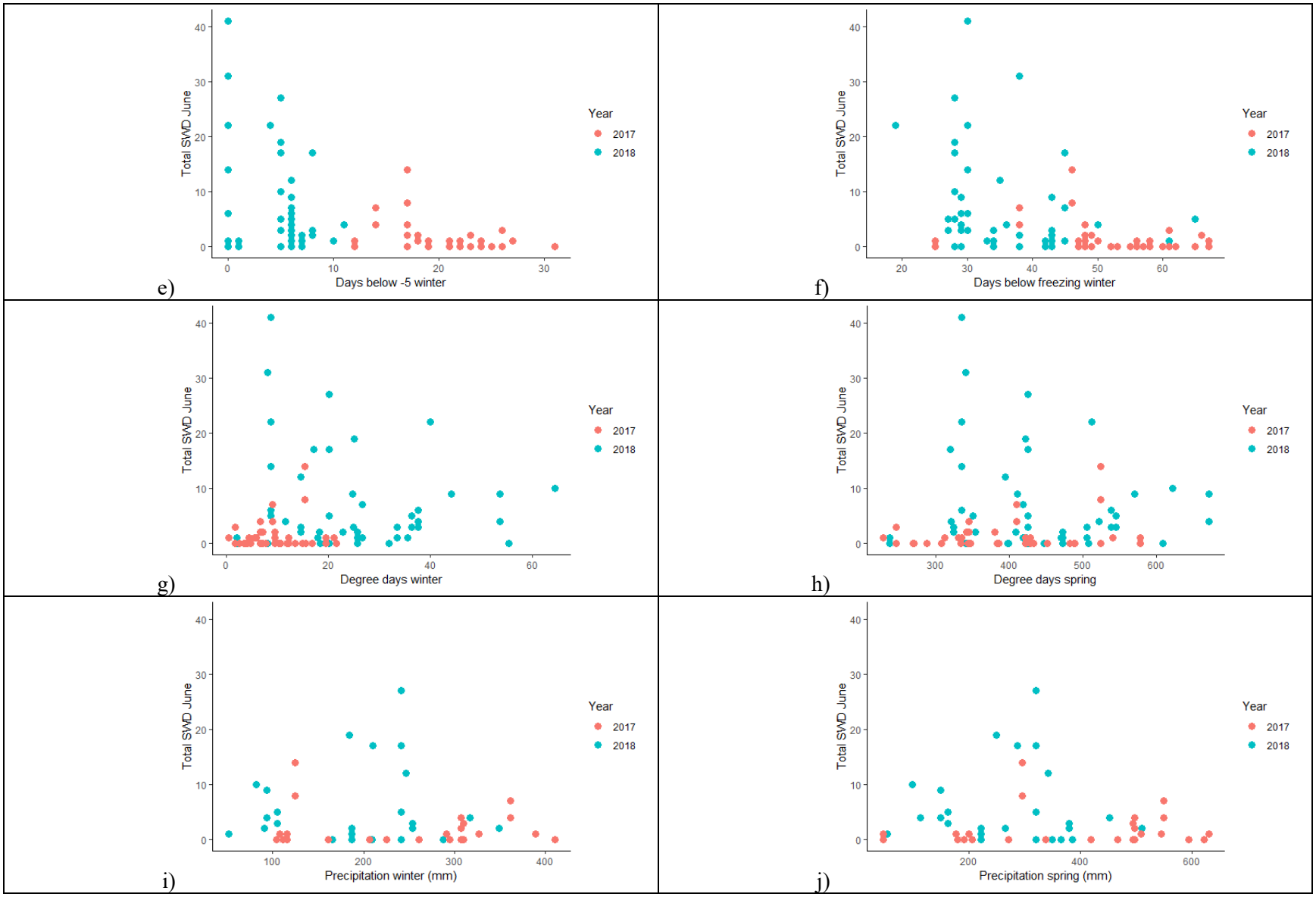
There is not a clear-cut threshold on how spring DD can affect SWD abundance in cold years, or winter precipitation in mild years. After a cold winter, sites with less than 400DD accumulated by the end of the spring equinox (~ 20 June), will likely have very low occurrence of SWD. After a mild winter, sites with more than ~7in (200mm) of rain accumulated during winter solstice (until March 20), or sites near forested areas may be at higher risk of SWD infestation. It is important to emphasize that there is still a lot of variation unaccounted for that none of these variables can predict, so there is inherent risk in relying on these models.

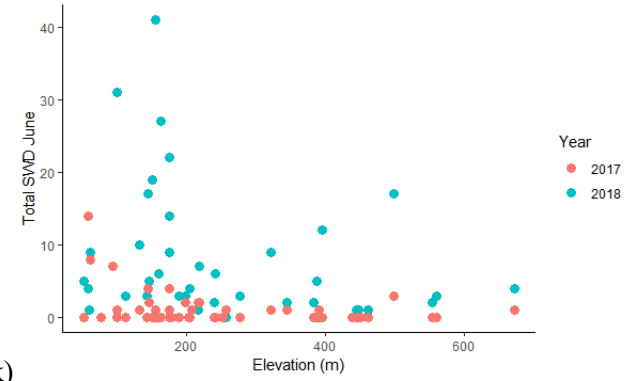
Implications for SWD management

This study highlights the importance of keeping track of environmental conditions when assessing risk of SWD infestations. We found that in years with cold winters, it could be possible to skip early insecticide sprays, and it is important to keep track of DD accumulation during spring to make application decisions. In years with milder winters, DD accumulation during spring becomes an unreliable predictor for SWD abundance.

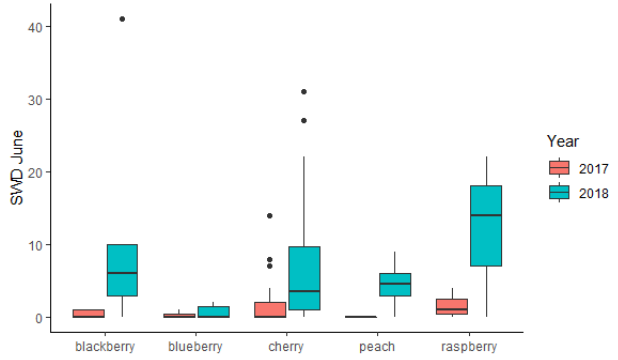
Figure 2. Environmental and categorical variables plotted against total number of SWD collected in June in 2017 and 2018 in Hood River and Wasco counties.



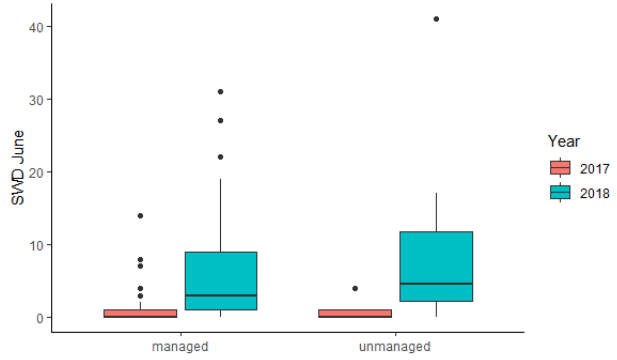

e)
Total SWD June
Days below -5 winter
Year
2017
2018
f)
Total SWD June
Days below freezing winter
Year
2017
2018
g)
Total SWD June
Degree days winter
Year
2017
2018
h)
Total SWD June
Degree days spring
Year
2017
2018
i)
Total SWD June
Precipitation winter (mm)
Year
2017
2018
j)
Total SWD June
Precipitation spring (mm)
Year
2017
2018


Total SWD June
Elevation (m)
Year
2017
2018

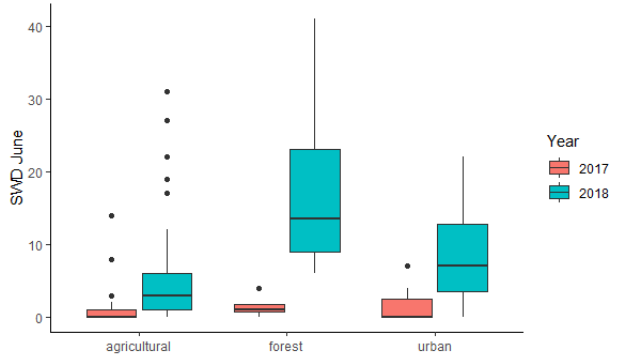
k)


SWD June
blackberry
blueberry
cherry
peach
raspberry
Year
2017
2018

l)


SWD June
managed
unmanaged
Year
2017
2018

m)


SWD June
agricultural
forest
urban
Year
2017
2018

n)

2) Which environmental factors best describe the abundance of SWD in unmanaged forest areas (Mt. Hood national forest)?

To collect in Mt. Hood national forest we deployed live traps at 9 sites on the northern and southern slope during summer. We measured the following variables:

- Elevation
- Fruit host (huckleberry / thimbleberry / native blackberry)
- Average maximum temperature during summer solstice
- Average minimum temperature during summer solstice

Additionally, we collected ripening fruit from each site at various times during the summer, and reared SWD from them. We also measured brix from each fruit, to determine if there was a relationship between brix and fruit infestation.

To be able to collect live flies for SNP molecular analysis, the traps used for the forest sites were different from the traps used in the Hood River/ Wasco trapping network, and did not have a drowning solution. We had tested these traps previously in 2016, but this year, we found that these traps were not very effective to collect SWD. As a result, many sites had low or zero catch, and we were not able to perform analyses to select the best predictive variables. The data presented here is only descriptive, and summarizes what we found in orchard and forest areas with live traps (Table 2). Data from live traps should not be used for monitoring or analysis purposes.

Table 2. Total SWD caught in live traps between 27 June and 17 Oct 2018 in various forest and orchard sites.

Elevation (ft)	Setting	Total SWD traps
292	Orchard	0
394	Orchard	0
528	Orchard	0
643	Orchard	0
669	Orchard	0
758	Orchard	0
761	Orchard	2
810	Orchard	0
866	Orchard	2
909	Orchard	8
925	Orchard	1
1089	Orchard	0
1204	Orchard	0
1263	Orchard	0
1483	Orchard	3
1486	Orchard	1
1732	Orchard	0
1880	Forest	2
2008	Orchard	0
2024	Forest	2
2028	Forest	2
2211	Orchard	2
2618	Forest	0
3025	Forest	50
3196	Forest	10
3563	Forest	3
3957	Forest	0
4518	Forest	0
4669	Forest	2

We reared SWD from huckleberry (*Vaccunium ovalifolium*) and thimbleberry (*Rubus parviflorus*) collected from Mt. Hood NF. There was no relationship between location or fruit brix on the number of SWD reared from forest berries.

Female flies collected alive in these traps during 2018 will be sent to UC Davis for SNP analysis.

EXECUTIVE SUMMARY

We aimed to identify which environmental factors best predict the total number of SWD trapped in various areas in Hood River and Wasco counties during 2017 and 2018. To answer this question, we used the data from the trap network deployed by OSU extension in 2017 and 2018 in Hood River and Wasco counties. As an assessment of risk infestation, the response variable selected was total number of SWD collected in each trap during June of each year. For each trap, we selected the nearest weather station from the U.S pest website. From each weather station, we extracted multiple environmental variables.

After performing a principal component analysis to eliminate redundant variables, we selected three environmental predictor variables: 1) "average maximum temperature during winter solstice", 2) "precipitation during winter solstice" and 3) "degree-days accumulated during spring equinox". Additionally, we selected the following categorical variables:

- Fruit host (cherry / blackberry / peach)
- Setting (agricultural / urban / forest)
- Management (managed / unmanaged)
- Lure type (Apple cider vinegar ACV / ACV+ Trece commercial lure).

The models that best described SWD collected during June varied by year. In 2017, the best predictors were DD accumulated at the end of spring equinox, mean maximum temperature during winter solstice, and cumulative winter precipitation. In 2018, environmental predictors were weak, but the best ones were setting (forest/agricultural/urban), and cumulative winter precipitation.

The difference between predictors in both years is likely due to differences in winter temperatures. For instance, there was a clear cut difference in number of days below 23F between 2017 and 2018. In years with cold winters, DD spring accumulation can provide information about SWD in late spring/early summer, but in years with milder winters, DD spring is not a reliable predictor.

Keeping track of winter temperatures in local weather stations as winter progresses is a useful tool to estimate which environmental predictors will become important to predict SWD abundance. We recommend taking note of how many days below 23F have occurred, and then keeping track of spring DD. There is not a clear-cut threshold on how spring DD can affect SWD abundance in cold years, or winter precipitation in mild years. After a cold winter, sites with less than 400DD accumulated by the end of the spring equinox (~ 20 June), will likely have very low occurrence of SWD. After a mild winter, sites with more than ~7in (200mm) of rain accumulated during winter solstice (until March 20), or sites near forested areas may be at higher risk of SWD infestation. It is important to emphasize that there is still a lot of variation unaccounted for that none of these variables can predict, so there is inherent risk in relying on these models.

We used live traps in some forest and orchard sites to be able to preserve flies for molecular analyses, but live trapping was very inefficient and unreliable for monitoring and analysis. It is unclear whether flies migrate between these sites; future molecular SNP analyses can help determine whether these are separate populations.

FINAL PROJECT REPORT

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

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

Budget History:

Item	Year 1: 2016	Year 2: 2017	Year 3: 2018
WTFRC expenses			
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
Plot Fees			
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 marketing issue when PNW sweet cherries are subject to long-distance ocean shipping (3-5 weeks). The goals of this project were: 1) to understand the mechanisms of cherry flavor deterioration; 2) identify pre- and postharvest factors affecting flavor deterioration and internal browning; and 3) develop commercially feasible protocols to maintain postharvest quality of PNW sweet cherry cultivars.

SIGNIFICANT FINDINGS

Mechanisms of flavor deterioration

- Desirable flavor was closely associated with the accumulation of malic acid (titratable acidity, TA), not soluble solids content (SSC).
- Bland flavor mainly resulted from the loss of 2-hexen-1-ol (fruity odor) and accumulation of benzaldehyde (almond-like odor).
- During storage, TAC (total antioxidant capacity), DPPH (2, 2-diphenyl-1-picrylhydrazyl radical-scavenging capacity), FRAP (ferric-reducing antioxidant power), and TP (total phenolic content) decreased significantly, while MDA (malondialdehyde) and O-quinones increased.
- Loss of TAC, DPPH, FRAP, and TP were associated with reduction in TA.
- The decrease of TP was accompanied by the increase of O-quinones. Severe internal browning (IB) and bitter flavors were associated with high O-quinones level.
- MDA was significantly correlated with flavor deterioration, IB, and bitter taste. It may be a good indicator for evaluation of cherry quality.

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

- Bland flavor was positively correlated with TA reduction, and negatively correlated with SSC/TA ratio. If fruit had < 0.6% TA or > 30 SSC/TA ratio, bland flavor developed.
- Fruit harvested in the morning had greater fruit firmness (FF) and lower risk of fruit flesh extrusion.
- Sustained high temperatures for three days before harvest significantly decreased FF at harvest, but did not affect flavor degradation during fruit storage.
- A pre-harvest simulated rain event did not affect ‘Lapins’ and ‘Skeena’ fruit quality parameters, but a rainfall event in 2016 totaling 0.34 inch resulted in high rates of fruit cracking.
- Fruit calcium (Ca) levels affected flavor deterioration. Fruit with higher calcium concentration were more resistant to softening, loss of TA, and flavor deterioration.
- Fruit harvested at optimum maturity (i.e. Ctifl color score of 6 for ‘Bing’) had extended fruit storage and flavor life.
- Ultraperf modified atmosphere packing (MAP) liners with an equilibrated O_2 7-8% + CO_2 8% maintained flavor and desirable qualities of cherries better than the same liners with an equilibrated O_2 11-13% + CO_2 7% after 5 weeks at 32 °F.
- Rapid forced-air cooling immediately after packing was extremely important to maintaining TA, flavor and other quality attributes during subsequent storage.
- Storage temperatures ≥ 36 °F accelerated quality and flavor loss. ‘Regina’ was more sensitive to high storage temperature than ‘Lapins’.

Determine reliable predictors for cherries with long postharvest flavor life

- For ‘Skeena’, flavor life was significantly positively correlated with TA, TAC, P, K, Ca, and Zn.
- For ‘Lapins’ and ‘Sweetheart’, flavor life was positively correlated with TA and TAC.
- Regardless of orchard location, Ca level was significantly positively correlated with TA in ‘Sweetheart’ and ‘Skeena’. Fruit with high Ca levels had longer flavor life.
- TA and TAC may be good indicators of the potential for long-lasting postharvest flavor.

RESULTS

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

a. Changes in SSC and TA

During 2016-2018, 'Skeena' was harvested 1 day before commercial harvest date from the experimental orchard at the MCAREC. TA (malic acid) degraded at a relatively fast rate following 5 weeks of storage at 32 °F. However, SSC did not change significantly between harvest and week 5 (Fig. 1A). The positive correlation was significant between flavor and TA (r = 0.845), while no significant correlation was observed between flavor and SSC (r = 0.213) (Fig. 1B). These results indicated that good flavor development was dependent on the levels of TA. Fruit with greater TA retained good flavor for longer periods.

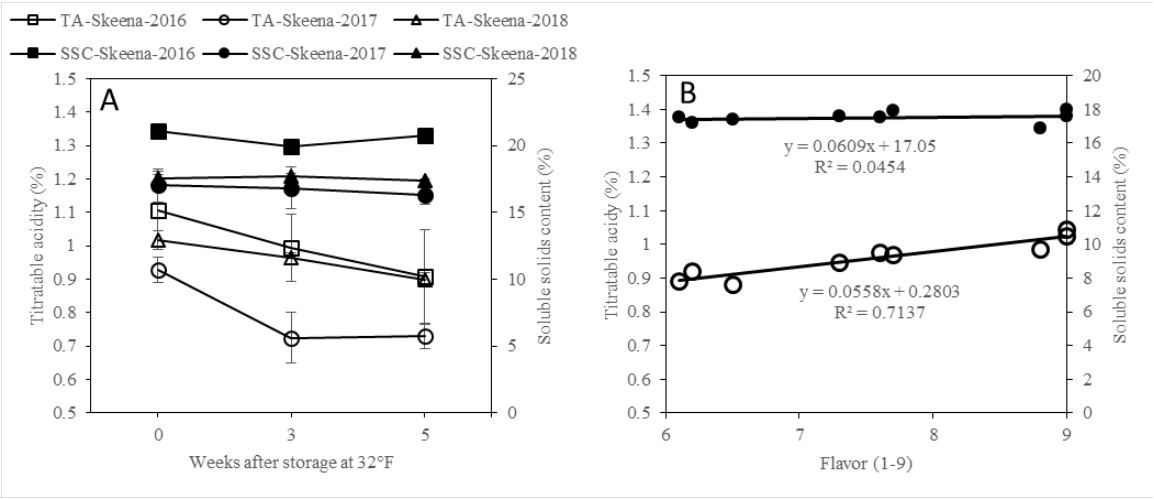

Fig. 1 *(A) Changes in TA (open symbols) and SSC (closed symbols) of 'Skeena' during 5 weeks of storage at 32 °F in 2016-2018; (B) The correlation between flavor and TA (open circles) or SSC (closed circles) in 2018.*

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

Volatile aroma compounds of 'Skeena' harvested from five orchards were analyzed at harvest and after 5 weeks of storage. Nineteen volatile aroma compounds were identified by GC-MS (Table 1). These compounds consisted of 7 alcohols (1-penten-3-ol; 3-methyl-3-buten-1-ol; 2-penten-1-ol; 2-methyl-2-buten-1-ol; 2-hexen-1-ol; hexanol; benzyl alcohol), 8 aldehydes (acetaldehyde; pentanol; 2,4-hexadiene; 3-hexenal; hexanal; 2-heptenal; 2-hexenal; benzaldehyde), 1 alkane (dodecane), 1 ester (butyl butyrate), 1 furan (2-ethyl furan), and 1 terpene (limonene). The major compounds are 2-hexanal, hexanal, 2-hexen-1-ol, and hexanol. Compared to levels at harvest, acetaldehyde, pentanol, 2, 4-hexadiene, 2-penten-1-ol, 2-hexen-1-ol, benzyl alcohol, and limonene decreased significantly during storage, while benzaldehyde increased. As a result, the loss in 2-hexen-1-ol (fruity odor) and accumulation of benzaldehyde (almond-like odor) imparted bland flavor.

c. Dynamics of TAC, DPPH, FRAP, MDA, TP, O-quinones

After cherries were packed and placed into cold storage, lipid peroxidation and reactive oxygen species (ROS) gradually increased and induced oxidative damage in fruit. MDA levels were associated with lipid peroxidation and indirect loss of membrane integrity in storage. Levels of TAC, DPPH, and FRAP reflected the capacity of fruit to scavenge ROS. During storage at 32 °F for 5 weeks, TAC, DPPH, and FRAP in 'Skeena' significantly decreased, while MDA increased (Table 2), indicating that the amount of MDA and ROS generated in storage reduced fruit antioxidant capacity, which was followed by rapid deterioration of fruit quality. The mechanism of IB development is unknown but it is generally accepted that oxidation produces TP, identified as O-quinones, which

give fruit a brown color and bitter taste. We found that the decrease of TP was associated with an increase of O-quinones. The severe IB and bitter flavor were accompanied by a relatively high level of O-quinones (Table 3).

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

Compound	Orchard 1-H	Orchard 1-5w	Orchard 2-H	Orchard 2-5w	Orchard 3-H	Orchard 3-5w	Orchard 4-H	Orchard 4-5w	Orchard 5-H	Orchard 5-5w
Alcohols										
1-Penten-3-ol	0.41	0.48	0.85	0.54	0.69	0.67	0.82	0.9	1.05	0.9
3-Methyl-3-Buten-1-ol	1.93	2.49	3.2	2.28	2.2	2.73	2.22	2.99	1.34	1.82
2-Penten-1-ol	0.25	0	0.35	0	0.25	0	0.58	0	0.31	0
2-Methyl-2-buten-1-ol	2.81	2.02	3.52	1.47	1.65	2.13	2.13	1.32	1.27	1.3
Benzyl alcohol	4.33	2.75	5.2	2.97	7.11	4.27	13.88	9.76	2.11	1.43
Hexanol	21.9	18.39	37.99	22.88	18.17	23.64	26.79	23.16	22.53	17.33
2-Hexen-1-ol	71.07	40.35	100.92	48.76	91.95	48.71	83.54	47.43	55.95	37.66
Aldehydes										
Acetaldehyde	0.73	0.65	1.05	0.45	1.06	0.56	1.02	0.69	0.83	0.6
Pentanol	0.43	0.38	0.54	0.26	0.49	0.3	0.47	0.3	0.41	0.22
2,4-Hexadiene	1.33	0.76	2.06	1.03	1.86	0.96	1.81	1.13	1.3	0.86
3-Hexenal	2.74	2.16	2.06	2.41	3.2	4.19	3.15	2.77	3.94	3.22
2-Heptenal	3.75	3.19	2.58	3.73	4.95	6.81	6.16	4.18	6.43	5.07
Benzaldehyde	4.72	7.47	7.25	10.84	8.51	16.21	18.83	29.21	6.22	14.11
Hexanal	44.38	46.97	39.47	40.94	53.52	55.17	66.4	47.45	56.58	57.44
2-Hexenal	132.9	114.29	108.56	127.68	161.76	192.67	171.61	135.52	187.64	151.62
Alkane										
Dodecane	0.81	0	0.53	0	0.54	0	0	0	0	0
Ester										
Butyl butyrate	7.22	6.74	6.93	5.01	4.69	5.98	7.21	5.8	6.91	5.25
Furan										
2-Ethyl furan	0.43	0.47	0.73	0.45	0.88	0.72	1.42	0.85	0.88	0.97
Terpen										
Limonene	20.76	0.78	9.55	5.2	4.76	1.86	5.87	1.27	5.57	1.37

H: at harvest; 5w: after 5 weeks of storage. Data were presented by total ion 10^7.

Table 2. *Changes in TAC, DPPH, FRAP, MDA, TP, and O-quinones of 'Skeena' at harvest and after 3 and 5 weeks of storage at 32 °F.*

	TAC (mM g^{-1})	DPPH (mM g^{-1})	FRAP (mM g^{-1})	MDA (mM g^{-1})	TP (mg g^{-1})	O-quinones (ΔOD)
At harvest	14.77 ± 0.49 a	623.31 ± 58.38 a	1.51 ± 0.12 a	16.73 ± 1.56 c	2.11 ± 0.12 a	0.14 ± 0.02 b
3 Weeks storage	12.91 ± 1.46 b	480.91 ± 27.26 b	0.80 ± 0.08 b	21.54 ± 3.92 b	1.89 ± 0.08 b	0.17 ± 0.01 a
5 Weeks storage	12.84 ± 0.11 b	465. 49 ± 49.46 b	0.65 ± 0.08 c	36.98 ± 8.97 a	1.79 ± 0.09 b	0.18 ± 0.01 a

Data within columns with different letters are significantly different by Fisher's protected least significant difference test (LSD) at $P < 0.05$.

d. Correlation of fruit physiology and biochemistry with fruit flavor evaluations

For 'Skeena', the development of desirable flavor was positively correlated with TA, TAC, DPPH, FRAP, and TP, while negatively correlated with MDA, O-quinones, IB, and bitter taste (Table 3). The development of IB was positively correlated with MDA and bitter taste, while negatively correlated with FRAP, TP, and flavor score. The development of bitter taste was positively correlated with MDA and IB, while negatively correlated with flavor score. TA was significantly correlated with TAC, DPPH, FRAP, and TP in fruit. Additionally, MDA was negatively correlated with flavor score, and positively correlated with IB, and bitter taste. MDA may be a good indicator of cherry quality.

Table 3. *Correlations among fruit physiology, biochemistry, and fruit flavor evaluations in 'Skeena'.*

	FF	Size	TA	SSC	TAC	DPPH	FRAP	MDA	TP	O-quinones	Flavor	IB	Bitter
FF	1	-0.614	-0.133	**-0.851****	0.399	-0.050	0.071	0.185	0.275	0.049	-0.117	0.092	0.186
Size		1	0.606	**0.717***	0.077	0.591	0.303	-0.009	0.049	-0.146	0.267	-0.082	-0.120
TA			1	0.396	**0.717***	**0.956****	**0.903****	-0.506	**0.696***	**-0.727***	**0.820****	-0.569	-0.488
SSC				1	-0.151	0.371	0.083	-0.219	-0.212	-0.097	0.295	-0.226	-0.274
TAC					1	**0.756***	**0.801****	-0.541	**0.729***	-0.645	**0.719***	-0.522	-0.436
DPPH						1	**0.845****	-0.481	0.665	-0.562	**0.782***	-0.602	-0.540
FRAP							1	**-0.679***	**0.921****	**-0.864****	**0.910****	**-0.730***	-0.640
MDA								1	-0.601	0.662	**-0.850****	**0.863****	**0.838****
TP									1	**-0.783***	**0.807****	**-0.736***	-0.658
O-quinones										1	**-0.852****	0.627	0.541
Flavor											1	**-0.903****	**-0.848****
IB												1	**0.983****
Bitter													1

*, ** Indicate significant at 0.05 and 0.01 *P* level.

2. Identify pre- and postharvest factors affecting flavor deterioration

a. Cultivars

During 2016-2018, TA and SSC/TA ratio were measured in five cultivars ('Bing', 'Lapins', 'Regina', 'Skeena', 'Sweetheart') after 5 weeks of storage at 32 °F. The development of bland flavor was positively correlated with the levels of TA (r = 0.935) in fruit, while negatively correlated with SSC/TA ratio (r = 0.810) (Fig. 2). For example, fruit with TA < 0.6% or SSC/TA ratio > 30 cherries developed bland flavor. Thus, TA or SSC/TA ratio may be good indicators of the potential for bland flavor development.

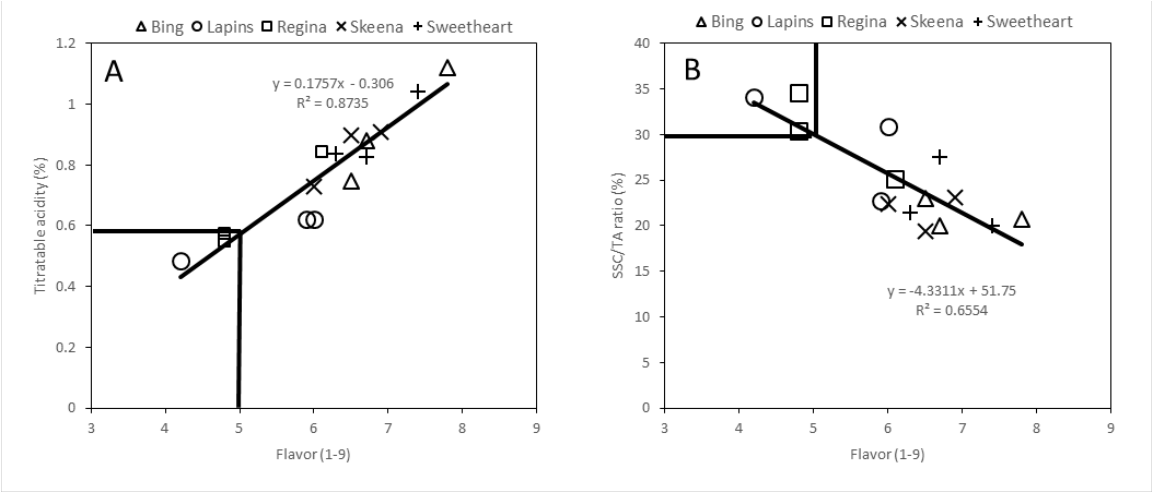

Fig. 2. *(A) Correlation between flavor and TA; (B) SSC/TA ratio of 'Bing', 'Lapins', 'Regina', 'Skeena', and 'Sweetheart' after 5 weeks of storage at 32 °F.*

b. Ambient temperature at harvest

On July 2, 2018 the high temperature was 84 °F; the low was 58 °F. 'Lapins' cherries were harvested at 9 AM when the ambient air temperature was 62 °F, or harvested at 3 PM when the ambient air temperature was 82 °F. Fruit harvested at 9 AM had higher FF than fruit harvested at 3 PM, but no significant differences were observed in SSC or TA (Table 4). After fruit was cooled and stored at 32 °F for 5 weeks, there were no differences in FF, SSC, TA, or flavor. Results indicated that harvesting cherries in the morning is generally preferable to the afternoon, because high FF

provided a benefit in resisting fruit flesh extrusion during harvest, though no differences were observed in fruit quality attributes or flavor after storage.

Table 4. *Effect of ambient temperature at harvest on quality attributes and flavor of 'Lapins' at harvest and after 5 weeks of storage at 32 °F.*

Treatments	At Harvest			Week 5			
	FF (g mm^{-1})	SSC (%)	TA (%)	FF (g mm^{-1})	SSC (%)	TA (%)	Flavor (1-9)
9 AM – 62°F	**298.30 ± 4.76 a**	18.27 ± 0.75 a	0.98 ± 0.11 a	338.07 ± 25.22 a	17.70 ± 0.78 a	0.67 ± 0.05 a	5.83 ± 0.25 a
3 PM – 82°F	**273.03 ± 3.76 b**	18.33 ± 0.45 a	1.05 ± 0.06 a	323.07 ± 10.50 a	17.93 ± 1.24 a	0.66 ± 0.01 a	5.90 ± 0.36 a

Data within columns with different letters are significantly different by Fisher's protected LSD test at $P < 0.05$.

In 2018, the commercial harvest date for 'Regina' was July 9 at MCAREC. Fruit were collected at ~9 AM on July 2, 5, and 8; daily maximum and minimum temperatures are listed in Table 5. Early harvest resulted in greater FF at harvest and after 5 weeks of storage, slightly higher SSC, while no effects were observed on TA and flavor (Table 6). A 4 day heat spell (July 4 through July 7) resulted in a greater rate of loss of FF for fruit harvested on July 8. Thus, sustained high temperature before harvest decreased fruit FF, but had no effect on flavor during fruit storage in this study.

Table 5. *Harvest date (red) and daily temperature (°F) before 'Regina' harvest in 2018.*

	July 2	July 3	July 4	**July 5**	July 6	July 7	**July 8**
High temperature	**74**	84	91	**94**	91	87	**95**
Low temperature	**58**	55	54	**57**	67	61	**54**

Table 6. *Effect of harvest date on quality attributes and flavor of 'Regina' at harvest and after 5 weeks of storage at 32 °F.*

Date	At Harvest			Week 5			
	FF (g mm^{-1})	SSC (%)	TA (%)	**FF (g mm^{-1})**	SSC (%)	TA (%)	Flavor (1-9)
July 2	**278.13 ± 7.58 a**	21.40 ± 0.46 a	0.92 ± 0.02 a	**336.17 ± 4.31 a**	22.07 ± 0.21 a	0.85 ± 0.05 a	6.10 ± 0.10 a
July 5	**268.67 ± 2.52 b**	21.33 ± 0.42 a	0.95 ± 0.04 a	**324.67 ± 5.51 b**	21.47 ± 0.55 b	0.83 ± 0.03 a	5.97 ± 0.15 a
July 8	**249.17 ± 4.19 c**	20.67 ± 0.40 b	0.97 ± 0.06 a	**302.80 ± 10.9 c**	21.20 ± 0.26 b	0.84 ± 0.02 a	5.80 ± 0.20 a

Data within columns with different letters are significantly different by Fisher's protected LSD test at $P < 0.05$.

c. Simulated rain

In 2017, 8 gallons of tap water were sprayed with a hand-pressure sprayer over the course of 25 minutes on 'Lapins' and 'Skeena' trees, 1 day before commercial harvest. The ambient air temperature was ~87 °F and the water temperature ~69 °F. Fruit were harvested before this simulated rain, and then again after 8 h. No significant differences were observed in FF, SSC, TA, or flavor between fruit treated or not treated with simulated rain (Table 7). In 2016, a rainfall event totaling 0.34 inch occurred 9 d before the commercial harvest. Fruit cracking was 20% and 49% for 'Lapins' and 'Skeena', respectively.

Table 7. *Effect of simulated rain on quality attributes and flavor of 'Lapins' and 'Skeena' at harvest and after 5 weeks of storage at 32 °F.*

Cultivar	Treatment	At harvest			Week 5			
		FF (g mm^{-1})	SSC (%)	TA (%)	FF (g mm^{-1})	SSC (%)	TA (%)	Flavor (1-9)
Lapins	Before rain	331 ± 9	16.1 ± 1.1	0.53 ± 0.02	363 ± 32 a	16.0 ± 1.3 a	0.46 ± 0.03 a	6.1 ± 0.2 a
Lapins	After rain	-	-	-	360 ± 15 a	16.3 ± 1.2 a	0.45 ± 0.06 a	6.1 ± 0.2 a
Skeena	Before rain	354 ± 7	17.0 ± 1.2	0.55 ± 0.03	359 ± 22 a	16.3 ± 0.7 a	0.49 ± 0.02 a	6.8 ± 0.3 a
Skeena	After rain	-	-	-	353 ± 31 a	16.3 ± 0.8 a	0.48 ± 0.02 a	7.1 ± 0.2 a

Data within columns and each cultivar with different letters are significantly different by Fisher's protected LSD test at $P < 0.05$.

d. Fruit calcium content

In earlier studies, we determined a Ca spray rate of 0.1-0.15%, application timing starting at pit-hardening, and frequency of 6 applications at weekly intervals from pit-hardening to harvest were optimal for increasing fruit tissue Ca content. In 2017, $Ca(NO_3)_2$ was applied at the rate of 0.42% (Ca rate at 0.15%, 3.5 lb/acre, 100 gal/acre) 6 times at weekly intervals from pit-hardening to harvest. These pre-harvest Ca applications significantly increased tissue Ca content and FF of 'Skeena', 'Lapins', and 'Regina' cherries at harvest (Table 8). After 5 weeks of storage, Ca-treated fruit maintained higher FF, TA, and flavor. Thus, pre-harvest Ca sprays may improve fruit quality and extend flavor life.

Table 8. *Effect of preharvest $Ca(NO_3)_2$ sprays on tissue Ca content, quality attributes, and flavor of 'Skeena', 'Lapins', and 'Regina' at harvest and after 5 weeks of storage at 32 °F.*

Cultivar	Treatments	At Harvest				Week 5			
		Calcium content (mg kg^{-1} DW)	**FF (g mm^{-1})**	SSC (%)	TA (%)	**FF (g mm^{-1})**	SSC (%)	**TA (%)**	**Flavor (1-9)**
Skeena	Control	**400 ± 100 b**	**418 ± 21 b**	21.10 ± 0.85 a	1.10 ± 0.11 a	**485 ± 20 b**	20.77 ± 1.21 a	**0.78 ± 0.02 b**	**7.0 ± 0.2 b**
	$Ca(NO_3)_2$	**517 ± 73 a**	**477 ± 21 a**	20.63 ± 2.97 a	1.15 ± 0.03 a	**533 ± 18 a**	20.13 ± 1.54 a	**0.99 ± 0.03 a**	**7.6 ± 0.3 a**
Lapins	Control	**433 ± 58 b**	**376 ± 2 a**	14.67 ± 0.15 b	0.73 ± 0.01 b	**355 ± 32 b**	16.63 ± 0.15 a	**0.60 ± 0.03 b**	**6.5 ± 0.3 b**
	$Ca(NO_3)_2$	**750 ± 93 a**	**374 ± 19 a**	15.20 ± 0.44 a	0.82 ± 0.05 a	**471 ± 24 a**	15.13 ± 0.40 b	**0.68 ± 0.04 a**	**7.5 ± 0.3 a**
Regina	Control	**533 ± 58 b**	**361 ± 8 b**	17.00 ± 0.44 a	0.68 ± 0.05 a	**410 ± 23 b**	17.43 ± 0.42 a	**0.57 ± 0.01 b**	**6.2 ± 0.2 b**
	$Ca(NO_3)_2$	**922 ± 54 a**	**427 ± 12 a**	17.30 ± 0.61 a	0.71 ± 0.00 a	**458 ± 15 a**	17.03 ± 0.25 a	**0.63 ± 0.04 a**	**7.2 ± 0.2 a**

Data within columns and each cultivar with different letters are significantly different by Fisher's protected LSD test at $P < 0.05$.

e. Harvest maturity

'Bing' fruit were harvested when average skin color had a Ctifl color score of 4, 5, or 6. Fruit with Ctifl color score of 6 had higher FF, SSC, and TA than early harvested fruit (Table 9). Furthermore, these fruit maintained higher FF, SSC, TA, as well as good flavor after 5 weeks of storage at 32 °F. Harvest at Ctifl color score of 7 or higher resulted in lower TA, and fruit were more susceptible to flavor deterioration, softening, pitting, pedicel browning, and fruit skin luster loss (data not shown). Thus, for 'Bing', harvesting fruit at optimum maturity, i.e. Ctifl color score of 6, can prolong fruit storage and flavor life.

Table 9. *Effect of harvest maturity on quality attributes and flavor of 'Bing' at harvest and after 5 weeks of storage at 32 °F.*

Ctifl color	At Harvest			Week 5			
	FF (g mm^{-1})	SSC (%)	TA (%)	FF (g mm^{-1})	SSC (%)	TA (%)	Flavor (1-9)
4	257 ± 4 b	17.87 ± 0.15 b	0.57 ± 0.09 c	264 ± 14 b	17.43 ± 0.51 b	0.44 ± 0.01 c	5.5 ± 0.2 c
5	272 ± 29 ab	17.90 ± 0.30 ab	0.75 ± 0.02 b	276 ± 33 ab	17.77 ± 0.32 b	0.70 ± 0.04 b	6.2 ± 0.4 b
6	**284 ± 15 a**	**20.23 ± 0.47 a**	**0.86 ± 0.02 a**	**295 ± 36 a**	**19.33 ± 0.31 a**	**0.82 ± 0.04 a**	**6.9 ± 0.3 a**

Data within columns with different letters are significantly different by Fisher's protected LSD test at $P < 0.05$.

f. Commercial MAP liners:

O_2 and CO_2 concentrations for Ultraperf MAP liners with low, moderate, and higher gas permeability tested for storage quality and flavor of 'Regina' are shown in figure 3. Compared to the control, a MAP liner with an equilibrated O_2 level of 7-8% + CO_2 level of 8% retained higher TA and better flavor during 5 weeks at 32 °F (Table 10). In contrast, the MAP liner with an equilibrated O_2 level of 11-13% + CO_2 level of 7% had little effect on cherry flavor.

Table 10. *Effect of Ultraperf modified atmosphere packaging (MAP) liners on quality attributes and flavor of 'Regina' after 5 weeks of storage at 32 °F.*

MAP liner	FF (g mm^{-1})	SSC (%)	TA (%)	Flavor (1-9)
Control	368 ± 15 b	18.6 ± 0.30 a	0.23 ± 0.02 c	6.2 ± 0.5 c
Ultraperf 1	**397 ± 13 a**	**18.9 ± 0.53 a**	**0.35 ± 0.02 a**	**8.5 ± 0.5 a**
Ultraperf 3	373 ± 17 b	18.5 ± 0.49 a	0.25 ± 0.01 b	7.1 ± 0.5 b
Ultraperf 4	389 ± 12 ab	18.9 ± 0.49 a	0.27 ± 0.03 b	7.0 ± 0.2 b

Data within columns with different letters are significantly different by Fisher's protected LSD test at $P < 0.05$.

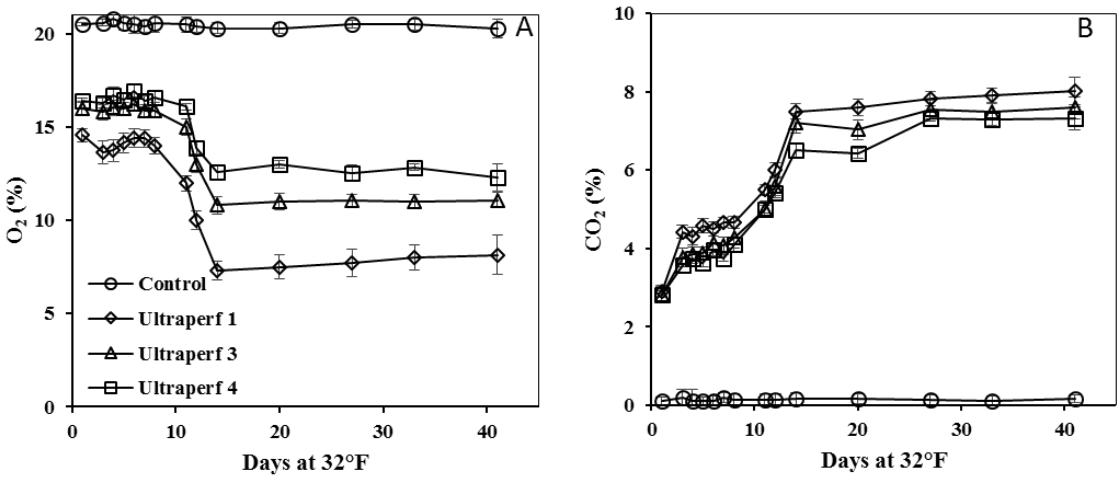

Fig. 3 *O_2 and CO_2 concentrations in Ultraperf modified atmosphere packaging (MAP) liners with 'Regina' cherries at 32 °F.*

g. Post-packing forced-air cooling

In an earlier survey, we determined that fruit pulp temperature at the time of box-filling in some packing houses ranged from 38-45 °F. Under these conditions, pulp temperature was not efficiently reduced to 32 °F in the containers due to the barrier created by the MAP liner reducing heat exchange and retention of heat produced by fruit respiration. In this study, compared to 32 °F, fruit stored at 38 °F or 45 °F had lower FF, and TA as well as greater stem browning and decay after 5 weeks in storage (Table 11). Therefore, rapid forced-air cooling immediately after packing is crucial for flavor (TA) maintenance and other fruit quality characteristics.

Table 11. *Effect of post-packing forced-air cooling on FF, SSC, TA, and flavor of 'Lapins' after 5 weeks of storage at 32 °F.*

Temperature	FF (g mm^{-1})	SSC (%)	TA (%)	Flavor (1-9)	Stem browning (1-5)	Decay (%)
32°F	415 ± 9 a	17.8 ± 0.22 a	0.74 ± 0.01 a	6.1 ± 0.4 a	4.5 ± 0.2 a	1.8 ± 0.2 a
38°F	374 ± 18 b	17.9 ± 0.36 a	0.69 ± 0.02 b	5.5 ± 0.2 b	4.1 ± 0.1 b	5.8 ± 1.1 b
45°F	353 ± 17 c	17.6 ± 0.29 a	0.55 ± 0.03 c	5.1 ± 0.1 c	2.9 ± 0.2 c	15.1 ± 3.2 c

Data within columns with different letters are significantly different by Fisher's protected LSD test at $P < 0.05$.

h.Storage temperature

'Lapins' and 'Regina' were harvested 1 day before the commercial harvest date at MCAREC, then fruit were stored at 32 °F, 34 °F, or 36 °F for 5 weeks. After storage, no significant differences were observed in FF, SSC, TA, or flavor score in 'Lapins' cherries stored at 32-36 °F. However, 'Regina' that had been stored at 36 °F had lower FF, SSC, TA, and flavor score than fruit stored at 32 °F or 34 °F (Table 12). Thus, quality and flavor deterioration of 'Regina' was more sensitive to storage and shipping temperature.

Table 12. *Effect of storage temperature on FF, SSC, TA, and flavor of 'Lapins' and 'Regina' after 5 weeks of storage at 32 °F, 34 °F and 36°F.*

Cultivar	Treatments	FF (g mm^{-1})	SSC (%)	TA (%)	Flavor (1-9)
Lapins	32°F	311 ± 5 a	17.70 ± 0.78 a	0.66 ± 0.01 a	5.3 ± 0.3 a
	34°F	315 ± 9 a	17.99 ± 0.53 a	0.65 ± 0.05 a	5.4 ± 0.4 a
	36°F	309 ± 5 a	18.03 ± 0.35 a	0.66 ± 0.03 a	5.3 ± 0.2 a
Regina	32°F	303 ± 11 a	21.20 ± 0.26 a	0.85 ± 0.05 a	6.0 ± 0.2 a
	34°F	301 ± 9 a	21.45 ± 0.47 a0	0.82 ± 0.03 b	5.5 ± 0.3 b
	36°F	**278 ± 4 b**	**20.27 ± 0.25 b**	**0.76 ± 0.02 c**	**5.1 ± 0.1 c**

Data within columns with different letters are significantly different by Fisher's protected LSD test at $P < 0.05$.

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

In 2016, 'Skeena' from 5 Washington orchards were sampled for quality evaluation, flavor, and fruit nutrient content. Fruit were packed in MAP liners and stored at 32 °F. Results indicated that flavor intensity after 5 weeks at 32 °F was significantly positively correlated with TA, TAC, P, K, Ca, and Zn, and negatively correlated with SSC (Table 13).

Table 13. *The correlation among fruit quality attributes, flavor, production elevation, TAC, and fruit nutrients (N, P, K, Ca, Mg, B, Fe, Zn) of 'Skeena' after 5 weeks of storage at 32 °F from 5 orchards in 2016.*

	FF	Size	TA	SSC	Flavor	Elevation	TAC	N	P	Mg	K	Ca	B	Fe	Zn
FF	1	-0.219	-0.295	0.072	-0.165	-0.359	-0.239	0.169	-0.0173	-0.386	0.036	0.086	-0.094	-0.238	0.046
Size		1	-0.210	0.286	-0.411	-0.270	-0.359	-0.435	**-0.584***	-0.421	**-0.565***	**-0.682****	-0.213	**0.656****	-0.387
TA			1	**-0.585***	**0.951****	-0.250	**0.940****	0.140	0.438	0.433	0.477	**0.558***	0.171	0.019	**0.561***
SSC				1	**-0.606***	**0.535***	**-0.684****	**-0.574***	-0.451	-0.081	-0.333	-0.334	**-0.587***	0.118	**-0.646****
Flavor					1	-0.208	**0.970****	0.177	**0.523***	0.430	**0.568***	**0.660****	0.152	-0.131	**0.538***
Elevation						1	-0.272	-0.231	0.069	0.489	0.068	0.094	-0.167	-0.310	-0.371
TAC							1	0.260	**0.530***	0.394	**0.562***	**0.616***	0.229	-0.110	**0.585***
N								1	0.483	0.086	0.264	0.261	0.486	-0.255	**0.534***
P									1	**0.700****	**0.946****	**0.894****	0.477	-0.446	**0.792****
Mg										1	**0.682****	**0.714****	0.467	-0.249	0.480
K											1	**0.937****	0.321	-0.433	**0.762****
Ca												1	0.339	-0.442	**0.723****
B													1	0.035	**0.570***
Fe														1	-0.279
Zn															1

*, ** Indicate significant at 0.05 and 0.01 *P* level.

In 2017, 'Lapins' from 7 Washington orchards and 2 Oregon orchards were sampled for quality evaluation, flavor, and fruit nutrient content. Fruit were packed in MAP liners and stored at 32 °F. Results indicated that flavor intensity after 5 weeks at 32 °F was significantly positively correlated with TA and TAC (Table 14).

Similar 2018 results for 'Sweetheart' from 4 orchards in Washington and 1 in Oregon are shown in Table 15. None of the macronutrients or micronutrients measured were found to be associated with fruit flavor life in 'Sweetheart'. However, in 'Sweetheart' and 'Skeena', Ca was significantly positively correlated with TA. Thus, fruit with high Ca content generally had longer flavor life. TA or TCA can be a good indicator of a cultivar's potential for long postharvest flavor life.

Table 14. *Correlation among fruit quality parameters, flavor, production elevation, TAC, and fruit nutrients (N, P, K, Ca, Mg, B, Fe, Zn) of 'Lapins' after 5 weeks of storage at 32 °F from 9 orchards in 2017.*

	FF	Size	TA	SSC	Flavor	Elevation	TAC	N	P	Mg	K	Ca	B	Fe	Zn
FF	1	**0.625****	0.295	**0.567****	0.006	**0.603****	0.371	0.061	0.051	-0.091	-0.162	-0.191	**0.407***	0.096	0.295
Size		1	**0.406***	**0.691****	0.144	0.247	**0.642****	-0.347	0.316	-0.320	0.024	-0.183	**0.530****	-0.043	**0.390***
TA			1	**0.395***	**0.855****	0.012	**0.793****	-0.070	0.004	-0.269	0.034	-0.102	0.205	0.289	-0.094
SSC				1	0.213	0.211	**0.462***	-0.035	-0.006	-0.167	-0.004	-0.128	0.323	0.266	-0.044
Flavor					1	-0.099	**0.706****	-0.038	0.064	-0.094	0.160	0.052	0.129	0.202	-0.134
Elevation						1	0.033	**0.455***	-0.031	0.233	0.308	**-0.546****	0.297	0.102	0.257
TAC							1	-0.230	0.222	-0.321	0.014	-0.169	0.373	0.235	0.202
N								1	-0.022	**0.622****	0.379	-0.167	-0.032	0.354	-0.192
P									1	0.368	**0.480***	0.185	**0.698****	-0.195	**0.477***
Mg										1	**0.691****	0.262	0.257	-0.132	0.183
K											1	-0.113	**0.458***	0.053	0.213
Ca												1	-0.197	-0.272	0.223
B													1	-0.240	0.359
Fe														1	-0.357
Zn															1

*, ** Indicate significant at 0.05 and 0.01 *P* level, respectively.

Table 15. *Correlation among fruit quality parameters, flavor, production elevation, TAC, and fruit nutrients (N, P, K, Ca, Mg, B, Fe, Zn) of 'Sweetheart' after 5 weeks of storage at 32 °F from 5 orchards in 2018.*

	FF	Size	TA	SSC	Flavor	Elevation	TAC	N	P	Mg	K	Ca	B	Fe	Zn
FF	1	**0.825****	-0.317	**-0.701****	0.145	0.154	-0.419	0.218	**0.729****	-0.095	**0.773****	0.487	**-0.610***	0.165	-0.351
Size		1	0.095	-0.328	0.432	**0.517***	0.034	0.407	**0.720****	0.018	**0.696****	0.247	-0.194	0.370	-0.439
TA			1	**0.817****	**0.730****	**0.584***	**0.969****	0.065	-0.130	0.409	-0.106	**0.769****	**0.808****	0.189	-0.080
SSC				1	0.327	0.488	**0.887****	-0.189	**-0.602***	0.166	**-0.636***	**-0.846****	**0.771****	-0.067	0.094
Flavor					1	0.390	**0.612***	0.016	0.295	0.197	0.379	-0.248	0.413	0.294	0.128
Elevation						1	**0.635***	0.110	-0.075	-0.053	-0.084	**-0.524***	0.314	0.045	-0.247
TAC							1	0.026	-0.268	0.343	-0.268	**-0.823****	**0.849****	0.073	-0.081
N								1	**0.581***	0.213	0.475	0.099	0.181	**0.635***	**-0.581***
P									1	0.171	**0.954****	**0.535***	-0.250	0.436	-0.325
Mg										1	0.189	-0.254	0.403	0.265	-0.332
K											1	0.490	-0.254	0.377	-0.248
Ca												1	**-0.698****	0.250	0.225
B													1	0.155	-0.090
Fe														1	-0.188
Zn															1

*, ** Indicate significant at 0.05 and 0.01 *P* level, respectively.

Executive Summary

In the PNW, more than 1/3 of sweet cherries are exported each year. While airfreight requires 2-3 days, transit time to export markets by sea may range from 3-5 weeks after packing. With protracted transport, flavor deterioration can be a significant issue for PNW cherries upon arrival in export markets. Flavor deterioration includes bland flavor, bitter taste, and anaerobic aroma, and is often associated with IB. Other arrival issues include pitting, luster color loss, pedicel browning, splitting, and decay.

Understand the mechanisms of flavor deterioration

Our current research indicated that desirable fruit flavor was found to be positively correlated with TA (malic acid), but not SSC. Level of TA impacted flavor lifespan. By analyzing the volatile aroma compounds of cherries from five orchards during storage, we found that the loss of 2-hexen-1-ol (fruity odor) and accumulation of benzaldehyde (almond-like odor) imparted bland flavor. During storage, sweet cherries rapidly lost antioxidants, including TAC, DPPH, FRAP, and TP. Meanwhile, lipid peroxidation product (MDA) and browning product (O-quinones) increased and induced IB and bitter taste. MDA was negatively correlated with flavor score, and positively correlated with IB, and bitter taste. MDA may be a good indicator of cherry quality.

Identify pre- and postharvest factors affecting flavor deterioration

This study indicated that cultivar, air temperature at harvest, rain, Ca level, harvest maturity, MAP bag permeability, rapid forced-air cooling, and storage temperature affected the flavor of cherries:

- After storage at 32 °F for 5 weeks, cultivars with high TA level, such as 'Sweetheart' and 'Bing', or fruit with > 0.6% TA or <30 SSC/TA ratio, demonstrated additional duration of desirable flavor.
- Harvest in the morning was better than afternoon due to the higher fruit firmness and lower fruit flesh extrusion risk. Sustained high temperatures before harvest contributed to rapidly decreasing fruit firmness but did not affected flavor life.
- Simulated rain did not affect flavor life, but a natural rainfall event (0.34 inch in 2016) resulted in high rate of fruit cracking.
- Fruit with higher Ca levels were more resistant to softening, loss of TA, and flavor deterioration.
- Fruit harvested at optimum maturity had longer fruit flavor life.
- Ultraperf MAP liners with an equilibrated O_2 levels of 7-8% + CO_2 levels of 8% maintained better flavor and quality than liners with an equilibrated O_2 level of 11-13% + CO_2 level of 7%.
- Rapid forced-air cooling immediately after packing was highly important for maintaining TA and flavor and other fruit quality attributes during storage.
- Storage temperatures at or above 36 °F accelerated flavor deterioration. Some cultivars, such as 'Regina', were more sensitive to storage temperatures.

Determine reliable predictors for cherries with long postharvest flavor life

We determined the correlation among quality attributes, antioxidant, and fruit nutrients of three PNW cherry cultivars ('Skeena', 'Lapins' and 'Sweetheart') from 5-9 orchards located in Washington and Oregon. This work indicated that longer flavor life in 'Skeena' was associated with higher TA, TAC, P, K, Ca, and Zn. Flavor life in 'Lapins' and 'Sweetheart' was significantly positively correlated with TA and TAC and Ca played an important role in enhancing TA level. TA or TAC can be a good indicator of a cultivar's potential for longer postharvest flavor life.

FINAL PROJECT REPORT

Project Title: The hunt for leafhopper vectors of Western X in Washington cherries

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Cooperators: Washington cherry growers, Stemilt Growers, G.S. Long

Other funding sources

Agency Name: National Clean Plant Network – Fruit Trees
Amount awarded: NCPN-FT pays land rental fees and maintenance costs of the virus research block where field experiments are conducted, sources of plant material for research and plant maintenance. The estimated cost associated with this project is $22,300 and is a portion of a larger NCPN grant to WSU-Prosser.
Notes: WSU is including this information on other funding available for the support of similar research undertaken by the faculty member proposing this research. 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: **Year 1**: $33,740 **Year 2**: $34,603 **Year 3**: $35,499

Budget History:

Item	2016	2017	2018
Salaries[1]	$15,601	$16,226	$16,875
Benefits[2]	$5,939	$6,177	$6,424
Supplies[3]	$12,200	$12,200	$12,200
Travel	$0	$0	$0
Miscellaneous	$0	$0	$0
Plot Fees	$0	$0	$0
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
   - Field sampling supplies
   - Sample extraction and PCR assays
   - Leafhopper colony establishment and maintenance
   - Acquisition/retention of inoculated plants over 2 years

RECAP OF ORIGINAL OBJECTIVES

1. **Conduct survey of leafhoppers in Western X affected orchards.**
   As planned, we conducted surveys of leafhoppers in affected orchards in 2016 to 2018, beginning in March/April and concluding in October. While some blocks were sampled in three years, sampling had to be discontinued in other blocks removed by the grower. Molecular testing of leafhoppers to determine which species have the Western X (WX) phytoplasma was accomplished, concentrating on two abundant species, *Colladonus reductus* and *C. geminatus*.
2. **Conduct survey of host plants for leafhoppers and/or WX phytoplasma in affected orchards.**
   Potential host plants for leafhoppers were collected from field sites and identified; some were propagated in the greenhouse. Data from sticky card and sweep sampling inside and outside of the orchard also provided information on potential host plants.
3. **Examine the capability of selected leafhopper species to transmit WX phytoplasma.**
   Colonies of *C. reductus* and *C. geminatus* were established and maintained on celery seedlings. Acquisition and inoculation studies (n=75) were conducted in late season 2017 and 2018.

SIGNIFICANT FINDINGS

- Objective 1 (100% complete): Two leafhopper vector species, *C. 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.
- Objective 1 (100% complete): Molecular diagnostic testing showed that for *C. geminatus*, 40/519 samples (8%) from within affected orchards and 71/348 samples (20%) from extra-orchard habitats, were positive for Western X phytoplasma. Likewise, for *C. reductus*, 103/687 samples (15%) from within affected orchards and 20/204 samples (10%) from extra-orchard habitats were positive for WX. Our data show that there are two main time periods of WX-positive leafhopper activity in cherries—the spring and late summer into fall—although time periods may vary among orchards and year to year. Other infrequently collected potential vector species of leafhoppers tested for WX (number positive/sample size in parentheses) were: *Fieberiella florii* (1/12), *Osbornellus* sp. (1/23), *Scaphytopius* sp. (2/43), *Paraphlepsius* sp. (0/16), *Euscelidius variegatus* (1/35), *Macrosteles* sp. (0/15), *Circulifer tenellus* (0/22), and *Erythroneura* sp. (0/3).
- Objective 2 (100% complete): From collections of weed and sagebrush plants and data from sticky card and sweep samples, we completed our ecological survey and compiled a list of 33 potential host plant species for leafhoppers and/or Western X.
- Objective 3 (90% complete): In late season 2017 and 2018, we conducted a total of 75 acquisition and/or inoculation trials with *C. geminatus* and *C. reductus*. One transmission was successful: *C. reductus* collected from an infected cherry block transmitted WX to a previously WX-free *Prunus tomentosa* tree. Testing recipients for Western X is not complete.

RESULTS & DISCUSSION

Objective 1: Conduct survey of leafhoppers in Western X affected orchards.
Data from sticky cards and sweep net samples yielded information on leafhoppers present in cherry tree foliage as well as foliage in extra-orchard habitats (sagebrush, *Purshia* sp., clover, etc.). Overall, for the two most abundant species, 14% of *C. reductus* samples were WX-positive, and 13% of *C. geminatus* samples were WX-positive. We analyzed DNA samples of other potential vector leafhopper species for Western X (Table 1). One sample each of *Fieberiella florii*, *Osbornellus* sp., *Euscelidius variegatus,* and two samples of *Scaphytopius* sp. were positive for WX.

Table 1. Leafhopper species collected from sweet cherry orchards and extra-orchard habitats, 2016-2018. Overall percentages of WX-positive samples for each species are given here.

Leafhopper species	No. pooled samples	No. positive for Western X	Percent positive for Western X
Colladonus reductus	891	123	14
Colladonus geminatus	867	111	13
Euscelidius variegatus	35	1	3
Scaphytopius sp.	43	2	5
Osbornellus sp.	23	1	4
Macrosteles sp.	15	0	0
Paraphlepsius sp.	16	0	0
Fieberiella florii	12	1	8
Dikraneura sp.	10	0	0
Circulifer tenellus	22	0	0
Erythroneura sp.	3	0	0
Ceratagallia sp.	1	0	0

The Mattawa1 site was sampled in two different blocks because the 2016 block was taken out in late 2016. The same Mattawa1 block sampled in 2015 was studied in 2017. This location had the greatest abundance of *Colladonus* spp. leafhoppers with peak numbers occurring mainly in spring and late summer into fall (Fig. 1). *Colladonus* DNA samples showed a range of 4 to 14% positive for Western X, including orchard and sageland samples at the Mattawa1 site (Tables 2 and 3). Note that leafhoppers positive for WX were found mainly during spring and late summer into fall. See Figs. 2-5 for fairly similar results for Mattawa2, Mattawa3, Mattawa4, Granger, Benton City, and Selah.

For the Mattawa2 site, which was across the highway from Mattawa1, the orchard border was not sampled in 2016 (Fig. 2). The entire block was removed in late 2016 and apples were planted. The 2017 leafhopper sticky traps were in plants in the windbreak border. Less than 11% of DNA samples from leafhoppers collected at Mattawa2 tested positive for Western X (Tables 2 and 3)

The Mattawa4 site showed a greater percentage of *Colladonus* leafhopper samples positive for Western X for those from the neighboring sageland in 2016 (14 to 47%) compared with those collected in the orchard (0 to 8%; Tables 2 and 3; Fig. 3). In 2017, regular insecticide sprays kept leafhopper numbers low, and fewer leafhopper DNA samples were positive for Western X.

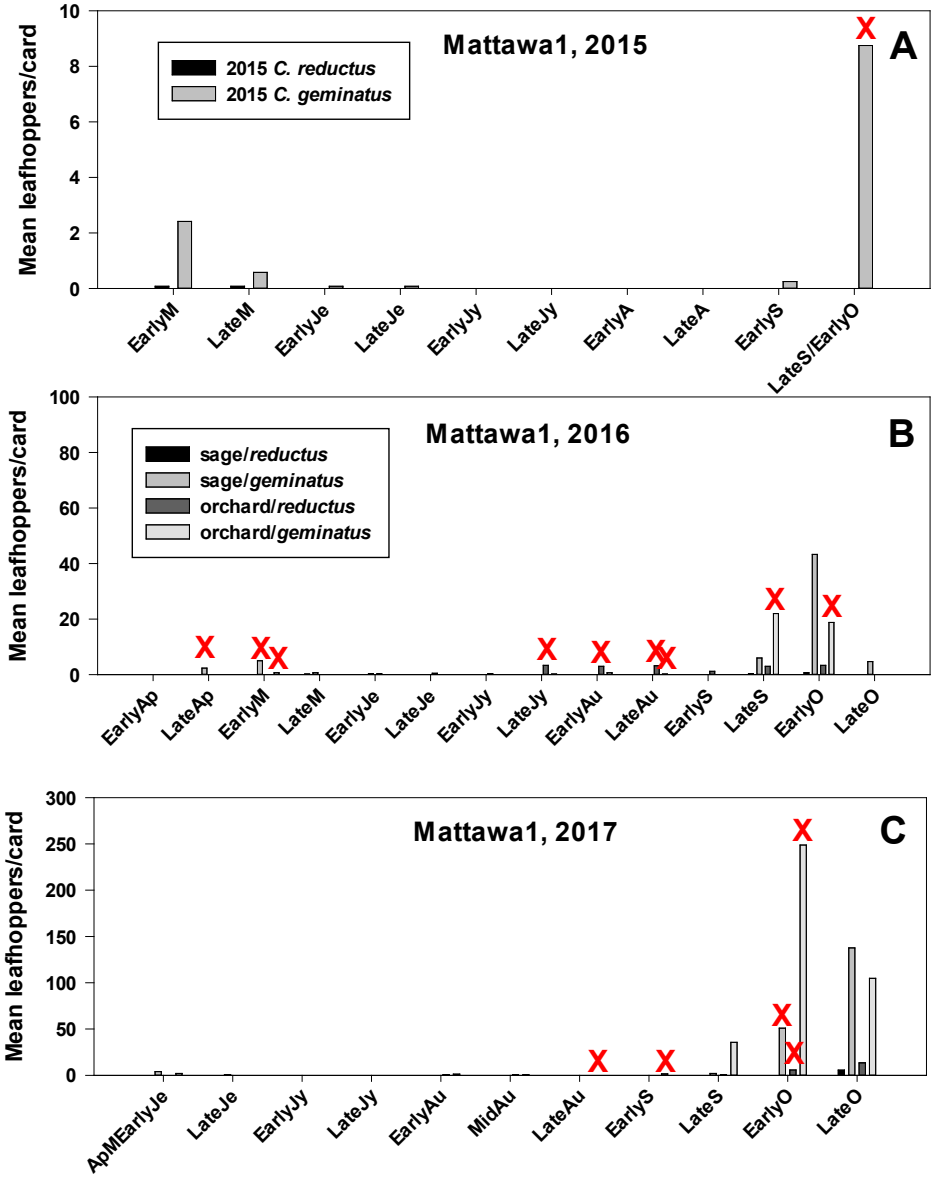

Figure 1. Seasonal abundance of and incidence of Western X (X over bar) in *Colladonus* spp. leafhoppers at the Mattawa1 site, 2015-2017.

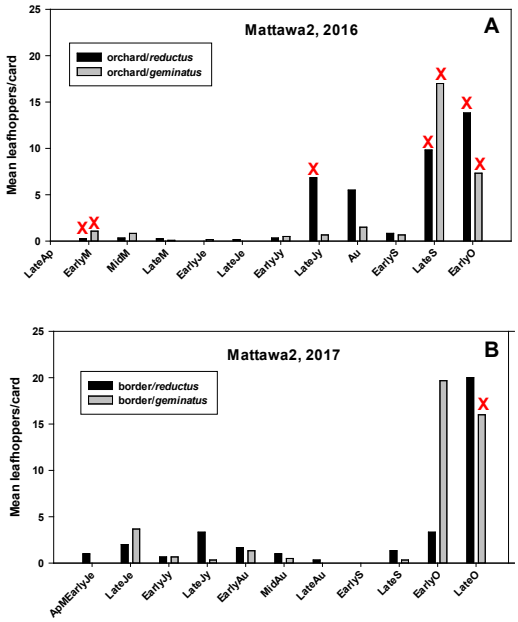

Figure 2. Seasonal abundance of and incidence of Western X (X over bar) in *Colladonus* spp. leafhoppers at the Mattawa2 site, 2016-2017.

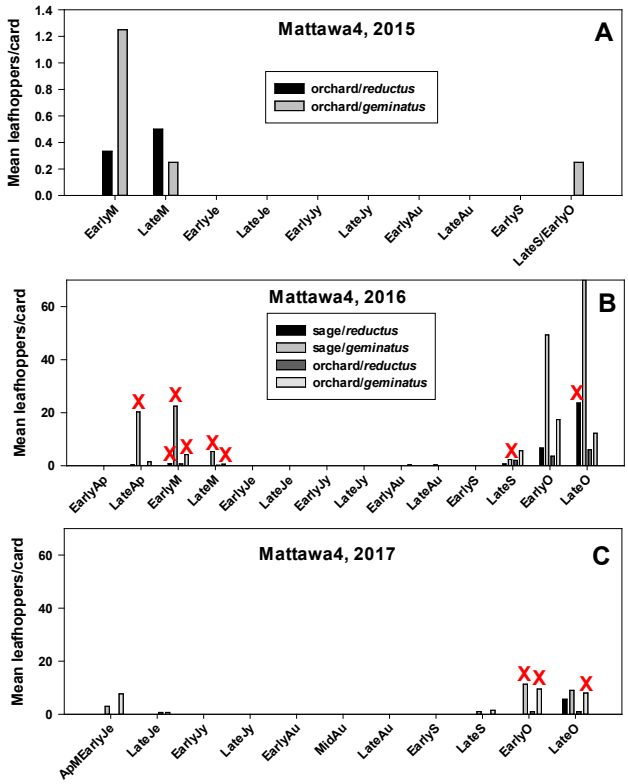

Figure 3. Seasonal abundance of and incidence of Western X (X over bar) in *Colladonus* spp. leafhoppers at the Mattawa4 site, 2015-2017.

While the Mattawa3 location had large *Colladonus* populations in fall of 2016, no leafhoppers were found to be positive for Western X in the fall samples from orchard and sageland collections (Fig. 4A and B, Tables 2 and 3). In 2017, frequent insecticide applications for Western cherry fruit fly made access to and sampling in those orchards difficult; three WX-positive samples came from the small sageland area outside of the orchard blocks. Only six WX-positive samples were found at the Benton City site; four of these samples were found in the sticky traps in border weeds (Fig. 4C). Orchard sampling at the Selah site had to be discontinued when the grower removed the trees in those blocks being sampled (Fig. 4D). A few weeks of orchard sampling took place in another block that was next to sageland recently burned off by a human-caused brush fire. Only one *Colladonus* sample taken from a *Purshia* sticky trap at the Selah site was found to be WX-positive (Tables 2 and 3).

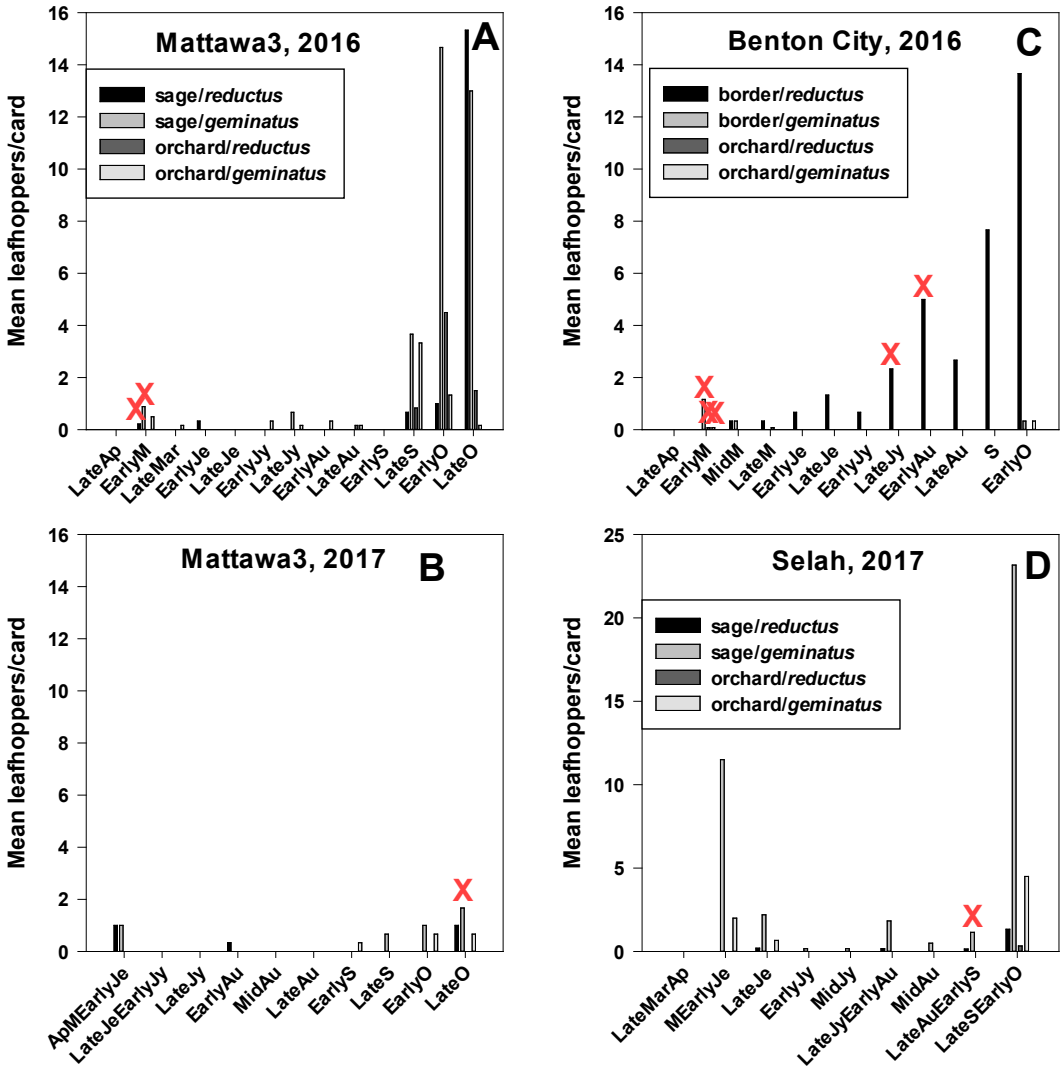

Figure 4. Seasonal abundance of and incidence of Western X (X over bar) in *Colladonus* spp. leafhoppers at the Mattawa3 site, 2015-2017; Benton City site, 2016; and Selah site, 2017.

Compared with other sites, the Granger site showed greater percentages of *Colladonus* leafhoppers positive for Western X in those that came from within the orchard (19 to 30%) as well as those collected from the neighboring alfalfa field (8 to 21%; Tables 2 and 3; Fig. 5). This site is a good example of an orchard with minimal pest and disease management.

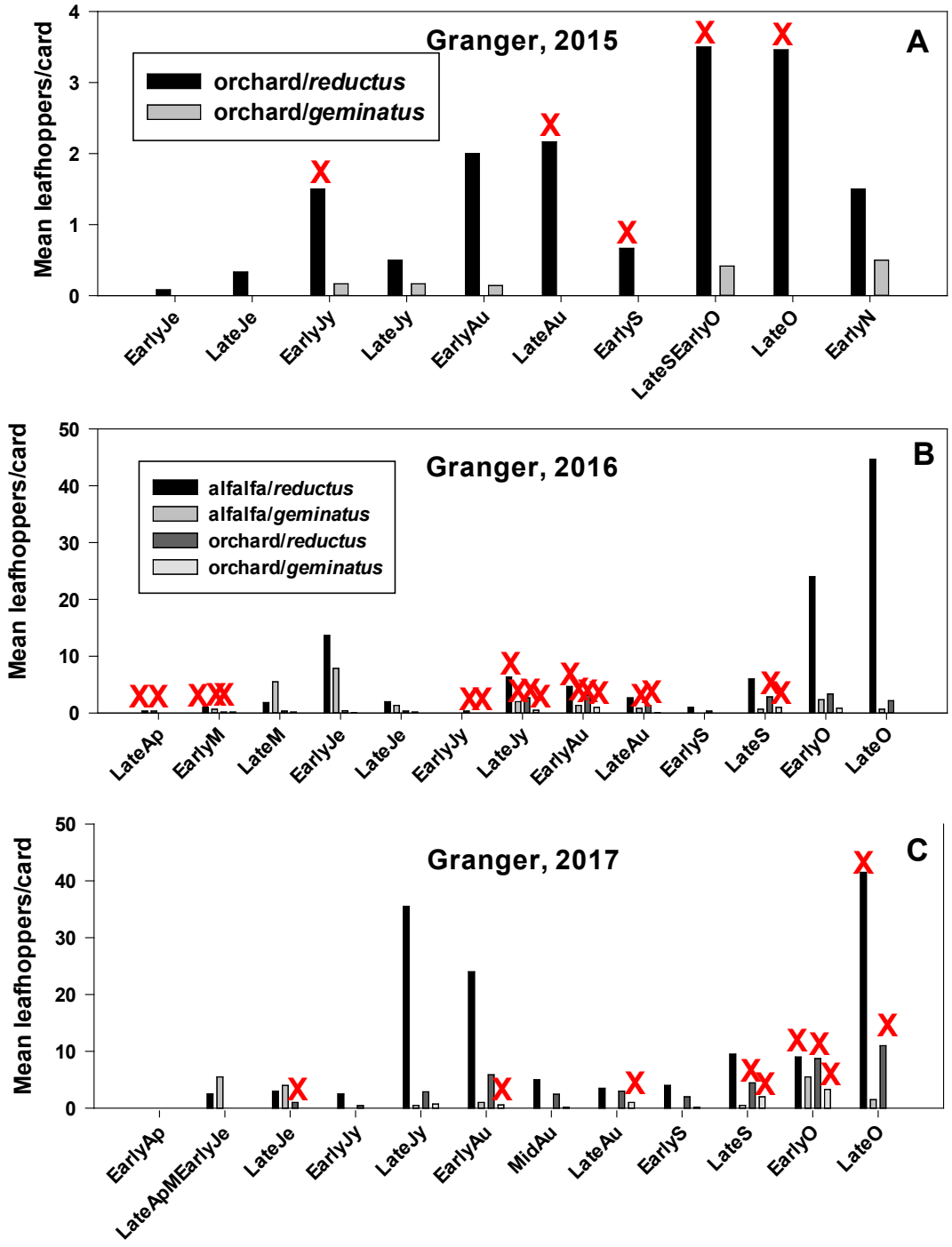

Figure 5. Seasonal abundance of and incidence of Western X (X over bar) in *Colladonus* spp. leafhoppers at the Granger site, 2015-2017.

For the Wapato site, *C. reductus* appeared to be relatively dominant over *geminatus*, and a greater percentage of *reductus* samples were positive for Western X (18% vs. 4%, Fig. 6, Tables 2 and 3). The drops in leafhopper numbers in early June and mid August 2018 were due to insecticide sprays. With G.S. Long collaboration, we continue to sample in the Wapato block through October and November. Note that in 2018, an additional study was conducted to compare sticky trap captures near ground versus tree trap captures. This would give us additional information on leafhopper movement among host plants.

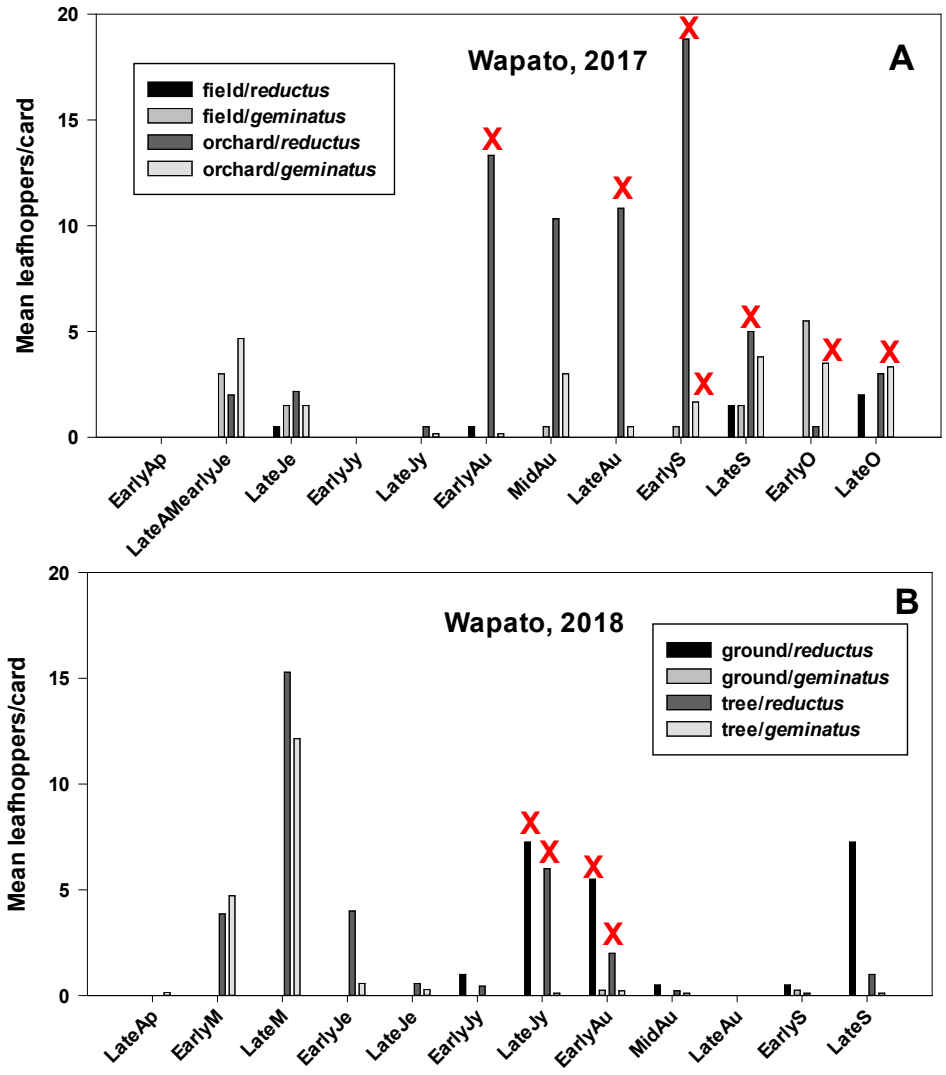

Figure 6. Seasonal abundance of and incidence of Western X (X over bar) in *Colladonus* spp. leafhoppers at the Wapato site, 2017-2018. Molecular analyses of late season 2018 samples are not complete.

Wenatchee sticky trap data in 2015 (collected in 2015 and analyzed during the grant period) and 2016 showed very low numbers of *Colladonus* leafhoppers throughout the growing seasons (data not shown). This was due to widespread spraying of cherry orchards in both years. One leafhopper sample from September 2016 was positive for WX.

Tables 2&3. Incidence of Western X in *Colladonus geminatus* (Table 2) and *C. reductus* (Table 3) collected in cherry orchard and extra-orchard habitats, 2016-2018. Extra-orchard habitats included alfalfa, sagebrush, *Purshia* sp., volunteer cherry, and herbaceous weeds.

Table 2. *Colladonus geminatus*						
	Cherry orchard			Extra-orchard habitat		
2016 sites	# samples	# positive	%	# samples	# positive	%
Mattawa1	100	4	4	71	7	10
Mattawa2	84	8	10	0	0	0
Mattawa3	29	0	0	20	3	15
Mattawa4	96	8	8	100	47	47
Granger	29	7	24	39	8	21
Benton City	3	1	33	7	1	14
2017 sites	# samples	# positive	%	# samples	# positive	%
Mattawa1	64	6	9	16	1	6
Mattawa2	--	--	--	20	1	5
Mattawa3	2	0	0	9	1	11
Mattawa4	11	1	9	9	0	0
Granger	10	3	30	13	1	8
Wapato	51	2	4	9	0	0
Selah	5	0	0	35	1	3
2018 sites	# samples	# positive	%	# samples	# positive	%
Wapato*	35	0	0	--	--	--

Table 3. *Colladonus reductus*						
	Cherry orchard			Extra-orchard habitat		
2016 sites	# samples	# positive	%	# samples	# positive	%
Mattawa1	104	15	14	10	0	0
Mattawa2	102	5	5	0	0	0
Mattawa3	23	0	0	13	1	8
Mattawa4	40	0	0	21	3	14
Granger	105	28	27	60	10	17
Benton City	2	1	50	31	3	10
2017 sites	# samples	# positive	%	# samples	# positive	%
Mattawa1	31	1	3	4	0	0
Mattawa2	--	--	--	16	0	0
Mattawa3	0	0	0	4	0	0
Mattawa4	4	0	0	1	0	0
Granger	36	7	19	39	3	8
Wapato	148	27	18	3	0	0
Selah	0	0	0	2	0	0
2018 sites	# samples	# positive	%	# samples	# positive	%
Wapato*	92	19	21	--	--	--

*Sample processing is not complete. Sticky card sampling continued into October 2018 but these samples have not yet been processed.

Objective 2. Conduct survey of host plants for leafhoppers and/or WX phytoplasma in affected orchards

We collected and identified 33 potential host plant species in our ecological survey. We attempted to maintain selected species in the greenhouse for host plant studies. Unfortunately, the numbers of leafhoppers required for these studies were not available. However, data from sticky trap and sweep net sampling in extra-orchard habitats provided host plant information.

Table 4. Plants identified in orchard and extra-orchard ecosystems that are potential host plants for *Colladonus* spp. leafhoppers and/or Western X phytoplasma.

Family	Species	Common name	Orchard floor/border	Sageland
Chenopodiaceae	*Chenopodium album*	Common lambsquarter	*	
Chenopodiaceae	*Atriplex spinosa*	Spiny hopsage		*
Chenopodiaceae	*Salsola tragus*	Russian thistle		*
Apiaceae	*Lomatium macrocarpum*	Large-fruit desert parsley		*
Apiaceae	Lomatium simplex	Great Basin desert parsley		*
Asteraceae	*Achillea millefolium*	Common yarrow	*	
Asteraceae	*Acroptilon repens*	Russian knapweed	*	
Asteraceae	*Arctium minus*	Common burdock	*	
Asteraceae	*Cyclachaena xanthifolia*	Burweed marsh elder	*	
Asteraceae	*Sonchus oleraceus*	Common sowthistle	*	
Brassicaceae	*Sisymbrium loeselii*	False London rocket	*	
Caryophyllaceae	*Arenaria serpyllifolia*	Thyme-leaf sandwort		*
Elaeagnaceae	*Elaeagnus angustifolia*	Russian olive	*	
Fabaceae	*Medicago lupulina*	Black medic	*	
Fabaceae	*Medicago sativa*	Alfalfa	*	
Fabaceae	*Melilotus officinalis*	Yellow sweet-clover	*	
Fabaceae	*Trifolium repens*	White clover	*	
Fabaceae	*Vicia villosa*	Hairy vetch	*	
Geraniaceae	*Geranium pusillum*	Small flower cranes-bill		*
Liliaceae	*Brodiaea douglasii*	Wild hyacinth	*	
Liliaceae	*Calochortus macrocarpus*	Sagebrush mariposa lily		*
Onagraceae	*Epilobium sp.*		*	
Onagraceae	*Oenothera villosa*	Common evening primrose	*	
Oxalidaceae	*Oxalis corniculata*	Creeping wood-sorrel	*	
Plantaginaceae	*Plantago major*	Common plantain	*	
Polygonaceae	*Persicaria maculosa*	Lady's thumb	*	
Polygonaceae	*Rumex crispus*	Curly dock	*	
Polygonaceaea	*Polygonum aviculare*	Prostrate knotweed	*	
Portulacaceae	*Portulaca oleracea*	Common purslane	*	
Rosaceae	*Amelanchier alnifolia*	Western serviceberry	*	
Rosaceae	*Prunus virginiana*	Chokecherry	*	
Rosaceae	*Purshia tridentata*	Antelope bush		*
Vitaceae	*Parthenocissus vitacea*	Virginia creeper	*	

Based on sticky trap and sweep samples, several species have been identified as probable host plants for *C. reductus* and *geminatus*. They are: alfalfa (*Medicago sativa*), clover spp. (*Trifolium* spp.), antelope bush (*Purshia tridentata*), and common mallow (*Malva neglecta*). If large stands or fields of these plants are found in orchard floor vegetation or next to orchard blocks, large populations of vector leafhoppers can build up, which creates a greater potential for WX transmission by leafhopper.

Objective 3. Examine the capability of selected leafhopper species to transmit WX phytoplasma. In 2016, we used several propagation methods (whip grafting, bottle grafting, sucker transplants) to establish an infected *Prunus* source of Western X but were unsuccessful. Acquisition and inoculation (transmission) studies were accomplished in 2017 and 2018. As we had no infected source of WX available to conduct the experiments in-house, we relied primarily on field-collected plant and leafhopper materials. We maintained clean, disease-free *C. reductus* and *C. geminatus* colonies in the laboratory on clean celery. Several approaches were taken to test the leafhoppers' potential to transmit Western X (Table 5). Either "clean" leafhoppers from the colonies or "dirty" leafhoppers from the field were used. In the no-choice trials, leafhoppers were confined to a small mesh bag over a clean plant shoot; this may have affected their natural flying and foraging behavior. In the "choice" experiments, leafhoppers could freely fly about in a dome-shaped mesh cage; this environment would be closer to a natural environment for them. One transmission was successful: *C. reductus* collected from an infected cherry block transmitted WX to a previously WX-free *Prunus tomentosa* tree. Recipient plants will continue to be tested for WX into the late fall/early winter.

Table 5. Western X acquisition and inoculation trials with the leafhoppers *Colladonus reductus* (n = 45) and *geminatus* (n = 30), 2017-2018.

Leafhopper vector source*	Acquisition source	Recipient plant	Choice or no-choice	No. trials	Transmissions observed?**
Colladonus sp. from colony	Cherry leaves from field	Celery seedlings	No-choice	20	none
Colladonus sp. from colony	Cherry leaves from field	*Prunus tomentosa*	No-choice	10	none
Colladonus sp. from field	From infected cherry trees	*P. tomentosa*	No-choice	6	1
Colladonus sp. from colony	Cherry leaves from field	*P. avium*	No-choice	12	none
Colladonus sp. from colony	Cherry leaves from field	Radish plants	No-choice	15	none
Colladonus sp. from colony	Cherry leaves from field	Radish seedlings	Choice of recipient	8	none
Colladonus sp. from field	From infected cherry trees	Celery seedlings	No-choice	4	none

*Summarized for brevity. Most studies involved either *C. reductus* or *geminatus* but not both.
**Trials are not complete. Additional testing will be done in fall and winter 2018-2019.

SIGNIFICANCE TO THE INDUSTRY AND POTENTIAL ECONOMIC BENEFITS

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 caused by Western X phytoplasma. Our data show that there are two main time periods of WX-positive leafhopper activity in cherries—the spring and late summer into fall—although time periods may vary among orchards and year to year. To adopt a more targeted approach to leafhopper management, chemical controls could be implemented during those time periods, which should help to reduce costs of spraying for vector control.

EXECUTIVE SUMMARY

This project aimed to conduct surveys of leafhoppers and their potential host plants in Western X affected orchards and determine the incidence of Western X phytoplasma in populations of different leafhopper species. Western X is a pathogen of sweet cherries with infected trees exhibiting symptoms such as undersized and bitter-tasting cherries. Recommendations for preventing the spread of this disease include leafhopper vector control, leafhopper host plant control, and removal of trees. We also sought to determine the potential for *Colladonus reductus* and *C. geminatus* to transmit the Western X pathogen to cherries.

We conducted surveys of leafhoppers in affected orchards in 2016 to 2018, beginning in March/April and concluding in October. While some blocks were sampled over three years, sampling had to be discontinued in other blocks removed by the grower. Two leafhopper vector species*, C. 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.

Molecular diagnostic testing showed that for *C. geminatus*, 40/519 samples (8%) from within affected orchards and 71/348 samples (20%) from extra-orchard habitats, were positive for Western X phytoplasma. Likewise, for *C. reductus*, 103/687 samples (15%) from within affected orchards and 20/204 samples (10%) from extra-orchard habitats were positive for WX. Other infrequently collected potential vector species of leafhoppers that were tested for WX (number positive/sample size in parentheses) included: *Fieberiella florii* (1/12), *Osbornellus* sp. (1/23), *Scaphytopius* sp. (2/43), *Paraphlepsius* sp. (0/16), *Euscelidius variegatus* (1/35), *Macrosteles* sp. (0/15), *Circulifer tenellus* (0/22), and *Erythroneura* sp. (0/3).

From collections of weed and sagebrush plants and data from sticky card and sweep samples, we compiled a list of 33 potential host plant species for leafhoppers and/or Western X. A new project on native hosts of Western X phytoplasma began in the 2018 field season.

In late season 2017 and 2018, we conducted a total of 75 acquisition and/or inoculation studies with *C. geminatus* (n = 30) and *C. reductus* (n = 45). One transmission was successful: *C. reductus* collected from an infected cherry block transmitted WX to a previously WX-free *Prunus tomentosa* tree. We continue to check recipient plants during the fall into winter. While 1 success out of 45 *reductus* experiments may seem low, it only takes one leafhopper to introduce the disease to a block. Also, if leafhopper populations are high, as they were during peak periods in Mattawa1, Mattawa4, and Wapato blocks, this may account for the rapid spread of WX.

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 caused by Western X phytoplasma. Our data show that there are two main time periods of WX-positive leafhopper activity in cherries—the spring and late summer into fall—although time periods may vary among orchards and year to year. To adopt a more targeted approach to leafhopper management, chemical controls could be implemented during those time periods, which should help to reduce costs of spraying for vector control.

To prevent the spread of Western X, management of leafhopper vectors is only one component of an integrated strategy. Growers should also consider other ways in which Western X is spread such as root grafting or planting trees that are not pathogen-free. Additional research is currently underway and ongoing at the Clean Plant Center Northwest to investigate other aspects of Western X pathology and transmission.

FINAL PROJECT REPORT

Project Title: Model reporting

PI:	David Gibeaut	Todd Einhorn
Organization:	OSU-MCAREC	Michigan State University
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Cooperators: Stacy Cooper and Drew Hubbard. Susan Bishop and Austin Wilson at Allen Brothers Fruit

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

Provide a predictive model of bud hardiness throughout floral bud development because real-time analysis requires two or three days--after the information was desired.

Use relative water content of floral buds as a measure of phenology and freeze tolerance.

METHODS

Models were constructed in Excel spreadsheets that contain extensive phenology measurements including DTA, visual bud assessment, relative water content, bloom progression, fruit size, weight, color. This year leaf out and specific gravity were included.

The VFB model was coded in PHP programming language by Sean Hill and Otari Iosliani. The programming is currently running in beta-testing mode for use by researchers and any potential grower/cooperators. We hope to enlist more growers.

Further sampling was done this year to follow vernalization of Sweetheart at Cooper Barn, The Dalles. Mid-winter to awakening for Regina also at Cooper Barn, and for Chelan and Bing at Pyles, The Dalles. Attika, Benton, Bing, Lapins, Rainier and Skeena at MCAREC were assessed from awakening to the loss of low temperature exotherms in DTA. 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.

RESULTS & DISCUSSION

Results of 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.

Extensive rewriting and additions to the VFB modelling prevented timely updates in 2018.

Visual assessment of floral bud phenology is too subjective for modelling purposes. A standard method is required. The relative water content of buds used in DTA assessments was plotted vs the determined lethal temperature. A plot comparing the actual values and those predicted by VFB show good correlation (Fig. 1).

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.

In 2017 (fig 2.), 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.

In 2018 (fig. 2), warming in early February was sufficient to begin bud swelling. Swelling was followed by a long period of chill weather that slowed bloom progression to near normal timing in

Hood River and The Dalles. There was a potentially lethal freeze in late February depending on the bud stage. At MCAREC we experienced about 10% damage to floral primordia.

We have begun to collect and analyze temperature data for the 2018-19 season.

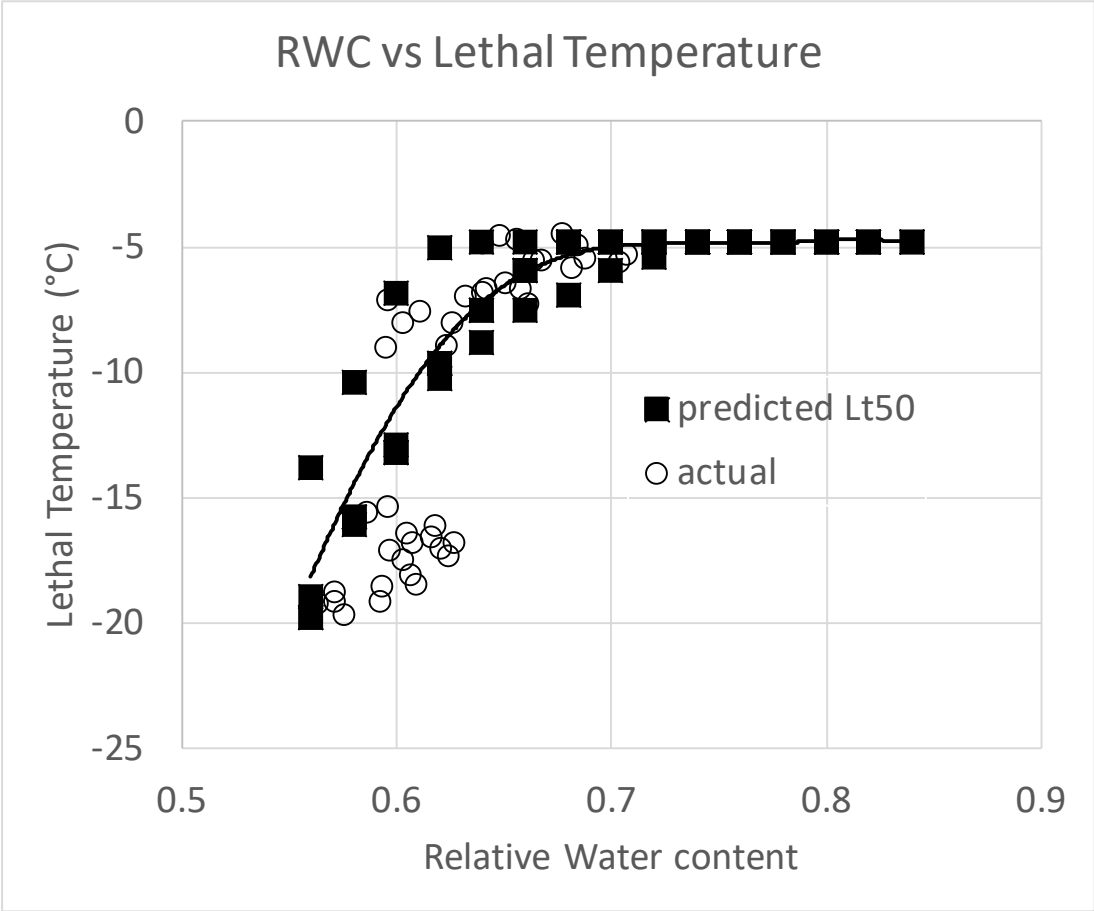

Figure 1. Phenology stage can be assessed as the relative water content of buds. Open circles are DTA estimates of the LT50. Closed squares are the VFB predictions of LT50. Note that at approximately 0.6 rwc buds are capable of acclimation and deacclimation. Once, over about 0.66 rwc, deaccliamtion is irreversible.

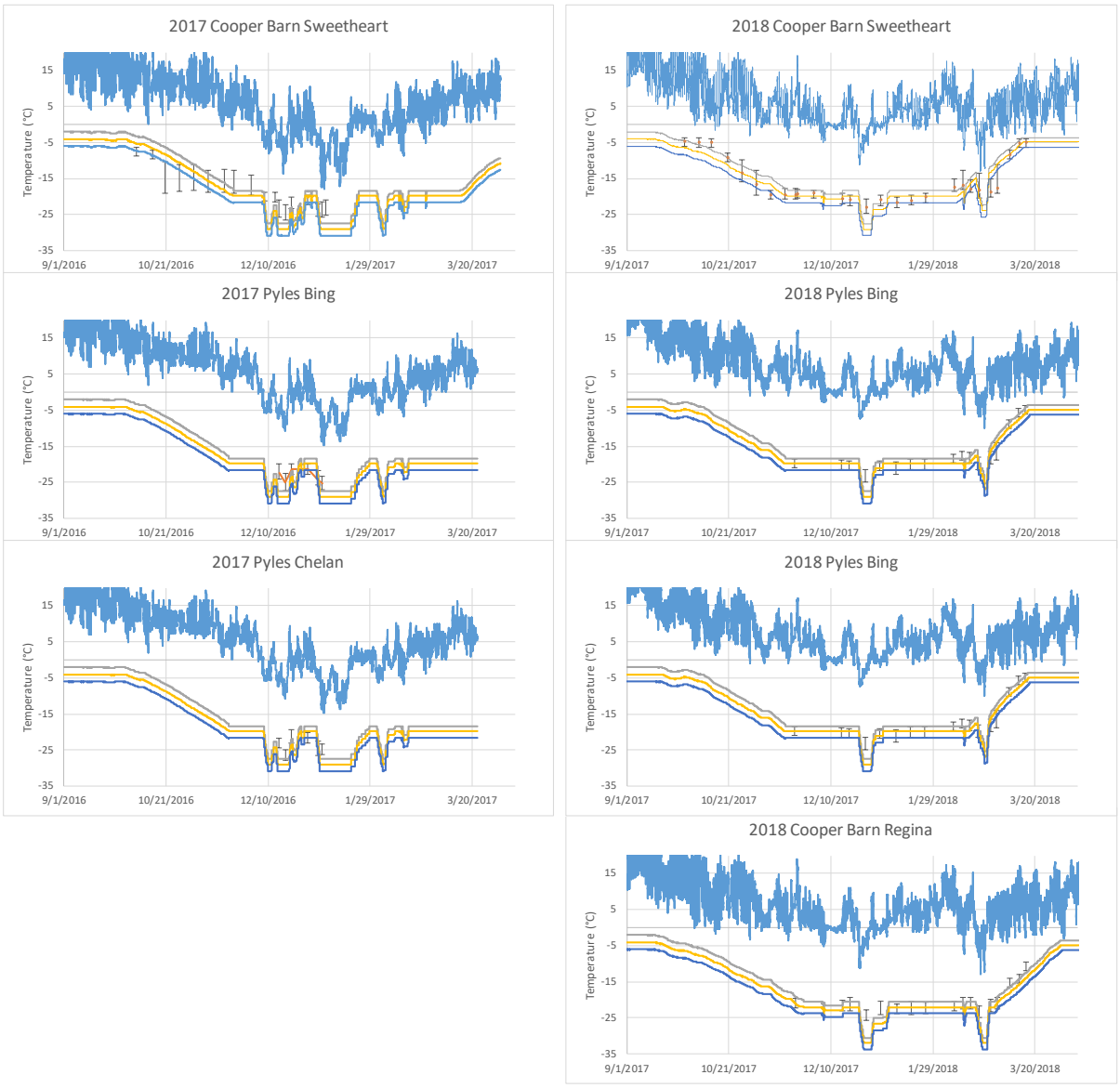

Figure 3. Years 2017 and 2018 at the same locations and cultivars. Air temperature (upper line), VFB predictions of lethal temperature (lines for Lt10, 50, 90%) and DTA assessment of lethal temperature (points with error bars).

EXECUTIVE SUMMARY

- Predictive modelling of Freeze Tolerance in Endo and Ecodormancy (FTEED) was published on website: blogs.oregonstate.edu/gdavs
- Vernalization modelling was added
- The awakening period after ecodormancy, was modelled by scaling the FTEED in relation to DTA assessments
- Relative water content is an objective measure of bud phenology and can be accurately related to FTEED preditions
- Three models; Vernalization, FTEED and Bloom, were combined to into one (VFB)
- PHP language coding was completed for use on the AgWeatherNet server
- I will bring full results of the VFB modelling to the meeting
- Growers, please bring any assessment of bud damage you may have from 2018

FINAL PROJECT REPORT

Project Title: *D.suzukii* transgenic, population replacement, eradication, suppression

PI: Omar Akbari
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Cooperators: Bruce Hay, Caltech

Other funding sources: None

Total Project Funding: $25,000

Budget History:

Item	Year 1:	Year 2:	Year 3:
WTFRC expenses			
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies	$25,000		
Travel			
Plot Fees			
Miscellaneous			
Total	$25,000		

ORIGINAL OBJECTIVES

Drosophila suzukii is a major invasive pest of ripening small fruit including raspberries, blueberries, strawberries, and cherries [1,2]. It has caused significant worldwide economic losses including significant damage in the berry- and cherry-growing industries of western North America [2–5]. Achieving effective control of *D. suzukii* has been difficult in a number of crop systems including cherries [6,7], and control measures have largely relied on prophylactic application of expensive broad spectrum insecticides [6–8]. This is problematic, as the repeated use of broad-spectrum insecticides has led to disruption of integrated pest management systems developed for crops such as cherries and berries, and has had a serious impact on beneficial arthropods, resulting, for example, in an increased use of miticides [4]. Additionally, broad use of insecticides makes it inevitable that resistance will become a major problem in the foreseeable future [8], increases the risk of residues on fruits [8], and arouses public concern [6]. However, there are no effective alternatives to managing *D. suzukii* infestation, and it is likely that, unless more effective control measures are developed, this pest will continue to spread [8].

An alternative, highly promising approach that could complement existing control methods is genetic pest management [9], which includes strategies such as gene drive [10,11] and transgenic-based sterile insect technique (SIT) [12,13]. In particular, engineered *D. suzukii* gene drive strains can be utilized to spread desirable genes (e.g., susceptibility to a novel bio-friendly pesticide) throughout, or to entirely suppress/eradicate, wild *D. suzukii* populations. Such an approach is catalytic, with release of only modest numbers of engineered insects required to spread desirable genes or achieve population suppression. Additionally, since such a system relies on only a few releases of transgenic insects to do the all of the work on an ongoing basis, it is cheap as compared to the use of insecticides, which need to be applied regularly. Finally, a major appeal of this approach is that it is environmentally friendly and entirely insect-specific, and would have no effect on crops or on beneficial organisms.

Our objective over the last year, therefore, was to make progress towards engineering gene drive systems in *D. suzukii*. Specifically, out of the multiple types of gene drive systems that can be utilized in a genetic pest management program [11,14], we decided to focus our efforts on developing *Medea* and Cas9-mediated systems. Our goals were to evaluate the feasibility of engineering each strategy in *D. suzukii*, and to take concrete steps towards developing a product (a genetically modified *D. suzukii*) that can be mass-reared and deployed into the wild to catalytically suppress, and completely eliminate, the wild populations of this significant pest.

SIGNIFICANT FINDINGS

I. Objective A - Development of CRISPR/Cas9-based drive systems in *D. suzukii*
   - A. Achieved an efficient means of transgenesis (required to test any gene drive components)
   - B. Developed and characterized multiple Cas9 transgenes in *D. suzukii* that are highly functional and enable efficient Cas9-mediated mutagenesis
   - C. Developed several ways to efficiently express gRNAs from the *D. suzukii* genome
   - D. Developed/optimized several components needed to build Y-gene drive
     1. Identified *D. suzukii* X and Y chromosome regions
     2. Identified putative X chromosome specific target sites
     3. Efficiently engineered the Y chromosome of flies
   - E. Developed/optimized several components needed to build Cas9-based suppression gene drive
     1. Identified promising suppression drive candidate target genes

2. Identified *D. suzukii* homologues of target genes and selected suitable gRNA target sites within these genes
3. Designed gRNA-expressing transgenes to test ability to target these genes
4. Built a proof of principle Cas9-based homing system in the *white* gene to test its ability to self-replicate

II. Objective B - Development of a *D. suzukii Medea*-based drive system

A. Finished characterizing and testing previously developed *D. suzukii Medea* drive system
   1. Characterized resistance to this drive system, which could hinder the spread of such a drive

B. Developed a modified version of this same system that should obviate the observed resistance
   1. Currently testing this system; preliminary evidence suggests that it does, as expected, function better that the original *Medea*

C. Developed a second-generation "reversal" *Medea* system that should be more robust in the face of genetic diversity in general and could be used to replace the original *Medea* in case a recall is necessary
   1. Currently testing this system

D. Identified several promising putative cargo genes that could be spread with the *Medea* gene drive to cause population suppression
   1. Currently testing these in *D. melanogaster* as proof of principle

RESULTS & DISCUSSION

(A) Development of CRISPR/Cas9-based drive systems

Summary

CRISPR/Cas9 technology has great applicability to the development of genetic pest management approaches, and can be used to build various gene drives - including Y-chromosome drive and Cas9-mediated homing-based drive - that can be employed to suppress and eliminate pest populations. We have made significant progress in developing the tools needed to engineer both of these types of gene drives in *D. suzukii*. Specifically, we have developed and characterized multiple Cas9 transgenes in *D. suzukii* that are highly functional and enable efficient Cas9-mediated mutagenesis in this pest. We have also developed several ways to efficiently express gRNAs from the *D. suzukii* genome. Together, these tools enable efficient CRISPR/Cas9-based manipulations of the *D. suzukii* genome, and provide the basis for building Cas9-based gene drives. Furthermore, we have developed/optimized several components needed to build Y-gene drive, including identifying *D. suzukii* X and Y chromosome regions, identifying putative X chromosome specific target sites, and efficiently engineering the Y chromosome of flies. Additionally, we have also taken steps towards engineering Cas9-based suppression gene drive, including: identifying promising candidate genes to be targeted by this drive; finding *D. suzukii* homologues of, and selected suitable gRNA target sites within, these genes; designing gRNA-expressing transgenes to test our ability to target these genes; and building a proof of principle Cas9-based homing system in the white gene to test its ability to self-replicate. We can now begin putting these components together to generate functional suppression gene drives in *D.suzukii*.

Background

The arrival of CRISPR technologies heralded a new era for traditional genome manipulation and site-specific transgenesis [15,16], and for advanced engineering of target genomes including the construction of gene drives [14,17]. Out of all the types of gene drives that have been proposed, drives

based on the CRISPR/Cas9 gene-editing system may be the simplest to build (especially given CRISPR's functionality in many insects [18–26]) and the most effective [11]. Most CRISPR technologies used in insects utilize a simplified two-component system consisting of a *S. pyogenes* Cas9 endonuclease (SpCas9) and a single chimeric guide RNA (gRNA) [27] that can generate DNA double-strand breaks (DSB) in a location of one's choosing. These breaks can them be repaired either randomly (via non-homologous end-joining, NHEJ) or based off a template (via homology-directed repair, HDR) [27,28]. The functionalities of CRISPR/Cas9 systems can be exploited to bring about gene drive-based population suppression.

For example, distortion of the sex ratio in favor of males can lead to a gradual population reduction and eventual elimination of a target population [29–32], and natural so-called meiotic driving Y-chromosomes have been described [33–35]. A system for sex-ratio distortion can also be engineered by designing CRISPR-based transgenes that target the X-chromosome during spermatogenesis [36,37](Figure 1). This Y-gene drive approach would depend on the destruction of X-bearing sperm to produce males that only give rise to male progeny [14,38], and would require the ability to meiotically express an X-chromosome targeting element from the Y-chromosome [36,39]. Importantly, CRISPR/Cas9 technology could straightforwardly be utilized to engineer Y-gene drive elements by designing gRNAs that target only the X chromosome [36,37]. Such a system has already been developed in one species of mosquito [36,40,41], and should be portable to *D. suzukii*.

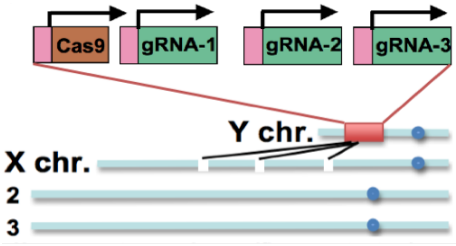

Fig. 1

Another way CRISPR/Cas9 can be utilized to bring about population suppression is via Cas9-mediated homing-based gene drive [14]. This concept is based on the idea of using homing endonuclease genes (HEGs) to manipulate populations [42]. These genes are extraordinarily selfish, and this property can be exploited for both population suppression and replacement. HEGs have the ability "cheat" during meiosis by converting their corresponding allele on the opposite chromosome into an exact copy of themselves, by encoding a sequence-specific endonuclease that severs and disrupts their competing chromosomal allele, which can force the call to use the HEG as a template for homology-directed repair (HDR), resulting in the HEG copying itself (i.e., homing) into its competing allele. If the latter repair option occurs in the germline, or early embryo, then the proportion of offspring that receive the HEG will be above that expected with normal Mendelian transmission (i.e., 50%), allowing for rapid invasion of the HEG into a target population [43]. A HEG can be used to spread a payload gene (replacement drive) or for population suppression and possibly eradication by homing into a target gene, the disruption of which leads to recessive lethality or sterility (Figure 2). In such a suppression approach, homing must be confined to the germline during gamete formation, leading to sterility/non-viability only in homozygotes that receive the HEG allele from both parents. Consequently the HEG can rapidly spread, and once a large fraction of the population is heterozygous, it can cause a population crash as heterozygote pairings will produce sterile/non-viable offspring. Although several proof-of-principle studies have shown the utility of HEGs as gene drives prior to the advent of CRISPR/Cas9 (e.g., [44]), this powerful system is enabling the efficient design of homing-based drive systems in many contexts [17]. Several replacement Cas9-mediated homing-based gene drives have been developed [18,45,46]; additionally, several Cas9-based suppression drive systems have recently been engineered in

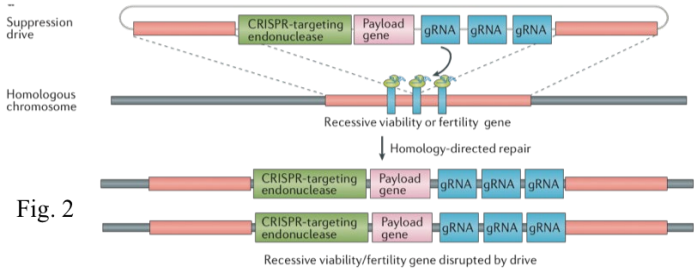

Fig. 2

fruit flies [47,48] and one species of mosquito [20,49], and should also be possible to transfer to *D. suzukii*. However, neither this approach nor Y-gene drive have been developed in this pest species.

Results and Future Directions

Efficient Transgenesis in *D. suzukii*

In order to engineer any type of gene drive system in *D. suzukii*, we first have to be able to efficiently generate transgenic flies. Although transgenesis in *D. suzukii* has been previously established [50], it is not very efficient [51], and we had previously struggled with obtaining *D. suzukii* transgenic fly lines. However, a recent work [52] described the generation of a "jumpstarter" *D. suzukii* strain that carries the *transposase* gene necessary for *piggyBac* transposition, and reported that performing germline transformation in this strain dramatically increased transgenesis rates (in some cases 40- to nearly 60-fold [52]). Since increased rates of transgenesis would help us accelerate our gene drive development efforts, this past year we obtained the USDA/APHIS permits necessary to acquire this transgenic strain from the researchers that developed it, have expanded the obtained stocks into a large colony, and are carrying out all microinjections for transgenesis into this strain. This has been greatly helpful, as we are now able to obtain transgenic lines with much greater efficiency.

Development of Cas9 Tools in *D. suzukii*

The development of both Y-gene drive and Cas9-mediated suppression drive in *D. suzukii* requires functional CRISPR/Cas9 tools in this fly. Although Cas9-mediated genome editing had been previously demonstrated in *D. suzukii* [53], it was carried out by microinjection of gRNAs and Cas9 protein into embryos. Conversely, the building of a gene drive requires a germline source of Cas9 and gRNAs driven by an effective promoter, typically a PolIII promoter such as U6.

Leveraging our experience in designing and optimizing CRISPR/Cas9 tools in *D. melanogaster*, we have generated both of these components. Specifically, we have generated four distinct functional transgenic Cas9 lines, where expression of Cas9 is driven by either strong female germline specific promoters (*BicC* and *Dhd*) or by male and female germline specific promoters (*vasa* and *nanos*) that have been previously validated in *D. melanogaster* [12,46]. We have tested these Cas9 lines, and have shown that all four work, with up to 100% mutagenesis efficiency (for *vasa*-Cas9). We have also generated several functional gRNA-expressing transgenes by targeting the *white* gene, which gives flies a red eye color, as a proof of principle. Specifically, after several failed attempts, we have demonstrated that a genomically encoded, PolIII U6:3 promoter-driven gRNA targeting *white* produces up to 100% mutated (white and mosaic-eyed) progeny when crossed to a Cas9 expressing line (Figure 3). We have also shown that a genomically encoded tRNA-gRNA expression cassette [54], driven by a PolII germline specific promoter, also functions to produce mutated progeny (albeit at a more modest frequency of ~15-30%).

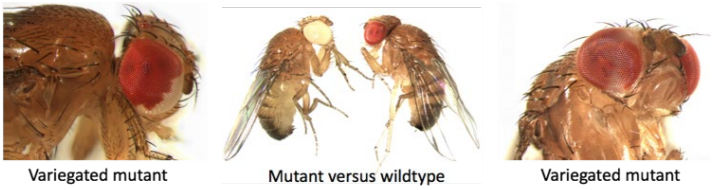

Fig. 3

The development of these tools lays the foundation for the ability to engineer Cas9-based gene drives in *D. suzukii*.

Engineering a Y-gene Drive System

Assuming that efficient CRISPR/Cas9 tools are available, the ability to build a Y-gene drive requires three further components: the ability to identify X and Y chromosomes in *D. suzukii*; the ability to insert large transgenes on the Y-chromosome; and the ability to target and cut sequences only present on the X-chromosome.

Identifying, and inserting genes on, the Y chromosome in D. suzukii

The current genome annotation of *D. suzukii* (http://spottedwingflybase.org) is divided into over 29, 000 contigs (independent fragments that have not been brought together to make a clear

linear sequence map of each chromosome), and it is not entirely clear which of these contigs comes from the *D. suzukii* Y and X chromosomes. Therefore, we have used a bioinformatic approach to try to identify fragments of these chromosomes. To do this, we took the entire *D. melanogaster* Y chromosome sequence and carried out a search for related sequences (a BLAST homology search) among the *D. suzukii* contigs; essentially, we looked for regions of *D. suzukii* that were nearly identical to those from the melanogaster Y chromosome, as these are likely to represent *D. suzukii* Y chromosome sequence. We identified a total of 134 contigs that had extremely high homology (E-value = 0) to the *D. melanogaster* Y chromosome. Given this high homology, we are confident that these contigs are pieces of the *D. suzukii* Y chromosome. From this data we have identified several regions of the putative *D. suzukii* Y chromosome that should be ideal locations for integrating an X chromosome targeting Cas9/gRNA cassette (outside of any known transcribed regions, in unique, non-repetitive DNA).

In order to assay whether we could use CRISPR/Cas9 to dock transgenes on the Y chromosome, we first set out to develop a CRISPR/Cas9-based technique for site-specific engineering of the *D. melanogaster* Y chromosome as a proof of principle [55], as it is much easier and faster to test and troubleshoot components in this species before porting them to *D. suzukii*. To do this, we engineered a vector comprising a fluorescent marker (tdTomato) driven by the eye-specific 3xP3 promoter and flanked by the gypsy and CTCF insulators, with unique restriction sites upstream and downstream for cloning specific homology arms (Figure 4). We then selected ten distinct intergenic regions spanning the Y chromosome for targeting, identified a suitable sgRNA target site in each region, and cloned in homology arms, corresponding to ~800-1,000 base pairs of sequence 5' and 3' of each selected target site, upstream and downstream of the insulator-flanked 3xP3-tdTomato element to generate ten unique Y chromosome targeting transgenes. Each transgene was then injected, along with the appropriate in vitro transcribed sgRNA and Cas9 protein, into a transgenic line expressing a germline source of Cas9 using standard procedures, and G1 progeny were screened for presence of the transgene marker. Two of the injected transgenes inserted in the correct positions on the Y chromosome, demonstrating that we can use the above approach to insert, and detect expression from, a fluorescently marked transgenic cassette at specific locations on the Y-chromosome in *D. melanogaster* using CRISPR/Cas9-mediated HDR.

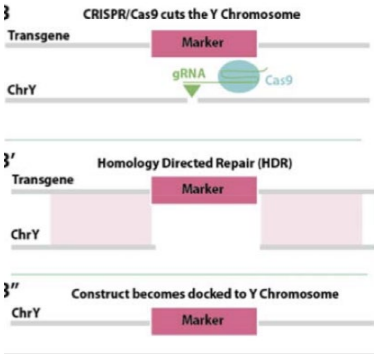

Fig. 4

We are now testing whether we can insert, and detect expression from, Cas9-containing transgenes at these same Y chromosome locations, as we will need to be able to express Cas9 cassettes from the Y in order for the Y gene drive approach to work. Once these experiments are complete, we plan to port this approach to *D. suzukii*.

Identifying and cutting the X chromosome in D. suzukii

We performed a similar bioinformatic analysis to the one described above identify the X chromosome of *D. suzukii*, and identified 388 contigs from *D. suzukii* as being X-linked. Then, to identify potential gRNA sequences specific to the *D. suzukii* X chromosome, and present in multiple copies, we first developed a program to predict all possible Cas9 cleavage sites on the X-chromosome by searching for the PAM motif (XGG in the target sequence N(21)XGG). Once potential X-chromosome cleavage sites were identified, they were aligned to the rest of the genome (all the other non-X contigs) and those that showed a sequence match to these contigs were eliminated. The final output of this program was a conservative list of X chromosome specific Cas9 cleavage sites.
From all of this, we conservatively predicted several potential target sequences repeated exclusively on the X chromosome in up to ten locations, making them ideal for the development of guide RNAs to cleave the *D. suzukii* X chromosome. However, our initial attempts at testing these gRNAs for their

ability to cut the X did not succeed because, as discussed above, our initial gRNA-expression configuration were not functional. However, now that we have a highly functional gRNA expression configuration, we can proceed to clone X chromosome-targeting gRNAs into our gRNA expression cassettes and test them.

Engineering a Cas9-mediated Suppression Drive System

To engineer a Cas9-mediated suppression homing drive, we need to introduce the coding sequence for Cas9 and gRNA into the genomic site targeted by the Cas9/gRNAs [11] to generate a self-replicating transgene that could continuously mutate a target gene every generation and/or carry a transgene into the population. This self-replicating (i.e., homing) Cas9-based transgene would need to be placed within a gene necessary for female fertility, so that eventually all of the females in a target population would become sterile and the population would collapse [49].

As described above, we now have working Cas9 and gRNA transgenes that we can utilize as the basis for such a gene drive. After analyzing recent efforts to develop such suppression drive systems in fruit flies [47,48] and mosquitoes [49], we have also identified several promising candidate target genes, including *dsx*, *tra*, *sxl*, and *zpg*, which are conserved in *D. suzukii*. After analyzing the sequences of the *D. suzukii* homologues of these genes to find regions that are highly conserved and thus unlikely to contain sequence variation, we have selected two gRNA target sites within each gene, and have engineered separate U6-driven gRNA transgenes targeting each gene to test whether the selected gRNA sequences will work to efficiently cut the selected targets. (We are currently working on obtaining transgenic lines for these transgenes.) After we verify that the gRNAs work, we will proceed to construct full Cas9-based suppression drive cassettes targeting the most promising candidates (based on gRNA function). In parallel, we are also testing a split Cas9-based gene drive cassette [56] targeting the *white* eye color gene as a proof of principle, to determine whether we can: a). dock transgenes in a site-specific location using CRISPR/Cas9 in *D. suzukii*; and b). observe the efficiency of self-replication/homing of this Cas9-based transgene in *D. suzukii*.

(B) Development of a *D. suzukii Medea*-based drive system

Summary

Previously, we had developed the first *D. suzukii* functional replacement gene drive system termed *Medea*, had rigorously tested it in laboratory cage populations, and had characterized it in different genetic backgrounds to determine effectiveness and fecundity (our results on this project were published in *PNAS* this year [57]). We found that this first-generation *Medea* system was capable of biasing Mendelian inheritance rates with up to 100% efficiency and could maintain itself at high frequencies in a wild population; however, drive resistance, resulting from naturally occurring genetic variation and associated fitness costs, was present and could hinder the spread of such a drive. Therefore, since mathematical modeling indicated that our *Medea* drive system could spread to fixation if either its fitness costs or toxin resistance were reduced [57], we have developed a modified version of this same system that should obviate the specific resistance that we observed, and have preliminary evidence to suggest that it does, in fact, function better that the original *Medea* we tested. We have also developed a second-generation *Medea* system in *D. suzukii* that should be more robust in the face of genetic diversity in general and could be used to replace the original *Medea* in case a recall is necessary. Finally, we have identified several promising putative cargo genes that could be spread with the *Medea* gene drive to cause population suppression, and are moving forward with testing them in *D. suzukii*.

Background

Medea was first discovered in the flour beetle [58], and multiple versions were later reverse engineered from scratch and shown to act as robust gene drives in the laboratory fruit fly, Drosophila melanogaster [59,60]. Such engineered *Medea* systems rely on a *Medea* element consisting of a toxin-antidote combination (Figure 5). The toxin consists of a miRNA that is expressed during oogenesis in *Medea*-bearing females, disrupting an embryonic essential gene. A linked antidote is expressed early

during embryogenesis and consists of a recoded version of the target gene that is resistant to the miRNA. This combination results in the survival of half of the embryos originating from a *Medea*-bearing heterozygous female, as those that do not inherit the *Medea* element perish. If a heterozygous *Medea* female has mated with a heterozygous *Medea* male, the antidote from the male will also take effect in the embryo, resulting in 3/4 of the embryos surviving. Therefore, *Medea* will rapidly spread through a population, carrying any linked genes with it.

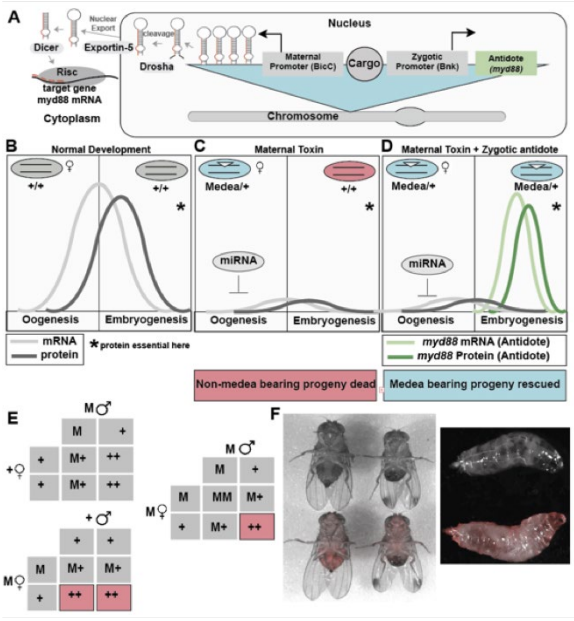

Fig. 5

In the case of *D. suzukii*, since elimination of the pest population is ultimately the goal, an engineered *Medea* system could spread a gene proffering susceptibility to a particular pesticide, or a conditional lethal gene that would be activated by some substance or environmental cue such as high temperature or diapause - a state that allows insects survive periods of adverse conditions such as cold [61]. For example, a *Medea* element can be used to spread a gene conferring sensitivity to a particular chemical that is normally innocuous, rendering such a chemical capable of being used as an environmentally-friendly, species-specific pesticide. Trigger-inducible transcription control elements – ones that turn on expression in the presence of a chemical such as tetracycline or vanillic acid [62,63] – can be engineered to drive expression of an insect-specific toxin (e.g., [64]). A *Medea* element can also be used to spread a gene under the control of a diapause-induced promoter that will splice to produce a toxin in females only, so that, upon the onset of the diapause-inducing environmental cue, all of the females will perish, causing a population crash [59]. Furthermore, a *Medea* element can be utilized to spread a thermally activated TRPA1 cation channel [65] that, upon exposure to a specific threshold temperature, renders flies paralyzed or dead. However, although transgenesis of *D. suzukii* has been established [50], no effective suppression gene drive systems in this major pest have yet been engineered.

Results and Future Directions

Generation of Synthetic *Medea* Gene Drive

To create a synthetic *Medea* gene drive in *D. suzukii*, we engineered a *piggyBac* vector comprising a miRNA toxin coupled with a toxin-resistant antidote, inspired by the architectures used to generate previous *Medea* systems in *D. melanogaster* [66,67]. We designed synthetic miRNAs to target *D. suzukii* myd88, a highly conserved gene shown to be maternally deposited and required for dorsal-ventral patterning in the early embryo in *D. melanogaster* [68]. We used the predicted *D. suzukii* female germline-specific bicoid (BicC) promoter to drive expression of a "toxin" consisting of a polycistronic array of four synthetic microRNAs (miRNAs) each designed to target the 5' untranslated region (UTR) of *D. suzukii* myd88 (Figure 5). Importantly, to ensure these miRNAs could target the desired sequence, we performed genomic DNA sequencing of the myd88 5'UTR target region in our reference *D. suzukii* strain (collected from Corvallis, Oregon) and designed the miRNAs against this sequence. This *Medea* drive also contained an "antidote" consisting of the *D. suzukii* myd88 coding region, insensitive to the miRNAs as it did not contain the miRNA-targeted 5'UTR, driven by the predicted *D. suzukii* early embryo-specific bottleneck (bnk) promoter, and two separate transformation markers – eGFP driven by the eye-specific 3xP3 promoter [69], and dsRed driven by the ubiquitous hr5-IE1 promoter [70].

Characterization of *Medea* Genetic Behavior

Following microinjection of the *Medea* transgene into *D. suzukii* embryos, a single G_1 transformant male was recovered, as identified by ubiquitous hr5-IE1 driven expression of dsRed (Figure 5), and weak eye-specific 3xP3-driven eGFP. When outcrossed to several wildtype (non-*Medea* bearing; +/+) females, this male produced roughly ~50% *Medea*-bearing and ~50% wildtype offspring, as would be expected from standard Mendelian segregation without biased inheritance. Resulting heterozygous G_2 *Medea*-bearing progeny were individually outcrossed to wildtype individuals of the opposite sex to determine inheritance patterns, and these individual outcrosses were continued for six generations (Table 1). Remarkably, until the G_5 generation, all heterozygous *Medea*/+ mothers (n = 91) produced 100% *Medea*-bearing progeny (n = 1028), while heterozygous *Medea*/+ fathers (n = 16) produced ~50% *Medea*-bearing progeny (n = 268). While the majority of heterozygous *Medea*/+ G_5 (23/31) and G_6 (16/25) generation females also produced 100% *Medea*-bearing progeny, some heterozygous G_5 (8/31), and G_6 (9/25) females unexpectedly produced a small yet notable number (52/1219) of wildtype offspring. Although the exact reason for the difference is unclear, later analysis suggested that resistance to the miRNA toxin might explain this unexpected observation. Notwithstanding, individually these G_5 and G_6 heterozygous *Medea*/+ females displayed significantly biased biased inheritance rates ranging from 76%-96%, with an average rate of 86.4%. Overall, in six generations of individual female outcrosses, the percentage of *Medea*-bearing progeny borne by single heterozygous *Medea*/+ mothers (n = 147) was 97.7% (2195/2247) as opposed to the 50% that would be expected with standard Mendelian segregation, indicating that the *Medea* drive is extremely functional at biasing inheritance.

To further characterize the genetics behind the highly biased inheritance patterns described above, additional crosses between individuals of various *Medea* genotypes were performed, and confirmed that *Medea* exhibits maternal-effect lethality and zygotic rescue. For example, matings between heterozygous *Medea*/+ mothers and wildtype fathers resulted in 55.63±0.76% total embryo survival with 94.20±1.33% of the progeny being *Medea*-bearing, while matings between heterozygous *Medea*/+ mothers and heterozygous *Medea*/+ fathers yielded 79.11±3.95% total embryo survival with 94.12±0.67% of the progeny being *Medea*-bearing. The higher-than-expected embryo survival is consistent with the observation that not all heterozygous *Medea*/+ mothers give rise to 100% *Medea*-bearing progeny, indicating that not all wildtype progeny from a heterozygous *Medea*/+ mother perish.

Medea Functionality in Geographically Distinct Populations

Fig. 6

To assess whether the *D. suzukii* *Medea* could function in geographically distinct populations that possibly harbor genetic variability in regions that canonically have less conservation such as the 5'UTR, heterozygous *Medea*/+ flies were tested in eight additional *D. suzukii* strain backgrounds. These strains were collected from various locations around the world, including: Mt. Hood, OR; Clayton, WA; Brentwood, CA; Tracy, CA; Watsonville, CA; Oahu, HI; Beltsville, MD; and Ehime, Japan. Interestingly, for 3/8 strains, the *Medea* inheritance rate from heterozygous *Medea*/+ mothers was 100%, while from 5/9 strains the inheritance rate ranged from 87.6% to 99.4%, with an overall transmission rate of 94.2% (Figure 6). These results strongly demonstrate that the *Medea* drive described here can dominantly bias transmission in diverse *D. suzukii* populations.

Long Term Population Cage Experiments

The above observations suggested that *D. suzukii Medea* should be able to drive robust population replacement. To test this prediction, we performed several long term multi-generational population cage experiments specifically challenging the *Medea* drive with a wildtype strain that harbored pre-existing resistance (Corvallis, OR). We set up these population cage studies after maintaining this population for approximately ten generations, we mated *Medea*-bearing fathers to wildtype Corvallis, OR, strain mothers at three distinct introduction (G_0) frequencies: low frequency (25 heterozygous *Medea*/+ and 25 wildtype +/+ males mated to 50 wildtype +/+ virgins, *Medea* allele frequency of ~12.5% and genotype frequency of ~25%); medium frequency (50 heterozygous *Medea*/+ males mated to 50 wildtype +/+ virgins, *Medea* allele frequency of ~25% and genotype frequency of ~50%); and high frequency (50 homozygous *Medea*/*Medea* males mated to 50 wildtype +/+ virgins, *Medea* allele frequency of ~50% and genotype frequency of ~50%). These experiments were conducted in separate bottles in biological triplicate for the low and medium threshold and quadruplicate for the high threshold drives, producing ten distinct populations with G_1 *Medea* allele frequencies ranging from ~12.5-50% and genotype frequencies ranging from ~25-100%. Altogether, these population cage experiments were followed for 9 generations (for lower allele frequency populations, as the *Medea* allele disappeared from the population by that time) or 19 generations (for higher allele frequency populations), counting the number of *Medea*-bearing adults each generation to determine the genotype frequency, as described previously [60,67]. Interestingly, the observed changes in *Medea* frequency over time indicated that, for release proportions (defined as the genotype frequency in the G_1 population) of 50% or smaller, the *D. suzukii Medea* drive was unable to drive into the wildtype population, likely because of selected drive resistance combined with high fitness costs outweighing the effect of drive. However, at higher release proportions of >90%, similar to classical chromosomal rearrangement thresholds [71], the drive largely compensated for the fitness cost, allowing the gene drive to remain in the population at high frequencies for the duration of the experiment (19 generations). Although unintended, the self-limiting dynamics of the generated *Medea* system may be useful in achieving a transient population transformation of the type associated with other proposed gene drives (e.g.,[72]).

Mathematical Modeling

To characterize the population dynamics observed in the above cage experiments, we fitted a mathematical model to the observed data in which the *Medea* drive had an associated fitness cost in heterozygotes and homozygotes and there was a *Medea*-resistant allele present in the population that reduced toxin efficiency. For the fitted model, the *Medea* drive was estimated to have a toxin efficiency of 93% in individuals homozygous for the resistant allele (95% credible interval (CrI): 90-95%) and was assumed to have a toxin efficiency of 100% in individuals lacking the resistant allele. The *Medea* drive was estimated to confer a large fitness cost on its host - 28% in heterozygotes (95% CrI: 27-30%) and 65% in homozygotes (95% CrI: 62-67%) - and the resistant allele was estimated to have an initial allele frequency of 78% in the population (95% CrI: 57-97%).

Predictive mathematical modeling based on these parameter estimates suggests that the *Medea* drive would spread to fixation in the absence of toxin resistance if released above a threshold frequency of 79%. Spread to fixation would also be expected if the fitness costs of the generated *Medea* drive were halved, even if all individuals in the population were homozygous for the *Medea*-resistant allele, provided the drive was released above a threshold frequency of ~25-27%. Consistent with the experimental results, a *Medea* drive with a large fitness cost in a *Medea*-resistant population is expected to be maintained at high frequencies through its drive; however, its eventual elimination is inevitable unless supplemental releases are carried out. However, for high release frequencies (90-95%), the drive may be maintained at high frequencies (>75%) for ~20 generations, which likely exceeds the duration required for agricultural impact.

Improved *Medea* Construct and Reversal *Medea*

Given our observations regarding resistance and its effect on *Medea* function, we set out to engineer improved *Medea* systems that could reduce the chances of resistance acting as an

impediment to spread. Specifically, we performed some sequencing-based characterization of naturally occurring genetic variation in various geographically distinct target populations to help guide selection of target sites that are well conserved across all populations in which the drive is intended to function. We then designed a modified version of the original *Medea* system that targeted different, conserved sequences (still in the 5'UTR of the *myd88* target gene), reasoning that such a *Medea* element should function very similarly to the original element but not be impeded by the resistance we previously observed. We have obtained transgenic lines for this improved *Medea* element, and preliminary data indicates that it works better than the original *Medea*, producing 100% inheritance bias. We will continue rigorously testing this second-generation *Medea* element in the coming year.

Additionally, we hypothesized that to reduce resistance, miRNA target site selection could be limited to the coding DNA sequence regions of a genome, which tend to be strongly conserved, as opposed to regions such as the 5'UTR, which canonically have higher tolerance for sequence variation. We have therefore also developed a second-generation *Medea* system in *D. suzukii* that should be more robust in the face of genetic diversity in general (because it targets coding DNA regions as opposed to the 5'UTR) and could be used to replace the original *Medea* in case a recall is necessary. Specifically, to reduce risk and mitigate the spread of the *D. suzukii Medea* system into wild populations, it is important to develop a reversal Medea (RM) system and demonstrate that it can function as predicted. Reversing the drive of a *Medea* system has been theorized; however it has never been experimentally demonstrated. Therefore, this should be of high impact and relevance when it comes to regulators assessing the risk associated with gene drives. We have finished designing and building a Reversal *Medea* system capable of spreading on its own and of replacing the first *Medea* described above, and are in the process of obtaining transgenic *D. suzukii* individuals containing this *Medea* and of rigorously characterizing this system.

Identification of Putative "Cargo" Genes

For *D. suzukii*, elimination of the pest populations is ultimately the goal. An engineered *Medea* system could achieve this by spreading a "cargo"gene proffering susceptibility to a particular pesticide, or a conditional lethal gene that would be activated by some substance or environmental cue such as high temperature or diapause. One promising type of candidate "cargo" gene is a thermally activated TRPA1 cation channel [65]. Specifically, TRPA1 is an ion channel located on the plasma membrane of many human and animal cells, and is finely tuned to detect specific temperatures ranging from extreme cold to noxious heat [65]. Upon exposure to a critical "threshold" temperature, this cation channel can "open" and modulate Ca2+ and Mg2+ entry into the cell [73]; when TRPA1 is overexpressed in an exogenous tissue (such as the fly brain, for example), this "opening" can lead to total fly paralysis and death. We therefore would like to engineer *D. suzukii* to express a specific TRPA1 channel in the brain, so that exposure of the engineered individuals to a threshold temperature (determined by the specific TRPA1 channel used) would paralyze/kill the flies. We should then be able to spread this temperature-activated "cargo" gene through wild populations by using our *Medea* system during cooler months, and achieve population suppression when the TRPA1 gene is activated in warmer months.

To achieve this, we are working to leverage data from the Montell lab (UCSD), which is developing this technology for mosquito control. The Montell lab is currently testing several TRPA1 channels with different activation temperatures (including rattlesnake TRPA1, python snake TRPA1, boa snake TRPA1 and fruit fly TRPA1) in *D. melanogaster* as a proof of principle, and has preliminary data indicating that at least some of the tested TRPA1 channels, when expressed in the fly brain, work as expected. Once we know which TRPA1 channel appears most promising, we will insert it into our best *Medea* element and begin testing this approach in *D. suzukii*.

EXECUTIVE SUMMARY

Drosophila suzukii is a major invasive pest of many small fruits, and has caused significant damage in agricultural industries of western North America. Control measures have largely relied on prophylactic application of broad spectrum insecticides, which is problematic, as repeated use of insecticides is expensive, has had a serious impact on beneficial arthropods, and makes it inevitable that resistance will arise in the foreseeable future. However, there are no effective alternatives to managing *D. suzukii* infestation, and it is likely that this pest will continue to spread.

An alternative, highly promising approach that could complement existing control methods is genetic pest management, which includes strategies such as gene drive. In particular, engineered *D. suzukii* gene drive strains can be utilized to spread desirable genes (e.g., susceptibility to a novel bio-friendly pesticide) throughout, or to entirely suppress/eradicate, wild *D. suzukii* populations. Such an approach is catalytic, with release of only modest numbers of engineered insects required to spread desirable genes or achieve population suppression, and can be cheap, since it relies on only a few releases of transgenic insects. A major appeal of this approach is that it is environmentally friendly and entirely insect-specific, and would have no effect on crops or on beneficial organisms. Our objective over the last year, therefore, was to make progress towards engineering *Medea* and Cas9-mediated gene drive systems in *D. suzukii*.

We had previously developed the first *D. suzukii* functional replacement gene drive system termed *Medea*, had rigorously tested it in laboratory cage populations, and had characterized it in different genetic backgrounds to determine effectiveness and fecundity (our results on this project were published in *PNAS* this year). We found that this first-generation *Medea* system was capable of biasing Mendelian inheritance rates with up to 100% efficiency and could maintain itself at high frequencies in a wild population; however, drive resistance, resulting from naturally occurring genetic variation and associated fitness costs, was present and could hinder the spread of such a drive. Therefore, since mathematical modeling indicated that our *Medea* drive system could spread to fixation if either its fitness costs or toxin resistance were reduced, we have developed a modified version of this same system that should obviate the specific resistance that we observed, and have preliminary evidence to suggest that it does, in fact, function better that the original *Medea* we tested. We have also developed a second-generation *Medea* system in *D. suzukii* that should be more robust in the face of genetic diversity in general and could be used to replace the original *Medea* in case a recall is necessary. Finally, we have identified several promising putative cargo genes that could be spread with the *Medea* gene drive to cause population suppression, and are moving forward with testing them in *D. suzukii*.

We have also made significant progress in developing the tools needed to engineer CRISPR/Cas9 based gene drives (including Y-chromosome drive and Cas9-mediated homing-based drive) in *D. suzukii*. Specifically, we have developed and characterized multiple Cas9 transgenes in *D. suzukii* that are highly functional and enable efficient Cas9-mediated mutagenesis in this pest. We have also developed several ways to efficiently express gRNAs from the *D. suzukii* genome. Together, these tools enable efficient CRISPR/Cas9-based manipulations of the *D. suzukii* genome, and provide the basis for building Cas9-based gene drives. Furthermore, we have developed/optimized several components needed to build Y-gene drive, including identifying *D. suzukii* X and Y chromosome regions, identifying putative X chromosome specific target sites, and efficiently engineering the Y chromosome of flies. Additionally, we have also taken steps towards engineering Cas9-based suppression gene drive, including identifying promising candidate genes to be targeted by this drive, finding *D. suzukii* homologues of and identifying suitable gRNA target sites within these genes, designing gRNA-expressing transgenes to test our ability to target these genes, and building a proof of principle Cas9-based homing system in the white gene to test its ability to self-replicate. We can now begin putting these components together to generate functional suppression gene drives in *D.suzukii*.

FINAL PROJECT REPORT

Project Title: Sweet cherry breeding: identifying genetically superior selections

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

Other funding sources

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 McCord and Peace.

Total Project Funding: $150,000

Budget History:WSU[1]

Item	Year 1:	Year 2:	Year 3:
Salaries	11,100		
Benefits			
Wages	29,466		
Benefits[2]	7,395		
Equipment	793		
Supplies			
Travel	3,579		
Plot Fees			
Miscellaneous[3]	34,396		
Total	86,729		

Footnotes: [1]Expenses recorded cover the period from 1 November 2017- 21 October 2018. [2] Combines benefits from both salaried and hourly employees. [3]Supplies and Miscellaneous combined.

Budget History: OSU-MCAREC[1]

Item	Year 1:	Year 2:	Year 3:
Salaries	6,005		
Benefits	4,985		
Wages	3,840		
Benefits	384		
Equipment			
Supplies	2121		
Travel			
Plot Fees			
Miscellaneous			
Total	17,335		

Footnotes: [1] OSU does differentiate amongst sub-accounts within overall projects, but funds were considered to be used in accordance with the proposed budget.

ORIGINAL OBJECTIVES

The Pacific Northwest Sweet Cherry Breeding Program (CBP) was established to provide superior sweet cherry cultivars for the Oregon and Washington industries. Since the departure of the previous breeder in 2016, the program has sought to improve horticultural practices and provide an improved framework for the eventual new breeder. Dr. McCord was hired as the new breeder in April 2018.

Overall goal: Develop superior new cultivars for the Pacific Northwest sweet cherry industry, using a streamlined breeding framework that is objective and resource-driven and quantitatively targets industry priorities

Specific objectives

1) Maintain a robust horticultural management system that efficiently raises and maintains healthy plant materials at all breeding stages
2) Incorporate new tools to deploy a robust performance evaluation system that effectively identifies superior selections in Phase 1 seedling trials and Phase 2 regional trials

SIGNIFICANT FINDINGS

- Automated irrigation timers were installed for the P1, P2, and RosBREED blocks, allowing for night-time irrigation.
- P1, P2, RosBREED blocks, and P1.5 trees (seedlings budded onto precocious rootstock) were fertilized with MAP and Zinc sulfate according to soil test results.
- Dr. McCord worked closely with WSU orchard staff to implement an appropriate spray regime.
- Virus screening of the orchard continued with approximately 400 trees screened via bioassay on 'Shirofugen' indicator. Fifty-seven trees tested positive, and will be removed this fall.
- Leaf tissue from forty cultivars and advanced selections was submitted to the Clean Plant Center Northwest (CPCNW) for virus screening via RT-PCR. These selections are being targeted primarily for use as parents in a protected crossing block comprised of trees in pots in a greenhouse.
- Evaluations were made of ten P2 selections. R19 (early mahogany) and R29 (mid-season mahogany) are of particular interest.
- The P2 site at the Roza orchard is poor (trees are stressed and have variable health). New P2 plantings will go into a different block.
- Field evaluations of P1 seedlings resulted in 58 selections being evaluated in the laboratory. However, none were judged sufficiently superior to warrant advancement to P2.
- Testing of the Mohr MDT-2 penetrometer indicates that its workflow is not compatible with a high-throughput breeding evaluation laboratory. In addition, preliminary analysis suggests that data captured by the instrument is not well-correlated with firmness as measured by the standard FirmTech device, nor is the data better able to discriminate perceived firmness (based on mouth feel) vs. the FirmTech.
- The crossing program was restarted in April 2018 very soon after Dr. McCord's arrival. Twenty-nine bi-parental crosses and ten open-pollinated crosses resulted in approximately 6,000 seeds produced. DNA information was used to guide the majority of bi-parental crosses made.
- Dr. McCord attended the OSU pre-harvest cherry tour in The Dalles in June 2018. He also visited private breeding programs, a commercial nursery, and the USDA-ARS germplasm collection in California (August), and the Agriculture and Agri Food Canada breeding program near Summerland, British Columbia (October)

RESULTS & DISCUSSION

1a) Irrigation and nutrient management, pesticide application

We installed electronic irrigation timers and associated solenoids on blocks C50-53 and B48, which encompasses the seedlings (P1), P2 selections, and the RosBREED collection of genetic stocks. This allowed irrigation to take place at night when it would not interfere with harvesting. Based on soil analysis, these same trees were fertilized with monoammonium phosphate and zinc sulfate, via banding into a shallow trench in the root zone. Promising seedling trees from the old F block that had been budded onto Gisela-6 (phase '1.5') were also fertilized. Dr. McCord worked closely with the WSU orchard manager to ensure that a spray program was followed that provided acceptable levels of control, while also allowing flexibility in harvesting. As in prior years, the RosBREED and adjacent P1 blocks were not sprayed for powdery mildew (PM), to allow for sufficient disease pressure to evaluate the seedlings for PM resistance, and for continued research on the genetics of resistance to PM infection.

1b) Virus screening
As a continuation of the more rigorous virus testing implemented in recent years, we sampled one tree from approximately 400 2-tree replications in the RosBREED block (C53). In late July 2018, a short section of current year's growth was sampled from four points throughout the tree. Bark from these sections was 'grafted' onto limb sections of *Prunus serrulata* 'Shirofugen'. This species is hypersensitive to ilarviruses, of which *Prune dwarf virus* and *Prunus necrotic ringspot virus* are of particular interest. In October 2018, these limb sections were sliced open with a knife to observe any hypersensitive reaction. A total of 57 trees tested positive, with a further 4 listed as questionable. The positive trees will be cut down to a stump, and glyphosate applied to identify any root-grafted neighboring trees that should also be removed. The questionable trees will be tested in the spring via RT-PCR or ELISA.

1c) New protected parental block
The CBP is in the process of transitioning from making crosses in the orchard, to potted trees in a greenhouse. This move is expected to provide greater flexibility in crossing, as well as protection from frost, birds, and vectors of pollen-borne viruses. As a first step, the first 40 potential parents have been identified. These individuals represent a range of genetic diversity as well as targeting high-priority traits such as fruit size, early/late maturity, and powdery mildew resistance. To ensure clean budwood and/or pollen, leaf tissue from these trees was sampled and sent to the CPCNW in October 2018, for RT-PCR testing of the following viruses: *Cherry leaf roll*, *Prune dwarf*, *Prunus necrotic ringspot*, *cherry virus A*, *cherry rasp leaf*, *little cherry virus* 2, and Western X (phytoplasma).

2a) Phase 2 evaluations
The P2 selections evaluated in 2018 are listed in Table 1. Fruit from P2 selections were evaluated for fruit size (weight and diameter), firmness, color, soluble solids content, and harvest defects (primarily bruising, pitting, and doubles. After two weeks of refrigeration, the samples were evaluated for the same criteria as at harvest, plus storage defects (loss of luster, shrivel, and stem browning).

Table 1. Evaluation status of current P2 selections.

Original name	Selection	Class[1]	Prosser 2018	Pasco 2018	Hood River 2018
FR14T012	R19	EM	Full	Full	
FR09T049	R3	EM	Full	Full	
FR11T059	R16B[2]	EB	Full		Full
FR51T113	R28B	EB	Just bearing	Full	
FR01T002	R1	MM	Full	Full	
FR44T083	R17	MM	Full		
FR36T035	R21	MM	Just bearing	Full	
FR52T095	R29	MM	Just bearing	Full	
FR01T070	R5B	LB			Full
FR01T074	R6	LM	Full		Full

Footnotes: [1]EM= early mahogany; EB= early blush; MM= mid-season mahogany; LB= late blush; LM = late mahogany. [2]B = blush.

Of the ten selections, two of them show the most promise. 'R19' is an early-season mahogany selection, with similar timing to 'Chelan'. However, it is showing larger fruit size, better firmness, and higher soluble solids content (Table 2). It appears to be more susceptible to birds than 'Chelan', possibly due to the higher SSC. DNA test results indicate that it is self-fertile. 'R29' is a mid-season mahogany selection. Data is only available for the Pasco site, as the trees in Prosser are just starting to bear. This selection is comparable to 'Bing', but has much larger fruit (Table 3), and is self-fertile.

Table 2. 2018 performance data for 'R19'.

ID	Harvest date	Fruit Wt. (g)	Fruit width (mm)	Fruit firmness (g/mm)	Juice SSC (ºBrix)	Year	Site
Chelan	12-Jun	8.0	25	297	15.9	2017	Pasco
R19	16-Jun	10.2	29	437	25.7	2017	Pasco
Chelan	8-Jun	7.3	24.5	272.0	18.7	2018	Pasco
R19	6-Jun	9.3	27.3	391.1	21.9	2018	Pasco
Chelan	7-Jun	7.1	22.7	249.8	18.4	2018	Prosser
R19	7-Jun	9.1	27.2	378.5	24	2018	Prosser

Table 3. 2018 performance data for 'R29'.

ID	Harvest date	Fruit Wt. (g)	Fruit width (mm)	Fruit firmness (g/mm)	Juice SSC (ºBrix)	Year	Site
Bing	26-Jun	12.5	30	336	20.6	2017	Pasco
R29	30-Jun	12.5	30	336	20.6	2017	Pasco
R29	21-Jun	13.9	31.0	293.6	21.5	2018	Pasco

2b) Phase 1 evaluations

The seedlings were evaluated generally twice per week. Field evaluations were based on fruit size, perceived firmness, and flavor. Of the approximately 1400 seedlings in the field, slightly more than half (~720) were potentially old enough to be fruiting. The majority of these were rejected for small fruit size. A total of 124 selections were sampled in the field, of which 58 were of enough interest in the field to be sent to the laboratory for evaluation. Three mahogany and four blush seedling selections showed consistently good performance over at least two seasons. However, based on BPAC input, none were deemed sufficiently superior to warrant advancement into P2. This was primarily due to lackluster fruit size, and generally midseason timing. Dr. McCord is focused on making new crosses that target fruit size and early/late maturity. In the meantime, he is propagating 2 of the most promising seedlings onto Gisela 6 for additional observation.

2c) Mohr Penetrometer

The departure of Dr. Blakey in early 2018 made it more difficult to evaluate the Mohr MDT-2 penetrometer for firmness testing. However, we were able to complete a preliminary assessment of the machine that reveals the following:

- The MDT-2 is slower than the standard FirmTech, as each fruit must be manually presented to the penetrometer. This is currently incompatible with the workflow of the CBP lab evaluation pipeline.
- By measuring the same fruit with the FirmTech and the MDT-2, we determined that none of the MDT-2 parameters are well-correlated with the FirmTech firmness output (Table 4).
- The FirmTech is better able to distinguish 'softer' vs. 'firmer' fruit based on mouth feel as compared to the MDT-2, though additional data is probably needed to definitively answer this question (Table 5).

Table 4. Correlation between Mohr MDT-2 with FirmTech firmness.

Parameter	Firmness (mm/gr)
Firmness (mm/gr)	1
Diameter (mm)	-0.17
Max Hardness R1 gr	0.62
Average Hardness R1 gr	0.66
Max Hardness R2 gr	0.38
Average Hardness R2 gr	0.42
Force at the end of R2 (gr)	0.30
Crispness	0.13

Table 5. Ability of Firmtech and MDT-2 to distinguish between different firmness classes (based on bite experience).

Parameters Bite Experience	Firmness (mm/gr)	Max Hardness R1* (gr)	Max Hardness R2** (gr)	Crispness	Force at end R2** gr
Soft	218.4 a	646.6 a	826.3 a	20.6 a	502.4 a
Middle soft	230.2 b	669.2 a	873.2 ab	21.6 a	595.6 ab
Firm	239.3 b	666.3 a	905.3 b	21.1 a	660.2 b
p value	0.006	0.438	0.033	0.625	0.012

*R1; referred as the skin region of the fruit. **R2; referred to the flesh portion of the fruit. Note that the Mohr MDT-2 performs a destructive analysis, so the bite experience was done on other fruit from the same sample.

2d) Crossing
Dr. McCord purposely arrived on the job early (April 2018) in order to be able to make crosses, which had not been done since 2015. Using DNA information provided by Dr. Peace, he and CBP team members made 29 bi-parental crosses, including five self-pollinations. Crosses were made with emasculated flowers and collected pollen, limb cages (for small self crosses), and whole trees in insect-proof cages, with a small hive of bees and a bucket with branches of the pollen parent enclosed. Approximately 4250 seeds resulted from these crosses. Open-pollinated seed were collected from ten additional mother trees selected for their large fruit. This yielded more than 1700 additional seeds, for a total approximately 6000 seed.

As the new breeder, Dr. McCord also spent time familiarizing himself with the breeding program, and conducting strategic travel to meet with breeders and other industry experts. His attendance at the OSU pre-harvest tour at the Dalles in June was an important opportunity to meet many in the PNW cherry industry. In August, he traveled to California to meet breeders from International Fruit Genetics and Zaiger's Genetics where he gained valuable insights into the basics of cherry breeding. He also visited Sierra Gold Nurseries to learn about rootstock propagation and characteristics, and the USDA-ARS germplasm collection in Davis. In October, he traveled to British Columbia to visit the federal Canadian program near Summerland, and the Summerland Varieties Corporation which handles final variety development and licensing of Canadian varieties.

EXECUTIVE SUMMARY

The Pacific Northwest sweet cherry breeding program (CBP) is devoting significant efforts in its mission to develop superior cherry cultivars for the Oregon and Washington industries. Horticultural practices put in place in recent years are being maintained in order to reduce viruses, and enhance tree health and fruit quality. Promising P2 selections have been identified, and rigorous selection criteria are being applied towards P1 seedlings to ensure that only superior selections are advanced. The crossing phase of the breeding program was successfully relaunched, resulting in large numbers of seed from DNA-informed crosses. In addition, Dr. McCord has reached out to experts in the industry to gain relevant information that can be applied to the CBP.

FINAL PROJECT 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	
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	No-cost extension

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 after the freeze event and having free water does appear to increase disease development as opposed to prior to.

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.
- Inoculated can yard experiment yielded stunning results of defoliant treatments with presence of the pathogen. Most treatments did not yield the expected outcome, but the Lime Sulfur treatment appeared to be a complete success.

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 in 2016, but ACC had similar effects in 2017.

Results & Discussion

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 (75ºF daytime and 60ºF 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 (68ºF) 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).

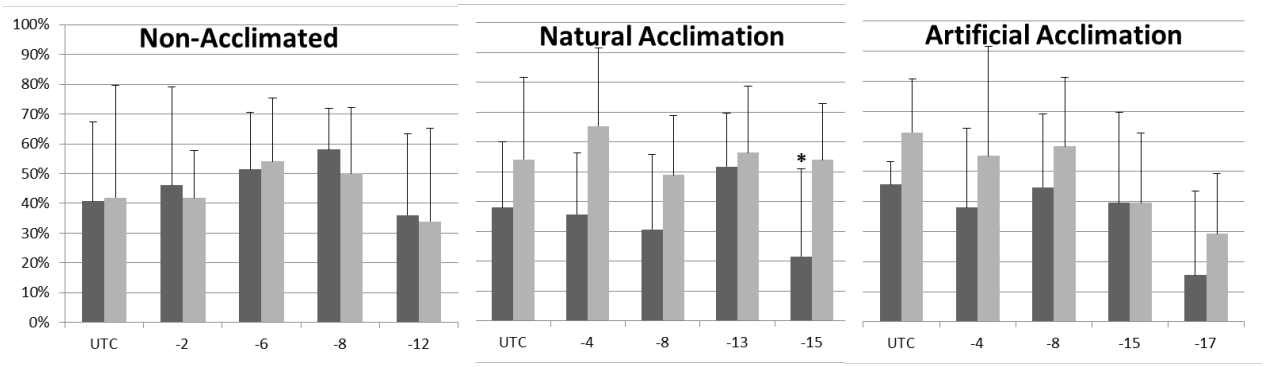

Figure 1. Percentage of relative regrowth of Gisela 6 rootstock of 3 levels of acclimation after being subjected to differing injurious temperatures. Dark bars represent inoculated treatments, while light bars are non-inoculated. Lines are top of bars are ± one standard deviation. * represents significance (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 needed to be regrown after a severe powdery mildew outbreak immediately following arrival at MCAREC defoliated many of them. Once growth had terminated, they were 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. This experiment was conducted twice on Gisela 6 and once Mazzard. Though we did not experience significant mortality at lower temperatures as we had hoped, we did begin to have effects at lower temperatures with significance being found at the lowest temperatures between inoculated and non-inoculated (Figure 2).

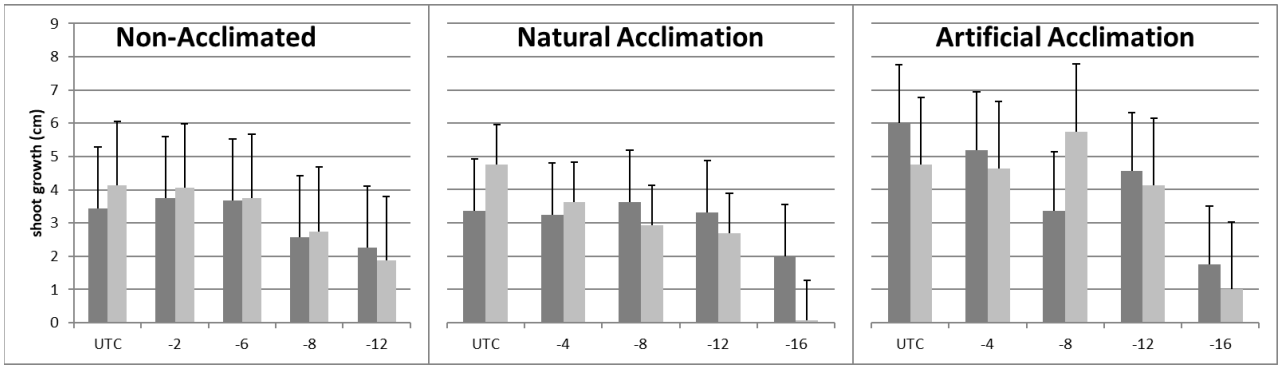

Figure 2. Growth of Mazzard rootstock of 3 levels of acclimation after being subjected to differing injurious temperatures. Dark bars represent non-inoculated treatments, while light bars are inoculated. Lines are top of bars are ± one standard deviation.

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 3).

Table 3. 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 4). 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 4. 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 5). 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 6). 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 5. 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 6. 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 were 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. Results from this trial are in table 7.

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.

For the defoliation trial, rates were similar to what had been seen before. Lime Sulfur began defoliation first but slowed once a period of time had been met. Treatments containing ACC were rapid once applications were made, although slightly faster if ABA had been applied prior to. ABA treatments did increase defoliation and were rate dependent. As seen in prior years, the number of recoverable exotherm peaks were insignificant across treatments with no differences in either the number or LT50 of the buds.

Table 7. Defoliation efficiency of chemical compounds over 4 weeks beginning 10 days post application in 2017

		Evaluation Date								
Treatments	Rate	10/10	10/17	10/24	10/31	11/3	11/7	11/14	11/21	11/28
UTC	---	3%	3% a[#]	4% a	5% a	30% a	39% a	48% a	82% a	100%
ABA (x3)	500ppm	3%	2% a	32% c	66% c	94% c	99% c	99% b	99% b	100%
ABA (x3)	250ppm	4%	2% a	16% b	39% b	68% b	71% b	79% b	93% b	100%
ABA then ACC	500ppm 1000ppm	3%	13% ab	93% e	96% d	100% c	100% c	100% b	100% b	100%
ACC	1000ppm	3%	3% a	4% a	95% d	100% c	100% c	100% b	100% b	100%
Lime Sulfur	10% (v/v)	5%	18% b	62% d	74% c	84% c	94% c	96% b	96% b	100%

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

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 2 commercial blocks will be evaluated for potential winter damage from freeze events next spring and evaluation of *Pss* symptoms. Results from these experiments were inconclusive as little to no winter injury or Pss symptoms developed

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 young potted trees also received these treatments in addition to 2 chemical treatments (LMA – 2% and Oxytetracycline – 200ppm + buffer). for *Pss* 2 days before and 2 days after inoculation occurred on November 7th, 2017. The potted tree experiment shows promise.

Table 7. Efficacy of chemical compounds for control of Pss on sweet cherry

			Shoots	Length (cm)	
Treatment	Rate	% dead	Total #	Total	Individual
UTC	---	25% a	4.9 abc[#]	49.7 ab	7.9 b
ABA	250ppm	63% c	3.0 ab	31.7 ab	4.5 a
ACC	1000ppm	38% b	4.6 abc	36.1 ab	4.9 ab
Oxytetracyline	200ppm	69% c	2.4 a	20.7 a	2.6 a
LMA	2% (w/w)	19% a	5.7 b	99.2 b	13.5 b
Lime Sulfur	10% (v/v)	13% a	7.6 ac	257.6 c	32.0 c

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

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 were not completed over the course of a year. After results were returned, discrepancies in the data had us resubmit samples that had been withheld. Given the multiple years of this study, we do not expect for variation from the 2 subsequent years.

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.

2017: Tissues were processed as in prior studies. As seen in 2016, Nitrogen remobilization appears to be the slowest (Figures 2). Upon analysis, we did not detect any significant differences for final concentrations of minerals in buds or spurs once dormancy was achieved.

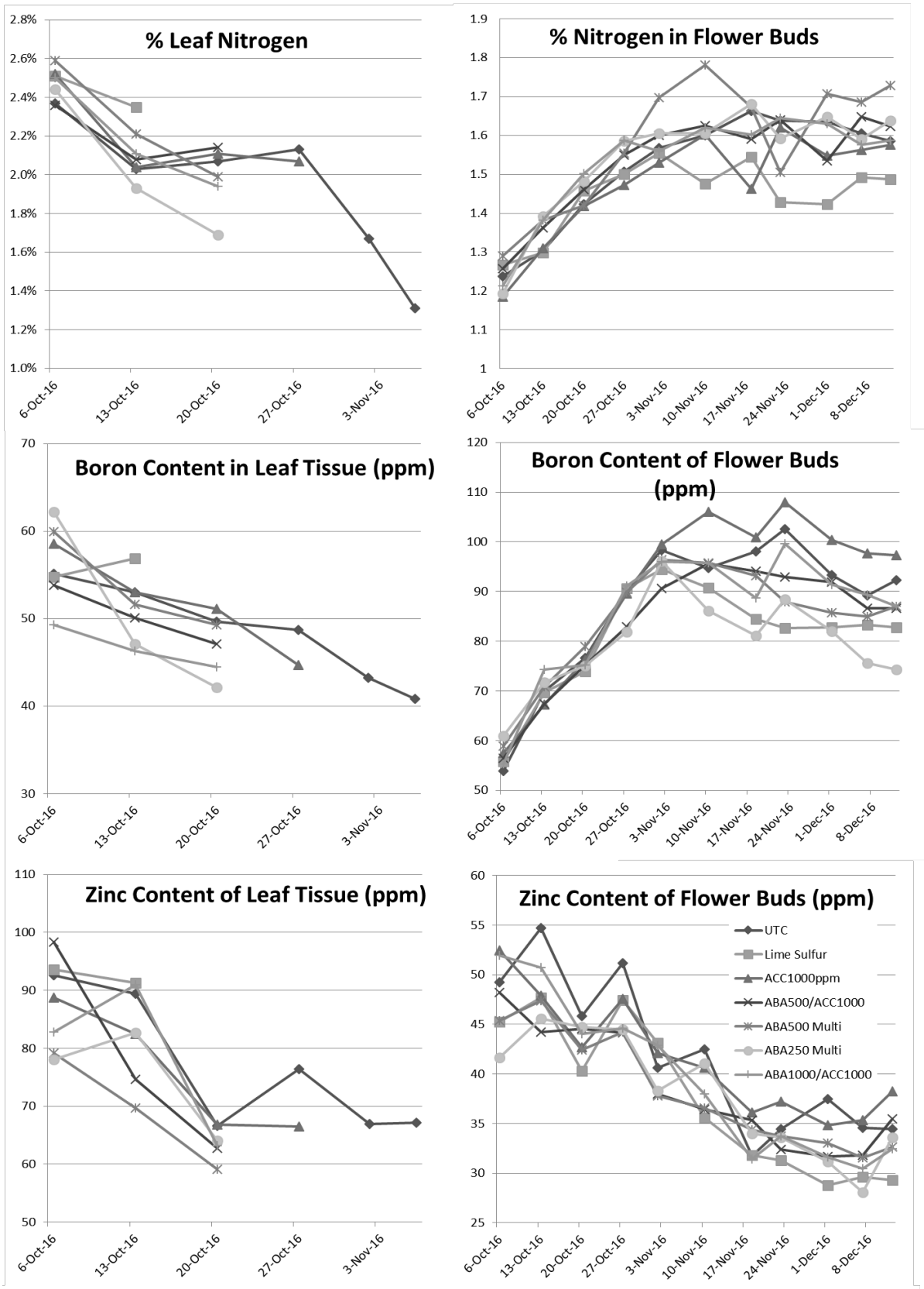

Figure 1. Nutrient levels in various 'Sweetheart' Sweet Cherry tissues from Fall 2016 (from left to right). Percent leaf Nitrogen. Percent Nitrogen content in flower buds. Leaf Boron content in leaf tissue in parts per million (ppm). Boron content (ppm) in flower buds. Leaf ZInc content (ppm) in leaf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

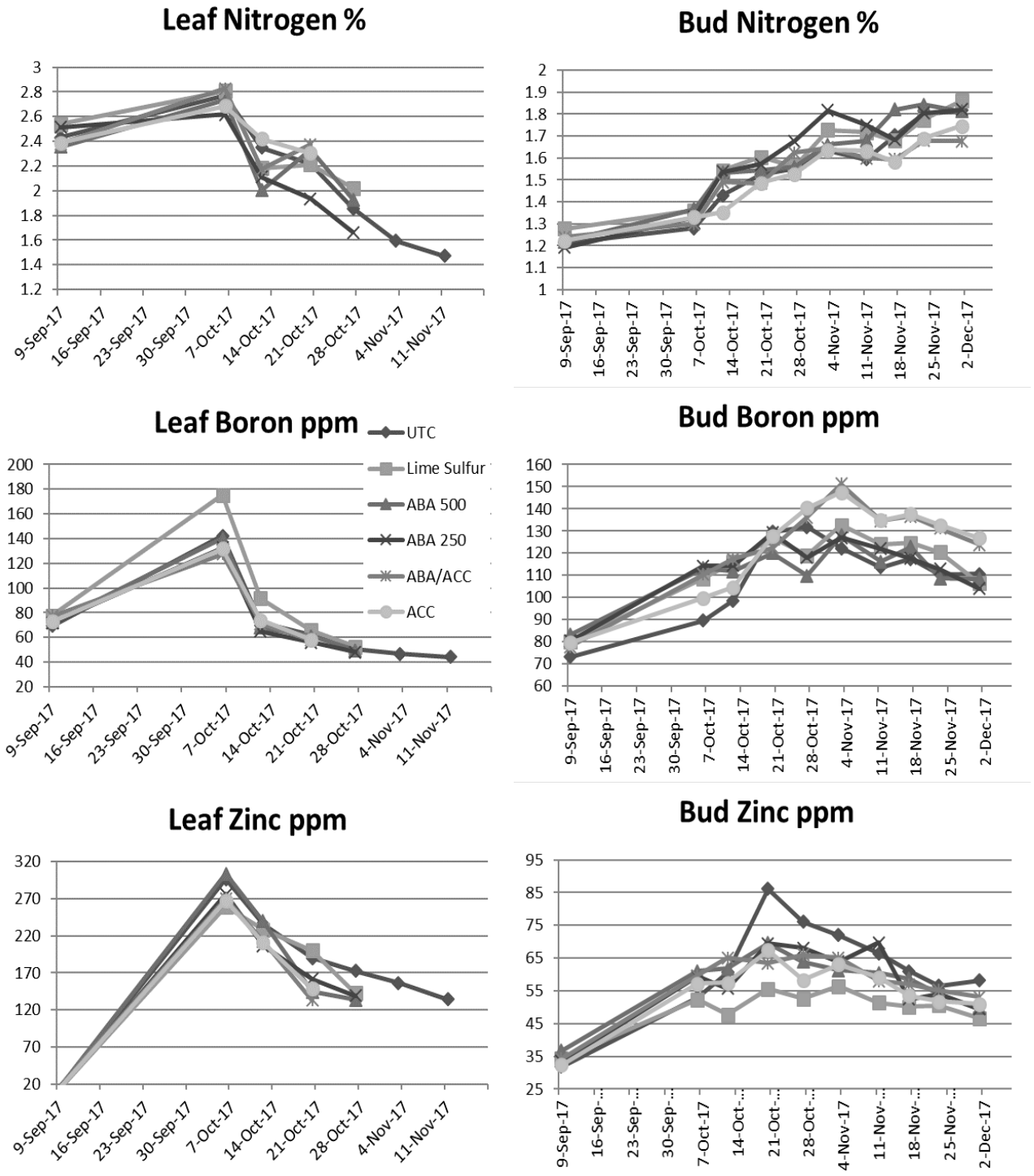

Figure 2. Nutrient levels in various 'Sweetheart' Sweet Cherry tissues from Fall 2017 (from left to right). Percent leaf Nitrogen. Percent Nitrogen content in flower buds. Leaf Boron content in leaf tissue in parts per million (ppm). Boron content (ppm) in flower buds. Leaf Zinc content (ppm) in leaf tissue. Zinc content (ppm) in flower buds. Treatment key in figure

Executive Summary

Objective 1) Development of our "model" system was wrought with problems, not all stemming from the experiment itself, but subsequent handling of plant material and inoculation protocols. These experiments are being conducted once more this year (no funding requested), so that we may better explain the variation seen across the experiments. Due to the challenge of working with *Pss*, these results were not surprising

Objective 3) These defoliation experiments should us a great deal in terms of effects from various compounds that had yet to be tested. We found ABA can induce something similar to natural defoliation and an slight increase in mineral remobilization. ACC shows great promise in future endeavors, though this compound has only recently begun to be studied in applied settings, future studies within plant science will develop it further. We did not find, however, any benefit in terms of gained cold hardiness from any one treatment. While this is discouraging, these data are only valid when using DTA to determine bud hardiness. Overall plant hardiness may still show a benefit.

The larger commercial plots, without bud sampling, showed there was no detriment to crop set the following year, but plots were harvested before samples could be taken for quality & mineral analysis. These experiments are being replicated this year, to answer that question (no funding requested). We also did not see any significant results from natural inoculation or winter damage in these plots (data not shown).

The small potted tree experiment did yield some startling results. Lime Sulfur appeared to have an, overall, positive effect on overall tree health & survival. Trees from all treatments had variation, but largely all grew very little the subsequent year and most had small, distorted foliage if they leafed out at all. LMA shows some promise, although the compound is neither available for commercial agriculture and it's residual for minimal applications will be another challenge going forward. The PGRs showed little in the way of a solution for bacterial canker, while ACC performed similar to the inoculated only treatment, the multiple applications of ABA showed an increased amount of infection and subsequent mortality. This, in combination with DTA data from the commercial experiments may show a brief lose in hardiness following an application, which disagrees with prior literature. This may be why mortality was so high. The treatment of Oxytetracycline is also troubling, as it had the highest mortality of the treatments. This could be for multiple reasons, but in prior experiments conducted in the Pacific Northwest it had performed well. We've recently discovered strains of the pathogen that are tolerant, if not resistant to the compound in the Mid-Columbia region, although not in the Hood River Valley. This may no longer be the case. Samples have been given to OSU in order to determine if this was the case. This experiment is also being replicated again, but in Corvallis, to corroborate the data seen from the prior year (no funding requested).

Objective 4) Nutrient remobilization has only been studied in sweet cherry, primarily for Nitrogen. These studies show us the movement of other minerals, such as Zinc & Boron moving from leaf into bud & spur tissue. We saw a saturation effected for these minerals in various tissues. This should aid in validating Fall applications of other minerals outside of Nitrogen.

FINAL REPORT

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, Allan Bros. Inc., Shield Bags and Printing Company, Washington Fruit & Produce Co., WTFRC, 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:** katy.roberts@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.

2. SIGNIFICANT FINDINGS

Following are the significant findings of the research carried out:

1. Moisture management is an important factor in controlling the cherry cracking in the post-harvest phase. Excess moisture in the bags does negatively impact the quality of the Cherries. Too dry conditions is also not good, as it would cause the Cherries to shrink and most importantly would lead to the browning of the stems.

2. The air knives effectively remove excess moisture from the fresh cherries before packing. But, it very critical to have the right type of belt to facilitate proper drainage, with the forced air.

3. Removal of excess moisture from cherry surface contributes to the reduction of fruit cracking during refrigerated storage and also improves shelf-life.

4. Air dried cherries showed significant reduction in the cracks during storage studies by more than 60% by the end of 7 weeks compared to other treatments (Figure 11).

5. Air knife position over the belt and the drain belt type has significant impact on the efficacy of surface moisture removal. The drain belts with larger pore/hole sizes drain the water more effectively. The fine mesh belts did not prove to be effective in our trials (Figure 3).

6. Over the period of the last three years of this project, we tested over 16 different types of coatings.

7. From all the testing performed only the, Gum Acacia and the combination of Gum Acacia and Sodium Alginate/Agar showed consistent results both in terms of reducing the fruit splitting and pedicle browning (Figures 5, 6, 7, 8, 9, 10 and 11).

8. Coating of cherries with Gum Acacia (Gum Arabic) solutions consistently reduced the number of cracks during multiple trials.

9. Different concentrations of gum Acacia were tried over the last three years and the concentrations in the range of 0.5% to 1.0% were all found to have similar effects.

10. Packaging with desiccant significantly decreased the cracking during storage, in some experiments, but not all.

11. The treatment with "Gum Acacia" coating and the "surface moisture removal" together provided the best benefit with significant reduction in the number of cracks during storage and the pedicle browning. The percentage reduction of the cracks and pedicle browning at the end of 5 weeks storage were > 55% compared to other treatments (see Figure 7 and 8).
12. In addition to reduction in the number cracked cherries, the treatments also provided a benefit of reduced pedicle browning.
13. It is important to note that, in some trials we did not see significant benefits, especially when the excess moisture removal was not effective.
14. Gum Acacia (var. Senegal) mixed with the fungicide treatment provided by the facility was shown to be the most effective for fruit cracking and weight loss, during the last set of experiments conducted in the year 2018.
15. Gum Acacia (var. Senegal) with sodium alginate was observed to have the least amount of severe stem browning throughout the 3rd year experiments. The addition of the modifiers was not always effective over the three years of experiments.
16. Gum Acacia (var. Senegal) was shown to have the most average green stems after 6 weeks of storage (Figure 7).
17. Sensory analysis conducted for the last two years of work, showed that the consumer panel was not able to distinguish between the coated and uncoated varieties, after the 1st and the 4th week of storage periods (Table 3 and Figure 12).
18. Overall, we have shown that all the three approaches, i) edible coating application, ii) forced air to remove excess moisture/coating solution and iii) packaging with desiccant embedded in the plastic, can help reduce the postharvest cracking of the fruit to various degrees.

If any facilities would like to try in the production setting, we highly recommend ensuring that they have excellent moisture removal system, to see the intended results.

<u>Summary of the work conducted in the year 3 (2018):</u>

- The best coatings and packaging liners from the 2017 trials were used in this year's work.
- Studied the interaction effects of the select coatings with packing liners with 2% desiccant and liners provided by packaging facility.
- Conducted two trials on packing lines with Gum Acacia (var. Senegal) with modified agar and sodium alginate, fungicide treatment provided by the facility, and a combination of both Gum Acacia (var. Senegal) and fungicide with the cherry variety Skeena.
- Tested for physical parameters from the above coatings specifically the stem browning, fruit cracking, and overall weight loss over time.
- Performed sensory analysis on the coated cherries to see if consumers can detect any difference in the coated and uncoated cherries, after week 1 and week 4 of storage periods.

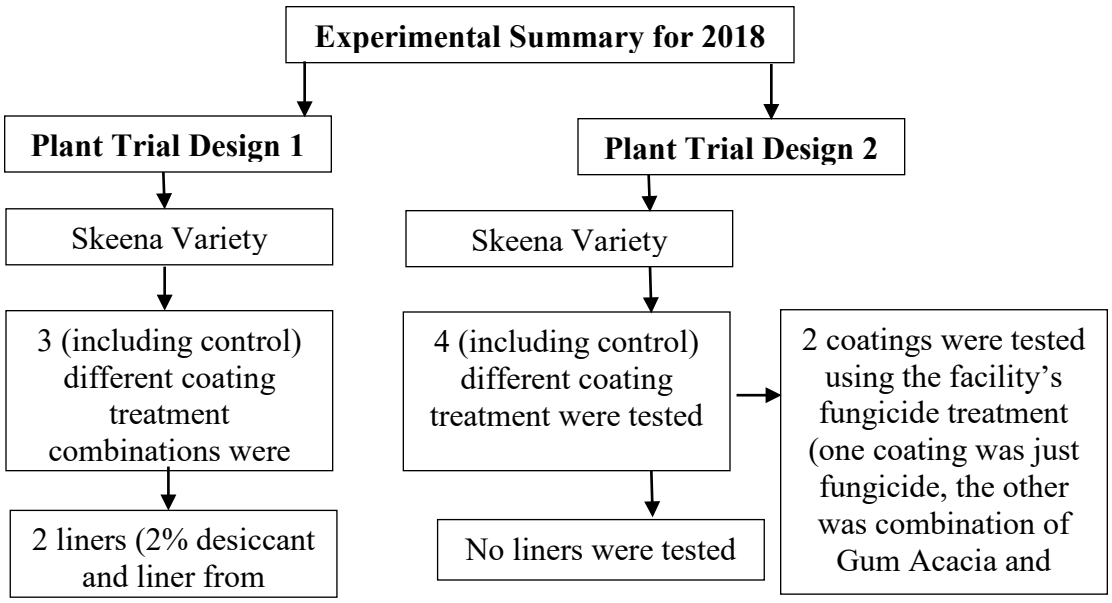


Figure 1. Flow chart showing the summary of the experiments conducted in 2018. All treated cherry samples were subjected to storage studies for 5 to 6 weeks.

Following are the significant findings categorized by Plant Trials:

2.2.1 Skeena Design #1 (Plant Trials) 3 levels of coatings and 2 levels of liners

- Gum Acacia (var. Senegal) (@ 0.5%) showed the best results for controlling stem browning and reducing cracking.
- Gum Acacia (var. Senegal) (@ 0.5%) combined with 2% loaded desiccant was less effective relative with the treatment combined with the regular packaging from the facility, for stem browning.

2.2.2 Skeena Design #2 (Plant Trials) 4 levels of coatings with single liner

- Gum Acacia (var. Senegal) (@ 0.5%) combined with fungicide treatment, had the least amount of stem browning.
- Gum Acacia (var. Senegal) (@ 0.5%) combined with fungicide treatment had least average cracking percentages than other coatings.
- Gum Acacia (var. Senegal) (@ 0.5%) was shown to have the most average green stems over the 6-week storage.

2.3 Sensory Analysis

No differences were observed in the parameters of aroma, sweetness, sourness, cherry flavor, stem color, texture, and overall acceptance and appearance amongst the i) Control (no coating) and ii) Gum Acacia (var. Senegal) at 0.5% concentration for treatments of Skeena cherries after 4 weeks of storage.

3. METHODS:

Note: *Materials and Methods described in this section are for the year 2018. For the whole project similar materials and methods were used. In the year 2018, only the Skeena variety was tested. While in the previous years, the other varieties tested included, Chelan and Sweethearts.*

3.1 Materials:

For the year 2018, two plant trials for *var.* Skeena, was conducted with the help of a packing facility in Wenatchee WA. These two trials were conducted in the same packaging environments in the same facility. The first comprised of 3 coatings with a control (water/no coating) treatment and 2 liner types with varying desiccant levels. The second study had 6 different coatings including control (water/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 stored in Johnson Hall's ground floor refrigerators at Washington State University (WSU), Pullman. These cherries were stored on in a refrigerated environment on average of 37.1^{o}F & 96% RH. These two trials have been referred to as Design #1 and Design #2 respectively throughout this report.

3.2 Coating Application:

For both designs, the cherries were coated using the dunking method, where the cherry feed drops the cherries into the coating solution for immersion and then taken by the conveyor belt to the packaging part of the line. The excess coating was drained off with the help of the air knife attached to the processing line before the cherries are packaged. Depending on design, the cherries were packaged in standard bags and boxes with some containing either a 2% desiccant liner or liners already provided at the facility. Following this, the cherries were transported to WSU and stored in the walk-in refrigerator for the storage studies. These studies were carried for 6 weeks, with quality parameter testing once every week and sensory testing after week 2 and week 4.

3.3 Coatings Used:

Table #1: Details of coatings used throughout the experiment

Sr. no.	Code	Coating	Solution Conc. %
1	C1	No Coating/ Water	
2	C2	Gum Acacia Senegal 100%	0.5%
3	C3	Gum Acacia Senegal 80% & Sodium Alginate 20%	
4	C4	Gum Acacia Senegal 90% & Agar 10%	
5	F1C2	Gum Acacia Senegal 100% with Fungicide Treatment	
6	F1	Fungicide Treatment	

Table #2: Details of packaging liners used

Sr. no.	Code	Liner with % desiccant loading
1	P1	2%
2	P2	Provided by facility

a) Dipping method

b) Waterfall Method

Figure 2. Coating application methods used in different plant trials.

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). For the year 2018, we only the stem browning, fruit cracking and weight loss were measured.

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

From the experiments conducted during the year 2018, the following are the key points,

- During the first set of trials in 2018, we did not have effective surface moisture removal, which led us not to rely on that set of data, as high moisture in the packages led to mold growth.
- The second set of trials were much better, although there was still some moisture left in the packages
- Gum Acacia (var. Senegal) performed well with the packing liner loaded with 2% desiccant in terms of reducing cracking.
- The effect of the modifiers with Gum Acacia (var. Senegal), did not show any significant added benefits with desiccant embedded packaging.
- Gum Acacia (var. Senegal) which had proven to have the best effects on reducing cracking and stem browning in previous year's study did perform well again this year.
- In the plant trial, modifiers; Agar and Sodium Alginate, with Gum Acacia (var. Senegal) were tested to check if any synergistic effect to improve the fruit quality. Gum Acacia (var. Senegal) alone provided better results relative to the combinations.

a) Belt with larger pore sizes

b) Mesh belt with smaller pores

Figure 3. Different types of drain belts used in the plant trials.

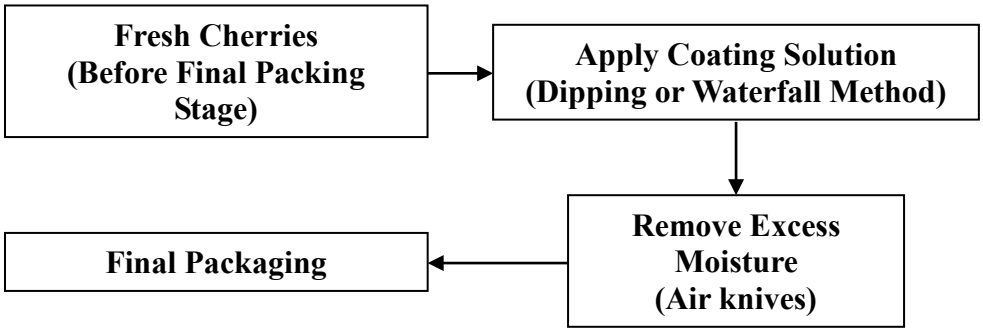


Figure 4. The flow chart showing the process of application of the coatings.

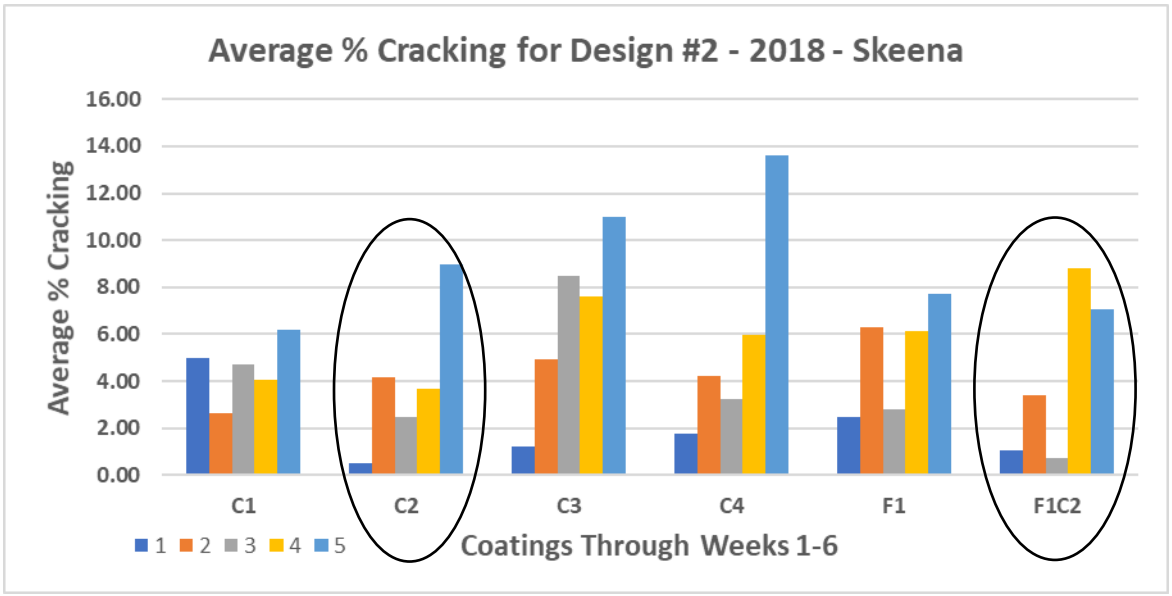


Figure 5. Cherry cracking data over the storage period of 5 weeks. Numbers 1 to 5 indicate the weeks of storage.

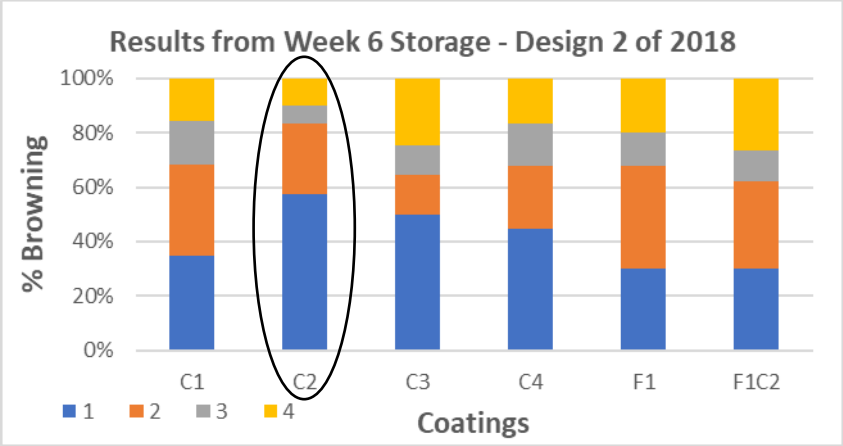


Figure 6. Stem browning results from Design 2, of the 2018 Trials for Skeena variety. (1 to 4, represent the varying stem color from green to brown, with 1 representing very green and 4 representing very brown)

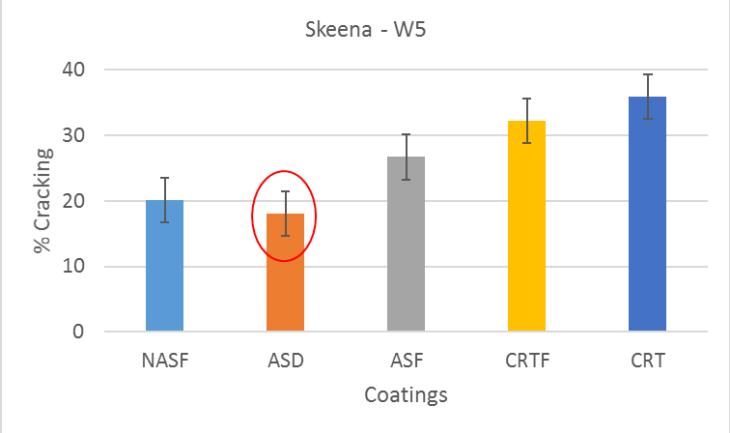


Figure 7 and 8, show the results on the % cracking and pedicle browning in the cherries, from the plant trials in <u>2016.</u>

It is clear from the Figure 7 that all the coatings showed a reduction in the cracking compared to the control treatment (CRT) and the fungicide treatment (CRTF).

Figure 7. Percent cracking in cherries with difference coatings on Week 5 (W5) for the plant trials with Skeena variety. ASD - Gum Acacia Senegal (100%) at 0.5% concentration.

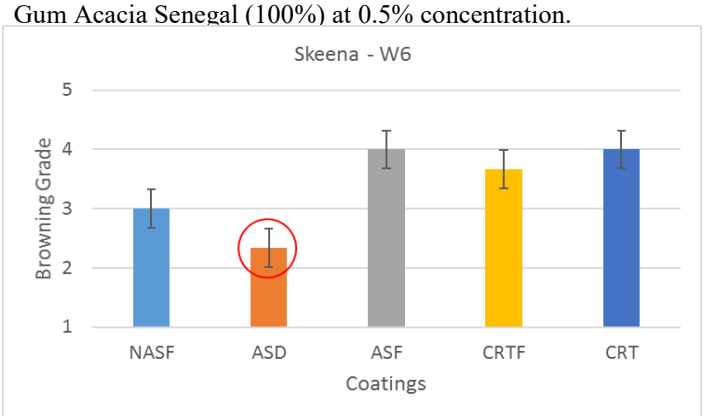


Figure 8. Pedicle browning in cherries with difference coatings on Week 6 (W6) for the plant trials with Skeena variety. ASD - Gum Acacia Senegal (100%) at 0.5% concentration.

The pedicle browning was significantly reduced by the coating "Gum Acacia – Senegal". It was the most effective. The coating, NASF (Gum Acacia – Seyal + Sodium Alginate), also was effective in reducing the pedicle browning (Figure 8).

These results show the effectiveness of the selected edible coatings in reducing the cracking and the stem browning.

Figure 9 and 10, show the results on the % cracking and pedicle browning in the cherries, from the plant trials in 2017. A significant reduction in cracking was observed at week 3 with Gum Acacia Senegal with Liner embedded 0% desiccant.

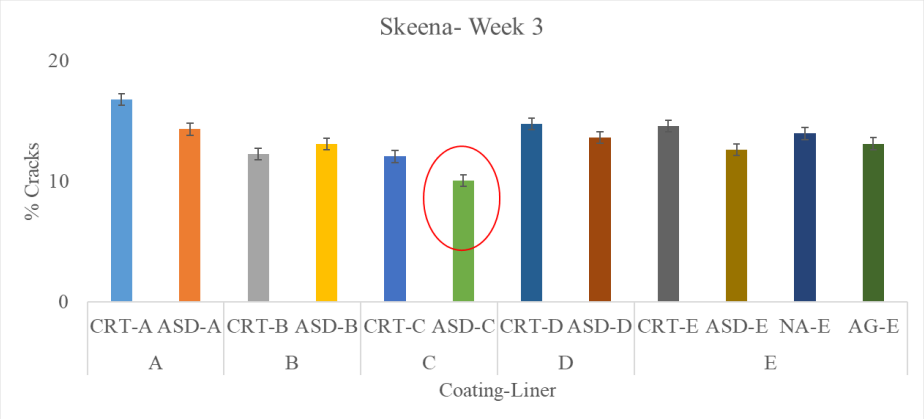
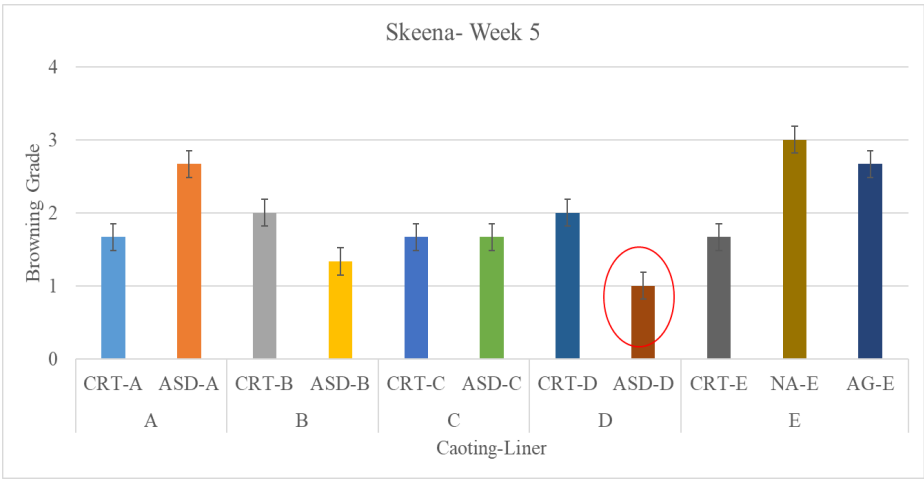


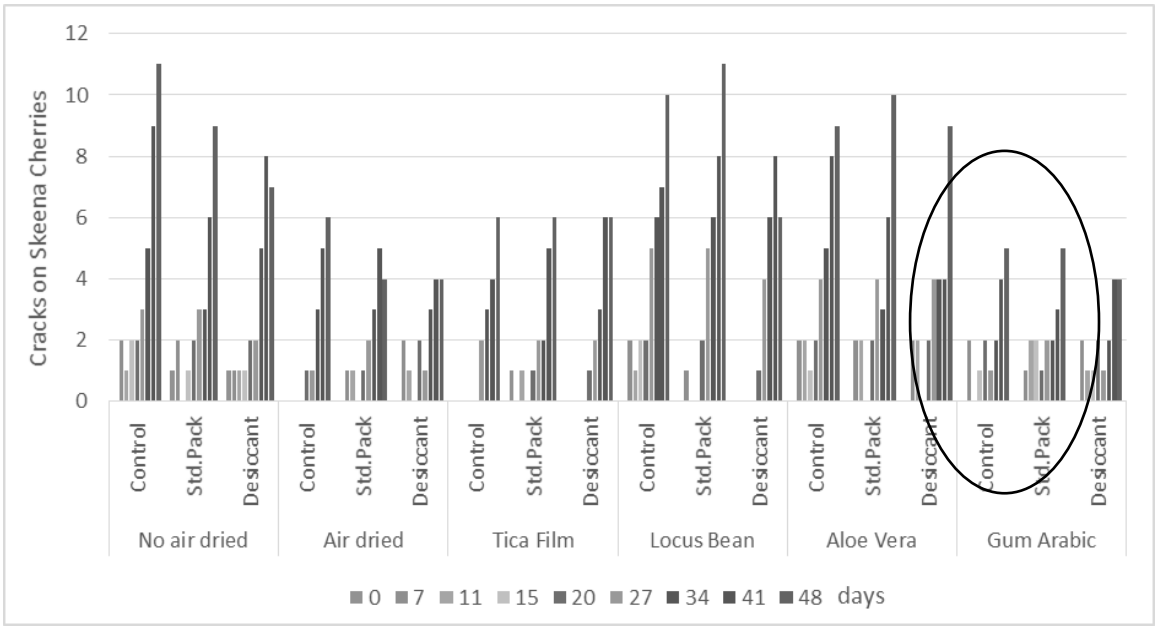
Figure 9. Percent cracking in cherries with ASD and Control at various liner levels at Week 3 for the plant trials with Skeena variety. ASD - Gum Acacia Senegal (100%) at 0.5% concentration. Liner C – 0% desiccant loading.



The pedicle browning was significantly reduced by the coating "Gum Acacia – Senegal" with Liner having 6% desiccant loading.

Figure 10. Pedicle browning in cherries with difference coatings on Week 5 for the plant trials with Skeena variety. ASD - Gum Acacia Senegal (100%) at 0.5% concentration. Liner D – 6% desiccant loading.

These results show the effectiveness of the selected edible coatings in reducing the cracking and the stem browning.



(2014 Trial)

Figure 11. Cracks observed in Skeena Cherries during storage studies (plant trial)

Table #3: Mean hedonic scale values for sensory attributes of fresh cherries for appearance, aroma, sweetness, sourness, cherry flavor, texture and overall acceptability. Each value is mean of 120 responses and represents a value along a 9-point hedonic scale. Values within a row followed by the same letter are not significantly different ($p<0.05$).

Week 1

Attribute	Coating	Control
Overall Appearance	6.54b	7.11a
Color of Fruit	7.17a	7.31a
Size	7.64b	7.82a
Stem Color	5.83a	5.99a
Aroma	5.80a	5.63a
Texture	7.10a	7.38a
Juiciness	7.17a	7.24a
Sweetness	6.99a	6.91a
Sourness	6.57a	6.63a
Cherry Flavor	6.86a	6.90a
Overall Acceptance	7.28a	7.27a

Week 4

Attribute	Coating	Control
Overall Appearance	6.54 b	7.11 a
Color of Fruit	7.17 a	7.31 a
Size	7.64 b	7.82 a
Stem Color	5.83 a	5.99 a
Aroma	5.80 a	5.63 a
Texture	7.10 a	7.38 a
Juiciness	7.17 a	7.24 a
Sweetness	6.99 a	6.91 a
Sourness	6.57 a	6.63 a
Cherry Flavor	6.86 a	6.90 a
Overall Acceptance	6.75 a	7.05 a

(a) **(b)**

Figure 12. Pictures of cherries during sensory evaluation, a) Grocery shelf-style bags of cherries for individual evaluation; b) Soufflé cups of cherries in serving order and organized by time pulled from refrigeration to ensure equitable panelist experience.

5. IMPACTS TO THE CHERRY GROWERS

The results from the project provide potential solutions for the post harvesting fruit cracking and stem browning issues. These solutions may not provide 100% reduction in cracking and stem browning, but does provide significant reduction, that maybe enough in some cases to increase the shelf-life by a few days in the market.

If the process of application of the coatings and excess moisture removal is followed carefully,

- The solutions can aid in reducing the cracking and the pedicle browning, there by extending the shelf life for the Cherries to be sold in the market by retaining the fruit quality.

- For the international markets, where the Cherries are cooled further using forced air cooling, this solution can help reduce the stem browning that occurs due to drying during forced air cooling process.

- This will potentially help increase the income for the Cherry growers, due to extended shelf-life of the Cherries.

Executive Summary

Postharvest cherry cracking and stem browning are major issues for fresh cherry markets. These issues can lead to significant economic losses, due to deteriorating fruit quality. It has been hypothesized that the excess moisture in the packaging can lead to its absorption into the fruit. This absorbed moisture along with the change in the physiology of the fruit can lead to the cracking of the fruit. If the conditions are too dry that can lead to the browning of the stems. Both the fruit cracks and brown stems can lead to lower consumer acceptance.

In this project, we tested various edible coatings (both lipid and water soluble) to evaluate their efficacy in reducing the fruit cracking in the post-harvest storage. From the three years of rigorous testing both on the laboratory and production scale, we were able to conclude that the, Gum Acacia (Gum Arabic) was the most effective in reducing the cracking and delaying the stem browning. This coating showed the best results compared to all other coatings that were tested.

The coating can be applied on the packing line, towards the end of the line, just before the last step of packing process. Either the dipping method or the waterfall method of application can be used, like the way the fungicide is applied in some packing facilities. Immediately after the application of the coating, a set of air knives can used to remove as much of the surface moisture as possible. In this process of surface moisture removal, the use of proper drain belt is critical. If the surface moisture is not removed properly then the benefits of the coating were not seen, as the excess moisture leads to mold growth. After the moisture removal, the cherries can be packaged in the regular packaging.

During the last two years of the project, sensory evaluation of the cherries was conducted, to determine if the consumers can differentiate between the coated and uncoated cherries. The cherries (coated and uncoated) that were in the storage for 1 week and 4 weeks, were tested. Based on the testing conducted, the consumer did not notice any obvious differences between the treatments.

Due to the size of this project, all the studies conducted were applied in nature. It is recommended that further studies be conducted to understand the fundamental aspects behind the observed results. Further studies are needed to evaluate the performance of the coatings in the industrial setting and during the typical transportation conditions, both at the national and international levels.

FINAL PROJECT 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
Wages	0	0	0
Benefits	0	0	0
Equipment	0	0	0
Supplies	1,008	388	585
Travel	3,892	6,672	8,340
Miscellaneous	0	0	0
Plot Fees	0	0	0
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
Wages	0	0	0
Benefits	0	0	0
Equipment	0	0	0
Supplies	5,000	6,408	6,230
Travel	0	0	0
Plot Fees	0	0	0
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)*
   The initial barrier to screening SWD adults was the need to capture them live from the field; monitoring traps, whether they used baits, synthetic lures or sticky panels were designed to kill the flies entering the trap in order to retain them. Despite the technical difficulties, this approach was deemed preferable to collecting infested fruit and rearing out larvae.
2. *Develop discriminating doses of insecticides to test susceptibility of SWD populations (yr 1)*
   The discriminating dose approach (in preference to probit bioassays) was chosen in order to screen more populations and insecticides. The discriminating dose requires only 100 subjects (vs 700) per bioassay. This also allowed us to perform bioassays on F1 progeny from field collections, instead of having to rear through multiple generations to obtain sufficient flies.
3. *Complete development of primers for genetic analyses of SWD alleles that confer resistance (yr 1)*
   The appropriate primers (those encoding for resistance mechanisms) were necessary before genetic analyses could be performed in Obj. 5. Primer design was greatly facilitated by the publication of the entire SWD genome.
4. *Screen SWD from multiple districts in CA, OR and WA for insecticide susceptibility (yr 2-3)*
   In addition to developing the methodology, our goal was to use it to establish the resistance status of SWD populations in sweet cherries at this point in time (ca. 7-8 years post-detection).
5. *Correlate results from discriminating-dose and genetic studies (yr 2-3)*
   Based on the information found in the bioassays, the genetic studies would reveal which alleles would most likely confer resistance in the future.

SIGNIFICANT FINDINGS

- Several styles of traps and techniques are effective for capture of live SWD.
- The numbers of founding females was below optimal in several orchard due to low underlying SWD density.
- In 105 bioassays, there were 10 instances of surviving females in the diagnostic dose screenings, including some in those re-tested due to initial survivorship. This may be indicative of the early stages of resistance, or issues related to the diagnostic dose bioassay (dose selected, evaluation interval, etc).
- Two populations with possible resistance showed ca 10-fold reduction in a cytochrome P450 gene expression. Functional experiments will be necessary to confirm that differential expression of this particular P450 gene confers resistance.

Note: an extension was requested to collect more SWD populations in Washington in the fall of 2017; rearing and bioassays on these populations were completed by February 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 was used as a control. For each concentration there was a minimum of 40 adult female SWD. Flies were treated using a Potter Spray Tower, and mortality was assessed 24 h post-treatment. The probit bioassays were 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,* adult specimens from the field-collected populations were collected and sent to the Chiu/Zalom lab at UC Davis. PCR-based assays and primers were developed to amplify genomic regions that are associated with development of resistance. Research in this proposal focused 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 to produce 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)
A target level of 100 adult female SWD (and associated males at a ratio of about 2 males/5 females) were collected from each orchard screened (Table 1) using traps or sweep nets. These females were used to produce cohorts of F_1 progeny for use in the diagnostic dose screening. Only females were used in the diagnostic dose screening. About 100 females (5-12 days old) from each population was exposed to the diagnostic dose of each of the five insecticides from Obj. 2. The females were 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 was repeated. If there were still survivors in the repeat bioassay, a full probit line was 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 were 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
		Tri-Cities

5. Correlate results from discriminating-dose and genetic studies (yr 2-3)
Genomic data were correlated with the results of the insecticide bioassays performed on the corresponding fly strains, and compared 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- to 3-day period. A second technique was found to be a practical means of collecting flies, viz., sweep netting beneath the trees (this technique was used successfully in OR). 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
2017	WA	Col. Basin	IR	Org	10/10/2017	56
2017	WA	Col. Basin	JM	Conv	10/10/2017	97

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.

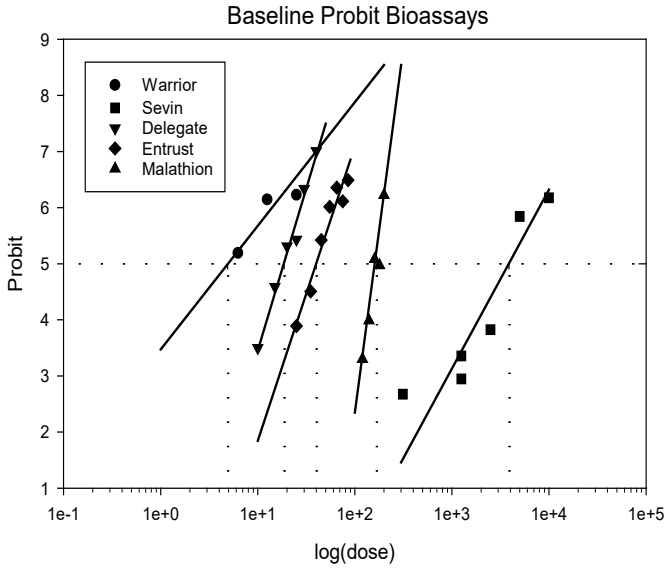

Fig. 1. Baseline probit bioassays for five candidate SWD pesticides.

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

A total of 12 populations of SWD have been sequenced. We focused on the identification of differentially expressed genes (DEG), in particular genes that are 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 did not yield single nucleotide variants (SNVs) in protein coding regions that are known to confer target-site resistance. We therefore concluded that any observed resistance in the populations we have examined are likely due to metabolic resistance.

Obj. 4. Screen SWD from multiple districts in CA, OR and WA for insecticide susceptibility (yr 2-3)

To date, 23 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). In 2016, none of the WA populations tested had survivors, and in 2017, one of the population had survivors.. A probit bioassay was conducted for this population (JM), and compared to the original probit used to develop the diagnostic dose, and a contemporaneous bioassays of the OSU colony. The POLO-Plus run of the JM and OSU original probit data indicated rejection of the hypotheses of equality and parallelism. However, the JM LC_{50} was slightly lower (128 ppm AI) than the original Shearer baseline for malathion (167 ppm AI), but due to the difference in slopes, the LC_{99} for JM was much higher (497 ppm AI) than the original Shearer baseline (262 ppm AI). The OSU malathion bioassay was repeated at a lower dosage range to prevent the high mortality levels in C1-C3; the previous bioassays used the diagnostic dose as the

high rate. The repeat bioassay yielded an LC_{50} of 112 ppm AI, and an LC_{99} of 336 ppm. The POLO-Plus comparison of these two bioassays rejected the hypothesis of equality, but did not reject the hypothesis of parallelism. However, only the LC_{10}s were significantly different. The Lethal Concentration Ratio test appears to be overly sensitive to differences, and thus may be too conservative an estimate of shifts in susceptibility.

Table 3. Percentage mortality of female SWD in diagnostic dose screening of five candidate 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
	2017	IR	Org	100	100	100	100	100
		JM	Conv	100	100	97	100	100
		JM rep	Conv	100	100	98	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 did not yield known mutations that confer target-site resistance, differential gene expression analysis identified a large number of genes that are up- and down-regulated in the various populations of SWD as compared to the SWD genome strain (Tables 4, 5). Although some of these differentially expressed genes could be the result of local adaptations and genetic variations, it is likely differentially expressed metabolic detoxification genes might have contributed to the observed changes in insecticide response as shown in Table 3.

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. It seems counter-intuitive that a reduction in a cytochrome P450 gene can promote insecticide resistance, but since the exact molecular substrate of Cyp312a1 is known, this remains a possibility and will have to be tested through functional experiments in the future. 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). Elevated expression of cuticular proteins is a widespread mechanism that confers insecticide resistance in addition to metabolic and target-site resistance.

Executive Summary

This project provided a blueprint for determining resistance in SWD populations in the future, as well as a snapshot of the current status. The methodology developed can be easily used by different research groups with commonly available equipment (synthetic lure traps, Potter Spray Tower). The bioassays of the OSU (reference) colony serve as a type of baseline; the insects (and their recent ancestors) were not likely unexposed to insecticides, but this colony represents the status of susceptibility prior to intense selection in western US specialty crops.

The assumption of a diagnostic dose bioassay is that there will be 100% mortality. This did not occur in all cases (10 of 105 had <100%). However, the levels of mortality and the proscribed follow-up probit bioassays do not present a compelling case for resistance at this point. Some of the sub-100% bioassays deviated from the protocol in terms of the numbers of founder females and the numbers dosed, but a few others had persistent survivorship or difference in lethal concentration ratios of the follow-up bioassays.

Resistance development in SWD continues to be a concern given the lack of alternative IPM tactics in use in sweet cherry. There is a reluctance to use thresholds for determining the need of spray applications because of the potentially severe negative consequences. This has promoted an over-reliance on insecticidal control, much of which is prophylactic. The economics of this course (high value of cherry crops relative to the cost of insecticides) will continue to favor preventive pre-harvest sprays to protect fruit from infestation. Although several different modes of action are in common use, rotation of materials may not provide insurance against resistance.

CONTINUING PROJECT REPORT **YEAR**: 3 of 3 (No-cost extension)
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: 509-293-8755
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/Kim Rains
Telephone: 509-335-2885/ 509-663-8181 **Email:** arcgrants@wsu.edu/kim.rains@wsu.edu

Item	2016	2017	2018	2019
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	0	0	0	
Supplies[5]	5,000	1,000	1,000	
Travel[6]	1,150	1,150	1,150	
Miscellaneous	0	0	0	
Plot Fees[7]	2,500	2,500	2,500	
Total	$35,924	$57,932	$60,064	No-Cost Extension

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	2019	2019	2019	2019
Salaries[1]	0	0	0	
Benefits[2]	0	0	0	
Wages	31,320	0	0	
Benefits	10,187	0	0	
Equipment	0	0	0	
Supplies[3]	3,411	0	0	
Travel[4]	1,582	0	0	
Plot Fees	3,000	0	0	
Miscellaneous	0	0	0	
Total	49,500	0	0	No-Cost Extension

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.* There is an ongoing need to determine efficacy of insecticides on SWD. Establishing the length of residual control, both for mortality and prevention of ovipositions, will aid in optimal timing and sequencing of products. New products must be screened to expand the selection of modes of action available for resistance management.
2. *Test chemical control products to determine ability to kill early stages of SWD in fruit.* If preventive sprays are not applied early enough, fruit infestation may occur under high pressure situations. Killing eggs or larvae in fruit will prevent the development of a complete generation of SWD that can attack nearby vulnerable crops (e.g., blueberry) or later maturing cultivars of cherry.
3. *Test provisional spray thresholds to determine initial and subsequent spray timing in commercial orchards.* In order for IPM to be implemented, action thresholds are necessary. Developing a sensitive and reliable monitoring tool is an essential first step in this process. Historic trap catch data coupled with records of infestation inform the need for insecticidal controls.

Significant Findings:

- Entrust and Delegate consistently provide long-term (up to 21 days) mortality of SWD
- GF-120 is still the most attractive/lethal attracticide tested, although new formulations may provide higher levels control in the field
- Some organic materials may provide overall control either by topical toxicity or oviposition deterrence, but lab studies need to be followed by field tests including phytotoxicity
- The spinosyns (Delegate and Entrust) have consistently provided the longest residual control of SWD, but the pyrethroids and diamides can play a role in resistance management
- The Scentry lure is the most attractive lure, but also has the most by-catch if used in a jar trap. Using this lure in combination with a species- and sex-specific trap (yellow sticky trap) may improve user-friendliness and ultimate adoption
- High pressure years for SWD can be predicted by the use of traps and help guide decisions on the intensity of control measures

Methods – *Field trial.* A field trial was performed in a research orchard to assess insecticide efficacy against SWD. Five insecticides were compared to an untreated check in a randomized complete block design, using single tree plots and rows as replicates. There were buffer rows and trees between the treated plots. Insecticides were applied a single time 2 weeks before harvest using an airblast sprayer calibrated to deliver 100 gpa. Four hundred fruit/replicate were collected and placed in plastic containers, and held at 72 °F for 10 days. The number of adult SWD that emerged from the fruit were recorded.

Results – *Field trial.* Fruit were infested to some degree prior to treatment, thus this was a test of both the insecticide's ability to kill pre-imaginal stages in fruit and prevent further infestation. The presence of buffers ensured a high level of pressure in this test. Entrust, Delegate, Exirel, and Warrior all provided similar levels of control. Admire Pro was not significantly different than the check (Fig. 1). This field test confirms observations in previous work that

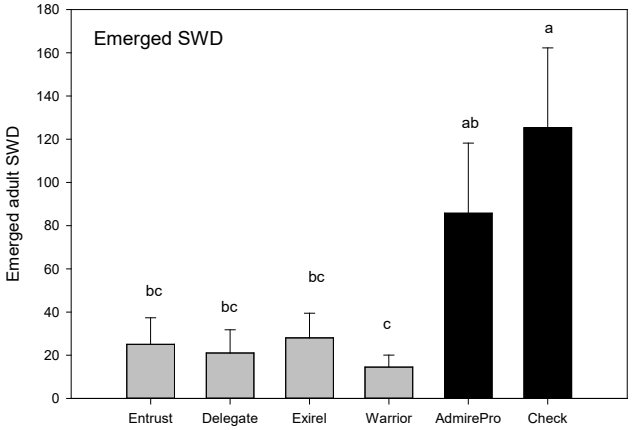

Fig. 1*. Number of flies emerging from field sprayed fruit.*

imidacloprid is a weak material against SWD, and the spinosyns, Entrust and Delegate, provide high levels of control. This is the first field trial of a diamide, and it performed better than expected based on field-aged residue bioassays. The Warrior treatment had the lowest number of SWD adults in this trial, confirming the high levels of activity of this pyrethroid.

Methods - *Field-aged residue bioassays.* A series of experiments was performed using similar methods to test the longevity and efficacy of insecticide residues. Insecticides were applied to a 1-acre block of 'Sweetheart'/Mazzard cherries at the WSU Sunrise orchard near Rock Island, WA. The trees were planted in 2007 at a 10 x 14 ft spacing. Replicates consisted of 2-3 trees per treatment, with sufficient fruit on the trees for the bioassay samples. Pesticide treatments were applied with an airblast sprayer calibrated to deliver 100 gpa.

Five replicates of undamaged fruit and leaves for the bioassays were collected 1, 3, 7, 14 and 21 DAT. The fruit and leaves were placed in paper bags and kept cool during transport to the laboratory. SWD used in the bioassay were from a laboratory colony originally collected from a cherry orchard in the fall of 2017. Flies were reared in 30-ml polystyrene vials with commercial *Drosophila* medium. The colony was reared in a controlled temperature room at 22 °C with a photoperiod of 16:8 L:D.

The bioassay arena consisted of a 32-oz plastic container lined with treated cherry leaves held in place with staples, and a 1-oz plastic portion cup was glued to the bottom of the arena. Five cherries were suspended from the lid of the container by inserting the stem through slits in the top and securing them with hot-melt glue (Fig. 2); allowed females access to all surfaces or the fruit for resting or oviposition. A 10-mm diameter hole was cut in the lid and covered with surgical tape for ventilation. Ten mated female flies were anesthetized with CO_2 and introduced into the arenas. After 16 h, the lid with the suspended cherries was removed and replaced with a standard lid (with honey agar and ventilation). At that time, a second 1-oz cup containing *Drosophila* medium was placed inside the glued cup to provide a food and moisture source in the absence of cherries. After the cherries were removed, the oviposition punctures were counted and recorded. After 48 h, mortality of females was evaluated, after which the females and the original bioassay container with leaves was discarded. The lids with fruit were incubated at 22°C for 16 d, and the number of emerged adults was recorded at this time. Mortality, oviposition and emergence data were analyzed using a mixed-model ANOVA and the Tukey-Kramer mean separation test.

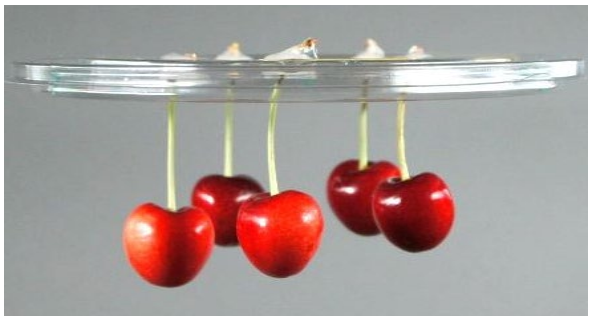

Fig. 2. *Sweetheart cherries suspended from lid of arena.*

Results - *Field-aged residue bioassay Trial 1.* Entrust caused 100% mortality through 14 DAT, and remained high at 21 DAT (Fig. 3). Fruit damage (ovipositions) were significantly lower than the check on 1, 3, and 14 DAT, and numerically lower on other dates. The total numbers of emerged flies (data not shown) was reduced commensurately with mortality and ovipositions, but the numbers of adults successfully emerging from the ovipositions was only occasionally different than the checks (3 and 21 DAT). The two rates of Cormoran behaved similarly, although the lower rate (21 fl oz) was not infrequently better than the

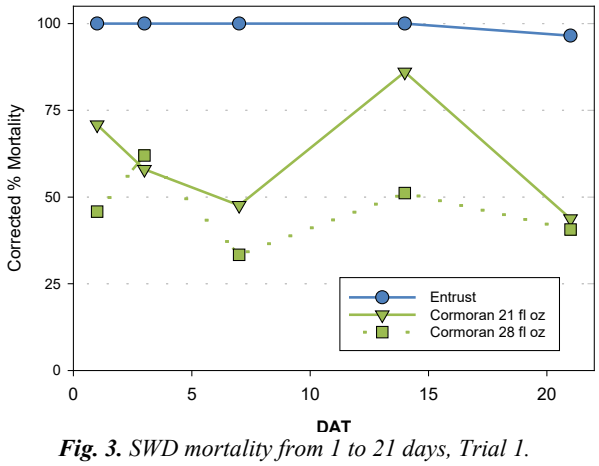

Fig. 3. *SWD mortality from 1 to 21 days, Trial 1.*

higher rate (28 fl oz). Mortality was moderate on 1 DAT, and while significantly higher than the check, it was also significantly lower than Entrust. Mortality in the Cormoran treatments continued higher than the check on 3 and 14 DAT, generally staying between 40 and 90%. Surviving females were able to oviposit normally, and ovipositions resulted in adult development, indicating no effects on egg hatch or larval development.

Results - *Field-aged residue bioassay Trial 2.* Delegate and Entrust caused high levels of mortality (90-100%) through 21 DAT (Fig. 4). Exirel (cyantraniliprole) and Minecto Pro (a mixture of cyantraniliprole and abamectin) caused high levels of mortality initially (3 DAT), which decreased (50-70%) at 7 and 14 DAT, and increased (>80%) again at 21 DAT. V-10433 (a formulation of sabadilla) at 8 fl oz caused ≈70% mortality at 3 DAT, but was low thereafter. This initial high level appears doubtful give that the higher rate (22 fl oz) was low throughout the test. Azera did not cause any mortality of SWD at any point in time. Ovipositions (a measure of fruit damage) were highest throughout the test period in the check V-10433 and Azera treatments, and lower in the Delegate, Entrust, Exirel, and Minecto Pro treatments (Fig. 5).

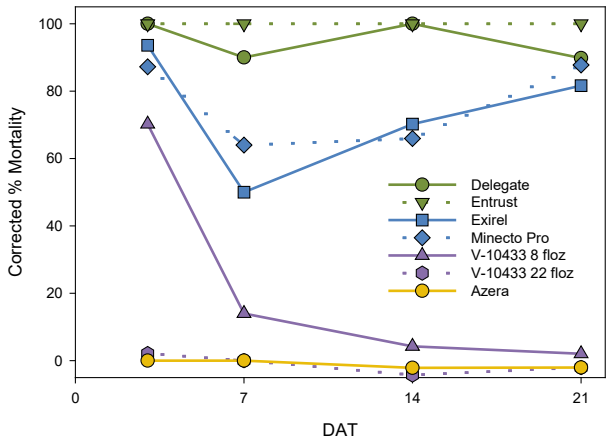

Fig. 4. *SWD mortality from 3 to 21 days, Trial 2.*

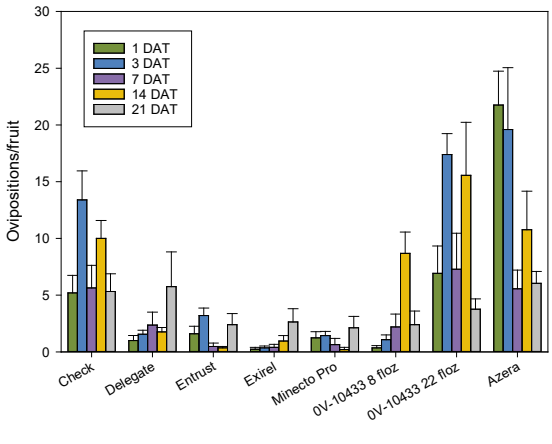

Fig. 5. *Ovipositions per fruit, 1 to 21 DAT, Trial 2*

Methods - *Laboratory bioassays.* Multiple experiments were conducted to determine if products either deterred female SWD from ovipositing, or killed eggs and larvae in fruit, thus preventing adult emergence. Three of the experiments tested the effects of two IGRs, Rimon and Dimilin, on SWD adults using different routes of exposure (topical, residual, ingestion). In these experiments, untreated leaves and fruit were collected from a research orchard, and treated with the two insecticides in the laboratory. The bioassay arenas were the same as described for the field-aged residue bioassays. For topical exposure, adults were anesthetized and transferred to a 14.7 ml plastic cup. Each group of SWD was sprayed with the specified rate of insecticide in a laboratory sprayer using 2 ml solution at 6.5 psi. Flies were transferred to prepared arenas with untreated leaves and fruit. For residual exposure, previously untreated cherry leaves were sprayed on a metal tray, and then stapled to arenas in groups of 3 leaves. Fruit suspended from arena lids were sprayed in a bucket sprayer with a laboratory mister, turning the lid ¼ turn after each spray in order to cover all fruit surfaces. Fruit and leaves were allowed to dry, and untreated adults transferred to the arena. For the ingestion treatments, the specified rates of the two insecticides were mixed in a 1:1 ratio with corn syrup. Twenty 25-µl droplets were applied to the leaves in the arena, and untreated adults transferred into it. Data collection and analysis of mortality, fruit damage, and adult emergence was the same as for the field-aged residue bioassays (above). In the first experiment, flies were 7 days old when treated; in the second experiment, they were 1 day old; and in the third experiment, they were 7 days old at the time of treatment, but a reduced rate of Rimon was used.

Results - *Rimon/Dimilin results.* *Bioassay #1.* Adult female mortality was low regardless of route of exposure, which is typical for IGRs. Oviposition level was quite variable, but lowest in the Rimon/Topical treatment. The most interesting result is the complete shut-down of adult emergence in the Dimilin/Residual treatment, despite the high levels of oviposition and only 16 hours of exposure. The exact mechanism for this shut-down (activity on eggs, larvae, or pupae) cannot be determined from this bioassay, but it represents the potential for an additional tool to suppress SWD populations and prevent fruit cullage. *Bioassay #2.* Both Rimon and Dimilin in the residual and ingestion treatments suppressed oviposition and completely shut down adult emergence when the flies were exposed at a young age. This experiment confirms the results of the first bioassay, which used the same treatments and methods except for the age of the flies at time of exposure (Fig. 6). The impact appears to be greater on flies exposed immediately after eclosion than those that are 7 days old. *Bioassay #3*. Fly mortality, ovipositions/fruit and emerged adults were not significantly different than the check in this test. The Dimilin/residual treatment had the lowest adult emergence, but due to variability, a statistical difference was not detected. This may be an indication that while the higher (Dimilin 16 fl oz/acre, Rimon 40 fl oz/acre) rates were effective, the lower rate of Rimon (24 fl oz) was not; in theory, Dimilin at the same rate should have performed similarly to the first bioassay.

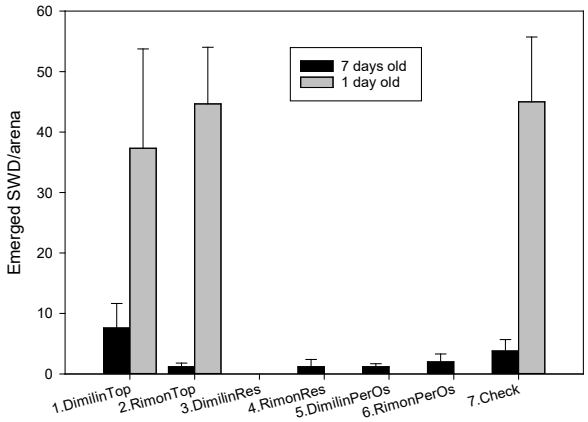

Fig. 6. *1 and 7 day old SWD emerging from fruit treated with Rimon or Dimilin (3 routes of exposure)*

Methods - *Bioassays of organic insecticides.* Additional bioassays were performed to determine if other pesticides (especially those registered for organic production) were effective against any stage of SWD. Contact bioassays were performed on 7-10 day old females in plastic Petri dishes, and sprayed with 2 ml of the solution at 15 psi. Residual control and oviposition deterrence bioassays were performed using the same bioassay arena as in previous experiments with untreated leaves, but with fruit sprayed with the candidate insecticides using a laboratory sprayer. The sprayed fruit were suspended from the lid of the bioassay arena, and 10 female SWD introduced. Mortality and ovipositions were assessed after 24 h, and adult emergence after 15 days.

Results - *Bioassays of organic insecticides.* Cinnerate at all rates tested (25-40 fl oz/100 gal) does not appear to have any contact toxicity to SWD, but the contact-only bioassay is a severe test. Use of plant materials or repellency/oviposition deterrent may be more successful. In tests of oviposition deterrence of organic materials, only Ecotrol (0.5%) and methyl benzoate (1%) reduced the numbers of ovipositions in relation to the check; petroleum oils had generally lower numbers of ovipositions, but differences were not statistically significant. Lavender oil residues caused high levels of mortality at 1-5% concentration; the 0.5% level caused a moderate amount of mortality. Conversely, topical applications of sabadilla caused little contact mortality to adult female SWD.

Methods - *Attracticides.* One category of insecticidal control, attracticides, was pursued in hopes of reducing the non-target impacts of full canopy sprays. A candidate material made by Scentry consisted of an attractant only, to which different insecticides might be added for resistance management schemes. Initial tests were done in laboratory assays, examining fly mortality when exposed to bait droplets in 1-liter arenas. Further tests were conducted in a field-aged residue bioassay where bait+insecticide droplets applied to leaves in a research cherry orchard, then collected at intervals to determine attractiveness and lethality. In all cases, the toxicant added to the Scentry attractant was spinosad (Entrust), and the comparison material used was GF-120 (which also has

spinosad as the toxicant). In addition, control with attracticides was tested in whole-tree field cages, where known numbers of SWD adults could be added to control the amount of insect pressure. This trial compared an airblast application of Entrust to GF-120 bait spray.

Results – *Attracticides*. *Laboratory bioassays.* Neither of the attracticides caused a significant amount of mortality after 6 h, but levels increased at 24 and 48 h. After 24 h, the GF-120 treatment had significantly more mortality than the Scentry attracticide treatment, but they were not different at 48 h (Fig. 7). When field-aged residues were tested, the Scentry attracticide had lower mortality initially than GF-120, and decreased rapidly thereafter (Fig. 8). Overall, the goal of developing an attracticide that is more effective than GF-120 has not been realized. *Field cage trials*. Damage was low overall, and while not significantly different from the check, fruit damage was numerically lower in both the Entrust and GF-120 treatments (Fig. 9).

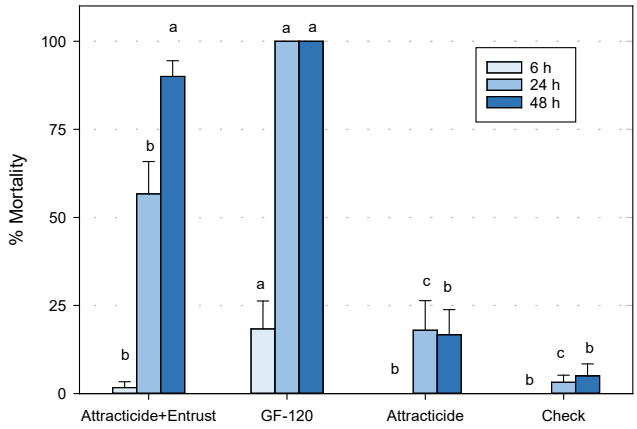

Fig. 7. *Percent mortality of female SWD following exposure to attracticides*

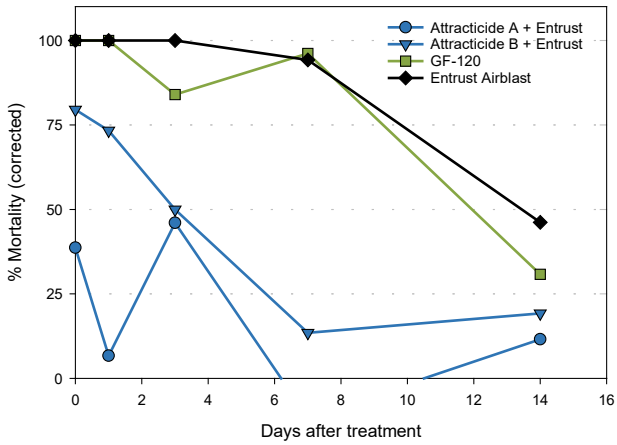

Fig. 8. *SWD mortality from 1 to 14 days, following exposure to attracticide*

Monitoring. The efficacy of traps and lures were tested over a 4-year period, and provide insight into the most sensitive monitoring tool for SWD. Early trap and bait systems were adequate to detect range expansion, but monitoring for action thresholds will require a trap that is sensitive and (if possible) user friendly. The Scentry lure has consistently provided the highest trap captures of SWD, whether during low density periods (cherry maturation period) or higher densities (fall post-harvest period). This lure also has the highest levels of by-catch, which is problematic with drosophilids, which are small and similar in appearance. Only the spots on the wings of the males distinguishes SWD from non-pest species, but the females (the damaging stage) are difficult to differentiate. The liquid-based traps overall provide the highest level of catch, but the poorest level of user-friendliness. Specifically, the in-house fabricated PBJ trap (Fig. 10) consistently captures the most SWD; the Scentry trap (of the commercial traps) has also performed well. Sticky traps offer an easy-to-use alternative to the liquid traps, but their primary limitation is that while males are relatively easy to identify, the females are more problematic in field counts. Of the sticky traps, the yellow card has a slight edge over other colors. An additional unresolved problem is the preponderance of females in the population in the early season, when spray

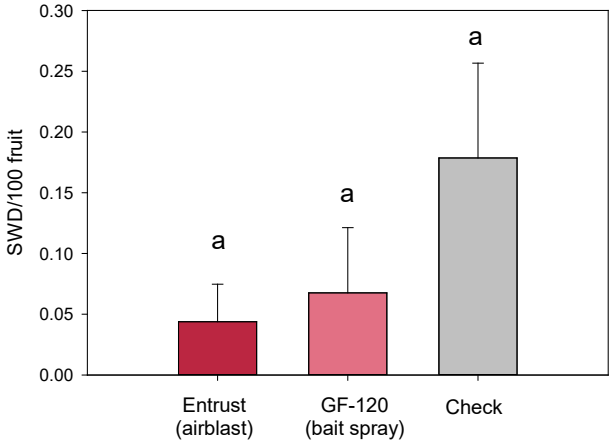

Fig. 9. *Fruit infestation by SWD following exposure to Entrust or GF-120*

decisions for cherries are usually made. Whether male capture on these traps is sufficiently sensitive to be used as an action threshold remains to be determined.

Regional trends. SWD traps were deployed in orchards in North Central Washington from 2012 to 2018 in the same locations each year to track year-to-year variation in trap capture (Fig. 11). Starting in 2013, we coordinated sample collection and rearing with the WSDA cherry packinghouse inspection program to rear larval *Drosophila* species and determine if they were SWD (Fig. 12). This information provides insight into the relevance of cherry season trapping to regional reports of damage. While there many factors (including weather interfering with insecticide applications) that influence the efficacy of control measures, it is clear that high trap numbers in the summer of 2015 corresponded with high numbers of SWD finds in the packinghouse. The packinghouse finds have been fairly steady in the last three seasons, indicating growers have made a reasonable response to moderate insect pressure. This level of pressure likely represents an average for this region, and more importantly, high pressure years can be predicted as early as May in order to intensify control measures.

***Fig. 10**. Clockwise from left: PBJ trap, yellow sticky card, and male SWD on a sticky trap with wing spots clearly visible*

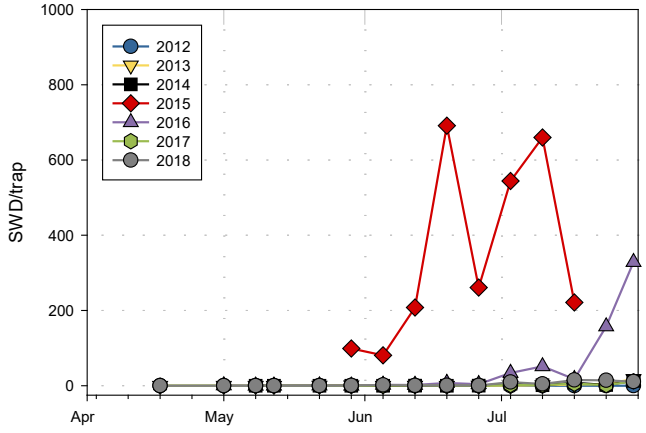

***Fig. 11.** SWD trap captures during cherry maturation and harvest, 2012-2018*

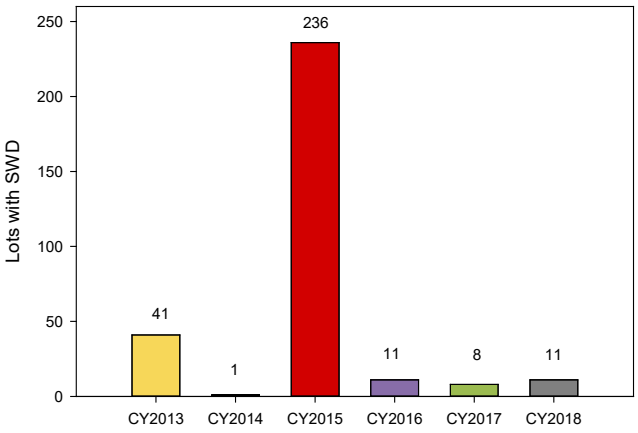

***Fig. 12.** WSDA larval SWD detections in packinghouses, 2013-2018*

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

Project Title: Non-nutritive sugar-based control strategy for spotted wing drosophila

PI: Man-Yeon Choi
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Cooperator: Dr. Ramesh Sagili, Associate professor, Oregon State University, Corvallis, OR

Total Project Request: $82,720 **Year 1**: $38,060 **Year 2: $44,660**

Other funding sources

Agency Name: USDA-ARS (Innovation-fund)
Amt. awarded: $25,000

BUDGET
Organization Name: Agricultural Research Foundation
Contract Administrator: Dr. Russ Karow
Telephone: 541-737-406 **Email address:** Russell.karow@oregonstate.edu

Item	2018	**2019**
[1]**Salaries**	$25,000	$25,750
[2]**Benefits**	$4,380	$4,510
[3]**Wages**	$2,880	$9,600
Equipment	$0	$0
[4]**Supplies**	$5,000	$4,000
[5]**Travel**	$800	$800
Plot Fees	$0	$0
Total	$38,060	**$44,660**

Footnotes: USDA ARS in-house fund supports for equipment, facilities and supplies for this project. [1]Salaries & [2]Benefit, 0.5 FTE Postdoc; [3]Wage, student assistant, $12/h x 10/w@4x6m; [4]Materialsand Supplies, non- & nutritive sugars; other supplies for bioassay, large potted blueberry plants, and Insect rearing materials and supplies; [5]Travel, PI and/or postdoc to attend and present results in the cherry commission or entomology meetings each year.

OBJECTIVES

Our approach for SWD control strategy is based on our current non-nutritive sugar study and previous research results. We recently discovered a variety of dosages of erythritol and other sugars in mixed or separate solutions had significantly reduced the survival of SWD adult flies, and suggested that erythritol alone or with sucrose had potential insecticidal activity. We have also found the impacts on fecundity and mortality from testing at a larger scale in greenhouse cages, and examined the nutritional pathway of ingested erythritol in the fly body. Undoubtedly, erythritol combined with sucrose reduced the survival and fecundity of SWD, which is caused by the physiological imbalance with the sugar osmolarity in the body. Based on those results, we proposed a possible mode of action of erythritol for insecticidal activity.

For practical applications, the erythritol formulation mixed with sucrose can be used as a potential insecticide or as a delivery agent combined with other biological insecticides such as RNAi (RNA interference) and microbial pathogen for SWD. To develop this new control method for cherry growers, we need to identify the mode of action of the erythritol formulation in SWD, evaluate the control efficacy from large scale tests, and investigate if negative impact(s) present on non-target insects. To achieve this goal, these specific objectives need to be accomplished in this project:

1. Test the efficacy of the erythritol formulation on SWD in a greenhouse (Yr. 1) – *completed*
2. Test the efficacy of the erythritol formulation on SWD in a field (Yr. 2) – *partially completed*
3. Evaluate the impact of the formulation applied on honeybees (Yr. 2)

SIGNIFICANT FINDINGS (Year 1)

- The erythritol formulation was significantly to reduce larval infestation and adult oviposition.
- The erythritol formulation reduced up to 90% larval infestation in the greenhouse trial.
- The erythritol formulation reduced up to 78 % and 49 % infestation in the early and late fields.

METHODS

The project team (PI/Co-PI) and collaborators have expertise in specialized areas for SWD molecular physiology (Choi), biology & IPM (Lee), field assay (Ahn), and honeybee (Sagili). Research results have been published and demonstrated the combination of sugars on survival, various types of feeding assay, carbohydrate analysis by Anthrone test & gas chromatography, and greenhouse test on SWD (Choi et al., 2017; Tang et al., 2017; Tochen et al., 2016; Wong et al. 2018).

1. Test the efficacy of the erythritol formulation on SWD in a greenhouse

Four potted blueberry plants (2 Bluejay, 1 Bluecrop, 1 Elliot plants) were placed in each of three greenhouses. Pre-bagged ‘Reka’ or ‘Elliot’ were collected from the field, they were bagged while green to prevent SWD infestation. Each blueberry cluster was placed in a water wick and clipped onto each blueberry bush, four clusters per greenhouse. Bushes within a greenhouse either received 100 mL of a water spray, an erythritol:sucrose formulation (2.0 M:0.5 M) called ‘E+S’ spray or a sucrose-only spray. Because this trial was done in the greenhouse with no other food sources, we set up sucrose as a positive control to ensure that SWD would survive in the greenhouse. An equal number of flies were released in each greenhouse for a given trial, depending on fly availability it varied between 396-600 flies per greenhouse.

Blueberries were exposed for 3 days, and collected. At this time, one trap was set up per greenhouse baited with 40:60 apple cider vinegar: wine. The trap was collected a few days later when a new trial was about to be set up. Blueberry fruits were incubated for 2-3 days in the lab and then checked for larvae by salt float. In the next week, another trial was set up and treatments were rotated to a different greenhouse. This was repeated for 10 trials, one trial was omitted due to no surviving SWD larvae from the high heat.

2. Test the efficacy of the erythritol formulation on SWD in a field

2-1 Bag method: Field plots were either treated with 2 litter of E+S, water, or nothing as a dry control. Erythritol was sprayed in Reka blueberries on June 11, June 18, and June 25. Water was sprayed on E+S plots on July 2 in order to wet the sugar for SWD feeding. Water was sprayed on water plots from June 11 to July 2, 2018. Field plots consisted of 8 Reka plants in a row, and plots were on the four corners of the field to maximize distance and prevent cross-treatment of flies. To measure oviposition, mesh bags were placed over a hanging ripe blueberry cluster and 10 females and 10 males ~4-7 d old were introduced. Clusters were selected based on having 10+ ripe berries to ensure adequate ovipositional substrate. Bags were all given a spritz of water to ensure that flies did not immediately die from field heat. After 24 h, bags were recovered from the field. Bags were set up on June 25, and for the next three weeks on new clusters. The first date was immediately following E+S spray. No further E+S sprays were made prior to bag set-up on the other weeks since the sprayed erythritol was considered sufficient and should not have degraded. Since there was no observable field infestations during the time, it was not necessary to pre-bag the clusters before the experiment. [Note: when experiments are run when the field is infested, it is necessary to bag green clusters to keep the cluster fly-free for the experiment]. Three bags were placed per plot, with 3 bags x 2 plots per treatment = 6 bags per treatment per week.

In week 2, 2 E+S bags were found in adjacent plants, and not actually treated with E+S, so those 2 bags were included in the dry treatment because berries were actually untreated. Once bags were taken to the lab, the number of live females and males were counted. The number of blue or ripening fruit were counted and checked for eggs under the microscope.

2-2 Field: On July 20, 2018, four Elliot plots were sprayed with 2 litter of E+S solution, and four were left untreated (Fig. 1). Since we found no indication of differences between water and dry controls in the bag trial, we decided to only test the dry control in which nothing is done. A grower would not treat the field with water. Plots were spaced apart as much as possible in the field to minimize cross-treatment movement. Treatments were 24-48 ft apart. Each week, ripe blue fruit were collected from each plot. At first, we collected ~500 g per plot, and then by week 4 August 17, we collected ~1.2 kg per plot. In the lab, fruit were weighed. Then fruit were placed in mesh container to allow any SWD to develop. After 2 weeks, fruit were checked for adult SWD. On sunny mornings, we took 2 1-minute observations of a plot counting the number of honeybees and yellow jackets on the bush.

Figure 4. Photo of the erythritol formulation spraying blueberry plants. Each plot was sprayed with 2 litter solution.

RESULTS & DISCUSSION

An erythritol formulation mixed with sucrose has reduced the lifespan and fecundity of SWD in the lab and greenhouse cages. Yet, the impacts of the formulation have not been tested in the field or at a larger scale, and on honey bees. For these objectives, we conducted full arena greenhouse and field studies to evaluate the effectiveness of this non-toxic alternative for year 1.

1. Greenhouse trial

We evaluated the erythritol formulation to reduce infestation by SWD exposed among blueberry bushes in the greenhouse. We found 86-90% reduction of SWD larvae infestation on blueberries collected from bushes treated with E+S than the bushes treated with the water or sucrose controls (P = 0.0003) (Fig. 2).

We also found fewer adults were trapped post-fruit removal in the greenhouse when bushes were treated with E+S than water, but there were also fewer adults with sucrose than water. We know that sucrose feeding extends the longevity of SWD (Tochen et al. 2016), and that satiated SWD are less likely to be trapped with fermenting odors than hungry SWD (Wong et al. 2018). Therefore, the lowered trap counts following sprays with sucrose-only may reflect fed SWD not being as attracted to the trap to get trapped.

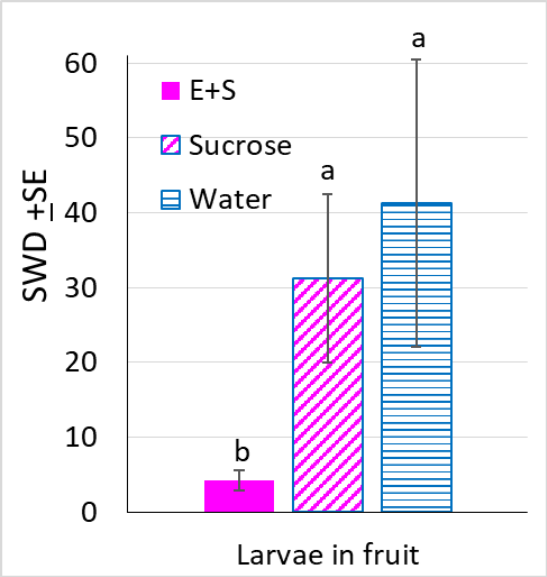

Figure 5. Numbers of SWD larvae infesting blueberries collected from bushes treated with E+S, sucrose or water.

2. Field trial

The E+S formulation was sprayed on blueberry bushes in the field, and evaluated the efficacy for SWD infestation.

2-1. Bag trial for Reka

The field trial in 'Reka' blueberries in June and July occurred before SWD infestation was prevalent yielding minimal data. So, we conducted bag trials in Reka to assess whether SWD laid on field-treated blueberries. The erythritol formulation was evaluated the oviposition reduction by SWD exposed to field conditions. We found about 72-78% fewer eggs were laid in berries treated with erythritol (Fig. 3).

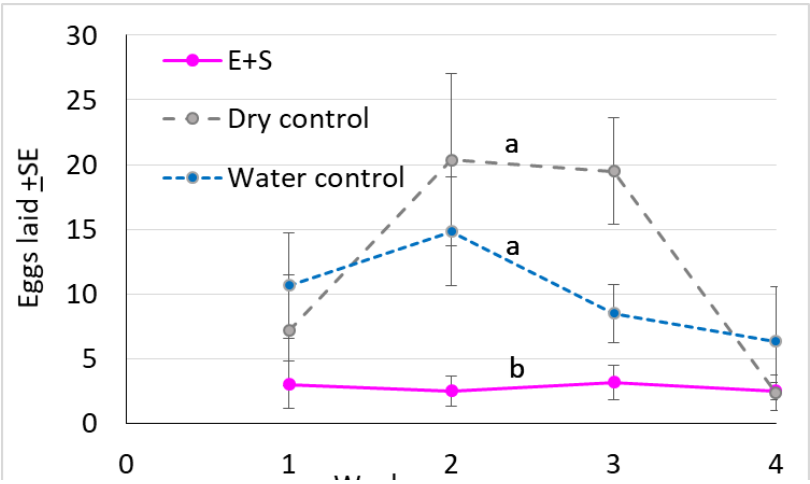

Figure 6. Numbers of eggs laid in blueberry fruits collected from plants treated with E+S, water control or dry control.

2-2. Field trial for Elliot

The E+S formulation was sprayed in blueberry plants (Elliot plots) to reduce SWD infestation in the field. We found that SWD larvae was reduced with an overall 49% among E+S plots. The difference was most pronounced at week 2 with a 96% reduction (Fig. 4).

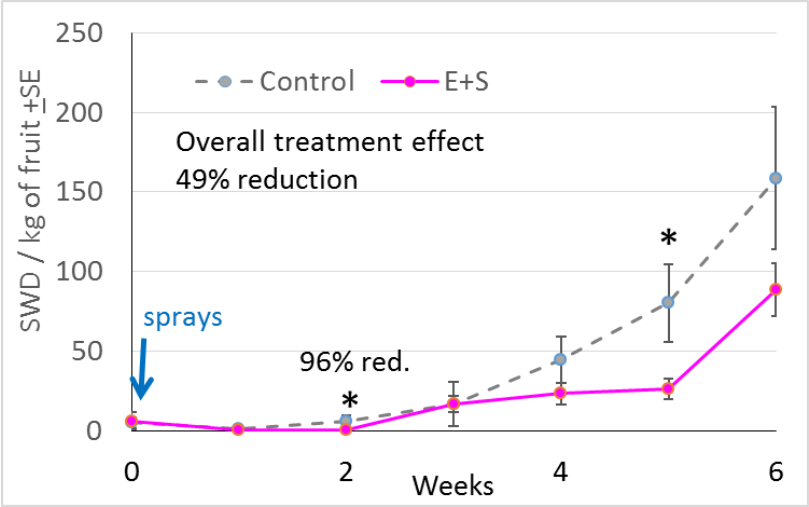

Figure 7. SWD infestation in blueberry fruits collected from plants treated with E+S or dry control.

Continue and ongoing study

For next year we will repeat the evaluation of Objective 2: Test the efficacy of the erythritol formulation on SWD in a larger field, and test for Objective 3: Evaluate the impact of the formulation applied on honeybees.

CONTINUING REPORT **No-Cost Extension**

Project Title: Electronic sensors to capture spatiotemporal population density of SWD

PI: Joanna C. Chiu
Organization: University of California Davis
Telephone: (530) 752-1839
Email: jcchiu@ucdavis.edu

Cooperators: Eamonn Keogh (UC Riverside, Dept. of computer science and engineering)

Budget: **Year 1**: $31,384

Percentage time per crop: Cherry: 100%

Budget
Organization Name: UC Davis **Contract Administrator: Yang Yeh**
Telephone: (530) 752-3794 **Email address:** ypyeh@ucdavis.edu

Item	2017-18	2018 -2019	
Salaries	$16,016		
Benefits	$8,168		
Wages	-		
Benefits	-		
Equipment	-		
Supplies	$5,200		
Travel	$2,000		
Miscellaneous			
Plot Fees	-		
Total	$31,384	No-cost extension	

Footnotes:
Salaries and Benefits are for one SRAI (technician) for sensor testing and insect collection (33.3% time)
Supplies include funding to construct 20 sensors for testing ($4000) and for insect capture and maintenance ($1,200)
Travel funds ($2,000) are requested for SRAI to travel to Washington or Oregon to conduct field sensor testing

JUSTIFICATION

Sensor technologies and automated insect identification models are currently being developed for the control of insects that spread human diseases. Our cooperator Dr. Keogh, a computer scientist at UC Riverside, has recently developed inexpensive pseudo-acoustic opto-electronic sensors and accompanying classification algorithm that can accurately classify multiple species of mosquitoes that vector pathogens such as Zika and West Nile virus (Chen et al. 2014) by using wing-beat frequencies, daily activity patterns, and geographical distribution. The ability to remotely capture real-time measurements and forecast insect density in a spatiotemporal manner allows for efficient and precise insect control response that could prevent public health crisis. ***The overall goal of this proposal is to adopt and translate this technology to optimize insect pest management programs and benefit agricultural stakeholders.*** We propose to develop and ultimately deploy opto-electronic sensors that can accurately identify Spotted Wing Drosophila (SWD) and differentiate it from other insect inhabitants of cherry orchards.

SWD are a highly invasive pest species that cause up to $500 million in annual losses in the western United States by ovipositing their larvae into marketable, ripening fruit (Goodhue et al. 2011, Wiman et al. 2016). An insect sensor utilizing wing beat frequency for classification can theoretically be applied to identify any flying insect, but the substantial economic loss caused by SWD warrants the prioritization of optimizing this new technology for its control. It is important to stress that the electronic sensor technology we propose to develop and optimize for SWD is not simply a modernized version of insect traps currently used for population monitoring. Besides supplanting conventional monitoring tools and greatly reducing the time necessary to process trap contents, we anticipate that the capability of the sensors to classify insects in real-time will revolutionize pest management research and lead to developments in precision agriculture. For example, current monitoring tools lack spatial and temporal resolution as conventional traps do not provide time-stamps for insect catches. Our sensors on the other hand can ultimately be connected to a central network and will be capable of reporting real-time movement between crop and non-crop host plants, providing opportunities to target SWD for sprays at times when they are at maximum density in non-crop plants. This can reduce insecticide residues on crops, a major concern for export markets.

OBJECTIVES

Objective 1:

Measure wing beat frequency and circadian activity pattern of SWD to improve insect identification algorithm. Opto-electronic sensors will be installed in insect cages that house SWD to measure wing beat frequency and daily activity patterns simultaneously. Since biological parameters, e.g. sex, age, and seasonal morphology, may alter wing beat frequency and activity patterns, we plan to evaluate male and female SWD, different ages of SWD, and summer and winter forms of SWD. Various abiotic factors can also affect wing beat frequencies so we will evaluate recordings in a range of environmental conditions.

Objective 2:

Field recording to assess opto-electronic sensor and insect identification algorithm. We will deploy opto-electronic sensors housed in McPhail traps to assess the capability of the sensors to accurately identify SWD from other inhabitants of Cherry orchards.

METHODS

Objective 1: Refining insect identification algorithm using opto-electronic sensors

Overview: In order to automate the process of insect identification based on wing beat frequency, an algorithm must be created and refined to take into account biotic and abiotic factors that may result in changes to insect wing beat frequency and activity pattern. Our cooperators have already created an algorithm to accurately identify insects down to species and sex using wing beat frequency in controlled environments, which they have tested on mosquito species (Chen et al. 2014). To refine

this algorithm for SWD and use in the field, wing beat frequency of SWD and other insects commonly found in cherry orchards will be recorded in different environmental conditions (temperature, light cycle, humidity, etc.). The data acquired from these species in controlled environments will be sent to our cooperators to be incorporated in their insect identification models and refinement of the algorithm.

Collection of data for insect identification algorithm refinement

Flies of a known species and sex (N=60) will be placed into a modified McPhail trap outfitted with an opto-electronic sensor ring and connected to a recording device. This setup will then be placed into a Digitherm incubator (Tritech Research) that allows us to control the environmental conditions. Using this setup, we will record wing beat frequency data in different temperatures, humidity, light-dark cycles with different photoperiods, etc. as well as wing beat frequency of different species and sexes. The data collected in these controlled environments can be visualized using analysis programs written by our cooperators using MATLAB (Mathworks). General trends can be visualized using these analysis tools. Comparison between SWD and the closely related *Drosophila melanogaster* in controlled conditions show distinct wing beat frequency patterns. Based on live capture in field in CA, we identified several closely related *Drosophila* species such as *D. simulans*, *D. biarmipes* and *D. tristis*. Recording using these different species and other relevant species present in cherry orchards will be generated in order to refine the algorithm and improve identification accuracy.

Refinement of insect identification algorithm

Our cooperators have created an insect classification algorithm which they have used to accurately identify disease carrying mosquito species based on wing beat frequency alone (Chen et al. 2014). When more species were added or environmental conditions were changed the classification model was less accurate. Due to the large diversity of species present in the field and the heterogeneity of environmental conditions, it is important to have accurate classification established on a wide range of fluctuating parameters and species to mimic field scenarios. By creating a training dataset using the data we collect from flies in various conditions, our cooperators will be able to "train" the classification model to accurately identify insect pests in vastly different environments. Our cooperators have already "trained" the insect classification model based on geographical and circadian rhythm data to increase the accuracy of the model in identifying mosquitos down to the species level (Chen et al. 2014). By "training" the insect classification model to correctly identify insects using a larger number of variables we will be able to increase the accuracy of our identification process in the field. This will be an iterative process of testing and refinement.

Objective 2: Assessment of insect identification algorithm and field deployment of sensors

Overview: With current monitoring methods, it is extremely time consuming to monitor insect pest species in the field because it requires the presence of a specialist to manually identify individuals. In addition, the time lapse between trapping and identification constitutes an important limitation to initiate a quick and appropriate response to slow down crop infestation. Our goal in refining the insect identification algorithm is to develop an automated identification process that is easier and faster to identify insect pests then is possible with current pest capture and identification processes. We will be assessing the ability of the sensors to correctly identify and monitor pest species both spatially and temporally in and around cherry fields.

Deployment of insect sensors in the field

Once the classification algorithm is found to be highly accurate (>99%), we will deploy our system in cherry fields. We will use baited McPhail traps outfitted with sensors in the opening at the bottom to record the wing beat frequency and relevant environmental variables (temperature, humidity, time, etc.) of any insect that enters the trap and identifying them in real time. By deploying

multiple trap/sensor setups in and around cherry fields, we will be able to track the movements of SWD throughout the day, e.g. from crop to non-crop hosts. This will allow for the development of more precise strategies of pest management than are possible through conventional monitoring techniques using traps and manual identification. The automated process of insect identification also means that there will be far less processing time required to identify flies allowing growers and researchers to respond to the presence of pests as soon as they arrive and are detected in their fields.

RESULTS:

Objective 1:

Hardware optimization for insect sensors

We successfully went through several interactions of design and testing of the sensors. We have converged on a solution that we feel is robust, maintainable and cheap to produce in large numbers. Briefly, we use IR emitters and phototransistors working at a wavelength of 940nm, which is outside the visible light spectrum. Our emitters (OSRAM SFH 4043) and phototransistors (Everlight PT19-21C) use around ~20mA. There may be some other low power emitters and phototransistors out there that we can used to further improve our design in the future. We are currently using a cortex M4 MCU, which runs at 80Mhz. This chip has 32K of flash and 2K of RAM. It consumes around 5.5mA when running at 8Mhz but we can put it to sleep when there is no activity, in sleep mode it only consumes few micro amps. For transmission we are using Long Range Wide Area Network (LoRaWAN) technology. LoRaWAN is a wireless standard designed for long range communications at a low bit rate on a very low power budget. We use Semtech SX1272 LoRa module which has a range of 2 miles in non line-of-sight environment and up to 15 miles in line-of-sight environment. It can achieve data rates up to 50 kbps. SX1272 consumes ~15mA while transmitting/receiving and a negligible power (1.5 uA) in idle state. We have started to install solar panel to the sensor unit, so that the sensors can be left unattended in the field for weeks at a time (Figures 1 to 3).

Development of species ID algorithm

We have now completed most of the activity and wing beat frequency recordings for 5 different *Drosophila* species (*D. simulans, D. tristis, D. suzukii, D. biarmipies, D. melanogaster*) at various temperature and photoperiod (Figure 4). The accuracy of the resulting species ID algorithm is easily over 90% accuracy, and will continue to improve as we finished our planned recordings.

Objective 2:

Field deployment and testing

With the outfitting of the sensors with solar panel and cellular data transmission for remote sensing, we are ready for field testing of the sensors in this upcoming year of the project. Field testing and validation will be performed as described in the Methods section.

LITERATURE REVIEW:

There have been some efforts in identifying insects based on recordings of their wing beat frequencies and these attempts date back to the advent of commercially available computers and audio recording devices (Reed et al. 1942, Foster and Robinson 1991, Moore and Miller 2002, Raman et al. 2007). These attempts have not been successful in creating an automated and accurate identification process based on recordings of wing beat frequencies. In most studies, wing beat frequency has been recorded using acoustic microphones, which are susceptible to noise from the wind as well as any ambient noise in the environment (Reed et al. 1942, Mankin et al. 2006, Raman et al. 2007, Villarreal et al. 2017). This made it very difficult to get quality recordings of insect wing beat frequency with acoustic recording devices. Because of this difficulty, wing beat frequency data is sparse, low quality, and typically recorded in unnatural conditions (Moore et al. 1986). Despite the sparseness and low quality of available insect wing beat frequency data, some researchers have

attempted to create insect identification models with 300 or less recordings (Moore 1991). It is difficult to create models with such sparse data and this will cause the models to have very low accuracy in identifying insects (Banko and Brill 2001, Halevy et al. 2009). This is compounded by the fact that most attempts at classification of insects by recording wing beat frequency have used just one variable (wing beat frequency). Other environmental factors that cause wing beat frequency to change have also been ignored (Chen et al. 2014). By using pseudo-acoustic opto-electronic sensors, we will be able to record higher quality data. We will also be able to record larger volumes of data in more natural conditions than has been possible in the past, which will allow us to create a highly accurate insect classification model that can be used to identify SWD and differentiate it from other species in the field.

LITERATURE CITED:

1. Banko M, Brill E. (2001) Mitigating the paucity of data problem: Exploring the effect of training corpus size on classifier performance for natural language processing. 1st-ICHLTR 1:1-5.
2. Chen Y, Why A, Batista G, Mafra-Neto A, Keogh E. (2014) Flying insect detection and classification with inexpensive sensors. JOVE-J Vis Exp 92.
3. Foster JA, Robertson RM (1992). Temperature dependency of wing-beat frequency in intact and deafferented locusts. J Exp Biol 162:295-312.
4. Goodhue RE, Bolda M, Farnsworth D, Williams JC, Zalom FG. (2011) Spotted wing drosophila infestation of California strawberries and raspberries: economic analysis of potential revenue losses and control costs. Pest Manag Sci 67:1396-1402.
5. Halevy A, Norvig P. Pereira F. (2009) The unreasonable effectiveness of data. IEEE Intell Syst 24(2):8-12.
6. Mankin RW, Machan R, Jones R. (2006) Field testing of a prototype acoustic device for detection of Mediterranean fruit flies flying into a trap. 7th-ISFFEI 7:165-169.
7. Moore A, Miller JR, Tabashnik BE, Gage SH. (1986) Automated identification of flying insects by analysis of wingbeat frequencies. J Econ Entomol 79:1703-1706.
8. Moore A. (1991) Artificial neural network trained to identify mosquitoes in flight. J Insect Behav 4(3):391-396.
9. Moore A, Miller RH. (2002) Automated identification of optically sensed aphid (Homoptera: Aphidae) wingbeat waveforms. Ann Entomol Soc Am 95(1):1-8.
10. Raman DR, Gerhardt RR, Wilkerson JB. (2007) Detecting insect flight sounds in the field: implications for acoustical counting of mosquitoes. Trans ASABE 50(4):1481-1485.
11. Reed SC, Williams CM, Chadwick LE. Frequency of wing-beat as a character for separating species races and geographic varieties of Drosophila. Genetics 27:349-361.
12. Villarreal SM, Winokur O, Harrington L. (2017) The impact of temperature and body size on fundamental flight tone variation in the mosquito vector *Aedes aegypti* (Diptera: Culicidae): implications for acoustic lures. J Med Entomol 54(5):1116-1121.
13. Wiman NG, Dalton DT, Anfora G, Biondi A, Chiu JC, Daane KM, Gerdeman B, Gottardello A, Hamby KA, Isaacs R, Grassi A, Ioriatti C, Lee JC, Miller B, Stacconi MVR, Shearer PW, Tanigoshi L, Wang X, Walton VM. (2016) *Drosophila suzukii* population response to environment and management strategies. J Pest Sci.

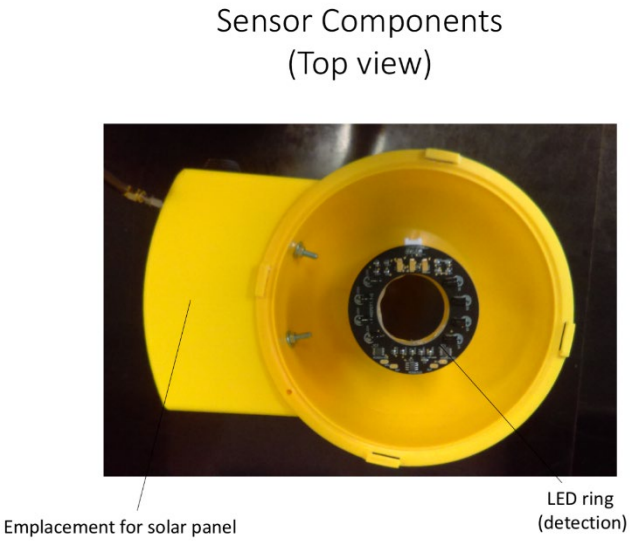

Figure 1: Top view of the modified Mcphail trap outfitted with the LED sensor ring, showing emplacement for solar panel.

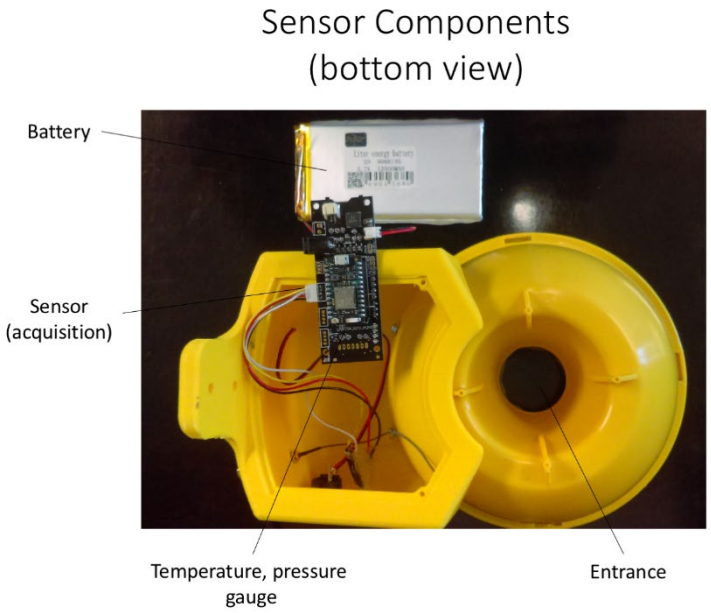

Figure 2: Bottom view of the modified Mcphail trap showing the battery unit, the data acquisition unit, the environmental measurement unit, and the entrance of the trap.

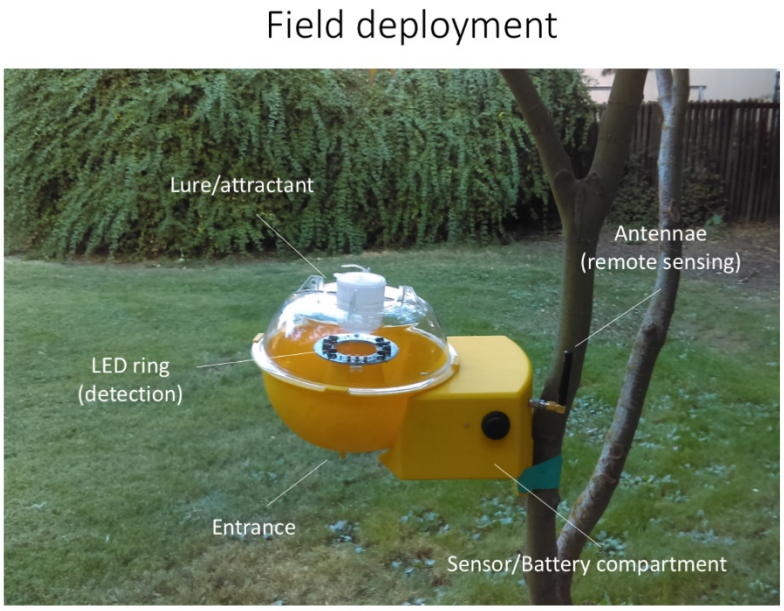

Figure 3: The modified Mcphail trap holding the sensor unit in a field setting. The antennae for transmitting remote sensing data is shown.

Temperature:		20C	25C	30C
D. sim	Male	x	x	x
	Female	x	x	x
D. tris	Male	x	x	x
	Female	x	x	x
D. suz	Male	x	x	x
	Female	x	x	x
D. biar	Male	x	x	x
	Female	x	x	x
D. mel	Male	x	x	x
	Female	x	x	x

Photoperiod		12:12
D. sim	Male	x
	Female	x
D. tris	Male	x
	Female	x
D. suz	Male	x
	Female	x
D. biar	Male	x
	Female	x
D. mel	Male	x
	Female	x

Figure 5: Temperature and photoperiod conditions for wing beat frequency recordings. Conditions marked with pink have been completed. *D. simulans (D. sim); D. tristis (D. tris); D. suzukii (D. suz); D. biarmipes (D. biar); D. melanogaster (D. mel).*

CONTINUING REPORT **YEAR**: 1 of 3
WTFRC Project Number: CH-18-101

Project Title: How do Western X phytoplasma and LChV-2 cause little cherry disease?

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Cooperators: *None*

Total Project Request: $114,872 **Year 1:** $41,058 **Year 2: $38,058** **Year 3:** $35,756

Other funding sources
None.

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

Item	2018	**2019**	2020
Salaries	$12,106	$20,145	$20,951
Benefits	$4,152	$7,313	$7,605
Wages	0	0	0
Benefits	0	0	0
Equipment	0	0	0
Supplies	$24,800	$10,600	$7,200
Travel	0	0	0
Miscellaneous	0	0	0
Plot Fees	0	0	0
Total	$41,058	**$38,058**	$35,756

Footnotes: 1. Salary & Benefits at 0.25 for the first year and 0.4 FTE thereafter for Dr. Wright
2. Supplies include laboratory consumables and reagents, and sequencing/HPLC services.

OBJECTIVES

1. *Perform transcriptomic analysis to compare healthy cherry trees, asymptomatic Western X & LChV-2 infected trees, and symptomatic Western X and LChV-2 infected trees to identify genes that may lead to symptom development.*

Western X infected trees, LChV-2 infected trees, and healthy trees were identified for use in this study. For the first year, we chose to focus only on symptomatic and healthy trees. RNA was extracted from fruit stem and from leaf/midrib tissue for sequencing. RNA isolations were performed at a single time point prior to harvest. Sequence data from these samples is currently being analyzed. Based on the results of sequencing, a schedule for collection and a determination of which tissue to examine will be established for next season.

2. *Address differences in physiology of the trees in objective one by determining rate of photosynthesis and sugar content in phloem exudate in those trees.*

Sugar content of both phloem exudate and cherry pulp was measured, approximately one week before harvest, for each tree used in sequencing. In place of HPLC, enzymatic reactions and spectrophotometry were used to determine the quantity of sucrose, glucose, and fructose in phloem exudate and cherry pulp. Depending on the results of the transcriptomics analysis, HPLC may be used next year to examine not only sugar content but also to detect and quantify other metabolites. Photosynthetic rates were not measured this year. Instead, we propose grafting buds from each tree onto rootstock and raising the trees in one central location to minimize any variables other than infection status that might influence photosynthetic rate.

3. *Determine relevant protein-protein interactions between Western X phytoplasma/LChV-2 protein products and protein products of selected genes from objective one using yeast two hybrid and bimolecular fluorescence complementation technologies.*

This objective is dependent upon the results from the transcriptomic studies in objective one. Based on the findings of the transcriptomic analysis, yeast two hybrid studies will begin in year two.

SIGNIFICANT FINDINGS

- In the cherry pulp, sucrose was barely detected in LChV-2 infected trees, compared to Western X infected trees and healthy trees. Fructose was also reduced in LChV-2 infected trees. There was no significant difference for any of the three sugars when the phloem exudate was examined and for both phloem exudate and cherry pulp, there was no significant difference between Western X infected trees and healthy trees.

METHODS

1. *Perform transcriptomic analysis to compare healthy cherry trees, asymptomatic Western X & LChV-2 infected trees, and symptomatic Western X and LChV-2 infected trees to identify genes that may lead to symptom development.*

To determine what genes may be involved in development of little cherry symptoms, a transcriptomics study will be conducted. Healthy cherry trees and symptomatic trees harboring Western X and LChV-2 were selected for the study. The presence of Western X has been confirmed by PCR and LChV-2 by RT-PCR. In June fruit stem and leaf/midrib tissue was collected from each tree and total RNA has been extracted from the tissue and samples have been submitted for library preparation and sequencing. The resulting data will be analyzed to generate a transcriptome against which individual samples can be compared for differential gene expression analysis. This analysis will be performed to identify transcripts that are upregulated or downregulated between samples. Differentially expressed transcripts will be assigned a function, if possible, based on homology to sequences with known function. These transcripts will be examined to determine which pathways may be altered in cherry when infected with Western X phytoplasma or LChV-2, particularly with reference to fruit development. Also, expression

of pathogen encoded genes will be examined. Transcripts of interest for both cherry and the pathogens will be examined further in objective 3.

2. *Address differences in physiology of the trees in objective one by determining rate of photosynthesis and sugar content in phloem exudate in those trees.*
Production of poor fruit in infected trees may be a result of physiological changes induced by the virus. To investigate this, the rate of photosynthesis and sugar content of phloem exudate and cherry pulp will be determined for the trees described in objective one. For all trees, buds will be grafted onto rootstock and the trees will be grown in the same location under the same conditions. This should remove most of the variables that would have been encountered in the field that would influence the rate of photosynthesis. Measurements will be performed on the scion material and will be performed in triplicate to determine the photosynthetic rate. To determine sugar content, phloem exudate was collected using the centrifugation technique described by Hijaz and Killiny (2014). Cherry pulp was collected by quartering multiple cherries and homogenizing the tissue with a mortar and pestle. Both phloem exudate and cherry pulp were collected one week prior to harvest. In lieu of HPLC, an enzymatic assay was chosen for the initial sugar content assessment because the assay was faster and less expensive than HPLC, allowing for the processing of more samples. Based on the findings of objective one, HPLC may be used next year not only to assess sugar content but also the accumulation of any secondary metabolites of interest.

3. *Determine relevant protein-protein interactions between Western X phytoplasma/LChV-2 protein products and protein products of selected genes from objective one using yeast two hybrid and bimolecular fluorescence complementation technologies.*
Symptom development for little cherry disease may be a result of protein-protein interactions between cherry proteins and pathogen proteins. To investigate this, relevant genes identified in objective one for both cherry and the pathogens will be selected for a yeast two hybrid screen. For Western X phytoplasma, which has a much larger genome than LChV2 (~1200 compared to LChV2's 10 genes) it will be necessary to use the differential gene expression data from objective one, as well as information regarding gene function, to select genes of interest. Yeast two hybrid analysis will be performed using the Clontech Matchmaker® gold yeast two hybrid system and will identify proteins that have the potential to interact. The yeast two hybrid system is a relatively quick means of identifying potential protein-protein interactions, however it occurs in artificial environment. To rule out any false positives, protein-protein interactions identified in the yeast two hybrid assay will be further investigated using bimolecular fluorescence complementation assays. These assays examine protein-protein interactions in plant cells, creating a more realistic environment than the yeast two hybrid assay.

RESULTS & DISCUSSION

1. *Perform transcriptomic analysis to compare healthy cherry trees, asymptomatic Western X & LChV-2 infected trees, and symptomatic Western X and LChV-2 infected trees to identify genes that may lead to symptom development.*
Symptomatic and healthy trees were selected for the study this year. Fruit stem and leaf/midrib tissues were harvested from these trees for the transcriptomic study. Fruit stem was chosen because for LChV-2, this tissue appears to have the greatest and most consistent accumulation of virus during fruit development. Western X has also been detected in fruit stem tissue. Leaf and midrib tissue was chosen because LChV-2 is also detected in the midrib and leaf tissue serves as a source of photosynthate for fruit development. These tissues were collected in June. RNA was isolated and sent for library preparation and sequencing. Analysis of the sequence data will determine if these tissues are best tissues to examine and at what time points RNA should be collected from these tissues.

2. *Address differences in physiology of the trees in objective one by determining rate of photosynthesis and sugar content in phloem exudate in those trees.*

Photosynthesis was not assessed this year as it was felt that the trees were in sufficiently different environments that environmental variables would overshadow any effects LChV-2 or Western X presence might have on the photosynthetic rate. To remove these variables, material from each tree will be grafted onto rootstocks and leaves from that scion material will be tested next year for photosynthetic rate.

Sugar content of both phloem exudate and cherry pulp was assessed for infected and healthy trees. Phloem exudate and cherry pulp was collected one week prior to harvest. In the phloem exudate, there was no significant difference between Western X infected trees, LChV-2 infected trees, and healthy trees (Figure 1A, C, and E). Sucrose did appear to be slightly higher in the phloem exudate of Western X infected trees. There were differences in sugar content of the cherry pulp (Figure 1B, D, and F). Sucrose was barely detectable in the cherry pulp of LChV-2 infected trees. Fructose was also significantly lower in the cherry pulp for LChV-2 infected trees. These differences in sugar content may account in part for the bitter taste of cherries of LChV-2 infected trees. However, there is no difference in sugar content for cherry pulp between Western X infected and healthy trees, suggesting that other mechanisms may be responsible for the poor taste. Repeating the sugar content assays next year will confirm if these differences in sugar content are consistent for LChV-2 infected trees. Also, the results of the transcriptomics study may provide additional targets for HPLC analysis.

3. *Determine relevant protein-protein interactions between Western X phytoplasma/LChV-2 protein products and protein products of selected genes from objective one using yeast two hybrid and bimolecular fluorescence complementation technologies.*

Candidate proteins for yeast two hybrid screening will be identified from the transcriptomics analysis. Work on this objective will begin during year two.

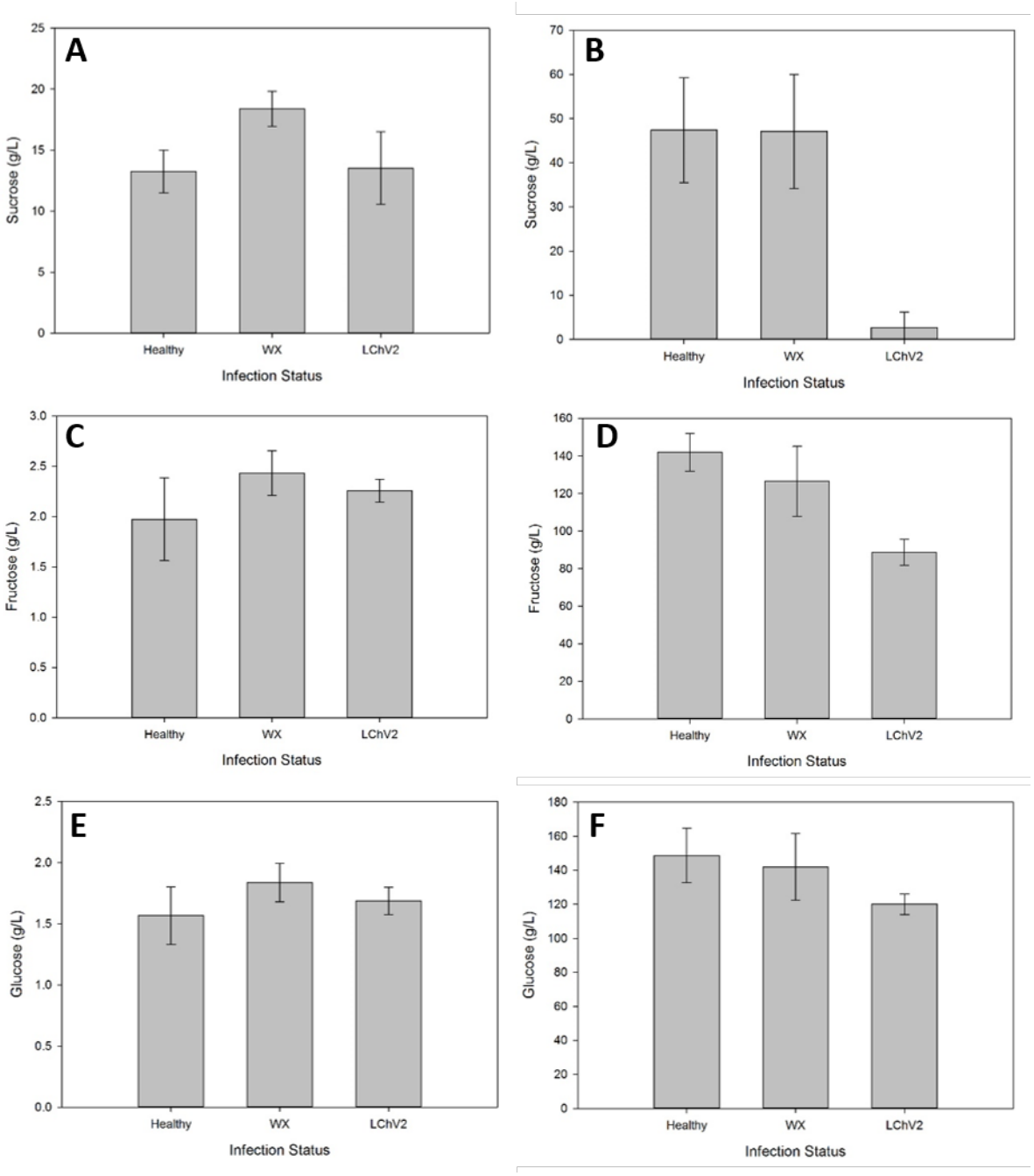

Figure 1. Sucrose, fructose, and glucose content of A), C), and E) phloem exudate and B), D), and E) cherry pulp. No significant differences in sugar content were observed for the phloem exudate, however sucrose and fructose content was significantly lower in cherry pulp of LChV-2 infected trees.

CONTINUING REPORT **YEAR**: 1 of 2
WTFRC Project Number: CH-18-102

Project Title: Native hosts of the Western X phytoplasma

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Cooperators: Washington cherry growers

Total Project Request: $76,840 **Year 1:** $38,107 **Year 2: $38,733**

Other funding sources: *None*

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

Item	2018	**2019**	
Salaries[1]	$12,106	$20,145	
Benefits	$4,152	$7,307	
Wages[2]	$9,840	$0	
Benefits[3]	$932	0	
Equipment	0	0	
Supplies[4]	$9,577	$10,281	
Travel[5]	$1,500	$1,000	
Miscellaneous	0	0	
Plot Fees	0	0	
Total	$38,107	**$38,733**	

Footnotes:
1. 0.25 FTE Salary for Dr. Wright the first year, and 0.4 FTE for the second.
2. Wages for a field worker for sample collection.
3. Benefits calculated at standard WSU rates.
4. Supplies include: Field sampling supplies and laboratory consumables and reagents
5. Travel covers mileage to reach field sampling sites.

OBJECTIVES

1. Survey plants both within and in the vicinity of cherry orchards for Western X phytoplasma.

Plants were collected around central and eastern Washington, both within cherry orchards afflicted with Western X phytoplasma and from locations distant from orchards. Plants were photographed for later identification and tissue was harvested for DNA extractions. Plants were screened for the presence of phytoplasma using a generic phytoplasma qPCR assay. Positive plants were further screened for the presence of Western X using a qPCR assay. A total of 923 plants, including trees, shrubs, broadleaf weeds, and grasses, were tested for the presence of Western X. Only10 tested positive for phytoplasmas and of those 4 were positive for Western X.

2. *Identify potential reservoirs and sources of spread for Western X phytoplasma in Washington.*

This objective was initially proposed to examine leafhopper spread of Western X from herbaceous weeds. However, the low incidence of Western X found in weeds during the survey indicates that weeds alone cannot explain the incidence and severity of Western X in cherry orchards, and studying leafhopper spread from weeds would not be informative. Therefore, this objective is being refocused on three alternative reservoirs: 1) Cherry planting stock, for symptoms are only visible on fruit and nursery stock is rarely, if ever, tested for the presence of this phytoplasma. 2) Other *Prunus* species, for we have recently detected Western X in peach and nectarine orchards in Washington, and 3) Pome fruit orchards in the vicinity of cherry orchards, for Western X has been reported to infect apples in other states.

SIGNIFICANT FINDINGS

Less than two percent of plants tested for the presence of phytoplasma were positive and only a subset of those contained Western X phytoplasma, suggesting that non-crop plants in and around orchards may not be the source of Western X phytoplasma.

METHODS

1. *Survey plants both within and in the vicinity of cherry orchards for Western X phytoplasma.*

In the first year, plant specimens were collected from within and near cherry orchards and identified. DNA was extracted from these plants and the presence or absence of phytoplasma determined by qPCR. Phytoplasma positive plants were further screened for the presence of Western X. As only four plants, belonging to three species, tested positive for Western X, in the following year we will limit our investigations to those weed species to confirm their role as potential reservoirs.

2. *Identify potential reservoirs and sources of spread for Western X phytoplasma in Washington*.

Due to the low number of Western X phytoplasma positive plants in the environment, we are proposing refocusing this objective to examine other sources of Western X, specifically cherry planting stock, other *Prunus* species, and pome fruits. In examining cherries, we will screen nursery tissue for the presence of Western X phytoplasma using qPCR. Participating volunteers will remain anonymous, with the location and identifier information removed from reports. Plants from all three sources will be screened for phytoplasma and Western X presence as described above.

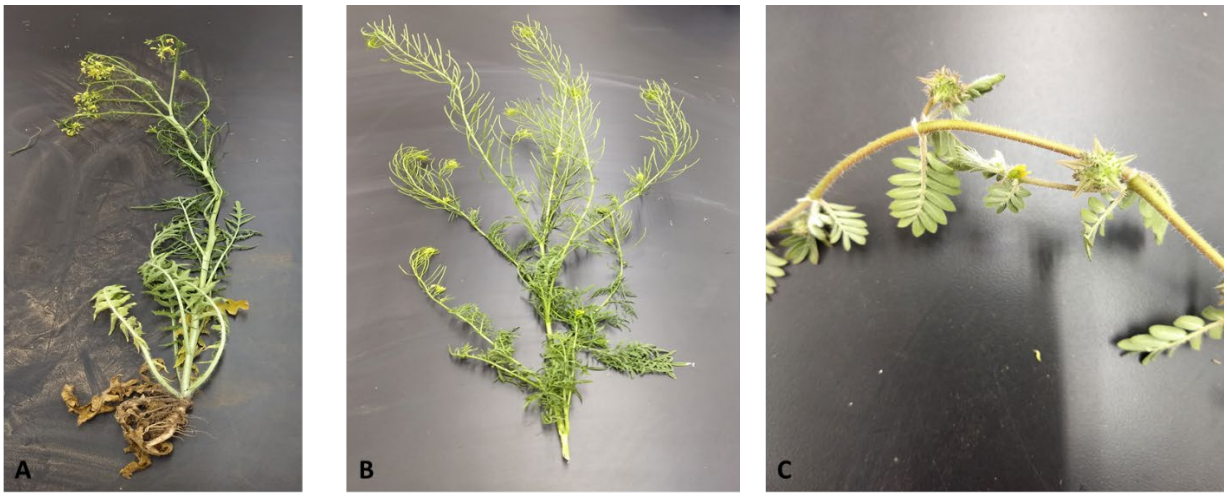
RESULTS AND DISCUSSION

1. Survey plants both within and in the vicinity of cherry orchards for Western X phytoplasma.

In the first year, tissue was collected from 923 plants for DNA extraction and phytoplasma testing. These plants were collected from across central and eastern Washington, both within afflicted orchards and farther afield. The plants examined included trees, shrubs, broadleaf weeds, and grasses. Of the 923 plants, ten came back positive for phytoplasma -1.08% of all plants screened. The plants that were positive included common mullein (2), common purselane (1), flixweed (2), puncturevine (1), Russian thistle (1), shepherd's purse (1), and tumble mustard (2). Of these only four tested positive for Western X: both tumble mustards, one flixweed, and the puncturevine. A total of 45 tumble mustards, 12 flixweeds, and 22 puncturevines were tested indicating that within each species the incidence of Western X phytoplasma is low. Representatives of these plants are pictured in Figure 1. With only four plants testing positive, the percentage of screened plants positive for Western X phytoplasma is 0.43%. This is a very low number, suggesting that Western X phytoplasma may not be prevalent among weeds in and around cherry orchards. This further suggests that we may be searching in the wrong place for the source of Western X phytoplasma spread. Efforts in the second year will focus on the above identified native hosts in the Columbia valley area, a hotbed of Western X, whereas further north in Chelan and Okanogan counties, Little cherry virus-2 predominates.

2. *Identify potential reservoirs and sources of spread for Western X phytoplasma in Washington*.

Because we doubt that we have located the source of Western X phytoplasma in the environment, examining leafhopper spread from non-crop hosts would be uninformative. Instead we propose to refocus on alternative reservoirs and sources of spread of Western X phytoplasma: cherry planting stock, other *Prunus* species, and pome fruits. A preliminary examination this year found Western X phytoplasma in 15 samples from two peach orchards, one of which is located in the vicinity of heavily infected cherry orchards. Determining if one of more of these potential sources is a reservoir for the spread of Western X phytoplasma may aid in restricting spread of the disease.



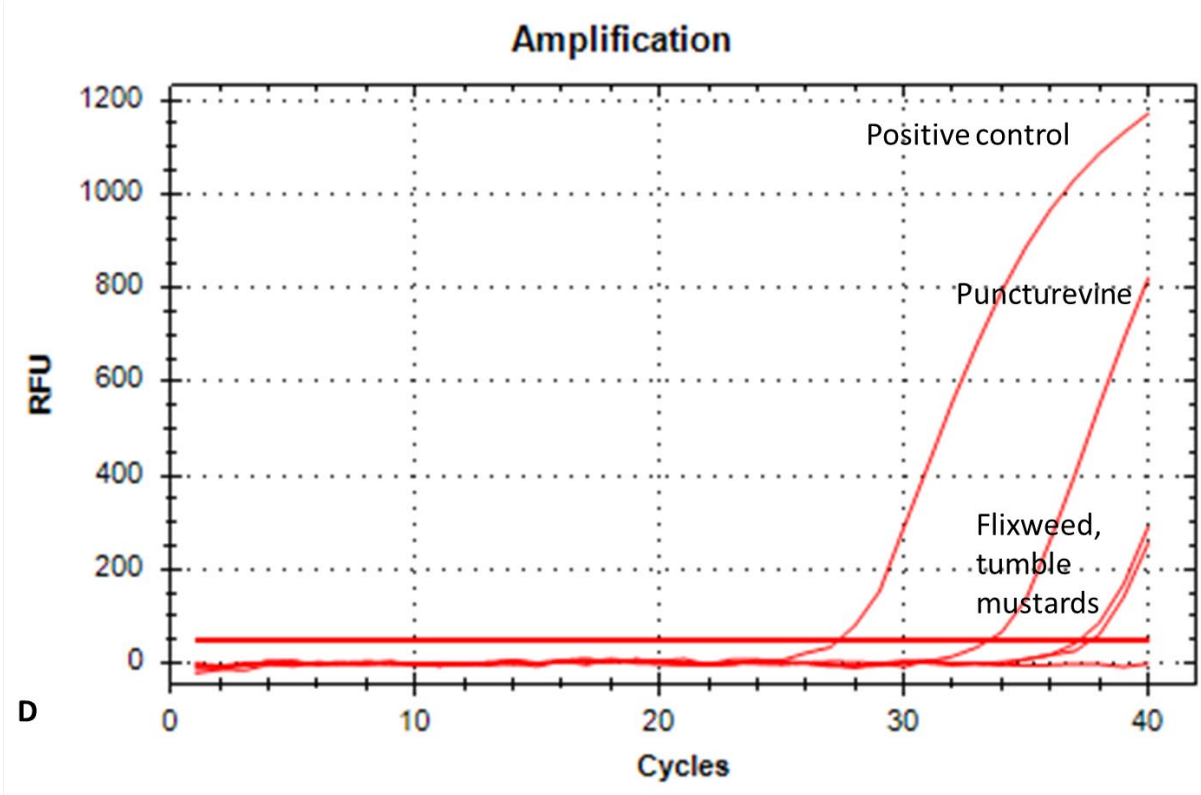


Figure 1. Representatives of plant species that tested positive for Western X phytoplasma: A) tumble mustard, B) flixweed, and C) puncturevine. D) Quantitative PCR results for the Western X phytoplasma assay. The red lines show amplification for, from left to right, the positive control, puncturevine, and the flixweed and tumble mustards.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: CH-17-103

Project Title: Orchard management practices for *little cherry virus 2*

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Cooperators: None

Total Project Request: $170,770 Year 1: $57, 512 Year 2: $55,716 Year 3: **$57,542**

Other funding sources
None.

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

Item	2017	2018	**2019**
Salaries[1]	$7,361	$7,655	$24,213
Benefits[2]	$2,157	$2,243	$8,329
Wages	$3,120	$3,245	0
Benefits	$324	$337	0
Equipment	0	0	0
Supplies	$44,550	$42,236	$25,000
Travel	0	0	$0
Plot Fees	0	0	$0
Miscellaneous	0	0	$0
Total	$57,512	$55,716	**$57,542**

Footnotes:
1. 0.15 FTE for year 1 and 2, and 0.5 FTE for year 3.
2. Benefits calculated at standard Washington State rates.

OBJECTIVES

1) *Examine the effect of rootstocks on the concentration of LChV-2*.

Mazzard, Krymsk 5, Krymsk 6, Gisela 6, and Gisela 12 rootstocks, inoculated with LChV-2 isolate LC-5, were screened for virus presence in April 2018. Canindex, Bing, Chelan, and Sweetheart scions were grafted onto the plants in September. Monitoring of virus concentration will follow, as described in the methods. Due to the delays in obtaining the various rootstock/scion combinations infected with LChV2 we propose to discontinue the mealybug transmission portion of the objective. Given the long acquisition and transmission periods of semi-persistent transmission by mealybugs, studying the transmission efficacy of the virus from the various rootstock/scion combinations would not provide significant results.

2) Quantify the accumulation of LChV-2 in different host tissues throughout the growing season.

In 2019, monitoring of virus distribution and titer will continue throughout the year as described in the methods section. Sampling in 2018 provided information on how the virus concentration and distribution changes in the host. In addition, for the August time point, two infected Bings were included in sampling to test for variation between cultivars. As this objective has provided useful information on what tissues should be tested for LChV-2 throughout the year, we are proposing an addition to this objective. Since Western X phytoplasma is prevalent in eastern Washington, we propose to examine titer and distribution for this pathogen within cherry trees. Recent data has indicated that distribution among tissues is not uniform, suggesting that if the right tissue at the right time is not selected for testing, false negatives may be generated. As with LChV-2, knowing which tissues to select for Western X phytoplasma testing will allow for improved detection of the pathogen.

3) *Determine the population structure of LChV-2 within Washington cherry production regions*.

The population structure of LChV-2 is being examined using samples from the 2017 and 2018 growing seasons. Data collection began in November-December 2017 and has continued throughout the year. Single-stranded conformation polymorphism (SSCP) has been used successfully to identify isolates that differ in genomic sequence. PCR products from representatives of these isolates have been Sanger sequenced, confirming that the isolates are different. Selected samples, particularly those representing unique isolates, will be sent out for sequencing.

SIGNIFICANT FINDINGS

- Titer of LChV2 varies throughout the year. Titer was highest, but most variable, in wood scrapings in the winter and spring. Titer was lowest in the buds and roots. In the spring, during fruit development, the titer was highest in the fruit stem. In the fall, titer was highest in the midrib.
- In examining the population structure of LChV-2 in Washington, variation was observed, both in SSCP and Sanger Sequencing. Six unique isolates were found from Washington samples that do not match known isolates.

METHODS

1. *Examine the effect of rootstocks on the concentration of LChV-2.*

In this study, the rootstocks Mazzard, Krymsk 5, Krymsk 6, Gisela 6, and Gisela 12 were bark patch inoculated with LChV-2 isolate LC5. The indicator cultivar ‘Canindex1’ and three commercial cultivars ‘Bing’, ‘Sweetheart’ and ‘Chelan’ have been grafted onto the rootstocks. Following establishment of the scion material, 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.

2. *Quantify the accumulation of LChV-2 and Western X phytoplasma in different host tissues throughout the growing season.*

For this part of the study, a Lambert tree with a known and established LChV-2 infection that is maintained in CPCNW field block has been used. Appropriate tissues have been collected throughout the growing season, for two years and the concentration of LChV-2 was 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). These time points and tissues have been sampled from August 2017 through August 2018 and will continue throughout the next year of the study.

The titer and distribution of Western X phytoplasma will be assessed as described above for LChV-2. To do so, a qPCR assay will need to be developed for Western X phytoplasma. Only part of one isolate of Western X phytoplasma has been sequenced. To develop a robust assay, multiple isolates of Western X phytoplasma from eastern Washington will be sequenced, including trees that exhibit symptoms but do not show up as positive with existing assays. Following sequencing and assay development, infected trees will be sampled throughout the year to determine how Western X phytoplasma titer and distribution varies among tissues and seasons.

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 are being screened by single stranded conformation polymorphism (SSCP) analysis of two 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 of LChV-2.*

Rootstocks for this objective have been established in the greenhouse (field plants were discontinued due to poor health and poor acquisition of the virus through grafting). In September 2017 the rootstocks were grafted with stem tissue containing the LC5 isolate of LChV-2. Plants have been screened for the presence of LChV-2. Scion material was grafted in September of 2018. In 2019 titer and distribution of LChV-2 throughout the canopy will be determined.

2. *Quantify the accumulation of LChV-2 in different host tissues throughout the growing season.* In 2017, a qPCR assay was developed for LChV-2 and two reference genes. From August 2017 through August 2018, sampling occurred as described in the materials and methods. This has provided a full year of data and a repeat of the August time point (Figures 1 and 2). Although at some time points the LChV-2 titer in the wood scraping was highest, it was also the most variable. In May, virus titer appeared to be highest in the fruit stem. In August, excepting the wood scraping values, concentration was highest in the midrib.

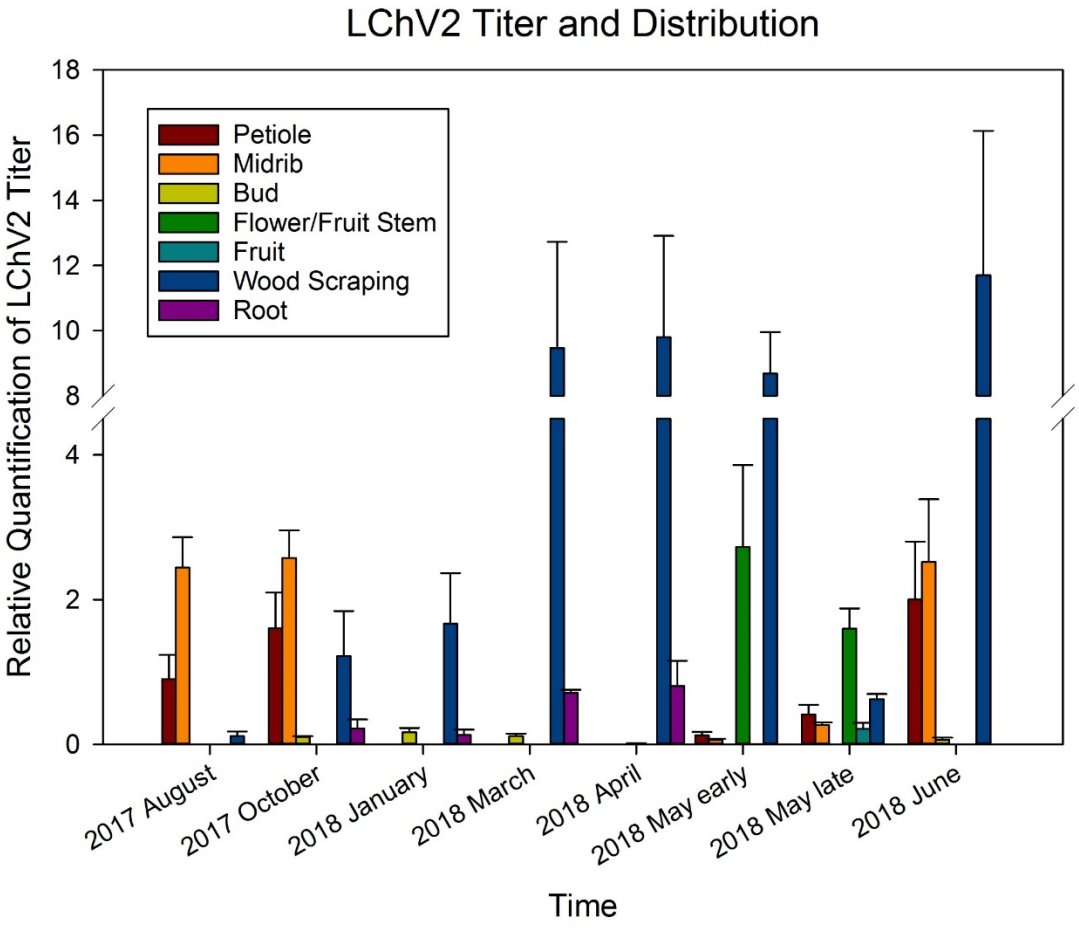

Figure 1. Quantification of LChV-2 concentration in midrib, petiole, wood scraping, bud fruit stem, fruit and root tissues throughout the year.

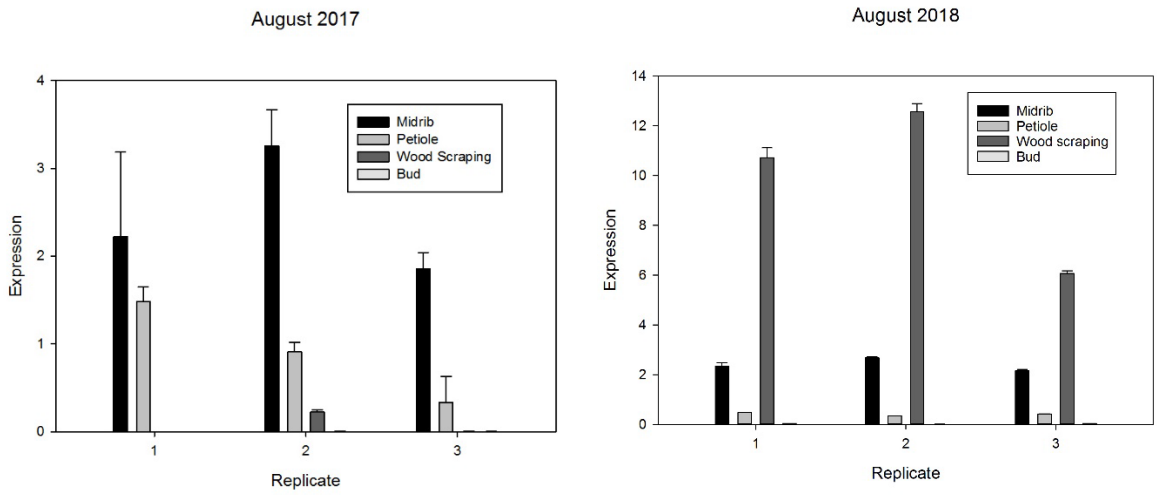

Figure 2. Comparison of 2017 and 2018 August time points for titer and distribution of LChV2.

Preliminary data indicate that Western X phytoplasma is not evenly distributed among tissues in infected trees (Figure 3). In testing infected trees, it was found that in some trees only fruit stem was positive for Western X phytoplasma while leaf and midrib tissue of the same branch on the same tree was negative. If only leaf and midrib tissue had been tested for these trees, they would have been considered negative, allowing infected trees to persist in the field and contribute to the spread of the disease. Developing a robust Western X phytoplasma assay and assessing titer and distribution throughout the year will identify which tissues growers should select for testing, thereby reducing the incidence of false negatives.

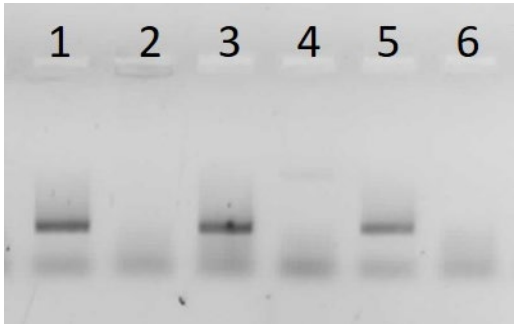

Figure 3. Detection of Western X phytoplasma in infected trees. Samples 1 and 2, 3 and 4, and 5 and 6 each represent a tree. Samples 1, 3, and 5 are fruit stem tissue and samples 2, 4, and 6 are leaf and midrib tissue. The upper band indicates that Western X phytoplasma is present.

3. *Determine the population structure of LChV-2 within Washington cherry production regions.*
SSCP is being used to assess population structure for LChV-2. This technique uses gel electrophoresis to detect the presence of sequence differences between samples (Figure 4). Two sets of primers have been selected for SSCP. These primer sets target the helicase and the RdRP. Unique isolates have been identified by this technique. The primer pair targeting the helicase identified five different isolates, three of which did not match existing sequences for LChV-2. For the primers targeting RdRP, seven different isolates were identified, four of which did not match existing LChV-2 sequences. Additional SSCP screening will take place this year and isolates will be selected for next generation sequencing to sequence the genomes of these unique isolates.

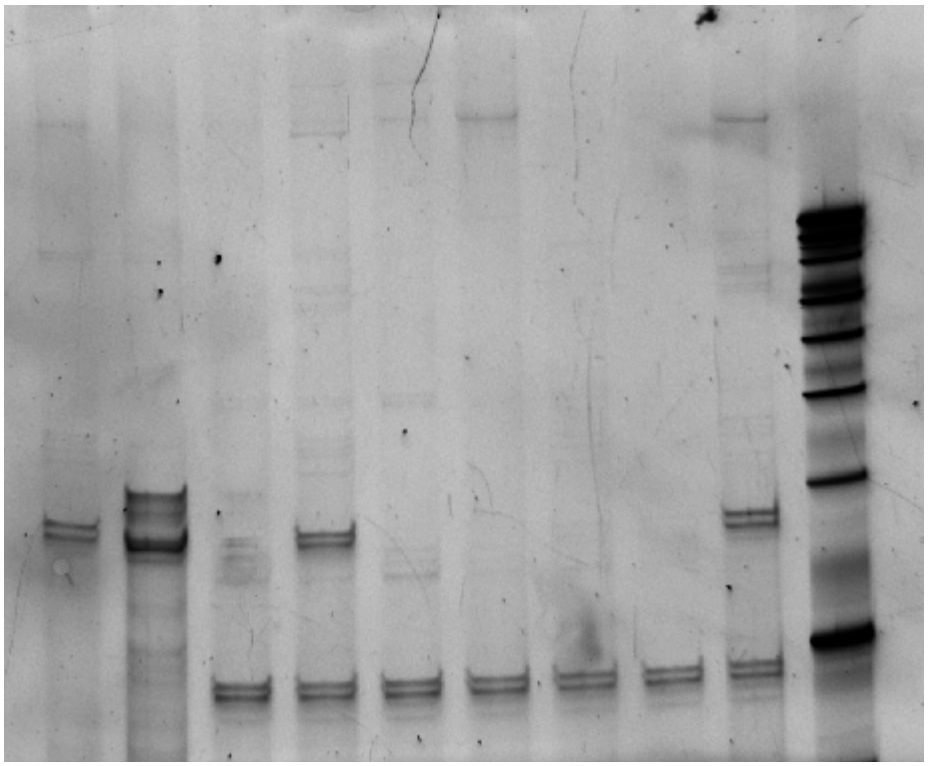

Figure 4. Example of SSCP used to identify different isolates of LChV-2. Different patterns of bands indicate unique isolates. The lane on the far right is the molecular marker.

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

Project Title: Non-toxic RNAi-based biopesticide to control spotted wing drosophila

PI: Man-Yeon Choi
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Cooperators: 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 $102,500 (2015-2018)

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) – *completed*
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) - *completed*

3-2. Feed dsRNA to larvae and adults, and evaluate RNAi impacts (Yr. 3)

SIGNIFICANT FINDINGS (Year 2)

- Constructed and biosynthesized double-stranded RNAs (dsRNAs) of 32 SWD RNAi targets.
- Screened 3 potential RNAi targets from 13 potential RNAi targets from SWD.
- Three housekeeping RNAi targets have been knock downed by dsRNA injection into SWD.
- Selected and found a significant target from three additional receptors genes in 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 140 ºF 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 – Year 2: For optimal impact of dsRNA delivered to target cells through feeding, RNAi target genes should focus on 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.

Objective 1. Cloning and identify potential RNAi target genes from SWD (Yr. 1): We selected and identified 32 SWD genes including 17 housekeeping, 10 neurohormone receptors, 3 neurohormone, and 2 chemosensory genes, for potential RNAi targets (Table 1).

Table 1. SWD RNAi candidate genes, 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. 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.

Objective 2. Construct, design and biosynthesis dsRNAs for target genes (Yr. 2)
The dsRNAs for each SWD genes were designed and synthesized approximately 250 nucleotides from parent genes (Fig. 1).

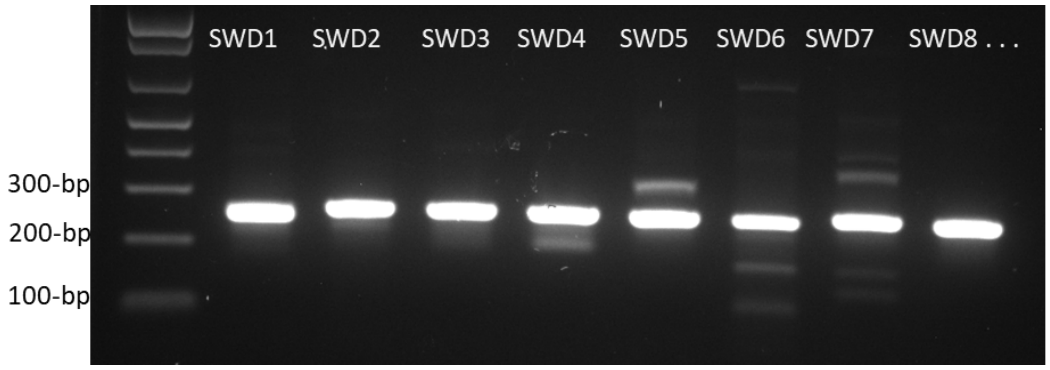

Figure 8. Examples of dsRNA synthesized for SWD RNAi test on injection and feeding.

Objective 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: During two years the 1st screening with more than 13 RNAi candidates was completed with over 3,000 nano-injections to flies. We found effective phenotypic impacts, mainly mortality, from some of the RNAi injection into SWD flies (Fig. 2).

Figure 9. Photos of dead SWD flies after RNAi injected into adults within 48h.

We found the maximum mortality up to about 60% on SWD flies injected with specific dsRNA within 48h (Fig. 3).

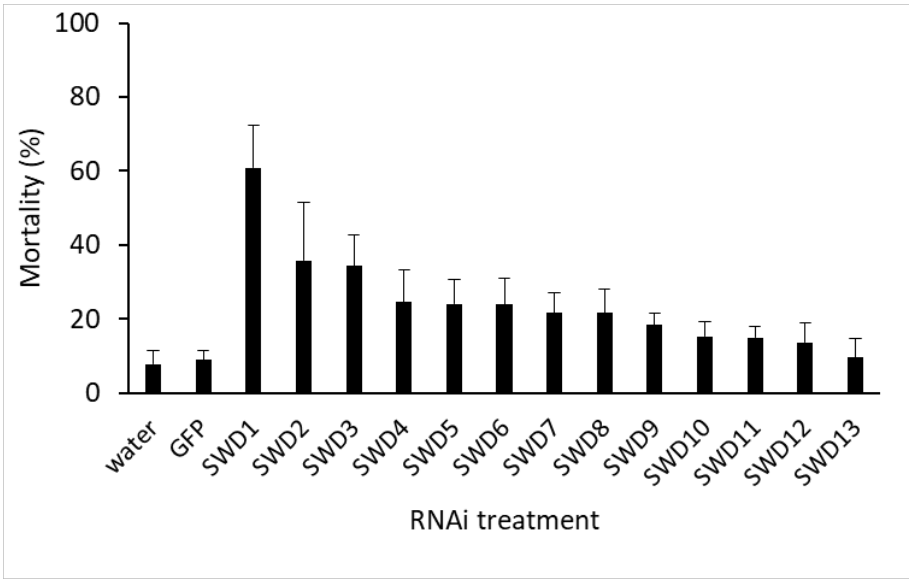

Figure 3. The mortality of SWD flies injected with different RNAi treatments.

Genotypic impact of the housekeeping genes for RNAi targets

We investigated the gene expression levels to find whether those genes are being suppressed or not after target RNAi (dsRNA) injected into SWD. Using the quantitative gene analysis we found all three RNAi target genes have been knock downed by dsRNA introduction to SWD (Fig. 4).

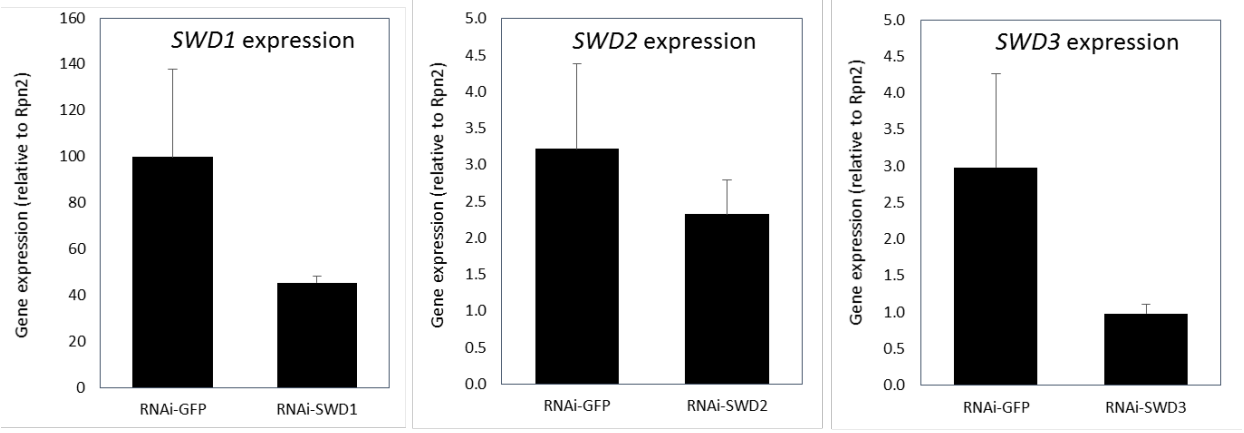

Figure 4. Knock-down of housekeeping genes expression by RNAi. The mRNA expression levels of *SWD1*, *SWD2*, and *SWD3* were compared between RNAi-GFP and RNAi-target in SWD 12h after dsRNA injection of *SWD1*, *SWD2*, *SWD3*, and *GFP*. Gene expression estimates are given per a copy of mRNA for the reference gene *Rpn2*.

RNAi impact of a neurohormone receptor on SWD adult

We examined the gene expression levels of neurohormone receptors after target RNAi (dsRNA) applied to SWD. Using the quantitative PCR gene analysis (qPCR) we found the receptor was significantly suppressed by dsRNA introduction in SWD adults (Fig. 5).

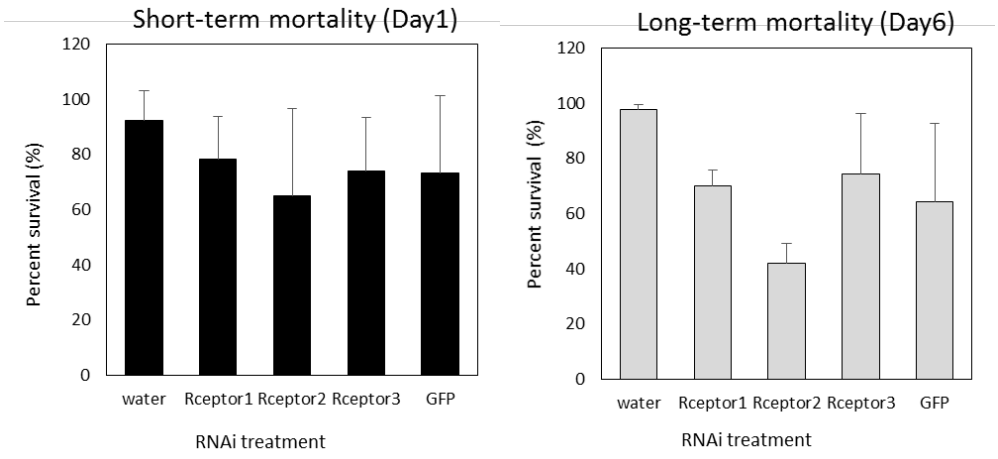

Figure 5. Knock-down of receptor genes expression by RNAi. The mRNA expression levels of Receptor 1, 2 &3 were compared between RNAi-GFP and RNAi-target for SWD 24h and 6 days. Gene expression estimates are given per a copy of mRNA for the reference gene Rpn2.

Continue and ongoing study

For next year we will continue the evaluation of Objective 3-1: Inject RNAi into adult flies and monitor RNAi impacts (*i.e*. fecundity or mortality) on SWD, and to move for Objective 3-2: Feed RNAi selected into larvae and/or adults, and monitor RNAi impacts on SWD.

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

Project Title: Mid-Columbia survey for sweet cherry viruses and vectors

PI: Jay W. Pscheidt, Extension Plant Pathology Specialist
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City/State/Zip: Corvallis/Oregon/97331-2903

Cooperators: Lauri Lutes (OSU), Steve Castagnoli (OSU), Ashley Thompson (OSU), Drew Hubbard (GS Long), Jeff Heater (The Dalles Fruit Co.), Inga Zasada (USDA-ARS)

Total Project Request: Year 1: $49,106 **Year 2**: **$52,200**

Other funding sources

Agency Name: OSU Extension Service
Amt. requested: $4,000
Notes: Annual discretionary statewide travel funds can be used to get to sampling sites.

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

WTFRC Budget: None

Budget 1
Organization Name: Agricultural Research Foundation (Oregon State University)
Contract Administrator: Russ Karow
Telephone: (541) 737-4066 **Email address:** Russell.Karow@oregonstate.edu
(Submit Proposals To: arf@oregonstate.edu)

Item	(2017-2018)	(2018-2019)*
Salaries (GRA Stipend**)**	23,776	24,242
Benefits (Health Insurance**)**	6,720	6,855
Wages		
Benefits (OPE**)**	100	103
Equipment		
Supplies		
Travel		
Miscellaneous (OSU fees**)**	18,520	21,000
Plot Fees		
Total**	$49,106	**$52,200**

Footnotes:
*Anticipating 2% increase for 2018-2019 school year.
**Anticipating tuition remission for both 2017-2018 and 2018-2019 school terms and thus reducing total to $32,654 and $35,419, respectively.

OBJECTIVES

Objective 1: Determine areas with sweet cherry trees expressing symptoms associated with Little Cherry Disease (caused by the pathogens *Little cherry viruses 1 & 2* and/or X-Disease phytoplasma) in Oregon's Mid-Columbia region.

Objective 2: Conduct regional Mid-Columbia survey for *Cherry leaf roll virus* and the *Cherry leaf roll virus* complex with *Prune dwarf virus* and *Prunus necrotic ringspot virus* on sweet cherry.

Objective 3: Survey Mid-Columbia sweet cherry orchards for nematode-transmitted viruses (such as *Cherry rasp leaf virus, Tobacco ringspot virus,* and *Tomato ringspot virus*) and their vectors.

Objective 4: Investigate spread and diversity of *Tomato ringspot virus* isolates from cherry in known-infected areas.

SIGNIFICANT FINDINGS

- **X-Disease found associated with little cherry symptoms in The Dalles, Dallesport, and Mosier.**
- **Several known *Cherry leaf roll virus*-infected trees removed in Oregon.**
- **Nematode virus vector, *Xiphinema americanum*, found in commercial orchard in association with *Tomato ringspot virus.***

METHODS

Objective 1: Determine areas with sweet cherry trees expressing symptoms associated with Little Cherry Disease (caused by the pathogens *Little cherry viruses 1 & 2* and/or X-Disease phytoplasma) in Oregon's Mid-Columbia region.

Local cooperators were informed about this survey at the Mid-Columbia Cherry Day on February 2, 2018, and again at the Pre-harvest Tour on June 5, 2018, when a presentation was made and a "wanted" poster was distributed to aid in the identification of symptoms associated with Little Cherry and X-Disease (little, immature fruit lacking flavor). Growers and field representatives were provided with contact information to report symptomatic trees for sampling and diagnostics testing. Growers/field representatives would use flagging tape, provide GPS coordinates, or identify the symptomatic trees in-person. Samples were collected from Hood River, Mosier, and The Dalles, OR, as well as Dallesport, WA.

Ten leaves and ten fruit stems were collected per tree for use in diagnostic testing. Nucleic acid kits (modified Qiagen RNeasy Plant Mini Kit) were used to extract total nucleic acid from the leaf and stem tissues. No RNase treatment was used, so DNA would be present for phytoplasma testing, as well as RNA for *Little cherry virus* testing. A Nanodrop spectrophotometer was used to confirm the presence and quality of nucleic acid in all extracted samples. Diagnostic testing was conducted using a general phytoplasma real-time PCR assay for the detection of the X-Disease phytoplasma, *Candidatus* phytoplasma pruni (Ito and Suzaki 2017) and a specific one-step real-time RT-PCR assay for *Little cherry virus 2* (Harper, unpublished).

Objective 2: Conduct regional Mid-Columbia survey for *Cherry leaf roll virus* and the *Cherry leaf roll virus* complex with *Prune dwarf virus* and *Prunus necrotic ringspot virus* on sweet cherry.

Requests were made to identify trees expressing symptoms associated with *Cherry leaf roll virus* (dieback, decline, rosetting of leaves due to shortened internodes, and/or enations). If suspect trees were found, leaf samples (ten leaves per tree) were to be collected in pairs from symptomatic and asymptomatic trees and screened using a double-antibody sandwich ELISA with CLRV-specific antisera (DSMZ, Braunschweig, Germany).

If a sample tests positive for CLRV, the sample were to be tested for *Prune dwarf virus* (PDV) and *Prunus necrotic ringspot virus* (PNRSV) to identify if the virus complex that induces rapid decline in sweet cherry is present.

Objective 3: Survey Mid-Columbia sweet cherry orchards for nematode-transmitted viruses (such as *Cherry rasp leaf virus, Tobacco ringspot virus,* and *Tomato ringspot virus*) and their vectors.

Local cooperators were instructed to identify orchards in the Mid-Columbia region with foliar enations (a gall-like formation on the underside of the leaf), rosetting (bunching of leaves due to shortened internodes), dieback and rapid decline symptoms associated with nematode-transmitted viruses. Ten leaves per tree are to be collected from a symptomatic and asymptomatic tree pair and screened by virus-specific double-antibody sandwich ELISAs (Agdia, Inc., Elkhart, IN) for *Tobacco ringspot virus* (TRSV) and *Tomato ringspot virus* (ToRSV), as well as an RT-PCR assay for *Cherry rasp leaf virus* (James et al. 2001).

For samples testing positive for one of these viruses, nematode extraction and identification will be performed on each composited soil core sample to identify the presence of dagger nematodes (*Xiphinema* spp.), the known vector of the nematode-transmitted viruses of interest. If found, dagger nematodes will be archived for future studies relating to virus detection and nematode characterization.

Additionally, for samples testing positive, nucleic acid will be extracted for downstream reverse transcription polymerase chain reaction (RT-PCR) and sequencing to identify which isolate or virus strains are present.

Objective 4: Investigate spread and diversity of *Tomato ringspot virus* isolates from cherry in known-infected areas.

A commercial orchard in The Dalles known to have *Tomato ringspot virus* was scouted for foliar enations (a gall-like formation on the underside of the leaf), rosetting (bunching of leaves due to shortened internodes), dieback and rapid decline symptoms associated with nematode-transmitted viruses. Visual assessments of ~1500 trees were made based on these symptoms, which were ranked in severity on a scale of 0-4 (0 = no symptoms, 4 = most severe). A map was generated using this information to identify a subset region, which included a range of disease severity in a “hot spot” area. Ten leaves per tree were collected from a block of 400 trees in this region for diagnostic testing using a *Tomato ringspot virus*-specific double-antibody sandwich ELISA (Agdia, Inc., Elkhart, IN).

Within the block of 400 trees, a smaller region of 50 trees was selected for nematode sampling based on the representation of varying degrees of disease severity. Soil samples were collected June 2018 using a 1-inch soil probe to remove 4 cores from around the base of each tree. Nematode extraction by sugar flotation and microscopic identification were performed on each composited soil core sample to identify the presence of dagger nematodes (*Xiphinema* spp.), the known ToRSV vector. Samples were collected and processed again using the same methods in October when it was expected that more adults than juveniles would be present, which allows for more specific characterization.

A second known ToRSV-infected commercial orchard will be assessed in 2019 to similarly assess the correlation of visual symptoms with diagnosis and nematode vector presence.

In the coming year, nucleic acid will be extracted from tree and weed samples for downstream polymerase chain reaction (PCR) and DNA sequencing for strain determination.

At the Mid-Columbia Agricultural Research and Extension Center, areas have been identified that have ToRSV-infected trees and the nematode vector, *X. americanum*. To test the natural transmission of the ToRSV strain on sweet cherry to other tree and small fruit hosts, a variety of hosts (apple, peach, cherry, blueberry, raspberry, and grape) were interplanted in 8 regions of the orchard block that were known to have ToRSV and where the vector was present. Samples were collected before planting to ensure the hosts were free of ToRSV upon planting by diagnostic testing using the ToRSV-specific double-antibody sandwich ELISA (Agdia, Inc., Elkhart, IN).

RESULTS & DISCUSSION

Objective 1: Determine areas with sweet cherry trees expressing symptoms associated with Little Cherry Disease (caused by the pathogens *Little cherry viruses 1 & 2* and/or X-Disease phytoplasma) in Oregon's Mid-Columbia region.

With the help of local cooperators, 27 symptomatic and 6 asymptomatic samples were collected from the Mid-Columbia region for diagnostic testing associated with Little Cherry Disease and X-Disease. All 27 of the symptomatic samples expressing little, unripened fruit tested positive for the X-Disease phytoplasma, *Candidatus* phytoplasma pruni, using a general phytoplasma real-time PCR assay (Table 1). Six asymptomatic samples tested negative for the X-Disease phytoplasma. All samples tested negative for *Little cherry virus 2.*

Table 1: 2018 Little Cherry/X-Disease Survey Results

			Number of positive samples	
Region	Year	# symptomatic /total	LChV2 qRT-PCR	Phytoplasma qPCR
Hood River, OR	2018	0/1	0	0
Mosier, OR	2018	5/5	0	5
The Dalles, OR	2018	20/24	0	20
Mill Creek		9/12	0	9
3 Mile		11/11	0	11
15 Mile		0/1	0	0
Dufur		0/1	0	0
Dallesport, WA	2018	2/2	0	2
TOTAL		**27/33**		

These results indicate that the X-Disease phytoplasma is present throughout cherry production region in The Dalles and Mosier, OR, as well as across the river in Dallesport, WA. Awareness of this disease and removal of infected trees should help control spread throughout the region.

A subsequent survey in 2019 will allow for more samples to be collected in regions that were not represented in the 2018 sampling (Hood River, Skyline and Dry Hollow region of The Dalles, and White Salmon, WA) to determine if these diseases are present.

Objective 2: Conduct regional Mid-Columbia survey for *Cherry leaf roll virus* and the *Cherry leaf roll virus* complex with *Prune dwarf virus* and *Prunus necrotic ringspot virus* on sweet cherry.

Past surveys revealed the presence of *Cherry leaf roll virus* (CLRV) in The Dalles. One of the CLRV-infected orchards was removed and replanted in 2017. Another CLRV-infected orchard was removed in 2018. No new CLRV symptoms were reported in 2018. Growers with the last few remaining trees known to have CLRV will be encouraged to remove them. Symptomatic trees that come to our attention in 2019 will be tested.

Objective 3: Survey Mid-Columbia sweet cherry orchards for nematode-transmitted viruses (such as *Cherry rasp leaf virus, Tobacco ringspot virus,* and *Tomato ringspot virus*) and their vectors.

No enation symptoms were submitted in the Mid-Columbia region this year. Symptomatic trees that come to our attention in 2019 will be tested.

Objective 4: Investigate spread and diversity of *Tomato ringspot virus* isolates from cherry in known-infected areas.

Commercial Orchard in The Dalles

On May 21, 2018, a commercial cherry orchard in The Dalles was surveyed for symptoms associated with *Tomato ringspot virus* (ToRSV). A total of 1,952 trees were surveyed for enations, dwarfed leaves, rosetting and dieback and rated on a 0-4 scale to indicate severity (0 = no ToRSV symptoms (rosetting, enations, 1 = Minimal ToRSV symptoms present, 2 = Moderate ToRSV symptoms present, 3 = Obvious ToRSV symptoms present, 4 = Tree stump with ToRSV symptoms on leaves). Trees were marked with an "X" if they had been removed partially (stump) or with a "M" if they were missing entirely. The orchard was primarily cv. 'Bing'. Pollinators were used as reference points and indicated by their marking "Y" for yellow indicating cv. 'Van' and "R" for red indicating cv. 'Rainier'. A "+" was used to note if suspected virus symptoms were observed that were not expected to be associated with ToRSV. Leaf samples were collected from a subsection of 400 trees (large rectangle, Figure 1) for testing by ToRSV ELISA. Due to missing trees, a total of 379 samples were tested. Of the samples tested, 92.9% of the samples expressing symptoms associated ToRSV tested positive for ToRSV, 6.6% tested positive when no visual symptoms were observed, and 0.5% tested negative when symptoms were observed.

Soil samples were collected on June 5, 2018, from a smaller subsection of 50 trees (5 rows by 10 trees in each row), as well as two small sections in areas where symptoms were not observed (medium and small rectangles in Figure 1) for identification of nematodes. Dagger nematodes were identified in 77.6% of the sites in the subsection of 50 trees, and in 25% of the samples from the regions where symptoms are not present on the outer edge of the orchard. All dagger nematodes present in June were juveniles.

Soil samples were collected again on October 17, 2018 from the same regions. Samples will be analyzed for the presence of adult dagger nematodes, which can be used for more detailed characterization. Dagger nematodes will be archived for future studies relating to virus detection and nematode characterization.

Visual assessments will be made again at the same orchard in 2019. Changes in disease severity from one year to the next will help determine the rate of tree decline/death from ToRSV infection. Another commercial orchard will be similarly surveyed for symptoms, ToRSV diagnosis, and dagger nematodes for comparison. Correlating dagger nematode presence with disease symptoms throughout

the orchard in symptomatic and asymptomatic areas will help determine a relationship between nematode presence, disease severity, and vector movement in an orchard.

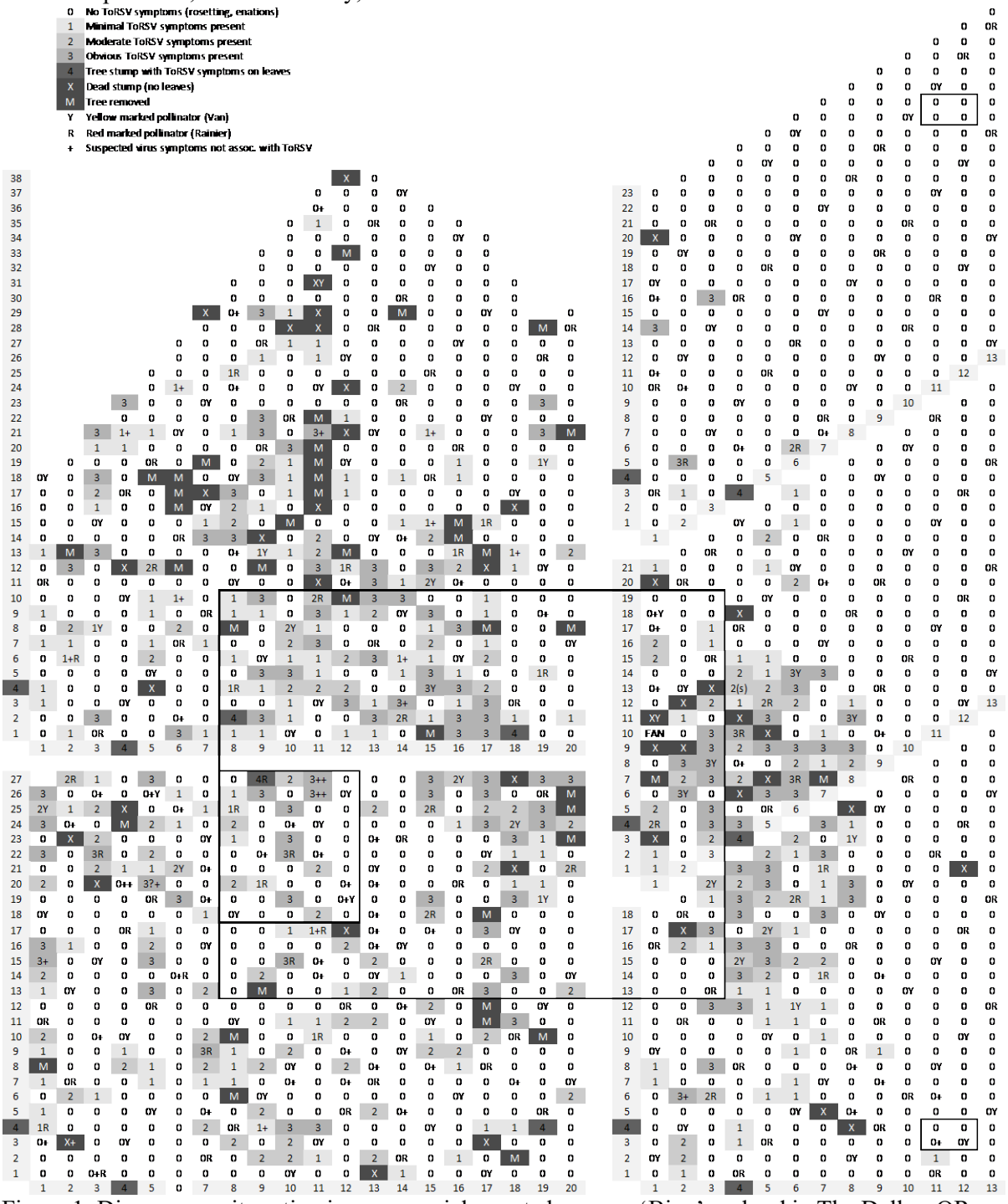

Figure 1: Disease severity rating in commercial sweet cherry cv. 'Bing' orchard in The Dalles, OR

Mid-Columbia Research and Extension Center

Soil samples were collected on March 20, 2018, at a known *Tomato ringspot virus* (ToRSV)-infected research block at the Mid-Columbia Research and Extension Center. Samples were processed for the extraction and identification of dagger nematodes (*Xiphenema americanum*), the known vector of

ToRSV. Matching the presence of nematodes in the orchard with locations of known ToRSV positive trees, allowed for the determination of eight areas where the virus and vector were present to test for the natural transmission of ToRSV to a variety of hosts. The following hosts were planted on April 13, 2018: apple (Scarlett Spur on MM106 rootstock), blueberry (Legacy), cherry (Lapins and G6), grape (self-rooted Chardonnay), peach (Loring on seedling rootstock) and raspberry (Meeker).

Bud, leaf, and/or root tissue were sampled from all hosts at planting and tested for the presence of ToRSV using ToRSV-specific ELISA. All samples tested negative for ToRSV (Table 2). Each tissue type (apple, blueberry, cherry, grape, peach and raspberry) was spiked with ToRSV-positive material in a 1:10, 1:100, 1:1000 serial dilution to account for possible inhibition of the test. None of the tissue types indicated the presence of inhibitors. Eight out of ten grape plants did not survive and were re-planted on June 4, 2018. Testing via ToRSV ELISA was performed again on root and leaf tissue to ensure plants were ToRSV negative (Table 2). Leaf samples were collected in September 2018 to test if transmission had occurred. None of the hosts tested positive, indicating no natural transmission had occurred.

Samples will be collected from each host again in Spring and Fall 2019. Mature trees that tested positive for ToRSV in 2017 will be tested again in spring 2019 to confirm ToRSV presence. More comprehensive testing of known weed hosts will be collected in all eight plots.

Identifying whether the ToRSV strain on cherry can naturally infect other tree, small fruit or weedy hosts can help inform disease management practices, especially upon re-planting.

Table 2: Testing results for samples collected at Mid-Columbia Research and Extension Center

		4/13/18 (Pre-plant)		6/4/2018 (Re-plant)		7/19/18		9/17/18	
Host	Tissue Type	n	# ToRSV + ELISA	n	# ToRSV + ELISA	n	# ToRSV + ELISA	n	# ToRSV + ELISA
Apple (Scarlett Spur	bud	9	0	-	-	-	-	-	-
on MM106)	leaf	-	-	-	-	-	-	9	0
	root	9	0	-	-	-	-	-	-
Blueberry (Legacy)	leaf	10	0	-	-	-	-	10	0
	root	10	0	-	-	-	-	-	-
Cherry (G6)	bud	10	0	-	-	-	-	-	-
	leaf	-	-	-	-	-	-	10	0
Cherry (Lapins)	bud	6	0	-	-	-	-	-	-
	leaf	-	-	-	-	-	-	6	0
	root	6	0	-	-	-	-	-	0
Grape (Chardonnary,	leaf	-	-	8	0	-	-	10	0
self-rooted)	root	10	0	8	0	-	-	-	-
Peach (Loring	bud	9	0	-	-	-	-	-	-
on seeding)	leaf	-	-	-	-	-	-	9	0
	root	9	0	-	-	-	-	-	-
Raspberry (Meeker)	leaf	10	0	-	-	-	-	9	0
	root	10	0	-	-	-	-	-	-
Dandelion	leaf	-	-	-	-	1	0	-	-
Plantain	root	-	-	-	-	1	0	-	-

REFERENCES

Ito, T., and Suzaki, K. 2017. Universal detection of phytoplasmas and Xylella spp. by TaqMan singleplex and multiplex real-time PCR with dual priming oligonucleotides ed. Ruslan Kalendar. PLoS ONE. 12:e0185427.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2 Years
WTFRC Project Number: CH-17-100

Project Title: Advancing precision pollination systems to improve yield security

PI: Matthew Whiting
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Cooperators: Finley Cherries, Olsen Brothers, Hayden Farms, Russ LeSage, David Green, Chisa Fruit Co., Firman Pollen Co., On Target Spray Systems, Cameron Peace, Katie Taylor, Connor Dykes

Total Project Request: Year 1: 74,566 **Year 2: 74,624**

Other funding sources
Firman Pollen company will provide pollen for this project at no cost – estimated at $5,000; OnTarget 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**
Salaries	$40,856		$44,191
Benefits	$5,482		$6,053
Wages	$12,480		$13,500
Benefits	$1,248		$1,350
Equipment	$0		$0
Supplies	$8,000		$2,500
Travel	$6,500		$7,030
Plot Fees			
Miscellaneous			
Total	$74,566		**$74,624**

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

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 pollinizers 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

Significant findings:

Overall:
There is high variability within and among orchards in fruit set
Pollination treatment effects should be assessed on large-scale

Finley 'Chelan'/Mazzard, mature block

- Low fruit set overall, ca. 9%
- High variability in branch-level fruit set (0% - 35%)
- No statistically significant effect on fruit set and yield

Finley 'Chelan'/Mazzard, young block

- High fruit set overall, ca. 50%
- No statistically significant effect on fruit set and yield
- Application timing likely late (ca. 95% open flowers)

Brewster 'Chelan'/'Gisela6'

- Moderate fruit set overall at ca. 20%
- High variability in branch-level fruit set (2% - 48%)
- No statistically significant effect on fruit set
- 10% increase in yield overall; 20% yield increase in top of tree

Angol, Chile 'Regina'

- Moderate fruit set overall at ca. 29%
- Range in branch-level fruit set of 15% - 40%
- Significant increase in fruit set from ca. 15 and 30 g pollen/ac

Pollen viability

- Viability may be reduced by electrostatic and over time in suspension media
- Viability varies among cultivars and between years

METHODS

1. Refine pollen rate and application timing

In 2018, we conducted 4 trials in 'Chelan' blocks: two near Finley, one near Benton City, and one near Brewster (Table 1). In 2017, we conducted a trial in 'Regina' in Chile and two trials in 'Early Robin' in 2017. In each trial, pollen rates and experimental design were different.
In 2018, each of the key issues identified by industry was addressed – the effects of the pollen suspension media alone, the effect of application technology (i.e., electrostatic vs. airblast), and the role of pollen rate. To address the effect of application timing we flagged replicate branches and made visual assessments of the stage of flowering on the day of application. This was expressed as a percent of flowers that were open, and estimated to the nearest 5%.

Table 1. Summary of precision pollination trials.

Trial number & site	Cultivar	Pollen treatments (g/acre)	Date(s) of application(s)	Open flowers on day of application	Sprayer*
1. Finley	'Chelan', old block	0, 15, 30	4 April 7 April	71% 78%	100-gal ES @ 30 gal/ac
2. Finley	'Chelan', young block	0, 15, 30, 60, mix only	7 April 11 April	72% 94%	50-gal ES @ 15 gal/ac
3. Benton City	'Chelan'	0, 30	9 April 11 April	86% 89%	50-gal ES @ 15 gal/ac, 400-gal AB @ 85 gal/ac
4. Brewster	'Chelan'	0, 30	23 April	86%	50-gal ES @ 15 gal/ac
5. Angol, Chile	'Regina'	0, 15, 30, 60		60-70%	100-gal ES @ 15 gal/ac

*ES=OnTarget Spray Systems electrostatic, AB=airblast

Preparation of pollen suspension. In each trial we pre-mixed pollen with dissolved suspension media in about 1 gallon of water. This pollen suspension was poured into the sprayer through a wire sieve (to strain any pollen clumps) and the system agitation was run for about a minute on low speed, until the suspension was uniform and there was no foaming. During application, agitation was turned off. Depending on the trial, we prepared either 5 gallons or 10 gallons total pollen suspension for application. The exception to that protocol was the older 'Chelan' block in Finley where our cooperator used his equipment, labor, pollen, and mix, to apply the treatments. We marked treatment rows and were on-site, with the grower, at applications and harvest for the block. In addition, prior to each trial application the sprayer was calibrated by collecting nozzle output over 30 second intervals and adjusting boom pressure to achieve the target volume of 15 gallons per acre.

Trial design. Each trial differed in the treatments compared and experimental design (Table 1). Treatments in trial 1 were made to 3 replicate blocks of two complete rows. Treatment blocks were separated by two rows. Yield data were collected from each set of two rows by weighing bins. In trial 2 we setup a completely randomized trial with 5 replications of 3 trees down a single orchard row. Yield and fruit set data were collected from the middle tree in each replication. In trial 3 treatments were made to single blocks of 3 contiguous rows, with 6 rows as buffer between treatments. Fruit set data were collected from 15 replicate limbs in the middle row. Yield data were not collected due to the low yield overall. In trial 4, treatments were made to 6-row sections, with 3 replicate sections per treatment. Fruit set data were collected from 10 replicate limbs per section, and yield data were collected from bin weights from each section. Fruit were harvested on two dates (6/20 and 6/23) as fruit matured in the upper canopy were harvested first. The trial in Angol, Chile was applied by cooperators, using their equipment and operators. Treatments were made to entire rows, with several untreated rows between treatments. Fruit set data were collected from 6 replicate limbs along the row. No yield data were collected.

Assessing fruit set. To attain fruit set data, we labeled replicate branches at the popcorn stage and counted flower number. Just prior to harvest we returned and counted fruit number on the same branches. We collected fruit set data from both east- and west-facing branches with a minimum of 10 branches per treatment, thus 20 samples (minimum) per treatment group for fruit set analysis (the trial in Chile was the exception with 6 replicate limbs). On each marked branch, the number of fruit (*Fr*) at harvest divided by the number of flowers (*Fl*) at popcorn stage determines fruit set (*FS*), the rate of successful pollination or fertilization (i.e., *FS = Fr/Fl)*

Sed genotyping trial. To assess the efficacy of supplemental pollination, we setup a trial in a 'Sonnet' (S_1S_3) orchard with 'Skeena' (S_1S_4') pollenizers near Brewster. Supplemental pollination was applied to 4 rows on 28 April at 15 g/acre of previously collected 'Cowiche' (S_5S_9) and at a volume of 15 gallons/acre using the 50 gallon On Target Spray System electrostatic sprayer. Just prior to commercial harvest, we collected 60 fruit from treated trees and 60 fruit from untreated trees (at least 5 rows separated from treatment). Seeds were collected from these fruit and stored at IAREC. At the time of preparing this report, the S-allele genotyping of the seed was being conducted by Dr. Peace's lab in Pullman. Results of seed S-allele genotyping will be presented at the research review.

2. Optimize pollen suspension constituents

The goal of this objective is to extend pollen viability and to improve suspension uniformity. We are conducting on-going trials to assess pollen viability in the suspension media mixed in simulated field conditions. Using the electrostatic sprayer, pollen, and suspension media, we are assessing viability from the tank and from the charged spray. The pure pollen is tested prior to mixing by sprinkling a sample onto 5 agar mediums (18% sucrose) to attain a baseline viability measure. Immediately following mixture into the agitating spray tank, solution is extracted from the tank and spray is collected from a nozzle for the same measure of pollen viability by germination. Samples from each (tank and spray) are collected at 30 minute intervals for 3 hours. For tank sampling: a 20 mL sample is collected from the middle of the tank; from that, 20µL is pipetted onto a germination media petri dish. Simultaneously, for spray sampling: a 10- second spray expulsion is collected into a beaker; from that, 20µL is pipetted onto the germination media petri dish. To assess viability, the ration of germinated pollen granules to total pollen granules is counted within a random sample of the petri dish with a dissecting microscope (6 x magnification).

Trials will continue to be conducted with adjustments to the liquid suspension media in efforts to extend pollen viability. Potentially limiting factors to viability being recorded are starting pollen viability, temperatures of air and solution, sunlight, time, and suspension media constituents. Additionally, similar trials will be conducted with the goal of analyzing suspension uniformity by pollen deposition prior to 2019 field trials. Adjustments are being made to the suspension media in efforts to improve pollen uniformity for sufficient pollen distribution onto stigmatic surface.

In a field trial in 2019, we will collect flowers one hour after spray application to assess pollen density on the stigmatic surface. The stigmatic surface of collected flowers will be preserved with mineral oil and pollen granules will be counted using a dissecting microscope.

3. Investigate pollen production systems

This objective has two parts: 1) identify pollen variability among cultivars and growing seasons, and 2) consider tree training systems for maximum pollen yield. We collected pollen from ten cultivars in 2017 and 2018 ('Benton' 'Bing', 'Chelan', 'Lapins', 'Santina', 'Selah', 'Skeena', 'Sweetheart', 'Ulster', 'Van') located in the WSU Roza experimental orchards. We plan to collect pollen from the same trees using the same method in 2019. Five branches at approximately 20% full bloom were cut from identified trees and brought to the lab. Popcorn stage flowers were selected from each branch. Pre-dehiscent anthers were plucked off filaments and left for 24 hr to dehisce at room temperature. The pollen is stored in a freezer until viability analysis by germination, as previously described. Briefly, pollen viability is assessed by dusting collected pollen on agar media. Plates are evaluated about 8 hr after dusting by counting ungerminated and germinated pollen in several replicate fields of view. Pollen is considered germinated (i.e., viable) if a pollen tube has grown longer than the length of the pollen grain.

The second component to this research objective is to begin the development of tree training systems for maximum pollen yield. This will be accomplished using orchards at the WSU Roza farm and a new orchard planted by Firman Pollen Company near Yakima. Our team of collaborators will meet to discuss potential training systems in these orchards including issues of trellis vs. free-

standing, horizontal vs. vertical limbs, methods for rapidly filling space, light relations, limb spacing, etc. Training decisions will be put into practice in these research orchards and, when possible, whole-tree harvest of flowers (pollen) will be timed and assessed for pollen yield. Data will be collected on bloom progression (i.e., time between first and final flowers opening), flower density, pollen yield, harvest efficiency, and number of passes required to harvest all flowers.

4. Use this work to strengthen larger proposals

The success of federal and regional grant proposals is improved with the presentation of strong preliminary results and the support of stakeholders. The data we generate from the research proposed herein will be used to support funding for further development of precision pollination systems.

RESULTS & DISCUSSION

1. Refine pollen rate and application timing

Trial 1 (Finley, Old 'Chelan'/Mazzard): In this trial we compared two pollen rates (15 and 30 g/acre) with untreated control. The grower collaborator used their electrostatic sprayer (100 gallon tank @ ca. 30 gallons/acre). Two applications were made (4 and 7 April) to 3 replicate blocks of two complete rows. Overall, fruit set was low in this block at about 9% across treatments (Table 2). Among the branches we evaluated for fruit set there was tremendous variability – a range from 0% - 35%. This variability was not associated with branch location (E vs. W sides of tree) nor the bloom density. There was no significant treatment effect on fruit set. Average fruit set was 8.9, 9.5, and 8.2% for the control, 15 g/acre, and 30 g/acre treatments, respectively. This lack of treatment effect may be related to the environmental conditions during application (Table 4). It was cold during the first application (51F) and it rained following the second application. Low temperatures reduce pollen tube germination and growth (Zhang et al., 2018).

Fruit yield was similarly unaffected by pollen application. We weighed all fruit harvested from each two-row block on the day of commercial harvest. Tree yield was determined from total weight from each block divided by the number of trees per block. Yield varied slightly among treatments: 32, 33, and 27 lbs per tree for control, 15 g/acre, and 30 g/acre, respectively. These data translate to 4.3, 4.5, and 3.6 tons per acre.

Trial 2 (Finley, Young 'Chelan'/Mazzard): In this trial we compared 3 rates of pollen, the pollen suspension media alone, and a water-sprayed control. Pollen was applied on two days, the 7th and 11th of April. Unfortunately the pollen rates on the first application date were miscalculated and applied at ¼ of the intended rate (i.e., about 4, 8, and 15 g/acre instead of 15, 30, and 60 g/acre). The proper rates were applied on the second date, so our analyses are from those data only. We again recorded a tremendous variability in fruit set among branches. Irrespective of treatment, the range was about 23% - 83%. Overall, across treatments, fruit set was high in this trial – just under 48%. This is particularly interesting because the same genotype in an adjacent block (older Chelan block data above) exhibited much lower fruit set (about 9% overall). Bloom timing, tree age, pollinizer density, pollinator activity, etc., all will impact fruit set (Whiting et al., 2005; Sagredo et al., 2017). Climatic conditions influence ovule viability and longevity, pollen viability, pollen deposition, and stigma receptivity (Zhang et al., 2018). The effective pollination period, estimated at 3-6 days in sweet cherry (Sanzol and Herrero, 2001; Ughini and Roversi, 1996; Sagredo et al., 2017) was cool and windy, with some precipitation. Temperatures were between 50° - 55° F (Table 4). Rain occurred on the day of application in approximately half of our trials, and wind visibly influenced spray deposition in several of our trials. Further research is necessary to identify the key factors influencing fruit set across years and locations.

Yield per tree was variable in this block, ranging from 20 to 56 lbs per tree. The highest mean yield was for the 15 g/acre treatment at 43 lbs/tree. Other treatment yields were 42 lbs (60 g/acre), 37 lbs/tree (30 g/acre), 36 lbs/tree (control), and 34 lbs/tree (suspension media only) though differences among treatments were not statistically significant.

Table 2. Summary of fruit set (% available flowers) across all trials in 2018.

	Finley young 'Chelan'*	Finley old 'Chelan'	Brewster 'Chelan'	Benton City 'Chelan'	Angol 'Regina'
Control	48%	9%	19%	5%	21% b
Mix Only	55%				
15 G	48%	10%			34% a
30 G	45%	8%	20%	2%	32% a
30 G AB**				3%	
60 G	58%				24% b
Overall	48%	9%	20%	3%	29%

Fruit set = # fruit at harvest/ # flowers at full bloom. Finley young *n = 20, p = 0.19; Finley old n = 20, p =0 .71; Brewster n = 30, p= 0.36; Angol n = 25, p=0.02.* *second application only; **AB=airblast (all other pollen treatments applied with electrostatic sprayer).

Trial 3 (Benton City, 'Chelan'/Mazzard): In this trial we compared treatments of 30 g pollen/acre applied by electrostatic sprayer (ca. 15 gallons/acre) and airblast sprayer (ca. 85 gallons/acre) with untreated control. Fruit set was particularly low in this trial – an average of 3% across all treatments; ranging from a low of 0 to 17%. This is likely related to relatively poor environmental conditions during bloom and the lack of pollenizers. 'Index' trees were planted as every third tree in every third row as pollenizers and these trees were weakened with poor bloom density (one of the reasons we selected this block for a trial). Poor fruit set in treated blocks may be related to timing of application. During the first and second applications, the percent of bloom on labeled branches was 87% and 89%, respectively. There was a slight negative linear relationship between fruit set and the percent bloom during application. This block was left unharvested due to poor yield.

Trial 4 (Brewster, 'Chelan'/'Gisela6'): In this trial we compared a single application of 30 g pollen/acre vs. water-sprayed control. The application was made on 23 April using the 50 gallon electrostatic system at ca. 15 gallons/acre. Fruit set in this block was moderate overall at ca. 20%. This may be related to the relatively high pollinizer ratio of 33%. The block was setup with 6 rows of 'Chelan' and 2 rows of 'Santina'. Among the labeled branches, irrespective of treatment, we documented a large variability in fruit set, from 2 – 48%. This is consistent with the range observed in other blocks. There was no statistical difference in fruit set between treated (20%) and control (19%) branches (Table 1). This may be related to the timing of application – average percent open flowers on labeled branches was ca. 86%.

Yield data were collected by weighing bins of fruit during commercial harvest. Trees were harvested over two dates (20 and 23 June) with the first pick targeting ripe fruit in the upper portions of the canopies. From the treated blocks, about 65% of the fruit were harvested on the first date, from the tops of the trees. In control trees, about 58% was harvested on the first date. This difference may have been due to pollen application since our treatment would have been most effective in the upper regions of the trees – flowers in the lower halves of the trees were fully open and likely past optimum receptivity. Extrapolated to a per acre basis, there was about 680 lbs more fruit from the upper portions of treated trees compared to control though this was not statistically significant ($p = 0.35$). Again, the lack of a clear improvement in tree yield may be related to the effect of application timing, or, in this case, insufficient replication. We observed a negative linear relationship between the percent open flowers during pollen application and final fruit set – this suggests that applications made earlier may have been more effective.

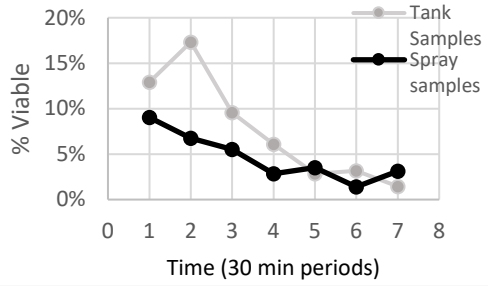
Trial 5 (Angol, Chile, 'Regina'/Colt): This trial was setup with collaborators who used their own application equipment (On Target Systems electrostatic sprayer). A single application (made at 60-70% full bloom) of ca. 15, 30, or 60 g/acre of pollen was compared to the untreated control. Fruit set

overall was moderate at about 29% across treatments, with a range in set among individual branches of about 15 – 40%. Fruit set was improved significantly by treatments with both 15 and 30 g/acre of pollen, but interestingly, not with the highest pollen rate (Table 2). Compared to control fruit set of 21%, treatment with 15 or 30 g/acre led to fruit set of 34% and 32%, respectively. This represents an improvement of about 59% over the control. It is possible that the positive treatment effects in this trial were due to the timing of application. Again, our results suggest that applications made at later stages of flowering are less effective. Our trials in 2019 will evaluate this issue of timing further by targeting applications at 50-60% flowering.

2. Optimize pollen suspension constituents

Prior to mixing pure pollen ('Cowiche') into the liquid suspension media in the sprayer, viability was determined to be approximately 55%. After sampling at 30-min intervals, viability decreased (Fig. 1). There are several reasons for the decrease in viability over time - pollen viability is lost in liquid, and/or our samples may have been a poor representation of the pollen population, which indicates unequal distribution of pollen throughout the solution. Our previous results showed extended viability – this issue requires further investigation.

Figure 1. Pollen viability over time



However, further development of the suspension media will occur privately, not through this project – in 2018 PI Whiting licensed the IP through WSU and is preparing it commercially (the budget request for 2019 has been reduced to reflect this change).

3. Investigate pollen production systems

Preliminary results suggest there are significant differences in pollen viability among cultivars and growing seasons (Table 3). In 2019 we will add a third year of analyses to examine trends and identify genotypes that may be particularly useful in pollen production systems. In addition, in 2017 and 2018 we worked with Firman Pollen Co. in establishing two new orchards for the purpose of pollen production. We are developing pruning and training strategies to promote high flower production and pollen yield. We anticipate the first significant harvest in 2020 from the 'Regina' and 'Black Gold' orchards planted in 2017 on 'Gisela5' rootstock. A block planted in 2018 is comprised of 'Regina' and 'Benton', both on 'Gisela5'.

Genotype	**Pollen viability**	
	2017	2018
Skeena	38%	32%
Van	31%	21%
Benton	26%	13%
Selah	18%	60%
Bing	21%	29%
Ulster	10%	15%
Santina	NA	42%
Lapins	NA	16%
Sweetheart	NA	21%
Chelan	NA	10%

Table 3. Viability of sweet cherry pollen sampled from the WSU-Roza farm.

4. Use this work to strengthen larger proposals

We submitted a proposal to the Washington State Specialty Crop Block Grant program (request of ca. $250,000) to expand our research into precision pollination systems and the potential to improve yield security in tree fruit. The proposal was well-rated but not selected for funding.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3 Years
WTFRC Project Number: CH-17-101

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

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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: **WSU** **Contract Administrator:** Katy Roberts
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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

- Bing, Chelan and Sweet heart models are available for WA on AWN.
- FTEED is coded on AWN
- Forecast is linked to both models FTEED and AWN's models.
- DTA data was collected in WA to compare rates of freezing for Bing, Chelan and Sweet heart.

RESULTS & DISCUSSION

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

Data has been collected in both OR and WA during 2017-2018 season and the analysis of the data is in progress for OR and results will be presented at the meeting in November for WA.

Several studies have been conducted in cherries and some other crops using different cooling rates (Table 1). In Washington, Whiting, M., et al (2018), in his nanocrystal study to reduce cold damage to reproductive buds in fruit crops, reported an standard cooling rate of 4◦C/h; Salazar, M.R. et al (2014) in her study about Variation in cold hardiness of sweet cherry flower buds through different phenological stages as well as Kader and Proebsting (1992) in their study about screening sweet cherry selections for dormant floral bud hardiness also stated a cooling rate of 4°C/h. In Ohio, Mathers used a rate of 5°C/h in sour cherry flower buds supercooling and cold hardiness. In Montana, Callan, evaluated the dormancy effects on supercooling in deacclimated tart cherry flower buds using different rates for different years; 2°C/h 1986-1987; 1°C/h to -10°C 2.5°C/h in 1987-1988. So far, there is not a unique recommendation about the rate to be used in cold hardiness studies. According to the literature reviewed (Table 1.), it has been observed that the rate varies depending on crops, cultivars, location, freezing events and even in observed temperature values during the day where temperature decrease to a critical damage.

Procedures.
In WA, the hardiness of the buds was estimated with DTA for previous years, using a rate of 7.2°F/h (equivalent to 4°C/hour). In OR, a rate of 1.8°F (equivalent to 1 °C/hour) has been used for data collection. An evaluation of both rates of cooling 7.2°F/h and 1.8°F was performed in WA (Fig 1), to determine the variation and compare the hardiness in order to standardize the procedure for the hardiness determination. For Chelan, the hardiness were not constant during the period evaluated, the estimated value for hardiness using DTA rate of 7.2°F/h were lower than the hardiness estimated rate of 1.8°F (Table 2.).

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.

We are working on two model approaches for the benefit of the industry because of the data collection in both locations OR and WA, did not follow the same protocol and the conceptual models were different. Both models will be valuable tools for future evaluation by the industry.

In WA, seasonal models have been develop for Chelan, Bing and Sweet heart using the data previously collected (in preceding projects supported by the TFRC from 2012-2015).

A nonlinear model was fitted for Chelan, Bing and Sweet heart including the lethal temperature of the two previous days prior to freezing and the accumulative day for the period and the maximum temperature. The model can be expressed by the following equation:

$$Y_{jk} = \beta_0 + \beta_1 Y_{j-1k} + \beta_2 Y_{j-2k} + \beta_3 d_{jk} + \beta_4 d_{jk}^2 + \beta_5 mxt_{jk} + \epsilon_{jk}, \quad (1)$$

where Y_{jk} represents the temperature threshold at the maximum rate of injury of any LT_{10}, LT_{50}, LT_{90}, or in general LT_j (j=10, 50, 90) and k is the accumulated Julian day from the first to the last measurement in each season. β_0, β_1, β_2, β_3, β_4, and β_5 are parameters and ϵ_{jk} is the error term. β_0 is the intercept, β_1 and β_2 are the coefficients of the first order lag Y_{j-1k} and second order lag Y_{j-2k}, value of previous day and the value of two days before correspondingly, d_{jk} is cumulative Julian day of each season, and mxt_{jk} is the maximum temperature at time k of each season.
For each j (=10, 50, and 90) the parameters were estimated separately (scientific paper in progress).

The correspondent steps of model calibration, model evaluation and sensitivity analysis were performed for Bing, Chelan and Sweet heart. Lethal temperatures observed and predicted as well as and lower and upper limits of the models for each cultivar are presented in Figure 2.

A rate based model was developed in Oregon FTEED is presented on http://blogs.oregonstate.edu/gdavs/. Analysis for previous season about the assessment of the acclimation to freeze tolerance during the period between late summer and the acquisition of dormancy will be presented at the review meeting in Nov 2018. AWN, will continue supporting the inclusion of this model on our web portal, with the purpose to better satisfy the needs of the growers and industry.

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.

For the last year of the project 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 in WA (Fig 3)

and OR (Fig 4), its inclusion and improvement into the AgWeatherNet Portal will be a continuous activity for the following year of the project. Seasonal models developed for Chelan, Sweet heart and Bing were included in the AgWeatherNet for Beta-testing, and forecast has been linked to the models for further evaluation (Fig 3).

Table 1. Biographical references of rate evaluation

Author	Year	Title of study	Crop-cultivars	Rate
Jassim O. Alhamid , Changki Mo, Xiao Zhang, Peipei Wang, Matthew D. Whiting , Qin Zhang (Washington)	2018	Cellulose nanocrystals reduce cold damage to reproductive buds in fruit crops	Cherries and grapes	Hold 4 °C for 1 h. then temperature drops until reaching -40°C within 11 h. with the rate of temperature drop 4°C within 11 h with the rate of temperature from 4°C to -40°C hold for 1 h then back to 4°C within 10 h .
Melba R. Salazar-Gutierrez, Bernardo Chaves, Jakarat Anothai, Matthew Whiting, Gerrit Hoogenboom (Washington)	2014	Variation in cold hardiness of sweet cherry flower buds through different phenological stages	Cherries and apple	Standard cooling rate of 4◦C/h decline which the freezer hold at 4◦C for 1 h and then drop to −40◦C in 11 h, at that moment return to 4◦C in 10 h.
H. M. Mathers (Ohio)	2004	Supercooling and cold hardiness in sour cherry germplasm: Flower buds	Sour Cherry	Holding period at -3 °C for 10 h followed by a temperature drop of 5 °C/ h from -5 to -50 °C.
Kadir, S.A and Proebsting, E. L	1994	Screening sweet cherry selections for dormant floral bud hardiness	Sweet Cherry	Buds were held in place with aluminum foil. Heat flux plates were frozen as reported by Kader and Proebsting (1992) at a cooling rate of 4°C/h.
N.W. Callan (Montana)	1990	Dormancy effects on supercooling in deacclimated tart cherry flower buds	Tart cherry flower buds	2°C/h 1986-1987 1°C/h to -10°C 2.5°C/h in 1987-1988
Jason P. Londo and Alisson P. Kovaleski (Geneva, NY)	2017	Characterization of wild North American grapevine cold hardiness using differential thermal analysis	33 different grapevine genotypes, representing six wild North 19 American grapevine species, one wild Asian grapevine species, and six hybrid grapevines	Using a temperature ramp from room temperature to 4 °C, hold for 1 hour, and ramp to -40°C over 13 hours, followed by a slow ramp back to room temperature. This program results in a cooling rate of ~0.06 °C/minute or 3.4 °C/hour.
Jun Yu, Ju Young Hwang, Sun Woo Chung, Hee Duk Oh, Seok Kyu Yun,Hee Jae Lee (Korea)	2017	Changes in cold hardiness and carbohydrate content in peach (Prunuspersica) trunk bark and wood tissues during cold acclimation and deacclimation	Peach trees	To various freezing temperatures during cold acclimation and deacclimation and assessed the extent of freezing injury by analyzing low-temperature freezing exotherms (LTEs 2°C/hr. until the target temperature was reached, and maintained at the target temperature for 2 h. Five target temperatures were selected in ranges of −2 to −24◦C in September 2014,−4 to −30◦C in November 2014 and March 2015, and −8 to −36◦Cin January 2015. The frozen samples were thawed at 4◦C.
S. Palacio, A. Lenz, S. Wipf, G. Hoch, C. Rixen (Switzerland)	2015	Bud freezing resistance in alpine shrubs across snow depth gradients	Alpine species, eight species.	Seven different freezing temperature treatments were applied concurrently, with three samples per species in each of the treatments. After an initial reduction to 4 °C, the temperature were decreased within each freezer by 3 Kh^{-1} and then maintained the temperature at the target value for 4 h.
Michael Wisniewski,∗, Lawrence Gusta, Gilbert Neuner	2014	Adaptive mechanisms of freeze avoidance in plants: A brief update	A shoot of Arctostaphylos uva-ursi (Bearberry)	Cooling rate of 3 ◦Kh−1
Lawrence V. Gusta and Michael Wisniewski	2013	Understanding plant cold hardiness: an opinion	Winter cereals, spring cereals	Only develop limited freezing tolerance (e.g. LT50, −9◦C) to an acute short-term freeze (2◦C h−1 cooling rate) but do not develop resistance to a chronic, prolonged freezing.
MIKKO RA¨ ISA¨ NEN,*, TAPANI REPO	2006	Effect of Thawing Time, Cooling Rate	Norway spruce (Picea abies)	2003-During the first 4 h of thawing, the rate of dehardening was 6 °C h^{-1} 2005—39°C to -35 °C at a

and TARJA LEHTO (Finland)		and Boron Nutrition on Freezing Point of the Primordial Shoot in Norway Spruce Buds		rate of 0.7 °C h^{-1}. In 2003, different cooling rates of 1–5 °C h^{-1} had a minor effect on Tf but in 2005 with slow cooling rates Tf decreased. In both samplings, at cooling rates of 2 and 1 °C h^{-1}, Tf was slightly higher in B-fertilized than in non-fertilized trees. By contrast, at very short thawing times in 2003, Tf was somewhat lower in B-fertilized trees.
Wisniewski and Bassett	2003	An Overview of Cold Hardiness in Woody plants: Seeing the forest through the trees	Trees and perennials	LTEs reported in many studies employing a cooling rate of 1 °C/h or greater may be the result of inadequate time for water in the xylem ray parenchyma cells to migrate to extracellular ice. Many of the early studies on deep super cooling employed a cooling rate of 10-40 °C/h
R. A. Hamman (Colorado)	1996	Seasonal carbohydrate changes and cold hardiness of Chardonnay and Riesling grapevines	Riesling, CH	2°C/hr. Four samples were removed at 2.5°C intervals, at each of four stress temperatures chosen to span the probable lethal temperature at a particular sampling date.
Pauliina Palonen - Deborah Buszard	1996	Current state of cold hardiness research on fruit crops	Tree fruit and berry crops	Different rates, several crops a review.
Barney, Mancuso, Finnerty	1994	A computerized multiple chamber controlled freezing system	Raspberry	The samples and chambers are equilibrated at about 4°C- samples were cooled at 5°C/h. Samples were held at their min temp for 2h and then warmed to 0°C-10°C
H.A. Quammec	1991	Application of thermal analysis to breeding fruit crops for increased cold hardiness	Apple	Furthermore, it has been difficult to maintain a constant temperature drop at slow rates of cooling (5°C/hr or less). As a result, most thermal analyses have been conducted at cooling rates (15°C/hr or greater) that are more rapid than those that occur in nature frozen to – 39°C at 1C/hr
Andrews, Proebsting and Campbell	1983	An exotherm sensor for measuring the cold hardiness of deep supercooled flower buds by DTA	Peach- Prunus persica (L.) Batsch cv. Redhaven	Thermos bottles cooled at 1°C/hr. in a freezing chamber
John Alden and R. K. Hermann	1971	Aspects of the Cold-Hardiness Mechanism in Plants	Winter wheat.	Reducing sugars did not accumulate in his experiments with either slow cooling at a rate of 4°C per 24 hours or rapid cooling at a rate of 2°C/h until -18°C was reached, although some increase was observed at temperatures above -8°C.

Table 2. Parameters and rate hardiness comparison for Chelan.

	Logistic Parameters*								Celsius				Farenheit			
Date	Rate	d1	c	k	g	Standard Error	Lower Limit	Upper Limit	LT10%	LT50%	LT80%	LT90%	LT10%	LT50%	LT80%	LT90%
12/5/2018	4	1	0.06	-1.05	-20.84	0.07	-20.89	-20.79	-18.8	-21.0	-22.5	-23.9	-1.9	-5.7	-8.5	-11.0
12/6/2018	1	1	0.05	-1.19	-24.03	0.02	-24.03	-24.00	-22.2	-24.1	-25.4	-26.4	-8.0	-11.4	-13.7	-15.5
1/9/2018	4	1	0.07	-0.88	-20.74	0.07	-20.87	-20.61	-18.3	-20.9	-22.8	-24.4	-1.0	-5.6	-9.0	-12.0
1/10/2018	1	1	0.08	-1.87	-22.53	0.02	-22.57	-22.50	-21.4	-22.6	-23.6	-24.7	-6.5	-8.7	-10.4	-12.5
2/5/2018	4	1	0.09	-1.12	-18.16	0.04	-18.23	-18.08	-16.3	-18.3	-20.0	-22.8	2.7	-1.0	-3.9	-9.0
2/6/2018	1	1	0.06	-1.63	-19.46	0.01	-19.49	-19.44	-18.2	-19.5	-20.5	-21.3	-0.7	-3.2	-4.9	-6.4
2/12/2018	4	1	0.08	-1.64	-17.99	0.02	-18.03	-17.96	-16.7	-18.1	-19.2	-20.5	1.9	-0.6	-2.5	-4.9
2/13/2018	1	1	0.03	-1.47	-19.81	0.01	-19.83	-19.79	-18.2	-19.8	-20.8	-21.5	-0.8	-3.7	-5.5	-6.7

*LT = c + (d1-c)/(1 + exp(-k(t-g))) Eq. to estimate Lethal temperature.

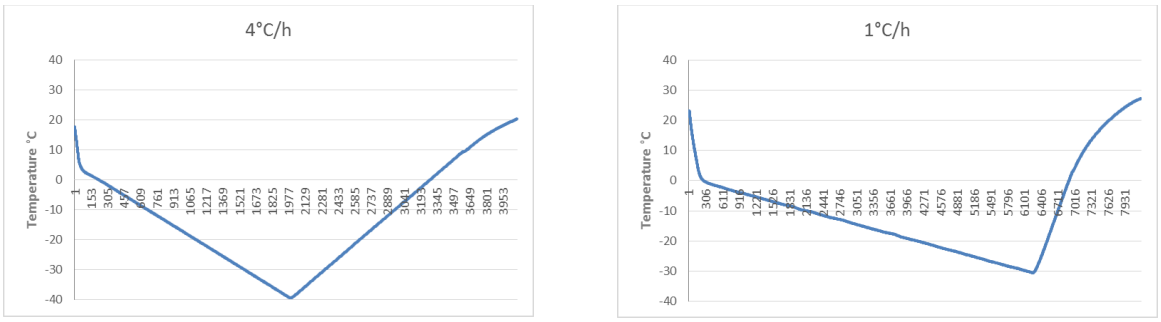

Figure 1. Rates tested during 2017-2018 for cold hardiness determination

Figure 2. Lethal temperatures observed, predicted and lower and upper limits of the models for each cultivar.

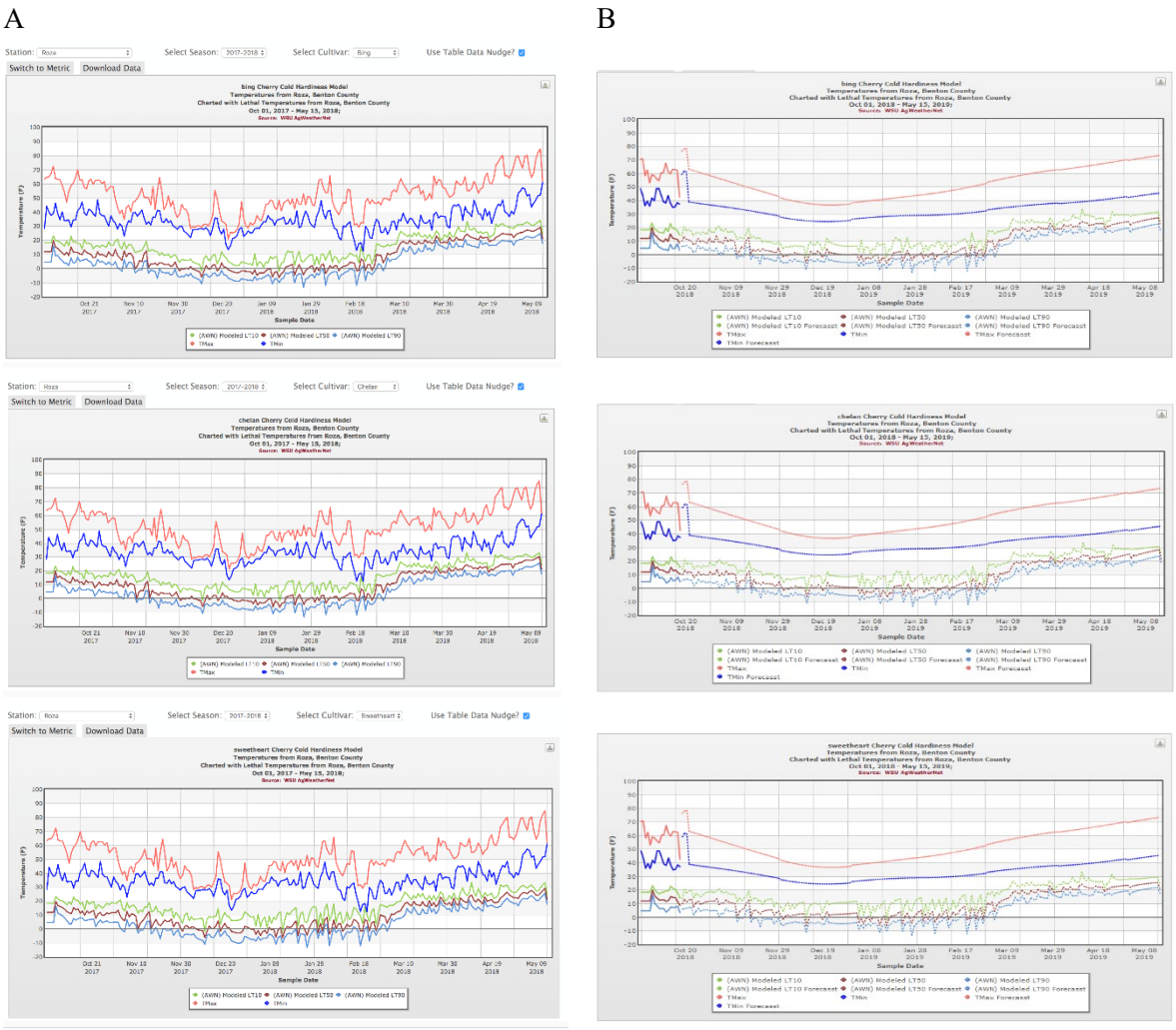

Figure 3. Flower bud hardiness of Sweet Cherry models developed with data collected in WA for 2017-2018, location Roza, maximum and minimum temperature, LT10, LT50 and LT90 are presented in Column A and forecast add it to the model is presented in Column B.

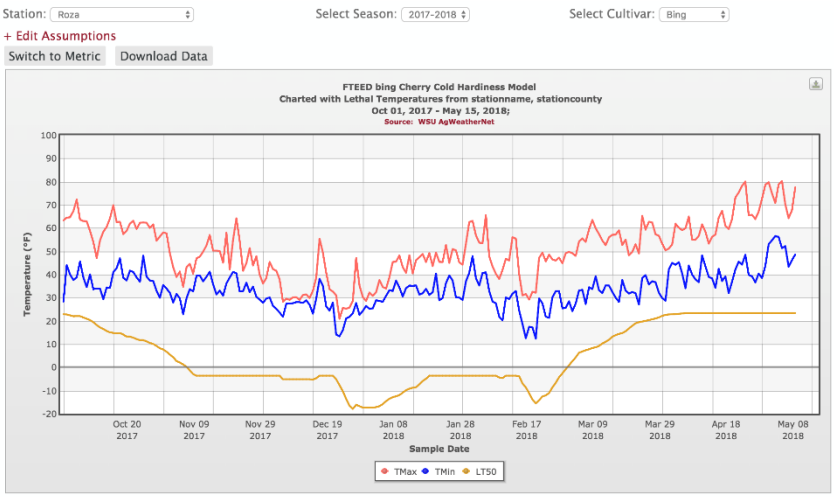

Figure 4. Freeze Tolerance in Endo - and Ecodormancy (FTEED) of Sweet Cherry Floral Buds. (Model developed in OR).

CONTINUING PROJECT REPORT **YEAR**: Year 2 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:** $**84,913**

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	**$ 19,062**

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.

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 three 2015 plantings, trees on the MSU rootstocks were significantly smaller than trees on the control rootstocks [Gisela 5 (Gi5), Gi6, Krymsk 5 (K5) and K6], except for Clinton at Wenatchee (Fig.1). The K5 and K6 trees are too vigorous and not sufficiently precocious for the ultra-high density systems.
- For the 2015 planting at The Dalles where 'Early Robin' was trained to a KGB, the highest fruit set was obtained with Clinton, Gi5 and Gi6 due to heavy fruiting on spurs (Table 2). This trend of individual tree yields was also observed with 'Regina' and 'Sweetheart'; yet, the yield predictions tended to level out when the per acre yields were calculated based on higher densities for the smaller rootstocks (Tables 3-5).
- Trees with higher crop loads tended to have smaller fruit (Tables 3-5). There does not appear to be a genetic tendency for the small MSU rootstock trees to have smaller fruit independent of crop load.
- For the 2017 plantings, in general, the tree sizes for 'Regina' and 'Sweetheart' on Gi5, Clinton and Crawford were similar. Promalin-scoring techniques resulted in successful lateral shoot development.
- Project team members visited grower plots that collectively 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 different scions, orchard systems and environments.
- 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.

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

2015 plantings: Replicated rootstock trials including three scions, four MSU rootstocks and three controls were planted at 3 locations (Fig. 1). For all three sites, trees were harvested to obtain individual tree yields. The fruit from the Mattawa and East Wenatchee plots were transported to the Sallato laboratory in Prosser for fruit quality evaluations. The Oregon fruit was transported to the OSU cherry laboratory located at the Extension office in Wasco County. After harvest, tree diameters were measured 10 cm above the graft line. Per acre yields were calculated using tree spacings based on projections of rootstock canopy size. Similar evaluations will be conducted in 2019.

2017 plantings: Replicated rootstock trials including two scions ('Regina', 'Sweetheart') and the fifth cherry rootstock, Crawford, in comparison with the most similar rootstocks, Clinton and Gi5 were planted in three locations. In 2018, trunk diameters were measured. In 2019, data collection will consist of trunk diameter and fruit measurements.

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. Replication size was capped at 200 trees per combination due to the experimental status of the MSU rootstocks. To establish these plots, all of which are on vertical or V-trellis, the growers used various Promalin – scoring techniques. The project team will continue 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 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 Iezzoni lab will continue to 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.

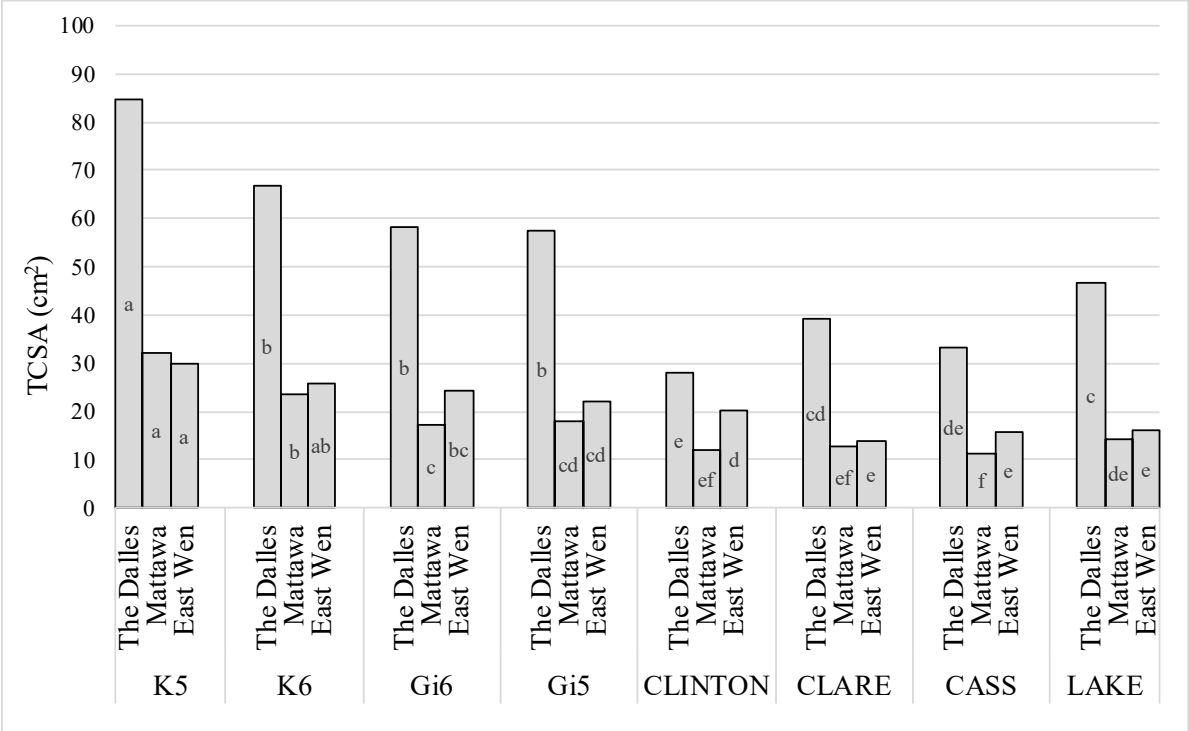
RESULTS & DISCUSSION:

Obj. 1. 2015 plantings: Trees on the MSU rootstocks were significantly smaller than trees on the control rootstocks [Krymsk 5 (K5), K6, Gisela 5 (Gi5), Gi6] measured as TCSA (Fig.1). The only exception was at Wenatchee where trees on Clinton were not significantly smaller than trees on Gi5. Among the four MSU rootstocks, Lake tended to result in larger trees at The Dalles and Mattawa, while Clinton resulted in the largest trees in Wenatchee (Fig. 1). Fruit for all three scions were harvested from the plots in The Dalles and Mattawa; however, 'Early Robin' was not harvested from Wenatchee due to severe bird damage. At Mattawa and Wenatchee the control trees of 'Regina' on Gi5 and Gi6 unfortunately turned out not to be 'Regina' so those comparisons are not available. Fortunately, a comparison of 'Regina' on Gi5 is included in the 2017 planting.

At The Dalles, the crop loads for 'Early Robin' and 'Sweetheart' trained to KGB were particularly high and therefore the fruit was hand-thinned. Crop load ratings for 'Early Robin' were taken prior to thinning to capture the fruit set, in particular the low fruit set on K6 (Table 2). The highest fruit set was obtained with Gi5, Gi6 and Clinton due to heavy fruiting on spurs. Similar high yields were observed at this site with 'Regina' and 'Sweetheart' (Table 3). For all three plots, yields per acre were calculated based on different tree spacings. The different tree spacings that contributed to these production calculations tended to equalize the yields among the rootstocks, compared to yields based on individual trees (Tables 3-5). In general, fruit size (measured as row size), fruit firmness and soluble solids content decreased with increased crop load (Table 3-5).

The higher pre-thinning yields of 'Early Robin' on Clinton compared to K6 and Lake from The Dalles were consistent with the findings from Mattawa (Table 3 and 4). For 'Regina', the crop load at Wenatchee was low due to poor weather during bloom. However, for 'Regina' at Wenatchee and Mattawa, Clinton and Clare tended to have higher yields, and all the MSU rootstocks yielded significantly higher than trees on K6 (Tables 4 and 5). In Wenatchee, 'Sweetheart' yielded lowest on K5 with Clinton, Gi5 and Gi6 exhibiting the highest yields. However, at Mattawa, the four MSU rootstocks had yields that were not significantly different from each other or Gi5, but significantly higher than yields on K5. For the SSA trees at Mattawa and Wenatchee, fruit weight did not significantly differ among rootstocks but row size was more variable and the significant differences were not consistent (Tables 4 and 5). At Wenatchee, across the rootstocks there were no significant differences in brix or firmness, whereas at Mattawa, fruit from 'Regina' on Lake was significantly sweeter and firmer compared to the other rootstocks. Across all plots there were no consistent trends in fruit color, except that Lake tended to have more advanced fruit color compared to the other rootstocks.

Fig. 1. Mean comparison of trunk cross-sectional area (TCSA; cm^2) in Sept. 2018 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).[1,2]



[1]Means that are significantly different for 2018 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 for 'Early Robin' and 'Sweetheart' contributed to the larger trunk sizes compared to the SSA plots in Wash.

Table 2. Crop load ratings for 'Early Robin' grown on four MSU rootstocks, Gi5, Gi6, and Krymsk 6 from trees planted in 2015 and trained to a KGB. These crop load ratings were taken prior to fruit thinning. Fruit samples were picked on June 12, 2018, with commercial harvests on Jun13, 17 and 24.

Rootstock selection	The Dalles Crop Load Rating[1]
Gi5	8.0 ab[3]
Gi6	8.3 ab
K6	5.3 d
CASS	7.2 bc
CLARE	7.3 bc
CLINTON	8.6 a
LAKE	6.5 c

[1]Crop Load Rating scale is as follows: 0=no crop to 7=perfect crop load
[2]Means that are significantly different ($P < 0.05$) are denoted by different letters.

Table 3. Individual tree yields, yield per acre, fruit weight, row size, firmness, color and brix for 'Early Robin', 'Regina' and 'Sweetheart' grown on four MSU rootstocks, Gi5, Gi6, K5, and K6 and trained using a KGB (ER, SH) or SL (R) from trees planted in 2015 at The Dalles, OR. The tree spacings (ft) associated with the projected yield/ac are 519 (6×14), 622 (5×14), and 778 (4×14).

Rootstock selection	Tree yield (lb)	Trees per acre	Yield per acre (tons/acre)	Fruit weight (g)[2]	Row size (FirmTech)[3]	Firmness (g/mm)[3]	CTIFL Color[3]	Brix (%)[4]
				Early Robin				
Gi5	41.5 ab[5]	622	12.9 a	8.9 ab	10.7 b	314 bc	2.6	14.5 c
Gi6	45.3 a	662	14.1 a	8.9 ab	10.5 b	308 c	2.5	14.4 c
K6	37.3 abc	662	11.6 a	10.0 a	9.5 a	346 a	2.6	16.5 ab
CASS	28.2 c	778	11.0 a	8.6 b	9.9 a	328 abc	2.9	16.5 ab
CLARE	35.1 abc	778	13.7 a	9.7 ab	9.7 a	316 bc	2.5	16.1 b
CLINTON	31.3 bc	778	12.2 a	8.7 b	10.8 b	319 abc	3.0	14.3 c
LAKE	28.0 c	778	10.9 a	9.4 ab	9.4 a	338 ab	3.0	17.6 a
				Regina				
Gi5	23.8 ab	622	7.4 a	10.9 a	9.8 ab	277 a	5.4 bc	19.6 a
Gi6	30.1 a	622	9.4 a	9.9 ab	10.1 ab	275 a	5.4 bc	18.5 a
K6	22.2 ab	622	6.9 a	9.9 ab	9.7 ab	308 a	5.2 c	20.7 a
CASS	23.6 ab	778	9.2 a	11.3 a	9.8 ab	255 a	6.4 a	20.7 a
CLARE	13.6 b	778	5.3 a	11.2 a	9.6 a	272 a	5.8 abc	20.2 a
CLINTON	22.7 ab	778	8.8 a	9.5 b	10.2 b	286 a	5.4 bc	20.1 a
LAKE	15.0 b	778	5.9 a	11.3 a	9.7 a	274 a	5.9 ab	20.4 a
				Sweetheart				
Gi5	51.2 a	622	15.9 a	8.9 ab	10.1 b	318 a	5.4 c	18.2 c
Gi6	45.2 ab	622	14.1 ab	9.0 ab	10.0 b	322 a	5.5 bc	19.1 bc
K5	19.0 d	519	4.9 c	10.0 a	9.5 a	346 a	5.8 ab	20.5 ab
CASS	27.8 cd	778	10.8 b	8.6 b	10.2 b	329 a	5.8 abc	20.7 a
CLARE	29.5 cd	778	11.5 b	9.7 ab	9.8 ab	327 a	5.8 abc	20.8 a
CLINTON	32.9 bc	778	12.8 ab	8.7 b	10.2 b	310 a	5.5 bc	19.7 ab
LAKE	34.2 bc	778	13.3 ab	9.4 ab	9.9 ab	322 a	5.9 a	20.8 a

[1]Fruit were harvested on the following dates: 'Early Robin' – June 12, 'Regina' – June 27, and 'Sweetheart' – July 9, 2018

[2]Fruit weight were measured from 100.

[3]Fruit firmness, row size, color were measured from 50 fruit per replicate. For 'Early Robin' the color scale was utilized to determine percentage of color; 1=1-25%, 2=26-50%, 3=51-75%, and 4=76-100%.

[4]Brix º (SSC) were measured from 25 fruit per replicate.

[5]Significantly different means ($P < 0.05$) are denoted by different letters.

Table 4. Individual tree yields, yield per acre, fruit weight, row size, firmness, color and brix 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 Mattawa, WA. The tree spacings (ft) associated with the projected yields are 2,420 (1.5×12), 2,904 (1.25×12), 3,630 (1×12).

Rootstock selection	Tree yield (lb)	Trees per acre	Yield per acre (tons/acre)	Fruit weight (g)[2]	Row size (FirmTech)[3]	Firmness (g/mm)[3]	CTIFL Color[3]	Brix (%)[4]
				Early Robin				
Gi5	6.7 ab[5]	2,904	9.7 a	8.7 a	9.9 ab	351 bcd	2.02	15.3 a
Gi6	7.3 a	2,420	8.8 ab	8.7 a	9.9 ab	337 cd	1.87	14.3 a
K6	5.1 cd	2,904	7.4 b	9.0 a	9.4 a	410 ab	2.44	15.5 a
CASS	5.5 bcd	3,630	10.0 a	9.5 a	9.5 ab	392 abc	2.03	14.9 a
CLARE	5.5 bcd	3,630	10.0 a	8.9 a	9.6 ab	395 abc	2.64	16.3 a
CLINTON	6.0 abc	2,904	8.8 ab	7.4 a	10.7 b	319 d	2.05	13.6 a
LAKE	4.6 d	3,630	8.3 ab	10.5 a	9.9 ab	431 a	2.31	15.3 a
				Regina				
K6	1.4 b	2,904	2.0 c	9.7 a	9.6 a	294 b	4.6 c	18.8 ab
CASS	2.5 b	3,630	4.6 b	8.7 a	10.1 a	285 b	5.1 b	16.2 bc
CLARE	4.3 a	3,630	7.7 a	10.0 a	10.0 a	287 b	5.3 ab	15.1 c
CLINTON	4.1 a	2,904	5.9 ab	9.5 a	10.1 a	302 b	5.2 b	18.1 ab
LAKE	2.4 b	3,630	4.3 b	11.1 a	9.7 a	342 a	5.5 a	19.9 a
				Sweetheart				
Gi5	10.9 a	2,904	15.9 a	7.2 ab	11.1 c	334 a	5.0 c	20.6 a
Gi6	9.1 ab	2,420	11.0 b	8.5 ab	10.4 ab	341 a	5.3 ab	22.3 a
K5	2.5 c	2,904	3.6 c	8.9 a	10.5 b	346 a	4.9 c	21.2 a
CASS	7.3 b	3,630	13.3 ab	6.7 ab	11.1 c	308 a	5.3 ab	21.4 a
CLARE	9.2 ab	3,630	16.7 a	7.8 ab	10.5 b	345 a	5.3 ab	19.1 a
CLINTON	8.1 b	2,904	11.8 b	6.4 b	11.5 d	332 a	5.0 c	20.2 a
LAKE	7.7 b	3,630	14.0 ab	8.1 ab	10.2 a	348 a	5.5 a	20.7 a

[1]Fruit were harvested on the following dates: 'Early Robin' – June 14; 'Regina' – June 29; and 'Sweetheart' – July 10, 2018.
[2]Fruit weight were measured from 100.
[3]Fruit firmness, row size, color were measured from 50 fruit per replicate. For 'Early Robin' the color scale was utilized to determine percentage of color; 1=1-25%, 2=26-50%, 3=51-75%, and 4=76-100%.
[4]Brix^{o} (SSC) were measured from 15 fruit per replicate.
[5]Significantly different means ($P < 0.05$) are denoted by different letters.

Table 5. Individual tree yields, yield per acre, fruit weight, row size, firmness, color and brix for '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, WA. The tree spacings (ft) associated with the projected yields per acre are 1815 (2×12) and 3,630 (1×12). 'Regina' yields were reduced due to poor weather at bloom time.

Rootstock selection	Tree yield (lb)	Trees per acre	Yield per acre (tons/acre)	Fruit weight (g)[2]	Row size (FirmTech)[3]	Firmness (g/mm)[3]	CTIFL Color[3]	Brix (%)[4]
				Regina				
K6	0.3 d[5]	1815	0.3 c	10.2 a	10.0 a	186 a	5.6 c	16.8 a
CLARE	2.3 b	3630	4.1 a	10.3 a	10.1 a	197 a	6.4 a	19.1 a
CLINTON	4.7 a	1815	4.3 a	10.8 a	9.9 a	189 a	6.2 b	17.8 a
LAKE	1.2 c	3630	2.2 c	10.2 a	9.9 a	189 a	6.5 a	16.1 a
				Sweetheart				
Gi5	11.7 bc	1815	10.6 b	9.0 ab	10.4 a	223 a	4.9 ab	18.8 a
Gi6	14.5 ab	1815	13.1 ab	9.4 a	10.4 a	228 a	4.7 c	19.0 a
K5	1.1 e	1815	1.0 c	8.5 b	10.2 a	224 a	4.8 bc	20.4 a
CASS	8.7 cd	3630	15.8 a	8.9 ab	10.0 a	200 a	4.9 abc	20.0 a
CLARE	9.5 cd	3630	17.2 a	9.0 ab	10.4 a	217 a	4.9 abc	20.5 a
CLINTON	15.6 a	3630	14.2 ab	9.2 ab	10.4 a	243 a	5.0 a	19.3 a
LAKE	6.3 d	3630	11.4 b	8.7 ab	10.4 a	233 a	4.9 abc	20.3 a

[1]Fruit were harvested on the following dates: 'Regina' – July 2 and 'Sweetheart'– July 19, 2018.
[2]Fruit weight were measured from 100.
[3]Fruit firmness, row size, color were measured from 50 fruit per replicate.
[4]Brix^{o} (SSC) were measured from 15 fruit per replicate.
[5]Significantly different means ($P < 0.05$) are denoted by different letters.

Obj 1. 2017 plantings: In general, the tree sizes for 'Regina' and 'Sweetheart' on Gi5, Clinton and Crawford were similar (data not presented). The trees were notched and treated with Promalin resulting in good lateral shoot production.

Obj 2. Project team members visited the plots 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.

Obj. 3. All five MSU cherry rootstocks have been virus certified by the CPCNW-FT including testing negative for CVA, trademarked under the name Corette™, and licensed to commercial nurseries. The goal was to have virus certified genetically verified nursery stock available for limited plantings. 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 provided DNA diagnostic support as needed at no cost to the collaborating nurseries to assure rootstock trueness-to-type.

CONTINUING PROJECT REPORT **YEAR**: 3 of 3 (no cost extension approved)
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:** Karen Kniep
Telephone: 509-786-2226 **Email address:** prosser.grants@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:**

   Isolate and characterize cherry powdery mildew populations in commercial orchards in WA and OR

   i. 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:**

   Evaluate response of powdery mildew spores to fungicide sprays before fruit infection is established (visible) using viability qPCR

   i. Identify critical spray periods in which fungicidal protection is most needed to suppress onset or minimize the severity of fruit infection

Significant Findings

ADAPTATION

- Fifty-five new powdery mildew isolates were collected in 2018 in addition to 93 fungal isolates that were collected in 2017 from Washington and Oregon cherry growing locations. The focus of 2018 collection effort was to include isolates from distinct geographic locations throughout WA and OR.
- Internal transcribed spacer (ITS) region analysis in 2017 did not exhibit enough sequence (genetic) variation but in 2018, one of the isolates found out to contain genetic variability (Okanogan county isolate). ITS sequences from most of the isolates were cloned in 2018.
- Several representative isolates were used in cloning and sequencing of *Cytochrome b* gene, a target gene of QoI class (FRAC Group 11) fungicides. This served two purposes; 1. An additional gene for isolate characterization and 2. Sequence analysis of the *Cytochrome b* gene to look for any mutations that correspond to fungicide resistance.

BEHAVIOR

- Two distinct foliar mildew growth habits were discovered. Fruit mildew growth patterns were different than those found on leaves.
- We found evidence of mildew infection on leaves (most common), fruit (often seen) and fruit stems.
- Chasmothecia, the overwintering propagules were collected from all locations.

CONTROL

- We detected fungicide resistance in one of the isolates. The resistant FRAC groups included 3 (DMI) and 11 (QoI).
- Foliar and fruit mildew disease pressure was monitored throughout the season. Two distinct peaks were identified in fruit. This information may eventually be used to critically reduce mildew pressure.

Methods, Results & Discussion

Adaptation and Behavior

In addition to previous powdery mildew isolate DNA collections, mildew isolates were obtained at several new sites. During morphological analysis of mildew isolates, two distinct foliar mildew growth habits were observed. They were often present in mixed populations in most locations. Morphological observations such as conidia branching, length, and width of each conidial type were investigated. A-type mildew was identified as being highly branched conidia arrangement. The conidia were larger in size compared to B-type (Figure 2). B-type was characterized as most widely present mildew with many conidia in linear chains. Each conidiophore contains only one conidia chain. The comparison is shown in Figure 1. Each isolate collected in 2018 was morphologically characterized (Table 1).

CPM-A *Globular* CPM-B *Filamentous*

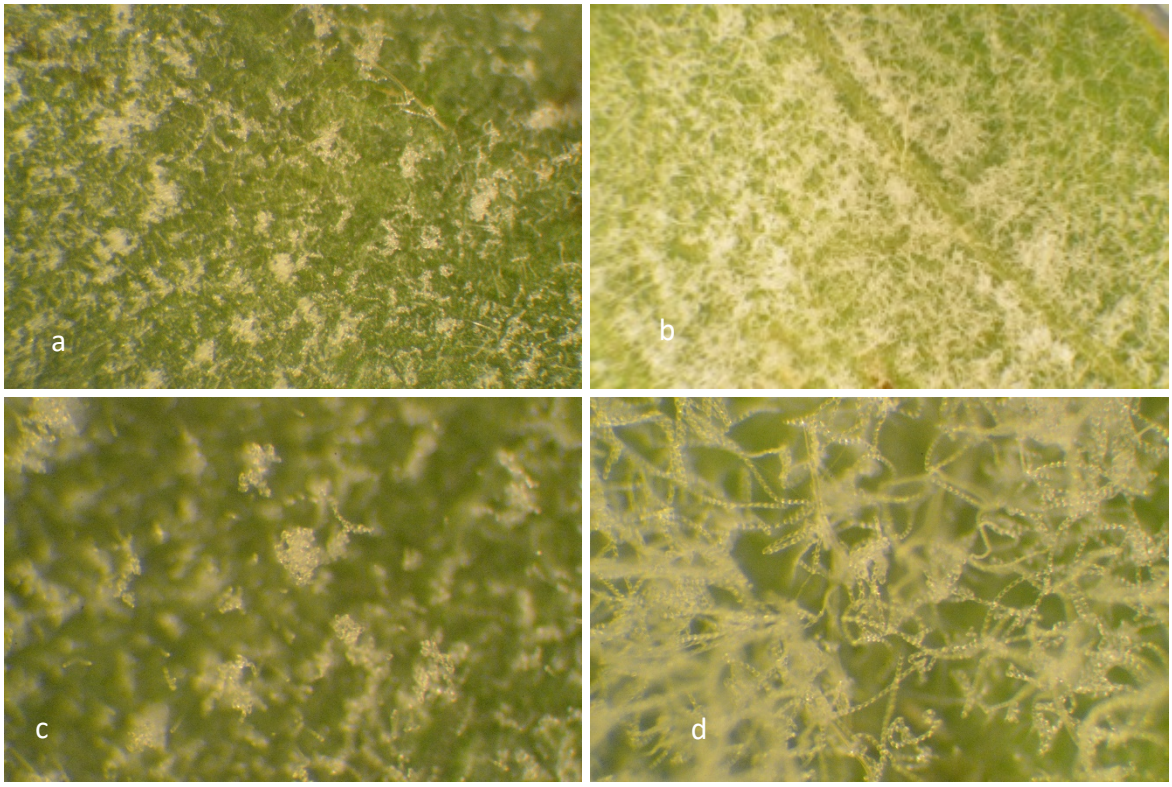

Figure 1. Two Powdery mildew growth patterns on cherry leaves. Note globular and highly branched CPM-A (a, c). Long linear chains observed in CPM-B (b, d).

Table 1. Cherry powdery mildew (*P. clandestina*) collections in 2018.

Region	Code	Host variety	Mildew type	ITS confirmation
Okanogan/ North Central Washington	BP-1	Lapins	A+B	Yes
	BP-2	Lapins	B	Yes
	OV-1	UN	B	No
	BR-1	UN	B	No
Chelan	MS-1	Bing	B	Yes
	MS-2	Bing	B	Yes
	MS-3	Bing	A+B	Yes
	MS-4	Bing	B	No
	MS-5	Bing	B	Yes
	MS-6	Skeena	B	Yes
	HF-1	UN	A+B	No
	MA-1	UN	B	No
Wenatchee	St1	Skeena	A+B	Yes
	St2	Skeena	A+B	Yes
IAREC Headquarters (Prosser, WA)	Roza-1	Bing	A	Yes
	Roza-2	Bing	B	No
	C-9	Bing	Fruit PM	No
Yakima Valley	MH-1	Lapins		Yes
	MH-2	Rainier	A+B	Yes
	MH-5	Bing	B	Yes
	MH-6	Bing	B	Yes
	MH-7	Lapins	B	No
	HT-1	Rainier	B	Yes
Columbia Basin	JP-1	Bing	A+B	Yes
	HL-1	Tieton	B	Yes
	HL-2	Santina	A+B	Yes
	HL-3	Santina	B	Yes
	HL-4	Bing	B	Yes
	HL-5	Rainier	B	Yes
	HL-6	Rainier	A+B	No
Lower Columbia Basin	DH-1	Bing	B	Yes
	DH-2	Bing	B	Yes
	DH-3	Bing	B	Yes
	DH-4	Bing	A+B	Yes
	DH-5	Bing	B	Yes
Dallesport area/ Oregon State	TP-1	Skeena	A+B	No
	SC-2	Skeena	B	Yes
	SC-4	Sweetheart	A+B	Yes
	SC-6	Rainier	A+B	Yes
	SC-7	Rainier	A+B	Yes
	AR-3	Bing	A+B	No

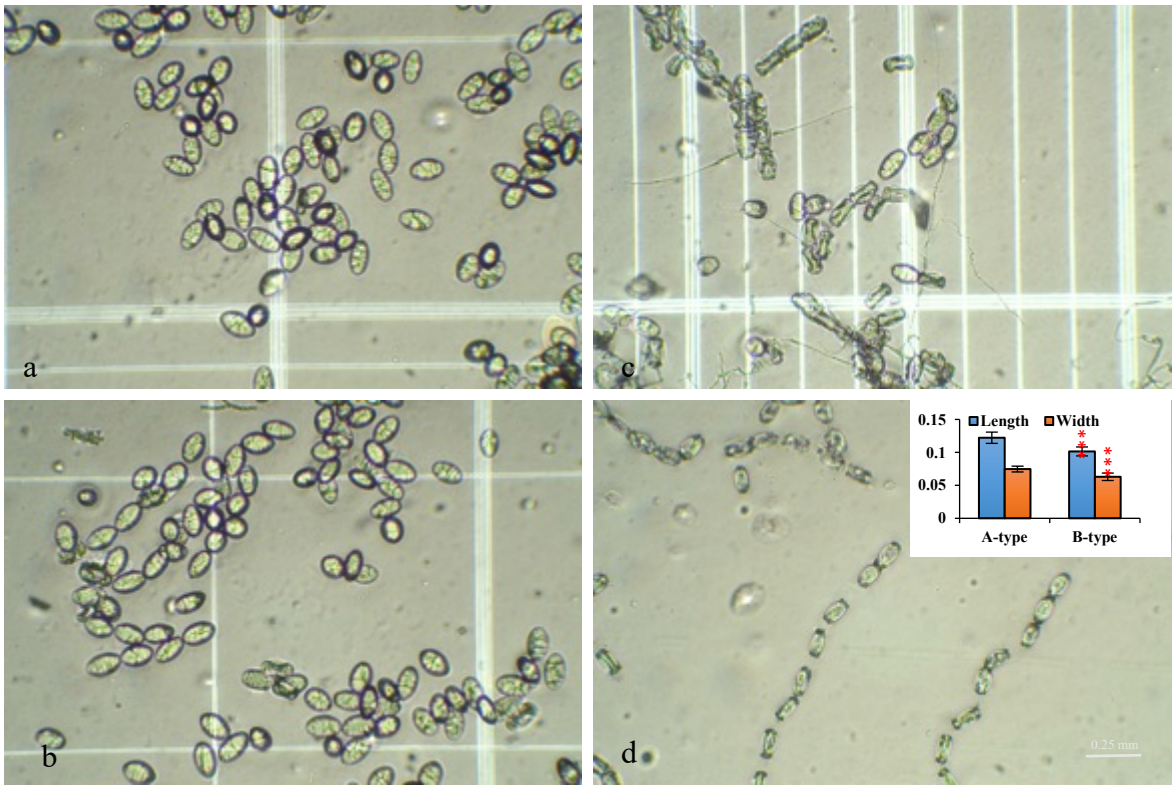

Figure 2. Growth habits in two types of powdery mildew conidia on cherry leaves. The sticky tape analysis exhibit slightly longer (and thicker) conidia of CPM-A (a, b) compared to CPM-B (c, d, e). Note the arrangement of conidia in both types. Significant differences between A- and B-type conidia are represented by asterisks (*) as determined using *t-test* ($p<0.001$).

Control

Fungicide resistance

We investigated the occurrence of fungicide resistance of a powdery mildew isolate collected at Washington State University's Roza farm in 2018. The powdery mildew was subjected to inoculations on leaf disks treated with a different group of fungicides, including control that received no fungicide. These fungicides include myclobutanil (Rally, FRAC group 3), trifloxystrobin (Gem, FRAC group 11), penthiopyrad (Fontelis, FRAC group 7), quinoxyfen (Quintec, FRAC group 13), a combination of trifloxystrobin and fluopyram (Luna Sensation, FRAC group 11 and 7, respectively), a combination of fluopyram and tebuconazole (Luna Experience, FRAC group 7 and 3, respectively), a combination of azoxystrobin and difenoconazole (Quadris Top, FRAC group 11 and 3, respectively) and, a combination of pyraclostrobin and boscalid (Pristine, FRAC group 11 and 7, respectively). Fourteen days after inoculation, the cultured leaf discs were microscopically examined for the presence of infection. Our results indicate that fungicide products containing quinoxyfen and penthiopyrad were most effective. As it may be expected, a combination of synthetic chemicals with different FRAC groups was most effective in inhibiting powdery mildew on cherry leaf discs.

In our analysis, powdery mildew colonies grew on leaf discs treated with myclobutanil (Rally), trifloxystrobin (Gem) and a combination of pyraclostrobin and boscalid (Pristine) (Table 2). Although the colonies were smaller than the untreated controls (leaf discs), they produced abundant conidia. To

confirm the resistant colonies, the colonies were re-inoculated onto fungicide-treated leaf discs and observations were made after 14 days.

Table 2. Summary of fungicide resistance assay of Roza mildew isolate.

Common name	Trade name	FRAC group	Rate of application*	Total leaf discs	Resistant leaf discs
Penthiopyrad	Fontelis	7	20 fl oz.	80	0
Myclobutanil#	Rally	3	6.0 oz.	80	5
				100	14
Trifloxystrobin#	Gem	11	3.8 fl oz.	80	22
				100	23
Quinoxyfen	Quintec	13	7 fl. oz.	80	0
Fluopyram/ tebuconazole	Luna Experience	7/3	8.6 fl. oz.	80	0
Azoxystrobin/ difenoconazole	Quadris Top	11/3	14 fl. oz.	80	0
Trifloxystrobin/ fluopyram	Luna sensation	11/7	7.6 fl. oz.	80	0
Pyraclostrobin/ boscalid#	Pristine	11/7	14.5 oz.	80	17
				100	19

*Rate of application per acre assuming 400 g spray material per acre.
#Fungicides were screened in two independent experiments.

Monitoring foliar and fruit mildew inoculum

Foliar and fruit infections (or conidial density) was monitored throughout the growing season. Ten leaves and 50 fruits were processed using laboratory protocols. Two conidial suspensions was split into two aliquots with one of them receiving PMA-treatment. After DNA extractions, quantitative PCR (qPCR) was performed using PCR primers specifically developed for short fragment of ITS gene (improvement to previous qPCR efforts). The results indicated a steady increase in live conidia on leaves while live conidia accumulated on cherries just one week before harvest (Figure 3).

Spray coverage

The spray coverage on large cherry trees was determined in the year 2018. Pyranine dye was sprayed at 400 gallons per acre. Fruit samples were collected immediately after drying. The spray coverage was noted in different canopy levels (Figure 4). Results indicate that spray coverage on large trees (the traditional type with Mazzard rootstock) was inadequate at a spray volume of 400 gallons per acre. The coverage significantly decreases at higher canopy levels suggesting a need for improvement in current spray technologies. This experiment also explains a high number of cherries being infected with mildew in the higher canopy levels.

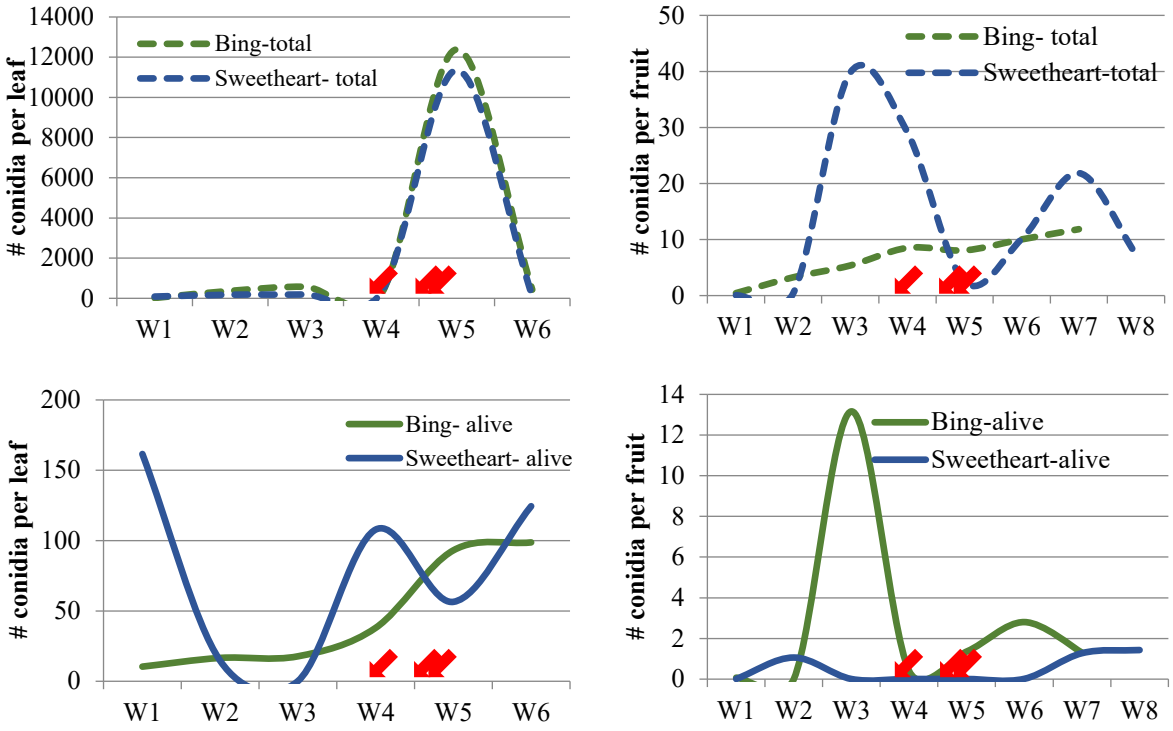

Figure 3. Conidial density of *Podosphaera clandestina* on cvs. 'Bing' and 'Sweetheart' trees. Leaves and fruits were analyzed separately from each tree. Red arrows indicate precipitation events.

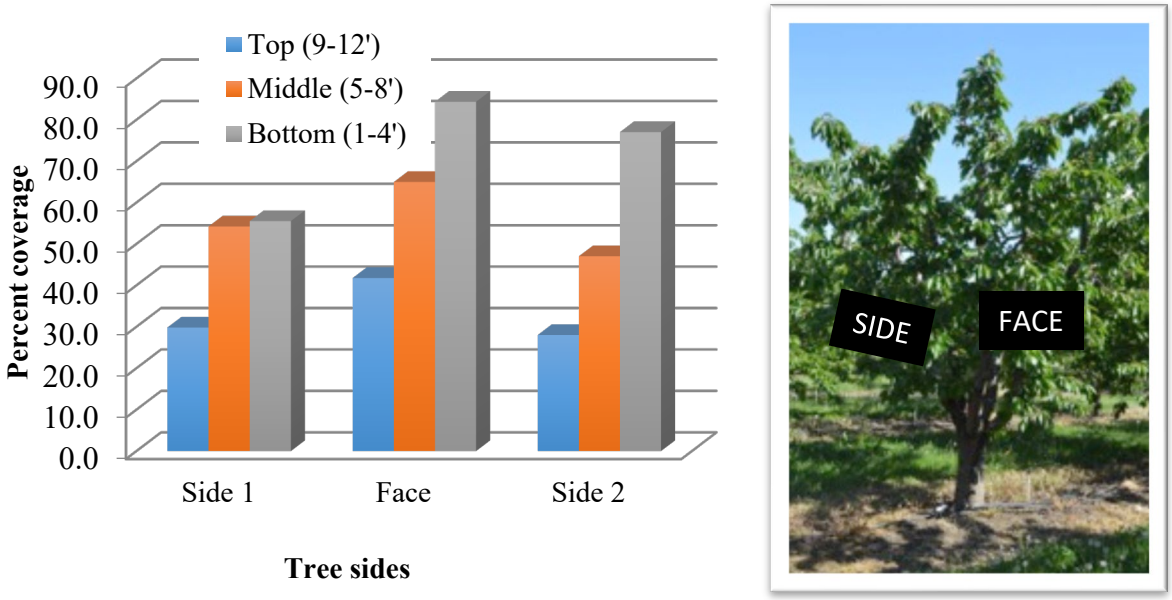

Figure 4. Spray coverage analysis at different canopy levels. Pyranine dye was quantified using images taken under UV-light and image processing was accomplished using online software tools.

Ongoing research

1. **Multi-gene characterization of Washington and Oregon mildew isolates is in progress**
2. **Next-generation sequencing is ongoing. This will enable us to identify several key genes to identify genetic variability among various powdery mildews affecting different species**
3. **Cloning, sequencing, and characterization of mating type locus (loci) are proposed to fulfill objective 2c.**
4. **Fungicide response to mildew conidia is being evaluated using qPCR and qualitative scoring methods.**
5. **Two types of mildew growth habits need further evaluations using molecular approaches.**
6. **Further investigations on spray coverage will be addressed under new collaborative projects.**

2018 WTFRC CHERRY PESTICIDE RESIDUE

For the eighth 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.

TRIAL DETAILS

- Mature 'Bing'/Mazzard multiple leader open vase trees on 10' x 20' spacing near Orondo, WA (a different block than was used in previous WTFRC studies)
- 10 insecticides/acaricides & 3 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
- Most applications made by Rears PakBlast PTO-driven airblast sprayer with 8 oz Regulaid per 100 gal water/acre; applications of 16 oz/acre Fyfanon ULV-A (malathion) made by helicopter
- Roughly 1.6" cumulative total rainfall recorded on 8 separate days during study: heaviest rain events were approximately 1.2" which fell May 17-20 (42 to 39 days before harvest), 0.2" on June 8 (20 dbh), 0.16" on June 15 (13 dbh), and 0.08" on June 25 (3 dbh)
- Samples submitted overnight to Pacific Agricultural Labs (Sherwood, OR) for chemical analysis

Airblast application @ 400 gal/acre

RESULTS & DISCUSSION

As before, this study generally 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: Bexar, Agri-Mek 0.15SEC, Mustang MAX, Baythroid XL, Danitol 2.4EC, Perm-Up 3.2EC, Carbaryl 4L, Onager
Fungicides: TopGuard, Orbit
Fertilizer: 19% potash + 33% phosphite foliar fertilizer

Moderate drip from blast zone cherries after 200 gal/acre spray

Application dates for this study are typically set by estimating the commercial harvest date and then setting the spray schedule based on label preharvest and retreatment intervals. Due to rapidly advancing fruit maturity, the grower had to pick the trial block earlier than anticipated, forcing a compression of the spray schedule for the week prior to harvest; as a result, some materials may have been applied within standard timing intervals, resulting in slightly elevated residue levels.

Concentrate applications (200 gal water/acre) produced a moderate drip in the sprayer blast zone with very little drip in the tree tops; in contrast, dilute applications (400 gal water/acre) produced a heavy drip in the blast zone and light drip in tree tops. **Pesticide residues were consistently 10-80% higher on fruit sprayed concentrate vs. dilute**; this trend corroborates the results of a 2017 apple study comparing residues from spraying at 100 vs. 200 gal/acre, but contradicts the findings of a similar cherry study in 2016, where 400 gal/acre applications generally produced higher residues than 200 gal/acre. However, that earlier cherry study was conducted in a block of larger trees where higher carrier volumes were likely needed for thorough wetting of the entire canopy. This suggests that growers seeking to optimize the efficacy of their pesticide

applications would be well served to customize their sprayer calibration to the specific needs of individual blocks rather than rely on generic assumptions regarding effective carrier volumes. We plan to repeat this study in the same trees to develop a more robust data set.

For the first time in three years of testing, helicopter applications of Fyfanon ULV-A produced a measurable malathion residue in one of four samples, but was less than Europe's stringent MRL of 0.02 ppm. For the third consecutive year, application of a potash & phosphite fertilizer produced residues exceeding the EU's MRL for fosetyl-Al; cherry growers should avoid these products if they hope to export fruit to Europe.

Measured residue levels vs. MRLs for pesticides applied in 200 vs. 400 gal water/acre with 8 oz/100 gal water of Regulaid. 'Bing'/Mazzard, Orondo, WA. WTFRC 2018.

Common name	Trade name	Application rate	Application timing(s)	Measured residue at 200 gal/acre	Measured residue at 400 gal/acre	US tolerance[1]	Lowest export tolerance[2]
		per acre	*days before harvest*	*ppm*	*ppm*	*ppm*	*ppm*
phosphite	33% phosphite fertilizer	64 oz	40, 26, 12	**19**	**11**	na	2 (EU)*
tolfenpyrad	Bexar	27 oz	26, 12	**0.76**	**0.49**	2	0.01 (many)
abamectin	Agri-Mek 0.15SEC	20 oz	19	**0.024**	**0.018**	0.09	0.01 (EU)
zeta-cypermethrin	Mustang MAX	4 oz	19, 12	**0.16**	**0.14**	1	0.1 (Can)
beta-cyfluthrin	Baythroid XL	2.8 oz	19, 5	**0.064**	**0.052**	0.3	0.01 (Tai)
spirotetramat	Ultor	14 oz	19, 5	0.020	0.014	4.5	3 (many)
flutriafol	TopGuard	14 oz	12, 5	**0.64**	**0.39**	1.5	0.01 (Jap)
metrafenone	Vivando	15.4 oz	12, 5	0.075	0.041	2	1 (Tai)
myclobutanil	Rally 40WSP	6 oz	12, 5	0.79	0.46	5	1 (Can, Tai)
fenpropathrin	Danitol 2.4EC	21.3 oz	12, 3	**1.7**	**1.4**	5	0.01 (EU)
permethrin	Perm-Up 3.2EC	8 oz	12, 3	**0.70**	**0.42**	4	0.05 (EU)
carbaryl	Carbaryl 4L	96 oz	8, 3	**7.8**	**3.8**	10	0.01 (EU)
propiconazole	Orbit	4 oz	8, 1	**0.42**	**0.27**	4	0.01 (EU)
hexythiazox	Onager	24 oz	5	**0.37**	**0.21**	1	0.1 (Kor)
malathion**	Fyfanon ULV-A	16 oz	8, 1	0.012		8	0.02 (EU)

16 July 2018. http://nwhort.org/export-manual/comparisonmrls/cherry-mrls, https://www.globalmrl.com

[2] Major export markets for Pacific Northwest cherries; 16 July 2018; 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)

** Fyfanon ULV-A applied as formulated (16 oz/acre) by helicopter

WASHINGTON TREE FRUIT RESEARCH COMMISSION

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.