Northwest Cherry & Stonefruit Research Review
Thursday, 11/12/20

Time	Page	Presenter	Project Title	Yrs
8:00		Doornink & Thompson	Welcoming statement	
8:10		Hanrahan	Meeting etiquette & housekeeping	
			Continuing & No-Cost Extension (NCE) reports 8:20 - 11:30	
8:20	1	Akbari	Engineered transgenic D. suzukii for wild population suppression	19-21
8:30	8	McCord	Supporting a robust PNW sweet cherry breeding and genetics program	19-21
	16	McCord	Equipping the re-launched PNW sweet cherry breeding program ***(NCE w/above)***	19
8:40	20	Walton	A novel attract-and-kill technique to manage SWD	20-21
8:50	27	Nottingham	Insecticidal control of leafhopper in cherry: ***(O)***	20-21
9:00	34	Harper	Understanding little cherry disease pathogenicity	20-22
9:10	41	Northfield	Identifying sources of X disease in cherry orchards	20-22
9:20	49	DuPont	Awareness and application to stop little cherry disease: ***NCE***	20
9:30	55	Northfield	Field evaluation of leafhopper controls for X disease management	20-22
9:40	62	Thompson	Rootstock sensitivity to X disease	20-21
			Break	
10:30	65	Brown	Modeling PNW sweet cherry bud phenology and cold hardiness: ***(O)***	20
10:40	72	Peace	Durable genetic solutions to powdery mildew infection in sweet cherry: ***NCE***	19-20
10:50	78	Smith	Canine detection of Western X disease in controlled and field settings: ***(O)***	19-20
11:00	85	Schmidt	Cherry pesticide residue study	20-21
11:10	88	Lee	Erythritol formulation for SWD control: field trial and mode of action: ***(Written report only)***	20
11:20	92	Grove	Fungicide resistance: a vital need to protect PNW cherries from mildew; ***NCE***	19-20
			Final project reports	
	99	Gibeaut	Development index model of sweet cherry ***(Written report only)***	19
			Stone Fruit Reports - 11:30 - 11:40	
11:30	102	Harper	Understanding decline in peach trees infected by multiple phytoplasmas: ***NCE***	19-20

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

Project Title: Engineered transgenic *D. suzukii* for wild population suppression

PI: Omar Akbari
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Total Project Request: **Year 1:** $46,609 **Year 2:** $50,946 **Year 3: $52,445**

Other funding sources: **Awarded**
Amount: Approx. $75,000
Agency Name: California Cherry Board
Notes:

WTFRC Budget: none

Budget 1
Organization Name: UC San Diego **Contract Administrator:** Susan Pastell
Telephone: 858-534-4896 **Email address:** spastell@ucsd.edu
Station Manager/Supervisor: N/A **Email Address:** N/A

Item	2019	2020	2021
Salaries	$31,555	$35,221	$36,437
Benefits	$6,383	$7,104	$7,387
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies	$8,050	$8,000	$8,000
Travel			
Plot Fees			
Miscellaneous	$621	$621	$621
Total	$46,609	$50,946	**$52,445**

Objectives: Spotted wing Drosophila, *D. suzukii*, is a major worldwide crop pest of various soft-skinned fruits. A highly promising approach to *D. suzkuii* control that could complement existing control methods is genetic pest management, which includes strategies such as gene drive and precision-guided sterile insect technique (pgSIT)[1,2]. SIT has been a successful technology for insect population suppression, which is achieved by introducing large number sterile males into a target population. While the classic irradiation-based SIT presents an environment-friendly method of a local population suppression, it is not technically feasible or scalable for the control of most insects. PgSIT, on the other hand, is a simplified way to generate sterile males and should be less expensive and labor intensive than irradiation-based SIT even at scale.

We also propose to engineered *D. suzukii* gene drive strains, which can be utilized to more rapidly 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 all of the work on an ongoing basis, it is affordable as compared to the use of insecticides, which need to be applied regularly. Finally, such an approach is environmentally friendly and entirely insect-specific and would have no effect on crops or on beneficial organisms.

Our objective is to therefore engineer *D. suzukii* gene drive strains that could be utilized as part of current integrated pest management programs to control wild *D. suzukii* populations. Specifically, out of the multiple types of gene drive systems that can be utilized in a genetic pest management program, we aim to develop a pgSIT system in *D. suzukii* using the design principles we have optimized in *D. melanogaster*[2]. We also aim to develop synthetic *Medea* elements that can be used to suppress wild *D. suzukii* populations[1]. Ultimately, our goal is to develop 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.

Objective A - Refinement of a *Medea* drive system for *D. suzukii* population suppression. We have developed a synthetic *Medea* gene drive system for population suppression[6]. Engineered *Medea* systems rely on a *Medea* element consisting of a toxin-antidote combination. 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.

We have already engineered a first-generation *Medea* system in *D. suzukii*[1], which is the first functional gene drive developed in this pest. We had rigorously tested it in laboratory cage populations, and had characterized it in different genetic backgrounds to determine effectiveness and fecundity. 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 indicates that our *Medea* drive system could spread to fixation if resistance was reduced[1], we need to engineer a second-generation *Medea* system that should obviate the specific resistance that we observed. To safeguard, reduce risk, and mitigate the spread of the *D. suzukii Medea* system into wild populations, we also aim to develop a reversal *Medea* (RM) system that can be used to replace the original *Medea* in case a recall is necessary. Finally, in order to use *Medea* to bring about population suppression, we need to link it to a cargo gene capable of killing *D. suzukii* under specific conditions to bring about a population crash. We have already identified several promising putative cargo genes and are testing them in *D. melanogaster*, a closely related species to *D. suzukii* that is easier to work with and provides a useful

testing platform for transgenes. However, we will still need to build and test them in *D. suzukii*. Successful completion of the above objectives would lead to the development of a genetically modified *D. suzukii* strain (carrying a synthetic *Medea* element) that can be mass-reared and deployed into the wild to catalytically suppress, and completely eliminate, wild populations of *D. suzukii*.

Objective B: Precision guided sterile insect technique (pgSIT) for *D. suzukii* population suppression. The sterile insect technique (SIT) is an alternative, proven pest management approach that could complement existing control methods. SIT involves the mass-production and release of sterile males, and has historically been used to control, and eradicate, insect pest populations dating back to the mid-1930s[10–14]. Traditional SIT methodologies have relied on DNA-damaging agents for sterilization, substantially reducing overall fitness and mating competitiveness of released males. A next-generation highly-efficient technology that can be used for biocontrol of *D. suzukii* is precision guided SIT (pgSIT). PgSIT functions by exploiting the precision and accuracy of CRISPR to simultaneously disrupt genes essential for either female viability or male fertility. It utilizes a simple breeding scheme requiring two homozygous strains - one expressing Cas9 and the other expressing double guide RNAs (dgRNAs). A single mating between these strains mechanistically results in synchronous RNA-guided dominant biallelic knockouts of both target genes throughout development, resulting in the complete penetrance of desired phenotypes in all progeny. We have previously built pgSIT in *Drosophila melanogaster*, a model organism that is closely related to *D. suzukii*, and shown that it is extremely robust at genetically sexing and simultaneously sterilizing resulting progeny reproducibly with 100% efficiency, and that pgSIT sterile males are fit and can compete for mates[2]. We therefore aim to develop pgSIT technology in *D. suzukii* (Objective B). Successful development of this technology would produce a genetic-based sterile insect strain that can be mass-reared and released to reduce populations of *D. suzukii* in a straightforward manner with respect to regulations.

Significant Findings:

Objective A:

- We have developed a modified version of our original *Medea* system that is designed to reduce resistance to the drive. We are currently rigorously testing this second-generation *Medea* element and planning for longer term population cage studies.
- We have 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. We are currently testing this system and planning for longer term population cage studies.
- We have identified several promising putative cargo genes that could be spread with the *Medea* gene drive to cause population suppression. Multiple genes have been tested in *D. melanogaster* as proof of principle and are now being transitioned to *D. suzukii*.

Objective B:

- Designed and injected constructs that express gRNAs targeting the female viability genes and *beta tubulin* (***β-tub***), a male fertility gene. We are expanding these lines and will test them in crosses to multiple Cas9 expression lines to determine the most efficient gRNA and Cas9 line combinations to generate sterile male progeny.
- Established six transgenic gRNA lines targeting both *sxl* and ***β-tub*** simultaneously.
- Generated homozygous pgSIT lines that consistently produce sterile males when crossed (**Table 1**).
- We engineered new sex sorting systems in *D. suzukii* to make it easier to set up pgSIT crosses.
- We developed a new pgSIT system to eliminate the need for gRNA and Cas9 crosses. We recently developed a novel Temperature-Inducible pgSIT (TI-pgSIT) genetic system and demonstrated its proof-of-concept in *Drosophila melanogaster* (**Figure 1**).

Methods:

Objective A - Refinement of a *Medea* drive system for *D. suzukii* population suppression. We have developed the first proof of concept *Medea* drive in *D. suzukii*[6]. Given our observations regarding resistance and its effect on *Medea* function, we now need to engineer improved *Medea* systems that could reduce the chances of resistance acting as an impediment to spread. So far, we have 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 are now obtaining transgenic lines for this improved *Medea* element, and preliminary data indicates that it works better than the original *Medea*, producing 100% inheritance bias. We are continuing to rigorously test this second-generation *Medea* element to characterize its function and ability to bias inheritance 100% in geographically distinct populations. We also will need to perform multiple long term multi-generational population cage experiments to determine whether this *Medea* can drive robust population replacement.

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 "reversal" *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. 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.* Once we have transgenic lines for this construct, we need to rigorously test them for their ability to bias inheritance in both wild type and original *Medea* backgrounds. We will then need to perform multiple long term multi-generational population cage experiments to determine whether this *Medea* can actually spread and replace the original *Medea*.

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. 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. Upon exposure to a critical "threshold" temperature, this cation channel can "open" and modulate Ca2+ and Mg2+ entry into the cell[16]; 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 have started 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.

Developing a field-ready strain: Similar to the other suppression drives, when we build an optimized *Medea* drive, we will also need to conduct laboratory and caged field trials to determine mating competitiveness, longevity, and fitness of these strains. This data will be used and fed into mathematical models to predict the numbers of flies we will need to release to achieve suppression.

Objective B: Precision guided sterile insect technique (pgSIT) for *D. suzukii* population suppression. In order to construct a pgSIT system, we need functional Cas9 tools (including gRNA lines that target genes essential for female viability and male sterility and Cas9 expressing lines (Figure 2) in *D. suzukii*. We have now developed multiple transgenic lines that express Cas9 (*bicC-cas9, dhd-*

cas9, vasa-cas9, nanos-cas9, ubiq-cas9). Also, essential to building a pgSIT system are guide RNA (gRNA) lines that target genes essential for female viability and male fertility. We have previously identified genes essential for female viability or male fertility in *D. melanogaster* and have shown that disrupting these genes via CRISPR/Cas9 produces the desired results (e.g., female death or conversion of females into sterile intersex individuals for the former group, male sterility for the latter. Since *D. melanogaster* is closely related to *D. suzukii*, we reasoned that disruption of these same genes would have a similar effect in *D. suzukii* and are focusing our efforts on these validated target genes. Specifically, to disrupt female viability, we are targeting several sex-specifically alternatively spliced sex-determination genes including *sex lethal* (*sxl*), *transformer* (*tra*), and *doublesex* (*dsxF*), as well as *zero population growth* (*zpg*), a germline-specific gap junction gene. So far, we have identified *D. suzukii* homologues of all of these genes and have carefully selected two gRNA target sites in each gene that are highly conserved and thus unlikely to harbor sequence variation. We have generated multiple transgenic lines for each gRNA target and we are currently in the process of crossing each one separately to our five Cas9 strains to see whether the combinations of Cas9+gRNA will produce female lethality and male sterility. So far, we have multiple gRNA lines that generate the expected 100% sterile male phenotype (**Table 1**). We are now rigorously testing these strains to ensure these results are reproducible over many replicates. We are also conducting male competition and fitness studies to ensure the sterile males are fit to compete in field conditions.

Efficient sex sorting:In order to be easily implemented, the pgSIT approach also requires the ability to efficiently separate animals by sex to set up appropriate crosses (i.e., crossing Cas9 and gRNA parents together) for sterile male generation[2] . Therefore, we are also testing a sex-specific fluorescent reporter transgene that can facilitate automated sex sorting. Specifically, we have designed a transgene that contains a fluorescent marker (dsRed) under the control of a ubiquitous promoter. This transgene includes a female-specific intron from the Drosophila *transformer* (*tra*) gene that can be processed only in female flies. Linking this intron to a fluorescent marker should generate a transgene where successful splicing and expression of dsRed occurs exclusively in females, therefore generating a system where only females express a fluorescent marker.

Developing a field-ready strain: Once all of these components are individually validated, we can proceed to assemble a single transgene that, coupled with a Cas9 strain, can be used to generate a pgSIT strain ready for use in the field for *D. suzukii* biocontrol. Laboratory and caged field trials will also be conducted on this strain to determine mating competitiveness, longevity, and fitness compared to wild flies. This data will be used and fed into mathematical models to predict the introduction frequencies we will need to use to achieve suppression. Gene drive experiments will be initiated at various introduction frequencies to characterize the population suppression dynamics. Modeling work will occur in collaboration with Dr. John Marshall (UC Berkeley), a mathematical biologist with whom we have worked on a number of modeling studies.

Since the ultimate goal here is to develop a product (a genetically modified *D. suzukii*) that can be mass-reared and deployed into the wild to suppress, and completely eliminate, the wild populations of *D. suzukii*, we will need regulatory bodies to permit such releases. In brief, we have requested a field cage study permit from USDA-APHIS BRS/PPQ. APHIS is responsible for issuing permits for the import, transit and release of regulated animals, animal products, veterinary biologics, plants, plant products, pests, organisms, soil, and genetically engineered organisms. We have a permit for a BRS 2000 (Application for Permit or Courtesy Permit for Movement or Release of Genetically Engineered Organisms), which has been uses in past and ongoing SIT programs. Some key advantages of the pgSIT approach will be that only males will need to be released (so crops will not be damaged); that it is very species-specific, since the released males will be sterile and not capable of mating with wild *D. suzukii* or any other species; and that the approach is self-limiting, which makes it a safer alternative than self-sustaining approaches and thus more likely to win public and regulatory approval.

Results and Discussion: Objective A: We have developed a modified version of our original *Medea* system that is designed to reduce resistance to the drive. 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 are currently rigorously testing this second-generation *Medea* element and planning for longer term population cage studies.

We have 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. 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. We are currently testing this system and planning for longer term population cage studies.

We have identified and are characterizing several promising putative cargo genes that could be spread with the *Medea* gene drive to cause population suppression. We are exploring 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*. However, multiple genes have been tested in *D. melanogaster* as proof of principle and are now being transitioned to *D. suzukii*.

Objective B: We generated homozygous pgSIT lines that consistently produce sterile males when crossed. We established homozygous vas-Cas9 and gRNAs lines (pure-breeding). The trans-het offspring generated from vas-Cas9 and gRNAs lines are entirely 100% sterile males (no females) **(Table 1).** To make it easier to set up pgSIT crosses, we engineered new sex sorting systems in *D. suzukii*. We designed a construct that will generate female specific red fluorescent makers (Opie2-RFPTraF). So far, we have built and injected four different plasmids with this construct and now we are screening for Opie2-RFPTraF transgenic flies.

In this reporting period, we also confirmed the efficiency of our Cas9 lines by testing the efficiency of four Cas9 lines to completely knockout target genes in both somatic and germ cells. This will take two generations and will be replicated multiple times to ensure we identify the most robust Cas9 lines for the pgSIT system.

We have also developed methods to do more comprehensive population cage studies. We developed a strategy to assess the pgSIT males population suppression using stable cage populations in the lab (fitness and competitiveness of pgSIT males). Now, we are expanding the homozygous vas-Cas9 and gRNA, as well as wildtype (wt) lines for these experiments. We also continued pgSIT fitness studies. We are in the process of setting up assays to compare the longevity of pgSIT males vs *wt* males.

We developed a new pgSIT system to eliminate the need for gRNA and Cas9 crosses. We recently developed a novel Temperature-Inducible pgSIT (TI-pgSIT) genetic system and demonstrated its proof-of-concept in *Drosophila melanogaster* (**Figure 1**). The TI-pgSIT address one shortcoming of the pgSIT, i.e. requirement to maintain two lines and their sex sorting to generated F1 eggs in the lab. The TI-pgSIT relies on the maintenance of a single transgenic line and Temperature-Inducible activation of the pgSIT system. We are now transferring the TI-pgSIT system into *D. suzukii*. Different versions of TI-pgSIT systems have been engineered and injected into *D. suzukii* embryos. We have already begn screening for TI-pgSIT transgenic lines in *D. suzukii*.

Additional Items:

References: 1. Buchman, A., Marshall, J. M., Ostrovski, D., Yang, T. & Akbari, O. S. Synthetically engineered Medea gene drive system in the worldwide crop pest Drosophila suzukii. *Proc. Natl. Acad. Sci. U. S. A.* 115, 4725–4730 (2018). **2.** Kandul, N. P. *et al.* Transforming insect population control with precision guided sterile males with demonstration in flies. *Nature Communications* vol. 10 (2019).

Triple gRNAs construct	Line #	Replicate #	Total # of F_1♀ *gRNAs/+; Cas9/+*	Total # of F_1♂ *gRNAs/+; Cas9/+*
gRNA^{Slx}, gRNA$^{\beta Tub\#1}$, gRNA$^{\beta Tub\#2}$	1, #50	14	0	158 sterile ♂
gRNA^{Slx}, gRNA$^{\beta Tub\#1}$, gRNA$^{\beta Tub\#3}$	2A, #51	8	0	121 sterile ♂
gRNA^{Slx}, gRNA$^{\beta Tub\#1}$, gRNA$^{\beta Tub\#3}$	2B, #52	5	0	44 sterile ♂
gRNA^{Slx}, gRNA$^{\beta Tub\#1}$, gRNA$^{\beta Tub\#3}$	2C, #53	6	0	78 sterile ♂
gRNA^{Slx}, gRNA$^{\beta Tub\#1}$, gRNA$^{\beta Tub\#4}$	3A, #54	6	0	105 sterile ♂
gRNA^{Slx}, gRNA$^{\beta Tub\#1}$, gRNA$^{\beta Tub\#4}$	3B, #55	4	0	63 sterile ♂

- The best fitness (#51), easy to maintain in the lab. ✓
- Lines #51 & 54 are now homozygous ✓

Table 1: Assessment of triple gRNA transgenic lines with vasa-Cas9

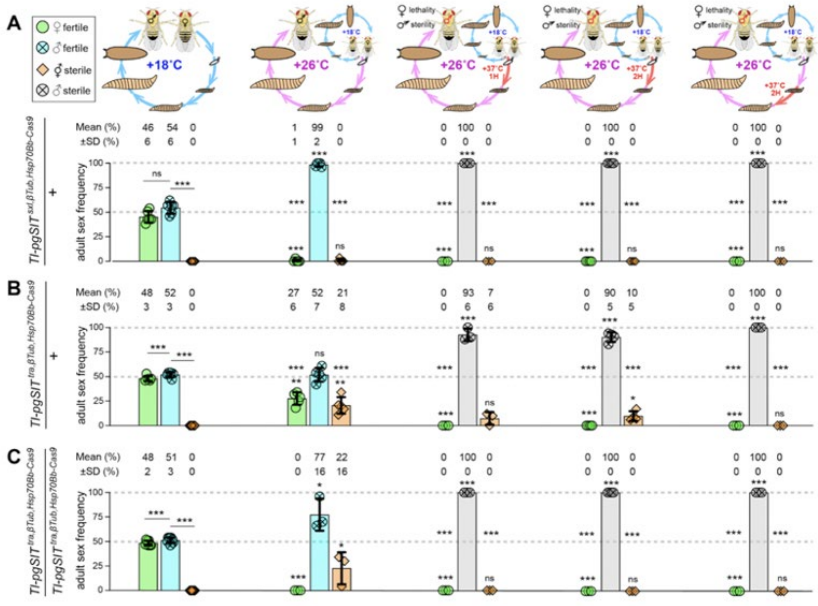

Figure 1: TI-pgSIT data from *D. melanogaster*. Temperature treatments of two single locus TI-pgSIT cassettes: *TI-dgRNA$^{sxl,\beta Tub,Hsp70Bb\text{-}Cas9}$* and *dgRNA$^{tra,\beta Tub,Hsp70Bb\text{-}Cas9.}$* These generate 100% sterile male progeny only with TI-pgSIT cassette when flies raised at 26˚C with an additional heat-shock at 37˚C during the first days of development. **This simplifies pgSIT system by eliminating need to maintain, sex separate and cross separate gRNA and Cas9 lines.**

COVID update: From March 20th, 2020 to June 1st the Akbari laboratory was shut down due to the COVID19 crisis. During this time, we were required to cease all experiments and we were only allowed to take care of minimal *Drosophila suzukii* lines. As of June 1st, the lab has partially opened allowing us to again expand our stocks in preparation for larger experiments, but we are still working at 25% capacity.

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

Project Title: Supporting a robust PNW sweet cherry breeding and genetics program

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Cooperators: Allan Bros. Fruit, Cherry River Farms, Custom Orchards, Inc. Orchardview Farms, Stemilt Growers, Breeding Program Advisory Committee (BPAC) members

Total Project Request: **Year 1:** $48,623 **Year 2:**$174,559 **Year 3: $183,584**

Other funding sources: **Awarded**
Amount: $188,165 (2019-2022)
Agency Name: WSDA Specialty Crop Block Grant
Notes: "Reducing Cold Damage in Tree Fruit". Co-PI: Matt Whiting

Awarded
Amount: $79,000 (2019, no-cost extension 2020)
Agency Name: WTFRC/OSCC
Notes: "Equipping the re-launched PNW cherry breeding program"

Awarded
Amount: $88,000 (2019-2020)
Agency Name: WTFRC/OSCC
Notes: "Durable genetic solutions to powdery mildew infection in sweet cherry". PI: Cameron Peace. Co-PIs: Per McCord, Prashant Swamy.

Awarded
Amount: $458,022 (2020-2022)
Agency Name: WTFRC/OSCC
Notes: "Understanding little cherry disease pathogenicity". PI: Scott Harper. Co-PIs: Alice Wright, Per McCord.

Requested
Amount: $88,000
Agency Name: WTFRC/OSCC
Notes: "Micropropagation and preservation of PNW sweet cherry germplasm". PI: Cameron Peace: Co-PIs: Amit Dhingra, Per McCord, Scott Harper

Requested
Amount: $310,000 (2021-2024)
Agency name: US-Israel Binational Agricultural Research and Development Fund (BARD)
Notes: "Developing phenotypic and molecular tools for breeding pitting-resistant sweet cherry cultivars". Co-PIs: Per McCord, Cameron Peace, Shaul Naschitz (Israel)

WTFRC Budget: None

Budget 1
Organization Name: Washington State University **Contract Administrator:** Katy Roberts
Telephone: (509) 335-2885 **Email address:** arcgrants@wsu.edu
Station Manager/Supervisor: Naidu Rayapati **Email Address:** naidu.rayapati@wsu.edu

Item	(2019)	(2020)	(2021)
Salaries[1]	$45,760	$37,440	$38,938
Benefits	$19,493	$16,230	$17,327
Wages[2]	$31,200	$32,450	$33,750
Benefits[3]	$10,564	$5,390	$5,606
Equipment			
Supplies[4]	$9,760	$33,325	$52,363
Travel	$4,000	$5,500	$6,100
Miscellaneous[5]	$40,000	$19,259	$2,500
Plot Fees	$4,275	$7,630	$8,800
Total	$32,387	$157,224	**$165,384**

Footnotes: [1]Includes Horticultural Support in 2019 (only), plus 1.0 FTE research technician. [2]Includes temporary labor for crossing, harvesting, seed extraction/transplanting, plus farm crew wages. [3]Reduction of benefit costs for 2020-21 reflects a more accurate estimate based on actual 2019 expenses. [4]Supplies for fruit evaluation, DNA extraction/genotyping, embryo rescue, propagation supplies/services, orchard maintenance. Amount is increased from original request as a result of more detailed expense information. [5]Irregular expenses. Amount is reduced from original request as a result of more detailed expense information.

Budget 2
Organization Name: OSU-MCAREC **Contract Administrator:** Russell Karow
Telephone: 541-737-3228 **Email address:** russell.karow@oregonstate.edu
Station Manager/Supervisor: Steve Castagnoli

Item	2019	2020	2021
Salaries[1]	$5,405	$6,005	$6,305
Benefits	$4,486	$4,985	$5,234
Wages[2]	$3,840	$3,840	$4,032
Benefits	$384	$384	$403
Equipment			
Supplies			
Travel			
Plot Fees			
Miscellaneous[3]	$2,121	$2,121	$2,226
Total	$16,236	$17,335	**$18,200**

Footnotes: [1] Estimated salary for technician to complete pruning, thinning and data collection. [2] Wages for one part-time employee ($16/hr) to assist with orchard activities. [3] Fees include per-acre research plot fees ($3104/acre), 2 months cold storage room fee ($1.24/square foot) and miscellaneous lab supplies.

Objectives

1. Build a well-trained support team to maintain and improve horticultural practices in the breeding orchard and maximize breeding efforts
2. Continue to rigorously evaluate existing selections in Phase 2 (P2) and seedlings in Phase 1 (P1). *Advance selections as warranted to Phase 3 (P3)*
3. Increase the number of targeted crosses made, seeds germinated, and seedlings transplanted
4. Enhance precocity and reduce external variation in the seedling blocks (*Delayed 1 year from 2019*).

Significant Findings

- Hired new WSU startup-funded technician in October 2020
- Continued to fertilize orchard blocks guided by soil and foliar analyses. Fertigation for new P1 plantings
- Limited testing (vs. 2019) for X-disease, Little cherry virus-2 and other viruses
    - X-disease is present, but early data suggests low level of infection
    - Additional samples collected, will be processed during the fall and winter
- Removed *Prune dwarf virus*-positive trees from A37 block
- Evaluated 169 P1 and P1.5 selections
- Advanced 2 P1 selections to P2 (1 early mahogany, 1 late mahogany)
- Evaluated 7 mahogany P2 selections (4 multi-location, 3 only at Prosser)
- Significantly expanded new P1 block at Prosser IAREC headquarters with 2,466 additional seedlings planted
- Produced an estimated 3,788 seed from 48 bi-parental crosses and 9 open-pollinated families
    - Caged crosses with orchard bees not as successful as 2019 season (poor emergence of bees)
- Determined that using ReTain to boost seed set in emasculated, hand-pollinated crosses can be effective, but is cross-specific
- Continued the use of embryo rescue for early crosses, culturing approximately 800 embryos
    - Removal of seed coat greatly reduces stratification time
    - Recovery of embryos on track to be higher than 2019 (first year attempted)
- Spring budded approximately 85 Gisela-6 rootstocks with P1 scions (2018 seedlings), with 9% take
- Budded approximately 100 Gisela-12 rootstocks in the greenhouse with newest seedlings (from 2019 crosses), but buds were too immature to take

Methods

1. Support team and horticultural practices

Dr. Juhi Chaudhary joined the breeding program in October 2020. Her primary responsibilities will be the molecular laboratory, greenhouses, and data management. Corina Serban continues to lead harvest and orchard management activities. We continue to consult with Bernardita Sallato with respect to nutrient management, and have received training from both Bernardita and Dr. Matt Whiting on pruning. Horticultural practices were implemented generally as follows:

- Nutrient management: standard practices for soil and foliage analysis to guide fertilizer applications. As for last year's P1 planting, a double drip system (micro-sprinklers and drip tape) was installed in the 2020 P1 planting to allow fertigation.
- Weed control was maintained through a combination of mowing, herbicide spraying, and manual weeding.

- In the absence of a dedicated orchard manager, spray recommendations were provided by a commercial crop consultant (Jeff Sample) and implemented by the farm manager and his crew after consulting with Dr. McCord.
- The Hood River (MCAREC) and Sagemoor blocks were pruned during the winter, as well as portions of the Roza (RosBREED, younger P1s, P2s, and main parental blocks). Summer pruning was undertaken at the Roza (particularly the younger P1s and RosBREED blocks), and last year's P1 planting at IAREC headquarters.
- Thinning to ~30 fruit/foot was performed as needed on P2 selections and standard cultivars.
- Virus monitoring and control: Three trees displaying symptoms of little cherry disease (LCD) were sent to the Clean Plant Center NW (CPCNW) for diagnosis. Fifteen additional samples from parental trees were also screened for LCD by CPCNW. Following training on LCD symptom recognition, pedicel samples were taken from 337 trees throughout the P1, P1.5, P2, RosBREED, and parental block B53. In addition, 164 trees in the RosBREED and B53 blocks were sampled for *Prune dwarf virus* (PDV).

2. *P1 and P2 evaluations*

As in prior years, BPAC members were invited to inspect P1 seedlings during the fruiting season. Walkthroughs were conducted 1-2 times per week, with BPAC members visiting once per week. Selection criteria in the field was based on fruit size, firmness, and flavor. Fruit from selected P1 seedlings, all current P2 selections, and standard cultivars were evaluated in the laboratory for defects (harvest and post-harvest), weight, diameter, firmness, stem pull force (P2 only), color, Brix, and titratable acidity.

In order to maximize efficiency, P1 selections that did not meet the thresholds of weight (minimum 9 grams) or firmness (minimum 270 g/mm) generally were not evaluated for downstream traits. An 'induced pitting' protocol for post-harvest analysis was implemented by putting fruit in a bucket on an orbital shaker platform for 3 minutes at 200 rpm. When sufficient fruit was available, we also performed an induced cracking test based on a 4-hour soak in deionized water. Fruit sampled for post-harvest analysis was placed in modified-atmosphere packaging and stored in a walk-in cooler for 4 weeks at approximately 35°F.

3. *Crossing and seedling production*

Our goal is to produce 10,000 seed annually with an overall germination of 50%. In 2020, the majority of crosses were made using emasculated, hand-pollinated blossoms. Pollen was tested for viability by an *in vitro* germination screen. Crosses were also made with caged trees and either orchard bees (*Osmia ligaria*) or honeybees. Some seed was also collected from open-pollinated trees with desired characteristics (primarily early ripening and fruit size). The experiment from 2019 testing the effects of ReTain (aminoethoxyvinylglycine) was repeated. ReTain was sprayed on flower buds either 1 or 2 days before pollination. For flowers being pollinated the following day, emasculation occurred the same day as the Retain treatment. For flowers being pollinated 2 days later, emasculation occurred the day after product application. A control group of flowers on each tree in the experiment was left unsprayed. Fruits were counted in May, once it could be reliably determined which flowers had set fruit.

Fruits were harvested before full maturity. The pits were cracked with anvil pruners to extract the seeds, which were then soaked in a 10% bleach solution for 10 minutes, followed by 2-3 rinses with deionized water. Seeds were dried briefly on paper towels, then placed into zipper lock plastic bags. Seeds were dusted with Captan fungicide, and moist vermiculite was added to the bags. Seeds were

stratified in a walk-in cooler until germination occurred (3-5 months), and then planted in a soilless potting mix in Ray Leach Cone-Tainers.

As in 2019, embryo rescue was utilized for early-ripening crosses, as well as a small number of interspecific crosses. Fruits were sterilized in a solution of 70% ethanol, with a few drops of dish detergent as a surfactant. A pair of anvil pruners was used to open the fruit and the stone to extract the seed. For the majority of embryos, the seed coats were also removed. Embryos were cultured in McCown's woody plant medium (WPM) supplemented with myo-inositol, sucrose, and agar as a gelling agent. The embryos were cultured in the dark in a walk-in cooler. Embryos were checked regularly for germination, and germinated embryos were moved to an LED light cart. Seedlings were transferred to a soilless potting mixture once true leaves and a well-developed root system were observed. Seedlings were acclimated in plastic boxes inside a greenhouse. After approximately 1 week, the boxes were opened for several hours each day. After approximately 2 weeks, the boxes were left open and acclimation was complete.

For seedlings from the 2019 crosses, DNA was extracted from dried leaf tissue and sent to Cameron Peace's lab in Pullman for DNA testing. DNA tests included self-fertility (S4') and powdery mildew resistance. Surviving seedlings were planted in the field in two groups. The main group was planted in May, and a smaller group was planted in early September. Row spacing was 12 feet, with 4 feet between plants. 'Black Pearl' and 'Skeena' were planted at intervals (generally 1 pair of trees at the head of alternating rows) to serve as standards for ripening time.

4. *Enhancing precocity and reducing variation in seedling blocks*

In the winter of 2020, approximately 100 Gisela-12 rootstocks were budded, using greenhouse grown 2019 seedlings as scions. The more mature wood from the base of the seedling was used as budwood. Separately, in March 2020, approximately 85 Gisela-6 rootstocks were budded in the field, using budwood from field-planted 2018 seedlings as scions. The budwood had been collected during the winter and stored in plastic bags in a freezer at approximately 28 °F.

Results and Discussion

1. *Support team and horticultural practices*

Dr. Juhi Chaudhary joined the CBP in October 2020, replacing Michael Stein who left at the end of June. Dr. Chaudhary has significant laboratory experience in the context of a breeding program, and she will be able to leverage her experience to increase the efficiency, accuracy, and throughput of the CBP. Corina Serban has strengthened her horticultural knowledge and expertise through both experience and continuing education. As a result, her ability to manage an ever-expanding orchard footprint has been enhanced. Through Ms. Serban's efforts and close coordination with our crop consultant (Jeff Sample) and the IAREC farm crew, we have been able to maintain good orchard management practices. Control of insect pests and powdery mildew was acceptable, and irrigation of established plantings was done in a timely manner. The new planting of seedlings from 2019 crosses suffered some transplant shock in spite of cool and cloudy weather at planting time. We will continue to improve our ability to get drip tape installed as soon as possible on new plantings. The number of seedlings planted in 2020 (2,488) was more than three times the number planted in 2019 (752).

Of the 18 samples submitted to CPCNW for LCD screening, three came back positive, all for X-disease phytoplasma. The pedicel samples collected by the CBP team for LCD screening have not been analyzed due to the presence of PCR inhibitors in the DNA extractions. We will resume analysis of these samples after the DNA has been further purified. The samples collected for PDV screening have been extracted and will be analyzed during the fall/winter via real-time PCR.

2. *P1 and P2 evaluations*

A total of 169 P1/P1.5 selections passed field criteria and were evaluated in the laboratory. Two of these have shown good performance over at least two seasons and are being advanced to P2. Performance characteristics of these two selections are in Table 1. Seven P2 selections were evaluated in 2020. Characteristics are in Table 2. Of this group, R19, R3, and R29 were all advanced last year to a Phase 3 trial in at least one location. While R19 has good harvest timing, good size (for timing), and excellent flavor and firmness, our data suggest problems with rain-induced cracking and decay in storage. The CBP does not currently use a fungicide treatment in its post-harvest analysis, so it is possible that this is a manageable defect. Because of its early ripening, rain covers may be an acceptable management tool to limit cracking. We will need to see how this variety does in the Phase 3 trials. While R3 has been considered an early selection, it was harvested this year only 1-3 days before 'Bing' at both Prosser and Hood River. R29 continues to yield very large fruit, as does R17. R29 has the advantage of being self-fertile. Both varieties, however, are mid-season, which is their greatest drawback, and there has been limited interest from BPAC members. The remaining P2 selections (R45-47 and R50) are currently only in Prosser. They will be part of a full multi-location trial in 2021. Moving forward, higher quality data from P2 trials will allow for more confidence in evaluating selections. For the new P2 trials, all plots will be randomized, and we have increased the number of trees per selection which will allow for replication using plot sizes of several trees per plot. The P2 site in Prosser will be relocated from the Roza to IAREC headquarters. We will also be adding an additional P2 trial site near Naches. Finally, we are following BPAC recommendations to include modern varieties as standards. 'Chelan', 'Bing', and 'Sweetheart' will continue to be planted in small numbers (primarily for harvest timing comparisons), but we will also include 'Benton' and 'Skeena'. We will continue to use 'Rainier' as the standard for blush selections.

Table 1. Characteristics of 'FR09T084' (vs. 'Chelan') and 'CR11T019' (vs. 'Sweetheart'), P1 selections advanced to P2. Both selections are mahogany. Unless otherwise noted, data are the average of 2 seasons (2019 and 2020).

ID	Timing	Fruit Weight (g)	Row Size/Diameter (mm)	Firmness (g/mm)	Brix/TA	Notes
FR09T084	Bing -10	9.7	9.5/27.6	352	18.7[1]/0.58[1]	Low field cracking (2020)
'Chelan'	Bing -~10	7.7	11/24.2	257	18.4/0.71	
CR11T019	Bing +26	9.6	9.5/27.6	335	23.1[2]/0.72[2]	
'Sweetheart'	Bing +18	7.8	11/24.1	379	23.2/0.55	

Footnotes:[1]2020 data. [2]2019 data.

Table 2. Characteristics of Phase 2 selections, including those currently only at Prosser. Except where noted, data is averaged across all available years and locations, including multiple picks. Prosser-only selections will be planted in multi-location P2 trials in 2021.

ID	Timing[1]	Fruit weight (g)	Row size/Diameter (mm)	Firmness (g/mm)	Brix/TA[2]	Notes	Status
R19	Bing -10	9.2	9.5/27.7	353	23.0/0.60	Rain cracking, post-harvest decay, bird-susceptible	In Phase 3

R3	Bing -5	10.4	9.5/27.9	332	20.6/0.47	Good texture	”
R29	Bing +5	12.3	9/29.7	307	20.9/0.35	Very large, self-fertile	”
R17	Bing +6	11.1	9.5/28.3	289	22.3/0.55	Very large, low stem pull force	Evaluate 1 more year
R45	Bing +16[3]	10.0	9.5/27.7	280	23.8/0.43	Low stem pull force	Full P2 2021
R46	Bing +2[4]	9.8	N/A	250[4]	20.8/0.69		”
R47	Bing -2	10.6	N/A	359	22.2/0.74	Very firm	”
R50	Bing +17	10.4	9.5/28.5	308	21.6/0.58	Not harvested in 2020 (small fruit)	”

Footnotes: [1]Where multiple picks occurred in a given year, the harvest date was chosen as the average of pick dates for that year. [2]TA data from 2019 and 2020 only. [3]Picked late (over-mature) in 2019. [4]Picked over-mature in 2020.

3. *Crossing and seedling production*

As in prior years, cross combinations were guided by DNA information and phenotypic performance. Major targets for crosses made included early/late maturity, fruit size and firmness, self-fertility, and powdery mildew resistance. As more information becomes available for potential parents, crosses will also be made targeting resistance/tolerance to LCD. Germination testing of pollen was effective in identifying and eliminating potential parents with sparse or weak/non-viable pollen.

Seed production for 2020 was approximately 3,800, significantly less than the nearly 7,000 seed produced in 2019. Frost damage did play a role, and COVID-19 labor restrictions limited the number of crosses we were able to make, but the largest factor was the poor performance of the orchard bees. The bees were used very successfully in 2019 (2667 seed from 9 crosses), and we intended to expand their use in 2020. However, we had very poor emergence of the bees from their cocoons, and only produced 1022 seed from 9 caged crosses (one additional cross was made with both honeybees and mason bees). We are hopeful that an upcoming orchard bee workshop will provide information on proper management of these bees. The majority of seed (64%) was produced from bi-parental crosses.

We repeated the experiment using ReTain (aminoethoxyvinylglycine) which was first conducted in 2019. Statistical analysis showed no overall treatment effect. However, when the effects of ReTain were compared within individual crosses, the picture was much more complex. In 2019, spraying ReTain 2 days before pollination (ReTain-2) significantly increased fruit set vs. control in two crosses (out of five attempted), while spraying 1 day before pollination (ReTain-1) actually decreased fruit set in three crosses (out of eight attempted). In 2020, ReTain-2 increased fruit set in three crosses (out of eight), while ReTain-1 increased fruit set in five crosses, and decreased fruit set in two crosses (out of nine). We conclude that ReTain can be effective in increasing fruit set in emasculated, hand-pollinated crosses, but it must be tested on a cross-by-cross basis. As these types of crosses will continue to be an important part of the CBP, we will continue efforts to test the effects on fruit set of other plant growth regulators as well as varying rates of ReTain.

Germination and emergence of seed continues to be a challenge. As stated earlier, our goal is 50%. Based on 2019 results (the most recent year of complete data), the program produced 6971 seed

resulting in 2863 seedlings, or 41%, which is significantly greater than 2018, which was approximately 25%. We have learned the importance of fungicide and proper moisture levels during stratification (which affect germination per se), and we are actively seeking strategies to improve emergence of sown seed.

We continue to improve our use of embryo rescue for early ripening crosses. Although we performed about the same number of rescues as for 2019 (approximately 800), our recovery rate was higher. In 2019, we recovered approximately 260 embryos (33%), while in 2020 we have already acclimated and tissue sampled 254, with at least another 150 undergoing acclimation (estimated recovery 51%). Harvesting the fruit sooner and removal of the seed coat may have contributed to the higher recovery. Seed coat removal did result in shorter stratification times. As a result, the majority of our embryo rescue seedlings have been planted before the conventional seedlings, rather than afterward. Moving forward, we will investigate treatments to further improve recovery, including with interspecific crosses.

4. *Enhancing precocity and reducing variation in seedling blocks*

We attempted to bud Gisela-12 rootstocks in the greenhouse during the winter, using greenhouse-grown seedlings (2019 crosses) as scions. Despite using the more mature wood at the base of the seedling, the buds were not sufficiently developed, and none took. Our attempt to spring bud Gisela-6 rootstocks in the field using winter-collected wood from 2018 crosses was also not very successful, with a bud take of only 9%. Additional practice should increase our bud take, and we will also attempt to produce more mature budwood on greenhouse grown seedlings by giving them a cold treatment after approximately three months' growth. We are also testing training systems to see if we can enhance the precocity of seedlings on their own roots.

In summary, the CBP continues to make progress in its objectives. Our team has enhanced its skill set, and horticultural management is improving. We continue to advance selections through the program. Our expansion of the seedling blocks has re-started the breeding pipeline, and our improved P2 design should yield higher quality data to guide crucial advancement decisions. Despite the challenges of the season, we produced an acceptable crop of seedlings primarily from data-driven bi-parental crosses and have improved our results with embryo rescue for early-ripening crosses. We continue to seek methods (via rootstocks and training systems) to enhance precocity in our seedling blocks. Our progress towards our objectives will yield results in the form of an accelerated pipeline of superior sweet cherry cultivars for Pacific Northwest growers.

CONTINUING PROJECT REPORT **YEAR**: No-Cost Extension

Project Title: Equipping the re-launched PNW cherry breeding program

PI: Per McCord
Organization: WSU Dept. Horticulture
Telephone: 509-786-9254
Email: phmccord@wsu.edu
Address: WSU-IAREC
Address 2: 24106 N. Bunn Rd
City/State/Zip: Prosser, WA 99350

Cooperators:

Total Project Request: **Year 1:** $79,000

Other funding sources: **Awarded**
Amount: $406,766 (2019-2021)
Agency Name: WTFRC/OSCC
Notes: "Supporting a robust PNW sweet cherry breeding and genetics program". Co-PIs: Cameron Peace, Bernardita Sallato, and Steve Castagnoli

Awarded
Amount: $188,165 (2019-2022)
Agency Name: WSDA Specialty Crop Block Grant
Notes: "Reducing Cold Damage in Tree Fruit". Co-PI: Matt Whiting

Awarded
Amount: $88,000 (2019-2020)
Agency Name: WTFRC/OSCC
Notes: "Durable genetic solutions to powdery mildew infection in sweet cherry". PI: Cameron Peace. Co-PIs: Per McCord, Prashant Swamy.

Awarded
Amount: $458,022 (2020-2022)
Agency Name: WTFRC/OSCC
Notes: "Understanding little cherry disease pathogenicity". PI: Scott Harper. Co-PIs: Alice Wright, Per McCord.

Requested
Amount: $88,000
Agency Name: WTFRC/OSCC
Notes: "Micropropagation and preservation of PNW sweet cherry germplasm". PI: Cameron Peace: Co-PIs: Amit Dhingra, Per McCord, Scott Harper

Requested
Amount: $310,000 (2021-2024)
Agency name: US-Israel Binational Agricultural Research and Development Fund (BARD)
Notes: "Developing phenotypic and molecular tools for breeding pitting-resistant sweet cherry cultivars". Co-PIs: Per McCord, Cameron Peace, Shaul Naschitz (Israel)

WTFRC Budget: None

Budget 1
Organization Name: Washington State University **Contract Administrator:** Katy Roberts
Telephone: (509) 335-2885 **Email address:** arcgrants@wsu.edu
Station Manager/Supervisor: Naidu Rayapati **Email Address:** naidu.rayapati@wsu.edu

Item	2019	2020	2021
Salaries			
Benefits			
Wages			
Benefits			
Equipment	$79,000		
Supplies			
Travel			
Miscellaneous			
Plot Fees			
Total	$79,000	0	0

Footnotes:

Objectives

The Pacific Northwest sweet cherry breeding program (CBP) was re-launched with the hiring of Dr. McCord in April 2018. This is a request to augment the investment the university has made to outfit the program with the equipment and infrastructure needed for a successful breeding and genetics program, namely:

1. A laminar flow hood and refrigerated incubator for embryo rescue of seedlings from crosses targeting early ripening, a major priority of the CBP and PNW cherry growers.
2. A tissue grinder to allow for high-throughput DNA extraction for marker-assisted selection of seedlings.
3. A hoop house or similar structure to allow for crosses to be made indoors, which increases flexibility and provides protection from frost and disease vectors.

Results and Discussion

1. *Flow hood and incubator*

   As a refrigerated incubator was determined to be unnecessary for embryo rescue, two flow hoods were purchased instead to allow for increased throughput. Embryo rescue was initiated as part of the 2019 crossing season and continued in 2020. Approximately 260 embryo-rescued seedlings were produced in 2019/20, and we are on track to produce over 400 such seedlings in 2020/2021. Having two hoods will allow for expansion of the program, and for the time-sensitive operation to be completed more quickly.

2. *Tissue grinder*

   Since the grinder was installed in spring 2020, we have utilized it to process more than 1,200 samples, supporting projects in fruit quality DNA marker development, genetic diversity of a crop wild relative, and pathogen detection. Beginning this fall, it will be used for processing the 2020 crop of seedlings (1,300-1,900 depending on germination), prior to running DNA tests for marker-assisted seedling selection (MASS). Since the relaunch of the CBP, MASS has been used to eliminate 7-19% of seedlings prior to transplanting, saving important time and resources.

3. *Crossing hoop house*

   The framework for a 30 x 96-foot poly-covered hoop house was donated in 2019, but delays in developing the scope of work in-house led to a no-cost extension (NCE) for 2020. The scope of work was submitted to the new IAREC facilities manager in October 2019. COVID-19 precipitated construction delays, necessitating a second NCE for 2021. The framework of the house has been erected (see photo below), and completion is expected by late 2020/early 2021. In anticipation of the hoop house's construction, we have begun assembling a parental collection of trees in pots, a number of which will begin flowering in spring 2021. With proper management, the trees should be small enough to be moved around the house to pair for crossing as desired yet produce sufficient fruit to generate reasonable family sizes for breeding. The trees will be protected from frost and disease vectors, and the smaller size of the trees will reduce or eliminate the need for ladders.

In summary, the equipment and improvements funded by this request have already made an impact on the work of the CBP, and this impact will continue well into the future. Embryo rescue is an important tool for generating early-ripening varieties, which are critical to the profitability of PNW cherry growers. The throughput for sample processing provided by the tissue homogenizer allows for thousands of seedlings and mature trees to be analyzed for important DNA tests and screened for diseases of concern. And the ability to make targeted crosses more flexibly and in a protected environment should allow the CBP to generate more seedlings of superior quality, resulting in superior new varieties for the industry and consumers.

CONTINUING PROJECT REPORT **YEAR: 2020**

Project Title: A novel attract-and kill technique to manage Spotted-Wing Drosophila

PI: Dr. Vaughn M. Walton
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Co-PI (3): Steve Castagnoli
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Contact information: Vaughn Walton, vaughn.walton@oregonstate.edu, 541-740-4149

Total Project Request: **Year 1:** $63,778 **Year 2: $65,422**

Other funding sources: None
WTFRC Budget: None

Budget 1:
Organization Name: Oregon State University **Contract Administrator:** Charlene Wilkinson
Telephone: 541-737-3228 **Email address:**charlene.wilkinson@oregonstate.edu

Item	2020	2021
Salaries	15,000	15,450
Benefits	9,375	9,656
Wages	14,000	14,420
Benefits	1,000	1,030
Equipment		
Supplies	9,443	9,726
Travel	6,000	6,180
Miscellaneous		
Plot Fees	8,960	8,960
Total	63,778	**65,422**

Footnotes:

OBJECTIVES

Spotted-wing drosophila (SWD) has emerged as a major pest of cherry since its establishment in the Pacific Northwest (PNW) in 2009. Due to the lack of effective alternatives, insecticides have been the mainstay of SWD management programs for PNW cherry growers. Multiple factors associated with reliance on insecticides (high cost, potential for resistance development, disruption of natural enemies, MRLs, etc.) make development of alternative approaches to SWD management imperative.

Behavioral controls offer an alternative to insecticides, especially if they can have a longer-term impact on SWD pest populations. Historically, attractant studies have focused on fruit blends, which are believed to outcompete synthetic blends. Several commercially available lures focus on attraction based on fruit-derived volatiles, while little attention has been given to the manipulation of oviposition behavior (Haye et al. 2016; Cloonan et al. 2019). There are several technologies making use of behavior manipulation to control insect pests (Lee et al. 2011; 2013; Iglesias et al. 2014; Evans et al. 2017; Kirkpatrick et al. 2017), including a recently developed novel, pesticide-free behavioral disruptor technology, which can compete with ripening fruit in modulating the SWD oviposition behavior (Tait et al. 2018; Rossi-Stacconi et al. 2020). Previous laboratory and field tests on cherry and soft-skin fruits showed that the disruptor technology, which is composed of a proprietary matrix, causes an alteration of SWD behavior. This alteration has been described as an arrestant, with the altered behavior ultimately resulting in SWD adults being sequestered and arrested close to and on the matrix.

The goal of this proposed project is to develop an effective attract-and-kill (A&K) technology for SWD for PNW cherry growers that are effective under field conditions beyond 21 days. Our trials will provide direct comparisons with the current grower standard. We anticipate the A&K technology will allow growers to reduce insecticide use by ~50%. The A&K technology will significantly reduce costs, with estimated savings ranging from 40-60%, but also other negative consequences of current practices, genetic resistance for instance (Gress & Zalom et al. 2019).

1) Evaluate multiple conventional and organic toxicants in combination with the arrestant under *laboratory* conditions in order to create an A&K tool for SWD.
2) Validate the new A&K formulation under *greenhouse conditions*.
3) Conduct long-term (21-day and beyond) *open-field efficacy trials* of the refined A&K tool, grower-standard (GS) pesticide applications, and integrated (INT, reduced insecticide reliance) as a direct comparison. We will assess the efficacy of this technology through fruit damage levels in cherries.

SIGNIFICANT FINDINGS

- We found in laboratory tests that the use of pesticide can be reduced by ~2000X when using the arrestant in combination with Entrust.
- Similar levels of fruit protection and SWD mortality was found under controlled laboratory conditions in comparison with insecticide only.
- In Hood River we found a trend of lower damage levels under open field conditions during the experimental period between untreated Control plots and Arrestant plots.
- In Salem, we found a significant reduction (~90% and ~50%) in damage levels in Buffer and A&K plots during the experimental period.

METHODS

1) Laboratory evaluation of toxicant/arrestant combinations. This experiment consisted of three treatments: Berries dipped in pesticide (a), Attractant treated with pesticide (b), and the untreated control (c).

a. Berries dipped in pesticide. We tested four toxicants: Spinosad, Grandevo, Erythritol and Venerate in order to allow for direct comparison with the A&K solution. Here, concentrations of insecticides were conducted at field rate (Al/ha): Entrust 105.4 g (Al/ha), Grandevo 1,005.9 g (Al/ha), Venerate 17.7 kg (Al/ha) and Erythritol (1.75 M) (Al/ha).
b. Attractant treated with pesticide. To obtain the optimal A&K formulation, the arrestant was combined with the four toxicants trialed above. Each toxicant was tested by mixing it at the equivalent of ~1/2000th of field rate.
c. Untreated control. No pesticide or A&K.
Pesticide-dipped berries and the A&K formulations were allowed to dry (1-1.5 hours) before they were placed within the test arenas.

The egg laying/mortality tests were conducted in the laboratory (72 ± 2 $^{\circ}$F, 62 ± 8 % R.H., and 14:10 L: D photoperiod) using 20 arenas (6-7 treatment containers per treatment) using ½ gal transparent Griffin-style graduated low-form plastic beakers (Nalgene, Rochester, NY), each with 9 ventilation holes (1/2 inch diameter) (Tait et al. 2018). The holes were covered with fine white mesh in order to prevent SWD individuals from escaping. The top of each beaker was drilled and were connected to a 0.5 cm diameter plastic tube providing a vacuum in order to create a constant and uniform air flow (1.5 L min^{-1}) within the containers. Beakers were placed upside down on a flat surface covered by white paper sheets. We placed three berries and one 3 oz plastic cup (Dart Container Corporation, Mason, MI) containing 3 gm of the A&K formulation containing each of the respective toxicants. Inside the arena, a ball of cotton soaked with water were provided as hydration to the test flies. Each container had ten mated females and ten mated males aged between 7 and 12 days. At 24h after the initiation of the experiment, the berries were inspected for ovipostion, and the number of eggs per berry and dead insects were reported. Untreated Control treatments (treatment c) consisted only of three berries inside each arena. For the cerries dipped in pesticide (i.e Entrust, treatment a), or the attractant treated with pesticide (treatment b), the residual effect of the toxin in the attractant was monitored for 6 days by recording the oviposition in the berries and the number of dead flies. For treatments a and b each container also had three berries.

2) Controlled greenhouse evaluations. These experiments were not conducted during 2020 because of COVID 19 restrictions during this period.

3) Field evaluations 2020.
Hood River: We conducted field trials to determine field-efficacy under standard commercial cherry production conditions at the Mid-Columbia Agricultural Research and Extension Center (MCAREC, 45°68'515''N, 121°51'67''W). There were two treatments with the experiment conducted over a period of 35 days starting on June 16 through July 22, 2020.
1) Untreated control (UTC). No insecticide was applied during the duration of the experiment.
2) Attract-and-kill (A&K). A&K treatments applied at day 0 (June 16), at the rate of 50 per acre. No additional chemical treatments.
There were 8 plots for the two treatments, each ~0.18 acres in size (~41 trees, Regina cv. sweet cherry each) within a total orchard of 2.8 acres (8 plots left blank). Each A&K plot therefore received a total of 8 dispensers. All plots were assigned in a gridded pattern for each treatment. Because volatile plumes from the A&K plots can be influenced by air movement, UTC plots will be situated upwind from the A&K plots to minimize interference caused by drifting volatile plumes originating from the dispensers placed in those plots. At first fruit color, we did supplemental releases of SWD in each plot. We released 200 mated 8-12 day-old SWD in the center of each plot (800 total) on a weekly basis in order to create a relatively even distribution of populations. These populations were released four times, on June 23, July 1, July 8, and July 15 of the experimental period. We collected cherries once per week. Each collection contained 10 cherries, respectively from the lower (3 ft), middle (5ft) and high (7ft) portion of the central two plants in each plot (30 per plant, 60 total per plot and 240 per

treatment). Assessment of oviposition was determined considering the number of eggs laid per berry and percent of infected berries.

Willamette Valley: Field trials were conducted at a grower orchard (8-year-old Regina cv. sweet cherry) in Salem, Oregon from June 23-July 21, 2020, with the last sampling date concluding at harvest. There were three treatments and the experiment was conducted over a period of 29 days. There were five evaluation dates i.e. June 23, 29, and July 12 and 21. Five berries each were collected from each of four trees within the center of each of the experimental plots (total 20 berries collected from each experimental plot). Experimental plots were ~0.2 acres each, replicated ten times within a randomized block design (30 plots total, 6 acres total). Plots were oriented so that A&K were downwind from the prevailing wind direction.
1) Grower standard (GS) (2 acres total). Two insecticide applications i.e. Rimon/Delegate were applied on all plots (GS, Buffer and A&K) on June 24, and July 4, 2020. The pesticide applications were done as a tank mix of Rimon/Delegate at registered field rates, concluding at the appropriate preharvest interval before harvest.
2) Buffer (2 acres total). Two insecticide applications i.e. Rimon/Delegate were applied on all plots on June 24, and July 4. These plots were 40-60 feet away from the dispensers placed in the A&K plots.
3) Attract-and-kill (A&K, 2 acres total). Two insecticide applications i.e. Rimon/Delegate were applied A&K plots on June 24, and July 4. A&K dispensers were applied on June 23, 2020. The A&K treatments consisted of placing the hemp fiber substrate (10x10x0.5 cm, BioComposit, Alberta, Canada) at the base of every 4th tree in a shaded position. The treatments were applied at the rate of 50 per acre (10 per 0.2 acres). Drip irrigation was supplied every day ~5pm from the initial placement up to July 12.
There were therefore 30 plots, each ~0.2 acres in size for a total of ~6 acres. Because volatile plumes from the A&K plots can be influenced by air movement, GS plots were situated upwind from the Buffer and A&K plots to minimize interference caused by drifting volatile plumes originating from the dispensers placed in those plots. Assessments of oviposition were determined by counting the number of eggs laid per berry, enabling determination of percentage of infected berries.
Environmental data was collected during the field trials using data loggers (HOBO U23 Pro v2 Temperature/%RH; Onset Computer Corp., Bourne, MA) placed in the bottom, middle and top part of the trees. The data loggers will measure ambient air temperature (°F), and relative humidity (%RH). These data will indicate how different SWD pressure levels in each of the microclimates are affected by the treatment.

Statistical Analysis
Data from laboratory double-choice experiments and oviposition trials was analyzed using a Kruskall Wallis test were applied to separate differences at $\alpha < 0.05$. Field trial data were analyzed using factorial ANOVA tests in R-studio.

Results:
1) Evaluate toxicants in combination with the arrestant under *laboratory* conditions in order to create an A&K tool for SWD.
Erythritol: in the egg laying/mortality test, both treatments. Erythritol alone and Attractant plus Erythritol (17.86, 33.14 eggs per berry respectively) resulted in significantly lower oviposition compared to the control (52.28 eggs per berry) treatments ($\chi^2 = 21.63$, $P < 0.001$). No dead flies were recorded after 24 hours of exposure in both control and treatments.

Entrust: in the egg laying/mortality tests, results from 1 day-exposure periods showed a significantly lower oviposition rate in Entrust and Attractant and Entrust, compared to the untreated control ($\chi^2 = 42.32$, $P < 0.001$). No statistical differences were recorded between the two treatments where toxicant

or no toxicant was used. Entrust alone and Attractant plus Entrust (8, 16.85 eggs per berry respectively) resulted in significantly lower oviposition compared to the control (74.57 eggs per berry) treatments ($\chi^2 = 3.655$, $P = 0.052$). The number of dead flies was 0 in the control, 70 (35 females and 35 males) in Entrust and 70 (35 females and 35 males) in the Attractant plus Entrust.
At 3 days of exposure, there was a significantly lower oviposition in Entrust and Attractant plus Entrust ($\chi^2 = 13.23$, $P < 0.001$). There were no statistical differences between the Entrust alone and Attractant plus Entrust (21.42, 14 eggs per berry respectively). These two treatments however resulted in significantly lower oviposition levels compared to the untreated control (30.28 eggs per berry, $\chi^2 = 4.359$, $P = 0.036$). The number of dead flies was 15 (5 females and 10 males) in the control, 70 (35 females and 35 males) in Entrust and 70 (35 females and 35 males) in the Attractant plus Entrust.
At 4 day-exposure there were significantly lower oviposition in Entrust and Attractant plus Entrust ($\chi^2 = 13.7$, $P < 0.001$). There were no statistical differences between the in Entrust and Attractant plus Entrust (23.85, 11.28 eggs per berry respectively). These two treatments however resulted in significantly lower oviposition levels compared to the untreated control (32.28 eggs per berry). Statistical mortality differences were recorded between the untreated control two pesticide-containing treatments ($\chi^2 = 10.05$, $P < 0.001$). The number of dead flies was 13 (8 females and 5 males) in the control, 53 (25 females and 28 males) in Entrust and 47 (23 females and 24 males) in the Attractant plus Entrust.
At 5 day-exposure data showed a significantly lower oviposition in the two pesticide-containing treatments (Entrust and Attractant plus Entrust) ($\chi^2 = 30.88$, $P < 0.001$). There were no statistical differences between the Entrust and Attractant plus Entrust (17.42, 20.71 eggs per berry respectively). These two treatments however resulted in significantly lower oviposition compared to the control (62.14 eggs per berry) No statistical difference were recorded between the two insecticide treatments ($\chi^2 = 0.240$, $P = 0.622$). The number of dead flies were 2 (2 females and 0 males) in the Untreated control, 60 (29 females and 31 males) in Entrust and 46 (22 females and 24 males) in the Attractant plus Entrust.

Grandevo: in the choice test, both treatments (only Grandevo and Attractant plus Grandevo) resulted in no significantly lower oviposition compared with the control ($\chi^2 = 4.878$, $P = 0.086$). No statistical difference was recorded between the two treatments ($\chi^2 = 1.949$, $p = 0.178$). The number of dead flies at 24 hours was 0 in the control, 4 in the Grandevo and 0 in the Attractant plus Grandevo. At 48 hours the number of death flies was 0 in the control, 46 in the Grandevo and 56 in the Attractant plus Grandevo.
Venerate: in the choice test, both treatments (only Venerate and Attractant plus Venerate) resulted in no significantly lower oviposition compared with the control ($\chi^2 = 7.691$, $P = 0.021$). No statistical difference was recorded between the two treatments ($\chi^2 = 0.126$, $p = 0.724$), The number of dead flies at 24 hours was 1 in the control, 0 in the Venerate and 3 in the Attractant plus Venerate. At 48 hours the number of dead flies was 0 in the control, 46 in the Grandevo and 56 in the Attractant plus Grandevo.

2) Validate the new A&K formulation under *greenhouse conditions*.
This portion of the experiment was not conducted because of COVID19 greenhouse facility shutdown during the planned experimental period.
3) Conduct long-term (21-day and beyond) *open-field efficacy trials* of the refined A&K tool, grower-standard (GS) which include pesticide applications, and buffer plots as a direct comparison.

Hood River:
Field experiments indicated a numerical (no statistical differences recorded) reduction of eggs laid. The overall reduction of eggs laid on fruits during the experimental period was 11% in the A&K plots compared to the Untreated Control plots ($F_{2,4} = 0.093$, p=0.91, Table 1). When looking at the

respective sampling dates, the A&K treatment plots resulted in a numerically lower level of eggs compared to the Untreated Control plots (Figure 1). There were three dates, i.e. on June 23 (7 days after placement), July 8 (21 days after placement) and July 23 (28 days after placement) during the experimental period when there were sizable reductions in egg laying. Here the reductions in SWD egg laying were 44, 30 and 28% on those dates respectively.

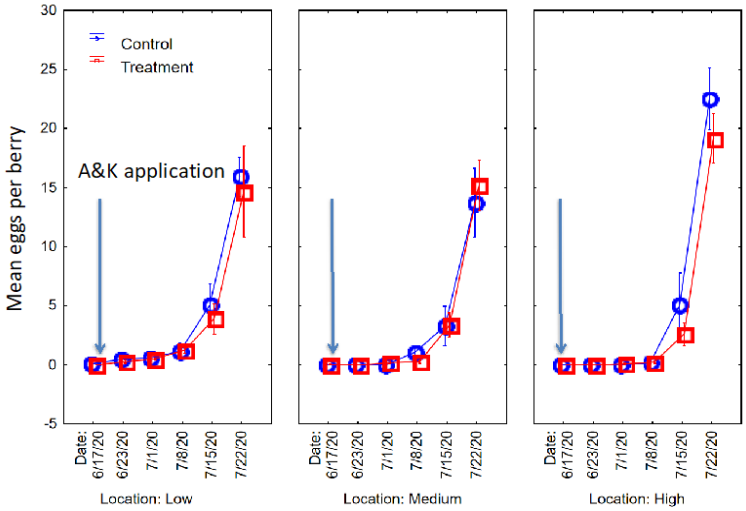

Figure 1. Mean number of *Drosophila suzukii* eggs per berry (±SEM) in a research cherry production block in Hood River, Oregon from June 17-July 22, 2020. The Attract and Kill (A&K) applications was done on June 17 (indicated by arrows).

Willamette Valley:

Field experiments indicated a statistical difference in the reduction of eggs laid in the Buffer compared to the Untreated Control plots. During the overall experimental period, reduction of eggs laid on fruit was 92.8 and 50% lower in the Buffer and A&K plots respectively compared to the Grower Standard plots ($F_{1,3} = 2.88$, $P < 0.022$, Table 1). There were 6.5 and nearly 2X times less infested fruit in Buffer and A&K plots compared to the Grower Standard plots. The majority of *D. suzukii* infestation happened during the last week before crop harvest, with 82% of eggs laid within the last week of the analysis in these trials.

Table 1. Mean number of *Drosophila suzukii* per berry (±SEM) and percent infested berries in a conventional cherry production block in Hood River and Salem, Oregon from June 17-July 22, and June 23-July 21, 2020 respectively. Numbers with different letters are statistically different

Treatment	**Mean eggs/berry**	**% Infested**
Hood River		
Untreated Control	3.8±0.81 ns	36
Attract and Kill	3.4±0.76 ns	34.9
Salem		
Buffer	0.011±0.004B	0.8
Grower Standard	0.152±0.058A	5.2
Attract and Kill	0.076±0.038A	3.1

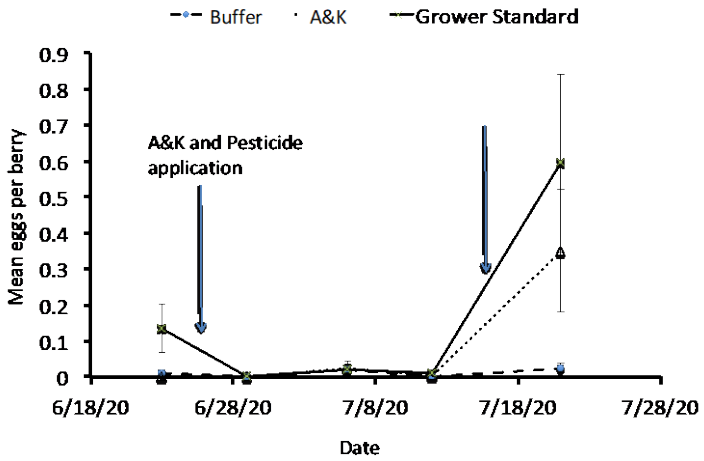

Figure 2. Mean number of *Drosophila suzukii* eggs per berry (±SEM) in a conventional cherry production block in Salem, Oregon from June 23-July 21, 2020. Pesticide and A&K applications are indicated by arrows. One A&K application was applied on June 24.

The combination of A&K with insecticide shows promise, potentially resulting in similar levels of control of SWD under field conditions. The Arrestant used alone under high pressure conditions in Hood river resulted in a trend of reduced damage due to SWD. In Salem, where growers used pesticides in combination with the A&K, the damage was lower in and adjacent to plots containing the arrestant. Data (not shown) generated by third parties in California, and Georgia resulted in similar reductions in SWD damage, attributable to the arrestant. The arrestant is currently undergoing EPA registration and commercialization.

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CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project: CH-20-103

Project Title: Insecticidal control of leafhoppers in cherries

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Cooperators: Scott Harper, WSU

Total Project Request: **Year 1:** $81,166 **Year 2: $84,185**

Other funding sources: **None**

WTFRC Budget: ***None***

Budget
Organization Name: Washington State Univ. **Contract Administrator**: Katy Roberts
Telephone: 509-335-2885 **Email address**: arcgrants@wsu.edu
Station manager: Chad Kruger **Email address**: cekruger@wsu.edu

Item	**2020**	2021
Salaries[1,2]	52,827	54,940
Benefits	18,373	19,108
Wages[3]	3,900	4,056
Benefits	366	381
RCA Room Rental		
Shipping		
Supplies[4]	4,500	4,500
Travel		
Plot Fees	1,200	1,200
Miscellaneous		
Total	81,166	**84,185**

Footnotes: [1] Research assistant professor (Nottingham) at 2% FTE of $7,612.5 per month for 12 months.
[2]Postdoc at 100% FTE of $4,250 per month for 12 months
[3]Summer time slip at 20 hours per week for 13 weeks at $15.00 per hour.
[4]Supplies including potted cherries, greenhouse and colony supplies (cages, soil, pots), bioassay supplies (pipette tips, paper cups, lab sprayer supplies), and PCR diagnostic services.

Objectives:

1. **Perform initial screening on a wide range of insecticides (broad spectrum-conventional, soft-conventional, and organic) against leafhoppers for mortality and feeding suppression.**
   Future goals: Continue screening products, particularly selective-conventional and organic insecticides. Develop methods for colony rearing to allow testing of insecticides on nymphs.
   Deviations: (1) Upon further research into stylet sheath assessment for measuring feeding suppression, we determined that this method may be too time-consuming to justify performing on all insecticides. Many insecticides were very toxic and immediately effective, so such elaborate assays are likely unnecessary. To better gauge the success of selective insecticides, instead, we think examining nymph mortality will provide more useful knowledge to the industry. Because nymphs feed in the ground cover, feeding success is not as important as mortality. (2) We were unable to establish a colony in the lab this year, potentially due to our limited knowledge of necessary plants for development. We suspect that a complex of wild weeds is necessary, such as common mallow, which is difficult to establish in colony given the lack of cultivation for seed. Instead, we relied on field collections for insecticide bioassays. This also proved difficult at first due to high mortality in transport, but we eventually developed a successful method allowing larger collections of leafhoppers from the field.

2. **Determine whether X infected leafhoppers are more susceptible to insecticides than uninfected leafhoppers.**
   Future goals: Continue to store adults that were killed in bioassays, then extract salivary glands for PCR diagnosis of the presence of phytoplasma.
   Deviations: Instead of performing separate bioassays for this hypothesis, we are utilizing individuals from insecticide screening in objective 1 to gain higher samples sizes, test more materials and save time.

3. **Determine residual control timelines for the most effective foliar products.**
   Future goals: Continue to perform residual time-line bioassays for more materials.
   Deviations: Due to lower ability to travel under COVID restrictions, bioassays were performed using potted cherry trees grown outdoors at the TFREC.

4. **Determine the potential for soil applications of systemic insecticides to provide long-term control of leafhoppers and disease transmission.**
   Future goals: Continue to test soil applied materials, potentially larger trees in 2020. Include an additional material, Safari (dinotefuran), which has a label for soil drenches and trunk sprays for cherries grown in nurseries and is known to control other leafhoppers species.
   Deviations: Due to low leafhopper numbers, we decided to eliminate a treatment, Verimark, which had the lowest likelihood for success.

Significant Findings:

- We identified conventional and organic insecticides that caused very high mortality of *C. reductus* (100% mortality across all reps) in direct contact spray bioassays within 24 hours of application.
  - Convention products resulting in 100% mortality were Asana, Malathion, and Actara. Transform WG resulted in ca. 92% mortality between two bioassays.
  - Organic products resulting in 100% mortality were Pyganic and Azera. Cinnerate and TetraCURB Organic resulted in >70% mortality assuming moribund as dead.
- Major improvements were made in collecting, transporting and assaying leafhoppers. Sweep netting with minimal sweeps per collection, storage and transport in mesh cages, and assaying with living plant material and soil all enhanced experimental viability.

- Another potentially important leafhopper species, *Euscelidius variegatus* was discovered in various field sites throughout the season in high abundance, especially in an organic apple block. Very little information on this species exists, but one prior study found it to be a competent vector of X-disease. Adults are larger than *C. reductus* and proved hardier in terms of collection, transport, and lab survival.
- Thiamethoxam and imidacloprid applied as soil drenches resulted in 50-70% mortality of *E. variegatus* leafhoppers 6 days following application.
- Thiamethoxam (Actara) and imidacloprid (Admire Pro) applied as foliar sprays also resulted in ca. 50-70% mortality of *E. variegatus* leafhoppers 6 days following application.

Methods:

Collection and Transport. Sweep netting was performed in commercial cherry and apple blocks near Rock Island, WA. Ten collection trips were made throughout the summer of 2020. Out of these attempts, just three resulted in experiments with usable data. Initially, we attempted aspirate leafhoppers directly out of sweep nets and into vials for transport to the lab. This resulted in very high mortality in transport and in experimental checks (untreated) for those that survived transport and were used in bioassays. To mitigate this issue, we stopped using aspirators and vials, and began dumping all contents from sweep nets directly into 12 x 12" mesh cages (Fig. 1B, in background). This was more efficient than aspirating leafhoppers and their survival in transport increased substantially. However, check mortality in bioassays remained higher than desirable (20-40%) for *C. reductus*. We then adjusted our sweeping technique to involve five sweeps maximum before dumping leafhoppers into mesh cages, to prevent sublethal injury. This was not necessary for *E. variegatus*, a larger and hardier species, but greatly improved the health and longevity of *C. reductus*.

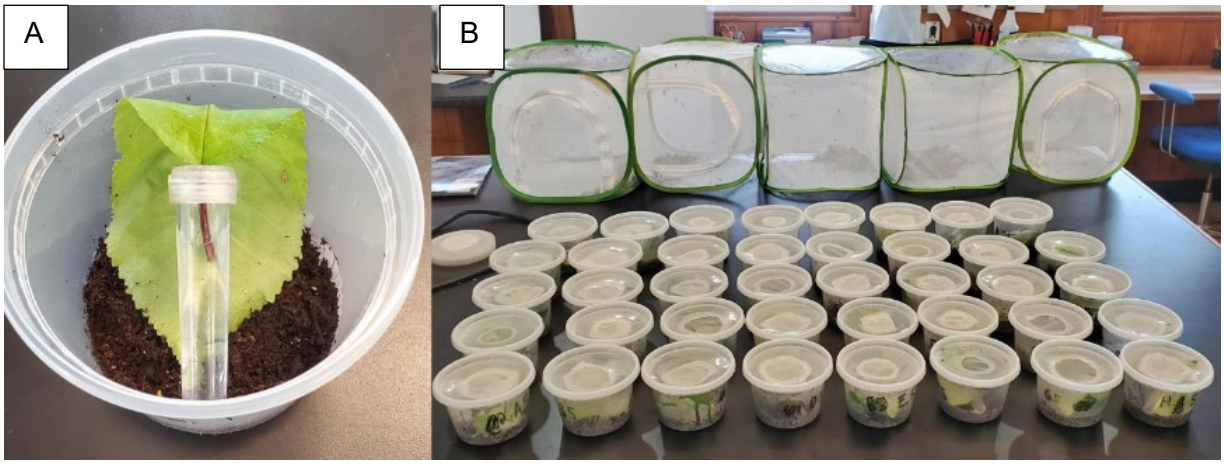

Fig. 1. Leafhopper bioassay arenas and collection cages. A) Closeup of one arena without lid to show cherry leaf in floral tube and soil. B) Multiple arenas with lids in foreground; field collection

Contact Spray Bioassays. Arenas were constructed using 8 oz plastic deli cups with slightly moistened soil and excised cherry leaves kept alive by inserting petioles into floral tubes with water (Fig 1A). Leafhoppers were aspirated from collection cages and moved into each arena (5-9 leafhoppers per arena). Each arena was sealed with a plastic lid with a mesh cutout. Once leafhoppers were in all arenas, treatments were applied using hand-pump aluminum spray bottles. Insecticide solutions were sprayed through mesh lids to contact the leafhopper, leaf, and soil, as would occur in the field. Containers with sprayed leafhoppers were then stored for 24 hours in a greenhouse prior to evaluation. To evaluate efficacy of insecticides, leafhoppers were rated as either alive or dead, to be considered dead, leafhoppers needed to be unable to walk. The distinction 'moribund' (impaired but alive) was used in the organic bioassay only (the final bioassay) but will be used in the future for

more detail on sublethal effects. Leafhoppers that were clearly alive and standing but could/did not hop when forcibly prodded with forceps were called moribund. Five spray contact bioassays were conducted, however, only two resulted in usable data (the other three had control mortality above 20%). The first conducted tested conventional insecticides (Table 1) and the second tested organic insecticides with the addition of Transform WG as a positive control (Table 2).

Table 1. Conventional Spray Contact Bioassay

Trt.	Per 100 gallons
UTC	-
Asana	14.5 fl oz
Malathion 5EC	44.8 fl oz
Bexar	27 fl oz
Actara	2.75 oz
Transform WG	2.75 oz
TetraCURB conc.	256 fl oz

Table 2. Organic Spray Contact Bioassay

Trt.	Per 100 gallons
UTC	-
Transform WG	2.75 oz
TetraCURB organic	2%
Cinnerate	60 fl oz / 100
Entrust SC	8 oz
Neemix 4.5	16 fl oz
Azera	56 fl oz /acre
Pyganic 1.4 EC	64 fl oz

Systemic Soil Drench and Spray Residue Bioassay. The experiment used Lapins cherry trees (3/4") on Mazzard rootstock planted in 3.6-gallon injection molded pots. Five cherry trees were assigned to each of five treatments: one untreated control (UTC), two soil applied insecticide treatments, and two foliar applied insecticide treatments (Table 3). Trees were not watered for 72 hours prior to applications. On 15 August, insecticide applications were made between 8:00 and 9:00 am. One-liter insecticide solutions were poured into each pot for soil treatments. Foliar treatments were sprayed using an arm-pump SOLO backpack sprayer, trees were sprayed to just before runoff, about 0.25 liters per tree. Soil drench treatments concentrations were made assuming 400 trees/acre. Soil drench mixes were made to use 1 liter of solution per tree while foliar applications were made to use 0.25 liter/tree (based on amount of spray needed to achieve full coverage). This resulted in soil applications using more A.I. per tree. Trees were lightly watered later that day, but not enough for water to run out from the bottom of the pots to avoid leaching insecticides out of the pots.

Table 3. Systemic Soil Drench Bioassay

Trt.	App.	Max label / acre	Per tree
UTC	-	-	-
Platinum 75 SG	Soil	3.67 oz / ac	0.26 g in 1 liter
Admire Pro	Soil	10.5 fl oz/ ac	0.77 ml in 1 liter
Actara	Foliar	2.75 oz /ac	0.194 g in 0.25 liter
Admire Pro	Foliar	2.8 fl oz / ac	0.21 ml in 0.25 liter

A leafhopper bioassay was conducted 48 hours after treatment applications using *C. reductus* and the same arena methods as the spray contact bioassays, but with the stated modifications to insecticide application method. This first assay resulted in very high check mortality, so the data were not usable. On 19 August, 4 days after treatment, another collection and bioassay attempt was made with *C. reductus* but with similar high check mortality. Luckily, in the same batch of collected insects was another leafhopper species in high numbers which we believe is the lesser known species, *E. variegatus* (Fig. 2C). We used these leafhoppers in a third bioassay beginning on 20 August. This bioassay was successful with almost no check mortality, leading us to further investigate this species. We found just one study in the literature examining *E. variegatus* (Jensen 1969), which determined it to be a competent vector of X phytoplasma, and one other with just a brief mention of its abundance in some orchards (Purcell and Elkinton 1980). We have saved many of these specimens to confirm the species ID and to run PCR analysis for X-phytoplasma.

Results:

Contact Spray Bioassays. Results for contact spray bioassays only include *C. reductus*. The conventional insecticides Asana (esfenvalerate), Malathion 5EC (malathion) and Actara (thiamethoxam) all resulted in 100% mortality of *C. reductus* leafhoppers 24 hours after treatment (Fig. 3). The organic insecticides Pyganic (pyrethrins 1.4%) and Azera (premix of pyrethrins 1.4% and azadirachtin 1.2%) both achieved 100% mortality 24 hours after treatment (Fig. 4). The conventional insecticide Transform WG (sulfoxaflor) resulted in 87.5% mortality in one bioassay (Fig. 3) and 96% mortality in a second when moribund individuals are considered dead (Fig. 4). The conventional materials Bexar (tolfenpyrad) and TetraCURB Concentrate (rosemary oil) provided marginal control at 66.7% and 52% mortality, respectively (Fig. 3). The organic materials Cinnerate (cinnamon oil) and TetraCURB Organic (rosemary oil) provided the next highest level of mortality for organic materials, both at ca. 72%, however many of these individuals were moribund (Fig 4.). The other organic insecticides Neemix (azadirachtin 4.5%) and Entrust SC (spinosad) provided marginal to poor control (Fig. 4).

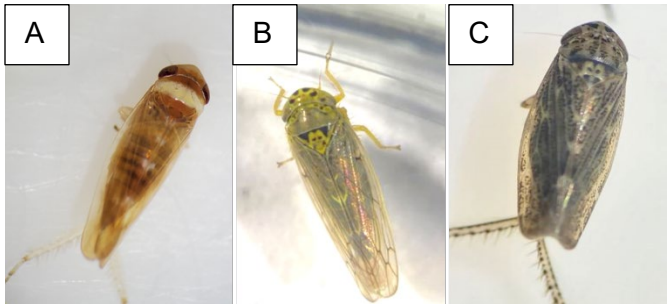

Fig. 2. Washington Leafhoppers: A) *C. reductus*, B) *C. geminatus*, and C) *E. variegatus* (seeking confirmation)

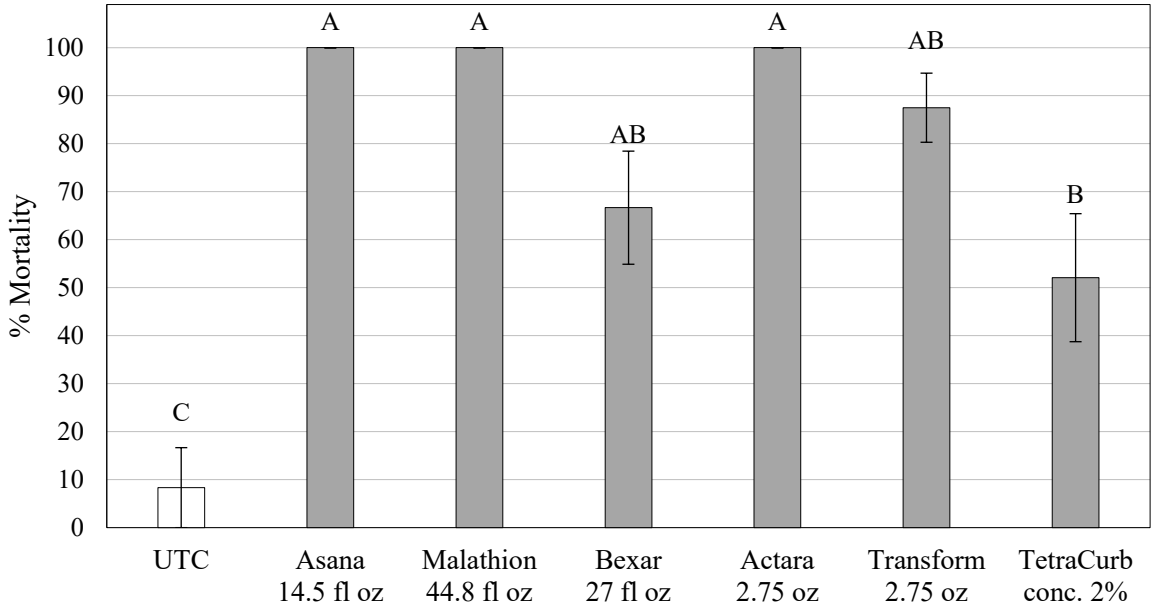

Fig. 3. Conventional Spray Contact Bioassay. Bars show average leafhopper mortality resulting from each insecticide. Bars not sharing a letter are significantly different according to Tukey's HSC ($P < 0.05$)

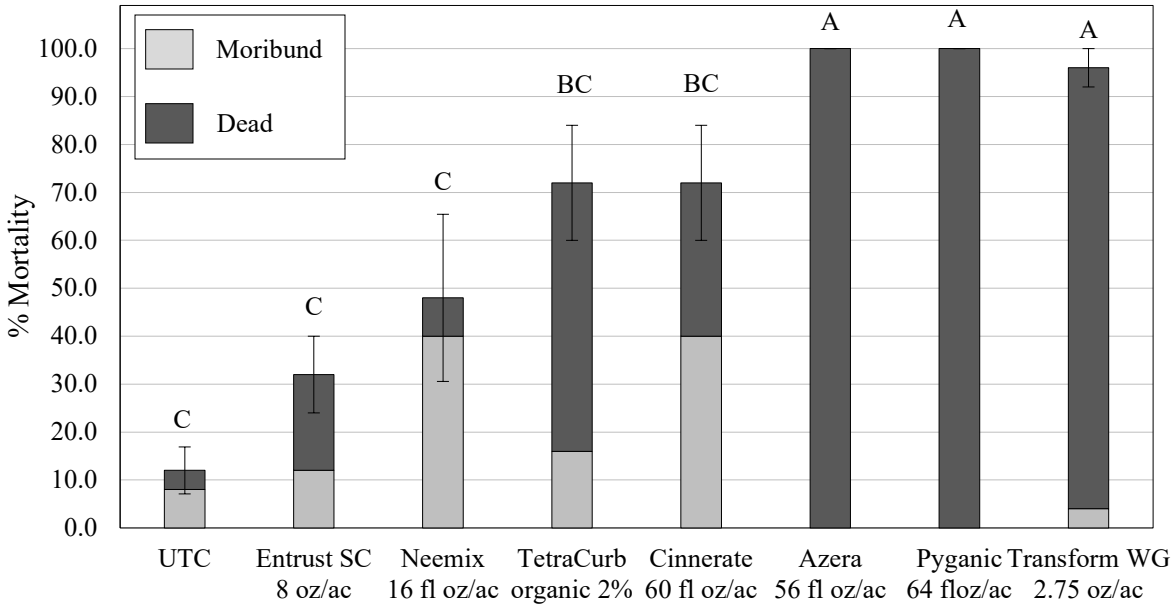

Fig. 4. Organic Spray Contact Bioassay. Bars show average leafhopper mortality resulting from each insecticide. Bars not sharing a letter are significantly different according to Tukey's HSC ($P < 0.05$)

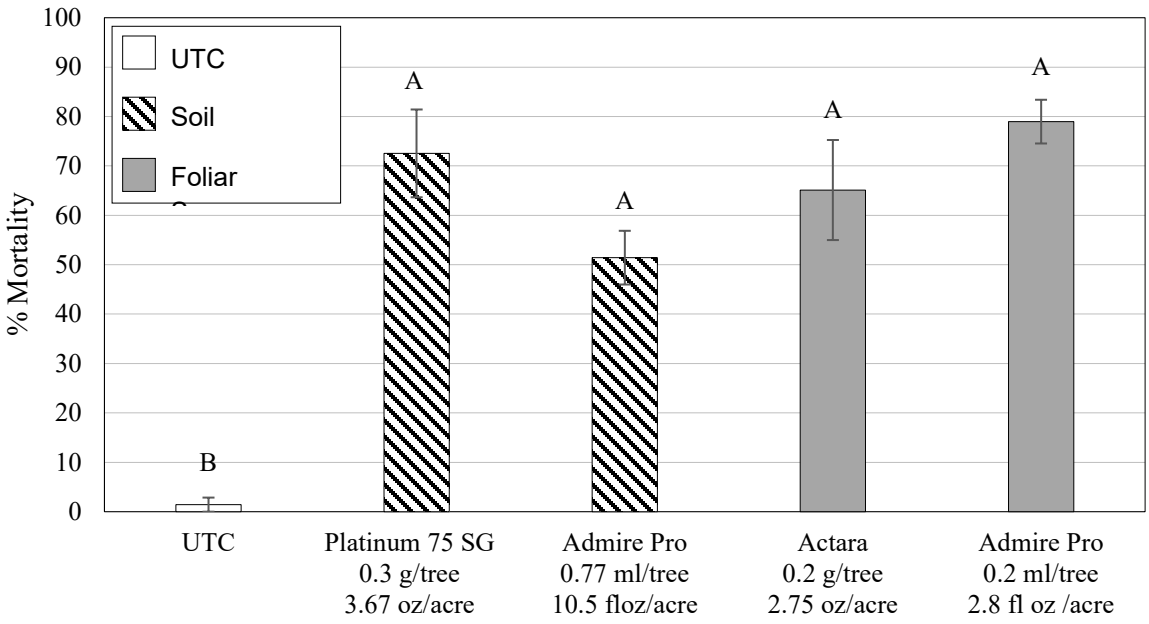

Fig. **5.** Systemic Soil Drench and Spray Residue Bioassay. Bars show average leafhopper mortality resulting from each insecticide product and application method. Bars not sharing a letter are significantly different according to Tukey's HSC ($P < 0.05$)

Systemic Soil Drench and Spray Residue Bioassay. Thiamethoxam and imidacloprid as both soil drenches and foliar sprays exhibited similar control of leafhoppers, which was significantly greater than the check but not overly impressive. However, these data are promising as a preliminary finding considering that this is a first attempt to use a soil drench for control of leafhoppers in cherries to our knowledge, so certain minor adjustments may improve outcomes. In addition, these data demonstrate that residual toxicity of both materials to leafhoppers one week after spray applications

was notably lower than direct contact. However, the leafhoppers tested against residues were *E. variegatus* while *C. reductus* was tested against direct sprays, decay information is not conclusive.

Discussion:

Through these 2020 experiments we have identified 5 insecticide materials, 3 conventional and 2 organic, that are highly toxic to leafhoppers upon direct spray contact. The design of contact spray bioassays did not necessarily produce perfect contact with all leafhoppers. Sprays were applied through screen lid cutouts into containers containing soil, large cherry leaves, and leafhoppers, so it is reasonable to suggest that perfect coverage (i.e., coating the leafhoppers with insecticide) did not always occur. We believe that this conservative approach will lend to more accurate predictions of field success.

While these data are preliminary given the few number of experiments, growers and advisors should still find these data useful in making certain spray decisions. Organic growers especially will find these data useful, as very little was known about which materials are toxic to leafhoppers. Pyrethrin containing products such as Pyganic and Azera are highly toxic to leafhoppers and may be used if infestations are high. It should be noted that Azera is a premix product, and the pyrethrin component is likely "pulling the weight". We can assume this because the other component is azadirachtin, the active ingredient in Neemix, which was only moderately toxic to leafhoppers. Pyrethrin products may have some risk for flaring secondary pest through disruption of biological control, however that risk is likely low due to the short residual of these materials. Future experiments should examine lower rates of pyrethrins against leafhoppers, which could lower risks of secondary pest outbreaks. These data also elucidate both effective and ineffective conventional products. Conventional growers will run the risk of flaring secondary pest with any of the products tested, so it is important in the future to develop strategic spray programs based on phenology or trapping to avoid over-spraying and causing secondary pest outbreaks.

Thiamethoxam and imidacloprid products (both soil drenches and foliar sprays) exhibited similar control, 50-70% mortality, of *E. variegatus* leafhoppers, which was significantly greater than the check but not overly impressive. However, these data are promising as preliminary findings considering that this is a first attempt to use a soil drench for control of leafhoppers in cherries, to our knowledge. Additionally, *E. variegatus* seems to be hardier than *C. reductus*, and therefore may also be more tolerant of insecticides. Certain minor adjustments may improve outcomes, such as testing *C. reductus*, increasing rates, or examining mortality 48 and 72 hours after exposure to allow more dying time. Soil drench information may be most important to nursery growers, as both active ingredients are allowable as soil drenches in non-bearing cherry trees (thiamethoxam as product Flagship). Admire Pro can be used as a soil drench in mature, bearing cherries, however this technique will require more testing on larger trees in the field. Additionally, we identified another product, Safari 20SG (dinotefuran), which is supposed to be highly effective on leafhoppers, more mobile in trees than the two products tested, and is legal to use on nursery cherries trees. This will be of interest to explore in 2021 experiments.

A very important finding for future research was determining proper methods for collection and experimenting with these leafhoppers. Now that methods are established and known to be successful, we will be able to conduct more, large scale experiments and provide greater amounts of control information to the industry.

References Cited

Jensen, D. D. 1969. Comparative Transmission of Western X-Disease Virus by Colladonus montanus, C. geminatus, and a New Leafhopper Vector, Euscelidius variegatus1. J. Econ. Entomol. 62: 1147-1150.

Purcell, A. H., and J. S. Elkinton. 1980. A Comparison of Sampling Methods for Leafhopper Vectors of X-disease in California Cherry Orchards1. J. Econ. Entomol. 73: 854-860.

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

Project Title: Understanding little cherry disease pathogenicity

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Cooperators: Washington cherry growers and extension agents.

Total Project Request: $458,022 **Year 1:** $155,882 **Year 2: $153,942 Year 3:** $148,198

Other funding sources: **None**

WTFRC Collaborative Expenses: None

Organization Name: WSU-IAREC **Contract Administrator:** Katy Roberts
Telephone: 509-335-2885 **Email address:** arcgrants@wsu.edu
Station Manager: Naidu Rayapati **Email address:** naidu@wsu.edu

Item	2020	2021	2022
Salaries	60,528	62,950	65,468
Benefits	23,034	23,956	24,915
Wages	4,650	4,836	5,030
Benefits	745	775	805
RCA Room Rental	0	0	0
Shipping	0	0	0
Supplies	64,850	59,350	49,905
Travel	1,500	1,500	1,500
Plot Fees	575	575	575
Miscellaneous	0	0	0
Total	155,882	**153,942**	148,198

Footnotes:

OBJECTIVES

Objective 1. *Establish and inoculate a field plot of representative cherry germplasm to screen for little cherry disease induction and potential sources of disease resistance/tolerance.*

During 2021 grafting of selection scions will continue, with planting planned for May followed by inoculation in June when pathogen titer is highest.

Objective 2. *Identify the physiological effects of little cherry disease of different cherry cultivars from experimental plots and field collected samples to determine a) whether there are different symptom patterns, and b) what effect these have on fruit quality and tree health using a physiological and metabolomics approach.*

In 2021 we aim to continue following the effects of LChV-2 and XDP on cherry cultivars in selected commercial orchards from bloom (March) through to harvest (June/July), collecting samples for diagnosis, phenotypic characterization, and for transcriptomic analysis. These data will be added to the 2020 results to separate seasonal trends from the effects of pathogen infection, and a) aid in producing a description of the effects of the two pathogens as infection progresses, and b) inform the transcriptomic studies.

Objective 3. *Examine the underlying genetic basis of little cherry disease through examination of transcriptomic changes during disease induction and identify potential effectors or interacting genes/proteins at the host level to develop a method to screen germplasm for tolerance/susceptibility.*

In 2021, we intend to continue to collect samples on a biweekly timeframe from bloom through to harvest to examine transcriptomic changes occurring during fruit development that are affected by LChV-2 and DXP infection. As in 2020, we are targeting generative tissues (fruit buds and developing fruit) and comparing those to somatic tissues (stems and leaves) to define host response to infection from disease expression pathways.

SIGNIFICANT FINDINGS

- Fruit shows increasing severity of symptoms with a higher concentration of either XDP or LChV2.
- In LChV2 infected Rainiers, a decrease in fructose, glucose, and sorbitol content was observed while citric acid and total phenolic content increased.
- Flowers and immature fruit were observed near pruning cuts in infected trees of Benton, Skeena, Santina and Cristalina cultivars at harvest, suggesting a broader deregulation of signaling.

METHODS

Objective 1. We will establish a 1-acre test block at WSU-IAREC consisting of 32 different cherry varieties (Table 1). This list includes commercially grown varieties, as well as cherries reported to have some level of tolerance or resistance to LChV-2 or X-disease, and several accessions that represent more unique genetic backgrounds. For each variety, we will plant eight trees, three of which are to be inoculated with LChV-2 or XDP respectively, with two non-inoculated controls. To promote early fruiting, we will bud the trees on a precocious rootstock Gisela-6. Budding will take place in greenhouse conditions during late winter/early spring, and in May the budded trees will be transplanted to the field. Inoculation for both diseases will be via chip budding of infected material in June when pathogen titer is highest. Orchard maintenance, including pruning, fertilization, pesticide application, and weed control, will be conducted according to current horticultural practices.

Table 1. List of germplasm to be screened for tolerance/resistance to LChV-2 and X-disease phytoplasma

Variety Name	Notes
Benton	Commercial cultivar
Black Pearl	Commercial cultivar
Chelan	Commercial cultivar
Coral Champagne	Commercial cultivar
Early Robin	Commercial cultivar
Rainier	Commercial cultivar
Santina	Commercial cultivar
Skeena	Commercial cultivar
Sweetheart	Commercial cultivar
Tieton	Commercial cultivar
Brooks	Genetic diversity
Kristen	Genetic diversity
Moreau	Genetic diversity
Black Spanish	Genetic diversity
Walpurgus	Genetic diversity
Yellow Glass	Genetic diversity
Lambert	Genetic diversity
Van	Genetic diversity
Yellow Spanish	Genetic diversity
Schmidt	Genetic diversity
PMR-1	Genetic diversity
Ambrunes	Genetic diversity
Cristobalina	Genetic diversity
Attika	Genetic diversity/commercial cultivar
Regina	Genetic diversity/commercial cultivar
Bing	Possible resistance to LChV-2/X-disease
Black Tartarian	Possible resistance to LChV-2/X-disease
Napoleon	Possible resistance to LChV-2/X-disease
Angela	Reported resistance to X disease
Sweet Ann	Reported resistance to X disease
Utah Giant	Reported resistance to X disease
Windsor	Possible resistance to X disease

Objective 2. Knowing how different cultivars respond to both LChV-2 and X-disease phytoplasma is essential to developing an accurate field guide for growers. Therefore, we propose to collect symptom development observations and physiological data from both the controlled field experiments and grower fields throughout the state. To do so we will focus on two areas:

1) Observation and recording of symptoms present on known infected trees under controlled conditions as the fruit develop from fruit set to harvest, collecting data on fruit size, weight, color, and seed size/maturation. This data will be collated by cultivar type, and infected status.
2) Collecting and recording biochemical data present in maturing fruit at the fruit set, straw/yellow and harvest phases by collecting fruit from different varieties, reducing to pulp via blending and separating the liquid exudate through filtration. This liquid will then be used for sugar content and metabolite analysis. Sugar, acid, and phenolic content analysis will be performed on cherry pulp using enzymatic and chemical assay kits.

Objective 3. The underlying genetic basis of LCD development will be examined in parallel with the physiological studies. Samples will be collected from different symptomatic and asymptomatic

cultivars in the controlled field trial described in objective 1 as well as from field samples. From the trees in the new research block, three different tissue types (fruit, fruit stem, and leaf tissue) will be sampled at three time points (fruit set, straw/yellow, and harvest), macerated and total RNA extracted. Samples will be samples submitted for library preparation and deep 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 the X-disease phytoplasma or LChV-2, and associated with disease expression, particularly with reference to fruit development.

Symptom development for little cherry disease may be a result of protein-protein interactions between cherry and pathogen proteins. To investigate this, relevant genes identified in the transcriptomics study described above for both cherry and the pathogens will be selected for a yeast two hybrid screen. 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 an 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 that the yeast two hybrid assay.

RESULTS AND DISCUSSION

Objective 1. *Establish and inoculate a field plot of representative cherry germplasm to screen for little cherry disease induction and potential sources of disease resistance/tolerance.*

Due to delays in obtaining rootstocks, the need to grow the rootstocks used to an adequate size for grafting, and scion material availability, preparation of the field block trees has been slower than anticipated with 7 of 25 cultivars completed. Furthermore, after consultation with participants in the RosBreed Project, 7 additional cultivars have been added to capture greater genetic diversity. Over the winter of 2020-2021 we are forcing growth of the budded plants and remaining stocks, and will collect budwood for the remaining trees during the winter to bud them in the greenhouse in early 2021.

Objective 2. *Identify the physiological effects of little cherry disease of different cherry cultivars from experimental plots and field collected samples to determine a) whether there are different symptom patterns, and b) what effect these have on fruit quality and tree health using a physiological and metabolomics approach.*

In 2020 we focused on collecting symptom data from commercial orchards across central Washington. At each site, healthy or asymptomatic trees were compared to symptomatic trees for the purpose of sample collection; in select sites, trees were selected at random at bloom, and followed through to harvest for tissue collection for objective 3.

All samples were tested for the presence of XDP and LChV-2 by qPCR or RT-qPCR, and pathogen load quantified (Table 2). We assessed fruit symptom severity, fruit size, and fruit color as shown in table 2, and collated the data for each cultivar based on infecting pathogen and titer, to represent the different stages of the infection cycle.

Table 2. Effect of XDP and LChV-2 titer on symptom severity and fruit characteristics in different cherry cultivars.

Cultivar	Pathogen	Titer	N	Symptom rating[a]	Fruit Size[b]	Fruit color[c]
Benton	XDP	Low	8	1.375	2	4.5
		Medium	8	2.375	1.938	2.875
Bing	LChV-2	Low	1	2.5	2	4.25
	XDP	Low	14	1	2.5	4.678
		Medium	9	2.667	1.722	2.77
	Both (L/X)	L/L	15	1.277	2.5	4.767
		L/M	2	3	1.5	2.5
		L/H	1	3	1.5	2.5
		H/L	2	2.5	1.75	4
Cristalina	XDP	Low	9	0.333	2.667	4.667
		Medium	15	2.93	1.633	2.467
Lapins	LChV-2	Low	1	0	3	5
	XDP	Low	4	0.5	2.625	4.875
	Both (L/X)	L/L	4	0.25	2.75	4.875
Santina	XDP	Low	8	0.5	2.625	4.438
		Medium	5	3	1.8	3.3
		High	1	3	1	1.5
Skeena	LChV-2	Low	6	1.667	2	4.25
	XDP	Low	4	0.75	2.5	5
		Medium	1	3	2	1.5
	Both (L/X)	L/L	19	1.421	2.105	4.131
		L/M	3	3	1.667	4
		L/H	1	3	1.5	4
Sweetheart	LChV-2	Low	4	1.5	2	4.125
	XDP	Low	13	1.769	2	4.115
		Medium	5	3	1.6	3.1
	Both (L/X)	L/L	10	2.1	1.909	3.7
		L/M	1	3	1.5	3
Rainier	LChV-2	High	2	2.5	1.5	3.25
	XDP	Low	22	1.27	2.34	3.54
		Medium	7	2.86	1.71	2.78
		High	2	3	1.25	2.5
	Both (L/X)	L/L	6	1.167	2.16	4
		L/M	1	3	1.5	3.5
		H/L	10	2.5	1.75	3.45
Early Robin	XDP	Low	5	0.4	2.6	4.6
Sour Cherry	XDP	Low	5	0.4	2.6	3.8
		Medium	3	3	3	2

a. Symptom rating: 0 = asymptomatic, 1 = mild, 2 = medium, 3 = severe.
b. Fruit size: 1 = small (<50% of normal), 2 = medium (75% of normal), 3 = normal.
c. Fruit color for dark cherries: 1 = green/yellow, 2 = mottled/blush, 3 = pink/light red, 4 = red, 5 = dark red. For yellow cherries: 1 = green, 2 = white, 3 = yellow, 4 = pale blush, 5 = deep blush.

As can be seen in table 2, the stage of infection (low titer representing a new or early infection, and medium or high titer representing an established infection), has a significant effect on the type and severity of symptoms. Depending on the cultivar infected, fruit size was more severely impacted than fruit color. Also, while sample size for LChV-2 infected cherries is lower, due to general lower incidence in the field, Bing and Skeena are more severely impacted by LchV-2 than XDP at equivalent

titers, whereas for Lapins and Rainiers, the opposite was observed. Sweetheart was comparable, regardless of pathogen. Interestingly, in Skeena, Sweetheart, and Rainier, infection with both pathogens produced slightly more severe symptoms on infected plants than single infection of either pathogen at equivalent titer.

Next, we began assessing the impact of pathogen infection on fruit quality though measuring the sugar and metabolite content of infected versus healthy or asymptomatic fruit at different stages of infection (determined as before, by pathogen titer). Due to reduced operating levels, these assays are ongoing, and will be completed over the winter. However, preliminary data from Rainier cherries infected with either LChV-2 or XDP compared to healthy fruit indicated that both LChV-2 and XDP reduced fructose, glucose, and sorbitol content in established, but not early, infections (Figure 1), whereas sucrose content increased in early-stage infections for both.

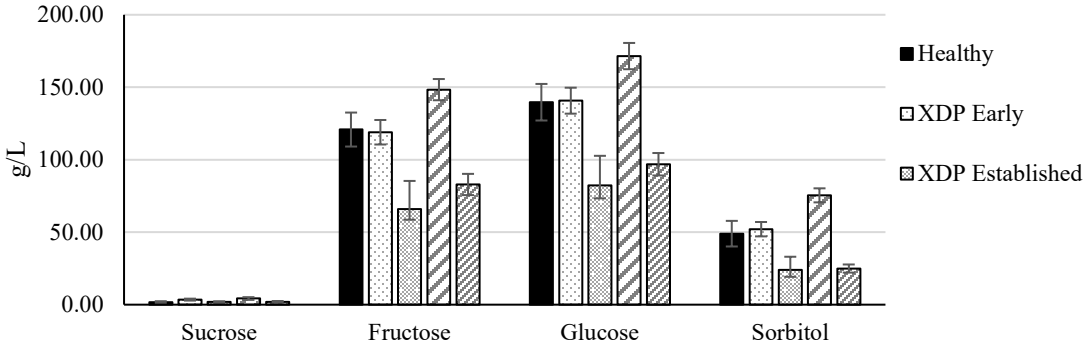

Figure 1. Sugar content of healthy rainier fruit compared to LChV-2 and XDP infected fruit at different stages of infection.

In contrast, citric acid content increased in LChV-2 infected fruit in established infections, whereas XDP fruit did not significantly differ from the healthy controls (Figure 2a). Total phenolic content increased in both LChV-2 and XDP fruit in established infections (Figure 2b).

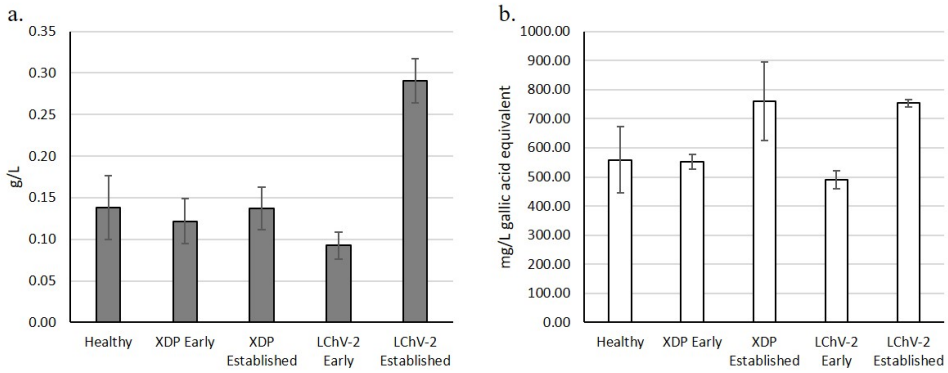

Figure 2. Citric acid (a) and total phenolic (b) content of healthy rainier fruit compared to LChV-2 and XDP infected fruit at different stages of infection.

Cumulatively these data suggest that there is a significant drop in fruit quality between the initial stages of an infection (the first 1-2 seasons), and when the infection of either pathogen becomes systemic and increases in titer (seasons 2-3 and beyond).

Objective 3. *Examine the underlying genetic basis of little cherry disease through examination of transcriptomic changes during disease induction and identify potential effectors or interacting genes/proteins at the host level to develop a method to screen germplasm for tolerance/susceptibility.*

In early 2020 we performed RNA-seq analysis on diseased and asymptomatic samples collected during the 2019 season, and identified eight genes of interest. RT-qPCR assays were designed for these genes to verify the differential expression observed from sequencing (Figure 2).

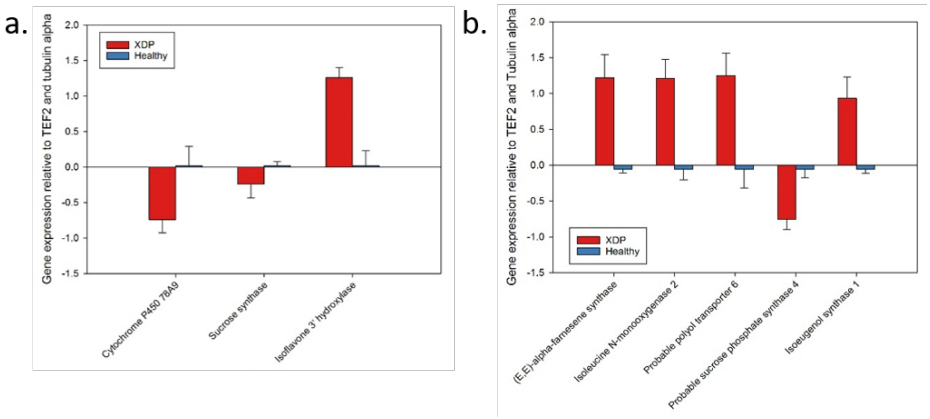

Figure 2. Expression of genes of interest in XDP positive trees compared to uninfected, healthy trees at shuckfall in a) developing fruit, and b) leaves.

We found that, interestingly, cytochrome P450 78A9, which regulates fruit size (Qi et al. 2017) and a sucrose synthase were downregulated in infected fruit. Isoflavone 3' hydroxylase, which is involved in isoflavonoid biosynthesis was upregulated. In leaves, a sugar transporter, polyol transporter 6, was upregulated in infected leaves while a sucrose phosphate synthase 4 was downregulated. (E,E)-alpha-farnesene synthase, which serves as chemoattractant for insects in apples (Bengtsson et al. 2001), was upregulated. Isoleucine N-monooxygenase 2 and isoeugenol synthase, which are involved in phenylpropene and cyanogenic glucoside biosynthesis, respectively, were upregulated. How differential expression of these genes may play into symptom development is not known and will need to be explored further.

Samples have been collected for RNA-seq during the 2020 season, although processing has been delayed due to covid-19 restrictions. Therefore, analysis of these samples will occur in late 2020 and early 2021.

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Qi X, Liu C, Song L, Li Y, and Li M. 2017. PaCYP78A9, a cytochrome P450, regulates fruit size in sweet cherry (*Prunus avium* L.). Frontiers in Plant Science. DOI: 10.3389/fpls2017.02076.

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

Project Title: Identifying sources of X disease in cherry orchards

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Cooperators: Garrett Bishop, Scott Harper, Tianna DuPont

Total Project Request: **Year 1:** $58,400 **Year 2:$55,849** **Year 3:** $53,707

Other funding sources: **Awarded**
Amount: $249,359
Agency Name: USDA/WSDA Specialty Crop Block Grant
Notes: The PI's on this grant are also on a USDA SCBG grant led by Scott Harper (Northfield and Cooper are co-PIs), that will build on the preliminary ground-truthing of gut content analysis from this grant in part to do fieldwork evaluating alternative host plant use by X disease phytoplasma vectors in the field. The SCBG grant is complementary, but not overlapping with this grant.

Budget 1
Organization Name: WSU-TFREC
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Station Manager: Chad Kruger

Contract Administrator: Katy Roberts
Email address: arcgrants@wsu.edu
Email Address: cekruger@wsu.edu

Item		2020	2021	2022
Salaries[1]		39,629	41,214	42,863
Benefits[2]		4,478	4,657	4,844
Wages				
Benefits				
Equipment				
Supplies[3]		7,000	4,000	4,000
Travel[4]		2,000	2,000	2,000
Miscellaneous				
Plot Fees				
Total		53,107	**51,871**	53,707

Footnotes:
[1] new student position
[2] 11.3%
[3] Research consumables (e.g., cages, pots, soil), + molecular tests for disease presence
[4] In state travel

Budget 2
Organization Name: USDA ARS **Contract Administrator:** Chuck Myers
Telephone: 509-454-4463 **Email address:** Chuck.Myers@ars.usda.gov

Item	2020	2021	2022
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies[1]	5,293	3,978	
Travel			
Plot Fees			
Miscellaneous			
Total	5,293	**3,978**	

Footnotes:
[1] Molecular supplies for gut content analysis

Objective Recap, Goals, and Anticipated Accomplishments:

Objectives

1. Conduct oviposition tests and life cycle analysis on leafhoppers on five host plants (cherry, clover, dandelion, peach, alfalfa).

Most knowledge we have about life history characteristics of the most common Washington leafhoppers that vector X disease (*Colladonus geminatus* and *C. reductus*) come from a single study on *C. geminatus* conducted in 1952 and 1953 in Dalles, OR (Nielson 1968). The author reared *C. geminatus* leafhoppers on alfalfa plants and peach trees and found that the generation time averaged across the two plant hosts was approximately 60 days. The authors stated that there were no statistically significant differences in the life histories for the two plants, but it would be helpful to get effective estimates for each host type and compare them to other common weeds that may host the X disease phytoplasma. It is also unclear what plants leafhoppers feed on or how other hosts affect their growth and reproduction. Furthermore, *C. reductus* was not included in the study, but is often far more abundant than *C. geminatus* in Washington orchards (*C. reductus* made up 97.5% of the *Colladonus* spp. in our surveys of Wenatchee and Yakima region orchards). Here, we originally set out to build on this research by evaluating the generation time for *C. reductus* and *C. geminatus* on 5 plant species: cherry, white clover (*Trifolium repens*), dandelion (*Taraxacum officinale*), peach and alfalfa. Understanding host plant use will help inform management plans. In our surveys of cherry farms in the Wenatchee and Yakima regions in this project and in the project title, "Field evaluation of leafhopper controls for X disease management" we rarely observed *C. geminatus*, with *C. reductus* being >95% of individuals collected by sweep nets and sticky traps. In response to the abundance of *C. reductus* and lack of knowledge, we focused our trials on this species. Furthermore, when collecting leafhoppers, we noticed they were commonly found on mallow plants, so we included mallow in our trials. In two attempts to start a colony of *C. reductus* with a diverse offering of plants (attempt 1: pea plants, clover, alfalfa; attempt 2: alfalfa, clover, mallow) the leafhoppers died as older nymphs or newly emerged adults, suggesting there was something missing in their diet, and that they may need a diverse diet. We are currently raising *C. reductus* leafhoppers on a combination of peach trees, mallow, alfalfa, dandelion, and clover. Given an apparent need for a diverse diet, we have focused trials on feeding behavior, and used an oviposition test to determine the number of generations per year for *C. reductus*, which is unknown (2 reported for *C. geminatus* in the 1950s), and is unclear from sticky trap data.

2. Evaluate incubation time and acquisition probability for leafhoppers feeding on each, cherry and peach trees and transmission likelihood to cherry, clover, dandelion, peach, and alfalfa.

In our evaluation of acquisition and transmission studies we will follow the methods of previous studies (Jensen 1971, Suslow and Purcell 1982), with the addition of molecular techniques to better evaluate acquisition and transmission success. To evaluate acquisition in year 2 of the project we will identify cherry and peach trees exhibiting X disease symptoms during harvest, and place *C. geminatus* and *C. reductus* leafhoppers in sleeve cages on the diseased trees. After 1 week of feeding (the maximum time needed according to previous research) we will cut the branch off the tree, keeping the sleeve cage intact and place the sleeve cage and branch immediately into a cooler with ice for transport back to the WSU TFREC without allowing leafhopper escape. The leafhoppers collected from cherry trees will then be transferred to greenhouse cages containing one of five potential host plants: cherry, peach, alfalfa, dandelion, or white clover, and replicated 8 times (40 total cages). Each cage will include 3 *C. geminatus* and 3 *C. reductus* leafhoppers, to focus on the potential of the plant to host the disease and allow for either leafhopper species to transfer the disease.

Note: These trials are planned for year two and have not yet been conducted. However, we have preserved the plants from the feeding trials for testing as alternative hosts. To our knowledge, only cherry, peach, and dandelion are known hosts, so testing the herbaceous hosts for phytoplasma after

the feeding trials with field-collected leafhoppers is an important step. These samples are currently awaiting molecular sequencing to test for phytoplasma presence.

3. Use molecular analysis on leafhoppers raised on different host plants to evaluate the reliability of gut content analysis to identify previous hosts of leafhoppers collected in orchards.

Research conducted by co-PI Rodney Cooper and colleagues on purple top disease in potatoes (Horton et al. 2018, Cooper et al. 2019), caused by a phytoplasma vectored by beet leafhoppers has included the development of molecular methods to identify previous plant hosts of leafhoppers collected from crops. While the methods have been focused on beet leafhoppers, rather than the *Colladonus* spp. that vector X disease, we expect the methods to be directly applicable to identifying non-cherry plants as sources of leafhoppers. Here, we will use leafhoppers arising from experiments described in objective 1 as a cost-effective evaluation of such methods for cherry-X disease research. These data can then be used as pilot research justifying federal funding identifying alternative leafhopper hosts and their potential importance for disease transmission in cherry orchards. Thus, at the end of the life cycle analysis in year 1 we will send leafhoppers from the field trials to the USDA lab in Wapato for molecular analysis to identify the host plant within the insect's gut. Assuming identification success in year 1, in year 2 we will collect adult leafhoppers from the end of experiments and place them on cherry seedlings, raised separately for each host plant. We will then collect 5 leafhoppers from each seedling at 0, 1, 2, and 3 weeks to identify the timeframe in which the previous host plant can be detected. We have stored leafhoppers from feeding trials and will conduct gut content analysis over the winter months.

Objectives timeline

Objective	Y1	Y2	Y3
1 Life history tests	x	x	
2 Transmission tests		x	x
3 Gut content analysis	x	x	

Significant Findings:

- Of the plants included in the trials (cherry, peach, mallow, alfalfa, white clover, and dandelion), *C. reductus* have a strong affinity for mallow, and to a lesser extent alfalfa. Given how common these plants are in orchard groundcover, these hosts should be considered in management strategies. *C. reductus* may also benefit from a diverse diet, that includes tree feeding.
- Leafhoppers feeding rates on cherry trees ranged from 14% to 51% of the observed feeding, depending the available herbaceous plants.
- Leafhopper feeding rates on peach trees ranged from 22% to 41% of the observed feeding, depending on the available herbaceous plants.
- We observed successful oviposition in August in field conditions, with adults emerging in October, suggesting there are three *C. reductus* generations in the Pacific Northwest. Two of these adult emergence periods typically occur after cherries are harvested.
- Leafhoppers are most active during daylight hours, and we did not observe evidence of leafhoppers moving into trees at night.

Methods:

Feeding trials

We initiated feeding trials in 24in × 24in × 56in (w × w × h) cages with a combination of white clover, alfalfa, dandelion, mallow, Early Red Haven peach trees, and/or Bing cherry trees, with each plant in a separate pot (Figure 1). Each trial lasted 5 days and each cage contained 10-15 leafhoppers, depending on mortality after collection. In the first trial, we conducted observations every two hours from 8am to 11pm. However, leafhoppers rarely moved in the span of the two-hour intervals and did not appear active in observations made at 9pm and 11pm, which were in the dark and made with red headlamps to avoid disturbing insects. Therefore, in subsequent trials, observations were made at 8AM, 1PM, and 6PM, doing 3-minute time searches in each cage. Trials were conducted in environmentally controlled growth rooms set at 75F, with a 16:8 L:D daylength. During each observation, we counted how many leafhoppers were on each plant, what plants they were on and if actively feeding or not by visually observing stylets piercing the plant. We present data only on actively feeding leafhoppers summarized across the insects within a cage.

Figure 1 Feeding trial cages in the growth room

The trials included the following treatments:

- 2 trials of cherry, alfalfa, clover, dandelion; each with 2 cages
  - Initiated June 11 and August 3, 2020
- 2 trials of peach, alfalfa, clover, dandelion; each with 2 cages
  - Initiated June 11 and August 3, 2020
- 1 trial of cherry, clover, mallow, dandelion; each with 2 cages
  - Initiated September 22, 2020
- 1 trial of peach, clover, mallow, dandelion; each with 2 cages
  - Initiated September 22, 2020
- 1 trial of peach, alfalfa, mallow, dandelion; each with 3 cages
  - Initiated August 22, 2020
- 1 trial of cherry, alfalfa, mallow, dandelion; each with 3 cages
  - Initiated October 6, 2020

Field oviposition test

Based on yellow sticky card data, in the Pacific Northwest *Colladonus* species leafhoppers typically have three periods of abundance: May, late July/early August, and October. However, it is difficult to determine the number of generations per year from yellow sticky card data. This is because the October generation may be the same generation as the August generation, just moving into orchards after loss of alternative host plants. Because leafhoppers overwinter as dormant eggs, we evaluated the potential for eggs laid in field conditions in August to hatch into nymphs. Development of these eggs would then suggest that the August adults represent a distinct generation that gives rise to the adults collected in October. Therefore, during the first week of August 2020 we collected *C. reductus* and placed them in cages 24in × 24in × 24in mesh cages with combinations of herbaceous plants next to the Brunner building at the WSU Tree Fruit Research and Education Center. The cages were monitored periodically to identify the emergence of nymphs and/or adults.

Additional Research: Leafhopper Location and Activity

Studies on the behavior of *Colladonus reductus* within orchards is lacking, leaving unknowns such as when they are most active throughout the day and where they are most abundant within a block. We did not observe activity during daylight hours in the feeding trials, but we were unable to replicate dawn or dusk in the growth rooms (due to non-dimming lights), so we sought to identify whether leafhoppers regularly move vertically from ground cover to canopies in four time periods: morning, mid-day, evening, and overnight. To begin addressing these unknowns we used yellow sticky cards (5 × 7 in) to examine leafhopper abundance at two heights, varying distances from the orchard border, and activity throughout a 24 hr period. In two cherry blocks at 6:00am Aug 5th, we deployed 32 sticky cards, one at each height at four distances from the orchard border (40, 80, 120, and 160 ft), and 16 sticky cards, one at each height. At each location, one trap was tied to a branch at 6 ft and another to a wooden stake at 2 ft from the ground. Traps were collected and replaced at 10:00am, 6:00pm, 10:00pm, and 6:00am the following morning, and *C. reductus* abundance was recorded by height, time, and distance from orchard border.

Figure 2 *C. reductus* feeding on mallow

Results & Discussion:

Feeding trials. We observed active feeding on all plants offered during the feeding trials (Figure 1). In the feeding trials that included cherry trees, the order of *C. reductus* preference appeared to be: mallow, alfalfa, cherry, white clover, and dandelion. Indeed, when offered mallow, alfalfa and a cherry tree we did not observe feeding on dandelion. In the feeding trials that included peach trees, the order of preference appeared to be: mallow, alfalfa, peach, white clover, and dandelion. However, interestingly, when offered mallow, alfalfa and peach together they fed more on peach than alfalfa. The fact that leafhoppers always fed on cherry or peach trees, regardless of what herbaceous plants were there begs the question of whether there is something important about feeding on trees that provide important nutrients to leafhoppers. However, future research is needed to determine whether this is the case.

Field oviposition tests: Adult *C. reductus* leafhoppers collected in the first week of August and introduced to outside cages with mallow and clover readily laid eggs that hatched into nymphs and began reaching the adult stage in October, suggesting that the August generation is a separate generation from the first generation that emerges in May from overwintering eggs and from the October generation that lays eggs that remain dormant for the winter. Given that these two later generations typically occur after cherry harvest, leafhopper control after harvest is likely critically important.

Additional Research: Leafhopper Location and Activity

Leafhoppers were most active mid day and in the evening from 6pm to 10am (Figure 2), although evening catch was much more skewed towards the low trap heights (2’ compared to 6’ height). We conducted this experiment in part to test the theory that leafhoppers move into the trees at night. However, these data do not seem to support this theory, as in the evening hours most trap capture occurred near the ground cover. In addition, we did not observe evidence that leafhoppers were moving in and out of the orchard during different periods of the day. There was consistent capture at the different distances from the edge in our different time periods (data not shown).

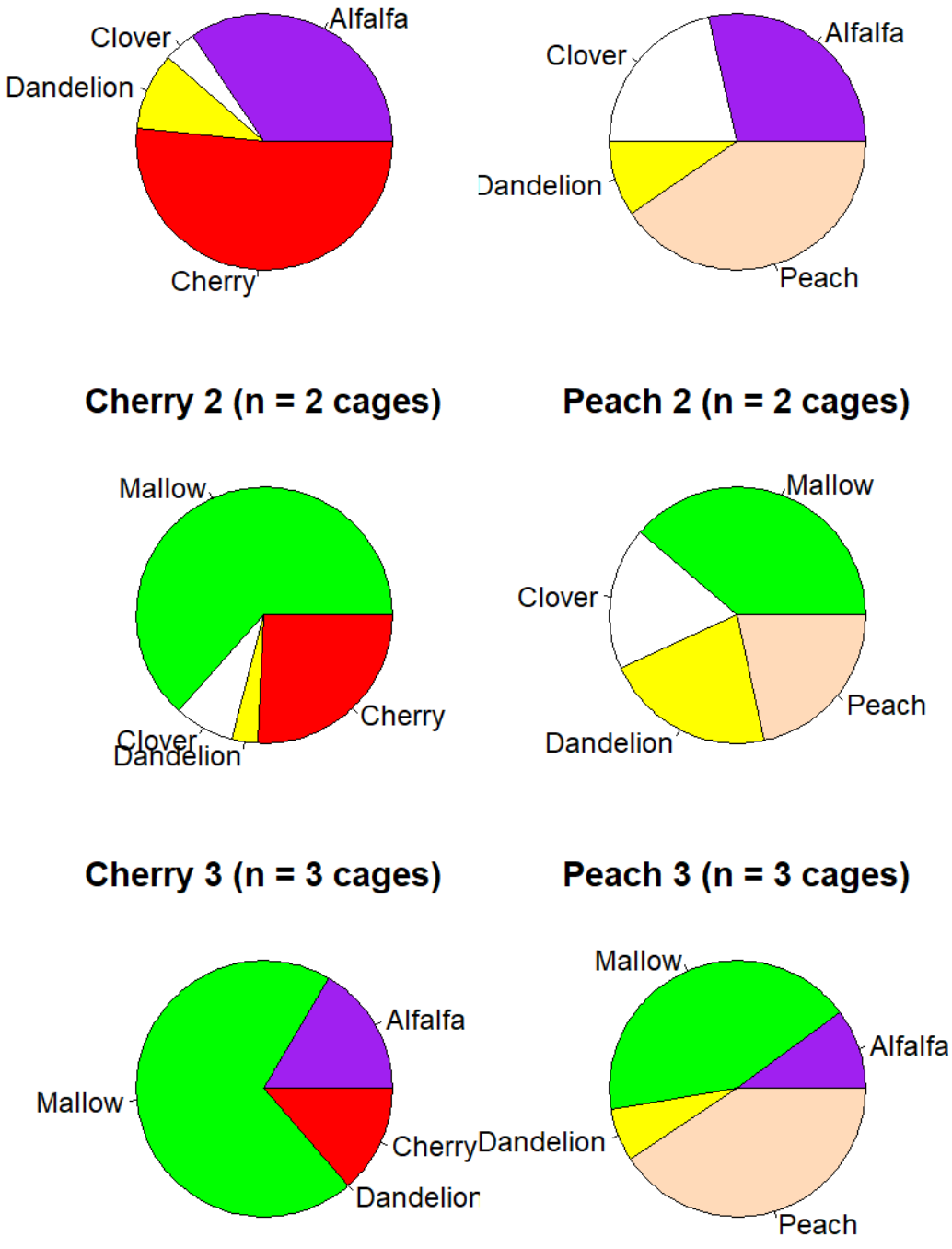

Figure 3 Proportion of feeding observations made on each of the different plant species in cages: cherry (red), white clover (white), dandelion (yellow), alfalfa (purple), peach (peach), or mallow (green) during feeding observations.

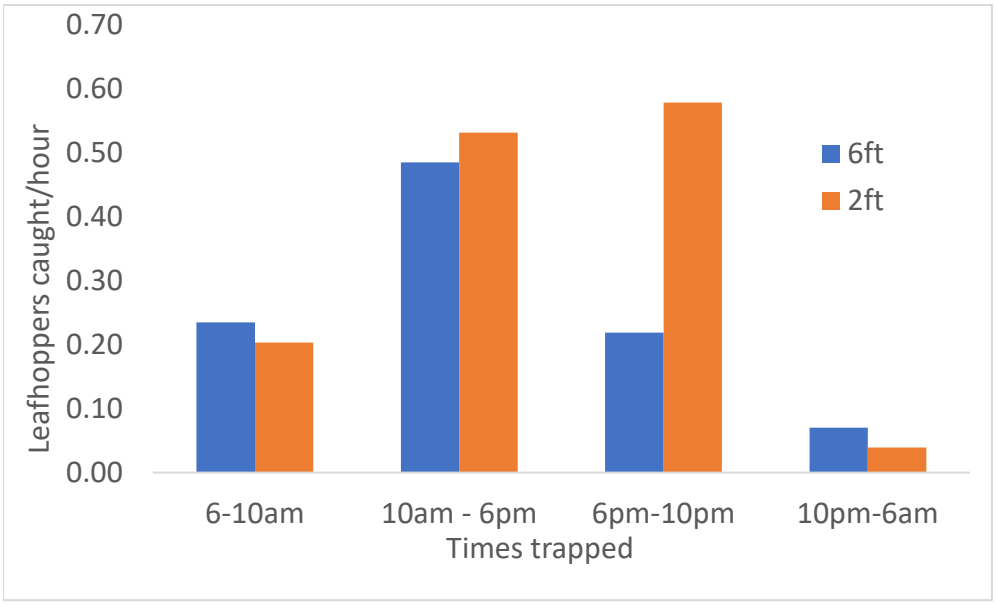

Figure 4 Mean *C. reductus* leafhoppers collected per hour (to account for variable trapping intervals) in a commercial Wenatchee region cherry block over a 24 hour period starting on August 5, 2020. No *C. geminatus* were collected. Traps were hung at 6 feet from a cherry tree or placed below the tree on a 2 foot stake. Data summarize 12 traps at each height

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CONTINUING PROJECT REPORT **YEAR**: No Cost Extension

Project Title: Awareness and application to stop little cherry disease

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Cooperators: Karen Lewis, Gwen Hoheisel, Jenny Bolivar, WSU Extension; Garret Bishop, GS Long

Total Project Request: **Year 1:** $45,689*

Other funding sources

Agency Name: WSU Tree Fruit Industry Endowment
Amt. $62,000 per year in salary and benefits for Extension Information Technology and Transfer (ITT) Coordinator

Agency Name: USDA Specialty Crop Block Grant
Amt. requested: $16,250 for Extension component
Notes: PI Scott Harper

Budget 1:
Organization Name: WSU **Contract Administrator: Katy Roberts**
Telephone: 509.293.8803 **Email Address: Katy Roberts**
Station Manager: Chad Kruger **Email Address:**

Item	2020	2021(No-cost extension)
Supplies [a, b, c, d]	$26,162	
Travel [c]	$8,590	
Miscellaneous [d]	$5,000	
Plot Fees		
Total	$39,752	0

Footnotes:
[a] **Scout training packets:**
Printing $4,670 per 1,000 scouting training 40-page spiral bound booklets x 2,000 = $9,340;
2000 color copies @$0.50 ea = $1,000;
Tree tags (thermal printed wrap around 9x1" @ $90 roll of 500 x 20 + $50 thermal transfer ribbon = $1,850;
[b] **Programing** for scouting phone ap development (57 hrs @ $96/ per hour) = $5,472;
[c] **Videographer** (includes editing and production) scout training video + two virtual field days = $6,000;
[d] **Other:** $1,000 camera Extension coordinator; $1,000 computer Extension coordinator; supplies to create innovative sample demonstration method= $500;
[c] **Travel:** Extension coordinator travel motor pool vehicle at $13.05/day plus $0.12 per mile = $6,347;
DuPont Travel. 3 trips to Yakima data/photo collection @ 360 miles x $.575/mi + 3 winter talks @ 200 miles x $0.575/mi = $966;
Sallato travel: Bi-weekly trips to orchards 77 mi x 12 x $0.575, 2 winter talks @ 100 miles x $0.575/mi = $633;
Bolivar travel: 2 trips to Yakima data/photo collection @ 360 miles x $.575/mi + 2 winter talks @ 200 miles x $0.575/mi=$644
[e] **Translational services** to put materials into Spanish and have an interpreter on hand for 1 event = $5,000.

Budget 2
Organization name: OSU-Wasco Co. Extension **Contract Administrator:** Rusell Karow
Telephone: 541-737-3228 **Email Address:** russell.karow@oregonstate.edu

Item	2020	2021 (No-Cost extension)
Salaries		
Benefits		
Equipment		
Supplies [a]	$5,420	
Travel	$518	
Plot Fees		
Miscellaneous		
Total	$5,938	0

Footnotes:
[a] Printing $4,670 per 1,000 scouting training 40-page spiral bound booklets x 1000 = $4,670. [b] $25 x 10 LCD tests for demonstration = $250.[c] Thompson Travel: Monthly trips to orchards (photos, sample collection for growers, etc) 100 mi x 6 x $0.575, 3 winter talks @ 100 mi x $0.575 = $518.

OBJECTIVES

Objective 1: Develop scouting training materials.

Objective 2: Increase awareness and management.

SIGNIFICANT FINDINGS

- A Scout Training Toolkit including a hard copy 26-page booklet and scouting fliers in English and Spanish, as well as tree tags was distributed to 1800 growers and consultants.
- Online training materials and information were produced, updated and distributed including 8 newsletter articles, 5 webpages, and 7 training videos reaching growers and consultants with 7,392 unique pageviews.
- Awareness of X-disease and Little cherry disease was increased to new audiences through Trade Journals and radio outlets including at least nine new coverage events.
- A Little Cherry Community with representatives from each region met monthly to increase discussion between growers, scientists and Extension and help distribute information and give WSU/OSU feedback on the latest needs.
- New agreements with local laboratories were created to provide new testing opportunities and information to populate maps of X-disease and Little cherry virus spread.

RESULTS

Obj. 1. Develop Scouting Training Materials

A scouting toolbox was designed and created. Version 1.1 included a scouting flipbook, a standardized scouting protocol, and standardized tree tags. Included in the scouting flipbook was a) photos of symptoms b) sampling procedures c) and a checklist of symptoms that could be confused with little cherry. Handy playing card size ID cards from OSU were included in packets. Sampling fliers were produced in English and Spanish. 1800 copies of scouting toolbox packets with hard copy flipbook, fliers and OSU cards were distributed with collaboration from major consulting companies during the first week in June 2020.

Scout training was conducted virtually due to CoVid-19 and via distribution of scouting material (above). Training included a webinar conducted in May, 2020 directly before scouting should have commenced during harvest.

Short training videos. Seven training videos were produced. Videos are available online at treefruit.wsu.edu and YouTube. Videos were shared via facebook and to 2048 Fruit Matters recipients. To date training videos have had 440 views. These videos will serve as an important resource to train growers on symptoms and vector management during winter virtual-trainings and in the coming growing season.

Provide scout training materials and trainings in Spanish. The Scouting flipbook, scouting fliers, as well as 3 videos and 1 newsletter article were produced in Spanish as well as English formats to ensure better access for Spanish speaking audiences.

Obj. 2 Increase awareness and management

Map. In order to increase awareness of the extent and location of the problem we maintain an online map which illustrates the general location (to city level) and incidence of positive X disease and Little Cherry Virus 2 trees. In order to maintain the online map during the 2020 season MOAs were created with two new labs doing X-disease and Little cherry virus testing: Cascade Analytical and AGNEMA. To create these relationships a large effort was needed as there was no existing protocols for proficiency testing, or data sharing. Multiple meetings were facilitated in order to troubleshoot testing results and methods in order to improve the quality of the data not only for mapping but also for those having samples tested. Updated maps for the 2020 season will be available after receipt of testing results from labs in November/December 2020.

Tours and trainings providing information on new research. In addition to scout training (see objective 1) trainings were designed to provide information on the latest in research results from Co-PIs Harper and Northfield. During 2020 a virtual training was created on Northfield's Kaolin and Reflective mulch trials in both English and Spanish which was viewed by unique 46 viewers.

Outreach to ensure effective management of X-disease vectors was also conducted by meeting with the field staff of major consulting companies. DuPont, Northfield and Nottingham met with the fieldstaff of Wilbur Ellis, Northwest Wholesale and Chamberlin to share the latest recommendations and answer questions at the beginning of vector management season.

Little Cherry Community X Phytoplasma and Little Cherry Virus is a rapidly evolving problem with new research, information and practice developing to keep pace. A Community of representatives of growers and consultants from each growing region was created in order to discuss the latest research and learn what questions are of most interest to growers at the moment. This group met monthly on second Thursdays at 3pm. This group drove outreach over the course of the season giving the little needed pushes. For example, in response to suggestions we made sure that vector management information was available on-time and pushed out through consultants of each of the major Wholesalers.

Timely newsletter articles will be pushed out to out to Fruit Matters e-news recipients (current subscribers 2,048). The team exceeded our goal of 4-5 articles during the season producing 8 articles which had over 969 unique readers.

Additionally, information was pushed out through trade journals and local newspapers, radio and television. Two articles and a video were published with the Good Fruit Grower with a distribution of 11,000. Associated press interview was picked up by KUOW 94.9, klcc.org, Spokane public radio, and The Register, Tri-Cities Business News among others. The Yakima Valley Herald and King 5 News also responded to press releases and covered the story.

X-disease Little Cherry Disease factsheets were updated to include any new management information available. Five major webpages related to Little cherry disease and X-disease were updated and had a combined number unique pageviews of 5,983 over the last year.

Trainings

Scouting and Sampling for Little Cherry and its Vectors. Webinar. Harper, S., Northfield, T., DuPont, S.T., Sallato, B., Thompson, A. May 22, 2020. http://treefruit.wsu.edu/article/wsu-osu-webinar-videos-scouting-and-sampling-for-little-cherry-and-its-vectors/

Training manual

X-disease Phytoplasma and Little Cherry Virus Scouting and Sampling Guide. DuPont, S.T., Harper, S., Sallato, B, Thompson, A. 1800 copies of distributed of hard copy flip book.

Newsletter articles

DuPont, S.T. **We need your help - X-disease, Little Cherry Disease Impact Survey.** Fruit Matters. September 26, 2020. http://treefruit.wsu.edu/article/x-disease-little-cherry-disease-impact-survey/

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A big concern: Cherry disease impacting iconic Washington crop. King 5 News. May 26, 2020. https://www.king5.com/article/tech/science/environment/cherry-disease-impacting-iconic-wa-crop/281-c80de091-0f8a-4ba2-89f1-0540e83d0d94

Training Videos

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Webpages

X-disease Phytoplasma and Little Cherry Virus Scouting and Sampling Guide. DuPont, S.T., Harper, S., Sallato, B, Thompson, A. June, 2020. http://treefruit.wsu.edu/crop-protection/disease-management/western-x/sampling-guide/

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X-disease and Little Cherry Disease Symptoms Guide**.** DuPont, S.T., Harper, S., Sallato, B, Thompson, A., Wright, A., Molnar, C. July, 2020. http://treefruit.wsu.edu/crop-protection/disease-management/western-x/symptoms-gallery/

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

Project Title: Field evaluation of leafhopper controls for X disease management

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Cooperators: Garrett Bishop, Jenna Bjur, Teah Smith, Scott Harper, Tianna DuPont

Total Project Request: **Year 1:** $79,864 **Year 2: $82,558**

Other funding sources

None

Budget 1
Organization Name: WSU - TFREC **Contract Administrator:** Shelli Tompkins
Telephone: 509-665-8271, ext 2 **Email address:** shelli.tompkins@wsu.edu

Item	2020	2021
Salaries[1]	50,039	52,040
Benefits[2]	17,325	18,018
Wages		
Benefits		
Equipment		
Supplies[3]	5,000	5,000
Travel[4]	7,500	7,500
Miscellaneous		
Plot Fees		
Total	79,864	**82,558**

Footnotes:
[1] New postdoctoral researcher position (100% FTE), Louis Nottingham (2%)
[2] 35% (postdoctoral researcher), 25.9% (Nottingham)
[3] Fieldwork consumables and X disease tests
[4] Domestic travel for research

Objective Recap, Goals, and Anticipated Accomplishments:

1. *Evaluate effects of kaolin clay applied post-harvest on X disease prevalence and density of leafhoppers and predators.*

   Kaolin clay application have shown to outperform insecticides for suppression of leafhoppers and Pierce's disease in California vineyards, cause direct mortality to leafhoppers, and even deter them from feeding to the point of starvation. As planned, we have finished the first year of the two-year trial evaluating the efficacy of kaolin clay to suppress the densities of leafhopper vectors of X-disease (*Colladonus reductus and C. geminatus*) in Yakima and Chelan county cherries blocks. Molecular testing of the leafhopper's guts will determine the efficacy of kaolin clay to deter feeding. Future greenhouse choice tests of clay sprayed vs. non-sprayed trees will assess leafhopper preference.

2. *Evaluate effects of UV-reflective mulch on X disease prevalence and density of leafhoppers and predators.*

   UV reflective polyethylene mulch use has demonstrated success in reducing the abundance of corn and potato leafhoppers even better than permethrin or thiomethoxrin. Our shipment of Extenday was delayed by 4 months due to COVID19 preventing us from deploying it in our experimental plots. Thankfully, the growers in Chelan county had Extenday in the cherry plots prior to harvest and graciously left it throughout the season for our experimental study. Therefore, in half of our cherry plots (Chelan county only) we were able to conduct the first year of evaluating Extenday for suppression and control of the X-disease leafhopper vectors. Our shipment has arrived and we will be able to include the other sites in Yakima county next year.

3. *Describe seasonal patterns of leafhopper abundance and map disease incidence in commercial cherry orchards.*
   A critical component to managing leafhoppers and X disease is understanding leafhopper phenology. We monitored leafhoppers in the blocks where we conduct the treatments described in objectives 1 and 2 to begin developing a general phenology for the growing regions of Wenatchee and Yakima valleys. We mapped disease incidence at harvest in our trial orchards, and will be able to identify patterns of disease spread within blocks throughout the following years.

Significant Findings:

- Surround reduced leafhopper numbers on 4 cherry plots in the Wenatchee Valley and 2 in Yakima County
- Extenday provided control surpassing Surround in 4 cherry plots in Wenatchee Valley
- Surround did not improve control in a trial in two Wapato nectarine plots, but leafhopper numbers were much lower than the other trials
- Optimal trap height for leafhoppers in cherry blocks depended on control method. In control sections traps at 6 ft high caught the most leafhoppers. However, in Surround and Extenday treated sections the most leafhoppers were collected at 2ft traps. 4 ft traps were often intermediate in each case.
- In no-choice tests, leafhoppers readily fed on Surround-treated leaves, suggesting that leafhoppers are able to detect leaves when presented with them.
- Phenology differed dramatically between Wenatchee Valley and Yakima County

Methods:

We evaluated two control methods (kaolin clay and Extenday groundcover) as additions to the spray rotation currently used on commercial cherry plots in the Wenatchee region (6 plots) and the Yakima region (2 plots) and evaluated kaolin clay in 2 Yakima region nectarine blocks. We targeted blocks with 1-10% disease prevalence to ensure that the block has disease to control, but that the disease prevalence is not high enough to risk block removal prior to the end of the experiment. Each replicate includes 12 rows with 200 feet of row, with three treatment locations randomized in a split-plot design. Thus, each plot included 36 rows, split in thirds for the three treatments. We evaluated leafhopper abundances and disease prevalence in the middle four rows and used the other rows as buffer rows to reduce spillover effects of the other treatments.

Figure 1 A 5 × 7 inch yellow sticky card placed at 4 ft on a cherry tree branch

Prior to harvest, disease incidence and location within the block was surveyed and recorded for the Wapato plots. After harvest completion, treatments were applied to assigned plots. Kaolin plots received four kaolin (Surround WP) sprays, one in July, August, September, and October (Table 1) on top of the grower's baseline insecticide program. Kaolin was sprayed at 50 lb/acre and 200 gal/acre. The postharvest Surround treatment aligns with a typical spray to reduce doubling, and doubling will be recorded in each plot next year. Our order of Extenday was delayed 4 months due to COVID19. Thankfully, our cooperator in Chelan County had Extenday which was deployed in our trial plots from May 27 – October 30. This gave us four replicates of Extenday for the 2020 season.

Figure 2 5x7 Yellow sticky cards suspended at 2, 4, and 6 ft from the ground.

After initial treatment application, leafhopper abundance in each treatment (Kaolin clay, Extenday, Control) replicate was monitored using 10 yellow sticky cards (5 × 7 inch) (Fig. 1) in the middle four rows. A yellow sticky card was tied to a cherry tree branch 4 ft from the ground and 25 ft in from each corner of the plot, and two sets of three yellow sticky cards were hung in the middle rows at 2, 4, and 6 ft from the ground using a bamboo pole and braided fishing line (Fig. 2-3). Sticky cards were deployed July 23rd in the Wenatchee region plots and July 31st in the Yakima region plots. Cards were collected and replaced every two weeks through October, and collected cards were returned to the lab to record leafhopper abundance by species (*Colladonus reductus* and *C. geminatus*). More than 99% of leafhoppers were *Colladonus reductus*, so we do not present *C. geminatus* data. Periodical beat sheet sampling was conducted to observe population densities within the tree canopy, but the low numbers relative to sticky cards suggested it was not an effective method of sampling. Throughout the winter sticky cards will be re-examined for natural enemy abundance including lacewings, ladybugs, and syrphid flies.

Table 1. Kaolin clay application timing and rate by county

	KC 1st app	KC 2nd app	KC 3rd app	KC 4th app	Rate
Chelan Co.	Jul 21, 2020	Aug 6, 2020	Sep 4, 2020	Oct 7, 2020	50 lbs/acre 200 gal/acre
Yakima Co.	Jul 29, 2020	Aug 10, 2020	Sep 9, 2020	Oct 15, 2020	50 lbs/acre 200 gal/acre

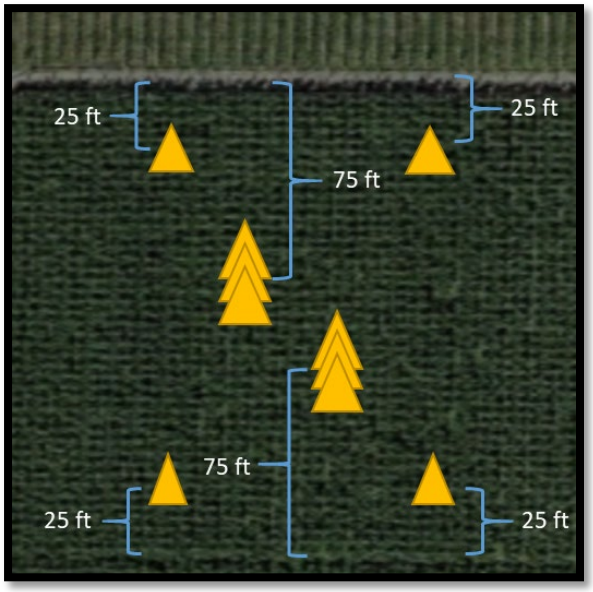

Figure 3 Trap deployment layout for each Surround, Extenday, and Control experimental plot.

No-Choice Surround Feeding Study

Kaolin clay (i.e. Surround) covered trees have been shown to reduce feeding and survivorship of other leafhoppers. However, while collecting traps in the Surround sprayed cherry plots, we observed leafhopper presence on leaves frequently. To empirically test if *C. reductus* leafhoppers will locate and feed on Surround covered cherry trees we conducted a no-choice feeding study. On Sep 29, we placed four field collected adult *C. reductus* in each of five cages with only Surround covered cherry tree leaves (collected from a sprayed experimental plot (Fig. 7)) and five cages with only non-sprayed cherry trees. We then observed leafhopper feeding behavior at 24, 28, and 46 hrs after initial set-up, recording the number alive, dead, on-plant, off-plant, and actively feeding (Fig. 8).

Results & Discussion:

Objective 1.

Two of the experimental plots in Wenatchee Valley were not analyzed, because we only observed a single leafhopper (1 *C. geminatus* in a control plot) all season across 60 traps. In 4 other plots in the Wenatchee region we observed generally lower numbers of leafhoppers in the Surround than control

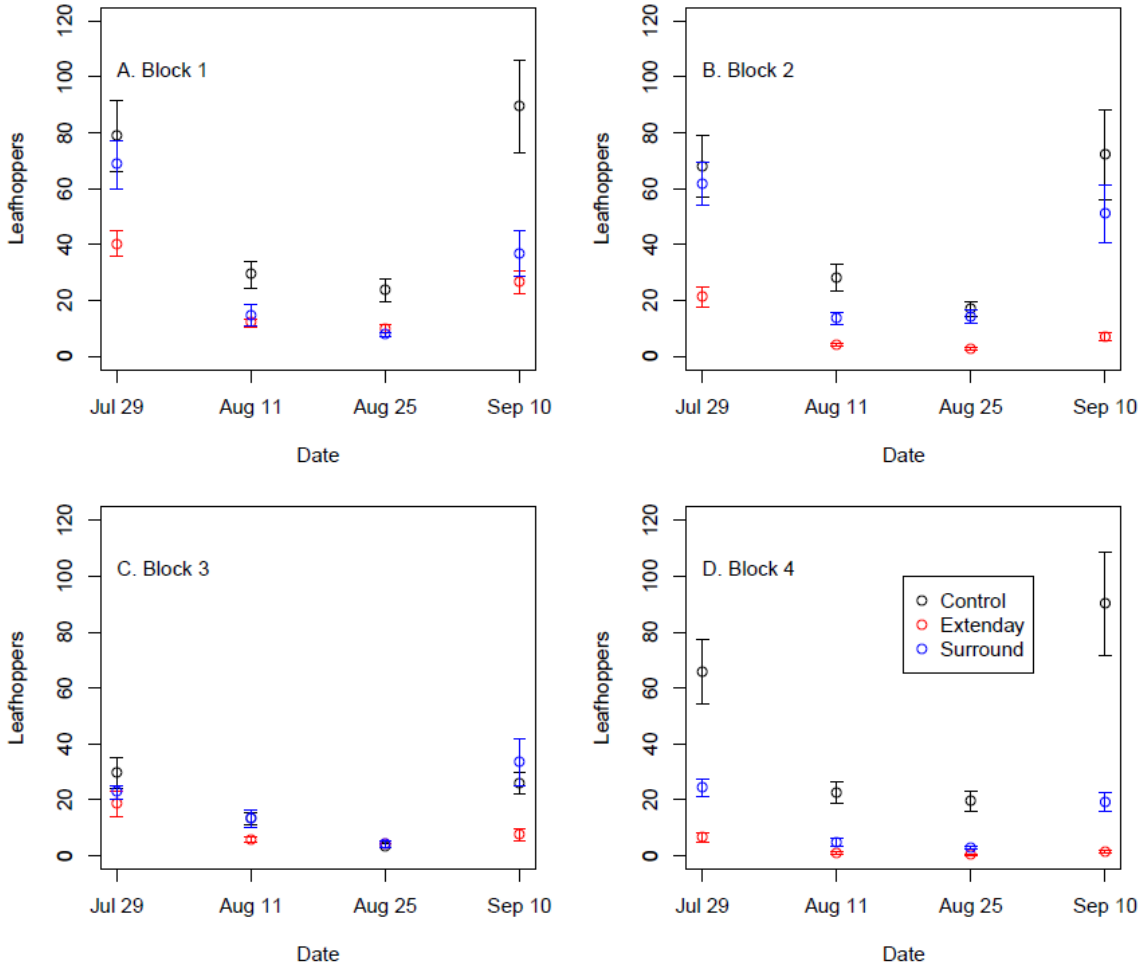

Figure 4 Mean *C. reductus* leafhoppers per trap in four plots in control (black), Extenday (red), or Surround (blue) subplots in Wenatchee region cherry blocks. Dates represent midpoints of 2-week sample periods, except for the September 10th date, which was a 3 week period.

plots (Figure 4). Similarly, Surround reduced leafhopper numbers on traps in 2 Wapato region cherry plots (Figure 5). In contrast, Surround did not improve control in nectarine plots with low leafhopper counts, with 2 and 1.25 leafhoppers per trap in the control plots and 1.875 and 3.62 leafhoppers in the Surround plots (averaged over 4 weeks of post-harvest sampling). In no-choice experiments *C. reductus* readily fed on leaves collected from one of our Wenatchee region Surround – treated plots, with similar mortality over 48 hours compared to untreated leaves, suggesting that kaolin clay does not inhibit leafhopper feeding.

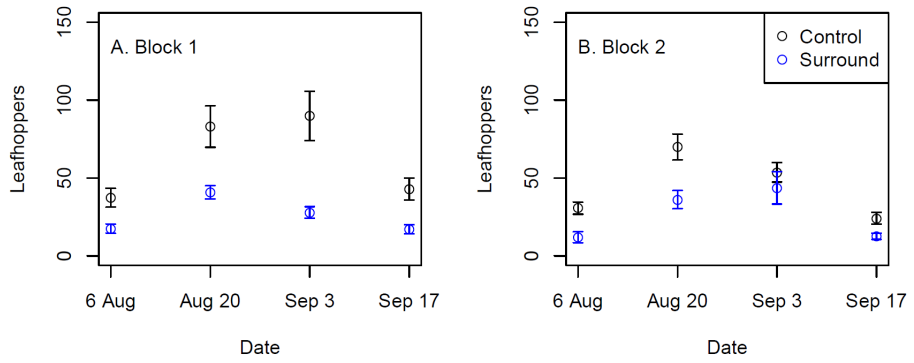

Figure 5 Mean *C. reductus* leafhoppers per trap in four plots in control (black) or Surround (blue) subplots in Yakima region cherry blocks. Dates represent midpoints of 2-week sample periods.

Objective 2.

Extenday reduced leafhopper numbers in the 4 Wenatchee region cherry plots, providing the best control (Figure 4). While further research is needed, it is likely that the control provided by Extenday is simply covering up the weedy hosts that leafhoppers commonly feed on (see continuing report on "Identifying sources of X disease in cherry orchards"). In some cases where leafhopper counts were higher than expected we observed weeds growing over the Extenday from the weed strip or it had come unattached and was pulled back, revealing ground cover.

Objective 3.

We observed different seasonal patterns of abundance in Wenatchee and Yakima regions, with leafhopper numbers highest in mid-August and early September in Yakima region plots, while Wenatchee leafhoppers were more abundant earlier and later. Sampling efforts in these plots are ongoing, as we anticipate leafhopper capture through the end of October. We did not observe strong edge effects in our blocks (data not shown) in leafhopper numbers.

Our interior traps included traps at 2ft, 4ft, and 6 ft, allowing us to evaluate optimal trap height for monitoring leafhoppers. These evaluations depended on the control method applied, presumably by altering the number of leafhoppers feeding on the trees versus ground cover. In control plots leafhopper counts were highest in the highest traps, whereas in Surround and Extenday plots leafhopper counts were highest in the 2 foot high traps (Figures 5,6).

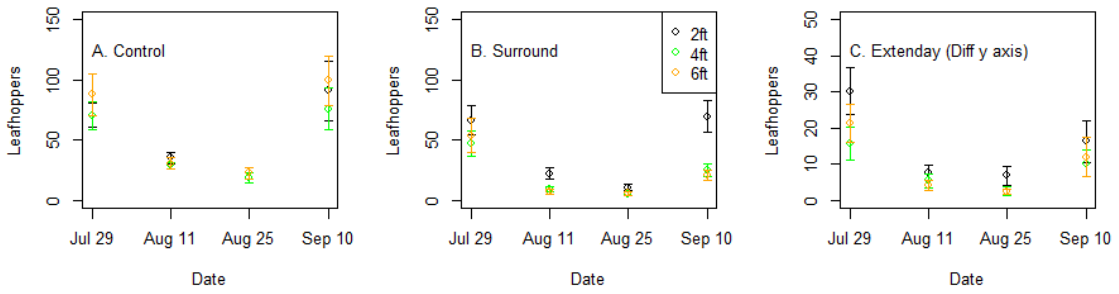

Figure 5 Mean *C. reductus* leafhoppers per trap averaged across 4 Wenatchee region cherry plots, at 2 feet (black), 4 feet (green), or 6 feet (orange) high in control (A), Surround (B), or Extenday (C) plots. Note the different axis for the Extenday plots (C).

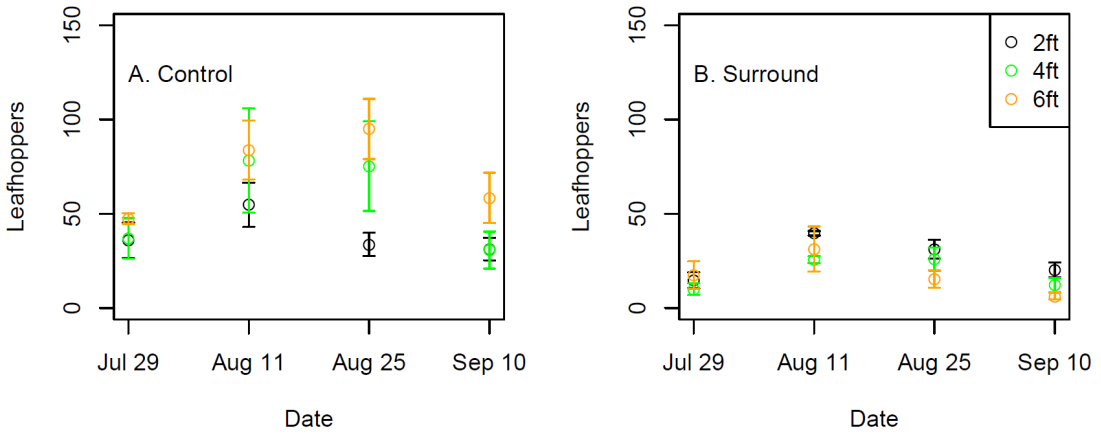

Figure 6 Mean *C. reductus* leafhoppers per trap averaged across 2 Yakima region cherry plots, at 2 feet (black), 4 feet (green), or 6 feet (orange) high in control (A) or Surround (B) plots.

Future plans: We are still collecting leafhoppers from traps in the 2020 season, which extends through October. Next year, we plan to continue trials on the identified plots to track disease progression from year to year, with a few key exceptions. First, now that we have Extenday we will implement it on all cherry blocks. Second, we will discuss the future of the blocks with grower cooperators and if any blocks are set to be removed we will identify other plots for research. We will also reconsider the plots where we did not collect leafhoppers, and search sticky traps on those plots for any potential vectors, which will guide future research. In addition, we have collected leafhoppers from the blocks and stored them in ethanol for gut content analysis after the season.

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

Project Title: Rootstock sensitivity to X disease

PI: Ashley Thompson
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Cooperators: Stacey Cooper, Steve Castagnoli

Total Project Request: **Year 1:** $35,450 **Year 2: $34,658**

Other funding sources: None

WTFRC Collaborative Expenses: None

Budget 1
Organization Name: OSU ARF **Contract Administrator:** Dan Arp
Telephone: (541)737-4066 **Email address:** Dan.j.arp@oregonstate.edu
Supervisor: Nicole Strong **Email address:** Nicole.strong@oregonstate.edu
MCAREC Director: Steve Castagnoli **Email address:** steve.castagnoli@oregonstate.edu

Item	2020	2021
Salaries[1]	$8,112	$8,112
Benefits[2]	$3,245	$3,245
Wages[3]	$8,320	$8,320
Benefits[4]	$5,824	$5,824
Equipment		
Supplies[5]	$5,693	$4,901
Travel[6]	$756	$756
Miscellaneous		
Plot Fees	$3,500	$3,500
Total	$35,450	**$34,658**

Footnotes:
[1] 1 month salary for Dr. Thompson
[2]OPE is calculated at 40%
[3]Wages for a BioScience II technician calculated at $16/hr for 40 hours a week for 13 weeks
[4]OPE is calculated at 70%
[5]150 trees at $15 each = $2,250; Grafting infected bud wood $500; pot-in-pot supplies $1985; Screen House; X disease molecular identification in 2020 20 samples at $35 + $258 set up fee = $958, X disease molecular identification in 2021 $3,500 + $258 set up fee; Pot-in-pot upkeep $1,143.
[6]Travel was calculated at $0.55/mile for 25 trips to the MCAREC from the Wasco County Extension office, which is 55 miles round trip.

OBJECTIVES:

1. Evaluate the response of five rootstocks, 'Maxma 14', 'Gisela 5', 'Gisela 6', 'Krymsk 5', 'Krymsk 6', 'Lake' and, 'Clinton' to the X disease phytoplasma.
2. Identify hypersensitive rootstocks that can be used to reduce X disease inoculum in cherry orchards.

2021 Goals:
March: Inoculate trees by grafting with X-disease infected buds in March.
July: Test all trees for X-disease and check for pits and grooves at the graft union.
October: Assess tree vigor by measuring the trunk cross sectional area and leader growth of each tree. Report findings from the X-disease molecular and physical tests to OSCC.

Deviation from original schedule:
I was unable to hire any additional staff due to COVID-19 restrictions put in place by OSU. This made it challenging to plant trees and obtain nursery materials (pots and soil) in a timely fashion. Due to these challenges, I decided to graft trees in March of 2021 to ensure good bud take and the best possible results. I will likely ask for a no-cost extension for this grant for 2022 to make sure we have enough time to observe infection in these trees. In addition, I have been unable to source 'Maxma 14', and I continue to look for this rootstock.

SIGNIFICANT FINDINGS:

I do not have any significant findings at this time.

METHODS:
Ten of each rootstocks grafted with sensitive varieties (Table 1.) were planted as a completely randomized design in 10-gallon pots filled with general purpose growing medium on 2 June, 2020 at the Mid-Columbia Agricultural Research and Extension Center. Rootstocks were uniformly watered three times weekly. A netted hoop house covering was erected over the trees to prevent the potential movement of X-disease to the surrounding orchard following X-disease infection.

Table 1. Rootstock treatments for this study were selected based on virus susceptibility characteristics and use in the Pacific Northwest.

Rootstock Treatments	**Notes**
'Mahaleb'	Positive control- exhibits a hypersensitive response (death) to X disease
'Mazzard'	Negative control- Susceptible to X disease
'Gisela 12'	Negative control- Susceptible to X disease
'Gisela 6'	Susceptible to pollen-borne viruses, but experiences reduced shoot growth when infected
'Krymsk 5'	Hypersensitive response (death) to pollen-borne viruses
'Krymsk 6'	Hypersensitive response (death) to pollen-borne viruses
'Lake'	Open pollenated with parental parent unknown
'Clinton'	Open pollenated with parental parent unknown

In March of 2021, half of the rootstocks will be infected by grafting three infected buds from confirmed X-disease infected trees will be grafted onto the scion. Uninoculated trees will serve as a control. To insure that the infected buds contain adequate X-disease phytoplasma to create an infection, bud wood was collected in the form of semi-hardwood stem cuttings from confirmed X-

disease positive trees was collected in late summer of 2020 when higher levels of X-disease phytoplasma are expected to be detected. Wood was rooted in a mixture of perlite and sphagnum moss using Hormodin rooting compound. Cuttings are being cared for indoors at the MCAREC.

RESULTS AND DISCUSSION:

I look forward to inoculating trees with X-disease in March, 2021 and reporting on the preliminary results of infection.

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

Project Title: Modeling PNW sweet cherry bud phenology and cold hardiness

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Co-PI(3): Todd Einhorn
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Co-PI(4): Clark Kogan
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City/State/Zip: Pullman, WA, 99164

Cooperators: Western Ag Improvement (Eric Shrum), GS Long (Garrett Bishop), Steve Castagnoli (OSU-MCAREC)

Total Project Request: $98,770 **Year 1:** $98,770

Budget 1
Organization Name: WSU
Telephone: 509-335-2885

Contract Administrator: Katy Roberts
Email address: ARCGrants@wsu.edu

Item	2020
Salaries	$ 62,526
Benefits	$ 7,357
Equipment	$ 4,000
Travel	$ 5,000
Total	$ 78,883

[1] Salaries include 1 months of postdoc time at AgWeatherNet for data processing, $24,005 in salaries and wages for staff in Prosser and Wenatchee to make phenology observations in the field and targeted freeze chamber measurements, and 270 hours of statistician time provided via the WSU Center for Interdisciplinary Statistical Education and Research (CISER).

[2] Benefit rates are budgeted for 10% to 44% depending upon the staff position.

[3] Equipment includes additional pods for freeze chamber measurements and misc supplies.

[4] Travel budgeted for travel to field sites for phenology observations and sampling for freeze chamber measurements.

Budget 2
Organization Name: OSU-MCAREC **Contract Administrator: Dan Arp**
Telephone: 541-737-4066 **Email address: Dan.J.Arp@oregonstate.edu**

Item	2020
Salaries	$5,806
Benefits	$4,581
Equipment	NA
Travel	$4,500
Total	$14,887

[1] Salary includes 0.15 FTE Bio Sci Research Tech in year 1.

[2] Benefit rates are budgeted for 79%.

3 Travel budgeted for travel to field sites and two round trips and associated expenses for Einhorn from Michigan to Washington State.

Budget 3
Organization Name: OSU-Extension **Contract Administrator: Dan Arp**
Telephone: 41-737-4066 **Email address: Dan.J.Arp@oregonstate.edu**

Item	2020
Salaries	$0
Benefits	$0
Equipment	NA
Travel	$5,000
Total	$5,000

[1] Travel budgeted for travel to field sites, industry education on use of AWN app for data collection, and collaboration meetings in Washington State**.**

OBJECTIVES

1. Acquire, organize, process and manage previously collected PNW region data on cherry phenology and cold hardiness.
2. Construct statistical models to estimate sweet cherry bud phenology (endodormancy, ecodormancy, dormancy break, and bud stages to bloom) and predict related lethal temperatures (10%, 50% and 90% mortality).
3. Validate model via a distributed, weekly field campaign focused on Fall 2020 and Spring 2021 to make systematic observations of bud stage and targeted freeze chamber measurements.

SIGNIFICANT FINDINGS

Preliminary analysis of data previously collected by Dr. Gibeaut suggests that:

- a low base temperature (2°C, 35.6°F) should be used for heat unit modeling of spring phenology; and
- the Dynamic Model and chill portions provide the best starting point for the spring heat unit modeling (vs. a fixed January 1 or chill units.)

METHODS

Methods to be used have not changed substantially relative to the proposal submitted in June of 2020. A summary is provided below.

Objective 1 – Data acquisition and processing

Data on hand

From April to May of 2020, Dr. Brown acquired substantial data from Dr. Gibeaut, including:

- A rich 2013 dataset of lethal temperature (LT) measurements associated with bud stage, some relative water content (RWC) measurements, and other related phenological measurements for four cultivars (Bing, Regina, Skeena, Sweetheart) and three locations (Upper Hood River, Lower Hood River, and The Dalles).
- For 2016-19, data was collected from more locations (28 orchards across eight areas, including Dallesport, Dufur, Hood River Lower Valley, Hood River Upper Valley, Mosier, The Dalles, Tri Cities, and Yakima Valley) and cultivars (Attik, Benton, Bing, Chelan, Early Robin, entLapins, Lapins, Rainier, Regina, Santina, Skeena, Sweetheart). But associated phenological information (e.g. bud stage) is not as rich for these LT measurements.

The project team have wrangled this data located in Excel spreadsheets into standard data files, processed weather data, clarified variable meaning, aligned time stamps, and organized metadata where available. This was a necessary first step to initiating modeling.

Other data

The project team continues to work on finding and organizing additional data described below.

Todd Einhorn, co-PI on this proposal, has additional data from 2010-15, similar to the 2013 data. It might also be possible to acquire data from students and technicians who worked on this project at MCAREC over this time period.

Dr. Gibeaut also collected LT and RWC data in 2017 and 2020 that should be passed to AWN. We will attempt to acquire this data though given the lack of bud stage information in 2016 to present datasets, it might be of limited additional value.

GS Long has quality archived freeze chamber data going back to 2015.

WSU researchers Per McCord and Matt Whiting have been making freeze chamber measurements for several years that can be evaluated for methodological consistency and potentially utilized for this modeling effort.

Objective 2 – Modeling

Meteorological drivers

Based upon established literature (Fadón et al, 2020), proven meteorological factors will be used as drivers for bud development (and related lethal temperatures). Though the goal is to estimate lethal temperature for the entire season from the onset of dormancy to bloom, our focus is on bud stages 1-8 in the spring, when buds are most vulnerable to frost damage. The transitions from stage 0 to 1 (dormant to bud swell) and 3 to 4 (green tip to tight cluster) are particularly important for the estimation of lethal temperatures.

- **Cessation of paradormancy and attainment of acclimation**. We will fit an empirical model to predict the timing of para-dormancy cessation and acclimation (as estimated from LT measurements). Drivers could include ***photoperiod*** (day length) and ***air temperature***.
- **Endo-dormancy**. We have compared two established chill calculations, the ***Utah chill units*** and the ***Dynamic Model chill portions,*** for use as a driver for acclimation and termination of endodormancy. As bloom date can be more accurately determined than dormancy break, this end point was used to evaluate chill models.
- **Ecodormancy → bud break**. Bud development after eco-dormancy is usually modeled as a function of ***Growing Degree Hours*** (GDH). There is evidence that ***photoperiod*** might be a factor in bud development at cooler temperatures, so we will include this variable in modeling efforts and evaluate importance. As with chill model evaluation, we used bloom dates to optimize GDH base temperature.

Phenology and lethal temperature

For any measurement of lethal temperatures with buds sampled at one time, location and cultivar, a range of lethal temperatures are obtained—commonly summarized with LT10, LT50 and LT90 values. This range of values is due to both natural variability of bud physiology and the fact that buds are always at a range of development stages. Bud-level phenology will be modeled as ordinal and we will characterize stage-specific probabilities conditional on variety and weather. All sites will be modeled under a multi-level modeling framework to allow for inference on new sites for which the data collection sites are thought to be representative. Site-level phenology will be conveyed from the model as the estimated probability that a bud of a particular cultivar exists in a particular stage at a particular time. This will support the estimation of a distribution of lethal temperatures at any particular time and location. From that distribution, LT10, LT50, and LT90 can be extracted.

Objective 3 – Validation

The model will first be constructed using available data, then externally validated using data collected over the 2020-21 dormancy season. Externally validating this kind of model with only one year is not robust, so we will follow up this exercise by refitting the model using all available data, and validating with a bootstrapping or cross-validation approach.

LT will be measured weekly in Fall 2020 (Sept 14-Nov 16) and in Spring 2021 (Feb 8-April 26) with an additional 4 measurements mid-winter.

Phenology will be recorded from mid-February (dormant) to April (bloom) in Wenatchee, Prosser, and Oregon. For each cultivar (Sweetheart, Regina, Chelan, and Bing), three sites will be identified with eight spurs/cultivar. Phenology will be recorded as number of buds in each stage.

All sampling or freeze measurement orchards will be selected for proximity to a quality weather station or an all-in-one METER Atmos 41 weather station will be installed to make accurate orchard temperature measurements from Sep. 1 to May 31.

The project team will also have access to two additional sources of validation data:

1) Lethal temperature and bud stage data collected by GS Long (archived and for 2020-21 season) from orchards in close proximity to AWN stations.
2) The new AWN Farm app directly solicits bud stage information from growers that can be used to crowd-source model validation and tuning to specific locations.

Project timeline

Objective 1 – Complete data acquisition and processing by September of 2020.

Objective 2 – Complete initial model construction by the end of November of 2020. Complete model revisions using Objective 3 data by June 2021.

Objective 3 – Complete data collection, processing and analysis by May of 2021.

The project is on schedule with Gibeaut data processed and initial model construction under way. It has been challenging to acquire additional data not previously provided by Dr. Gibeaut, so high quality 2020-21 data collection will be essential.

RESULTS AND DISCUSSION

We established preliminary models to predict 50% bloom timing using weekly observed bloom data. We compiled bloom and associated weather data for two cultivars with sufficient observed sites and seasons. We have 20 site-seasons for Sweetheart and 15 site-seasons for Bing, spanning 2013-2019.

To optimize Growing Degree Hour base temperature and chill model selection, we fit models with and without chill accumulation to assess whether inclusion of chill resulted in superior bloom prediction accuracy relative to initiating the spring model on January 1. For the January 1 model, we conducted a grid search over a range of base temperatures. Chill accumulation was computed both using the Utah chill model (chill hours) and the Dynamic Model (chill portions). A grid search across chill hour and chill portion thresholds was conducted and model performance was compared to the January 1 model.

Model performance was measured with the variability in the difference between actual bloom date and predicted bloom date. We established in-sample uncertainty in the error standard deviation by bootstrapping the field seasons. See Figure 1.

- For the January 1 models, the error standard deviation was similar across a large range of base temperatures. So, based upon published literature we decided upon a base temperature of 2°C (35.6°F). A greater geographic range of sampling and observations for 2020-21 could improve estimation of optimum base temperature.
- Using data available, we cannot predict bloom date to less than one week of accuracy—not surprising given the weekly field observation data. More frequent field sampling in the spring of 2021 could improve model performance.

- The Dynamic chill model (chill portions) yielded the lowest prediction errors for Bing, and possibly lower prediction errors for Sweetheart that were not statistically significant. In the 2020-21 field season, we will use field sampling and growth chambers to empirically estimate chill requirements (portions) for Sweetheart, Chelan, Bing and Regina cultivars.
- For Spring of 2021 Beta Model users, the AWNfarm app will require input of an initial bud swelling date for all blocks to be modeled.

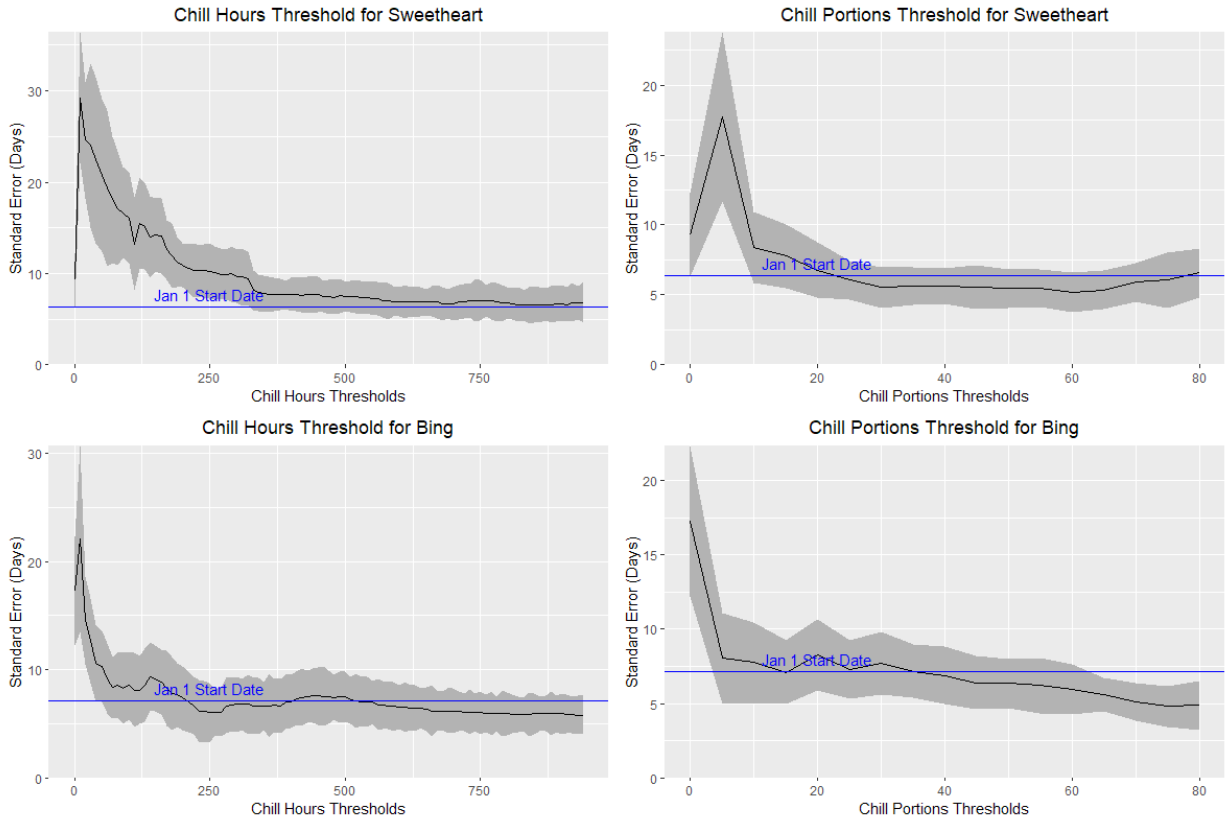

Figure 1. Model error for different chill thresholds.

Oregon Weather Stations

AgWeatherNet incorporated 10 private Oregon Atmos 41 weather stations in the spring of 2019. Recently, AWN has incorporated ~60 private Rainwise stations managed by the Columbia Gorge Fruit Growers. Staff are in the process of adding all WA and OR Agrimet stations to the AWN platform. All of these additions should allow Oregon cherry producers to utilize the AWNfarm platform with representative local weather data.

AWNfarm app

When the cherry cold hardiness model is developed sufficiently for beta release, the model output will be fed into a cherry cold hardiness module within a new free web- and mobile-app **AWNfarm** platform for weather-related decision-support (see Figure 2). A cherry cold hardiness module has been completed with cherry powdery mildew under development. Additional models are planned for release this winter. Growers can also access current, past and forecasted weather data, growing degree

days calculations. And the app will have a frost alarm feature that allows growers to receive a phone call and/or text when temperatures drop below a grower set point. This app was developed using prior cherry funding (collaborating with Dr. Gibeaut), internal funds, and two other sources of grant funding.

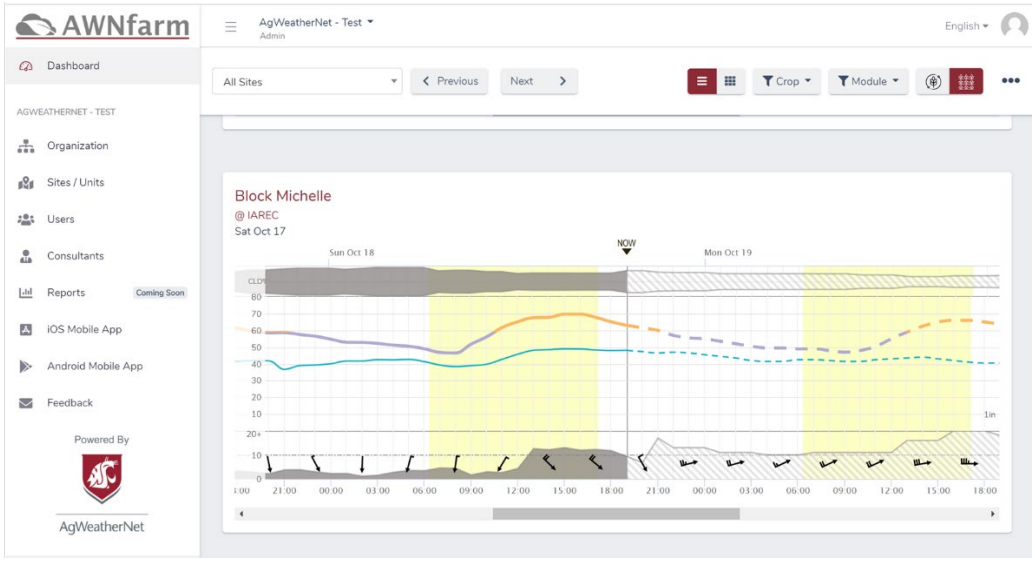

Figure 2. AWNfarm web app, meteogram module.

REFERENCES

Fadón E., et al. (2020). Chilling and Heat Requirements of Temperate Stone Fruit Trees (*Prunus sp*.) Agronomy 2020, 10(3), 409. doi: 10.3390/agronomy10030409

CONTINUING PROJECT REPORT **YEAR**: No Cost Extension

PROJECT TITLE: Durable genetic solutions to powdery mildew infection in sweet cherry

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City/State/Zip: Prosser/WA/99350

Cooperators: Alexandra Johnson (WSU graduate student – Horticulture, Pullman); Gary Grove (WSU – IAREC Plant Pathology, Prosser)

TOTAL PROJECT REQUEST: **Year 1:** $44,000 **Year 2:** $44,000 **Year 3**: $0

Other funding sources:

Agency Name: USDA Germplasm Evaluation Funds, Prunus
Amt. awarded: $28,000 (2020-2021)
Notes: "Germplasm evaluation for sweet cherry genetic diversity and disease resistance". PI: Cameron Peace. Co-PIs: John Preece, Stijn Vanderzande, Alexandra Johnson.

Agency Name: WTFRC/OSCC
Amt. awarded: $539,661 (2019-2021)
Notes: "Supporting a robust PNW sweet cherry breeding and genetics program". PI: Per McCord. Co-PIs: Cameron Peace, Bernardita Sallato, Mateus Pasa.

Agency Name: USDA NIFA – SCRI
Amt. awarded: $10 million (Sep 2014 – Aug 2019)
Notes: RosBREED 2 project for expanding DNA-informed breeding strategies, tools, and knowledge for rosaceous crops. PI: Amy Iezzoni. Co-PIs include Cameron Peace and Per McCord.

BUDGET

Organization Name: W.S.U. **Contract Administrator:** Katy Roberts
Telephone: 509-335-2885 **Email address:** katy.roberts@wsu.edu

Item	2019	2020	2021
Salaries[a]	$26,236	$27,285	
Benefits	$2443	$2541	
Wages	$5330	$5543	
Supplies[b]	$3516	$2156	
Travel[c]	$2000	$2000	
Plot Fees	$4475	$4475	
Total	$44,000	$44,000	No-Cost Extension

Footnotes
[a] Graduate student support for Alexandra Johnson
[b] Single use, disposable materials for sample collection and laboratory assays
[c] Pullman-Prosser return for approx. 4-5 multi-day trips during spring and summer each year

OBJECTIVES

1. Determine the long-term durability of Pmr1 for providing mildew resistance to the PNW industry
  a. Ascertain the pathogen's ability to overcome Pmr1 resistance
  b. Update knowledge about which selections and other PNWSCBP germplasm have Pmr1

2. Determine usability of alternative genetic sources for powdery mildew resistance
  a. Evaluate a diverse set of germplasm for degree of fruit powdery mildew resistance
  b. Identify other genetic factors capable of conferring mildew resistance in PNWSCBP germplasm
  c. Refine the DNA test for resistance to encompass new sources if they exist
  d. Identify which alternative genetic resistance factors are present in important germplasm individuals

SIGNIFICANT FINDINGS

- Two genetic factors at the Pmr1 genomic locus under examination for their influence on genetic resistance to fruit and foliar powdery mildew (PM) infection:
  - Pmr1a ('Moreau'/'Chelan'/PMR-1)
  - Pmr1b ('Hedelfingen'/'Venus' and Mildew-Immune Mazzards)
- Two genetic factors at the Pmr1 genomic locus under examination for their influence on susceptibility to powdery mildew (PM) infection:
  - Pmr1c ('Schneiders'/ 'Regina')
  - pmr1 – the common genetic factor associated with susceptibility
- Pmr1a: Confers complete resistance to PM infection, as concluded in previous years
- Pmr1b: Confers complete resistance to PM infection, as no infection was observed for a second year in detached leaf disk assay
- Pmr1a and Pmr1b offer durable resistance against low to moderate levels of mildew presence. Durability of Pmr1a and Pmr1b under high pathogen pressure will be tested in 2021 to identify differences between the two factors.
- Pmr1c: Not reliable for resistance to PM, as infection was observed for a second year in detached leaf disk assay. DNA test will be further refined to distinguish this genetic factor from Pmr1b.

METHODS

Three-year plan: **2019** – Use previously optimized foliar infection protocols (field and in vitro) for collection of a first season of data. Begin genetic dissection of fruit resistance. **2020** – Continue use of foliar infection protocols (field and lab) to collect a second season of data to validate 2019 results. **2021** – Develop a refined DNA test using data gathered over three years.

Germplasm use and tree management: Trees used for evaluation were growing at the Roza experimental orchard, part of Washington State University-Irrigated Agriculture Research and Extension Center (WSU-IAREC). Individuals selected for this study came from genetic stock trees in the RosBREED block (C53), breeding program mother block (B53), and the Toyama selection block (A37); all of which represent the diversity of the WSU sweet cherry breeding program (about 510 genetically distinct trees total). High-resolution, DNA-profiles of trees in the germplasm from the RosBREED project included those individuals thought to harbor PM-resistance factors.

Germplasm used in 2019 and 2020 included several offspring from resistance sources (15 individuals). These descendants along with their parental sources (40 trees, 25 individuals) included trees expected to be PM-resistant and others expected to be susceptible based on the genotypic presence/absence of Pmr1 and Pmr1-like genetic factors. Pedigree-connected cultivars known to be susceptible were included as positive controls, including 'Bing,' 'Rainier,' and 'Sweetheart.'

Management of orchard trees was conducted in accordance with standard practices of the WSU breeding program with the exception of a misapplication of fungicides mid-season in 2019.

Foliar PM-resistance evaluation – orchard: To assess initial infection within the orchard, chosen trees were observed for signs of mildew infection beginning with leaf emergence. Infection assessment was halted upon discovery of fungicidal applications in 2019. In 2020, chosen trees at the Roza were observed weekly from leaf emergence to early senescence for signs of mildew infection.

Foliar PM-resistance evaluation – lab: A lab-based detached leaf disk assay was performed on the chosen germplasm set for a second year. Briefly, this previously optimized assay began with collecting the first fully expanded leaf from a terminal shoot and transporting it to the lab for surface-disinfestation (10% bleach solution for 3 minutes followed by a quadruple rinse in sterile distilled water). From each leaf, a circular disk (12 mm in diameter) was excised and placed abaxial side up on a new well containing 500 µl water agar of a 24-well plate. Assays conducted for PM resistance/susceptibility consisted of two leaf disks from two independent leaves sampled from each germplasm individual. Conidial suspensions of *P. clandestina* were generated by gathering infected leaves from the mildew block of 'Bing' and 'Sweetheart' trees at the Roza, submerging them in a 0.01% TWEEN solution, and agitating the mixture until conidia were present in solution at sufficient numbers. A 10 µl conidial suspension of 20,000 conidia per mL (quantified through manual count using a hemocytometer) was administered to each leaf. Upon deposition on the leaf disks, conidia were allowed to settle for 5 minutes before residual moisture was wicked away using a sterile cotton swab. Settling time maximizes number of infectious propagules achieving contact with leaf surface, which in turn maximizes likelihood for infection establishment. Plates were subsequently sealed with parafilm to prevent contamination as well as moisture loss, and leaves with conidia were co-cultivated for 14 days in a plant growth chamber at 20°C and a 14 h light period. Plates were then viewed using a stereoscope and mildew presence/absence was assessed. A result was noted as positive if any signs of infection were observed, and negative only if zero mildew was found.

Foliar PM-resistance evaluation – durability under high pathogen pressure – lab: Testing of individuals harboring *Pmr1* and *Pmr1*-like alleles for resistance breakdown under high pathogen pressure was conducted using the standard detached leaf disk assay with different conidial concentrations. Conidia were collected from the mildew block of 'Bing' and 'Sweetheart' trees at the Roza and three concentrations of mildew suspension containing low (140 conidia per mL), medium (1,400 conidia per mL), and high (14,000 conidia per mL) levels of conidia were generated. These three concentrations of pathogen were applied to leaf disks according to the standard detached leaf disk assay protocol and infection presence/absence was assessed after 14 days. Any mildew growth was noted as positive for infection, and negative was recorded only if zero mildew was found.

Preliminary genetic dissection of resistance/susceptibility: Comparison of preliminary genome scan information gathered previously from the RosBREED project was used to facilitate the discovery of a genetic difference among the alleles located at the Pmr1 locus.

RESULTS & DISCUSSION

Summary: Resistance versus susceptibility to PM infection was discernable and repeatable for all individuals tested. Evaluations during the 2020 season verified findings from the 2019 season and substantiate the presence of a second mildew resistance factor. Beyond the previously known Pmr1 allele present in 'Moreau' (Pmr1a), there is compelling evidence for an additional mildew resistance allele (Pmr1b) present in 'Hedelfingen' and the MIMs that also offers complete mildew resistance. The allele present in 'Schneiders' (Pmr1c) has been found to be similar to Pmr1b but offers no mildew resistance, regardless of copy number (Figure 1). Therefore, mildew resistance is lineage-specific and genetic dissection during the 2021 season will elucidate the differences between the allele that confers mildew resistance, Pmr1b, and Pmr1c, which does not.

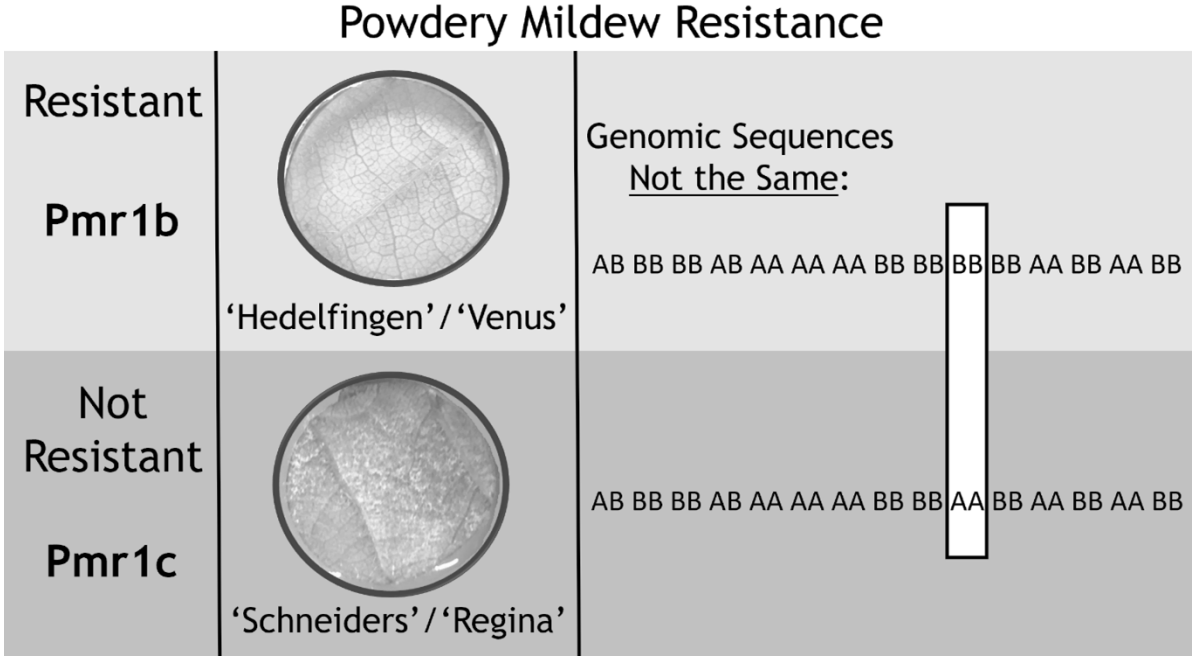

Figure 1. Grouping of mildew resistance determined by leaf disk assay results and DNA profiles

Foliar PM infection – in-lab evaluation: Resistance vs. susceptibility results from 2019 were verified in 2020 using the detached leaf disk assay. Consistent with previous findings, individuals containing one or two copies of Pmr1a (from 'Moreau' and its offspring) were observed to have no forms of mildew infection. Individuals harboring one or two copies of the Pmr1b allele from the 'Hedelfingen' and MIMs lineages were also observed to completely resist mildew infection, substantiating the strong evidence for the presence of this second resistance factor. However, individuals within the 'Schneiders' family (including its offspring 'Regina') with one or two copies of Pmr1c consistently developed PM infection, indicating Pmr1c is not effective for conferring mildew resistance.

Foliar PM infection – in-orchard evaluation: While orchard observations were conducted weekly in 2020, cool weather delayed mildew development in the orchard until the tree soft tissues were mature and therefore physiologically resistant to mildew infection. Infection was observed in some susceptible 'Bing' and 'Sweetheart' cultivars at the Roza orchard during the 2020 season, but no other mildew infections were identified. A third year of orchard observations in 2021, when mildew pressure could be present earlier, will be useful in validating previous season's mildew susceptibility/resistance results.

Durability of Pmr1 genetic resistance: Experiments conducted in 2020 using different levels of lab-standardized pathogen pressure – low (140 conidia per mL), medium (1,400 conidia per mL), and high (14,000 conidia per mL) – demonstrated the effectiveness of Pmr1a and Pmr1b in resisting mildew infection regardless of the quantity of conidia present. However, those individuals harboring Pmr1c were consistently susceptible to mildew infection regardless of conidial concentration. These results indicate that the newly discovered Pmr1b allele is likely just as robust as the previously identified mildew resistance allele Pmr1a in inhibiting mildew infection. Additional testing during the 2021 season will be useful in determining if mildew resistance from Pmr1a or Pmr1b can be overcome when significantly higher pathogen pressure can be produced via early season greenhouse culturing of mildew (20,000 conidia per mL or greater), or if both factors are equally effective in preventing mildew infection when presented with high pathogen pressure.

Genetic dissection of Pmr1b resistance: Further genetic dissection in 2020 revealed strong genetic similarity between the resistance-conferring Pmr1b allele from 'Hedelfingen' and MIM lineages and the ineffective Pmr1c allele from the 'Schneiders' lineage. Due to each group's phenotypic differences, a sensitive DNA test will need to be developed in 2021 to differentiate each factor. The currently available PM-resistance DNA test generates a positive response for Pmr1a, Pmr1b, and Pmr1c alleles, and therefore will need to be refined in 2021 to be sensitive enough for distinguishing the resistance factors from each other and from the similar yet susceptible factor. Newly acquired DNA profile data from the USDA Germplasm Evaluation project has substantiated information that test refinement is possible and has also provided new germplasm targets.

Refinement of the current DNA-based PM-resistance test is currently underway now that laboratory access restrictions due to the COVID-19 pandemic have eased. Assessment of the genetic sequences for Pmr1a, Pmr1b, Pmr1c, and pmr1 will be conducted in 2021 and should elucidate with greater precision the discernable differences among the factors. Phenotypic results of 2019–2020 (Table 1), along with closer dissection of the underlying genetics by assessing DNA-sequence data in 2021, should reveal the precise location of the *Pmr1* locus as well as sequence differences among the effective and ineffective resistance factors.

Variety	Resistance Factor Group	2019 Observations	2020 Observations	2021 Observations
'Moreau'	Pmr1a	Resistant	Resistant	To be determined
PMR-1	Pmr1a	Resistant	Resistant	
'Chelan'	Pmr1a	Resistant	Resistant	
DD	Pmr1a	Resistant	Resistant	
GG	Pmr1a	Resistant	Resistant	
'Hedelfingen'	Pmr1b	Resistant	Resistant	
'Venus'	Pmr1b	Resistant	Resistant	
MIM 17	Pmr1b	Resistant	Resistant	
MIM 23	Pmr1b	Resistant	Resistant	
'Schneiders'	Pmr1c	Susceptible	Susceptible	
'Regina'	Pmr1c	Susceptible	Susceptible	
'Bing'	pmr1	Susceptible	Susceptible	
'Sweetheart'	pmr1	Susceptible	Susceptible	
'Rainier'	pmr1	Susceptible	Susceptible	

Table 1: Resistance status of chosen varieties by year of field and lab evaluation

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

Project Title: Canine detection of Western X disease in controlled and field settings

PI: Heath Smith
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Co-PI(3): Suzie Marlow
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Co-PI (4): Jennifer Hartman
Organization: Rogue Detection Teams
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City/State/Zip: Rice, WA 99167

Cooperators: Washington Tree Fruit Research Commission (WTFRC); Scott Harper, Washington State University (WSU); Teah Smith, Zirkle Fruit Company; Hannah Walters, Stemilt Growers; Garrett Bishop, G.S. Long Company

Total Project Request: **Year 1:** $4,462 **Year 2:** $43,102

WTFRC Budget: None

Budget 1
Organization Name: Rogue Detection Teams (RDT) **Contract Administrator:** Mike Lammi
Telephone: 651-307-8415 **Email address:** mlammi@c2an.com
Station Manager/Supervisor: Heath Smith **Address:** heath.smith@roguedogs.org

Item	2019	2020
Salaries		
Benefits		
Wages	4462	25,000
Benefits		
Equipment		
Supplies		
Travel		
Miscellaneous		
Plot Fees		
Total	4462	$25,000

Footnotes:

Budget 2
Organization Name: WSU **Contract Administrator:** Katy Roberts
Telephone: **Email address:** arcgrants@wsu.edu

Item	2019	2020
Salaries		
Benefits		
Wages		
Benefits		
Equipment		
Supplies		
Travel		
Miscellaneous		
Plot Fees		
Total	2000	

Footnotes:

OBJECTIVES

Project Objectives

1) Ascertain if dogs can detect Little Cherry Disease, LCD-2. Completed
2) Ascertain if dogs can detect Western-X. Completed
3) Develop method for dog teams to detect infected trees in orchards. Incomplete

Anticipated Objectives for 2021

1) Visit 3 to 4 orchards during January 2021 when trees are being pruned
2) Visit orchards/nurseries to test nursery stock for infections, January 2021
3) Visit orchards in March/April just prior to planting new stock
4) Continue testing and developing an efficient method of collection for use in a controlled environment, Nov 2020-Aug 2021

Deviations from original objectives

Positive responses and the display of the desired behavior were rewarded as part of the operant conditioning program. The canine's proficiency was calculated and at least 80% proficiency was required and obtained completing the pilot study.

While teams have had success detecting both viruses in controlled environments and field visits to orchards, there still remain challenges in determining the best method of deploying teams.

SIGNIFICANT FINDINGS

In early spring, 2020, Research Scientists, Jake Lammi and Suzie Marlow with Rogue Detection Teams continued teaching RDT detection dog's recognition of infected samples in a controlled environment (at RDT HQ) using a box apparatus. This allowed RDT to present confirmed samples collected by Teah Smith, Hannah Walters and Garratt Bishop to the dogs in an maintained environment where the dogs could be directed to each sample individually. Dogs were worked using the box apparatus (Figure 6) prior to and following each field visit to an orchard. The apparatus allows for control over what samples the dogs are exposed to as well as to evaluate how they are reacting. While the apparatus can be used as a teaching tool it can also be used to recalibrate the dogs should there be confusion from a field visit where odors can be more elusive and harder for a detection dog handler (bounder) to reward. Many of the exercises with the box apparatus were filmed in order to watch later and observe from.

The bounders working the HQ trails include Heath Smith, Jake Lammi, Suzie Marlow, Collette Yee with detection dogs, Pips, Ranger, Skye, Zilly, Jack, and Dio.

Once bounders established that there were clear indications that the dogs were alerting to infected samples in a controlled environment, RDT wanted to further determine whether this would have an in-field application. As such, the next logical step was to introduce the dogs to a field setting. To be clear, while the final objective of the field visits is to have the dogs alert to infected trees, this was not the initial objective of the project at this stage. Initially, the goal is to have the dogs familiarize themselves with the novelty of a field location. Detection dogs need time to catalogue new odors and potential distractions. As such, this phase took several steps.

Initial field visits in February and March of 2020 yielded success with teams detecting infected trees leading us to conduct additional field visits during the summer growing season with hopes of doing full blown surveys for infections. These field visits included trips to two orchards (Rock Island and Wapato) in June 2020 over three separate visits. The bounders conducting the field visits were Jake Lammi and Suzie Marlow with detection dogs Ranger, Skye and Zilly.

On the first visit, RDT introduced the dogs to freshly pruned limbs from infected trees. The majority of the dog's exposure up to this stage had been from leaves in jars. From there, bounders began leading the dogs directly to marked infected trees and focused the dogs attention on leaves. While there were no clear indications at infected trees during these visits, again, it was more about introducing the area to the dogs. The visit did alert the team to some unexpected challenges including; difficulties searching rows due to the close proximity of trees to one another, concerns regarding odor movement between rows, and the inability to move quickly from row to row which ultimately reduced efficiency and efficacy of field surveys.

On the following two visits RDT changed how we approached our visits to incorporate what we had learned on our initial introduction, factoring in some of the aforementioned challenges. Rather than having the dogs search the rows, bounders picked fresh leaves from the trees and placed them in an apparatus we had brought with us. The dogs had no difficulty distinguishing healthy from infected plants using the fresh leaves. However, finding and collecting all of the materials for this exercise proved time consuming for the teams. One, they were not familiar with the layout of the orchard and trees. Additionally, they encountered hazards with high summer temperatures, as well as identifying ideal times to visit the orchards due to spraying schedules and increased human activity with harvesting schedules. With the challenges posed with working in an orchard effectively and efficiently in mind, we began brainstorming and then developing alternative ideas of detecting the virus using just leaf samples.

What we were learning is that we needed to discover a system that is efficient both on the collection side for growers but also be more efficient than our past experience with freezing and dehydrating samples, time consuming for all parties involved. Some of the ideas we implemented include, redesigning the apparatus we had been utilizing to be closer in design to our "wall" apparatus, with a vertical presentation of the odor for the dog. Built out of 8 panels and containing holes for 40 samples (5 per section), this wall forms an octagon that the dog works inside. As mentioned in past reports, the dogs are much more confident and accurate when there is not a beginning and an end of the exercise scenario and this new design (Figure 5) allows for just such a working concept.

Along with the construction of the new apparatus we also needed a way to tackle the storage of samples so they did not mold. A few challenges we faced include, we were quickly running out of freezer space for samples, dehydrating samples in a dehydrator was time consuming, as well ensuring samples were not contaminated in the process. We were learning that taken all together, there was less time for actually working with the samples and the dogs. RDT brainstormed an idea, a leaf pressing book. To help us determine if this was a viable and more effective option to continue to pursue, growers, Teah Smith, Hannah Walters and Garrett Bishop aided RDT in this venture by collecting samples and pressing them for us to use this fall (2020).

RESULTS & DISCUSSION

Our goal is for dogs to successfully alert to infected plants while effectively and efficiently surveying an entire orchard. This would allow diseased plants to be removed that currently would not start

showing signs of infection until 2-3 weeks prior to harvest. RDT's ultimate goal will be to deploy detection teams at orchards that can evaluate the health of each tree in the orchard, with trees targeted by the dogs being further tested for confirmation and/or removal.

As of October 2020, the dogs are successfully alerting to infected trees in both a controlled and field environment. However, we feel that in terms of accuracy of detection, using the dogs in a controlled environment with dried leaves is showing the most success. Working the dogs in orchards this past summer was difficult to implement due to frequent chemical spraying, high temperatures, activity at the orchards, and lack of movement through the rows.

Chemicals can be troublesome for two reasons; the dogs are using their nose constantly for the work and can inhale a substantial amount of chemicals quickly. While the danger in this is that it can pose long-term health risks, in the short term it may also serve to weaken their ability to differentiate odors, possibly limiting their ability to accurately detect the virus. Scheduling visits between sprays proved challenging and it is our recommendation that working around or during spraying schedules is not conducive to future survey efforts. We are hoping to find some successful alternatives this winter/spring that help avoid the difficult summer months.

With the hurdles we encountered during the field visits we began brainstorming alternate ideas for early detection of the virus. We have already seen success with the dried and crushed leaves in a controlled environment but found storing frozen samples and dehydrating them to be too time-consuming to be an efficient method. Additionally, dehydrating poses risks with sample contamination. Because of this, we decided to initiate a system of pressing leaves for long term storage and easier shipment. While the pressing of leaves has been successful for our purposes it does not sound like it is an efficient method, yet, for the growers to be able to sustain for the long term.

While our goal remains to find a repeatable, effective and efficient method of early detection using the dog teams, moving into 2021 we are focusing on three potential routes that avoid the difficulties we encountered in 2020.

1). One route is to take dog teams in just after pruning and flailing have taken place in January of 2021. This is based on the success we had in February of 2020 when RDT visited orchards and saw multiple instances where the dogs alerted to limbs and mulch from pruning/flailing. Our concern is that the virus has been shown to move into the roots during the winter and we are unable to ascertain at this moment how effective the dogs will be able to detect presence in limbs this late in the winter, based on the limited success we saw in February. The benefit of surveying during pruning and flailing however, lies in the increased volatility of compounds being released from the fresh cuttings. These fresh cuts may provide the dogs a better chance of detecting the odor from infected trees. However, in areas with large amounts of infection, this may also be overwhelming to the dog causing them to appear to shut down, showing no signs of detection. This happens when there is odor saturation and the dog fails in their ability to effectively pinpoint one single source of odor. A visual aid for this is to imagine a dog trained to detect cocaine; they have no trouble when there is a small amount hidden in a locker or car. However, if they are then taken to a warehouse full of cocaine they are faced with the dilemma of what to alert to first because there is simply too much odor to deal with. This can lead to the dog's shutting down.

2). Along with field visits during pruning in January 2021, we hope to also have access to nursery trees. With bare roots, the dogs may be able to quickly tell us if a planter box contains any infected trees or possibly laying trees out so the dogs are able to check each tree individually. This would be very similar to a controlled environment test and shows a lot of potential. If access to nursery stock is

not provided in January we could consider testing saplings directly prior to planting in March/April. We have built into the budget an extra trip if that is needed.

3). We currently have received a number of press books from Teah Smith, Hannah Walters, and Garrett Bishop (Figure 1) and funding that will allow us to continue working with the dogs through December of 2020. Not only does this provide additional material, which is imperative for the dogs to continue to catalog healthy versus infected for their knowledge, it allows RDT to continue developing the method in a controlled environment. While there remain some challenges to overcome on the efficiency of collection, this has proven to be the clearest and most accurate use of the dogs so far. Press books allow RDT to test samples any time of day, throughout the year, in an environment where we do not have to worry about temperature, wind, distractions or dangers. Samples can be stored indefinitely and do not need to be frozen or refrigerated. We are discussing ideas with orchards that will hopefully allow a person to walk down the rows of trees and collect leaves from multiple trees on one sheet, quickly cataloging the samples, and then turning to a new sheet and continuing on. We expect that a single binder could hold leaves from 200 to 300 trees (Figure 2). We want to make this method not only efficient but accurate in terms of designating which trees are labeled infected by the dogs.

Our apparatus (Figure 3) allows for 40 samples to be placed for inspection by the dogs with at least three, but up to five, dogs testing each sample. Having five dogs test each of those 40 samples would roughly take an hour. Once operating effectively, this would allow us to test 240 samples in a single day.

In 2021 we hope to;

1) Visit 3 to 4 orchards during January when trees are being pruned
2) Visit orchards/nurseries to test nursery stock for infections
3) Visit orchards in March/April just prior to planting new stock
4) Continue testing and developing an efficient method of collection for use in a controlled environment

FIGURES

Figure 1. Press books of leaves (infected and healthy) from collaborators, shipped from orchards to Rogue Detection Teams Headquarters.

Figure 2. Pressbook “binder design”, utilizing a deconstructed three ring binder with wood layers and ratchet strap to effectively separate healthy and infected leaves from various individual trees, and to allow for storage and proper drying.

Figures 3-5. Wall apparatus with detection dog, Dio. Note: These photos were for demonstrating use of the wall apparatus, as such there are no jars with samples in the wall.

Figure 6. Box apparatus

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

Project Title: Pesticide residues on WA cherries

PI: Tory Schmidt
Organization: WTFRC
Telephone: (509) 665-8271 x4
Email: tory@treefruitresearch.com
Address: 1719 Springwater Ave.
City/State/Zip: Wenatchee, WA 98801

Cooperators: Gerardo Garcia, Sandy Stone, Pacific Agricultural Labs, Northwest Hort Council, Doug Stockwell, Doyle Smith, various ag chemical companies

Total Project Request: **Year 1:** $4349 **Year 2:** $5450

Other funding sources: **Awarded**
Amount: **Chemical supplies**
Agency Name: **Various ag chemical companies**
Notes: **Registrants typically donate chemicals to be tested**

WTFRC Budget

Item	2020	2021 (est.)
Salaries		
Benefits		
Wages[1]	1269	1350
Benefits[1]	680	700
RCA Room Rental		
Shipping[2]	300	300
Supplies/Chemicals	300	300
Travel[3]	800	800
Plot Fees		
Analytical lab fees	~~4000~~ 1000*	2000
Total gross costs	~~7349~~ 4,349*	4,450
Anticipated Income (contracts and gift grants)	0	0
Total net costs	~~7349~~ 4,349*	**5,450**

Footnotes: Schmidt estimates 10% of his time is dedicated to this project on an annual basis
Most pesticides tested are donated by their registrants or an ag chemical supply company
1 Wages & benefits primarily for Garcia (spray applications), crew help for Garcia, and Stone (data entry & review)
2 Est. costs to ship cherries overnight to Sherwood, OR
3 Travel costs include hauling equipment to & from plots
*Note: actual lab fees were less than projected in the original budget ($4000) due to simplified trial protocol

2020 WTFRC CHERRY PESTICIDE RESIDUE STUDY

Since 2011, the WA Tree Fruit Research Commission has conducted annual studies of residues of commonly used pesticides on cherry fruit at harvest. Digital versions of this report and similar studies on apple and cherry including comprehensive summaries of multiple years' results are available at www.treefruitresearch.org. For current information on maximum residues levels (MRLs) and other regulatory issues, please consult the Northwest Horticultural Council website at http://nwhort.org/export-manual.

TRIAL DETAILS

- Mature 'Bing'/Mazzard multiple leader open vase trees on 10' x 20' spacing near Orondo, WA
- 11 insecticides/acaricides & 6 fungicides applied at or near maximum rates and minimum pre-harvest and re-treatment intervals
- Ground applications made by Rears PakBlast PTO-driven airblast sprayer of the same rate of product per acre with 8 oz Regulaid surfactant/100 gal water at 200 gal water/acre
- Approx. 0.4" of rain fell on trial block over the course of the study, including ¼ inch falling 12 days before harvest (May 30)
- Grower applied RainGard 4 times during the study (5/29, 6/5, 6/10, 6/19) to reduce cracking, which may have helped preserve residues of pesticides applied by WTFRC staff
- Samples submitted overnight to Pacific Agricultural Labs (Sherwood, OR) for chemical analysis

Airblast application @ 200 gal/acre

RESULTS & DISCUSSION

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

Cherries dripping after application

Insectides/acaricides: Bexar, Agri-Mek 0.15SEC, Transform WG, Danitol 2.4EC, Perm-Up 3.2EC, Carbaryl 4L, Onager

Fungicides: TopGuard, Torino, Gatten, Orbit, Topsin 4.5FL

Residue levels were generally higher in the 2020 study than in "typical" seasons, most likely due to mulitple applications by the grower of the anti-cracking agent RainGard, which may have provided a protective coating against rain and UV light which would typically degrade residues. Previous WTFRC studies (2013-2015) have also demonstrated a tendency for rain protectants (RainGard, Parka) to preserve residues on cherry. MRLs are known to change frequently and cherry producers should routinely monitor the most current information (http://nwhort.org/export-manual) to facilitate compliance with constantly shifting foreign standards.

Measured residue levels vs. MRLs for pesticides applied to cherry fruit at 200 gal water/acre. 'Bing'/Mazzard, Orondo, WA. WTFRC 2020.

Common name	Trade name	Application rate[1]	Application timing(s)	Measured residue	US tolerance[2]	Lowest export tolerance[3]
		per acre	*days before harvest*	*ppm*	*ppm*	*ppm*
tolfenpyrad	Bexar	27 oz	28, 14	0.77	2	0.01 (many)
abamectin	Agri-Mek 0.15SEC	20 oz	21	0.030	0.09	0.01 (EU)
zeta-cypermethrin	Mustang MAX	4 oz	21, 14	0.21	1	1 (Kor)
thiamethoxam	Actara	5.5 oz	21, 14	0.40	0.5	0.5 (many)
chlorantraniliprole	Altacor	4.5 oz	21, 10	0.18	2.5	0.5 (Kor)
acetamiprid	Assail 70WP	3.4 oz	21, 7	0.56	1.5	1 (Tai)
flutriafol	TopGuard	14 oz	14, 7	0.49	1.5	0.8 (Codex, Kor)
myclobutanil	Rally 40WSP	6 oz	10, 1	0.79	5	1 (Can, Tai)
sulfoxaflor	Transform WG	2.75 oz	14, 7	0.52	3	1.5 (many)
cyflufenamid	Torino	8 oz	14, 7	0.27	0.6	0.02 (Aus)
fenpropathrin	Danitol 2.4EC	21.3 oz	14, 3	1.7	5	0.01 (EU)
permethrin	Perm-Up 3.2EC	8 oz	14, 3	0.96	4	0.05 (EU)
carbaryl	Carbaryl 4L	96 oz	10, 3	7.4	10	0.01 (EU)
flutianil	Gatten	8 oz	10, 3	0.074	0.4	0.01 (many)
propiconazole	Orbit	4 oz	10, 1	0.48	4	0.01 (EU)
thiophanate-methyl*	Topsin 4.5FL	30 oz	10, 1	1.96	20	0.3 (EU)
hexythiazox	Onager	24 oz	7	0.29	1	0.1 (Kor)

[1] All materials were applied by Rears PakBlast sprayer with 8 oz Regulaid/100 gal water
[2] 5 Aug 2020. http://nwhort.org/export-manual/comparisonmrls/cherry-mrls/
[3] Major export markets for Pacific Northwest cherries; 5 Aug 2020; tolerances may be based on published MRLs or default values. http://nwhort.org/export-manual/comparisonmrls/cherry-mrls/
* Reported thiophanate-methyl values reflect sum total of thiophanate-methyl and carbenzadim residue levels

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

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.

CONTINUING PROJECT REPORT **YEAR**: No-Cost Extension

Project Title: Erythritol formulation for SWD control: field trial and mode of action

PI:	Jana Lee	**Co-PI:**	Man-Yeon Choi
Organization:	USDA-ARS	Organization:	USDA-ARS
Telephone:	541-738-4110	Telephone:	541-738-4026
Email:	jana.lee@usda.gov	Email:	man-yeon.choi@usda.gov
Address:	USDA ARS 3420 NW Orchard Ave. Corvallis, OR 97330	Address:	USDA ARS 3420 NW Orchard Ave. Corvallis, OR 97330

Cooperators: Chris Adams, Assistant Professor, Mid-Columbia Research and Extension Center

Total Project Request: **Year 1:** $35,800 **Year 2:** no-cost extension

Other funding sources: **Awarded**
Amount: $50,000, $14,800 subcontracted to OSU
Agency Name: NW Center for Small Fruits Research
Notes: Award notice in summer 2020, to be conducted in 2021. Titled "Sweet SWD control: non-target effects and field trials with erythritol." Focus on field trials in blueberries, and studying impacts on honeybees with Dr. Sagili.

Budget 1
Organization Name: USDA ARS **Contract Administrator:** ARF, Charlene Wilkinson
Telephone: 541-979-6672 **Email address:** Charlene.wilkinson@oregonstate.edu
Supervisor: Jana Lee **Email Address:** jana.lee@usda.gov

Item	2020	2021
Salaries	$16,400	
Benefits	$3,500	
Supplies	$3,007	
Travel	$1,000	
Miscellaneous		
Plot Fees		
Total	23,907	no-cost exten.

Footnotes:

Budget 2
Organization Name: OSU MCAREC **Contract Administrator:** Steve Castagnoli
Telephone: 541-386-2030 **Email address:** steve.castagnoli@oregonstate.edu
Supervisor: Chris Adams **Email Address:** chris.adams@oregonstate.edu

Item	2020		
Salaries	$7,000		
Benefits	$3,000		
Travel			
Plot Fees	$1,893		
Miscellaneous			
Total	$11,893	Total year 2	Total year 3

Objectives
This project addresses SWD management by using a non-toxic method safe for humans. While erythritol is promising, the formulations need to be improved for convenient field application and consider non-target effects.

Obj. 1a) Examine the efficacy of new formulations in sweet cherry trees for reducing SWD infestation.
Obj. 1b) Monitor visitation rates by honeybees.

Obj. 2a) Test the phagostimulative effect of the new formulation with sucralose.
Obj. 2b) Explore the mode of action of the new erythritol formulations, and whether erythritol ingestion interferes with the metabolism of other ingested sugars.

Significant Findings
- Flies consumed formulations readily even with the E+Sul which has no nutritional value.
- Flies excreted more when fed erythritol formulations, especially with E+Sul, suggesting that this causes osmolar imbalance quickly.
- Honeybees did not visit erythritol-sprayed plots more than control plots, suggesting a low non-target risk when erythritol is sprayed post-bloom.
- Late season infestation in cherries were marginally different between treatments, and were numerically lowest in E+Suc, intermediate in E+Sul treated fruit, and highest in controls.

Methods

1a. Examine the efficacy of the new formulations in sweet cherry trees for reducing SWD infestation
We sprayed: 1) 1.5 M erythritol: 0.5 M sucrose (E+Suc), 2) 1.5 M erythritol: 0.1 M sucralose (E+Sul), or 3) water control on cherry trees once they started ripening at MCAREC on June 3, 2020. Plots consisted of 3 cherry trees and each treatment was replicated in 5 blocks. Plots were separated by ~20 m. We and Sampson et al. (2018) have found significant treatment effects in similar plot arrangements in blueberries. Each week, we monitored the presence of SWD adults in baited apple cider vinegar: wine traps, and for larval infestation by collecting and rearing ripe fruit for adult fly emergence. SWD infestation and trap counts were compared with a repeated measures in Proc Glimmix in SAS; with treatment, week and their interaction as fixed effects, plot and block as random effects using the best fit distribution (Poisson, normal, lognormal).

1b. Monitor visitation rates by honeybees.
Our previous work indicates that honeybee adults survive similarly on erythritol as with other sugar solutions. Each week on a sunny and clear day, we took 2 minute observations per plot of honeybees, yellow jackets and other sugar feeding insects seen foraging. A similar repeated measures analyses compared honeybee and yellow jacket presence.

2a. Test phagostimulative effect of new formulation with sucralose
In progress In a choice assay, five flies were introduced into a plastic vial with a lid with and 2 glass capillary tubes with solutions of varying sweetness and nutritional value. Tubes contained water+erythritol, water+sucrose, water+sucralose, Ery+Suc, Ery+Sul, or Suc+Sul. Solutions were covered with a mineral oil to prevent evaporation. The amount in the capillary tube was measured before feeding and recorded daily for 72 hours.

In progress In a no-choice assay, one fly will be introduced into a plastic vial with a lid and 1 glass capillary tube. Treatment solutions are erythritol only, sucrose only, sucralose only and water only. The amount in the capillary tube and fly bodyweight will be measured before feeding, 24 hours after feeding

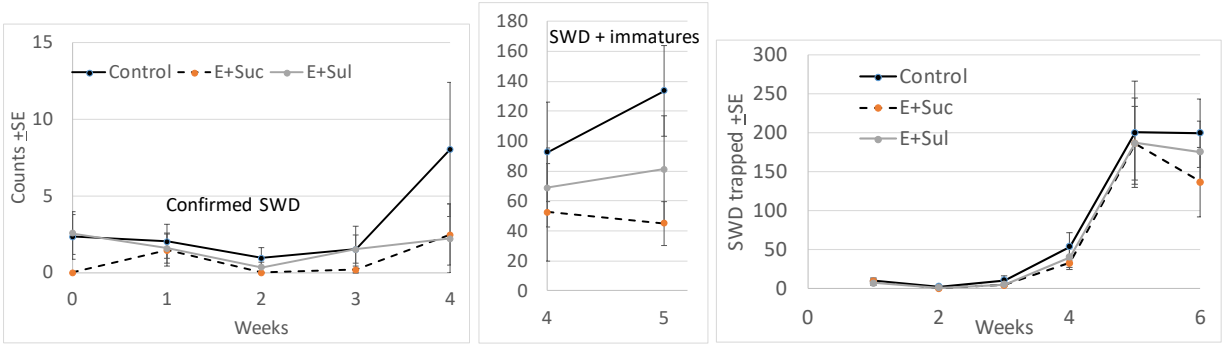
and 48 hours after feeding. An ANOVA will compare weight change and consumption by flies between treatments.

In progress *2b. Explore the mode of action of the new erythritol formulations, and whether erythritol ingestion continues to interfere with the metabolism of other ingested sugars*
To compare metabolism of ingested formulations into useable energetic reserves, 5-day old SWD will be fed: 1) E+Suc, 2) sucrose only, 3) E+Sul, 4) Sucralose only, and 5) water only for 2 days. Live SWD will be frozen and examined for sugar and glycogen levels (Wong et al. 2018). If erythritol interferes with metabolism, we will find different energetic reserves in flies given sucrose or sucralose with and without erythritol. If no energetic differences appear between E+S and S, this suggests that E+S is mainly detrimental by causing osmolarity imbalance, and not by starvation. To determine the location of sugar metabolism in the fly body, 20 females flies starved for 24 h will be fed solutions 2 days. The hemolymph and frass will be collected from fed-flies, and analyzed on a GC-MS

Results and Discussion
Obj. 1a Efficacy in sweet cherry

- Before cherry trees were sprayed at week 0, natural infestation was low and not different among assigned trees (LMM treatment $F_{2,8} = 1.5$, $P = 0.29$).
- After sprays were applied, from weeks 1 to 3, flies emerging from collected fruit were confirmed to be SWD. No significant differences were observed between treatments (LMM treatment $F_{2,12} = 0.77$, $P = 0.48$, week $F_{2,24} = 1.7$, $P = 0.20$, tr*wk $F_{4,24} = 0.14$, $P = 0.97$, Fig. 1a).
- During the late or post-harvest period, from weeks 4 to 6, collected fruit softened quickly and liquefied making it hard to identify SWD. Adults were identified as SWD, but larvae and pupae were not during weeks 4 and 5. Samples were too moldy from week 6 to get an accurate count, and not included in analysis. A marginal difference was observed during this late period between treatments (LMM treatment $F_{2,12} = 2.9$, $P = 0.097$, week $F_{1,12} = 0.38$, $P = 0.55$, tr*wk $F_{2,12} = 0.33$, $P = 0.73$, Fig. 1b).
- Lastly, adults captured in traps were not different between treatments (GLMM Poisson, treatment $F_{2,12} = 0.99$, $P = 0.40$, week $F_{5,60} = 99.0$, $P < 0.001$, tr*wk $F_{10,60} = 0.94$, $P = 0.51$, Fig. 1c). Pest presence was increasing by week 4, and nearly 200 flies were caught per trap at week 5 which includes flies trapped between weeks 4 and 5. This is consistent with heavy infestation found in collected cherries starting at week 4.

Fig. 1. Number of confirmed SWD emerging from collected cherries (a), SWD + immatures from cherries late/post-harvest (b), and SWD in traps (c).

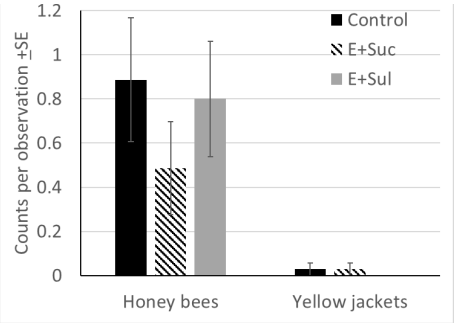


Depending on field availability, and resources, we may try to repeat this trial in cherries in 2021.

Obj. 1b Honeybee visitation

Few bees and even fewer yellow jackets were observed foraging on cherry trees post-bloom when observations were taken during warm sunny periods (Fig. 2). No bees were seen visiting freshly sprayed trees when trees were observed following the initial spray on week 0, and on week 2 when touch-up sprays were made after rainfall. There was no difference in visitation between treatments for honeybees (GLMM Poisson treatment $F_{2,12} = 1.9$, $P = 0.19$, week $F_{6,72} = 44$, $P < 0.0001$, tr*wk $F_{12,72} = 0.46$, $P = 0.93$), and yellow jackets (GLMM Poisson treatment $F_{2,12} = 0.43$, $P = 0.66$, week $F_{6,72} = 0.89$, $P = 0.50$, tr*wk $F_{12,72} = 1.28$, $P = 0.25$).

Fig. 2. Sugar-feeding visitors.

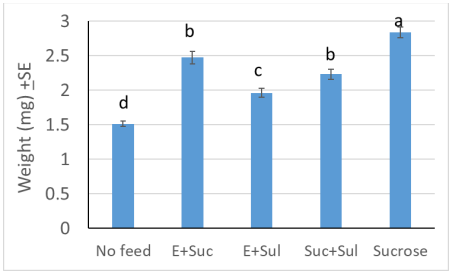


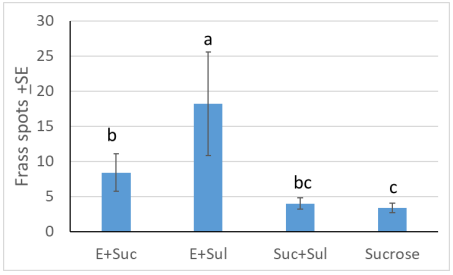
Obj. 2b Explore mode of action

SWD exposed to various sugar formulations all gained weight compared to the no feeding controls (GLM lognormal, treatment $F_{4,70} = 58.0$, $p < 0.0001$, Fig. 3a). Weight gain was greatest with flies given only sucrose, followed by mixtures with sucrose. The E+Sul, a non-nutritive yet sweet solution, still elicited feeding though it does not provide energy.

Excretion by SWD fed sugar formulations was measured by counting the number of frass deposits when flies were held in a vial with transparent film. Frass deposition varied by treatment (GLM Poisson, treatment $F_{3,16} = 23.3$, $P < 0.0001$, Fig. 3b). SWD fed E+Sul excreted the most suggesting that this formulation may lead to quicker osmolar imbalance.

Fig. 3. Adult SWD fed treatments and subsequent weight gain (A), and frass





CONTINUING PROJECT REPORT **YEAR**: No-Cost Extension

Project Title: Fungicide resistance: A vital need to protect PNW cherries from mildew

PI: Gary Grove, PhD
Organization: Washington State University
Telephone: 509-786-9283
Email: grove@wsu.edu
Address: 24106 N Bunn Road
Address 2: IAREC
City/State/Zip: Prosser, WA 99350

Co-PI (2): Prashant Swamy, PhD
Organization: Washington State University
Telephone: 509-786-9284
Email: prashant.swamy@wsu.edu
Address: 24106 N Bunn Road
Address 2: IAREC
City/State/Zip: Prosser, WA 99350

Cooperators: Tianna DuPont, Bernardita Sallato, Neusa Guerra

Total Project Request: **Year 1:** $60,175 **Year 2: $71,276** **Year 3:** $0

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

Item	2019	2020	2021
Salaries[a]	$18,405	$19,141	
Benefits	$8,958	$9,316	
Wages[b]	$11,520	$11,981	
Benefits	$1,152	$1,198	
Supplies[c]	$18,250	$27,750	
Travel[d]	$1,890	$1,890	
Total	$60,175	$71,276	**0**[5]

Footnotes:
[a] 0.5 FTE for an associate in research
[b] Time slip field and laboratory workers
[c] DNA extraction kits, DNA sequencing, Next-Gen sequencing, laboratory chemicals and supplies. Additional $4,500 is requested to cover supplies, kits and chemicals for additional study
[d] Travel to various orchard site for mildew collections, spore collections and follow up trips in WA and OR
[d] No cost extension

Budget 2
Organization Name: WSCPR Contract Administrator: WTFRC; 501 Consultants
Telephone: 509-665-8271 Email address:vicky@501consultants.com
Station Manager/Supervisor: Gary Grove Email Address: grove@wsu.edu

Item	(2019-2020)[a]
Salaries	**$15,241**
Wages	**$2,560**
Supplies	**$3,500**
Travel	**$1,500**
Total	**$22,801**

Footnotes:
[a] Funds were requested from Washington State Commission on Pesticide Registration to complement present study. The grant proposal was applied through Washington Tree Fruit Research Commission and awarded funding for CY2020 to carry out additional work on nursery and orchard isolates.

Recap of objectives

1. **Investigate the presence and extent of fungicide resistance in commercial orchards in the Pacific Northwest.** A total of 20 unique orchard sites (WA and OR) in 2019 and a collection of 11 orchard sites (WA and OR, 192 individual single colony isolates) and 5 nursery sites (WA alone, 80 single colony isolates) were used in the 2020 study. We have made extensive progress in both years and have conclusively identified the presence and extent of fungicide resistance to FRAC 3, FRAC 7 and, FRAC 11 group of fungicides.

2. **Identify and develop specific genetic markers for better identification of fungicide resistance.** We have been successful in designing, amplification, and sequencing of fungicide target genes of FRAC Groups 3, 7, and 11. The robustness of the assays was tested on several isolates and resistant colonies in 2019. In 2020 we identified genetic mutations in the FRAC group 7 target genes. This objective was successfully accomplished and at present, we have genetic information on three fungicide target genes.

3. **Develop alternative programs for disease management, if significant fungicide resistance is documented in this study (Conditional).** As we discuss the results below, insensitivity to some FRAC groups is present in both states. Our experimental data underscored the very problem of fungicide resistance in commercial orchard and nurseries. Moreover, given the extent of fungicide resistance to multiple groups of chemicals, there is an urgent need for alternative strategies to be implemented for managing powdery mildew. We initiated research in this direction, thanks to the additional collaborative funding from WSCPR.

Significant findings

Please note that the causal pathogen, Podosphaera clandestina is now identified as Podosphaera cerasi. The new nomenclature Podosphaera cerasi (P. cerasi) is used in this report.

- We accomplished the collection of 20 composite and 272 single colony *P. cerasi* isolates in diverse locations of cherry growing areas in WA and OR.
- DMI target gene was identified. Full-length gene was sequenced and assays to distinguish mutations corresponding to DMI -resistant and DMI-susceptible isolates were developed. The assay was used to identify the presence and extent of DMI resistance in nursery and orchard *P. cerasi* isolates. Overall, we found 28% isolates resistant to DMI fungicides (molecular-based detections).
- Major SDHI target gene, SDHB was identified using data from next-gen sequencing in 2018, and mutations corresponding to SDHI resistance were identified. Of the three fungicide groups (DMI, SDHI, and QoI), very few *P. cerasi* isolates were SDHI-resistant (10%).
- QoI target gene was tested in all cases and we found molecular evidence of fungicide resistance in 43% of isolates in 2019. Although there is no discernible pattern in the geographic distribution of isolates resistant to FRAC Group 11, we found a dramatic increase (87%) in QoI resistance in one of the orchards as a follow-up study.
- Leaf-disc bioassay confirmed our molecular data for FRAC Group 3 and 11 fungicides. Alternative mechanisms, other than genetic modification, may be responsible for the higher incidence of insensitive colonies in 2019 bioassay experiments.
- Several other FRAC Groups were also tested using the bioassays. In some cases, *P. cerasi* isolates were insensitive to fungicides from several FRAC Groups indicating that there is a good chance of fungicide resistance in FRAC Groups in addition to FRAC 3 and FRAC11. We

complemented the bioassay results with molecular confirmation on isolates resistant to DMI and QoI fungicides.

- In 2019, several *P. cerasi* isolates were incorrectly identified as resistant to FRAC 19 (polyoxin-D or PH-D) when applied as preventative dosage (leaves treated with polyoxin-D and later inoculated with *P. cerasi*). PH-D is a curative fungicide and was able to eliminate *P. cerasi* infections in bioassay results. Therefore, no further experimentation was necessary.

Methods used

- The methods for handling of *P. cerasi* isolates were followed as proposed in the project. In addition to the isolates from commercial orchards, several *samples* were collected from four independent nurseries and tested for the presence of DMI fungicide resistance in 2020. In 2019 all *P. cerasi* isolates were collected as composite isolates from each location. In 2020, individual colonies growing as foliar infections were collected directly in separate tubes and treated as single isolate (Table 1).

Table 1. List of *P. cerasi* isolates from orchard and nursery sites in Washington and Eastern Oregon.

						Collection method	
No.	**Code**	**Production Area**	**County**	**Variety**	**Management**	**2019**	**2020**
1	DH	Columbia Basin	Franklin	Bing	Conventional	Composite	Individual
2	CS	Columbia Basin	Franklin	Rainier	Conventional	Composite	
3	MH	Yakima Valley	Yakima	Bing	Conventional	Composite	Individual
4	HL	Columbia Basin	Grant	Rainier	Conventional	Composite	Individual
5	SC	Columbia Gorge	Wasco, OR	Rainier	Conventional	Composite	Individual
6	AR	Columbia Gorge	Hood River		Conventional	Composite	Individual
7	Roza	Yakima Valley	Benton	Bing	No fungicides	Composite	Individual
8	TP	Yakima Valley	Klickitat	Sweetheart	Conventional	Composite	Individual
9	RS	Wenatchee	Okanogan	Rainier	Organic	Composite	
10	BR	Wenatchee	Okanogan	Sweetheart	Conventional	Composite	
11	BO	Wenatchee	Chelan	Rainier	Conventional	Composite	
12	HF	Wenatchee	Chelan	Rainier	Conventional	Composite	Individual
13	ST-1	Wenatchee	Chelan	Sweetheart	Organic	Composite	Individual
14	ST-2	Wenatchee	Chelan	Bing	Organic	Composite	
15	BC	Yakima Valley	Benton	Bing	Conventional	Composite	Individual
16	BM	Columbia Basin	Grant	Bing	Conventional	Composite	
17	HT	Yakima Valley	Yakima	Rainier	Conventional	Composite	
18	OR	North Central WA	Okanogan	Lapins	Conventional		Individual
19	WD	Columbia Basin	Grant	Bing	Conventional	Nursery	Individual
20	ML	Columbia Basin	Grant	Bing	Conventional	Nursery	Individual
21	CO	Columbia Basin	Grant	Montmorency	Conventional	Nursery	Individual
22	CN	Columbia Basin	Franklin	Bing	Conventional	Nursery	Individual

- We found unexpectedly greater DNA sequence variability in the cytochrome b (cytb) sequence, the molecular target gene of Group 11 (QoI) fungicides (but the high similarity to deduced amino acid sequence). The approach to primer design for amplification of this gene from several samples is still a challenge. We used the data from the next-generation sequencing experiment to identify the full-length sequence but were unsuccessful to obtain (amplify) the complete sequence. Nonetheless, the partial cytb DNA sequence harboring mutations of interests were readily amplified using two sets of primer pairs.

- The CYP51 DNA sequences of myclobutanil and triflumizole- insensitive colonies (from bioassay experiments) were sequenced from several isolates. We found a single but less frequent mutation that correlated with bioassay results. The PCR analysis in 2020 was performed using an assay developed to identify the mutations of the target gene, CYP51. We used qPCR assays to distinguish between DMI-resistant- and susceptible isolate. Similarly, one of the major SDHI target genes, SDH-B was identified, and the information was used to obtain mutations corresponding to SDHI resistance in orchard isolates.

- We have initiated efforts to provide recommendations based on our findings in 2019 and 2020. In 2021, we will consult with growers, WTFRC, pesticide companies, and extension specialists to prepare recommendations for resistance management. The resistance assays developed herein can be communicated to interested parties for the possible commercialization of the molecular diagnostic assays.

Results and discussion

In the 2020 cherry growing season, several individual isolates of *P. cerasi* were collected from nursery and commercial orchards (including organic orchards) in all cherry growing regions of Oregon and Washington (Table 1). All isolates collected in 2019 were tested in mildew-susceptible leaf discs treated with candidate fungicides with an application rate equivalent to 200 gallons spray material per acre (please see 2019 continuing report). In 2020, all isolates were tested using molecular methods. Mutations corresponding to DMI, SDHI and QoI were identified using qPCR (DMI) and cloning and sequencing (SDHI and QoI) approach. In 2019, leaf discs treated with fungicides pointed out a potential problem of fungicide resistance in the PNW cherry orchards. Follow up with molecular targets and their underlying mutations corresponding to fungicide resistance confirmed that *P. cerasi* isolates were indeed resistant to either DMI or QoI fungicides. All isolates (composite, individual, and nursery) were subjected to qPCR assays to distinguish between DMI-resistant and DMI- sensitive *P. cerasi*

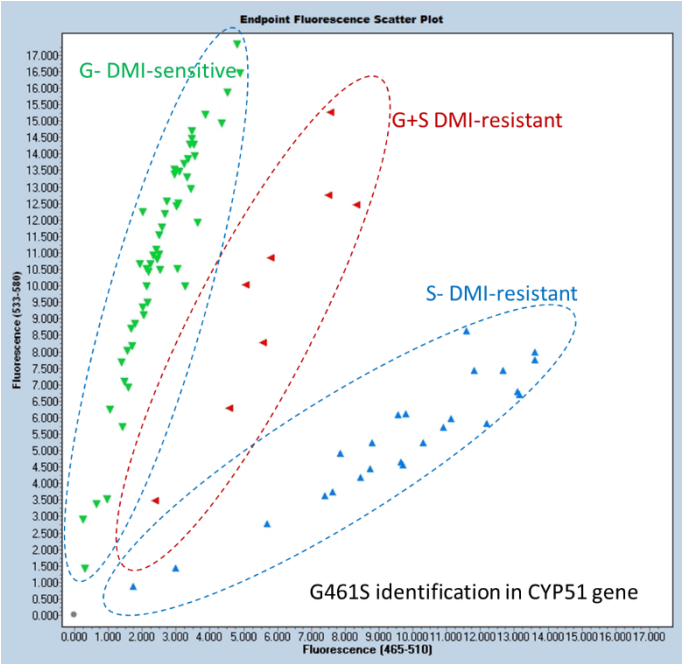


Figure 1. Probe-based qPCR assay to identify a point mutation in CYP51 gene corresponding to DMI resistance.

isolates using a probe-based qPCR assay (Figure 1). We found fungicide resistance as high as 45% in commercial orchards while 75% isolates were resistant to DMI fungicides in two nursery locations. We did not find resistance specific mutations in some orchard locations (Table 2). Our experiments indicated a potentially severe DMI resistance problem than originally anticipated. It should be noted however that, DMI fungicides belong to a broad class of antifungal agents with multiple modes of action on target pathogen. Additionally, pathogen resistance to one DMI class (e.g. triazoles) does not guarantee resistance to another DMI class (e.g. pyrimidines). For these reasons, each spray application involving DMI should be carefully evaluated. The fungicides should be removed and replaced with other effective groups from the mildew management in case of poor disease management.

Three target genes are targets of SDHI fungicides and mutations within these genes affect fungicide efficacy. Among those, mutations linked to SDH-B gene are of major significance. Using data from next-gen sequencing, we identified partial SDH-B gene and used this information to design and amplify gene target from several individual isolates collected in 2020. The SDH-B gene from a total of 58 isolates was sequenced which harbored region of interest potentially carrying all mutations correlated with SDHI resistance in several species. Of these isolates, 4 isolates contained H272R mutation while 3 independent isolates contained N230I/H mutation, both corresponding to SDHI resistance in other pathogen species. The percentage of mutation (or potential resistance) against SDHI fungicides appeared relatively lower in the cherry orchards suggesting a minimal risk of developing widespread resistance within a short period of time.

Analysis of QoI target gene, cytb was particularly challenging due to high heterogeneity in the DNA sequence. Partial gene sequencing using two independent sets of primer pairs revealed 40% resistance (G143A) in 2019. All resistance isolates from the bioassay experiments were confirmed to contain G143A mutation. We followed up the mutation rate in one of the orchard sites (Roza experimental orchard) in 2020 and the analysis revealed that as much as 87% (13 of 15) isolates contained G143A mutation (Table 3). Based on this study, QoI fungicides are clearly at risk of losing its efficacy either as single or premixed formulation.

Table 2. Analysis of DMI resistant in individual *P. cerasi* isolates from commercial orchard and nursery locations collected in 2020.

	CYP 51 alleles						
Isolates	**G**	**G+S**	**S**	**ND**	**Total**	**Resistant colonies**	**% Resistance**
Roza	14	2	0	4	20	2	10
MH	9	8	0	5	22	8	36
BC	7	0	0	1	8	0	0
DH	17	0	0	3	20	0	0
HL	2	0	0	0	2	0	0
HF	12	0	0	8	20	0	0
OR	12	2	4	2	20	6	30
ST	13	0	7	0	20	7	35
TP	12	0	3	5	20	3	15
SC	12	1	6	1	20	7	35
AR	8	5	4	3	20	9	45
WD	13	3	0	4	20	3	15
ML	19	1	0	0	20	1	5
CO	5	15	0	0	20	15	75
CN	5	15	0	0	20	15	75

Table 3. Summary of QoI resistant *P. cerasi* isolates in PNW.

Season	Isolate	Mutation	# resistant isolates	Percent QoI resistance
2019	Composite	G143A	6 of 15	40
2019	QoI resistant (bioassay)	G143A	9 of 9	100
2020	Individual	G143A	13 of 15	87

In anticipation of the widespread QoI resistance in PNW, we initiated a response program to identify efficacies of fungicide programs with or without QoI fungicides in a nursery trial. A nursery was chosen for the trial because of expansive planting, high powdery mildew disease pressure in every growing season, and ease of spray applications in nursery settings. We included three applications of QoI fungicides (single and premixed formulation) in regular intervals while non-QoI fungicide program included in another non-QoI treatment. At the end of nursery trials, the foliar disease incidence and severity were measured which indicated better disease management (less disease severity) in trees sprayed with non QoI fungicides (Table 4).

Table 4. Powdery mildew disease incidence and severity in the nursery spray trial involving QoI and non-QoI fungicides.

		Incidence (of 25 trees)			Severity*	
	Plot	22-Jun	6-Jul	24-Aug	6-Jul	24-Aug
Non-QoI	1	12	16	12	14	13**
	2	11	10	15	12	13**
QoI	1	8	11	17	11	18
	2	18	13	12	12	15
Control	-	16	19	25	15	33***

* severity is based on surface area colonized. ** significant compared to QoI applications. *** significantly higher incidence and severity in control, untreated trees.

Outreach

We have identified the presence and extent of DMI (FRAC Group 3), SDHI (FRAC Group 7), and QoI (FRAC Group 11) fungicide resistance in cherry orchards in *P. cerasi* pathogen. The results of the data are being communicated to the concerned growers, fieldmen, crop consultants, scientists, and commercial pesticide companies in the form of seminar/ technical talks. The results will be published in a peer-reviewed journal and as a trade magazine article to inform the experimental results to a wider audience.

Technical Publications:

Grove, G.G., Swamy, P., and Guerra, N. 2020. Current status of the powdery mildew fungicide toolbox in cherries. WSU Tree Fruit http://treefruit.wsu.edu/article/current-status-of-the-powdery-mildew-fungicide-toolbox-in-cherries/

Presentations describing data from this project:

- WSU Plant Pathology seminar series (2020)

Fungicide resistance in the *Prunus avium: Podosphaera cerasi* pathosystem. Virtual seminar April 13.

- APS (American Phytopathological Society) Plant Health annual meeting (2020).

The newly emerging powdery mildew management challenge of sweet cherry orchards: Fungicide resistance. Virtual seminar August 13.

- Semios Fieldsmen Workshops (2020)

Understanding fungicides and the risk of resistance development while managing the powdery mildew disease. Jan 16 (Yakima, WA) and Jan 17 (Wenatchee, WA).

- Orchard Pest and Disease Management Conference (2020)

Implications of fungicide resistance in the management of cherry powdery mildew in the Pacific Northwest. Portland, OR Jan 9.

- Field day at WSU's Roza research farm (2019)

Discussion on the powdery mildew disease management, fungicide spray coverage and fungicide resistance in the Washington cherry orchards in Prosser, WA July 23, 2019

- Cherry preharvest tour (2019)

Discussion on the importance of cultural controls, pesticide coverage and fungicide resistance for cherry powdery mildew disease management at Oroville, WA June 14, 2019

- Grower's meeting (2019)

Okanogan Horticultural Society- monthly meeting speaker, Omak, WA June 10, 2019

- Seminar (2019)

Understanding cherry powdery mildew in PNW and fungicide resistance. Presentation at Oregon State University (OSU) Cherry Day 2019 at The Columbia Gorge Discovery Center, The Dalles, OR

- Seminar (2019)

Cherry Powdery Mildew 2019, Presentation at the Okanogan Horticultural Society growers meeting, Omak, WA

- Fungicide Resistance in Pacific Northwest Cherries. 2019 Washington State Tree Fruit Association Annual Meeting, Wenatchee, WA.
- Fungicide Resistance in Pacific Northwest Cherries. 2020 NCW Cherry Day. Wenatchee, WA.

Conclusions

The project focused on identifying the presence and extent of DMI, SDHI, and QoI fungicide resistance in the commercial orchards of the PNW. Our results indicated an alarming situation for QoI fungicides and further demonstrated that if QoI become non-effective, they can be eliminated with other fungicides from different chemistries. DMI fungicides are also at risk but their efficacies need critical evaluation in every location owing to many chemistries within this group and multiple modes of action. SDHI fungicides are currently deemed safe due to marginal (10%) resistance based on molecular data. It is now critical to inform the results and information from the experiments to concerned parties to spread awareness on fungicide resistance and to advocate on being provocative on this issue in the PNW cherry orchards.

FINAL REPORT

Project Title: Development index model of sweet cherry

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Cooperators: Alan Reitz Mount, Adams Fruit; Garrett Bishop, GS Long; Mark Lapierre, Wilbur Ellis; Eric Shrum, Mike Omeg, Orchard View Farms

Total Project Request: Year 1: $131,909 **Year 2: $136,083**

Other funding sources: Year 1: Columbia Gorge Fruit Growers, $23,562; Washington Blueberry Commission, $5,250 **Year 2:** Columbia Gorge Fruit Growers, $23,562; Washington Blueberry Commission, $5,250

Budget 1
Organization Name: OSU-ARF
Telephone: 541-737-3228
Contract Administrator: Russ Karow
Email address: Russell.Karow@oregonstate.edu

Item	2019	2020
Salaries[1]	$39,026	$40,197
Benefits	$23,696	$24,407
Wages[2]	$17,213	$17,729
Benefits	$13,657	$14,067
Equipment	-	-
Supplies[3]	$1,000	$1,000
Travel[4]	$2,000	$2,000
Plot Fees	-	-
Miscellaneous	-	-
Total	$96,592	$99,401

Footnotes:
[1]Postdoctoral Research Associate: 0.7 FTE with 3% increase factored into Year 2.
[2]Biological Science Tech: 0.5 FTE with 3% increase factored into Year 2.
[3]Miscellaneous supplies for sample collection and preparation.
[4]Travel to grower field for sample collection.

Budget 2
Organization Name: WSU **Contract Administrator:** Karen Kniep
Telephone: **Email address: kmkniep@wsu.edu**

Item	2019	2020
Salaries	25,357	26371
Benefits	8,757	9,108
Wages		
Benefits		
Equipment		
Supplies		
Travel	1,203	1,204
Miscellaneous		
Plot Fees		
Total	35,317	36,683

Footnotes:
[1]**Systems Analyst:** 4.7 months at 1.0 FTE

PLEASE NOTE:
This report was originally funded for two years [June 2019- June 2021]. The reduction in time and funding by 12 months has severely curtailed result reporting. The following report is therefore a preliminary discussion of partial results.

JUSTIFICATION

Modelling is an extremely useful tool to assist scientists, growers and distributors with planning and execution of strategic objectives. This modelling is aimed at achieving the following:

- Predicting critical temperatures of **freeze tolerance** for flowers of sweet cherry (beginning in September and ending at bloom).
- Producing an optimized developmental model of sweet cherry (beginning in September and ending at fruit maturation) that is location and cultivar dependent. This will predict **dormancy acquisition**, **mid-winter hardiness**, **bloom** and, most importantly, **harvest time**. This uses a growing/degree/hour model employing air temperature data to assist growers in determining when and where to utilize specific management practices to prevent or minimize freeze damage and optimize cherry size / quality at harvest.
- Presenting these models on AgWeatherNet [AWN] for all to access.

- The freeze tolerance model will be available on AgWeatherNet in Spring 2019.
- The combined vernalization/freeze tolerance/bloom (VFB) model to be available end of 2020.
- Data collection and analysis will continue through 2019 and 2020 to allow for the creation of a maturation model. Data for numerous cultivars will be included to strengthen this model.

Results and Discussion

Cold-hardiness data was obtained by differential thermal analysis, bud and bloom phenology data by relative water content and bloom count. Collection of materials for this grant was accomplished by OSU-MCAREC (2017-2020). My thanks to Allan Bros Fruit who also collected material and provided relative water content data in the Tri-Cities (2018), G.S. Long in Yakima (2019) for cold-hardiness data and Mount Adams Fruit in the Columbia Gorge (2019) for providing material for relative water content analysis and bloom counts.

Pre-processing of cold hardiness and bud phenology data was performed by me to show a clearer picture of the trends in development. These data, encompassing 2013 to 2020, were shared with AWN beginning in 2017. Seminars were presented at AWN during the course of this funding explaining in full detail methods employed to gather and process the data. No cold-hardiness nor phenological data were collected by AWN during this time. All unprocessed data was also shared with AWN in spring 2020 and full instruction given as to the required preprocessing analysis.

Fruit maturation data from 2013 to 2019 was obtained by photography and gravimetry. These data were preprocessed and shared with AWN. During year 2 of this proposal these maturation data were to be included in the modelling.

Temperature modelling as explained by variable rate curves (as opposed to linear models) was encoded by AWN from my Excel spreadsheets. Unfortunately, the Python coding was not shared with me for evaluation, nor were the results of the Python programming for error checking.
A reset of 2020 spring model data due to unusual conditions was necessary but was not completed by AWN to reflect the current assessment of development at OSU-MCAREC.

Early indications showed that this modelling approach would provide a very useful tool for growers and marketers.

CONTINUING PROJECT REPORT **YEAR**: No-Cost Extension

Project Title: Understanding phytoplasmas infecting stone fruit trees in Washington state

PI: Dr. Scott Harper
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Co-PI (2): Dr. Alice Wright
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Address: 24106 N. Bunn Rd
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Cooperators:

Total Project Request: $91,835 **Year 1:** $46,380 **Year 2:** $45,455

Other funding sources: **None**

WTFRC Collaborative Expenses: None

Organization Name: WSU-IAREC **Contract Administrator:** Katy Roberts
Telephone: 509-335-2885 **Email address:** arcgrants@wsu.edu
Station Manager: Naidu Rayapati **Email address:** naidu@wsu.edu

Item	2019	2020	2021
Salaries	19,370	20,145	0
Benefits	7,510	7.810	0
Wages			
Benefits			
RCA Room Rental			
Shipping			
Supplies	19,000	17,000	0
Travel	500	500	0
Plot Fees			
Miscellaneous			
Total	46,380	45,4555	No-cost Extension

Footnotes:
Salaries and benefits for one 0.4X FTE postdoctoral researcher.
Supplies include laboratory consumables and sequencing services.
Travel is estimated mileage for field sampling.

OBJECTIVES

1. Determine which phytoplasmas are infecting stone fruit trees in Washington state and determine if multiple isolates are present by high throughput sequencing.

   Preliminary work has shown that cherries and peaches in the Columbia basin are infected with X-disease phytoplasma (XDP), and that peaches and nectarines are also infected with peach yellow leaf roll phytoplasma (PYLR). As of 2018, the incidence in peach and nectarine was unknown as they were detected that year in a brief survey in response to grower inquiries. This is a particular problem given the movement of material into and within the state. Therefore, we propose to survey stone fruit trees, including peaches, nectarines, apricots, and plums, to identify which phytoplasmas are present in Washington state. Sequencing will be performed to obtain draft genomes for these phytoplasmas, and marker-directed genotyping performed to see whether there is active movement of phytoplasmas from one stone fruit crop to another or from one county to another. These data will answer the questions of 'what' and 'where'.

2. Identify physiological markers associated with the disease by comparing fruit of infected and healthy trees.

   Both of the presently identified phytoplasmas, XDP and PYLR, can affect the quality of infected stone fruit trees, yet previous research is limited to a few varieties or species, and, for peaches and nectarines, is primarily from California. Moreover, no data has been collected on the effects of infection by multiple phytoplasmas, as we have observed in both peaches and nectarines in the Columbia basin. Here we propose to examine symptoms in fruit and phloem tissue of infected trees, and by comparing these to healthy trees in the same location, determining type and severity of disease caused by endemic phytoplasmas. This will identify which phytoplasma species, aside from XDP, are particularly problematic for the tree fruit industry in Washington.

3. Determine how the presence of multiple phytoplasmas affects symptom development by using transcriptomics to identify affected pathways.

   It is unknown how these phytoplasma species cause disease in infected stone fruit. Using transcriptomics, we will be able to determine which pathways have altered regulation in diseased trees and may be important to symptom development. Understanding which pathways are important to symptom development may one day help with breeding for tolerant trees.

SIGNIFICANT FINDINGS

- PYLR has been observed in peaches, nectarines, plums, pears, and apples. XDP has been observed in apricots, pluots, sour cherries, peaches, nectarines, pears, and cherries.
- A third phytoplasma, previously observed in Asia, may be infecting peaches in Washington state.
- Observations of fruit in infected trees continued for a second year and, as the year before, more small and misshapen fruit were found in infected trees compared to healthy.

METHODS

1. Determine which phytoplasmas are infecting stone fruit trees in Washington state and determine if multiple isolates are present.

Samples are to be collected from stone fruit trees of representative species and cultivars throughout Washington, from both those that are symptomatic or are in the vicinity of symptomatic trees. Trees will be screened for the presence of phytoplasmas by generic qPCR, with positives identified by

species-specific PCR. A subset of samples with different tree species and phytoplasma combinations will be sequenced using Illumina technology. The genomes will be used as a map to identify strain specific differences, with PCR-based screening used to identify specific variants. Cumulatively, the sequence data will provide information on which phytoplasma species are present in Washington state, which stone fruit trees they are present in, and how many genotypes of each phytoplasma are present. This will allow for the tracking of genotypes in fruit trees by species and geographic location, providing information on how widespread these pathogens are and where they are a problem.

2. Identify physiological markers associated with the disease by comparing fruit of infected and healthy trees.

   The screening in objective one will allow for the identification of infected trees from which we will conduct observations to determine the effects of different phytoplasmas on tree growth and fruit development. Tree growth, vigor, leaf shape and time of leaf drop will be assessed throughout the growing season. Fruit size, shape, and color will be assessed by comparing fruit between healthy and diseased trees. Sugar content of fruit, which is often affected in phytoplasma infected plants, will be determined using sucrose/D-fructose/D-glucose and sorbitol assays. Citric acid, malic acid, and total phenolics will also be assessed. Assessing these tissues will determine the pathogenicity and virulence of identified phytoplasma species and in which tree species they are a problem.

3. Determine how the presence of multiple phytoplasmas affects symptom development by using transcriptomics to identify affected pathways.

   The role of multiple infections in disease development will be assessed using a transcriptomics approach and will be compared to single-infected trees and healthy trees. As RNA from tissue showing symptoms is not of high enough quality for sequencing, samples will be collected throughout the season from suspected infected and healthy trees. This will allow for capture of the early stages of symptom development that lead to premature yellowing and shot-holing. RNA will be isolated from the leaf and midrib tissue of healthy and infected trees, libraries prepared, and NGS performed. Differential gene expression analysis will identify genes that are upregulated or downregulated in infected trees. This will be paired with the physiological data collected during objective two to identify differentially expressed transcripts that may have a role in symptom development, allowing the future development of disease markers and/or disease tolerance in breeding programs.

RESULTS AND DISCUSSION

1. Determine which phytoplasmas are infecting stone fruit trees in Washington state and determine if multiple isolates are present by high throughput sequencing.

This year we continued our survey of phytoplasmas in Washington state. Stone fruit species sampled included peaches, nectarines, plums, apricots, and pluots. Potential source neighboring apple and pear trees were also included in this survey. For each tree sampled, DNA was extracted from a wood scraping. Trees were screened for the presence of XDP with a specific qPCR assay and were then screened for PYLR using a PYLR specific end point PCR. XDP was detected in apricots, nectarines, peaches, plums, and pluots, while PYLR was detected in peaches and plums. Generic assays also indicated the presence of phytoplasmas in additional apricot, nectarine, peach, and plum samples, as well as one apple, however it is not known what species were present, therefore we are investigating this further by direct-sequencing of PCR markers. While this is ongoing, preliminary results indicate that the positive apple was PYLR.

Table 1. A summary of phytoplasma positive stone fruit trees identified sampled this year.

Host	No. Trees Sampled	PYLR Positive	XDP Positive
Apricot	9	0	2
Nectarine	49	1	9
Peach	121	7	48
Plum	20	4	5
Pluot	1	0	1

Draft genomes for Washington isolates of both XDP and PYLR have been obtained during 2020. The XDP draft genome consists of 397 contigs totaling 769,330 bp. Based on initial assembly, 879 potential genes were identified. For PYLR, 207 contigs were assembled, totaling 836,833 bp, with 1,034 potential genes. During 2021 we aim to use these contigs backbone for sequencing of additional isolates for genotypic analysis.

In parallel, we began screening samples collected during 2020 with published phytoplasma genotyping primers for the 16s rRNA, rp operon, secA, and secY genes, and sequencing the resulting amplicons. At time of writing this effort is underway, and only preliminary results for the rp operon from XDP isolates are available (Figure 1). Based on these results, it appears that there is a majority genotype infecting stone fruit across the state, as well as in Utah and California, with one aberrant type identified in Pasco.

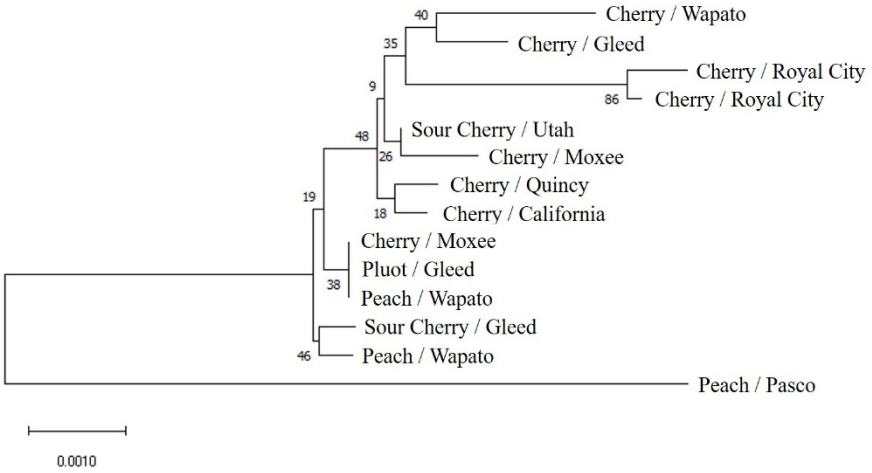

Figure 1. RP operon sequences from XDP isolates collected from stone fruit trees in Washington and other states in 2020.

Finally, a new phytoplasma may be infecting peach trees in central Washington. These trees show bleaching of the leaves but not the downward curling observed with other two phytoplasmas. The bleaching appears to worsen as the season progresses and while it appears similar to iron deficiency, testing by the owner eliminated this possibility. Finally, shoot-tip dieback was observed early in the season, leading to limb dieback by late season. A disease caused by a group Vb phytoplasma in peaches in Asia (Li et al. 2014) is very similar in appearance to the symptoms observed here in the field. We are in the process of screening samples collected from these trees for the presence of this phytoplasma.

2. Identify physiological markers associated with the disease by comparing fruit and phloem tissues of infected and healthy trees.

This season we focused on sampling and observing phytoplasma-like symptoms from a wide range of stone fruit cultivars and sites across central Washington. Fruit and foliar symptoms were assessed for all stone fruit collected, disease severity scores assigned and tabulated based on the variety and infecting pathogen(s) (Tables 2 and 3).

Table 2. Effects of phytoplasma infection observed on peach cultivars in Washington state.

Species	Cultivar	Pathogen	Titer	Avg. Symptom rating[1]	Leaf Yellowing	Enlarged Midveins	Leaf Shotholes	Dieback	Fruit Size[2]	Fruit Deformation[3]
Peach	Country Sweet	XDP	Low	1	Yes	No	No	None	2.5	2
			Medium	2	Yes	Yes	Yes	None	2.1	2.2
	Diamond Princess	XDP	Low	1	Yes	No	No	None	3	1
			Medium	2.17	Yes	Yes	Yes	Some	2.25	2
	Elegant Lady	XDP	Medium	2.5	Yes	Yes	Yes	None	2.08	2.17
	O'Henry	XDP	Low	2	Yes	Yes	Yes	None	2.83	2
		Both	Low/Low	2	Yes	Yes	Yes	None	2.5	2
	Regina	XDP	Low	1.5	Yes	No	No	None	2.25	2
			Medium	2	Yes	Yes	Yes	None	2.13	2
	Sierra Rich	XDP	Low	1	Yes	No	No	None	1	2
			Medium	2	Yes	Yes	Yes	None	2	2
		PYLR	Low	0	Yes	No	No	None	2.5	2
	Zee Lady	XDP	Low	1.5	Yes	Some	Some	Some	3	2
			Medium	2	Yes	Yes	Yes	Some	2.71	2
		PYLR	Low	1	Yes	No	No	None	3	2
		Both	Med/Low	2	Yes	Yes	Yes	None	3	2
			Med/Med	2	Yes	Yes	Yes	Some	3	2

1. Symptom rating: 0 – Asymptomatic, 1 = mild, 2 = moderate, 3 = severe.
2. Fruit Size rating: 3 = normal, 2 = 25% reduction, 1 = 50% reduction
3. Fruit Deformation rating: 3 = normal, 2 = bulge or lump on one side, 3 = complete deformation

From these observations we found that the titer of the infecting phytoplasma has a significant effect on the diversity of symptoms observed, with low titer, early-stage infections being largely limited to foliar symptoms with some effect on fruit size and/or deformation. Established infections with a higher titer resulted in the appearance of dieback and more severe impacts on fruit size and deformation. Fruit maturation was impacted with more severe infection. Interestingly, coinfection with both XDP and PYLR result in more severe symptoms than either pathogen alone at an equivalent titer.

As expected, XDP infection produced shotholing on leaves whereas PYLR infection did not, otherwise symptoms were largely indistinguishable between the two pathogens at early stages of infection. In general, established XDP infections produced more severe symptoms, particularly on fruit, than PYLR though small sample size of the latter should be considered. Similar effects were observed on peach, nectarine, and plum cultivars, whereas on apricots the effects were not as pronounced, suggesting that they may be more tolerant of phytoplasma infection.

Table 3. Effects of phytoplasma infection observed on stone fruit cultivars in Washington state.

Species	Cultivar	Pathogen	Titer	Avg. Symptom rating	Leaf Yellowing	Enlarged Midveins	Leaf Shotholes	Dieback	Fruit Size	Fruit Deformation
Nectarine	Grand Bright	PYLR	Low	2	Yes	Yes	No	None	2.5	2
	August Bright	XDP	Low	1	Yes	Yes	Yes	None	N/A	N/A
	Honeyhaven	XDP	Low	1	Yes	No	No	None	2.75	2.5
			Medium	2	Yes	Yes	Yes	None	2.17	2
	Summer Flair	XDP	Low	2	Yes	Yes	1	None	N/A	N/A
		PYLR	Low	2	Yes	Some	No	None	N/A	N/A
		Both	Low/Low	2	Yes	Yes	No	None	N/A	N/A
	Unspecified	XDP	Low	3	Yes	Yes	Yes	Some	N/A	N/A
		PYLR	Low	3	Yes	Yes	Yes	None	N/A	N/A
Plum	Friar	XDP	Low	0	0	No	No	None	2.5	1
			Medium	2	Yes	Some	Yes	None	2	1.67
		PYLR	Low	1	0.5	0.5	0.5	None	2.25	1
	Unspecified	XDP	Low	1	0	No	No	None	N/A	N/A
		PYLR	Low	2.67	Yes	Yes	Yes	N/A	N/A	N/A
Apricot	Unspecified	XDP	Low	1	0.5	No	No	Some	N/A	N/A

During this season we also collected fruit from diseased and healthy trees for sugar and metabolite analysis to gain a better understanding of the effects of phytoplasma infection on fruit quality. Due to the coronavirus pandemic, these analyses will be performed during the winter of 2020 and early 2021.

Once complete we aim to include this information, with images of the symptoms on each cultivar, into a grower guide.

3. Determine how the presence of multiple phytoplasmas affects symptom development by using transcriptomics to identify affected pathways.

The coronavirus pandemic affected our ability to collect samples for transcriptomic analysis during 2020. Based on results from our parallel project disease expression in cherry, gene expression is time critical and we were unable to access sites early enough to collect viable tissue this year. We aim to complete this objective in 2021, with additional staff and sites identified from the 2020 season to target known infected trees for sample collection.

REFERENCES

Li Z, Song S, Zhang L, Gao L, Wu Y. 2014. Identification of the phytoplasma associated with peach yellows disease in northwest China. Canadian Journal of Plant Pathology. 36:151-160.