# Northwest Cherry Research Review

Wenatchee Convention Center

Wednesday, 11/9/2016

| Time | Page | PI | Title | Funding period |
|---|---|---|---|---|
| 8:00 | | Willett | Welcome and introduction | |
| | | | Final project reports | |
| | 2 | Walse | Residue remediation to reduce MRL trade barriers: *Written report only* | |
| 8:15 | 4 | Peace | After RosBREED: developing/deploying new cherry DNA tests | 14-16 |
| 8:30 | 16 | Grove | Maintenance & evaluation of sweet cherry breeding program germplasm | 16 |
| 9:00 | 27 | Iezzoni | MSU sweet cherry rootstocks | 14-16 |
| 9:15 | 38 | Einhorn | New programs to increase fruit size & improve harvest quality | 15-16 |
| 9:30 | 50 | Wang | Improving shipping quality of cherry by pre-harvest Na and NaCl sprays | 14-16 |
| Group # | | | Continuing project reports 2:30 - 4:30 | |
| 1 | 61 | Wang | Strategies to reduce postharvest splitting of cherries | 16-18 |
| 1 | 68 | Wang | Ensuring long-distance ocean shipping arrival quality of PNW cherries | 16-18 |
| 2 | 76 | Probst | Sources of primary cherry powdery mildew inoculum - revisited | 16-18 |
| 2 | 83 | Probst | ABC of sweet cherry powdery mildew: adaptation, behavior and control | 16-18 |
| 2 | 86 | Probst | PM viability during postharvest handling of cherry fruit: *No-cost extension* | 15-16 |
| 2 | 93 | Pscheidt | Cherry virus diagnostic survey of Oregon | 16-17 |
| 2 | 101 | Villamor | Finding the Achilles' heel of a new virus infecting stone fruits | 15-17 |
| 3 | 106 | Beers | Integrated pest management of spotted wing drosophila in sweet cherry | 16-18 |
| 3 | 114 | Beers | Insecticide resistance of SWD in sweet cherry: *No-cost extension* | 14-16 |
| 3 | 123 | Bixby-Brosi | Developing a management strategy for little cherry disease: *No-cost extension* | 14-16 |
| 3 | 131 | Ferguson | The hunt for leafhopper vectors of Western X in Washington cherries | 16-18 |
| 3 | 138 | Hubbard | Managing acclimation, hardiness and bacterial canker | 15-17 |

**CONTINUING PROJECT REPORT** **No Cost Extension**

**Project Title:** Residue remediation for cherries: Breaking MRL trade barriers


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**Other funding sources:**
**Budget 1**
**Organization Name:** USDA-ARS **Contract Administrator:** Charles W. Myers
**Telephone:** 510.559.5769 **Email address:** chuck.myers@ars.usda.gov

**WTFRC Collaborative expenses:** None


**Budget:** **Year 1**: $5,000 (fruit) **Year 2**: $0 **Year 3**: $5,000 (fruit)

| Item | 2015 | 2016 | 2017 | 2018 |
|---|---|---|---|---|
| **Salaries (60% GS-5)** | | | | |
| **Benefits (included above)** | | | | |
| **Wages** | | | | |
| **Benefits** | | | | |
| **Equipment** | | | | |
| **Supplies (fruit)** | 5,000* | | | $5,000 |
| **Travel** | | | | |
| **Miscellaneous (shipping)** | | | | |
| **Plot Fees** | | | | |
| **Total** | $5,000 | **$0** | **$0** | $5,000 |

***Fruit was not purchased in 2015 or 2016 and money was not disbursed. It is requested that the funds be carried over to 2017 as a no-cost extension**

**Significant findings:**

- Proof of Concept demonstrated.
- Letter of support and in kind contributions received from TASC project received from California Raisins ($5,000), US Highbush Blueberry Council ($5,000), California Blueberry Commission ($5,000), California Cherry Board ($5,000), California Fresh Fruit Association ($5,000), California Pear Advisory Board ($5,000), and California Apple Commission ($5,000) – with more to come.
- Expected grant approval date, Spring 2017. Despite efforts to receive FY 2016 TASC monies, the funding was deterred due to scientific review conducted by the FDA. USDA-ARS, USDA-FAS, and industry continue to work together to try to get FDA approval for the project.

**Justification**:

Pesticides are applied throughout production and distribution channels to prevent reduction of commodity yield and quality, as well as to minimize food-borne health risks to consumers. The benefits of using pesticides for commercial applications must be balanced with unfavorable artifacts associated with commodity residues. Evolving environmental and public health concerns surrounding non-target exposure of consumers to residues, regardless of concomitant toxicological evidence, necessitates the development of safe and effective methodologies for residue reduction.

In this work, postharvest techniques will be developed to eliminate pesticide residues on sweet cherries, particularly those intended for export where minimum residue level (MRL) compliance is required.

**Objectives**:

1) Provide proof-of-concept research results to WTFRC for consideration.

2) Draft a USDA-FAS-TASC grant, with sponsorship from WTFRC, pertaining to the development of postharvest techniques to break MRL-trade barriers for the Northwest sweet cherry and apple industries.

3) Conduct research as outlined in the USDA-FAS-TASC grant. Use requested WTFRC/OSCC funding to purchase sweet cherries for research.

**FINAL PROJECT REPORT**

**Project Title**: After RosBREED: Developing and deploying new sweet cherry DNA tests


| | | | |
|---|---|---|---|
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| **State/Zip**: | WA/99163 | **State/Zip**: | WA/99350 |

**Cooperators**: Paul Sandefur (PhD student, WSU Pullman), Dorrie Main and Sushan Ru (WSU Pullman), Amy Iezzoni (Michigan State University), Fred Bliss (Davis, California)


## Other funding sources


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

**Agency Name:** USDA-NIFA Specialty Crop Research Initiative
**Amount awarded:** $2.74 M (Sep 2014 – Aug 2019)
**Notes:** "Genome Database for Rosaceae: Empowering specialty crop research through big-data driven discovery and application in breeding." PI: Main. Co-PIs include Peace.

**Agency Name**: WTFRC/OSCC
**Amount awarded**: $52,844 (2014–2015)
**Notes**: "New genomic regions controlling production and fruit disorder traits." PI: Oraguzie. Co-PIs include Peace.

**Agency Name**: WTFRC/OSCC
**Amount awarded**: $10,000 (2014-2015)
**Notes**: "Sweet cherry breeding toolbox." PI: Main. Co-PIs include Peace and Oraguzie.

**Agency Name**: WTFRC/OSCC
**Amount awarded**: $13,000 (2015-2016)
**Notes**: "Consulting for the sweet cherry breeding program" PI: Iezzoni.

**Agency Name:** WTFRC/OSCC
**Amount awarded:** $442,847 (2012–2014)
**Notes:** "PNW sweet cherry breeding and genetics program." PI: Oraguzie. Co-PI: Peace.

**Total Project Funding**:

**Budget History:**

| **Item** | **Year 1:** 2014 | **Year 2:** 2015 | **Year 3:** 2016 |
|---|---|---|---|
| **Salaries** | 17,651 | 18,440 | 19,265 |
| **Benefits** | 11,242 | 11,916 | 12,632 |
| **Wages** | | | |
| **Benefits** | | | |
| **Equipment** | | | |
| **Supplies** | 9,107 | 0 | 9,103 |
| **Travel** | 2,000 | 2,000 | 2,000 |
| **Plot Fees** | | | |
| **Miscellaneous** | | | |
| **Total** | 40,000 | 32,356 | 43,000 |

**RECAP ORIGINAL OBJECTIVES**

*Overall goal*: Improve prospects for sweet cherry breeding efficiency, accuracy, creativity, and speed by actively devising new predictive DNA tests that strategically target the region's valuable traits.

*Specific objectives*:

1. Begin with developing new DNA tests for **maturity time**, **fruit color**, and **fruit firmness** – those traits for which the most promising discoveries were made within the RosBREED project

2. Develop new DNA tests for **pitting** and **cracking incidence**, **fruit-pedicel abscission**, **resistance to bacterial canker** and **powdery mildew**, **sweetness**, and **acidity** – those traits for which discoveries are anticipated from other sources during the project period

3. Ensure appropriate use of new DNA tests by devising and trialing strategies for their routine deployment within the context of existing tests and ongoing Pacific Northwest Sweet Cherry Breeding Program (PNWSCBP) operations

**SIGNIFICANT FINDINGS**

- **Powdery mildew** (PM) resistance DNA test developed (foliar genetic resistance/susceptibility type**; might also target fruit incidence)

- **Maturity time** DNA test developed (early vs. late predisposition*)

- **Fruit color** DNA test developed (mahogany vs. blush fruit type)

- **Fruit-pedicel abscission** DNA test developed (PFRF level predisposition)

- **Fruit cracking** DNA test developed (presence vs. absence of resistance predisposition)

- **Fruit size & firmness** DNA test developed (size and firmness level predisposition)

- Improved maturity time DNA test under development – targeting multiple genomic regions with a single DNA test to maximize predictiveness

- Predictiveness of each DNA test established across PNWSCBP germplasm using new analytical method that provides greater accuracy by accounting for genetic background

- DNA information on **self-fertility**, **foliar PM incidence**, **fruit color**, **maturity time**, **fruit-pedicel abscission**, **fruit cracking**, **fruit size**, and **fruit firmness** obtained for <u>all</u> PNWSCBP selections and seedlings to aid in selection

- Four-stage deployment strategy devised to optimally utilize many DNA tests now available

* "predisposition" means the test is not deterministic but rather determines some degree of genetic propensity for certain trait levels

** "type" means deterministic: the DNA test appears to identify clearly one type vs. another

**RESULTS & DISCUSSION**

**Objectives 1 & 2: DNA test development**

Using genomic discoveries made within the RosBREED project, the WTFRC/OSCC project "New genomic regions controlling production and fruit disorder traits", and through international collaborations, DNA tests were developed for the PNW region's valuable market class-defining, essential, and enhancing traits levels. Adding to previously available DNA tests for self-fertility and fruit size, new DNA tests were developed for the traits of **fruit color**, **maturity time, foliar PM** incidence, **fruit size**, **fruit firmness**, **fruit-pedicel abscission**, and **fruit cracking** (Fig. 1).

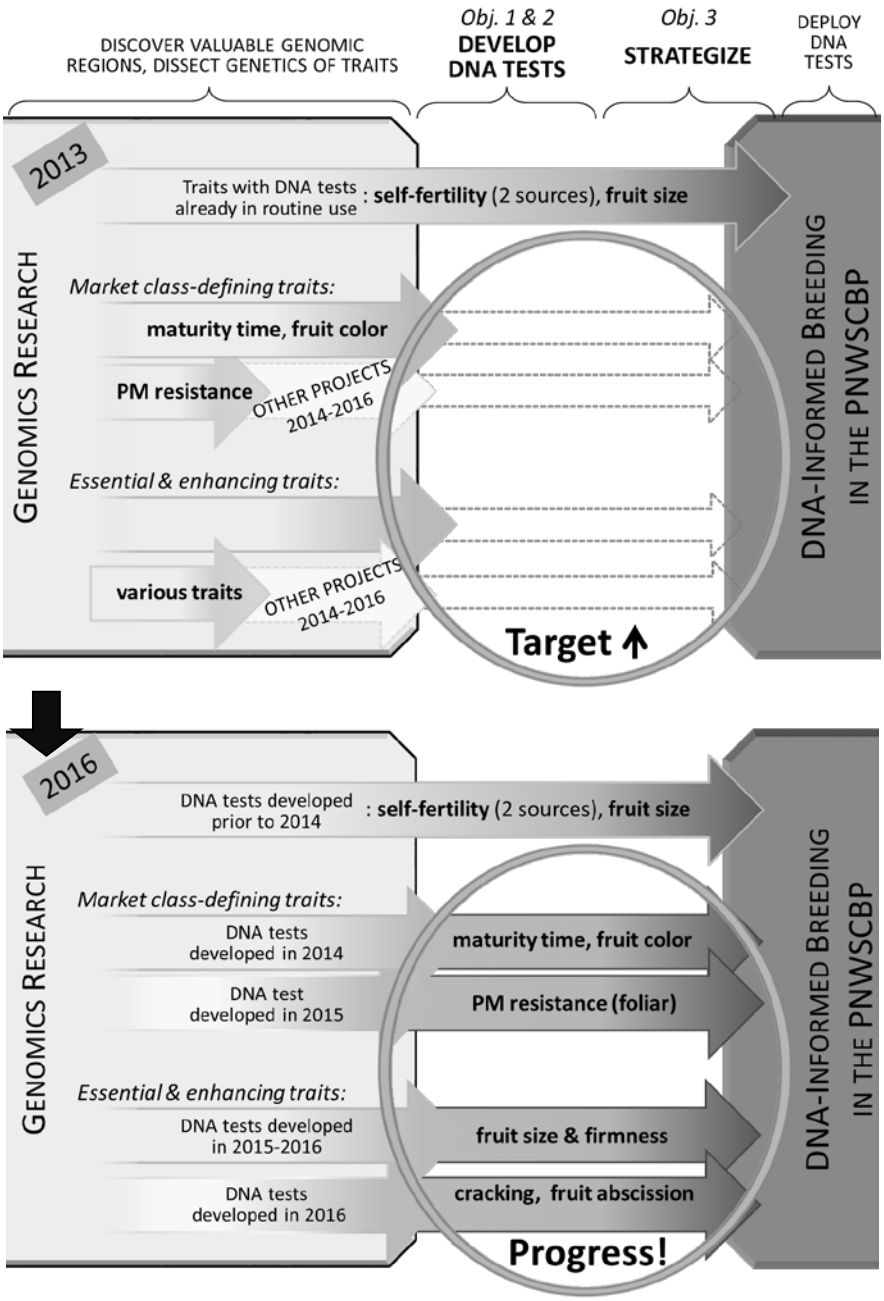


*Figure 1: Progress made during project, which has and will continue to improve the efficiency, accuracy, creativity, and pace of the Pacific Northwest Sweet Cherry Breeding Program (PNWSCBP) via DNA-informed breeding.*

*Foliar PM DNA test*

A new DNA test for routine prediction of **foliar PM** resistance/susceptibility was developed. This advance was made possible by the discovery of a genomic region associated with this trait in the WTFRC/OSCC-funded project, "New genomic regions controlling production and fruit disorder traits". The DNA test developed, "Pav-G5PM-SSR", is able to identify individuals with resistance to PM from the three sources, 'PMR-1', 'Moreau', and 'Mildew Immune Mazzard'. When screened across a large germplasm set representative of the breeding program, a significant, breeding-relevant difference was observed between those individuals with one or two resistance alleles compared to those without (Fig. 2). In PNWSCBP germplasm, this DNA test explains approximately 15% of the phenotypic and approximately 25% of the genotypic variation observed for foliar PM incidence. With Pav-G5PM-SSR, the costly and error-prone phenotyping that is traditionally used to evaluate foliar PM incidence can be avoided. Based on correspondence of DNA test genotypes among cultivars resistant vs. susceptible for fruit PM incidence, this test might also be effective to identify individuals with resistance to fruit PM; however, additional research, specifically the collection of new, accurate phenotypic data on fruit PM incidence, is needed to test this hypothesis. Pav-G5PM-SSR is currently being screened across all PNWSCBP seedlings to identify individuals resistant to foliar PM, especially in the late season mahogany market class where a new cultivar must be resistant to this costly disease.

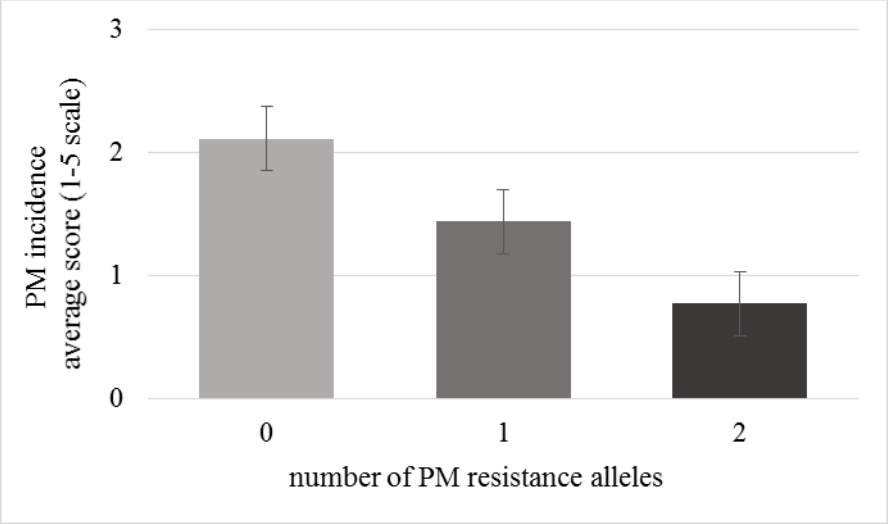


*Figure 2: Predicted difference in foliar powdery mildew infection between those individuals carrying no PM resistance alleles, one resistance allele, or two resistance alleles as revealed by the new DNA test, Pav-G5PM-SSR. Predictions based on three years phenotypic data using 1-5 scale (1 = 1–20%, 2 = 21–50%, 3 = 51–80%, 4 = 81–99%, 5 = 100%) and corrected for major influencing factors such as family (genetic background) and year effects.*

*Fruit maturity time DNA test*

A new DNA test for routine prediction of **fruit maturity time** was developed, Pav-Fht-SSR. The test targets the largest-effect genomic region associated with variation in sweet cherry fruit maturity time. In PNWSCBP germplasm, this DNA test explains approximately 27% of the phenotypic and approximately 35% of the genotypic variation observed for maturity time. This test reveals two functional alleles, termed "early" and "late", that in combination can predict differences in harvest

date of an average of approximately one week (Fig. 3); however, this test does not explain the early harvest date of 'Chelan'. Due to the importance of the 'Chelan' maturity time to the PNW industry, an effort was made in Year 3 of the project to identify additional genomic regions. New genomic regions on chromosome 1, 4, and 6 were found to be associated with additional variation in maturity time and preliminary analysis indicates that these regions, in combination with Pav-Fht-SSR, might provide additional genetic contributions to DNA-based predictions of maturity time. Development of a DNA test with improved predictivness for fruit maturity time is major focus of the remaining months of the project.

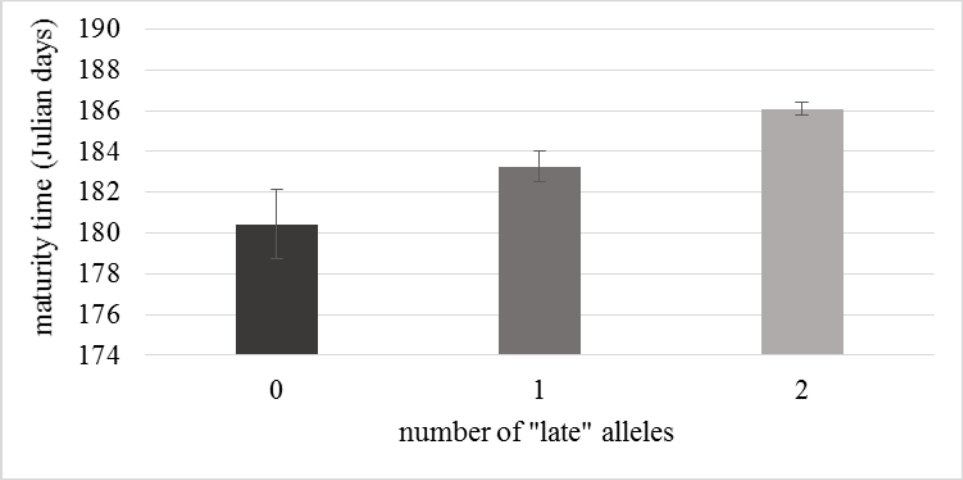


*Figure 3: Predicted difference in maturity time between those individuals carrying no late alleles, one late allele, or two late alleles as revealed by the new DNA test, Pav-Fht-SSR. Predictions based on two years phenotypic data measured as number of days to harvest from January 1 and corrected for major influencing factors such as family (genetic background) and year effects.*

*Fruit color DNA test*

A new DNA test for sweet cherry fruit color ("Pav-$R_f$-SSR") was developed. This DNA test clearly differentiates *blush* types from *mahogany* types, and can be used to differentiate "pure" mahogany types – those that possess two mahogany alleles ($R_f^{SSR}$) and tend to have a slightly darker color from those that possess only one mahogany allele and have a slightly lighter red hue (Fig. 3). A manuscript describing the development and utility of Pav-$R_f$-SSR was published in the journal Molecular Breeding [Sandefur P, Oraguzie N, Peace C (2016) A DNA test for routine prediction in breeding of sweet cherry fruit color, Pav-$R_f$-SSR. Molecular Breeding 36:33.].

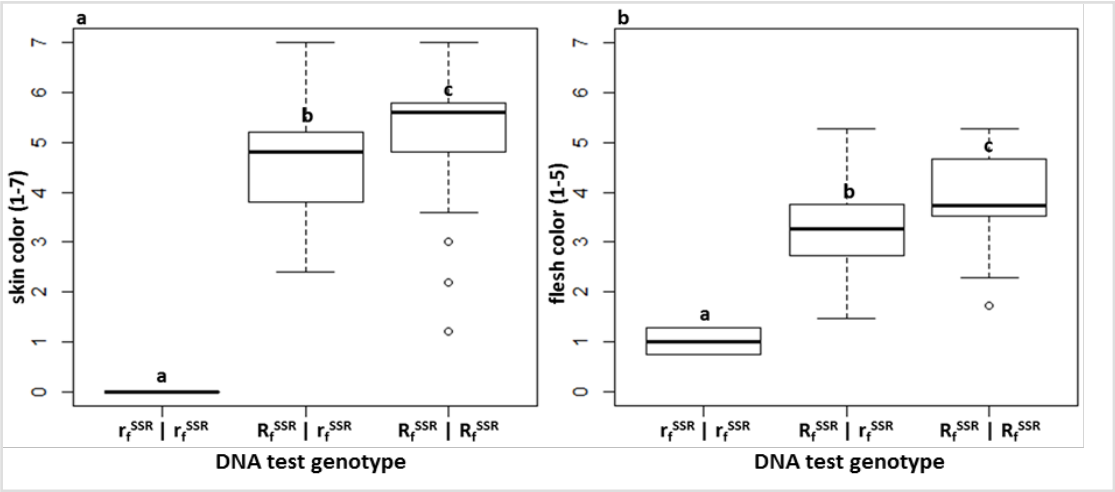


*Figure 4. Skin color (a) and flesh color (b) of Pav-$R_f$-SSR genotypes in PNWSCBP sweet cherry germplasm. $R_f^{ssr}$ is the dominant mahogany allele and $r_f^{ssr}$ is the recessive blush allele. Significant differences among groups are indicated by different letters (Kruskalmc multiple comparisons test; $P < 0.01$). The 2011 and 2012 data was combined after removing significant year effects*

*Fruit-pedicel abscission*

A new DNA test was developed for **fruit-pedicel abscission** [measured as "pedicel-fruit retention force" (PFRF)]. During Year 2, our collaborators in the WTFRC/OSC funded project "New genomic regions controlling production and fruit disorder traits" discovered a genomic region on chromosome 2 associated with PFRF that overlapped the region already targeted by Pav-G2-SSR for fruit size. However, additional analysis in Year 3 found that this region accounted for less of the phenotypic variation for PFRF in PNWSCBP germplasm than expected. It is still likely that in specific germplasm (e.g.in elite germplasm, such as 'Selah' and 'Cowiche') the DNA test detects a significant contrast in readiness of fruit to abscise from the pedicel. Additionally, the Spanish landrace 'Ambrunes' and its offspring in the breeding program carry a unique allele with breeding promise. A major focus of the remaining months of the project is to further investigate the utility of Pav-G2-SSR for PFRF in PNWSCBP germplasm.

*Fruit cracking*

A new DNA test was developed for rain-induced fruit cracking. Collaborating French researchers (led by Dr. Jose Quero Garcia, INRA) discovered a genomic region associated with rain-induced fruit cracking. This exciting discovery was made in a family with 'Regina', a low-incidence cracking cultivar, as a parent. In 2016, we confirmed the association between fruit cracking incidence and the newly discovered genomic region in germplasm of the PNWSCBP. Based on this confirmation, a DNA test was developed for performance prediction of this valuable trait (Fig. 5). The new DNA test, Pav-G5Crack-SSR, can be used to identify individuals highly resistant to rain-induced cracking. Although valuable, this test might only differentiate individuals that are highly resistant from individuals that have some susceptibility. Such a test would only be useful in some breeding applications, such as in parent selection and in seedling selection within families particularly targeted for genetic improvement in cracking incidence. If the test does not differentiate individuals that are highly susceptible (a fatal flaw) from those that are only moderately susceptible (not a fatal flaw), it should not be used on all seedlings. The DNA test might be effective for various cracking incidence levels; however, additional phenotypic data is needed to fully understand the predictiveness and maximize the utility of Pav-G5Crack-SSR.

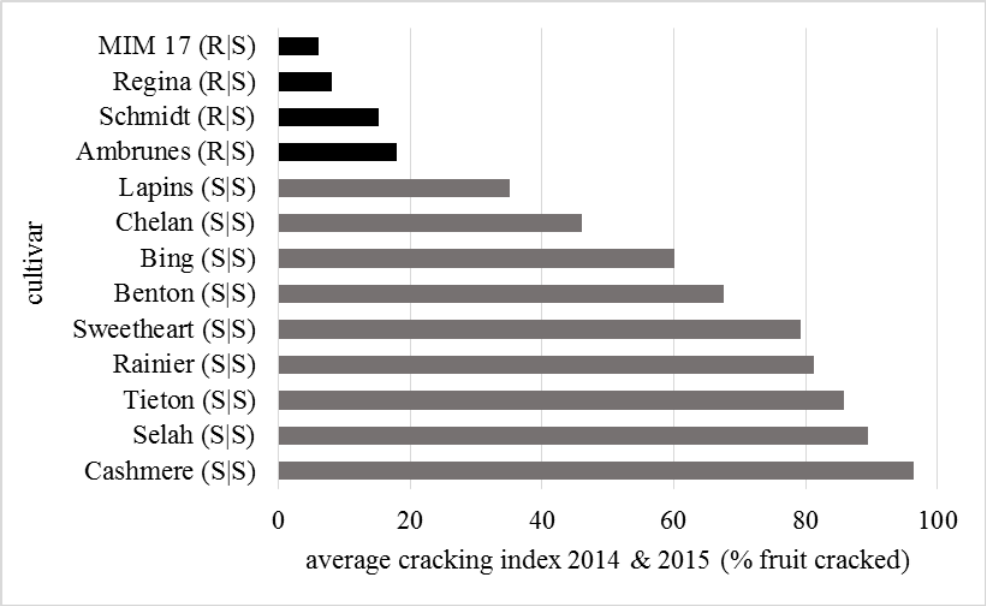


*Figure 5. Fruit cracking index (2014-2015 average) of some sweet cherry cultivars that carry a single resistance allele ("R") and a susceptible allele ("S") or two susceptibility alleles as revealed by Pav-G5Crack-SSR.*

*Fruit size & firmness*

DNA tests for fruit size and firmness have been refined by targeting additional genomic regions influencing these traits. Prior to 2014, the CPSCT038-BPPCT034 fruit size DNA test, targeting a region on chromosome 2 originally discovered by Amy Iezzoni's research group, was routinely used in the PNWSCBP. Since 2014, two more genomic regions associated with fruit size and firmness were reported by French (INRA) collaborators. We designed DNA-based assays to target these two new regions. We estimate that a single assay combining these multiple tests will explain approximately 20% of the phenotypic variation and 25% of the genotypic variation for fruit size and firmness. These proportions indicate that when used in seedling selection, only those seedlings with the worst genotypes should be discarded. In the remaining months of the project, we will finish the development of this combined DNA test and confirm its breeding utility.

*Further refinement of existing DNA tests*

In Year 3 of the project, quantitative statistical analyses were conducted to refine the prediction accuracy of all our DNA tests. The method used software developed by Dr. Craig Hardner within the WTFRC-funded apple project, "After RosBREED: Developing and deploying new apple DNA tests". The new analyses accounted for the genetic background of the PNWSCBP individuals, removing confounding effects associated with individual families, to more accurately estimate the outcomes of selecting for, or against, a specific DNA test allele or allelic combination. In addition, the outcomes were determined for selecting for, or against, a specific allele on other traits other than that targeted by a DNA test.

*Traits for which DNA tests were not made or that need additional confirmation*

Several traits were evaluated over the three years but effective DNA tests could not be developed. Bacterial canker resistance and pitting phenotypic data obtained in the PNWSCBP was insufficient for detection of robust genomic regions. Additional phenotypic data would be needed that adequately characterizes genetic susceptibility for these diseases. Although a fruit cracking DNA test was

developed, standardized phenotypic data on diverse breeding seedling families is needed to confirm the test's utility in the PNWSCBP. DNA test development for other traits of high priority for the PNW sweet cherry industry, such as incidence of fruit powdery mildew and the storage disorders of stem browning, shrivel, and luster loss, is also hampered by the lack of robust phenotypic data. DNA tests for these traits would be valuable because routine acquisition of performance data for these traits during breeding operations is expensive, logistically difficult to obtain, or both. A new proposal for 2017 has been submitted to the WTFRC/OSCC to fund the necessary phenotypic data collection so that new or refined DNA tests can be developed for resistance to fruit powdery mildew, cracking, pitting, shrivel, luster loss, and stem browning. Use of such DNA tests in the breeding program at the parent and/or young seedling stage, rather than older-plant fruiting stage, would reduce ongoing program operational costs.

**Objective 3: DNA test deployment**

Deployment strategies for all currently available DNA tests were developed and are ready for application. DNA information on prospective parents in the PNWSCBP for maturity time and fruit color, in addition to fruit size and firmness, self-fertility, and cross-compatibility, was used to guide 2014 crossing decisions. These same DNA tests were deployed for seedling selection in the families resulting from these targeted crosses, and resulted in a double deployment of DNA information thereby maximizing the genetic potential (new cultivar potential) of the offspring produced. Continued use of this double deployment is expected to improve the overall efficiency and impact of the PNWSCBP.

With so many DNA tests of different types now in the toolkit of the PNWSCBP (as described above) sophisticated deployment strategies are needed to best take advantage of the new opportunities. A four-step strategy was developed in this project.

*STAGE 1: Effectual parent combining*

With availability of the new **maturity time** and **fruit color** DNA tests, the PNWSCBP can more accurately align family sizes with established market class priorities. Creating the best cross combinations with predictable proportions of seedlings producing blush or mahogany and early-, mid-, or late-season fruit, and selecting those seedlings with the greatest potential to meet industry needs within each market class is more tractable than ever before.

A major market class-defining trait is **fruit color**, with 70% of the PNWSBP's resources to be allocated to mahogany-fruited individuals and only 30% to blush. Using the **fruit color** DNA test Pav-$R_f$-SSR, crosses producing a majority of mahogany offspring can now be targeted. Similarly, the DNA test Pav-G4Mat-SSR can be used to target another major market class-defining trait, **maturity time**, whereby crosses resulting in a majority of late-season and early-season individuals can be targeted. When combined, Pav-$R_f$-SSR and Pav-G4Mat-SSR provide the breeder with a convenient tool to create market class-targeted cross combinations.

For a new cultivar to be successful it must have the genetic potential to perform above a minimum level for many traits. Such minimums are what we call *essential attributes*. These essential attributes typically include **self-fertility**, **fruit siz**e >10 g, and **firmness** >300 g. The fewer seedlings that need to be culled for essential attributes, the greater the selection pressure that can be placed on all other traits, and greater selection pressure raises the genetic potential of remaining individuals. DNA information on parents can be used to identify parental combinations where little or even no culling for that trait is required on resulting families. For example, a cross using a homozygous (doubled-up) for the S4' self-fertility allele will only produce self-fertile seedlings – eliminating the need to cull any self-infertile seedlings. The parent selection stage is also a useful time to consider those parents possessing alleles for *enhancing* attributes. Enhancing attributes are those trait levels that enhance the value of a new cultivar but are not required for it to be viable in the marketplace, such as extra-large size, extra-firmness, or strong fruit-pedicel abscission. DNA tests now available

for **self-fertility, fruit size & firmness**, **fruit-pedicel abscission**, and **fruit cracking** and **powdery mildew resistance** will help to maximize effectual parental combining.

*STAGE 2: Punctilious seedling sorting*

After generating seedling families from superior parental combinations, specific proportions of industry driven market class individuals to be field-planted can be established by using DNA information for seedling sorting. After sorting, specific trait thresholds for each market class can be used to guide selection decisions.

*STAGE 3: Surgical seedling culling*

After careful consideration of parental combinations and targeted seedling sorting, the amount of seedling culling required can be reduced substantially and accurately applied to each market class. DNA tests that target essential attributes are first used to support "hard' selection decisions. For these "hard" selection decisions, the selection thresholds depend on the market class to which each seedling has been sorted. By deploying the current suite of sweet cherry DNA tests to target essential attributes, most seedlings field-planted and evaluated at adulthood for fruit are likely to meet target trait thresholds for each specific market class.

*STAGE 4: Versatile family enhancement*

Surgical seedling culling is followed by versatile family enhancement, where specific seedling families are screened with DNA tests for desired enhancing attributes to support "soft" selection decisions. Just like surgical seedling culling, the selection thresholds depend on the market class to which each seedling has been sorted, but here the selection is more nuanced with thresholds possibly varying from family to family. By following the Stage 3 "hard" selection decisions with "soft" selection decisions, most seedlings later evaluated for performance at adulthood are likely to meet essential target trait thresholds and also carry numerous enhancing attributes to suit desired targets and field capacity.

*DNA-based evaluation of Phase 2 selections*

DNA-based performance predictions were obtained for current Phase 2 selections by screening them with all available DNA tests. The resulting data are being used to aid in the selection process, providing confidence to decisions to remove selections from trials or to include them in 2017 phenotypic evaluations. Utilization of this new DNA information is a major focus of the remaining months of the project. This DNA information has made some intriguing revelations (Fig. 6). For example, 62% of P2 selections have mahogany fruit color but of those only ~25% carry two mahogany Pav-$R_f$-SSR alleles indicating a possible selection bias toward mahogany fruit with a lighter red hue [carrying only 1 mahogany allele (Fig. 4; Fig. 6)]. Only 23% of mahogany selections carry a single early-harvest-time allele (none have two) – thus there appears to be a large opportunity to develop earlier-season selections. For fruit size, only 17% of P2 selections carry the maximum of two of the CPSCT038-BPPCT034 large fruit size alleles, with only 43% carrying the self-fertile allele – thus there is still much opportunity to enrich future cohorts of P2 selections with desirable alleles.

Although sweet cherry fruit color, mahogany vs. blush, might seem like a trait that is easy to evaluate phenotypically, fruit color is directly influenced by fruit maturity (e.g., mahogany fruit can appear to be blush-type when not fully ripe) and environmental conditions (e.g., fruit might appear blush in low light or mahogany in high light environments). As a specific example, the selection R21 was classified phenotypically in 2014 as mahogany, in 2015 as blush, and in 2016 as "possibly blush, but likely mahogany". Using the Pav-$R_f$-SSR DNA test, we are now able to definitively say that R21 is mahogany, therefore having the genetic potential to produce fruit with a full red overcolor and red flesh when ripe. By using DNA test information on Phase 2 selections in this manner, we can reveal their true genetic potential.

One of the most important attributes to the success of a new cultivar in the PNW is self-fertility (Fig 6). Therefore, the self-fertility DNA test has been used since 2010 to screen all PNWSCBP seedlings to facilitate planting of only those that are self-fertile. However, many of the Phase 2 individuals were derived from seedlings planted prior to use of this DNA test and therefore are not necessarily self-fertile. By screening all Phase 2 selections with the self-fertility DNA test, we have found that only half of the current P2 selections are self-fertile. The genetic identification for each Phase 2 selection as self-fertile or non-self-fertile will help decide retention in vs. removal from field trials.

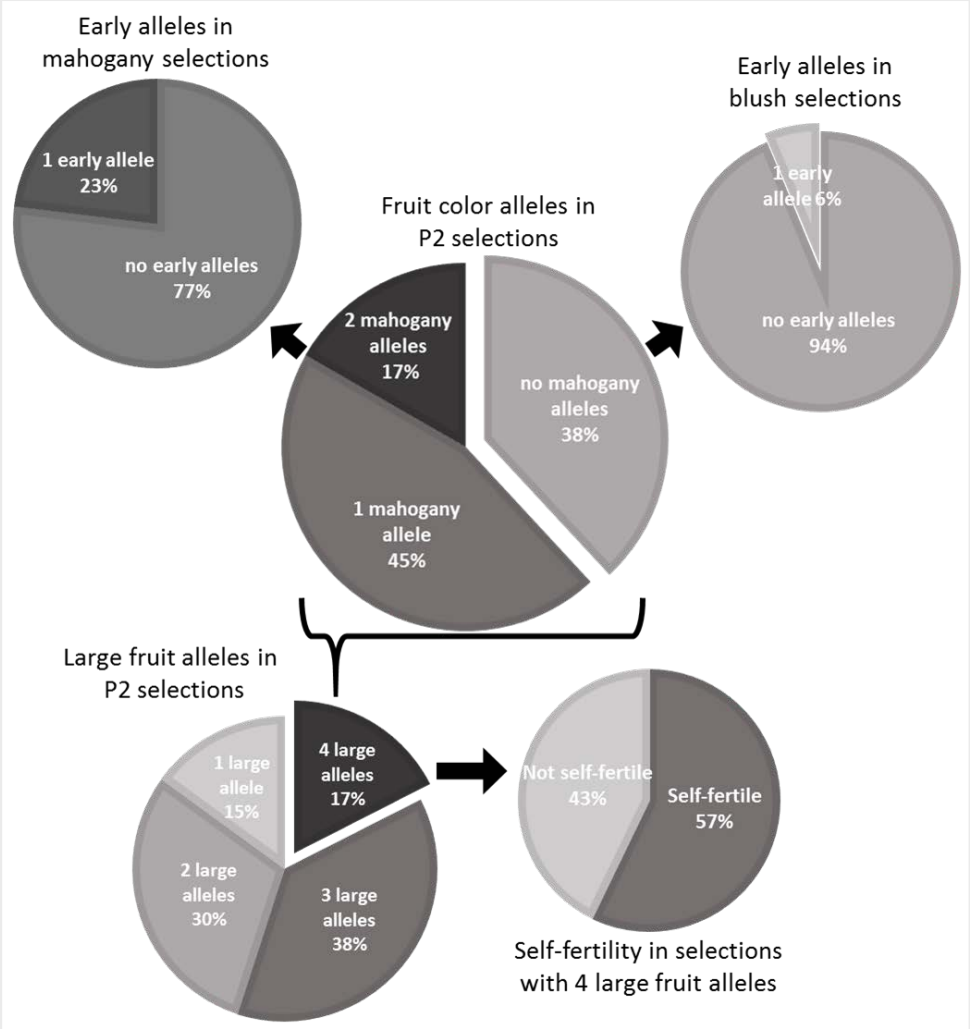


*Figure 6: Pav-Rf-SSR fruit color, Pav-Fht-SSR fruit harvest time, CPSCT038-BPPCT034 fruit size, and S4Pav fruit self-fertility DNA test results for PNWSCBP Phase 2 selections.*

*Evaluation of Phase 1 seedlings*

All Phase 1 seedlings are currently being screened with all available DNA tests. The resulting data will be used to aid in the selection process, providing confidence in decisions to remove seedlings from current trials, to advance seedlings to Phase 2, or to identify individuals that should receive additional phenotypic evaluation in 2017.

**EXECUTIVE SUMMARY**

Genetic improvement underpins the long-term economic sustainability of the Pacific Northwest sweet cherry industry. Focusing on industry priority traits, our goal was to deliver routinely implementable DNA tests to the Pacific Northwest Sweet Cherry Breeding Program (PNWSCBP). The two objectives of this project were to:

1. Develop new DNA tests for traits for which the most promising discoveries were made within the RosBREED project – maturity time, fruit color and fruit firmness and for which discoveries were anticipated from other sources during the project period – pitting and cracking incidence, fruit-pedicel abscission, resistance to bacterial canker and powdery mildew, sweetness, and acidity.
2. Ensure appropriate use of new DNA tests by devising and trialing strategies for their routine deployment within the context of existing tests and ongoing Pacific Northwest Sweet Cherry Breeding Program operations.

By successfully fulfilling these objectives, we expect to have improved the PNWSCBP's prospects for developing superior new cultivars that have fruit quality and disease/disorder resistance that is superior to the market-class standards. We believe that we can increase the PNWSCBP's development of such superior cultivars through the routine application of predictive DNA tests for the regional industry's priority traits. The appropriate deployment of trait-predictive DNA tests can efficiently reveal genetic potential for superior performance across the spectrum of breeding germplasm in each program phase. DNA tests for industry priority traits were developed and deployed on all PNWSCBP germplasm in this project.

- **Foliar powdery mildew** DNA test developed – this test can be used to identify individuals carrying the major-effect resistance allele that imparts resistance to foliar powdery mildew. This DNA test might also cover fruit PM incidence.
- **Maturity time** DNA test developed and improvement continues – this test can be used to differentiate individuals that will have, on average, up to one week difference in harvest date. However, this test does not explain the extra-early harvest of 'Chelan'. Additional genomic regions are being targeted to maximize predictiveness.
- **Fruit color** DNA test developed – this test can be used to differentiate individuals that will produce blush fruit from those that will produce mahogany fruit and can be used to identify individuals that will produce darker or lighter mahogany fruit.
- **Fruit-pedicel abscission** DNA test developed – this test should be useful in specific PNWSCBP families for differentiating fruit-pedicel abscission tendencies.
- **Fruit cracking** DNA test developed – this test appears to differentiate individuals that are highly resistant from those that are moderately to highly susceptible.
- **Fruit size & firmness** DNA test developed and improvement continues – multiple genomic regions are targeted with a single DNA test to maximize predictiveness.
- A new method was used that refines accuracy of DNA test predictions by accounting for genetic background and controlling non-genetic effects.
- DNA information on **self-fertility**, **foliar powdery mildew**, **fruit color**, **maturity time**, **fruit-pedicel abscission**, **fruit cracking**, **fruit size**, and **fruit firmness** was obtained for <u>all</u> PNWSCBP P2 selections and P1 seedlings to aid in the selection process.
- A four-stage deployment strategy (market class-defining in crosses then in seedling sorting, followed by hard selection for essential attributes within each market class, then soft selection for enhancing attributes) was devised to optimally utilize the many DNA tests now available.

In the remaining months of this project, we will conduct final refinements to DNA tests to streamline their future deployment and we will use the results from DNA-testing of all P2 selections and P1 seedlings to improve the accuracy of identifying germplasm with genetic potential for superior performance.

**FINAL PROJECT REPORT**

**Project Title**: Maintenance and evaluation of sweet cherry breeding program germplasm

**PI**: Gary Grove
**Organization**: WSU
**Telephone**: 509-786-9283
**Email**: grove@wsu.edu
**Address**: 24106 N. Bunn Road
**City/State/Zip**: Prosser, W 99352

**Collaborators:** Cameron Peace (WSU), Todd Einhorn (OSU),
**Cooperators**: Tom Auvil (WTFRC), Ines Hanrahan (WTFRC)

**Total Project Request: Year 1:** $129,350

**Budget 1: WTFRC Collaborative expenses**
**Organization Name:** WA Tree Fruit Research Commission (WTFRC)
**Contract Administrator:** Kathy Coffey **Telephone:** 509 665 8271
**Email address**: Kathy@treefruitresearch.com

| Item | Proposed 2016 | Actual 2016 |
|---|---|---|
| **Salaries** | | 22,767 |
| **Benefits** | | 5,758 |
| **Wages** | 8,366 | 15,145 |
| **Benefits** | 3,256 | 2,517 |
| **Supplies** | 200 | 277 |
| **Travel to plots** | 2,160 | |
| **WTFRC staff** | 1,500 | |
| **Total** | **15,482** | **46,464** |

**Budget 2:**
**Organization Name:** WSU Prosser **Contract Administrator:** Hallie Faulk
**Telephone:** 509 786 9283 **Email address**: prosser.grants@wsu.edu

| Item | 2016 |
|---|---|
| **Salaries**[1] | 38,250 |
| **Benefits** | 12,699 |
| **Wages**[2] | 2,640 |
| **Benefits** | 422 |
| **Supplies** | 3,053 |
| **Travel** | 1,000 |
| **Plot Fees**[4] | 9,025 |
| **Plot establishment /maintenance** | 64,500 |
| **Subtotal** | **$131,589** |
| **Carry over from 2015** | **$49,538** |
| **Total 2016 request** | **$82,051** |

[1] Salary and benefits for Associate in Research to supervise greenhouse, field, and lab activities; collect data and collate data; coordinate activities with WSU and WTFRC personnel. Additional salary will be provided by the WSU ARC.
[2] Wages and benefits for temporary employees to assist in foliar and fruit evaluations
4 Land use fee $475/acre.

**Budget 3:** Todd Einhorn
**Organization Name:** OSU-MCAREC **Contract Administrator:** Russell Karow
**Telephone:** 541-737-3228 **Email address:** Russell.Karow@oregonstate.edu

| Item | 2016 |
|---|---|
| **Salaries**[1] | 4,357 |
| **Benefits**[2] | 3,006 |
| **Wages**[3] | 13,000 |
| **Benefits**[4] | 1,084 |
| **Equipment** | 0 |
| **Fees and Supplies**[5] | 4,304 |
| **Travel** | 0 |
| **Miscellaneous** | 0 |
| | |
| **Total** | **25,751** |

[1]Estimated salaries for: 0.096 FTE (5 weeks) for full-time technician for orchard maintenance and data collection.
[2]Actual OPE rate 69%.
[3]Wages for 1,000 hours for three part-time employees ($13/hr) to assist with tree planting, weeding, bird netting, harvest, data collection and analyses.
[4]Benefits for part-time employees is 8.34%.
[5]Supplies include tree guards/paint, training materials (bamboo, spreaders, tape, fertilizer, filters and buffers for juice analysis, lab tape, and labels). Fees include per acre research plot fees: $3,104/acre and 2 months cold storage room fee ($0.94 per square foot).

**Budget 4**
**Organization Name:** Willow Drive Nursery Inc. **Contract Administrator:** Hal Leedy
**Telephone:** 509 787 1555 **Email address:** Hal@willowdrivenursery.com

| Item | 2016 |
|---|---|
| **Salaries** | |
| **Benefits** | |
| **Wages** | |
| **Benefits** | |
| **Equipment** | |
| **Supplies** | |
| **Tree propagation**[1]**:** | |
| **advanced selections** | |
| **Parents** | |
| **Miscellaneous** | |
| **Total** | 6,066 |

[1] Tree propagation fee is $11.23 per tree, with a target of 60 trees per genotype. Purchased trees include 5 PNWSCBP selections and 5 commercial cultivars.

## OBJECTIVES

The Pacific Northwest Sweet Cherry Breeding Program (CBP) was established to develop superior new cultivars for the Oregon and Washington State industries. Germplasm maintenance and breeding activities require consistent and effective management of greenhouse, lath house, field and lab operations. Furthermore, phenotypic evaluations of tree characteristics and fruit attributes must be well-documented and commercially relevant. The 2016 proposal aimed to continue the focus on improving efficiency and productivity and targeting genotypes in critical market classes.

*Specific objectives*

1. Continue improvement of horticultural practices in greenhouse, lath house, and field plots
2. Implement standardized phenotyping protocols in field and lab evaluations
3. Improve maintenance of P2 plantings at Pasco, Roza, and Hood River
4. Conduct tree, foliar and fruit evaluations of selected genotypes in P1 and P2 plantings

## SIGNIFICANT FINDINGS:

- Horticultural management practices were improved by applying contemporary horticultural techniques and regular observation of the blocks during the season.
- Guidance by representatives of the CBP Advisory Committee and WTFRC staff facilitated the assessment of overall condition and identify specific needs.
- By utilizing nutritional diagnostic tools, specific deficiencies, weak root development, and drainage problems were identified and are been remedied when possible.
- Identification of viruses (visual symptoms, Elisa and PCR techniques) allowed the identification viruses in the CBP which resulted in the removal of eight complete acres (F block) and another 80 trees in C block.
- In P1, 7% of 3710 seedlings were evaluated in the laboratory for fruit quality. Among the evaluated trees 37% meet the size and firmness thresholds and 8% (15 seedlings for Mahogany and 5 seedlings of blush) were considered very promising and will be followed in the coming years.
- In P2, a total of 39 selections (21 first bearing) were evaluated in three locations (Prosser, Pasco, Hood River) in 2016. Three blush and two mahogany selections (fully bearing) met minimum industry thresholds, but when considering additional horticultural traits, no recommendation for advancement to P3 is currently warranted.

## RESULTS & DISCUSSION

*Objective 1 (horticulture):*

1.a) The historical data base of field plots (maps) from 2009 to 2015 was analyzed and organized into a single data base, trees were identified and relabeled according to the location and available information of crossing, parents, planting year and historical evaluation data, for accurate field identification and laboratory analysis. Labels in the field contain bar code technology to track the identification throughout the field evaluation process and laboratory analysis which facilitated the identification process in the laboratory.

1.b) Horticultural practices in 2016 are detailed in Table 1 and were implemented by WSU with guidance from the WSU Cherry Breeding Program Advisory Committee (BPAC; D. Ybarra, J. Cleveringa, M. Hanrahan, E. Shrum, D. Crouse and M. Whiting) and input from WTFRC staff members (I. Hanrahan and T. Auvil). Blocks were inspected weekly to assess overall status and to ascertain plot-specific needs. A total of four written updates on program activities was prepared during the season and shared with BPAC.

Table 1. Timeline of 2016 crop management activities for WSU Prosser CBP field plots.

| Activity | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Pruning[1] | * | * | | | * | * | * | | | | |
| Weed control | | * | * | * | * | * | | | | | |
| Fungicide[2] | | | | * | * | * | | | | | |
| Insecticide[3] | | | | * | * | * | * | | | | |
| Irrigation | | | * | * | * | * | * | * | * | | |
| Planting[4] | | | * | | | | | | | | |
| Netting[5] | | | | * | | | | | | | |
| Harvest | | | | * | * | * | | | | | |
| Fertilizer[6] | | * | * | * | * | | | * | | | |
| Mowing | | | * | * | * | * | * | | | | |
| Tree removal[7] | | | | * | * | * | | | | * | * |

[1]Summer pruning was performed during harvest time with WSU crew to promote better light interception inside the tree and blocks. We incorporated mechanical pruning to reduce tree size.
[2]Fungicide spray in all Cherry Breeding blocks except in seedling (C 51-52 and F 12 -18).
[3]Insecticide application for WCFF, SWD and mites.
[4]Planting of 53 trees (B 52 Row 1)
[5] For P1 netting was performed in early maturing trees only.
[6] Foliar spray of Metalosate B and Metalosate Zn early spring. N-P-K (16-16-16) applied in new planting. Iron DP was applied to targeted trees through soil, and foliar spray of Zn sulfate donated by GS long. Elemental S was applied in Row 8 to the soil, product donated by Simplot.
[7] Tree removal was performed during the season as soon as identified as virus infected tree, bacterial canker (on the trunk), or weak trees. F block removal will be performed in November – December.

1.c) Health Diagnostics

We incorporated standard horticultural techniques for orchard diagnostic, which included visual analyses and symptomatology descriptions soil and foliar nutrient analyses, soil profile identification and laboratory testing for viruses. Nutritional deficiency symptoms were identified in individual trees. To associate the symptoms with nutrient deficiencies, absorption problems or diseases, we evaluated the roots and soil of both healthy and unhealthy trees. Soil and foliar samples were sent for chemical analyses.

After analyzing soil nutrient availability and the nutrient content in the tree, we observed zinc (Zn) deficiencies in leaves and generally low potassium (K) level. However, nutrient content in the soil showed high levels of phosphorous (P) and potassium (K). In some cases, we observed high levels of boron (B) in leaves. High levels of both P and K in the soil are a consequence of many years of fertilization, as both nutrients have low mobility in a silt loam type of soil. Even though high levels of P and K are not toxic to the plants, they can affect the absorption of other micronutrients and fruit quality. High levels of elements in the soil and low in the leaf indicates absorption issues associated with pH, deficient or excess of water in the root zone, pathogens, or other factors.

Root analyses revealed high variability in development and condition in the seedling blocks that were highly correlated with nutrient status. The smaller trees showed very low root development and lack of fine roots and root growth only within the potting substrate (mixture that differ greatly from the soil) while larger trees had better root growth.

A similar situation was observed in the young trees in C block where the trees were planted with the pot. Multiple trees were evaluated and in most cases the pot is still in good conditions (not degraded) and roots haven't been able to penetrate to the soil. Also, the soil of the pot and the site was very different in texture, porosity, density and organic matter, which generated a physical barrier for water

infiltration and roots growth. As a consequence, the roots are unable to grow beyond the pot and substrate.
Consequently, in the seedlings block the fertilization should be accomplished via foliar applications. For higher demand of macronutrients, the application should be made locally in the root zone. Unfortunately, it is likely that the root condition of these trees can hide the real potential of the seedling.

Trees with symptoms of viral infection were analyzed. In total, 13 samples were tested for PDV (Elisa), PNRSV (Elisa) and/or TriFoCap (PCR, broad spectrum test). Four samples were positive to PDV and six were positive to TriDoCap. Two samples were positive to both and no sample was positive to PNRSV. Virologists Bill Howell and Dan Villa or helped identifying symptoms in the field were we could find also Rusty Mottle Disease.

Trees from C block infected with virus or with clear virus symptoms were removed immediately and the trunk painted with glyphosate herbicide. Adjacent trees are continuously monitored, as both viruses can spread by grafting. The complete F block (eight acres) will be removed during winter to prevent disease spread and also to reduce the foot print of the CBP.

*Objective 2 protocol:* In 2016, all protocols utilized in 2015 were improved and further standardization of methods accomplished. For example, all selections in P2 were evaluated for doubling, received green fruit thinning followed by netting and finally an on-tree cracking evaluation if sufficient rain fall was received within two weeks of commercial harvest. All postharvest evaluations were coordinated between B. Sallato and the WTFRC team, based on agreed upon standardized procedures.

Four different protocols for evaluation of powdery mildew were developed and tested during the season: 1) orchard assessment of primary infection 2) assessment of secondary infection e to ascertain the spread of disease after primary infection, 3) a standardized leaf disk assay in the laboratory for foliar incidence and severity evaluation, and 4) evaluation of inoculated fruit in the orchard. As a result, all the protocols are developed and were able to give useful information regarding susceptibility. Orchard evaluations (protocols number 1 and 2) provided information regarding primary infection, which adds another layer to susceptibility/resistance evaluation. Protocol number 3 facilitated the standardization of assessments of foliar susceptibility, and protocol 4 (evaluation of inoculated fruit) permitted the identification of less susceptible selections. However, 2016 was an atypical powdery mildew year with late primary infection. It is unclear how many fruit from the breeding blocks 'escaped' infection.

*Objective 3 (maintenance of P2):* All horticultural practiced detailed in Table 1 were also performed in P2 blocks at Roza. Other P2 plantings at Sagemoor and Hood River were managed separately. All P2 plantings followed horticultural observations as detailed in the *WSU Cherry Breeding Protocol.* This series of protocols was developed collaboratively by WTFRC, WSU, OSU and others to ensure consistent and continued data collection, regardless of program staff turnover. Major tasks were synchronized across all locations include: full bloom timing observation, green fruit thinning and netting, preharvest evaluations (doubling, cracking, heat damage), harvest timing determination, harvest, and storage. Evaluated selections observed were not treated with GA. Fruit storage time was increased from two to three weeks in 2016. Fruit was held at 33F in clamshells.

*Objective 4 (P1 and P2 results):*
P1; B. Sallato, WTFRC staff and BPAC members inspected established P1 selections twice per week in order to identify selections for harvest and laboratory evaluations to make general orchard observations.

Fruit considered large and firm with less than 10% observed defects and good flavor were harvested for laboratory evaluation. Mahogany type fruit were harvested with color between 4 and 5 (according to a CTIFL chart) while blush types were harvested when more than 25% of the fruit had blush color and 17 brix. For trees with sufficient yield (more than 100 fruits), we performed up to two harvests. Of a total of 3710 productive trees in P1, 263 seedlings (7%), were selected for laboratory analysis (202 mahogany and 61 blush). The following figure shows a detailed sequence of trees and harvest date for mahogany and blush seedlings. Standards from the advance selection block (Jun 2: Early Robin, Chelan, June 6: Rainier, June 16: Bing, June 30; Sweetheart) were included as references.

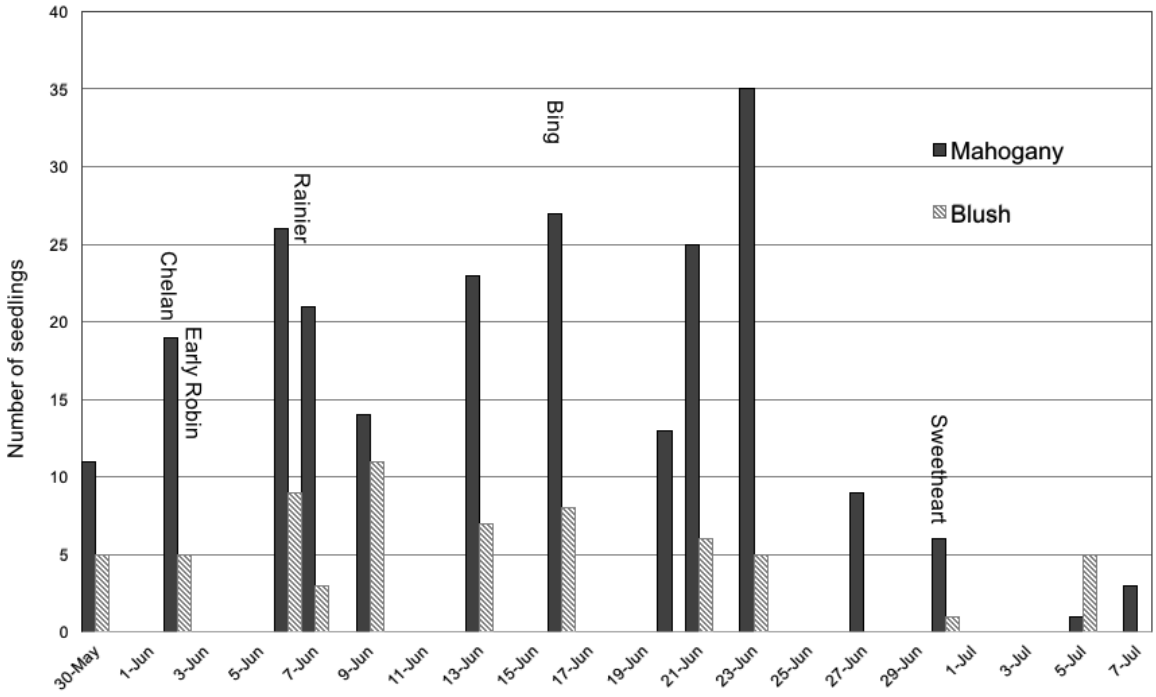


Figure 3. Timeline of number of seedlings by harvest date for mahogany and blush at Roza in 2016.

The percentages of early, middle, and late season mahogany were 38%, 39% and 23% respectively, and for blush cherries, were 48%, 34% and 18% respectively. The seedlings that met size and firmness BPAC thresholds, were 38% for mahogany and 36% for blush cherries (data not shown). Listed in Tables 2 and 3 are seedlings considered very promising for each market class. These selections met the size and firmness in 2016, plus additional traits like flavor, defects, soluble solid content and titratable acidity.

Table 2. Most promising selections for mahogany market class.

| Market Class | Harvest date | Location | Fruit Wt. (g) | Row size | Firmness (g/mm) | Pull Force (Kg) | Color (1-7) | SSC (Brix) | TA (% Malic) |
|---|---|---|---|---|---|---|---|---|---|
| ***Control*** | ***6/2*** | ***Chelan*** | ***8.1*** | ***10.5*** | ***312.0*** | ***1.60*** | ***4.9*** | ***18.3*** | ***0.9*** |
| EM | 5/30 | FR39T121 | 11.9 | 9.2 | 306.4 | 1.18 | 4.2 | 19.0 | 0.9 |
| EM | 6/9 | CR08T07 | 11.1 | 9.3 | 286.1 | 1.09 | 6.8 | 23.4 | - |
| ***Control*** | ***6/16*** | ***Bing*** | ***8.7*** | ***10.2*** | ***310.0*** | ***1.40*** | ***5.3*** | ***23.1*** | ***0.9*** |
| MM | 6/16 | CR6T22 | 10.8 | 9.4 | 468.4 | 0.86 | 4.2 | 22.3 | 0.9 |
| MM | 6/20 | FR39T117* | 15.2 | 8.5 | 326.5 | 0.76 | 6.1 | 22.6 | 0.8 |
| MM | 6/20 | FR39T112 | 13.6 | 8.9 | 286.4 | 0.84 | 7.0 | 23.2 | 0.9 |
| MM | 6/20 | FR40T112 | 16.5 | 8.5 | 308.1 | 0.59 | 5.9 | 21.5 | 0.9 |
| MM | 6/21 | FR52T60 | 11.4 | 9.6 | 478.7 | 0.89 | 4.2 | 18.9 | 1.2 |
| LM | 6/23 | CR1T79* | 12.3 | 9.1 | 409.2 | 0.75 | 5.0 | 21.1 | 0.9 |
| LM | 6/23 | CR10T11 | 11.1 | 9.4 | 355.9 | 0.98 | 5.0 | 23.8 | 1.0 |
| LM | 6/27 | CR1T69 | 12.4 | 9.3 | 306.4 | 0.47 | 4.5 | 17.6 | 0.7 |
| ***Control*** | ***6/30*** | ***Sweetheart*** | ***8.9*** | ***10.4*** | ***350.0*** | ***0.90*** | ***4.1*** | ***23.6*** | ***0.9*** |

*Selections considered good in others years.

The gray boxes in Table 2 indicate excellent values for size and firmness (row <8.5 and firmness > 400 g/mm). In these cases, even though some did not meet the BPAC firmness threshold (> 300 g/mm), they had a firm or crunchy taste. The advanced selections FR39T117, currently being propagated in the nursery, also showed excellent quality. Standards from the advance selection block (B48) were thinned (30 fruit per foot), while the seedlings were not thinned.

Table 3. Most promising selections for blush market class

| Market Class | Harvest date | Location | Fruit Wt. (g) | Row size | Firmness (g/mm) | Pull Force (Kg) | Color (1-7) | SSC (Brix) | TA (% Malic) |
|---|---|---|---|---|---|---|---|---|---|
| ***Control*** | ***6/2*** | ***Early Robin*** | ***12.0*** | ***9.5*** | ***362.0*** | ***1.90*** | ***B*** | ***17.1*** | ***0.6*** |
| ***Control*** | ***6/6*** | ***Rainier*** | ***7.5*** | ***10.5*** | ***257.0*** | ***1.50*** | ***B*** | ***18.4*** | ***0.7*** |
| MB | 6/9 | FR40T108 | 13.5 | 8.9 | 281.8 | 1.43 | B | NA | NA |
| LB | 6/13 | F070T089 | 14.0 | 8.5 | 320.0 | 0.82 | B | NA | NA |
| LB | 6/23 | C03T124 | 13.5 | 8.9 | 308.0 | 0.83 | B | 21.4 | 1.0 |

All promising selections will be followed next season and evaluated for other traits including storage, while the F seedlings will be propagated by WSU personnel on Gisela rootstock to preserve the material.

P2: In P2, 39 selections (21 first bearing) were evaluated in Prosser and Pasco led by Ines Hanrahan (WSTFRC), and 14 selections (R2,5,6,7,8,9,12,13,14,15 and 16) were evaluated by Todd Einhorn in Hood River (OSU).

During bloom, the WTFRC team visited the Roza and Sagemoor plantings twice a week to assess bloom stage, record full bloom dates and GDD's at full bloom (not shown). Briefly, we had a very narrow peak bloom distribution between cultivars. At Sagemoor, all selections reached full bloom within eight days, while full bloom dates at Roza were recorded in a mere three day span. At Hood River, full bloom was recorded between the 5$^{th}$ and 11$^{th}$ of April. In addition, full bloom fell in one of two basic patterns: 1) full bloom peaks at one observation date, 2) full bloom plateaus for up to 4 days (not shown).

Plantings were visited weekly after bloom. The amount of fruit doubles present in each selection was assessed on April 25-28. In general, the amount of doubles in 2016 was low. Several advanced selections showed doubling presence at similar rates to known standards (R 13,14,16,10) while another group of selections had no doubles (R 1,3,4,5,11,17,18). The WTFRC team performed green fruit thinning (30 fruit/foot) at Roza on May 5th, while Dave Allan directed green fruit thinning at Sagemoor.

In summary, R25, R2, R14, R10 and the standards Bing, Sweetheart and Rainier required heavy thinning, while R1 and R4 were not thinned due to low fruit set. All trees were pruned (topping) mechanically by Matt Whiting to facilitate netting. All trees were netted on May 11 under the leadership of Kyle Tynan utilizing both WSU and WTFRC staff. We recorded all rain events and assessed cracking percentage of fruit if more than 0.1 inches of rain (in a single event) was received within 14 days of respective harvest. Fruit maturation was tracked bi-weekly.

Table 4: Performance of all selections evaluated in 2016 at Roza P2 planting when considering minimum BPAC requirements (firmness and size)

| Harvest date | Cherry color | Selection | Market class | min. BPAC threshold met | Better than standard | Years observed* | Firmness | | Row size |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | g/mm | % > 300 | (8-13) |
| 6/2 | blush | Early Robin | early | standard | standard | 2 | 362 | 90 | 9.5 |
| 6/2 | blush | R16 | early | yes | no | 2 | 302 | 48 | 9.5 |
| 6/6 | blush | Rainier | midseason | standard | standard | 2 | 257 | 9 | 10.5 |
| 6/6 | blush | R10 | midseason | **yes** | **yes** | 2 | 315 | 60 | 9.9 |
| 6/13 | blush | R9 | late | **yes** | **yes** | 2 | 355 | 91 | 9.4 |
| 6/16 | blush | R7 | late | no | yes | 1 | 536 | 100 | 10.4 |
| 6/16 | blush | R11 | late | **yes** | **yes** | 1 | 461 | 99 | 10.0 |
| 6/16 | blush | R5 | late | **yes** | **yes** | 1 | 348 | 88 | 9.1 |
| 6/2 | mahogany | Chelan | early | standard | standard | 2 | 312 | 56 | 10.5 |
| 6/2 | mahogany | R25 | early | no | no | 2 | 313 | 62 | 10.6 |
| 6/6 | mahogany | R2 | early | no | no | 2 | 230 | 6 | 10.5 |
| 6/9 | mahogany | R14 | TBD | no | TBD | 2 | 284 | 34 | 9.8 |
| 6/16 | mahogany | R8 | midseason | **yes** | **yes** | 2 | 319 | 63 | 9.7 |
| 6/16 | mahogany | Bing | midseason | standard | standard | 2 | 310 | 54 | 10.2 |
| 6/16 | mahogany | R6 | midseason | **yes** | **yes** | 2 | 327 | 76 | 9.1 |
| 6/27 | mahogany | Selah | late | standard | standard | 2 | 315 | 59 | 9.1 |
| 6/27 | mahogany | Lapin | late | standard | standard | 2 | 353 | 88 | 9.7 |
| 6/30 | mahogany | Sweetheart | late | standard | standard | 2 | 350 | 85 | 10.4 |
| 6/6 | mahogany | R3 | early | no | no | 1 | 288 | 71 | 9.1 |
| 6/6 | mahogany | R15 | early | no | no | 1 | 252 | 1 | 10.0 |
| 6/9 | mahogany | R13 | TBD | no | TBD | 1 | 340 | 80 | 10.4 |
| 6/9 | mahogany | R12 | TBD | yes | TBD | 1 | 383 | 96 | 8.2 |
| 6/13 | mahogany | R1 | midseason | **yes** | **yes** | 1 | 347 | 80 | 9.1 |

None of the selections with fully mature trees at Sagemoor met all quality criteria set by BPAC for their respective harvest time frame (not shown). At Roza, selections that met all criteria including the blushes R16, 10, 9 and the mahogany R6, 8 (Table 4). Some selections bearing for the first time this year have shown promise by meeting all established benchmarks: R3 (mahogany, Sagemoor only), R24 (blush, Sagemoor only), R11 + R7 (blush. Roza); R1 (mahogany, Roza. Selections that met both thresholds at Hood River were the mahogany R6 and the blushes R12, R7, R9, however all selections showed high percentage of cracking (R6 and R12 over 49%; no data for R9; Table 6. More than 14 inches of rain were recorded at MCAREC recorded by Jun 6.

Table 5 shows all five selections harvested from mature trees that met minimum BPAC requirements (Roza data only) and the results for additional traits. When considering eight additional horticultural traits and six postharvest quality indicators, none of these selections warranted immediate advancement to P3. All exhibit at least two major flaws (i.e. performing below industry standard). R6 exhibited two potentially fatal flaws, by showing a propensity for) for pitting and shrivel when compared to Bing.

Table 5: Additional horticultural traits of P2 selections (Roza only) of all mature selections meeting minimum BPAC performance requirements in 2016

| Color | | Blush | | | Mahogany | |
|---|---|---|---|---|---|---|
| Selection | | R16 | R10 | R9 | R6 | R8 |
| Class | | early | midseason | late | midseason | midseason |
| Horticultural traits (units) | | | | | | |
| Harvest time | days ± the standard | 0 (Early Robin) | 0 (Rainier) | + 7 (Rainier) | 0 (Bing) | 0 (Bing) |
| 90% above 300g/mm | yes/no | no | no | yes | no | no |
| Self-fertility | DNA-tested | No | yes | TBD | Yes | TBD |
| Powdery mildew sensit. | yes/no | TBD | TBD | TBD | TBD | TBD |
| Cracking | % | 5.7 | 4.6 | 0 | 0.42 | 0.56 |
| Doubling | % | 1 | 0.6 | 0 | 0 | 0 |
| Flavor at harvest | better than standard | yes | yes | yes | yes | no |
| Thinning need | yes/no | yes | yes | yes | yes | yes |
| Better yield efficiency | yes/no | no | no | no | no | no |
| Postharvest | | | | | | |
| Pitting | better than standard | no | yes | yes | no | no |
| Induced pitting | better than standard | yes | yes | no | no | no |
| Stem browning | better than standard | yes | yes | yes | yes | yes |
| Shrivel | better than standard | yes | yes | yes | no | yes |
| Luster | better than standard | no | no | no | no | yes |
| Flavor | better than standard | yes | yes | yes | yes | no |
| | | | | | | |
| Fatal flaw* | | | | | pitting shrivel | |

*at least 100% worse than standard

Table 6. P2 Harvest data, MCAREC. Data are means of 5 reps. FF, fruit firmness; PRF, pedicel retention force; SSC, soluble solids; TA, titratable acidity. n.d, no data.

| Cultivar | Harvest Date | Yield (lbs/tree) | FF (g/mm) | Fruit diam. (mm) | Fruit sz (row sz) | Fruit wt. (g) | Skin color (ctifl) | Cracking (%) | PRF (g) | SSC (%) | TA (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Early Robin | 6-Jun | < 1 | 304.2 | 31 | 9 | 12.2 | BLUSH | n.d. | n.d. | 17 | 0.64 |
| R 16 | 6-Jun | 1.3 | 289.8 | 30.9 | 9 | 11.9 | BLUSH | 49.4 | 614.8 | 17.5 | 0.98 |
| FR1T5 | 9-Jun | < 1 | 339.3 | 29.2 | 9.5 | 10.7 | 4.7 | 100 | 681.3 | 18.7 | 0.74 |
| R 2 | 10-Jun | 2 | 234.4 | 26.4 | 10.4 | 8.8 | 4.7 | 29.6 | 701.8 | 17.3 | 0.88 |
| R 15 | 16-Jun | 3.7 | 248.3 | 28.7 | 9.6 | 10.2 | 5 | 50.5 | 638 | 18.2 | 0.96 |
| R 6 | 16-Jun | 7.7 | 321.3 | 31.8 | 8.7 | 11.4 | 4.5 | 27.5 | 776.5 | 19.2 | 0.88 |
| Rainier | 16-Jun | 7.4 | 222.3 | 30.3 | 9.1 | n.d. | BLUSH | n.d. | 633.8 | 17.8 | 0.46 |
| R 8 | 21-Jun | 5.3 | 274.9 | 29.3 | 9.4 | 10.9 | 4.7 | 28.7 | 812.4 | 16 | 0.68 |
| R 13 | 21-Jun | 32.4 | 277.1 | 27.7 | 9.9 | 9.8 | 5.6 | 22.2 | 595.1 | 16.3 | 0.68 |
| R 14 | 21-Jun | 14.4 | 244.8 | 30.2 | 9.1 | 12.3 | 5.3 | 19.6 | 595.7 | 17.5 | 0.91 |
| Bing | 21-Jun | 11.9 | 222 | 28.3 | 9.6 | 9.6 | 4.6 | 16 | 173.8 | 18.4 | 0.65 |
| R 5 | 22-Jun | < 1 | 261.7 | 29.7 | 9.3 | 11.1 | BLUSH | 49 | 457.2 | 17.6 | 0.71 |
| 49T83 | 22-Jun | < 1 | 375.3 | 31.8 | 8.7 | 13.4 | BLUSH | 37.5 | 798.5 | 17.3 | 0.44 |
| R 12 | 22-Jun | < 1 | 372.9 | 30.8 | 9 | 12 | BLUSH | 70.1 | 456 | 17 | 0.56 |
| R 7 | 22-Jun | < 1 | 401.4 | 30.6 | 9 | 11.1 | BLUSH | 18 | 773.2 | 17.6 | 0.68 |
| 13T4 | 30-Jun | < 1 | 318.5 | 31.6 | 8.7 | 13.7 | 4.6 | 39.5 | 552.5 | 20.2 | 0.77 |
| R 9 | 30-Jun | < 1 | 309.3 | 31.8 | 8.7 | 12.8 | BLUSH | n.d. | 744.8 | 18.8 | 0.73 |
| Sweetheart | 7-Jul | 2.3 | 355.2 | 29 | 9.5 | 10.7 | 4.5 | 7 | 372 | 20.7 | 0.82 |

Note: the cultivar identification has been change to Roza identification to facilitate the analyses.

## EXECUTIVE SUMMARY

The Pacific Northwest Sweet Cherry Breeding Program (CBP) was established to develop superior new cultivars for the Oregon and Washington State industries. Germplasm maintenance and breeding activities require consistent and effective management of greenhouse, lath house, field and lab operations. Furthermore, phenotypic evaluations of tree characteristics and fruit attributes must be well-documented and commercially relevant. The 2016 proposal aimed to continue the focus on improving efficiency and productivity and targeting genotypes in critical market classes.

Major accomplishments in 2016 include:

- Horticultural management practices were improved by applying contemporary horticultural techniques and regular observation of the blocks during the season.
- Guidance by representatives of the CBP Advisory Committee and WTFRC staff facilitated the assessment of overall condition and identify specific needs.
- By utilizing nutritional diagnostic tools, specific deficiencies, weak root development, and drainage problems were identified and are been taking care of when possible.
- Identification of viruses (visual symptoms, Elisa and PCR techniques) allowed the identification viruses in the CBP which resulted in the removal of eight complete acres (F block) and another 80 trees in C block.
- In P1, 7% of 3710 seedlings were evaluated in the laboratory for fruit quality. Among the evaluated trees 37% meet the size and firmness thresholds and 8% (15 seedlings for Mahogany and 5 seedlings of blush) were considered very promising and will be followed in the coming years.
- In P2, a total of 39 selections (21 first bearing) were evaluated in three locations (Prosser, Pasco, Hood River) in 2016. Three blush and two mahogany selections (fully bearing) met minimum industry thresholds, but when considering additional horticultural traits, no recommendation for advancement to P3 is currently warranted.

**FINAL PROJECT REPORT**

**Project Title**: MSU cherry rootstocks: Pre-commercialization


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**Other funding sources**

**Agency Name**: Michigan Economic Development Corporation
**Amount awarded**: $6,984
**Notes**: Funding was obtained to optimize the DNA diagnostic tests for commercial service providers.

**Total Project Funding**: $134,804

**Budget History:** Summed over budgets for MSU, WSU, OSU, and the WTFRC

| Item | Year 1: 2014 | Year 2: 2015 | Year 3: 2016 |
|---|---|---|---|
| **Salaries** | $23,359 | $ 10,844 | $12,086 |
| **Benefits** | $ 6,391 | $ 3,445 | $ 3,855 |
| **Wages** | | | |
| **Benefits** | | | |
| **Equipment** | | | |
| **Supplies**[a] | $1,900 | $ 12,050 | $ 5,550 |
| **Travel** | $ 5,000 | $ 7,040 | $ 9,040 |
| **Plot Fees** | $ 2,000 | $ 1,340 | $ 660 |
| **Miscellaneous**[b] | $ 11,800 | $ 18,344[c] | $ 100 |
| **Total** | $50,450 | $53,063 | $31,291 |

[a]Including plot establishment items
[b]Including liner and tree cost

**ORIGINAL OBJECTIVES RECAP:**

1. Compare the performance of the MSU cherry rootstocks to currently available sweet cherry rootstocks using intensive cherry production systems.
   - **A.** 2009 planting of 'Bing' on MSU cherry rootstocks (removed after 2014 season).
   - **B.** 2015 planting of 3 replicated rootstock trials each containing 4 MSU cherry rootstocks and appropriate check rootstock cultivars with scion cultivars 'Early Robin', 'Regina', and 'Sweetheart'.
   - **C.** 2016 planting of three small replicated rootstock trials alongside the 2015 trials to evaluate the 5th MSU cherry rootstock.
2. Collaborate with commercial nurseries in liner and finished tree production to determine the nursery performance of the MSU cherry rootstocks.
3. Collaborate with the CPCNW-FT and cooperating nurseries to insure MSU cherry rootstocks are available as certified virus tested and genetically verified.

**SIGNIFICANT FINDINGS:** (bullets by objective)

- **1.A.** Five MSU cherry rootstocks produce dwarf precocious sweet cherry trees with 'Bing' scion based on six years of evaluation of the trees planted at the WSU-Prosser Roza Station in spring 2009. These five rootstocks, which are named after Michigan counties, are Clinton, Cass, Clare, Lake and Crawford. The trees produced were significantly smaller than 'Gisela®6' (Gi6) but of similar size to 'Gisela® 5' (Gi5) measured as trunk cross-sectional area (TCSA).
- **1.A.** In 2014, 'Bing' fruit maturity date differed among the seven rootstocks tested at the Prosser plot with Cass, Clare and Lake ripening ahead of Clinton, Crawford, Gi5 and Gi6.
- **1.A.** In 2014, all five of the MSU candidate rootstocks had yield efficiencies (kg fruit/$cm^2$) that were higher than that of Gi5 and Gi6. However, the fruit size for Crawford was significantly less than that for Gi5 due to the high crop load on Crawford compared to Gi5 and insufficient thinning of Crawford. These results suggest that producing large fruit is possible on the MSU rootstocks given the proper training system and crop load adjustments. The plantings established in 2015 and 2016 and to be established in 2017, will address these management systems and include a wide range of scion cultivars.
- **1.B.** Three plots were planted in 2015 to compare four MSU rootstocks (Cass, Clare, Clinton, and Lake) with the Gisela (5 and 6) and Krymsk rootstocks using intensive orchard systems with 'Regina', 'Early Robin' and 'Sweetheart' scions. The plots are in The Dalles, Mattawa, and East Wenatchee.
- **1.B.** Across all three scions and locations, in general, the MSU rootstocks Cass and Clare are the smallest trees followed by Lake and then Clinton.
- **1.C.** Crawford was not included in the 2015 plantings as it was delayed being released from the CPCNW-FT and then there was a delay in liner production. Three smaller plots comparing Crawford with the most similar rootstocks, Clinton and Gi5, are on track to be planted in spring 2017.
- **2.** The five MSU rootstocks performed well in liner and finished tree production at commercial nurseries; therefore, no nursery barriers to commercialization were identified.
- **3.** Virus certification of all five MSU rootstocks was completed by the CPCNW-FT.
- **3.** Certified budwood of all 5 MSU rootstocks was sent to nine commercial nurseries, followed by experimental production of liners and finished trees. DNA testing of the MSU rootstocks was done at critical stages in budwood transfer and liner and tree production. To date, DNA testing has verified the trueness-to-type of the stock plants at the nurseries and the trees for the 2015, 2016 and 2017 plantings.

**RESULTS & DISCUSSION:**

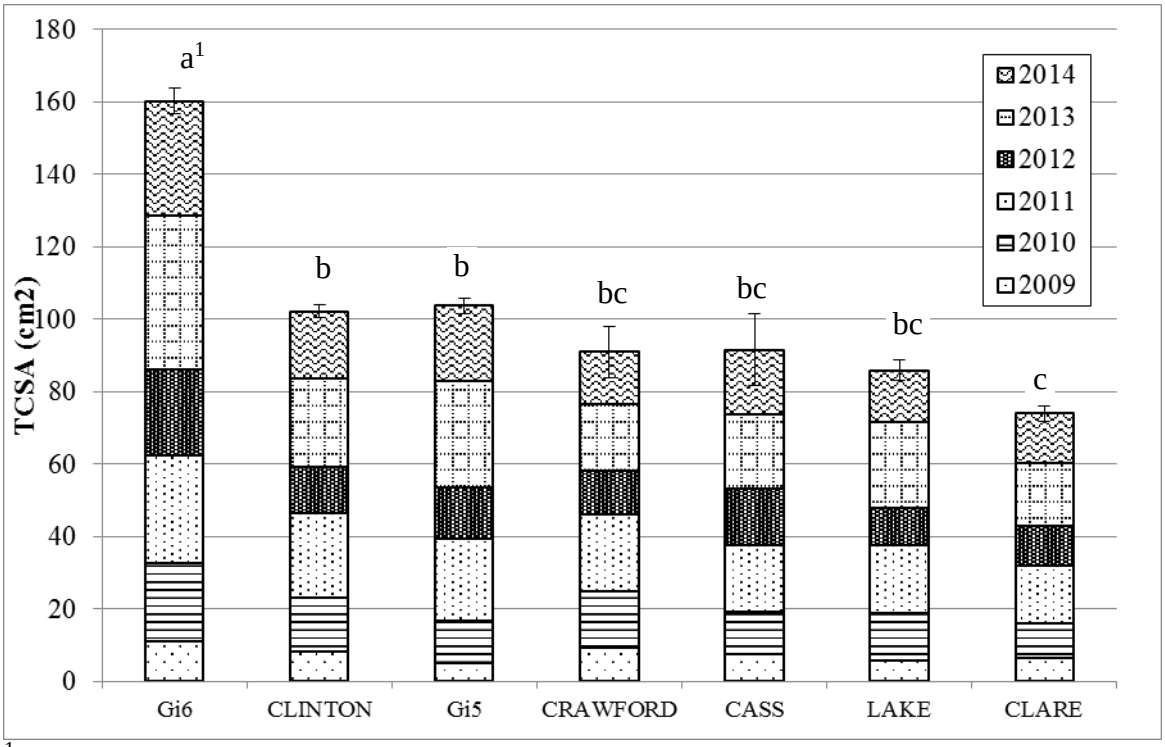
**1.** Compare the performance of the MSU cherry rootstocks to currently available sweet cherry rootstocks using intensive cherry production systems.

**A.** 2009 planting of 'Bing' on MSU cherry rootstocks (removed after the 2014 season)

This plot at WSU-Prosser Roza Station compared all 5 MSU cherry rootstocks and Gi5 and Gi6. It was planted in 2009 and removed after the 2014 season. Trees were spaced at 8 ft × 15 ft in five-tree replicates and trained to a multiple leader architecture.

All five MSU rootstocks produced 'Bing' trees significantly smaller than Gi6 but similar size to Gi5 measured as trunk cross-sectional area (TCSA), except Clare which produced trees significantly smaller than Gi5 (Fig. 1). In 2014, 'Bing' fruit harvest date differed among the seven rootstocks tested at the Prosser plot with Cass, Clare and Lake, ripening ahead of Clinton, Crawford, Gi5 and Gi6 (Table 1). The four day spread in harvest dates would likely have been more pronounced in a cooler June as the temperatures at Prosser were an average of 5 F above normal and June 19 had a maximum temperature of 85.7 F. In 2014, all five of the MSU candidate rootstocks had yield efficiencies (kg fruit/cm$^2$) that were higher than that of Gi5 and Gi6. However, fruit size for Crawford was significantly less than that for Gi5. This was due to the high crop load on Crawford compared to Gi5, where fruit thinning was not sufficient. These results suggest that producing large fruit is possible on the MSU rootstocks given the appropriate intensive training systems and crop load adjustments required for these dwarf precocious rootstocks. A series of additional plantings (Obj. 1B and 1C) were designed to address these management systems.

**Fig. 1.** Trunk cross-sectional area (TCSA; cm$^2$) of 'Bing' trees grafted on 5 MSU rootstocks, Gi5, and Gi6 for trees planted in 2009 at the WSU-Prosser. Boxes represent growth over one season. TCSA measurements in 2014 were taken on June 16. Bars represent standard error of the means for 2014 TCSA.




[1]Means that are significantly different for 2014 TCSA ($P < 0.05$) are denoted by different letters.

**Table 1.** Year 2014 average tree yield, yield efficiency, fruit weight and mean row size, for 'Bing' grown on five MSU rootstocks[1].

| Rootstock selection | Harvest date (June) | Average Tree Yield (lb) | Average Tree Yield (kg) | Yield efficiency ($kg/cm^2$) | Fruit weight (g) | Mean row size |
|---|---|---|---|---|---|---|
| Gi5 | 20th | 23.4 b | 10.7 b | 0.10 c | 10.4 a | 10.0 b |
| Gi6 | 20th | 26.2 ab | 11.9 ab | 0.07 c | 10.3 ab | 10.3 ab |
| CASS | 16th | 32.7 ab | 14.9 ab | 0.16 ab | 8.9 ab | 10.6 ab |
| CLARE | 16th | 25.1 ab | 11.4 ab | 0.16 ab | 9.3 ab | 10.5 ab |
| CLINTON | 20th | 28.8 ab | 13.1 ab | 0.13 bc | 9.8 ab | 10.5 ab |
| CRAWFORD | 20th | 33.2 a | 15.1 a | 0.17 a | 8.7 b | 10.9 a |
| LAKE | 16th | 23.9 ab | 10.9 ab | 0.13 abc | 9.2 ab | 10.6 ab |

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

**B.** 2015 planting of 3 replicated rootstock trials each containing 4 MSU cherry rootstocks and appropriate check rootstock cultivars with scion cultivars 'Early Robin', 'Regina', and 'Sweetheart'.

*Plot descriptions*: For the 2015 planting, Cass, Clare, Clinton and Lake liners were budded with three scions: 'Early Robin', 'Regina' and 'Sweetheart' at Willow Drive Nursery, Cameron Nursery and Willow Drive Nursery, respectively, along with Gisela and Krymsk rootstocks as controls (Table 2). The plots were planted in The Dalles (hosted by Tim Dahle), Mattawa (hosted by Wash. Fruit and Produce), and East Wenatchee (hosted by McDougall & Sons) using a range of tree spacings and training systems (Table 2). Of the 22 scion/rootstock combinations needed for the 2015 planting, only the combination 'Regina'/Cass was not included at one site (East Wenatchee) due to low tree numbers.

**Table 2.** Summary of rootstock plantings made in spring 2015 at three locations: The Dalles (TD), Ore., Mattawa (MA) & East Wenatchee (EW), Wash.

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

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

[b]Wires 2.3 (0.7m) apart vertically

*Tree survival*: On average the tree sizes on the MSU rootstocks at planting were smaller than that for trees on the Krymsk and Gisela rootstocks (Figs. 2, 3, and 4). The reduced size was especially pronounced for the 'Regina' trees. Overall, the poorest year 1 survival was for trees on Cass followed by Clinton suggesting that these trees are slower in recovering from transplant stress (data not presented). Additional trees of 'Regina' on Cass have been propagated for 2017 planting allowing a second evaluation for this combination, as the trees planted in 2015 were very small (mean TCSA of 0.7 $cm^2$) compared to trees on the other rootstocks and there were insufficient trees for the East Wenatchee planting. No trees died in 2016.

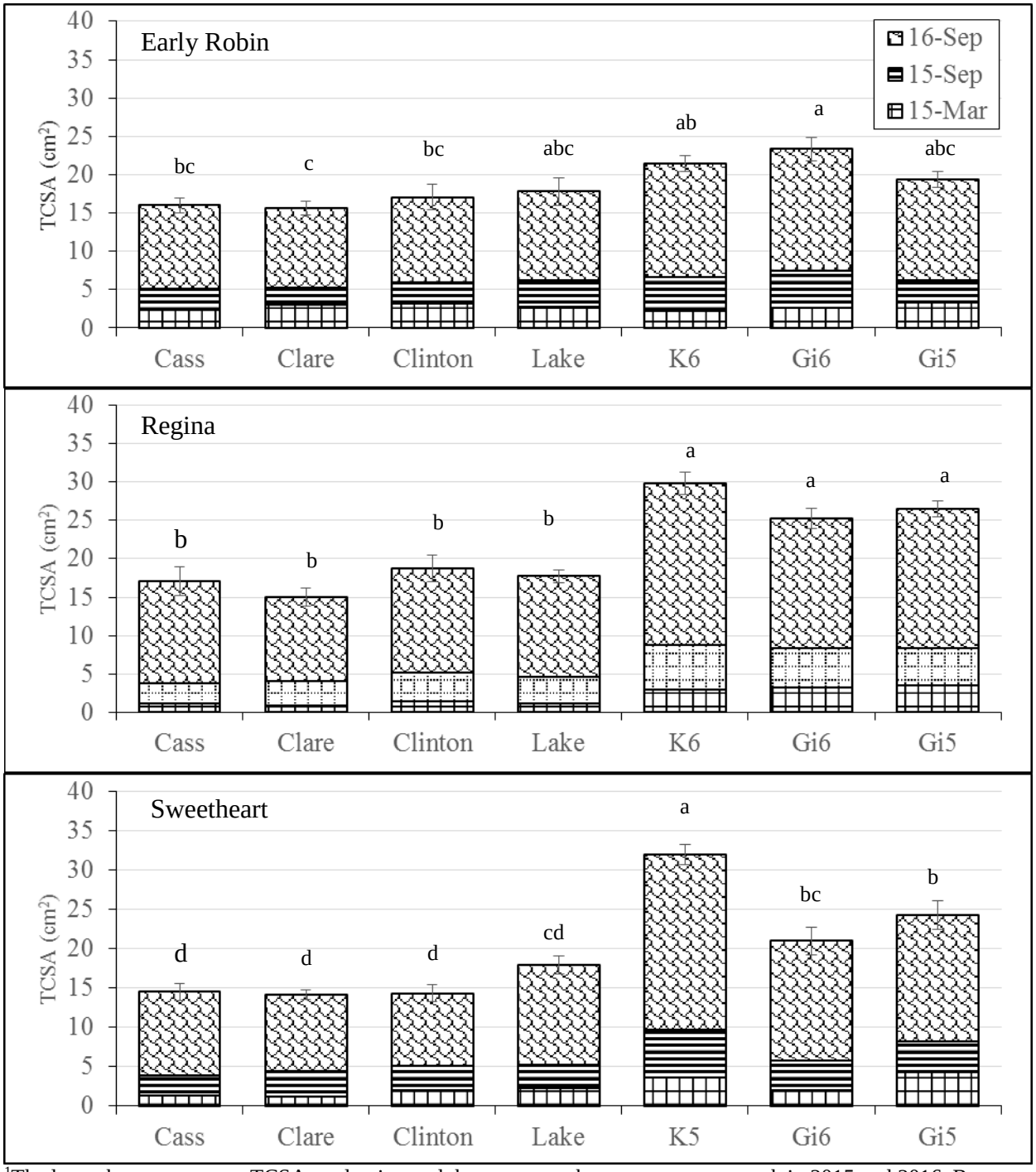
*Tree growth:* For the planting at The Dalles, the 'Regina' and 'Sweetheart' trees on the four MSU rootstocks (Cass, Clare, Lake and Clinton) were in general significantly smaller (measured as TCSA, $cm^2$) than the trees on the Krymsk and Gisela rootstocks (Fig. 2). A similar trend was apparent for 'Early Robin'; however, the differences were not as significant as in general 'Early Robin' on K6, Gi5 and Gi6 were smaller than 'Regina' and 'Sweetheart' on these rootstocks (Fig. 2). The TCSAs for 'Regina' and 'Sweetheart' on K6 and K5, respectively, were on average about two times larger than for trees on Cass and Clare. This result is consistent with K5 and K6 being vigorous rootstocks compared to the other rootstocks.

The relative sizes for the trees at East Wenatchee were similar to those at The Dalles, except for Clinton (Fig. 3). Three of the MSU rootstocks (Cass, Clare and Lake) had tree sizes significantly less than trees on the Krymsk or Gisela rootstocks, although trees on Lake tended to be larger than trees on Cass or Clare. The size reductions for 'Early Robin' and 'Sweetheart' on Clinton were not significant compared to the Gisela rootstocks, indicating that at this site, Clinton is a more vigorous rootstock than the other three MSU rootstocks. Similar to The Dalles, the combination of 'Sweetheart'/K5 was especially vigorous.

At the Mattawa planting, the results varied by scion (Fig. 4). For 'Regina' and 'Sweetheart' the four MSU rootstocks were significantly smaller than the trees on the Krymsk or Gisela rootstocks, with the smallest trees on Cass. For 'Early Robin', trees on Lake were not significantly smaller than trees on K6, Gi6 or Gi5. Gi6 and Clinton, showed more heat and sandy soil stress than K5, K6, Lake and Clare.

In summary, on average across all three scions and locations, the trees on the four MSU rootstocks were significantly smaller than those on the Gisela or Krymsk rootstocks. Among the MSU rootstocks, Cass and Clare produced the smallest trees followed by Lake and Clinton. The relatively larger TCSA for Lake at the sandy Mattawa location relative to the other MSU rootstocks agrees with results from Michigan that suggest that Lake may be more tolerant to sandy soils than Clinton. The poorest tree survival of trees on Cass and Clinton suggests that these trees may be slower in recovering from transplant stress. Additional trees of 'Regina' on Cass have been ordered for 2017 planting allowing a second evaluation for this combination which is under represented in the 2015 plantings.

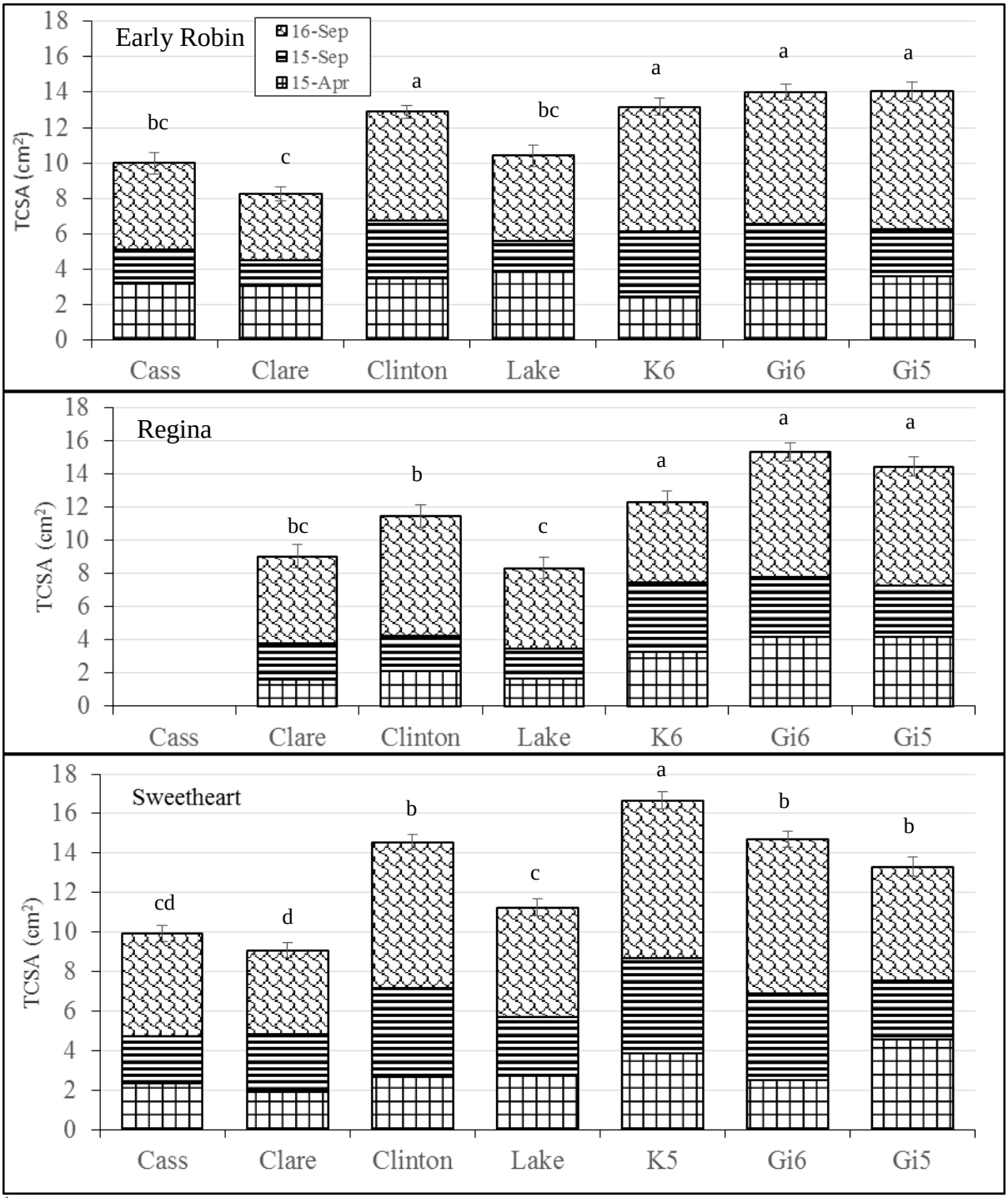
**Fig. 2.** Trunk cross-sectional area[1] (TCSA; $cm^2$) of 'Early Robin', 'Regina', and 'Sweetheart' trees grafted on 4 MSU rootstocks, Krymsk 5, Krymsk 6, Gi6, and Gi5 for trees planted in 2015 in The Dalles, OR. Measurements were obtained in Mar. and Sept., 2015 and in Sept., 2016.




[1]The lower boxes represent TCSA at planting and the two upper boxes represent growth in 2015 and 2016. Bars represent standard error of the means for September TCSA.

[2]Means that are significantly different for September 2016 TCSA ($P < 0.05$) are denoted by different letters.

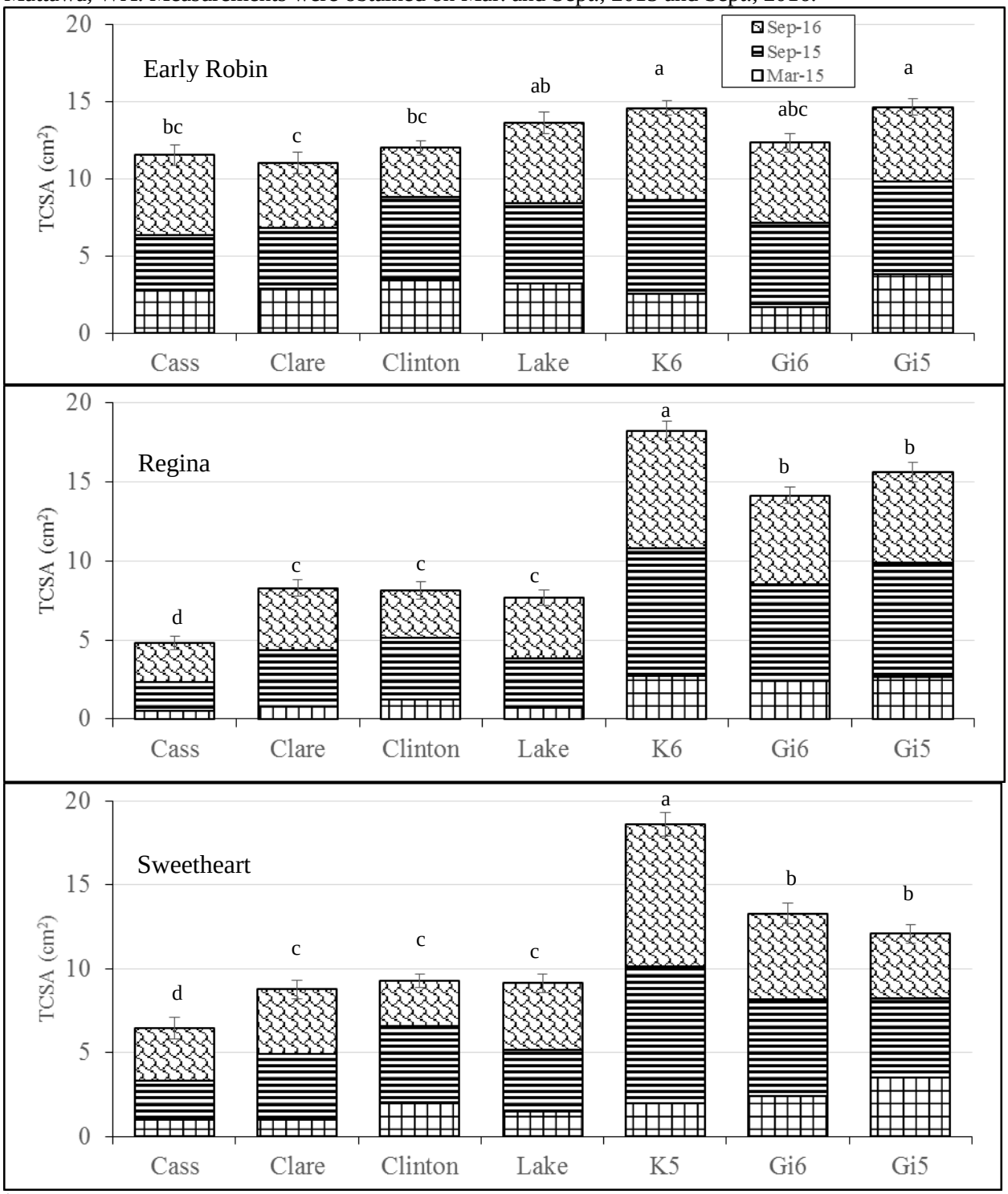
**Fig. 3.** Trunk cross-sectional area[1] (TCSA; $cm^2$) of 'Early Robin', 'Regina', and 'Sweetheart' trees grafted on 4 MSU rootstocks, Krymsk 5, Krymsk 6, Gi6, and Gi5 for trees planted in 2015 in East Wenatchee, WA. Measurements were obtained on April and Sept., 2015 and in Sept., 2016.




[1]The lower boxes represent TCSA at planting and the two upper boxes represent growth in 2015 and 2016. Bars represent standard error of the means for September TCSA.

[2]Means that are significantly different for September 2016 TCSA ($P < 0.05$) are denoted by different letters.

**Fig. 4.** Trunk cross-sectional area[1] (TCSA; $cm^2$) of 'Early Robin', 'Regina', and 'Sweetheart' trees grafted on 4 MSU rootstocks, Krymsk 5, Krymsk 6, Gi6, and Gi5 for trees planted in 2015 in Mattawa, WA. Measurements were obtained on Mar. and Sept., 2015 and Sept., 2016.




[1]The lower boxes represent TCSA at planting and the two upper boxes represent growth in 2015 and 2016. Bars represent standard error of the means for September TCSA.
[2]Means that are significantly different for September 2016 TCSA ($P < 0.05$) are denoted by different letters.

**C.** 2016 planting of three small replicated rootstock trials alongside the 2015 trials to evaluate the 5th MSU cherry rootstock (Crawford).

'Regina' and 'Sweetheart' trees grafted onto the MSU cherry rootstocks Crawford and Clinton, plus the control Gi5, along with 'Regina' grafted on Cass, will be dug at ProTree Nursery in fall 2016 for shipping spring 2017. DNA tests of rootstock liners sampled determined that the liners were labeled correctly (Obj. 3). It was initially anticipated that the trees would be budded in 2015 and planted in the test orchards in 2016. However, a delay in liner production pushed back the planting until spring 2017. The delay allowed the addition of 'Regina'/Cass, which is under-represented in the 2017 plantings. Two of the plots will be planted next to the 2015 plantings [The Dalles (hosted by Tim Dahle) and East Wenatchee (hosted by McDougall & Sons)] while the Mattawa plot will be hosted by Zirkle Fruit Co. due to lack of space next to the current Mattawa plot.

**2.** Collaborate with commercial nurseries in liner and finished tree production to determine the nursery performance of the MSU cherry rootstocks.

*Distribution of rootstock budwood for pilot propagation trials and limited liner production*: Nine commercial nurseries have all five MSU cherry rootstocks that originated from virus certified materials from the CPCNW-FT. These nurseries are gaining experience propagating these rootstocks. To date, liner production appears to be most efficient using tissue culture, while techniques to propagate from softwood cuttings are in development as an alternative to tissue culture. Since the rootstock materials established at the nurseries originated from virus certified and genetically verified plant material, liners from these plant materials could be commercialized if a decision is made to release one or more of the MSU cherry rootstocks.

*Finished tree nursery performance*: Liners of four of the MSU rootstocks (Cass, Clare, Clinton and Lake) were planted at three Washington nurseries in spring 2013. Because of the late delivery/planting time, only liners at one nursery were of sufficient size to bud in fall 2013. The other two nurseries budded in spring 2014. The scions used were those for the 2015 plantings: 'Regina', 'Early Robin' and 'Sweetheart'. Once the liners were established, they all had acceptable nursery characteristics, including sufficient apical dominance and minimal branching. For those liners that were of sufficient size to bud, the bud take was over 90% for all four MSU rootstocks across all three scion cultivars.

In addition, 600 liners of four of the MSU rootstocks (150 each - Cass, Clare, Clinton and Lake) were planted at one Washington nursery in spring 2014 to provide additional information on the performance of these rootstocks in a finished tree nursery. Budding with 'Skeena' was done in spring 2015. As in prior experience, the bud take was excellent. The bud take percentages for the four rootstocks were as follows: Lake 95%, Clare 93%, Clinton 96% and Cass 96%. In 2016, liners of all 5 MSU rootstocks, including Crawford, were planted at ProTree Nursery at their Davis, Calif. location for spring budding. As with the other nursery plot observations, no barriers to nursery performance were identified for any of the five MSU rootstocks.

**3.** Collaborate with the CPCNW-FT and cooperating nurseries to insure MSU cherry rootstocks are available as certified virus tested and genetically verified.

*Virus certification*: The virus certification of Crawford was completed at the CNCNW-FT and in 2015 budwood was sent to nursery collaborators who had not previously received this selection. All five MSU rootstocks are virus certified and plants are being maintained at the CPCNW-FT.

*Genetic verification*: The goal of this sub-objective is to assure that the genetic identities of the five MSU rootstocks are correct at key points in propagation and distribution. A DNA test, involving two

DNA markers, has been developed that distinguishes among all five MSU rootstocks along with Gi3, Gi5 and Gi6. This DNA test that was developed at MSU was verified by four other laboratories including two commercial service providers.

The MSU rootstocks that were used to make the trees for the 2015 and 2017 plantings were subjected to DNA testing by the MSU lab to confirm rootstock identify. These DNA diagnostic tests have confirmed that the MSU cherry rootstocks are labeled correctly. All the participating nurseries have been informed that MSU will perform DNA diagnostics on the MSU rootstocks at no cost to the nursery if there are any identity concerns. The goal of this strategy is to avoid any delays and financial losses at the nurseries that would be associated with a plant material mix-up.

## EXECUTIVE SUMMARY

**Project Title**: Establishment and testing of MSU sweet cherry rootstocks

Five MSU sweet cherry rootstocks were identified that induce precocious abundant flowering and significantly reduce tree size compared to Gi6. This result was based on a trial at WSU-Prosser with 'Bing' scion planted in 2009 and removed after the 2014 season. All five MSU rootstocks named after Michigan counties (Cass, Clare, Clinton, Crawford and Lake) produced trees of similar size to Gi5 or smaller. In 2014, 'Bing' fruit maturity date differed among the rootstocks tested at the Prosser plot with Cass, Clare and Lake ripening ahead of Clinton, Crawford, Gi5 and Gi6. Also in 2014, all five of the MSU candidate rootstocks had yield efficiencies (kg fruit/$cm^2$) that were higher than that of Gi5 and Gi6. However, the fruit size for Crawford was significantly less than that for Gi5 due to the high crop load on Crawford compared to Gi5 and insufficient thinning of Crawford. These results suggest that producing large fruit is possible on the MSU rootstocks given the proper training system and crop load adjustments.

Despite the potential of the MSU dwarfing cherry rootstocks to contribute to profitability due to precocious fruiting, and a reduced cost of harvest labor, critical performance-related questions have not yet been answered. These include performance with scions with different cropping potential, and suitability with different training systems, soils and growing conditions. All the fruit data for the MSU rootstocks from the Pacific Northwest is from one plot at WSU-Prosser with 'Bing' scion trained to a multiple leader architecture. Therefore plantings were established in 2015 and will be established in 2017 (see new proposal) to include a wider range of scions and management systems.

The 2015 plantings were established at three locations (The Dalles, Mattawa, and East Wenatchee), with four MSU rootstocks (Cass, Clare, Clinton, and Lake) plus Gisela and Krymsk controls, and three scion cultivars ('Regina', 'Early Robin' and 'Sweetheart'). In general, across all three scions and 2015 planting locations, the MSU rootstocks Cass and Clare resulted in the smallest trees followed by Lake and then Clinton. The first fruit data will be obtained in 2017 (see new proposal). Crawford was not included in the 2015 plantings as it was delayed being released from the CPCNW-FT and then there was a delay in liner production. Three smaller plots comparing Crawford in comparison with the most similar rootstocks, Clinton and Gi5, are on track to be planted in spring 2017 along with several other producer-led plantings. Collectively these plantings are designed to provide the information needed for producers to decide whether to plant trees on any of the new MSU cherry rootstocks.

In anticipation of commercializing one or more of the MSU cherry rootstocks, progress was made to enable an ample future supply of virus-certified and genetically verified plant materials. Virus certification of all five MSU rootstocks was completed by the CPCNW-FT. Certified budwood of these five roots was sent to nine commercial nurseries followed by experimental production of liners and finished trees. The five MSU rootstocks performed well in liner and finished tree production at commercial nurseries; therefore, no nursery barriers to commercialization were identified. DNA testing of the MSU rootstocks was done at critical stages in budwood transfer and liner and tree production. To date, DNA testing has verified the trueness-to-type of the stock plants at the nurseries and the trees for the past and future plantings.

**FINAL PROJECT REPORT**

**Project Title:** New programs to increase fruit size and improve harvest quality

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**Cooperators**: Matthew Whiting

**Total Project Request:** **Year 1:** $58,388 **Year 2:** $59,585

**Other funding sources**

None

**Total Project Funding**:

**Budget History:**

| **Item** | **Year 1:** | **Year 2:** | |
|---|---|---|---|
| **Salaries** | 30544 | 31460 | |
| **Benefits** | 19276 | 19557 | |
| **Wages** | 5850 | 5850 | |
| **Benefits** | 585 | 585 | |
| **Equipment** | | | |
| **Supplies** | 1000 | 1000 | |
| **Travel** | 1133 | 850 | |
| **Plot Fees** | | | |
| **Miscellaneous** | | | |
| **Total** | 58388 | 59585 | |

**Objectives:**

1) Large-scale pre-bloom PGR trials to enhance fruit size
2) Accurately reproduce the color and percent of full size cherry for a decision aid tool
3) Create a novel temperature-dependent model to predict phenology and fruit development of sweet cherry

**Significant Findings:**

1) We do not recommend use of giberellin or cytokinin based on our data
2) Anecdotal evidence for increased fruit size with 20ppm CPPU at full bloom exists
3) A 30-spur sampling method provided a good estimate of orchard condition
4) Pistil growth during dormancy break was quantified with Differential Thermal Analysis
5) Temperature controlled dormancy break experiments showed changing temperature responses as dormancy breaks
6) Relative water content (60-62%) of floral buds can be used as a field ready test for the break of dormancy
7) 50% maximum fruit weight was coincident with an increase in fruit density and darkening of the cherry
8) Two new phenology input values describing dormancy break and color development were determined for six varieties at locations throughout the Columbia Gorge

**Objective 1- PGRs: Sampling Methods**

One grower hosted non-crop destruct test trials in 2015. Fruit size, set and yield at harvest were measured for cultivars Chelan, Early Robin, Bing and Rainier treated with 250 ppm Promalin prior to bloom, between first white and full bloom. Test and control blocks comprised at least ten contiguous rows each (i.e., ~ 1 tank of Promalin per cultivar). Ten subsamples per cultivar were collected, each comprising the total fruit from 30 spurs. We have previously demonstrated that 30 spurs were an adequate sample size to estimate orchard variability and reduce experimental error so that treatment differences can be detected. At each sampling date, fruit were weighed in the field then photographed for later counting. The data provided good estimates of average fruit weight, set and yield per spur. Statistical analysis revealed no significant differences in weight, set or yield in Promalin versus control treatments, although a slight trend of lower set and larger size was observed. Inconsistent responses between this year and last year (i.e., a significant increase in 'Sweetheart' fruit size) may be attributed to the interaction of environmental factors and phenology stage at the time of application. Irrespective, the small effect on fruit size does not, at present, validate commercial applications

**Multiple Range Tests for fruit weight by Cultivar_ Treatment**

| PGR: | | 95.0 percent LSD | |
|---|---|---|---|
| *Level* | *Replicates* | *Mean* | *Groups* |
| Early Robin control | 10 | 9.57645 | a |
| Early Robin Promalin | 10 | 9.84101 | a |
| Bing Promalin | 10 | 9.84183 | a |
| Bing control | 10 | 9.97351 | a |
| Chelan control | 10 | 10.496 | b |
| Chelan Promalin | 10 | 10.5552 | b |
| Rainier control | 10 | 11.1059 | c |
| Rainier Promalin | 10 | 11.4334 | c |

A fourth trial was conducted at MCAREC with Regina and ten individual trees per treatment. In addition to a trial of 125 ppm Promalin, trials of NovaGib at 31, 62 and 125 ppm, and one trial of 250 ppm K-Salt were applied at an average bud phenology of first-white that was determined by counting bud phenology per spur. Spurs and the remaining fruit on the limb were collected and the fruit weighed individually for the best estimate of size distribution. All treatments showed a slight increase of fruit weight when spurs were sampled, and slightly more when all fruit including terminal fruit were sampled. Any gains were small and may be attributed to an influence on set and size. An additional objective to increase leaf area, based on visual observation of markedly greater leaf size from previous PGR trials, was also evaluated. No significant differences, however, were observed for any of the treatments relative to leaf area (data not shown).

Multiple Range Tests for weight by spur

Method: 95.0 percent LSD

| spurs | Count | Mean | Groups |
|---|---|---|---|
| Control | 559 | 9.27 | a |
| NAA, 250 ppm | 226 | 9.56 | b |
| Novagib, 125 ppm | 373 | 9.57 | b |
| Novagib, 31 ppm | 321 | 9.69 | b |
| Promalin, 125 ppm | 456 | 9.75 | b |
| CPPU/Novagib, 20/125 ppm | 250 | 9.78 | b |
| Novagib, 62 ppm | 404 | 9.82 | b |

Multiple Range Tests for weight by limb

Method: 95.0 percent LSD

| limbs and spurs | Count | Mean | Groups |
|---|---|---|---|
| Control | 1280 | 9.36 | a |
| Novagib, 125 ppm | 1045 | 9.49 | ab |
| Promalin, 125 ppm | 1257 | 9.60 | bc |
| NAA, 250 ppm | 591 | 9.64 | bce |
| Novagib, 62 ppm | 1066 | 9.73 | ced |
| Novagib, 31 ppm | 944 | 9.76 | ed |
| CPPU/Novagib, 20/125 ppm | 630 | 9.92 | d |

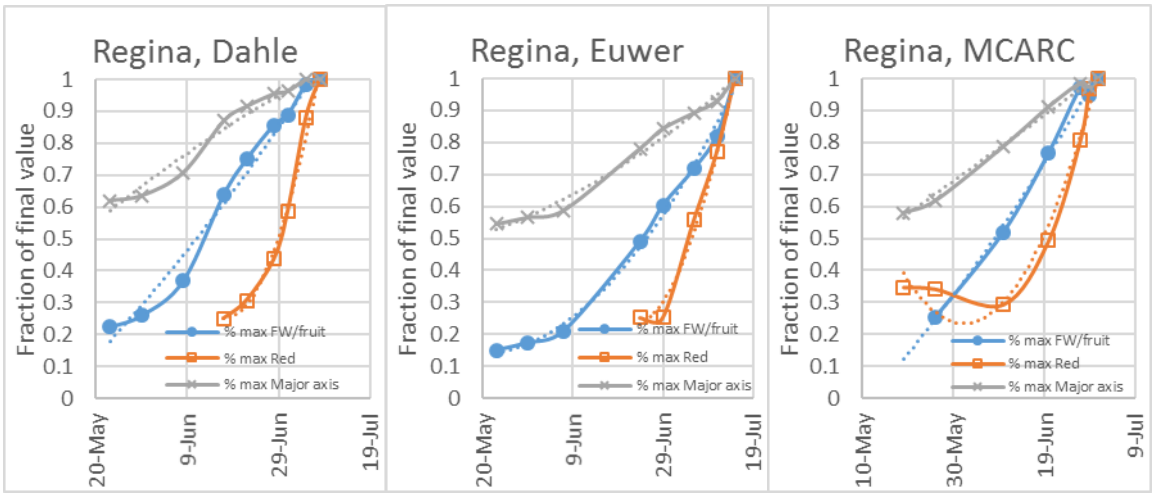
**PGR 2016**

Large scale trials were again employed in a Bing orchard in The Dalles. One application at three timings around bloom were done. Fruit from whole limbs were taken for size and fruit quality measurements. We found no significant effects of giberrellin (NovaGib, FAL-477) or the synthetic cytokinin, CPPU.

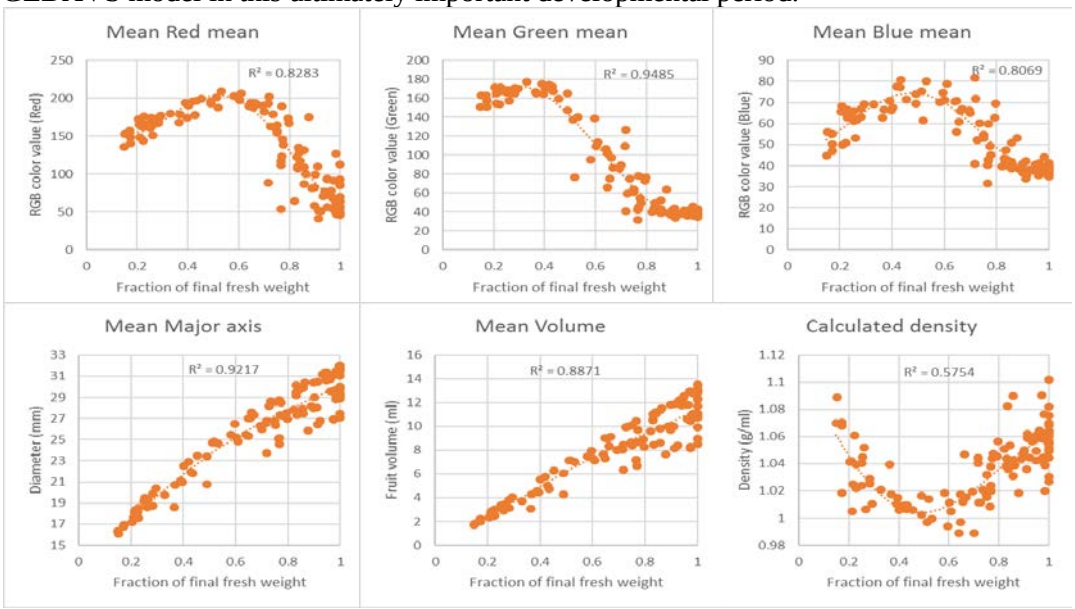
| Treatment | avg Fruit Set | Avg Weight (g) | Total Weight (g) | Avg TA | Avg SS | Avg CTIFL | avg ff | avg mm | avg Row size | Avg leaf Area | avg # leaves | Avg area per leaf |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| UTC | 63 | 9.5 | 11496.5 | 0.81 | 20.42 | 3.92 | 284 | 27.6 | 9.93 | 4541 | 151 | 30.0 |
| NovaGib 10L | 67 | 8.93 | 10623.0 | 0.68 | 21 | 4.14 | 264 | 27.0 | 10.1 | 4529 | 155 | 29.2 |
| NovaGib 10L | 63 | 8.83 | 10551.9 | 0.81 | 21.38 | 3.72 | 290 | 26.7 | 10.2 | 4762 | 159 | 30.0 |
| NovaGib 10L | 59 | 9.14 | 7205.08 | . | 23.18 | 4.23 | 293 | 27.1 | 10.1 | 4772 | 151 | 31.4 |
| NovaGib 10L | 79 | 9.47 | 9129.06 | 0.82 | 22.78 | 3.84 | 286 | 27.6 | 9.94 | 4685 | 156 | 29.8 |
| NovaGib 10L | 73 | 9.49 | 9317.31 | . | 21.3 | 3.84 | 293 | 27.7 | 9.9 | 4821 | 151 | 31.8 |
| FAL-477 | 68 | 9.38 | 11262.2 | . | 21.15 | 3.94 | 277 | 27.5 | 9.97 | 4871 | 152 | 32.0 |
| FAL-477 | 59 | 9.21 | 10886.9 | 0.81 | 21.47 | 3.97 | 284 | 27.3 | 10.0 | 4707 | 154 | 30.5 |
| FAL-477 | 64 | 8.91 | 8876.02 | 0.89 | 20.08 | 3.9 | 287 | 27.1 | 10.1 | 4272 | 138 | 31.0 |
| FAL-477 | 68 | 9.43 | 11268.4 | 0.82 | 21.52 | 4.12 | 284 | 27.4 | 9.98 | 4872 | 151 | 32.1 |
| CPPU | 66 | 9.09 | 10711.3 | 0.84 | 22.64 | 3.85 | 295 | 27.3 | 10.0 | 4996 | 149 | 33.4 |
| NovaGib 10L | 65 | 8.46 | 9955.48 | 0.84 | 22.08 | 3.93 | 288 | 26.6 | 10.2 | 5049 | 157 | 32.2 |
| CPPU | 55 | 9.23 | 10998.9 | . | 22.2 | 3.92 | 299 | 27.4 | 9.98 | 4728 | 157 | 30.1 |
| FAL-477 | 72 | 9.22 | 10853.9 | 0.96 | 22.05 | 3.82 | 296 | 27.5 | 9.98 | 4708 | 156 | 30.1 |
| CPPU | 74 | 9.3 | 10931.6 | . | 22.92 | 3.87 | 298 | 27.5 | 9.93 | 4621 | 152 | 30.2 |
| CPPU | 68 | 9.07 | 10501.2 | 0.8 | 22.18 | 4.13 | 295 | 27.2 | 10.0 | 4934 | 155 | 31.9 |
| CPPU | 76 | 8.9 | 10654.3 | 0.84 | 21.73 | 3.73 | 306 | 27.0 | 10.1 | 4212 | 145 | 29.0 |
| CPPU | 73 | 8.61 | 12077.7 | . | 23.69 | 4.07 | 348 | 26.6 | 10.3 | 4580 | 156 | 29.3 |
| CPPU | 76 | 9.18 | 10876.5 | 0.9 | 22.55 | 4.02 | 305 | 27.0 | 10.1 | 4701 | 139 | 33.6 |

**Objective 2- Develop appropriate sweet cherry color chips**

The first year of a color index of skin color and fruit size of cherry in the PNW focused on Sweetheart, Bing and Regina. This year we observed Bing, Chelan, Lapins, Regina, Skeena and Sweetheart in 15 of the combinations of cultivar/station that were observed for the RWC-dormancy test. Total fresh weight of the fruit from each sampling were measured, but individuals were measured photographically. Image analysis software was used for maximum and minimum diameters, and RGB color (totalling over 25,000 fruit). Data were analyzed as the fraction of the greatest measurement (generally the final date of sampling). An example for Regina at five locations is shown.




All cultivars were similar in color progression and final color; however, they varied in the duration of time required for growth and color development, largely dependent on location. Interestingly, we found a strong correlation of the progession of color in relation to a relative measure of fresh weight per fruit. Furthermore, at about 50% maximum fruit weight we observed the beginning of the increase in fruit density during final swell. From these curves the dates of 50% maximum fresh weight provides an important phenological input that can be used for all dark sweet cherries to improve the GEDAVS model in this ultimately important developmental period.



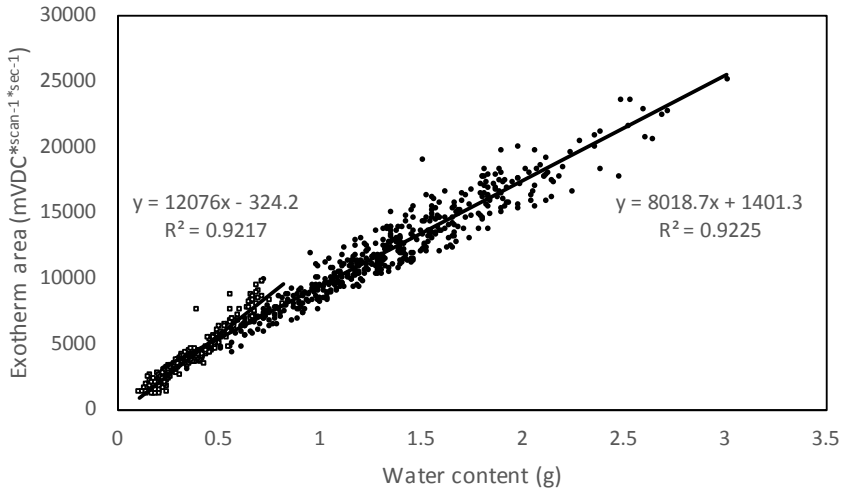
A color chart based on these findings will be presented in PowerPoint at the meeting.

| Fraction of final weight | Fraction of major diameter | Color chip | RGB Red | RGB Green | RGB Blue |
|---|---|---|---|---|---|
| .2 | .57 | | 157 | 163 | 57 |
| .3 | .65 | | 178 | 173 | 66 |
| .4 | .72 | | 190 | 141 | 73 |
| .5 | .78 | | 191 | 141 | 74 |
| .6 | .83 | | 184 | 111 | 70 |
| .7 | .88 | | 166 | 80 | 60 |
| .8 | .92 | | 139 | 54 | 49 |
| .9 | .96 | | 102 | 38 | 39 |
| 1 | 1 | | 55 | 40 | 39 |

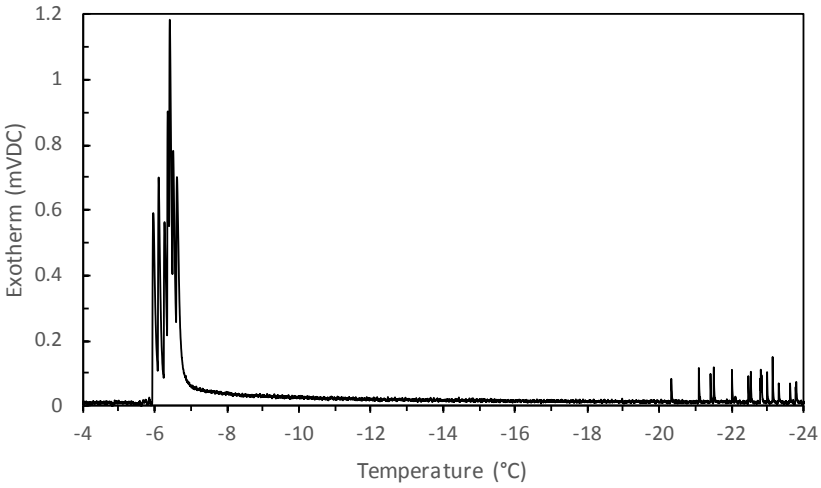
## Objective 3: Modelling of Sweet Cherry Development

### Controlled Environment Chamber: Dormancy Break Forcing

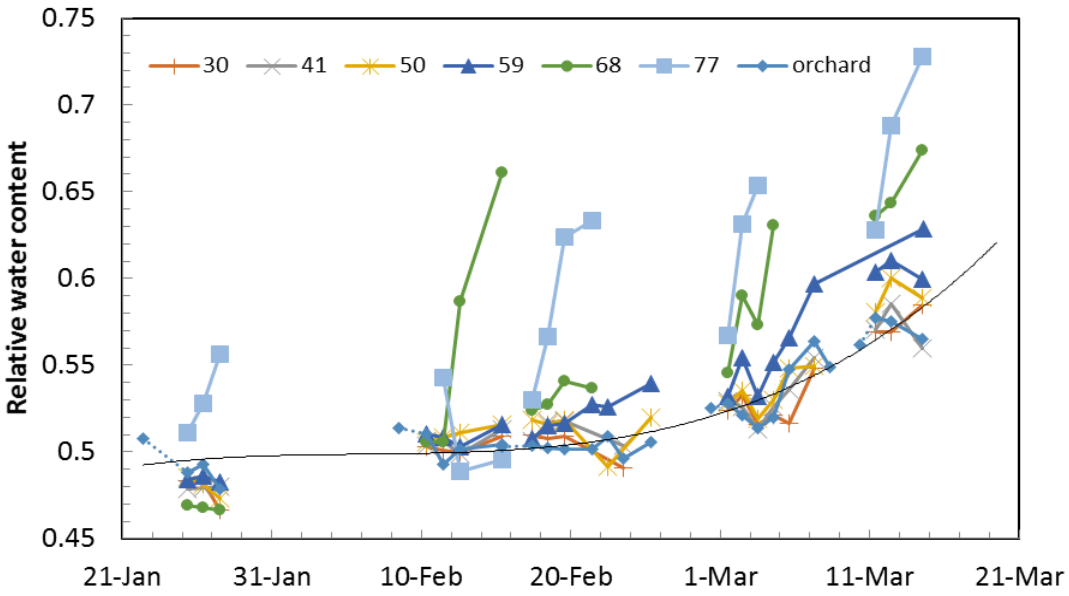
Of the three cardinal points of cherry development, dormancy break, bloom and maturation, dormancy break is the most difficult to determine. Loss of dormancy is deemed complete when the pistil loses the ability to supercool. This irreversible physiological process is assumed to be accompanied by growth of the pistil; however, evidence for the timing of dormancy, supercooling and growth are scant. Visible changes in bud size and color are too subtle to be reliable, and dissection of pistils for photographic measurement is far too tedious. We realized through our work with Differential Thermal Analysis (DTA) that pistil growth could be determined by measuring the height of the DTA response because of the direct and linear relation of water content and DTA response.



An Excel program was written to digitally remove noise and baseline drift and automate peak detection so that hundreds of pistils could be measured in a single analysis.

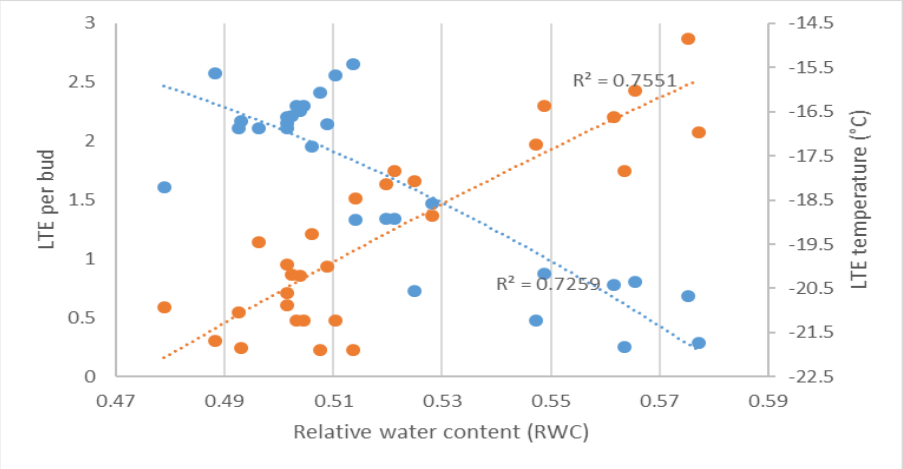



Similar to our bloom-forcing experiments of last year, we again used 1.5 ft-sections of fruiting wood placed in controlled environment chambers. Spur buds were analyzed by DTA and some dissection and measure of pistils was done to confirm the DTA results. Five separate experiments were set up as the season progressed from dormancy to bud swell. Each experiment was compared to the natural progression in the orchard. Forcing this material to develop at six temperatures in comparison to the orchard helped establish a developmental response curve needed in our improvement of the temperature dependent GEDAVS model.

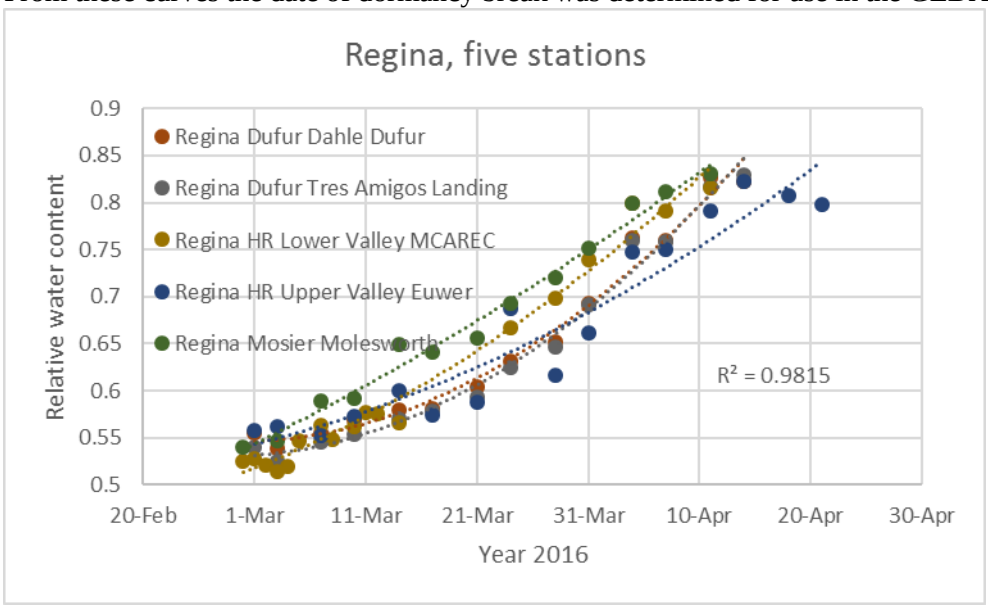


**Relative Water Content (RWC) of Floral Buds: A Field Ready Test for Dormancy**

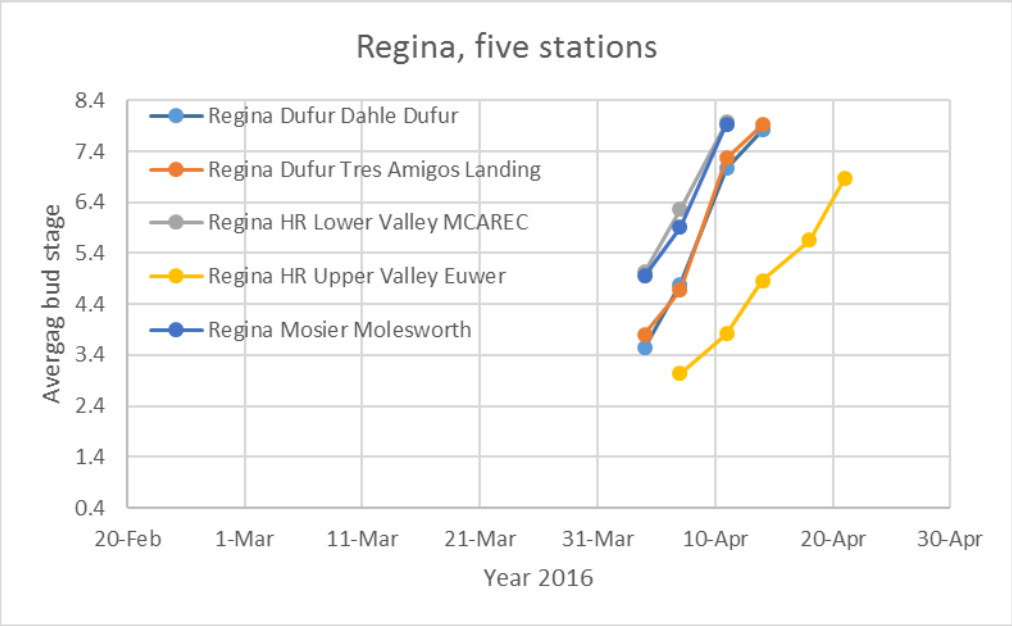
As useful as DTA was for determining dormancy break, it is still limited by the lengthy freezer-process run time and post-analysis. A simple measure that can be correlated to the loss of dormancy was needed so that the dormancy status of many orchards could be determined. RWC of floral buds was found to be such a measure. As the RWC increased from January to March in Regina at MCAREC, the LTE50 temperatures increased and the number of detectable peaks per bud decreased until approximately 60% RWC was reached and all DTA signal was lost. We concluded that 60-62% RWC is a good indicator of the irreversible loss in dormancy.




With this in mind we sampled orchards using one replicate of the 30-spur sampling method (described in Objective 1) of RWC as a stand-in measurement for the loss of dormancy. We visited 14 orhards ranging from 500 to 2000ft in elevation and in each region of the Columbia Gorge. Six cultivars in 21 combinations of cultivar/station were observed. Approximately twice weekly, buds from 30 spurs were photographed for individual bud size and color. The buds were then weighed in bulk for fresh weight and later for dried weight. An example for Regina at five locations is shown. From these curves the date of dormancy break was determined for use in the GEDAVS model.



**Open Bud Phenology and Bloom Timing:**
The 30-spur sampling was continued in these orchards to assess bloom timing. Once buds opened individual flowers were graded for phenology. Scores were given for each stage, 4 for tight cluster , 5-open cluster, 6-first white, 7-balloon and 8-bloom. Weighted scores were plotted versus date such that a weighted score of 7.5 was 50% bloom. An example for Regina at five locations is shown. From these curves the date of 50% bloom was determined for use in the GEDAVS model.



**GEDAVS: Gibeaut, Einhorn, Diurnal, Annual, Variation, Simulation (GEDAVS)**
The algorithm for a new growing degree model of sweet cherry growth is given below. Calculations are performed in Excel spreadsheets. Improvements to this model are underway and will include additional phenology date inputs and temperature response curves for dormancy break and maturation. Dates for dormancy break provide an end date for accumulating heat units required for the release of ecodormancy. A temperature response curve for this period of development was defined by the forcing experiments described above. Maturation in the current model was based upon growth in volume; however, with the new color and fruit density data the temperature response in the final days of ripening will be modified to better describe ripening.

PHENOLOGY DATE INPUTS
Start = starting date of simulation (can be set to 1-January)
Origin = date of 50%-anthesis, germination
Exponential = end date of increasing temperature indices
End = date of maturation, %growth

TEMPERATURE INPUTS

| | |
|---|---|
| Base temperature; | base; start, origin, exponential, end |
| Optimum temperature; | opt; start, origin, exponential, end |
| Critical temperature; | crit; start, origin, exponential, end |
| Negative temperature; | neg; start, origin, exponential, end |

DIURNAL SUNRISE-SUNSET
Naval observatory data location specific Sunrise Sunset tables (account for Day-light savings time and leap day)
Solar radiation time offset = typical time to positive net PAR (set to 3 hr)
Dawn = Sunrise + solar radiation time offset
Dusk = Sunset

ASSIGN DFA TO TIME STAMP
Create year specific date series in 1 day steps from 1-January, and variable DFA series
Lookup (time stamp, date step series, DFA series)
DFA = date step series – P1
Dusk = Sunset
Day-time > Dawn <= Dusk
Night-time > Dusk <= Dawn

GROWING DEGREE GD

Interval average temperature; int = $(\text{temperature}_1 + \text{temperature}_2) / 2$

PRE ANTHESIS
(P0 to P1)
IF, int < base, GD = (int – base) / (base – neg)
Else IF, int <= opt, GD = $((\text{opt} - \text{base}) / 2) \cdot (1 + \text{cosine}\ (\pi + \pi \cdot ((\text{int} - \text{base}) / (\text{opt} - \text{base}))$
Else IF, int <= crit,GD = $(\text{opt} - \text{base}) \cdot (1 + (\text{cosine}\ \pi) / 2) + \pi / 2 \cdot ((\text{int} - \text{opt}) / (\text{crit} - \text{opt}))$
Else IF,int > crit,GD = 0

DAY-TIME
(P1 to P3)

IF, int <base,  GD = 0
Else IF, int <= opt, GD = ((opt – base) / 2) · (1 + cosine (π + π · ((int – base) / (opt – base)))
Else IF, int <= crit, GD = (opt – base) · (1 + (cosine π) / 2) + π / 2 · ((int – opt) / (crit – opt)))
Else IF, int > crit, GD = 0

NIGHT-TIME
(P1 to P3)
(Exponential, Maturation)
IF, int <base, GD = 0
Else IF, int <= opt, GD = ((opt – base) / 2) · (1 + cosine (π + π · ((int – base) / (opt – base)))
Else IF, int <= crit, GD = (opt – base) · (1 + (cosine π) / 2) + π / 2 · ((int – opt) / (crit – opt)))
Else IF, int > crit, GD = (int - crit) / (crit – neg)

VARIABLE TEMPERATURE INDICIES
Create four columns of temperature (base, opt, crit and neg) for each growth phase (P0 to P1, P1 to P2, > P2)

IF DFA < P1, Trend (P0temp: P1temp, DFA)
P0temp = P0 (base, opt, crit)

IF DFA <= P2, Trend (P1temp: P2temp, DFA)
P1temp = P1 (base, opt, crit)

IF DFA <= P3, Trend (P1temp: P3temp, DFA)
P2temp = P3 (base, opt, crit)

IF DFA > crit

GROWING DEGREE HOURS
$GDH = GD \cdot (\text{time stamp}_2 - \text{time stamp}_1) \cdot 24$

GROWING DEGREE HOUR ACCUMULATED

IF P0 >= time stamp < P1
Sum PRE ANTHESIS

Else IF time stamp < P2
Sum DAY-TIME EXPONENTIAL
Sum NIGHT-TIME EXPONENTIAL

Else IF time stamp <= P3
Sum DAY-TIME MATURATION
Sum NIGHT-TIME MATURATION

GEDAVS Seasonal Growing Degree Hours Accumulated = PRE ANTHESIS + DAYTIME EXPONENTIAL + NIGHTTIME EXPONENTIAL + DAYTIME MATURATION + NIGHTTIME MATURATION

**Executive Summary**

Three years of trials in the use of PGRs near bloom has given mixed results. Any gains in size we observed may be related to the variability of fruit set on a given tree or limb. During the course of these trials we performed two sampling methods, whole limb sampling versus 30-spurs chosen at random. Size estimates were similar for both methods. Replication of the 30-spur samples up to 10 replicates reduced the variation of the means but can only differentiate a size differential of about 0.5 gram. The 30-spur technique has the advantage over whole limb sampling because of a broader sample pool for estimating orchard conditions. If a single replicate 30-spur method is employed over a time course of sampling such as twice weekly, very good estimates of developmental progression can be obtained. We encourage the adoption of the 30-spur method.

Dormancy break can be determined by a change in relative water content of buds from about 50-55% in dormancy to 60-62% at first swell. Differential thermal analysis of floral buds forced to break dormancy in controlled environments established this value and the changing developmental response to temperature during springtime bud development. The dates of this 60-62% relative fresh weight value will now be incorporated in the GEDAVS model of fruit development.

During our efforts to develop a robust, predictive fruit growth model we digitally imaged thousands of individual dark sweet cherries of six cultivars at 14 different locations to objectively identify their stage of maturation, according to skin color. This work resulted in 3 key findings: 1) The ctifl color chart ***does not*** adequately represent the progression of cherry skin color; and, 2) we can significantly advance the precision with which the industry assesses color development by producing a color wheel that optimizes harvest timing and fruit quality of cultivars commercially produced in the PNW. Additionally, we found the onset of the development of red color intensity was related—across cultivars and locations—to fruit development at 50% of the final fruit weight. The dates of this 50% fresh weight/fruit value will now be incorporated in the GEDAVS model of fruit development.

The GEDAVS model combines accurate phenology estimates with a new method of calculating growing degree accumulation that accounts for daily and annual variations and location. We have shown previously that temperature dependent difference on the predicted average harvest date of Sweetheart over several years and locations was about to +/- 1.5 days. Addition to the model of the dates for the attainment of 60% RWC in buds, and 50% final fruit weight will now be added to GEDAVS to more accurately predict the beginning and end of the sweet cherry season. Geographic latitude is accounted for in GDAVS by solar time tables; however, this effect has not been tested over a wide range of latitude. Further work in Washington will be needed to fully validate GDAVS.

**FINAL PROJECT REPORT** **YEAR**: 3 of 3 years

**Project Title**: Improving shipping quality of cherry by pre-harvest Ca and NaCl sprays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**Total Project Funding:** **Year 1:** $38,620 **Year 2:** $39,551 **Year 3:** $40,505

**Budget 1: Yan Wang**
**Organization Name:** OSU-MCAREC **Contract Administrator:** Russ Karow
**Telephone:** 541-737-4066 **Email address:** Russell.Karow@oregonstate.edu

| Item | 2014 | 2015 | 2016 |
|---|---|---|---|
| **Salaries** | 15,104[1] | 15,557[7] | 16,024[7] |
| **Benefits** | 2,688[2] | 2,769[7] | 2,852[7] |
| **Wages** | 6,810[3] | 7,014[7] | 7,224[7] |
| **Benefits** | 1,566[4] | 1,613[7] | 1,661[7] |
| **Equipment** | | | |
| **Supplies** | 8,000[5] | 8,000 | 8,000 |
| **Travel** | 500[6] | 500 | 500 |
| **Miscellaneous** | | | |
| **Total** | 34,668 | 35,453 | 36,261 |

**Footnotes:**
[1]Postdoctoral Research Associate: 800hr at $18.88/hr.
[2]OPE: $3.36/hr.
[3]Wages: 500hr for a Biological Science Tech. at $13.62/hr.
[4]OPE: 23% of the wage.
[5]Supplies: fruit, Ca analysis, gases (helium, nitrogen, hydrogen, standard gases), gas tank rental, chemicals, and MCAREC cold room and land use fees.
[6]Travel to grower's fields
[7]3% increase

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

| Item | 2014 | 2015 | 2016 |
|---|---|---|---|
| **Salaries** | | | |
| **Benefits** | | | |
| **Wages** | 3,510 | 3,645 | 3,780 |
| **Benefits** | 292 | 303 | 314 |
| **Equipment** | | | |
| **Supplies** | 150 | 150 | 150 |
| **Travel** | | | |
| **Miscellaneous** | | | |
| **Total** | 3,952 | 4,098 | 4,244 |

**Footnotes:**
[1]Wages: 270 hours $13/hour temporary labor for 2014, $13.50 for 2015, $14 for 2016
[2]OPE: 8.31% of the wage.

## OBJECTIVES

1. Study the effect of preharvest calcium (Ca) and salt (NaCl) treatments on shipping quality of PNW cherry cultivars.
2. Determine the response of fruit growth, fruit size, yield, and return bloom to Ca/NaCl sprays.
3. Develop a commercial Ca spray protocol to improve shipping quality of PNW cherry cultivars.

## SIGNIFICANT FINDINGS

### Fruit tissue Ca/N concentrations and shipping quality

1. Shipping quality of cherries ('Lapins', 'Sweetheart' and 'Skeena') sampled from different orchards was found to be correlated with fruit tissue Ca content (400-900ppm, dw), but not N content (0.9-1.1%).

### Ca spray

2. The optimum Ca spray rate for increasing fruit tissue Ca content was determined to be 0.1-0.15% $Ca^{2+}$. Higher Ca concentrations (i.e., 0.2%) might cause leaf burning or reduce fruit size. Lower $Ca^{2+}$ concentrations (i.e., 0.05% for 6 or 9 times) didn't increase fruit tissue Ca content at harvest.
3. The optimum Ca spray timing and frequency were 6 times at weekly interval from pit-hardening to harvest. Ca spray prior to pit-hardening was not found to increase fruit tissue Ca content at harvest.
4. Sprayed at 0.1-0.15% $Ca^{2+}$ for 6 times, all the Ca sources [$CaCl_2$, $Ca(NO_3)_2$, Ca citrate, $Ca(OH)_2$+organic acid (OA), amino acid (AA) chelated Ca, Ca carbonate, and Ca carbonate silicon] increased fruit tissue Ca content with little difference among the Ca sources.
5. Ca spray at 0.1-0.15% $Ca^{2+}$ for 6 times on leaves only didn't alter fruit tissue Ca content.
6. Sprayed at 0.1-0.15% $Ca^{2+}$ for 6 times, $CaCl_2$ and Ca citrate but not other Ca sources might reduce fruit growth rate and fruit size.
7. Ca spray at 0.1-0.15% $Ca^{2+}$ for 6 times tended to increase fruit SSC and TA slightly without affecting maturation (coloration) at harvest.
8. Ca spray at 0.1-0.15% $Ca^{2+}$ for 6 times increased fruit firmness at harvest, reduced pitting, splitting, stem browning, fruit internal browning and decay after storage/shipping.
9. For improving cherry shipping quality (not for fruit tissue Ca content), OA or AA chelated Ca formulations tended to perform better than other Ca sources; $Ca(NO_3)_2$ is better than $CaCl_2$.
10. None of the Ca treatments affected return bloom (buds per spur or flowers per bud) or fruit set of 2 and 3-year-old spur populations.

### NaCl spray

11. NaCl spray at 30, 60, and 120 ppm for 6 times at weekly interval from pit-hardening to harvest increased fruit tissue $Na^+$ content with a dose response, but didn't affect $Cl^-$.
12. NaCl spray at 60 and 120 ppm increased FF, SSC, and TA in 'Lapins' but not in 'Regina' at harvest and during storage.
13. NaCl spray at 120ppm but not 60ppm reduced fruit size in both 'Lapins' and 'Regina'. NaCl spray enhanced fruit color by increasing anthocyanin accumulation in both cultivars.
14. NaCl spray at 30, 60, and 120 ppm improved stem quality by enhancing cuticle and wax development on stem.
15. There was no synergistic effect between Ca and NaCl pre-harvest treatments on cherry quality and shipping quality.
16. NaCl spray may not be justified as a commercial application practice in cherry production.

**METHODS**

***1. The relationship of fruit tissue Ca and N contents with shipping quality.*** Fruit of different cultivars was randomly sampled from different orchards. Ca and N contents were determined and fruit quality were evaluated at harvest and after 3 and 5 weeks of storage.

***2. Ca and salt treatments.***

In 2014, treatments were designed to focus on optimizing the application rate, timing, and frequency. In 2015, treatments were designed to focus on optimizing application timing and Ca sources. In 2016, treatments were designed to optimize Ca sources and confirm the previous findings.

| ***Ca Treatments (2014)*** | ***% Ca*** | ***Application timing*** |
|---|---|---|
| $CaCl_2$ | 0.07 | 9x, beginning 1wafb |
| $CaCl_2$ | 0.07 | 2x, pit hardening + 1wbh |
| $CaCl_2$ | 0.15 | 2x, pit hardening + 1wbh |
| $CaCl_2$ | 0.07 | 2x, 1 and 2 wbh |
| $CaCl_2$ | 0.15 | 2x, 1 and 2 wbh |
| $CaCl_2$ | 0.05 | 6x, beginning pit hardening |
| $CaCl_2$ | 0.10 | 6x, beginning pit hardening |
| $CaCl_2$ | 0.15 | 6x, beginning pit hardening |
| $CaCl_2$ | 0.20 | 6x, beginning pit hardening |
| $Ca(NO_3)_2$ | 0.10 | 6x, beginning pit hardening |
| $Ca(NO_3)_2$ | 0.15 | 6x, beginning pit hardening |
| Ca citrate (“6% Calcium”) | 0.07 (upper label rate) | 6x, beginning pit hardening |
| Ca(OH)2 + OA (“Cal-8”) | 0.20 (within label rate) | 6x, beginning pit hardening |
| AA chelated Ca (Metalosate®) | 0.05 (upper label rate) | 6x, beginning pit hardening |
| ***Ca Treatments (2015)*** | ***% Ca*** | ***Application timing*** |
| $Ca(NO_3)_2$ | 0.10 | 9x, beginning 1wafb |
| $Ca(NO_3)_2$ | 0.15 | 9x, beginning 1wafb |
| $CaCl_2$ | 0.10 | 6x, beginning pit hardening |
| $CaCl_2$ | 0.15 | 6x, beginning pit hardening |
| $Ca(NO_3)_2$ | 0.10 | 6x, beginning pit hardening |
| $Ca(NO_3)_2$ | 0.15 | 6x, beginning pit hardening |
| Ca citrate (“6% Calcium”) | 0.10 | 6x, beginning pit hardening |
| Ca citrate (“6% Calcium”) | 0.15 | 6x, beginning pit hardening |
| Ca(OH)2 + OA (“Cal-8”) | 0.10 | 6x, beginning pit hardening |
| Ca(OH)2 + OA (“Cal-8”) | 0.15 | 6x, beginning pit hardening |
| AA chelated Ca (Metalosate®) | 0.10 | 6x, beginning pit hardening |
| AA chelated Ca (Metalosate®) | 0.15 | 6x, beginning pit hardening |
| ***Ca Treatments (2016)*** | ***% Ca*** | ***Application timing*** |
| $CaCl_2$ | 0.15 | 6x, beginning pit hardening |
| $Ca(NO_3)_2$ | 0.15 | 6x, beginning pit hardening |
| Ca citrate (“6% Calcium”) | 0.15 | 6x, beginning pit hardening |
| Microcal | 0.15 | 6x, beginning pit hardening |
| Mainstay | 0.15 | 6x, beginning pit hardening |
| $Ca(NO_3)_2$ on leaves only | 0.15 | 6x, beginning pit hardening |
| ***NaCl treatments*** 0 | | 6x, beginning pit hardening |
| 30ppm | | 6x, beginning pit hardening |
| 60ppm | | 6x, beginning pit hardening |
| 120ppm | | 6x, beginning pit hardening |

*Given the large number of treatments evaluated, for clarity of presentation data will only be shown from selected treatments.*

Ca solutions with a non-ionic surfactant at 0.1% were sprayed to whole tree canopies using a $CO_2$ pressurized hand gun sprayer to achieve uniform, complete coverage (i.e., sprayed to drip). Experimental units (trees) were arranged in a completely randomized design with 4 single-tree replications per treatment. The Ca sources, application rate, application frequency, application timing were tested and optimized on different cultivars. NaCl at 0, 30, 60, and 120ppm was applied every week after pit-hardening until commercial harvest (total of 6 applications).

***3. Nutrition and quality evaluations.*** Fruit tissue Ca, Na, Cl, N contents were measured by ICP-AES (Ca, Na), Lachat Quikchem autoanalyzer (Cl), and Kjeldahl (N) methods, respectively. Fruit quality at harvest and shipping quality after 2, 4, and 6 weeks of cold storage were evaluated.

***4. Horticultural evaluations.*** Fruit growth rate of 15 fruit per rep were tagged prior to treatment application and measured weekly using a digital caliper. Return bloom and fruit set were evaluated for two spur populations: Two-year-old spurs (representing spurs that were non-fruiting in 2014, 2015); and, three-year-old spurs (representing spurs that yielded fruit in 2014, 2015).

## RESULTS

### 1. Fruit tissue Ca & N concentrations with shipping quality

Fruit with varied tissue Ca content (300-800ppm dw) from different orchards had different pitting susceptibility and fruit quality after storage/shipping for Lapins, Sweetheart, and Skeena. A trend exits that the higher Ca content, the higher fruit firmness (FF) and better flavor and less pitting after 3-5 weeks storage/shipping. Fruit tissue N content was between 0.9 – 1.1% and was not correlated with fruit quality at harvest and after 3-5 weeks storage/shipping.

### 2. Ca spray

***a. The optimum application rate***

Applied 6 times at weekly interval from pit-hardening to harvest, $CaCl_2$ or $Ca(NO_3)_2$ at 0.1-0.15% $Ca^{2+}$ increased fruit tissue Ca content significantly ($p < 0.05$) from 436ppm (control) to 615-655ppm at the time of harvest (Fig. 1). Ca at 0.05% didn't increase fruit tissue Ca content. Ca at 0.2% had no additional benefit on increasing fruit tissue Ca content, but reduced fruit size and caused leaf burning. The trend was similar between $CaCl_2$ and $Ca(NO_3)_2$.

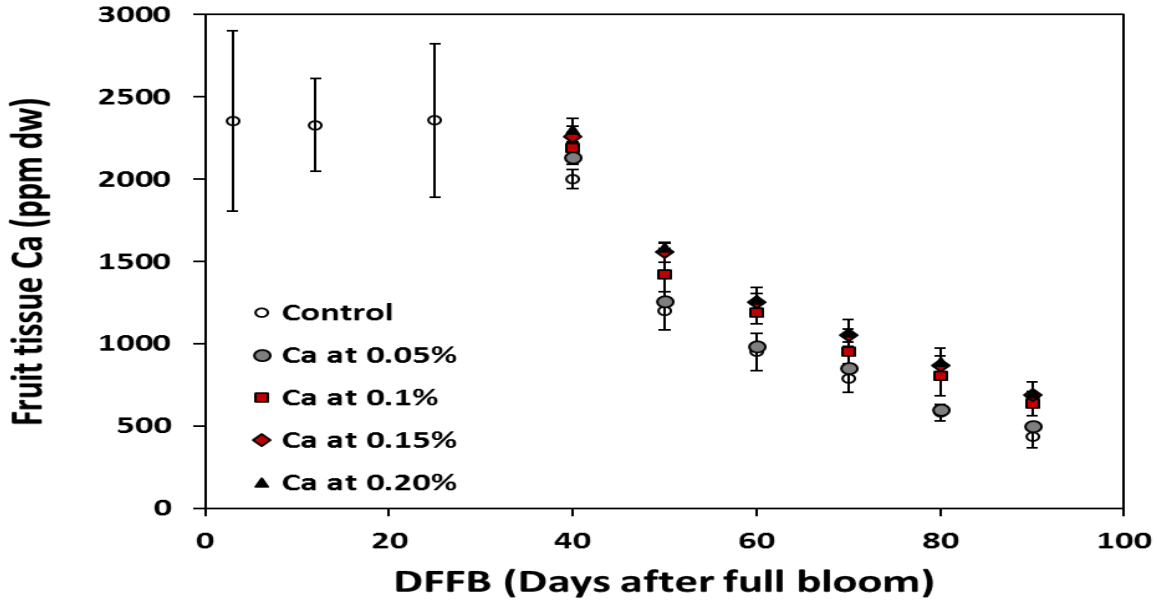



*Fig. 1. Effect of Ca application rate on fruit tissue Ca content of 'Lapins' sweet cherry.*

***b. The optimum application timing and frequency***

Compared to 6 times, spray frequency of Ca at 0.1% for 9 times at weekly interval did not improve cherry fruit Ca uptake significantly ($p < 0.05$) (Fig. 2A&B). Ca at 0.1% sprayed twice (pit-hardening + 1-week before harvest or 2-week + 1-week before harvest) did not increase fruit tissue Ca content at harvest compared to control (Fig. 2C&D).

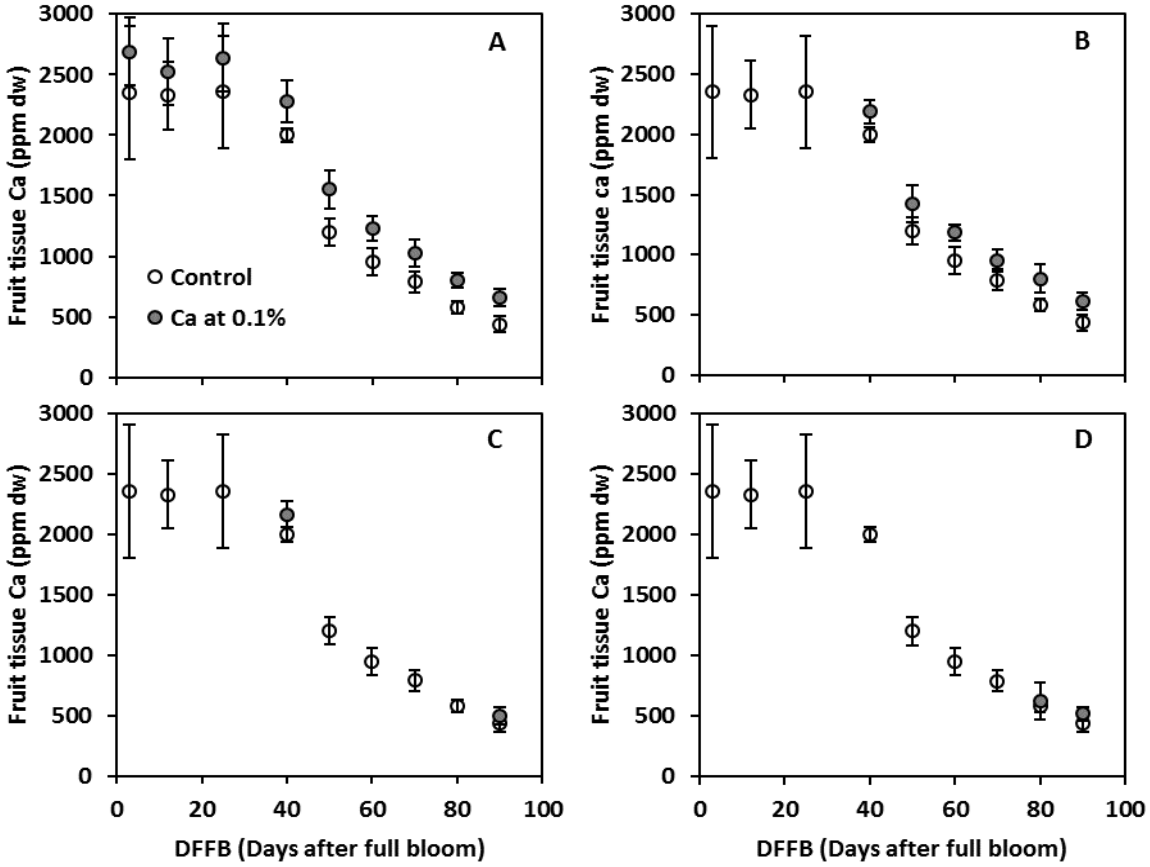
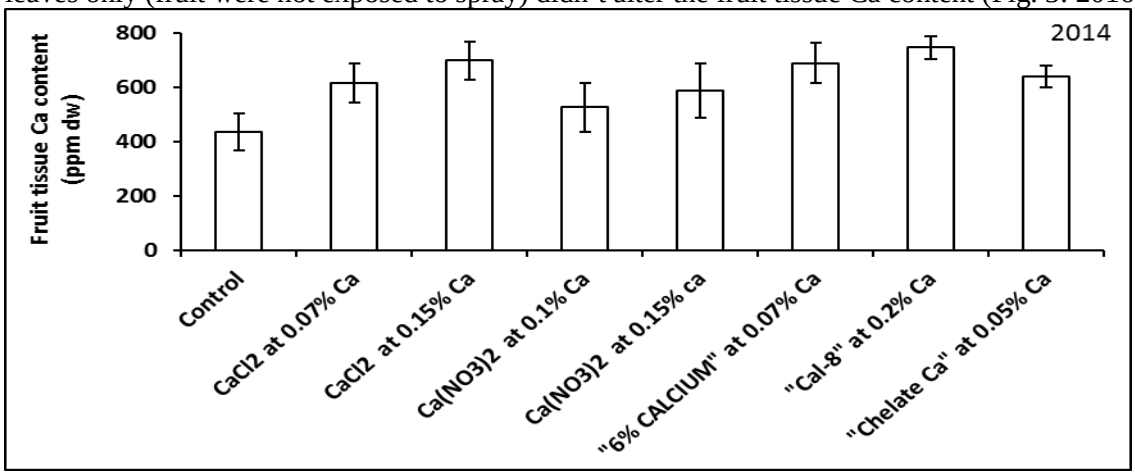



Fig. 2. Effect of frequency and timing of Ca spray on fruit tissue Ca content in 'Lapins' cherry.

***c. Ca sources on fruit tissue Ca content***

All the Ca sources tested at rates of 0.1-0.15% $Ca^{2+}$ for 6 times at weekly interval from pit-hardening to harvest increased fruit tissue Ca content at similar efficacy (Fig. 3). The Ca spray on leaves only (fruit were not exposed to spray) didn't alter the fruit tissue Ca content (Fig. 3: 2016).



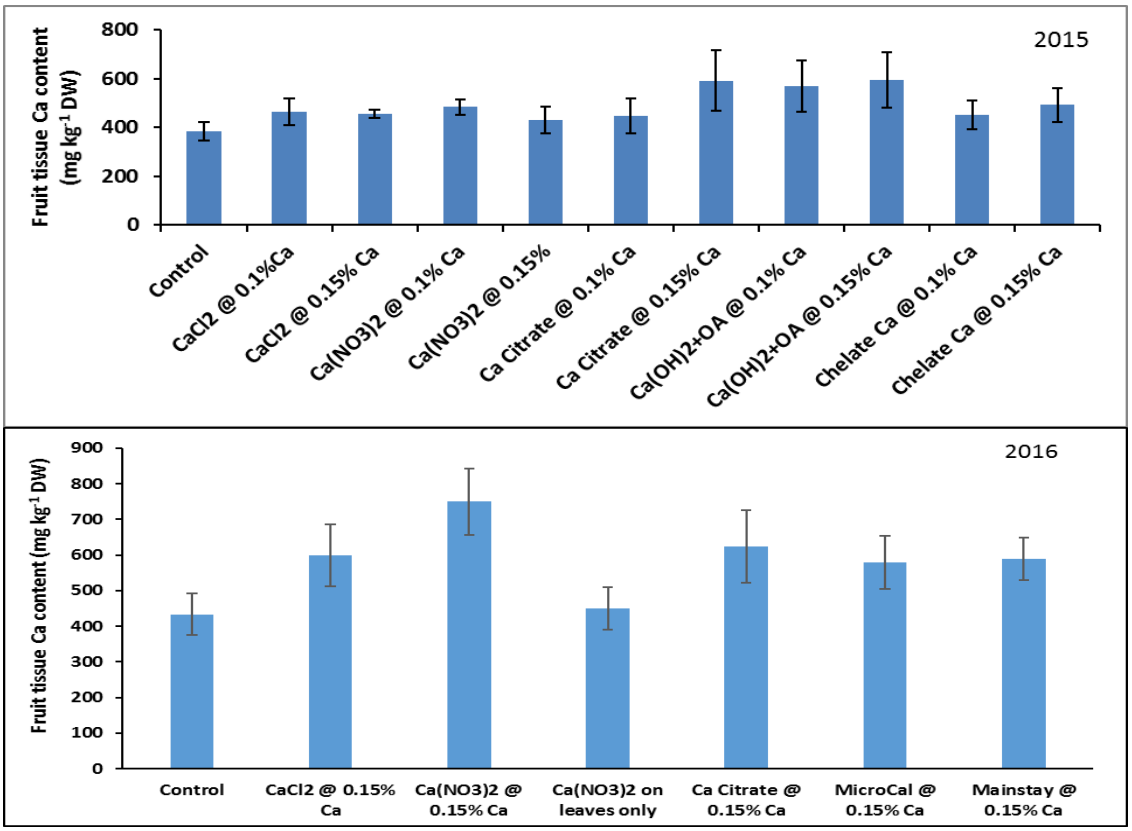



*Fig. 3. Effect of Ca sources on fruit tissue Ca content in 'Lapins' at the time of harvest.*

***d. Effect of Ca spray on fruit quality at harvest***

All the Ca sources at 0.1-0.15% Ca for 6x applications did not affect fruit maturity based on fruit color (data not shown). All Ca sprays increased FF ($p < 0.05$) compared to control (Fig. 4). Fruit growth rate and fruit size were unaffected by the Ca treatments at $p < 0.05$. The fact that fruit growth was adversely impacted by a few Ca treatments ($CaCl_2$ and Ca citrate) in 2014 but not in 2015 and 2016 might be attributed to the relatively light crop load in 2015 and 2016. Ca spray tended to increase SSC (Fig. 4) and TA slightly.

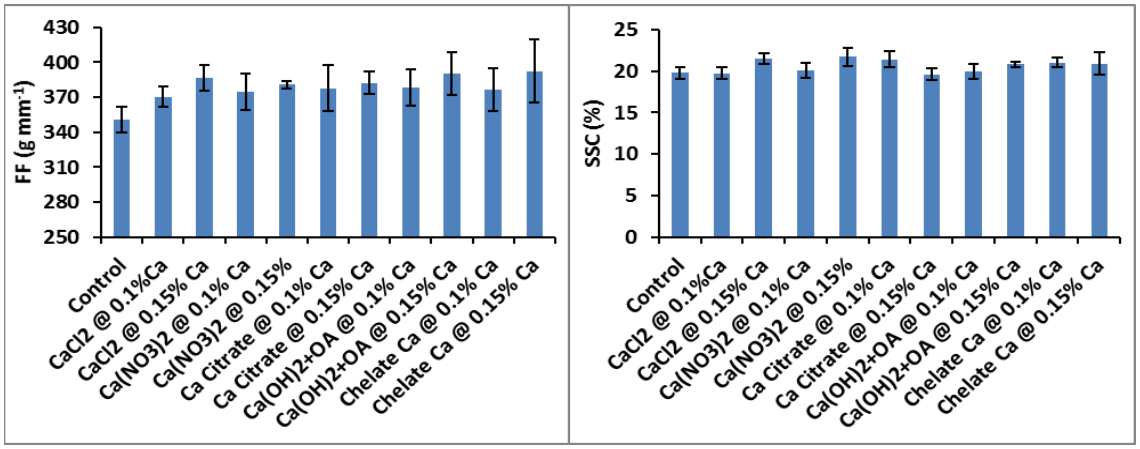



*Fig. 4. Effect of Ca spray on fruit firmness (FF) and soluble solid content (SSC) of 'Lapins' at the time of harvest.*

***e. Effect of Ca spray on shipping quality of sweet cherries after 3 weeks cold storage***

For 'Lapins', all the Ca sources at 0.1% $Ca^{2+}$ for 6x applications reduced pitting, decay, and stem browning incidences and retarded fruit skin darkening during 3 weeks storage (Table 1). 'Lapins' fruit treated with the Ca spays tended to have higher FF, SSC, and TA after storage. The Ca sprays reduced splitting, decay, and stem browning in 'Skeena' after storage (Table 1).

*Table 1. Effect of Ca sprays at 0.1% $Ca^{2+}$ for 6x applications on shipping quality of 'Lapins' and 'Skeena' after 3 weeks storage at 32°F. Different letters indicate significant differences between treatments according to Fisher's protected LSD test at $p < 0.05$.*

| | Natural pitting (%) | Splitting (%) | Decay (%) | Pedicel browning (%) | Fruit skin darkening (L*) | Fruit firmness (g $mm^{-1}$) | SSC (%) | TA (%) |
|---|---|---|---|---|---|---|---|---|
| | | | | Lapins | | | | |
| Control | 15.8a | 0 | 5.2a | 33.3a | 30.5b | 388b | 20.3b | 0.68b |
| $CaCl_2$ | 8.5b | 0 | 1.3b | 23.1b | 31.5a | 406a | 21.5ab | 0.71b |
| $Ca(NO_3)_2$ | 9.1b | 0 | 2.1b | 19.8b | 30.9a | 411a | 21.3ab | 0.73b |
| Ca citrate | 8.3b | 0 | 1.8b | 21.5b | 31.6a | 409a | 21.8ab | 0.76ab |
| $Ca(OH)_2$+OA | 6.6b | 0 | 1.6b | 18.6b | 31.2a | 416a | 22.3a | 0.78a |
| AA chelate Ca | 7.5b | 0 | 2.2b | 18.9b | 30.9a | 413a | 21.5ab | 0.79a |
| | | | | Skeena | | | | |
| Control | 5.8a | 6.3a | 4.8a | 10.0a | 30.2a | 422a | 22.3a | 0.78a |
| $Ca(NO_3)_2$ | 5.5a | 3.2b | 1.3b | 6.6b | 31.0a | 436a | 22.7a | 0.80a |

In 2016, fruit internal browning and sensory flavor were evaluated after 6 weeks at 32°F. Result indicated that a higher fruit tissue Ca content helped to reduce internal browning and maintain flavor after a simulated ocean shipping (Fig. 5).

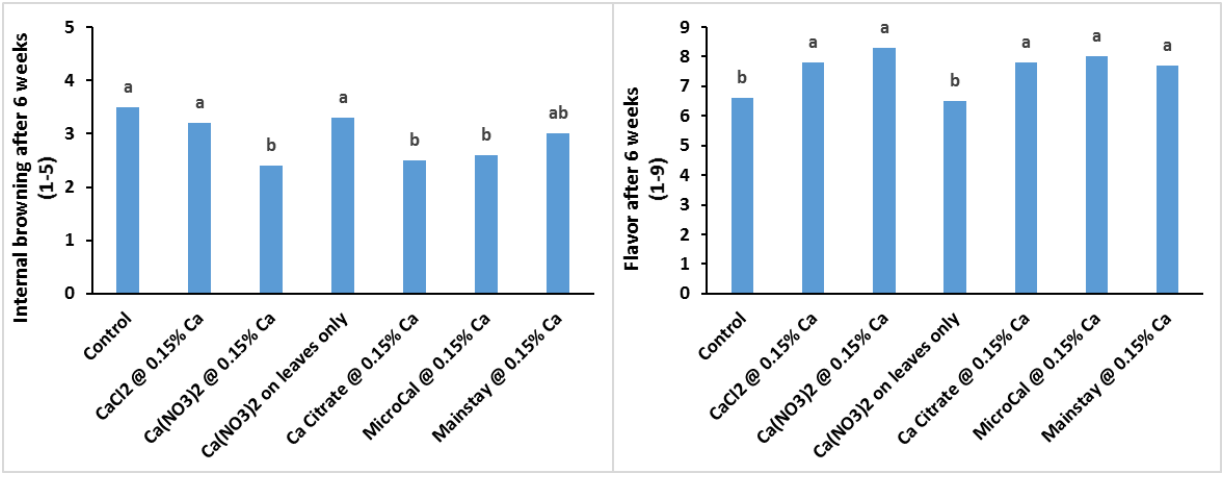



*Fig. 5. Effect of Ca spray on internal browning and flavor of 'Lapins' after 6 weeks at 32°F.*

***f. Effect of Ca spray on horticultural performance of Lapins trees***

In 2014, Lapins fruit growth was only affected by $CaCl_2$ and Ca citrate sources when applied 6 to 9 times (Fig. 6). Interestingly, $Ca(NO_3)_2$ applied at equivalent concentrations and frequency to $CaCl_2$ did not reduce fruit growth, likely due to the negative effects of Cl on cell processes. In 2015 and 2016, fruit growth was not affected ($p < 0.05$) by the Ca sprays (data not shown).

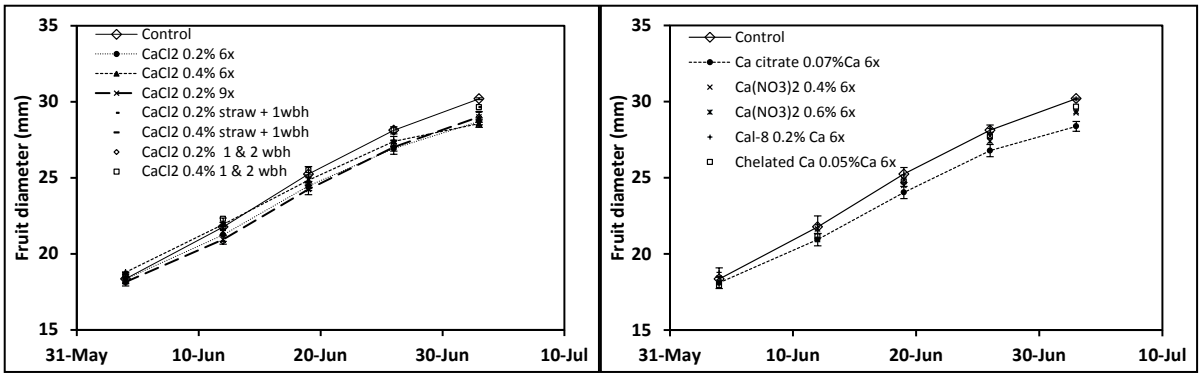



*Figure 6. Fruit growth of 'Lapins' sweet cherry as affected by Ca source, concentration and application frequency. All data are means of 3 replicates (n=15). Given the large number of treatments, the data were split between 2 graphs for clarity of presentation; control fruit are shown in each graph for comparative purposes. SE bars and lines are only provided for controls and those treatments that significantly differed from controls.*

Return bloom was not affected by 2014 Ca sprays when 2 and 3-year-old spurs were evaluated (Table 2). Average number of flowers per bud and buds per spur was 3, reflecting the non-productive characteristics of Mazzard rootstock. Fruit set was generally low for Lapins; however, the site sustained fairly high flower mortality following the 2014 November freeze event (3°F).

*Table 2. Return bloom of 'Lapins' sweet cherry affected by Ca sprays. Different letters indicate significant differences between treatments according to Fisher's protected LSD test at p < 0.05.*

| Treatment | Return Bloom | | | |
|---|---|---|---|---|
| | Average no. buds/spur | | Average no. flowers/bud | |
| | 2-yr-old spurs | 3-yr-old spurs | 2-yr-old spurs | 3-yr-old spurs |
| control | 3.1 | 3.6 | 3.1 | 2.8 de |
| CaCl2 0.1% 6 times | 3.0 | 2.8 | 3.1 | 2.9 de |
| CaCl2 0.15% 6 times | 3.3 | 3.4 | 3.1 | 3.1 abcd |
| Ca(NO3)2 0.1% 6 times | 2.9 | 2.9 | 2.9 | 2.8 e |
| Ca(NO3)2 0.15% 6 times | 3.2 | 3.0 | 3.0 | 2.8 e |
| Ca citrate 0.1% Ca 6 times | 3.2 | 3.1 | 3.2 | 2.9 bcde |
| Ca citrate at 0.15% Ca 6 times | 3.1 | 3.4 | 3.2 | 3.2 ab |
| Cal-8 at 0.15% Ca 6 times | 3.0 | 3.1 | 3.1 | 3.2 a |
| Cal-8 at 0.1% Ca 6 times | 3.0 | 3.3 | 3.1 | 2.9 de |
| Chelate Ca at 0.1% Ca 6 times | 2.9 | 2.9 | 3.0 | 2.8 e |
| Chelate Ca at 0.15% Ca 6 times | 3.0 | 3.2 | 3.0 | 2.9 de |
| Ca(NO3)2 at 0.1% 9 times | 3.1 | 2.8 | 3.0 | 2.9 cde |
| Ca(NO3)2 at 0.15% 9 times | 3.2 | 3.6 | 3.3 | 3.2 abc |
| *significance* | *n.s.* | *n.s.* | *n.s.* | *0.003* |

**3. NaCl spray (6 times at weekly interval from pit hardening to harvest)**

***a. Effect of NaCl spray on fruit tissue $Na^+$ and $Cl^-$ contents***

NaCl spray increased fruit tissue $Na^+$ content with a dose response, but did not affect fruit tissue $Cl^-$ content in 'Lapins' and 'Regina' (Fig. 7).

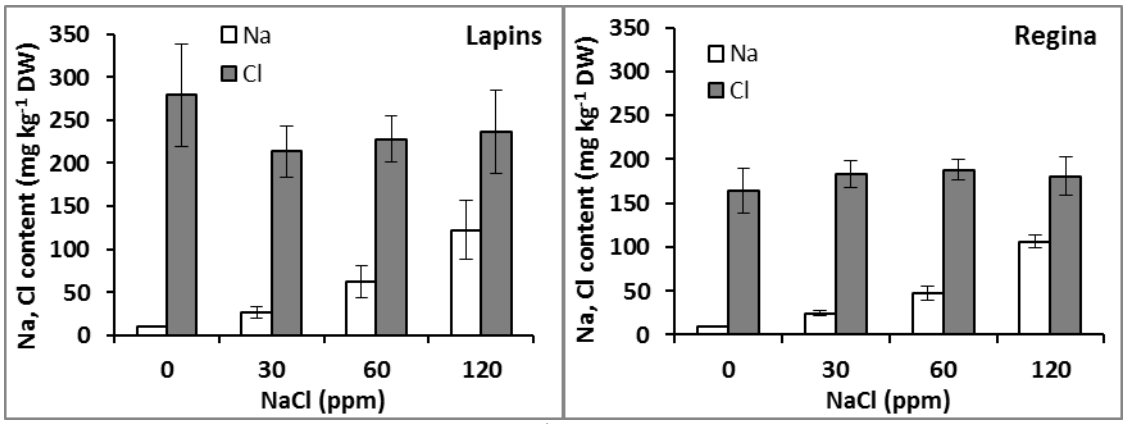



*Fig. 7. Effect of NaCl spray on fruit tissue $Na^+$ and $Cl^-$ contents in 'Lapins' and 'Regina'.*

***b. Effect of NaCl spray on fruit quality at harvest and after storage/shipping***

NaCl sprays at 30 and 60 ppm did not affect fruit size, but a higher rate at 120ppm reduced fruit size in 'Lapins' and 'Regina' (Fig. 8). NaCl sprays at all the application rates enhanced fruit color by increasing anthocyanin accumulation in fruit of both cultivars. The NaCl sprays did not affect fruit total antioxidant capacity (TAC).

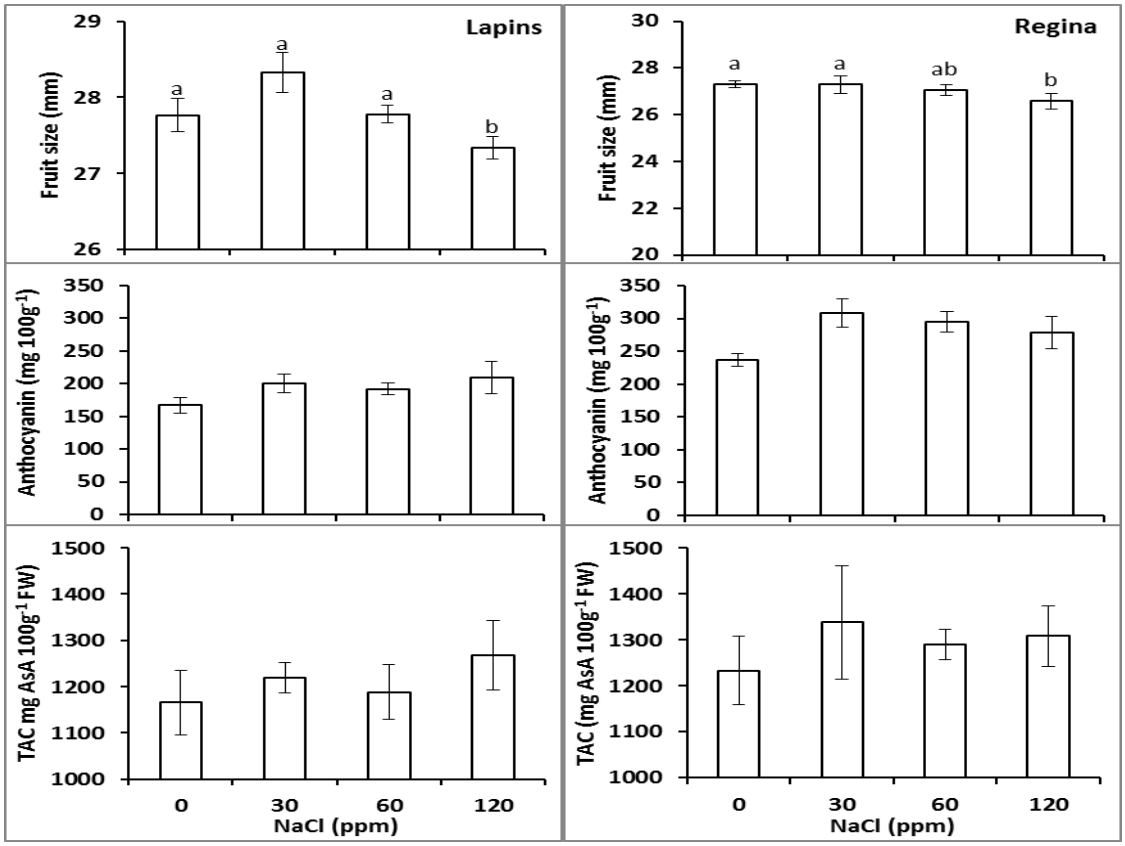



*Fig. 8. Effect of NaCl sprays on fruit size, anthocyanin content, and total antioxidant capacity (TAC) of 'Lapins' and 'Regina'.*

NaCl sprays at 60 and 120 ppm increased FF, SSC, and TA at harvest and during 3 weeks storage in 'Lapins', but not in 'Regina' (Fig. 9)

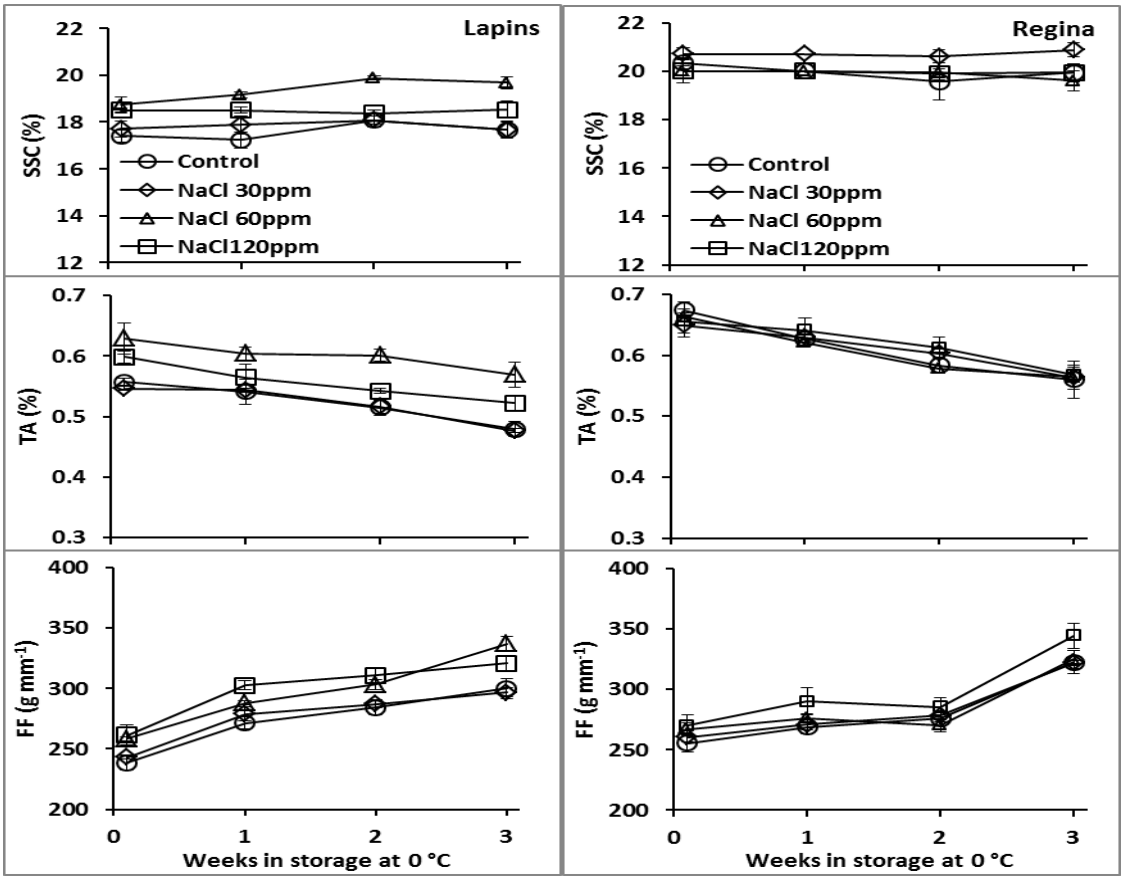



*Fig. 9. Effect of NaCl spray on soluble solid content (SSC), titratable acidity (TA), and fruit firmness (FF) of 'Lapins' and 'Regina' at harvest and during 3 weeks storage at 32°F.*

***c. Effect of NaCl spray on stem quality***

NaCl sprays at 30, 60, and 120 ppm improved stem quality by increasing green stem incidence in both 'Lapins' and 'Regina' after 3 weeks storage (Fig. 10). The NaCl sprays maintained higher moisture content in stems of both cultivars after storage. The natural cuticle wax content in NaCl treated stems was higher with a dose response than the control fruit (data not shown).

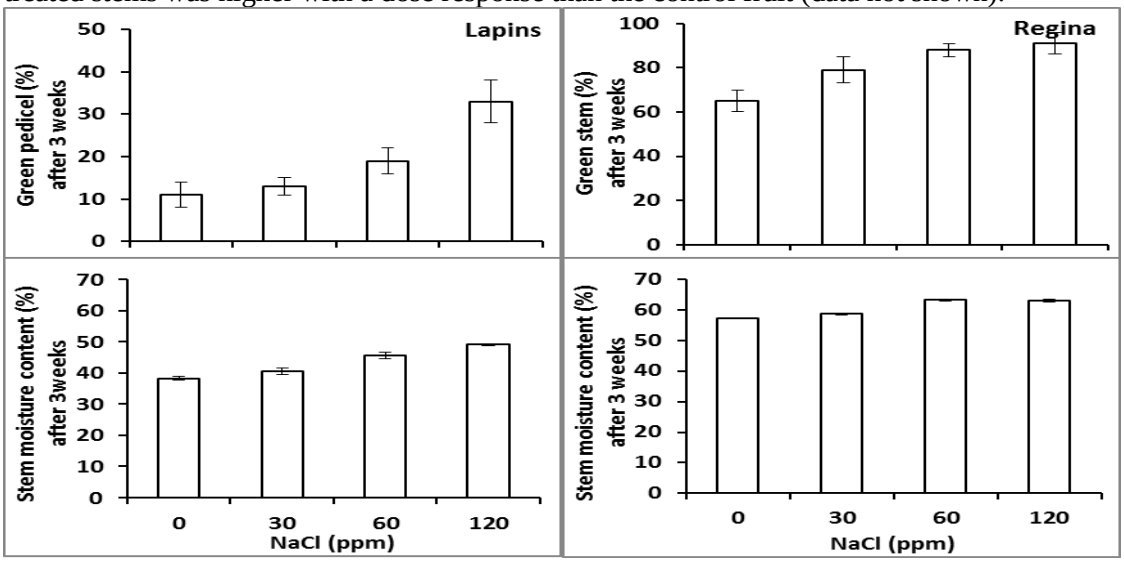



*Fig. 10. Effect of NaCl spray on stem quality of 'Lapins' and 'Regina' after 3 weeks storage at 32°F.*

**EXCUTIVE SUMMARY**

**Project title:** Improving shipping quality of cherry by pre-harvest Ca and NaCl sprays

In this study, fruit tissue Ca content is found to be positively correlated with shipping quality of three PNW cultivars surveyed from 5-9 orchards depending on cultivars. This study indicated that preharvest Ca spray at appropriate rate, timing and frequency is an efficient way to increase cherry fruit tissue Ca content and therefore improves shipping quality of the PNW cultivars. The optimum Ca spray rate is determined to be 0.1-0.15% $Ca^{2+}$. Higher Ca rates may cause leaf burning or reduce fruit size. Spray with lower $Ca^{2+}$ concentrations doesn't increase fruit tissue Ca content efficiently. The optimum Ca spray timing and frequency for increasing fruit tissue Ca content are 6 times at weekly interval from pit-hardening to harvest. Ca sprayings before the stage of pit-hardening were not found to increase fruit tissue Ca content. Ca spray at 0.1-0.15% $Ca^{2+}$ for 6 times increases fruit firmness at harvest, reduces pitting, splitting, stem browning, fruit internal browning and decay after storage/shipping. At these recommended application rates and frequency, $CaCl_2$ and Ca citrate, but not other formulations, may affect fruit growth rate and fruit size. These Ca treatments don't affect return bloom. When Ca spray at 0.1-0.15% $Ca^{2+}$ for 6 times, organic acid chelated or amino acid chelated Ca formulations are better than the other Ca sources; $Ca(NO_3)_2$ is better than $CaCl_2$ for improving cherry shipping quality. Ca sprayed on leaves doesn't remove to fruit.

NaCl spray at 60 ppm for 6 times at weekly interval from pit-hardening to harvest improves pedicel quality after storage/shipping. A synergistic effect between Ca and NaCl was not found on improving fruit quality at harvest and shipping quality of the PNW cultivars. Therefore, NaCl spray may not be justified as a commercial practice in cherry production.

**CONTINUING PROJECT REPORT** **YEAR:** 1 of 3
**WTFRC Project Number:** CH-16-100

**Project Title**: Enhanced strategies to reduce postharvest splitting of cherries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**Cooperators:** TIC Gums, Van Doren Sales, Inc., Chelan Fruit, Stemilt Growers LLC and Allan Bros. Inc., Shield Bags and Printing Company, Washington Fruit & Produce Co. and others.

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

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

| **Item** | 2016 | **2017** | 2018 |
|---|---|---|---|
| **Salaries** | $14,092 | $14,656 | $15,242 |
| **Benefits** | $1,235 | $1,285 | $1,337 |
| **Wages** | $9,055 | $9,417 | $9,794 |
| **Benefits** | $1,275 | $1,327 | $1,380 |
| **Equipment** | | | |
| **Supplies** | $5,000 | $5,000 | $5,000 |
| **Travel** | $750 | $1,500 | $2,000 |
| **Plot Fees** | | | |
| **Miscellaneous** | | | |
| **Total** | $34,107 | **$33,185** | $34,753 |

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

**1. OBJECTIVES**:

The original objectives proposed were:

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

<u>During the first year (2016) we worked on the following specific items:</u>

- Explored the mechanism of the coating on the cherry skin surface through microscopy work.
- Compared the effects of the coatings of different grades of Gum Acacia; Seyal (Grade 2, from previous years testing) and Senegal (Grade 1) in variable concentration individually and in combination, in lab trials, with Bing cherries.
- Tested the effects of other edible coating enhancers (Sodium Alginate and Agar) individually and with combination with Gum Acacia Seyal (Grade 2) which showed promise in previous year experiments to reduce cracking and pedicel browning, in lab trials with Bing cherries.
- Tested the select coatings (with enhancers and individually) on a packing line with 2 varieties of cherries (Sweetheart and Skeena), along with surface water removal by air knives.
- Performed preliminary sensory analysis on the coated cherries to see if consumers can detect any difference in the coated and uncoated cherries.

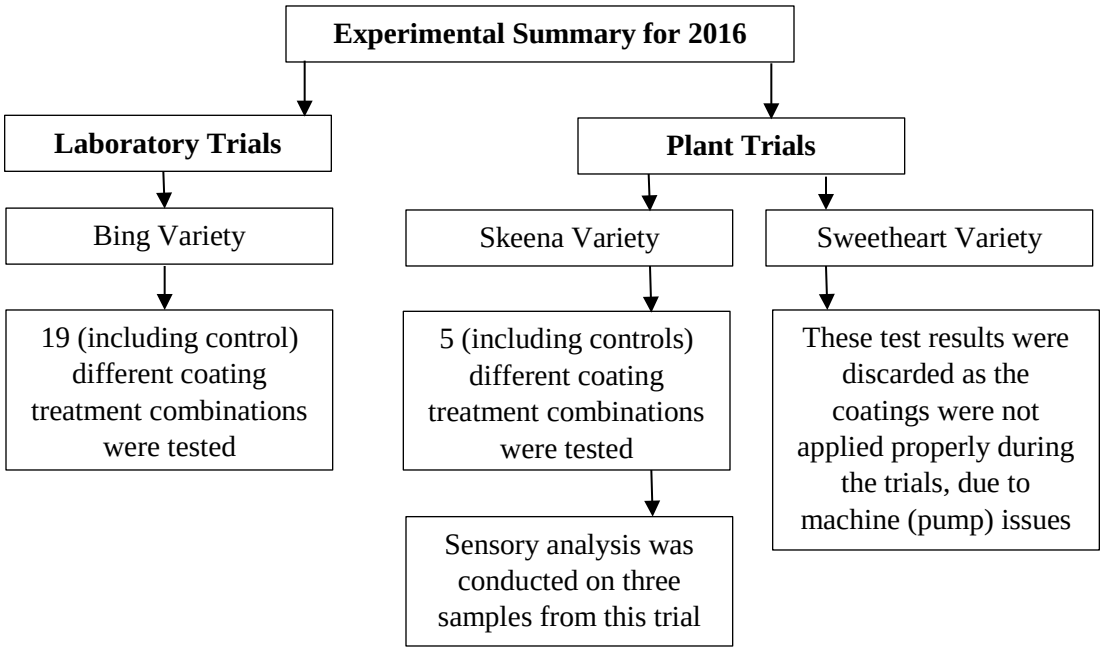



Figure 1. Flow chart showing the summary of the experiments conducted in 2016. All treated cherry samples were subjected to storage studies for 6 weeks (5 weeks in refrigerated storage and 1 week in room conditions).

## 2. SIGNIFICANT FINDINGS:

Overall findings:

- "Gum Acacia Senegal" showed best results in terms of reducing the cracking and pedicle browning, in the plant trials.
- "Gum Acacia Seyal + Sodium Alginate", also showed better results in reducing cracking and pedicle browning. Sodium Alginate, helped to strengthen the coating that showed the best results compared to the last year's results.

Following are the significant findings categorized for different Cherry varieties:

*2.1 Bing (Lab Trials)*

- "Gum Acacia Seyal (95%) & Sodium Alginate (5%)" at 0.5% concentration was the most effective in reducing cracking;
- "Gum Acacia Senegal (50%) & Seyal (50%)" at 0.5 & 3% concentrations and "Gum Acacia Senegal (100%)" at 3% concentration reduced the pedicle browning effectively;
- "Gum Acacia Seyal (100%)" at 3% concentration and "Gum Acacia Senegal (100%)" at 0.5% concentration, were effective for maintaining the fruit pH;
- "Gum Acacia (80%) & Agar (20%)" at 0.5% concentration showed the best Total Soluble Solids (TSS) retention;
- "Gum Acacia Seyal (100%)" at 0.5% concentration and "Gum Acacia Seyal (90%) and Agar (10%)" at 0.3% concentration maintained the acidity of the fruit well.

*2.2 Skeena (Plant Trials)*

- "Gum Acacia Senegal (100%)" at 0.5% concentration was effective in reducing, weight loss, cracking, pedicle browning, controlling TSS.
- "Gum Acacia Seyal (80%) & Sodium Alginate (20%) at 0.5% concentration for maintaining pH.

*2.3 Sweetheart (Plant Trials) – This Plant Trials data was Not completely reliable as the coating did not apply effectively during the whole trials.*

- "Gum Acacia Seyal (80%) & Sodium Alginate (20%)" at 0.5% concentration was effective in reducing cracking.
- "Gum Acacia Seyal (100%)" at 0.5% concentration, was effective in controlling the TSS.

*2.4 Sensory Analysis*

- No difference was observed in the in acceptance of aroma, sweetness, sourness and cherry flavor amongst the i) Control (no coating); ii) Gum Acacia Seyal (100%) at 0.5% concentration; and iii) Gum Acacia Seyal (80%) & Sodium Alginate (20%) at 0.5% concentration, treatments for Skeena cherries.

*2.5 Scanning Electron Microscopy (SEM)*

- Uncoated cherries, at week 2 & week 5 of storage, had open pores seen under magnification, which are absent in coated cherries.
- It appeared from the microscopic images that the coating effectively coated the surface of the cherries and closed the pores and cracks that are naturally present on the surface.

**3. METHODS**:

*3.1 Materials:*

Sweet cherries packed in carton boxes (*var.* Bing) was procured from a packing house in Chelan, WA. The cherries were stored in the pilot plant at Washington State University (WSU), Pullman. A total of 18 coatings, plus the control (19 treatments) were tested on these cherries. This trial has been referred to as "Lab Trial", throughout this report.

One plant trial for two varieties (*var.* Skeena and *var*. Sweetheart), was also conducted with the help of a cooperator in Naches, WA. Five different coatings were tested in this trial, including the fungicide and control treatments. The air knife treatment, to remove the excess coating from the surface was applied for all treatments in the plant. These cherries were then packed in carton boxes and brought to the pilot plant facility at WSU where they were stored in refrigerated storage ($42^0$F & 75% RH). This trial has been referred to as "Plant Trial" throughout this report.

*3.2 Coating Application:*

*3.2.1 Lab Trial*

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

*3.2.2 Plant Trial*

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

*a) Dipping in Coating Solution* *b) Waterfall Method* *c) Drying with air knife*

Figure 2. Coating application on Cherries (a) for Lab Trials; (b) & (c) for Plant Trials.

*3.3 Coatings Used:*

Tables 1 and 2, show the details of the coatings studied in lab trials and plant trials, respectively.

Table 1. Details of the coatings used in the lab trials with Bing cherry variety

| Serial # | Code | Coating | Solution Conc. % |
|---|---|---|---|
| **1** | **C1L1** | Gum Acacia Seyal 100% | 0.5 |
| **2** | **C1L2** | Gum Acacia Seyal 100% | 3.0 |
| **3** | **C2L1** | Gum Acacia Seyal 50% & Gum Acacia Senegal 50% | 0.5 |
| **4** | **C2L2** | Gum Acacia Seyal 50% & Gum Acacia Senegal 50% | 3.0 |
| **5** | **C3L1** | Gum Acacia Senegal100% | 0.5 |
| **6** | **C3L2** | Gum Acacia Senegal 100% | 3.0 |
| **7** | **C4L1** | Gum Acacia Seyal 80% & Agar 20% | 0.3 |
| **8** | **C4L2** | Gum Acacia Seyal 80% & Agar 20% | 0.5 |
| **9** | **C4L3** | Gum Acacia Seyal 80% & Agar 20% | 1.0 |
| **10** | **C5L1** | Gum Acacia Seyal 90% & Agar 10% | 0.3 |
| **11** | **C5L2** | Gum Acacia Seyal 90% & Agar 10% | 0.5 |
| **12** | **C5L3** | Gum Acacia Seyal 90% & Agar 10% | 1.0 |
| **13** | **C6L1** | Gum Acacia Seyal 95% & Agar 5% | 0.3 |
| **14** | **C6L2** | Gum Acacia Seyal 95% & Agar 5% | 0.5 |
| **15** | **C6L3** | Gum Acacia Seyal 95% & Agar 5% | 1.0 |
| **16** | **C7L1** | Gum Acacia Seyal 75% & Sodium Alginate 25% | 0.5 |
| **17** | **C8L1** | Gum Acacia Seyal 85% & Sodium Alginate 15% | 0.5 |
| **18** | **C9L1** | Gum Acacia Seyal 95% & Sodium Alginate 5% | 0.5 |
| **19** | **Control** | No Coating | |

**Footnote:** Seyal (Grade 2) – used in last year's trials. Senegal (Grade 1) – new type. C1L1 – Coating1Level1.

Table 2. Details of the coatings used in the plant trials with Sweetheart and Skeena cherry varieties.

| Serial # | Code | Coating | Solution Conc. % |
|---|---|---|---|
| **1** | **NASF** | Gum Acacia Seyal 80% & Sodium Alginate 20% | 0.5 |
| **2** | **ASF** | Gum Acacia Seyal 100% | |
| **3** | **ASD** | Gum Acacia Senegal 100% | |
| **4** | **CRTF** | Fungicide | |
| **5** | **CRT** | Control (no coating & no fungicide) | |

*3.4 Fruit Quality Testing Parameters*

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

*3.5 Sensory Analysis*

The Skeena variety of cherries coated with "Gum Acacia Seyal (80%) and Sodium Alginate (20%)" at 0.5% concentration, "Gum Acacia Seyal (100%)" at 0.5% and a "Control (with no coating)" were tested for differences. The panel consisted of untrained volunteers (n=120) that was carried out in a single day.

## 4. RESULTS & DISCUSSION

The key findings from the first year work are summarized in the initial part of this report. In addition to the details provided above the important things we have learnt from the first year of work is:

- The "Gum Acacia – Seyal" tested during the last year, still worked well with repeat tests this year.

- The efforts to increase the strength of this coating using other edible coatings such as the "Agar" and "Sodium Alginate" proved to be effective.
- The new version of the "Gum Acacia" tested called the Senegal, was found to be more effective compared to only "Gum Acacia – Seyal".
- These results suggest that these tested edible coatings can be effective in the reduction of the cracking and stem browning during 4 to 6 weeks of storage periods.

Table 3 and 4, below summarizes the results from the lab and the plant trials respectively. The tables provide the details of the specific coatings that were found effective in the storage studies with respect to the different cherry quality parameters that were studied.

Table 3. Summary of the effective coatings for various quality parameters tested for lab trials.

| Parameter Tested | Variety | Time (Week) | Effective Coating(s) |
|---|---|---|---|
| Cracking | Bing | 4 | C5L1 - Gum Acacia Seyal 90% & Agar 10% (0.3%)<br>C7L1 - Gum Acacia Seyal 75% & Sodium Alginate 25% (0.5%)<br>C9L1 - Gum Acacia Seyal 95% & Sodium Alginate 5% (0.5%) |
| Pedicel Browning | | 5 | C2L1 - Gum Acacia Seyal 50% & Gum Acacia Senegal 50% (0.5%)<br>C2L2 - Gum Acacia Seyal 50% & Gum Acacia Senegal 50% (3%)<br>C4L1- Gum Acacia Seyal 80% & Agar 20% (0.3%) |
| Weight Loss | | - | No Significant Differences |
| Firmness | | - | No Significant Differences |
| pH | | 6 | C1L1 - Gum Acacia Seyal 100% (0.5%)<br>C3L1 - Gum Acacia Senegal 100% (0.5%) |
| Total Soluble Solids | | 6 | C2L2 - Gum Acacia Seyal 50% & Gum Acacia Senegal 50% (3%)<br>C5L2 - Gum Acacia Seyal 90% & Agar 10% (0.5%) |
| Malic Acid | | 6 | C1L1 - Gum Acacia Seyal 100% (0.5%)<br>C5L1 - Gum Acacia Seyal 90% & Agar 10% (0.3%) |

Table 4. Summary of the effective coatings for various quality parameters tested for plant trials.

| Parameter Tested | Variety | Time (Week) | Effective Coating |
|---|---|---|---|
| Cracking | Skeena | 5 | ASD - Gum Acacia Senegal 100% (0.5%) |
| Pedicel Browning | | 6 | ASD - Gum Acacia Senegal 100% (0.5%) |
| Weight Loss | | - | No Significant Differences |
| Firmness | | - | No Significant Differences |
| pH | | 6 | NASF - Gum Acacia Seyal 80% & Sodium Alginate 20% (0.5%) |
| Total Soluble Solids | | 6 | ASD - Gum Acacia Senegal 100% (0.5%) |
| Malic Acid | | - | No Significant Differences |

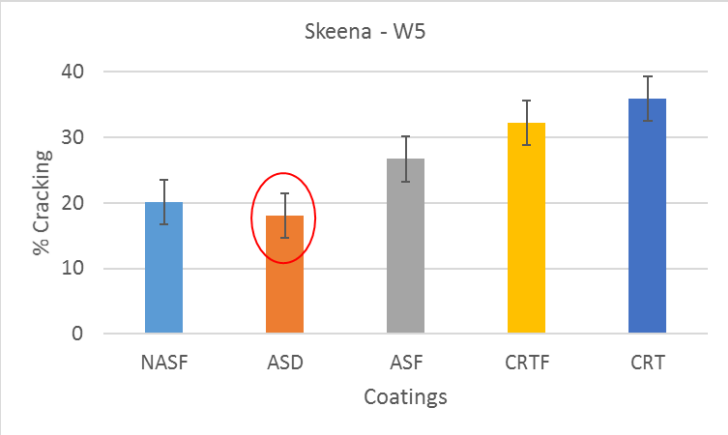



Figure 3. Percent cracking in cherries with difference coatings on Week 5 (W5) for the plant trials with Skeena variety. ASD - Gum Acacia Senegal (100%) at 0.5% concentration.

Figure 3 and 4, show the results on the % cracking and pedicle browning in the cherries, from the plant trials.

It is clear from the Figure 3 that all the coatings showed a reduction in the cracking compared to the control treatment (CRT) and the fungicide treatment (CRTF).

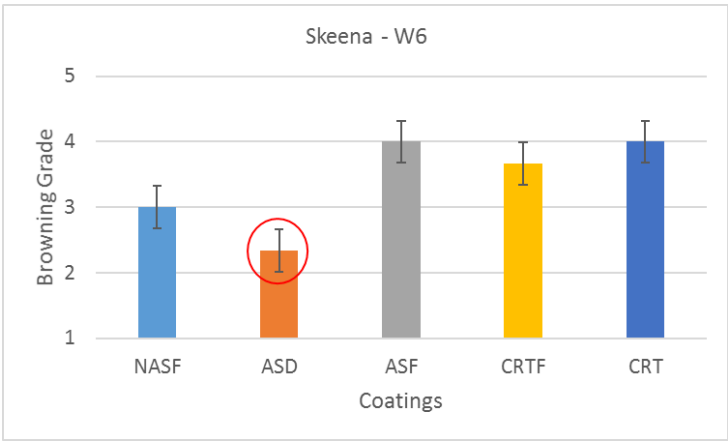



Figure 4. Pedicle browning in cherries with difference coatings on Week 6 (W6) for the plant trials with Skeena variety. ASD - Gum Acacia Senegal (100%) at 0.5% concentration.

The pedicle browning was significantly reduced by the coating "Gum Acacia – Senegal". It was the most effective. The coating, NASF (Gum Acacia – Seyal + Sodium Alginate), also was effective in reducing the pedicle browning (Figure 4).

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

**Sensory Analysis Summary**

- From the preliminary sensory analysis of the cherries with three different treatments, no differences were observed in the acceptance of aroma, sweetness, sourness and cherry flavor.
- Consumers significantly preferred the "Gum Acacia Seyal" at 0.5% and "Control" over the combination of "Gum Acacia Seyal (80%) & Sodium Alginate (20%)" solution at 0.5% concentration.
- For texture, the "Control" treatment was significantly preferred over the "Gum Acacia Seyal (80%) & Sodium Alginate (20%)" solution concentration 0.5% treatment, with the "Gum Acacia Seyal (0.5%)" treatment not significantly different from either of the other treatments.

## 5. IMPACTS TO THE CHERRY GROWERS

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

**CONTINUING PROJECT REPORT** **YEAR**: 1/3 years

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

**PI**: Yan Wang
**Organization**: MCAREC
**Telephone**: 541-386-2030 (ext. 38214)
**Email**: yan.wang@oregonstate.edu

**Cooperators**: Lynn Long, Todd Einhorn, Jinhe Bai, Johnny Gebbers, Yu Dong, Shunchang Cheng, Jiaming Guo

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

**Budget:**

**Organization Name: OSU-MCAREC** **Contract Administrator:** Russ Karow

**Telephone:** 541-737-4066 **Email address:** Russell.Karow@oregonstate.edu

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

**Footnotes:**

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

## OBJECTIVES

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

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

3. Identify pre- and postharvest factors affecting flavor deterioration and other shipping quality.

## SIGNIFICANT FINDINGS (year-1)

***Mechanisms of flavor deterioration***

1. Bland flavor mainly results from the losses of malic acid and possibly some volatile organic acids, but it is not correlated with sugars. Malic acid is the main substrate of respiration in sweet cherries.

2. The development of bitter taste and IB is positively correlated with cell membrane lipid peroxidation and an imbalance between oxidative and reductive processes.

3. Ethanol is an accurate indicator of anaerobic respiration in sweet cherries.

***Predictors for cherries with long postharvest flavor life***

4. Postharvest flavor life was positively correlated with fruit tissue contents of Ca, K, B, and soluble solid content (SSC), titratable acidity (TA), and total antioxidant capacity (TAC).

5. Postharvest flavor life was not found to be associated with fruit respiration rate, N, P, Mg, S, and other micronutrients.

***Factors affecting flavor deterioration and other shipping quality***

6. From one orchard, ‘Sweetheart’ had a longer postharvest flavor life than ‘Lapins’ and ‘Skeena’.

7. Harvest maturity affected flavor deterioration. Late-harvest fruit were more susceptible to flavor deterioration, softening, pitting, pedicel browning, and luster loss.

8. In controlled atmospheres (CA) storage, $O_2$ 5-8% maintained higher TA and better flavor than $O_2$ 10-21% in ‘Lapins’, ‘Sweetheart’ and ‘Regina’ during 5 weeks at 32°F.

9. Ultraperf MAP liners with an equilibrated $O_2$ 7-8% + $CO_2$ 8% maintained better flavor of cherries than that with an equilibrated $O_2$ 11-13% + $CO_2$ 7% during 5 weeks at 32°F.

10. A rapid forced-air cooling immediately after packing was extremely important for maintaining TA and flavor and other shipping quality during subsequent storage/shipping.

**METHODS**

***1. Understand mechanisms of flavor deterioration of cherry after 3-5 weeks of cold storage.***
   a. Changes of carbohydrates: SSC, TA,
   b. Changes of volatile aroma compounds: GC, GC-MS (mass spectrometry)
   c. Dynamic of TAC, individual antioxidants, MDA, TP, PPO, POD, O-quinones
   d. Correlate the physiology and biochemistry with sensory trained panel evaluations

***2. Determine predictors for cherries with long postharvest flavor life.***
   a. Fruit were collected from 5-10 orchards with distinct flavor deterioration during shipping.
   b. Measure fruit quality parameters: SSC, TA, TAC, individual antioxidants
   c. Measure fruit nutrition: macro (N, P, K, Ca, Mg, S) and micro (B, Fe, Zn, …) elements
   d. Correlate flavor life with theses quality attributes.

***3. Identify pre- and postharvest factors affecting flavor deterioration.***
   a. Cultivars
   b. Harvest maturity: based on ctifl (4, 5, 6, 7)
   c. Pre- and post-harvest Ca and GA3 treatments
   d. Controlled atmosphere storage (CA): $O_2$ 2, 5, 8, 10, 15%; $CO_2$ 10-15%
   e. MAP liners: low, medium, and high gas permeability
   f. With and without post-packing forced-air cooling
   g. Storage/shipping temperature: 32, 34, 36°F

**RESULTS**

***<u>1. Mechanisms of flavor deterioration</u>***

1. *Bland flavor*. TA in 'Lapins', 'Skeena', and 'Sweetheart' reduced significantly during 5 weeks at 32 °C. Low $O_2$ at 2, 5, and 8% maintained higher TA and better flavor than $O_2$ at 10, 15, 21% after 5 weeks at 32°F (Fig. 1). SSC didn't change significantly during 5 weeks at 32 °F. As a consequence, bland flavor is mainly a result of the TA loss. The decrease of some volatile organic acids (2-ethylhexanoic acid, octanoic acid, hexanoic acid, butanoic acid, 3-methylbutanoic acid, acetic acid) during storage/shipping might slightly contribute to the bland flavor development (data not shown).

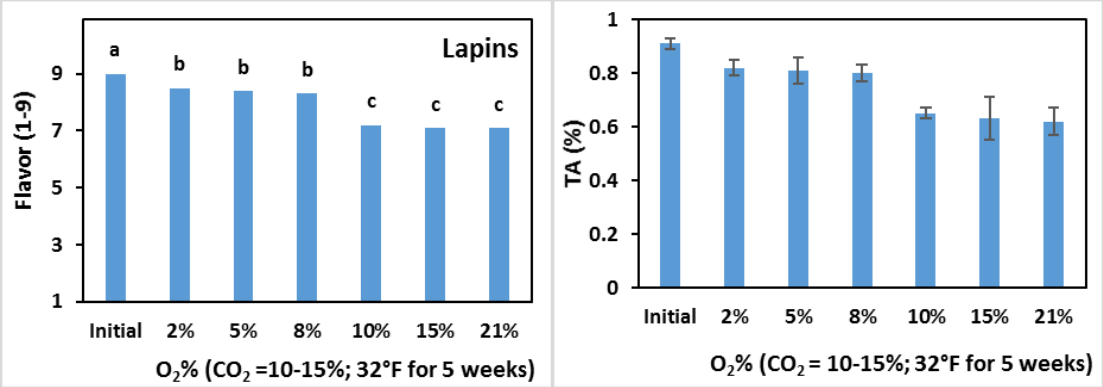



*Fig. 1. Flavor intensity of 'Lapins' cherries affected by $O_2$ concentrations in CA storage after 5 weeks at 32°F.*

Storage temperature affected flavor loss significantly in 'Lapins', 'Skeena', and 'Sweetheart' after 5 weeks at 32°F (Fig. 2). Flavor intensity was positively correlated with TA content after storage/shipping among the temperature treatments of 32, 35, and 38°F.

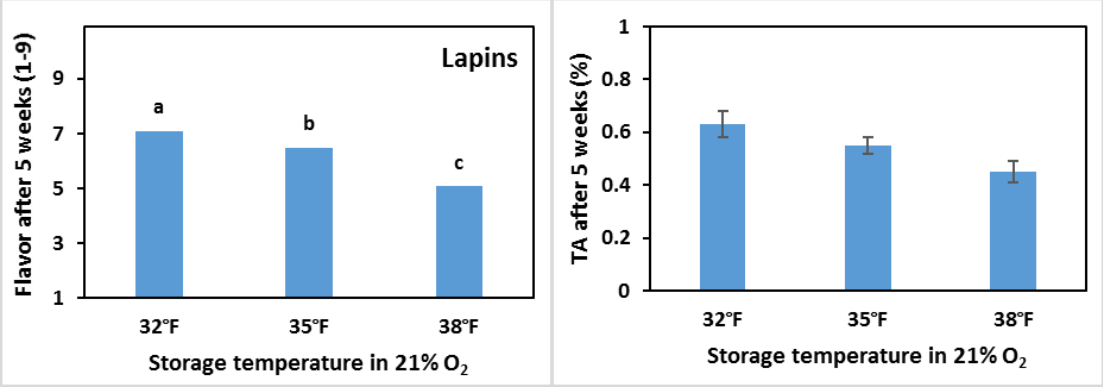
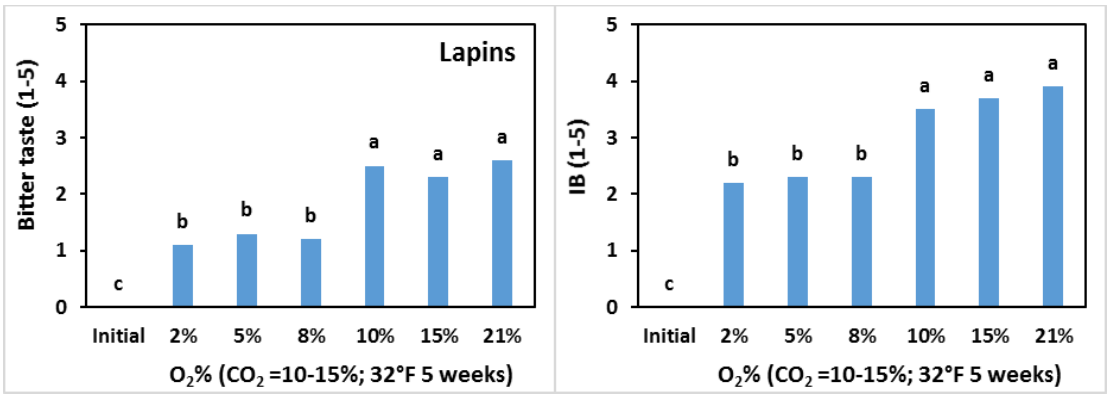
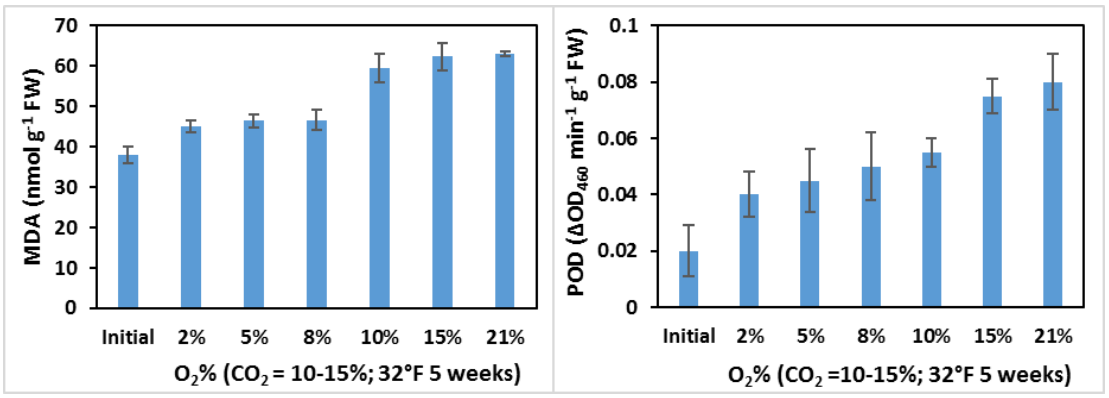


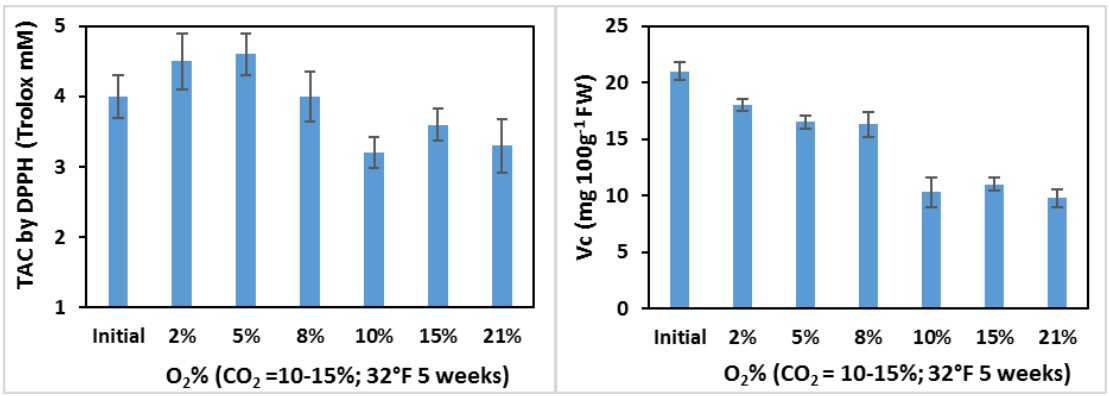

*Fig. 2. Flavor intensity of 'Lapins' cherries after 5 weeks at 32°F affected by storage/ shipping temperatures.*

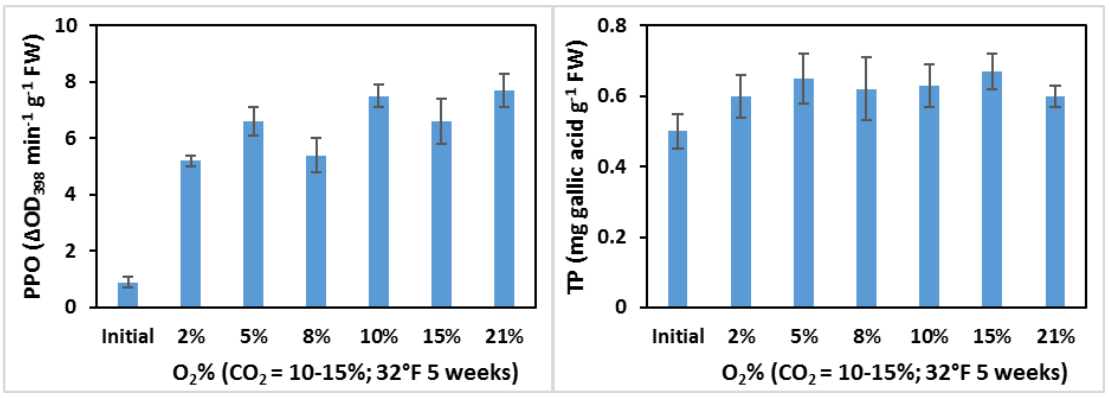
2. IB and bitter taste were developed significantly in one lot of 'Lapins' after 5 weeks at 32°F. $O_2$ at 2, 5, and 8% decreased IB and bitter taste significantly compared to $O_2$ at 10, 15, and 21% after storage/shipping (Fig. 3). The development of IB and bitter taste was correlated positively with malondialdehyde (MDA) and peroxidase (POD) activity, and negatively with the total antioxidant capacity (TAC), and Vc content. Interestingly, neither polyphenol oxidase (PPO) activity nor total polyphenol (TP) content was associated with IB.

3. Ethanol was confirmed to be a good indicator for anaerobic respiration in cherries (Fig. 3).


Lapins
Bitter taste (1-5)
IB (1-5)
Initial 2% 5% 8% 10% 15% 21%
O2% (CO2 =10-15%; 32°F 5 weeks)


MDA (nmol g-1 FW)
POD (ΔOD460 min-1 g-1 FW)
Initial 2% 5% 8% 10% 15% 21%
O2% (CO2 = 10-15%; 32°F 5 weeks)


TAC by DPPH (Trolox mM)
Vc (mg 100g-1 FW)
Initial 2% 5% 8% 10% 15% 21%
O2% (CO2 =10-15%; 32°F 5 weeks)


PPO (ΔOD398 min-1 g-1 FW)
TP (mg gallic acid g-1 FW)
Initial 2% 5% 8% 10% 15% 21%
O2% (CO2 = 10-15%; 32°F 5 weeks)

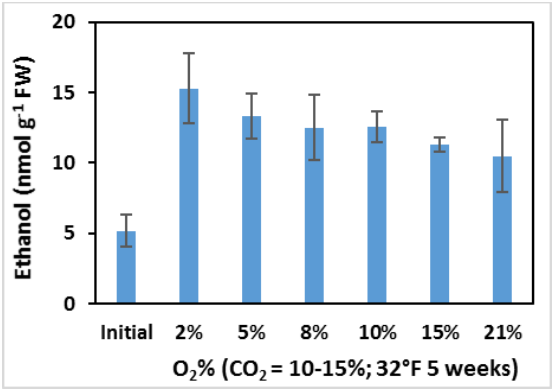
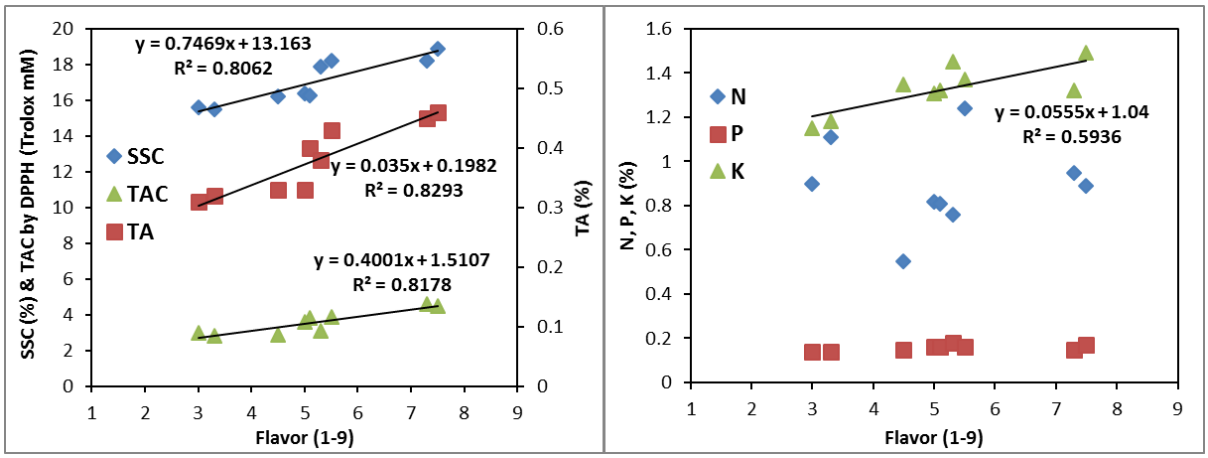



*Fig. 3. The mechanisms of IB and bitter taste development in 'Lapins' after 5 weeks at 32°F.*

## ***2. Predictors for cherries with long postharvest flavor life***

'Skeena' from 9 orchards with varied postharvest flavor life were sampled for quality evaluation and fruit nutrition measurement. Fruit were packed in MAP liners with equilibrated $O_2$ at 10-13% and $CO_2$ at ~8% and stored at 32°F. Results indicated that flavor intensity after 5 weeks at 32°F was positively correlated with FF, SSC, TA, TAC, and Ca, K, B. Fruit respiration rate, N, P, Mg, S, and other micronutrients were not found to be associated with fruit postharvest flavor life (Fig. 4). The flavor deterioration (bland flavor, bitter taste and IB) was positively correlated with MDA accumulation and POD activity, and negatively correlated with TAC and Vc (Fig. 4).



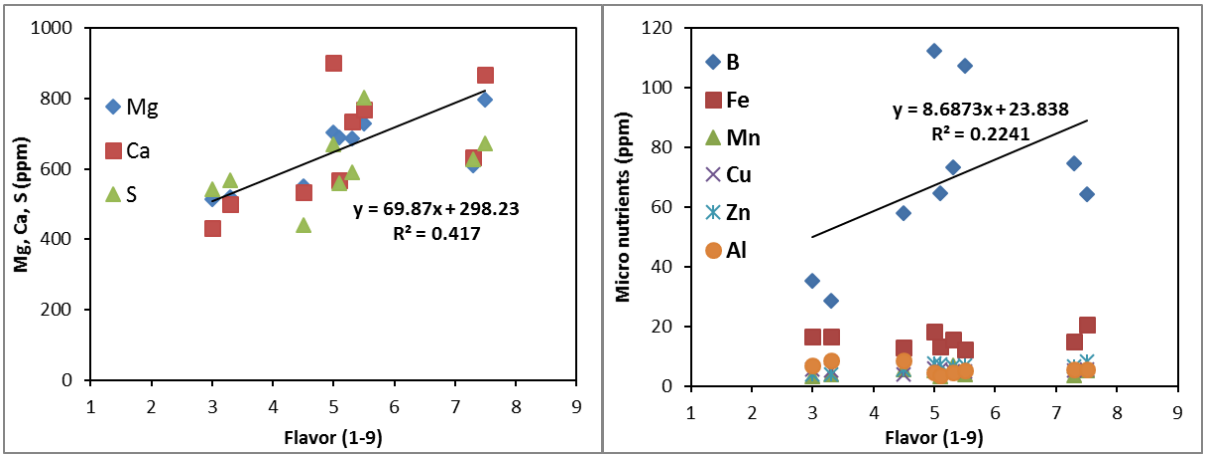



*Fig. 4. The relationship of flavor intensity after storage/shipping with fruit quality attributes and nutrition in 'Skeena' cherries from 9 orchards.*

## ***3. Factors affecting flavor deterioration and other shipping quality***

1. **Cultivar.** From one orchard, 'Sweetheart' kept a higher TA and better cherry flavor than 'Lapin' and 'Skeena' after 5 weeks at 32 °C. Data will be presented next year.

2. **Harvest maturity.** 'Lapins' and 'Regina' fruit were harvested when average skin color (ctifl) was 4.0, 5.5, 6.5, or 7. Compared to early harvests, the late harvested fruit had lower TA and sensory flavor score and higher bitter taste and IB scores after 5 weeks at 32°F. Late harvested fruit were more susceptible to flavor deterioration, softening, pitting, pedicel browning, and fruit skin luster loss. Data will be presented next year.

3. **Pre-harvest $GA_3$ application rates.**

4. **Pre- and post-harvest calcium treatments.**

5. **MAP.** Ultraperf MAP liners with low, moderate, and higher gas permeability were tested on flavor and shipping quality of 'Lapins', 'Sweetheart', and 'Regina' (Fig. 5). Compared to the control, a MAP liner with an equilibrated $O_2$ 7-8% + $CO_2$ 8% kept a higher TA and flavor score and inhibited the development of IB and bitter taste in 'Lapins', 'Sweetheart' and 'Regina' during 5 weeks at 32°F. In contrast, the MAP liner with an equilibrated $O_2$ 11-13% + $CO_2$ 7% had a little effect on cherry flavor. Data will be presented next year.

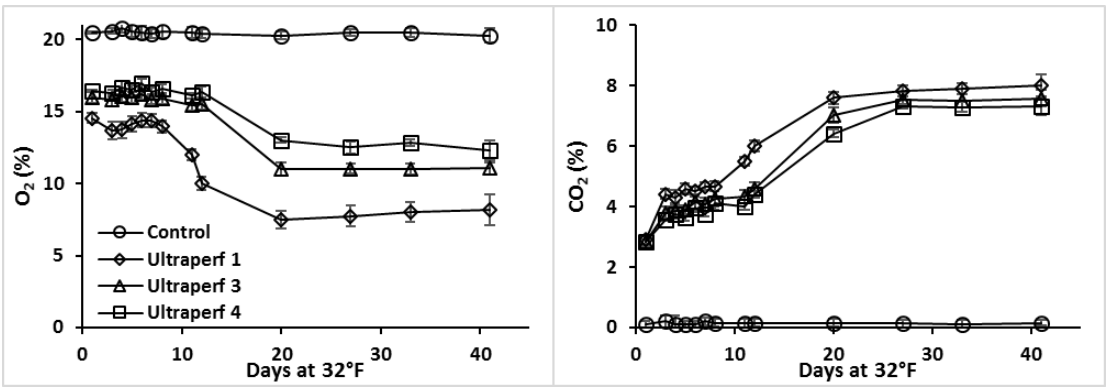



*Fig. 5. $O_2$ and $CO_2$ concentrations in Ultraperf modified atmosphere packaging (MAP) liners with 'Regina' cherries at 32°F.*

6. **Forced-air cooling after pacing.** It was determined that fruit pulp temperature at the time of box-filling in some of the packing houses was at 38-45°F. The pulp temperature at 38-45°F could not be reduced efficiently in the containers at temperature of 32°F due to barrier of the MAP liner for heat exchanging and fruit respiratory heat production within the MAP liner. Compared to 32°F, fruit stored at 38 or 45°F had lower FF and TA and higher stem browning and decay after 4-5 weeks storage/shipping (Fig. 6). Compared to 32°F, fruit stored at 35 and 38°F had inferior flavor after 5 weeks storage/shipping (Fig. 2). Therefore, a rapid forced-air cooling immediately after packing is extremely important for maintaining flavor (TA) (Fig. 2) and other shipping quality for long-distant ocean shipping (Fig. 6).

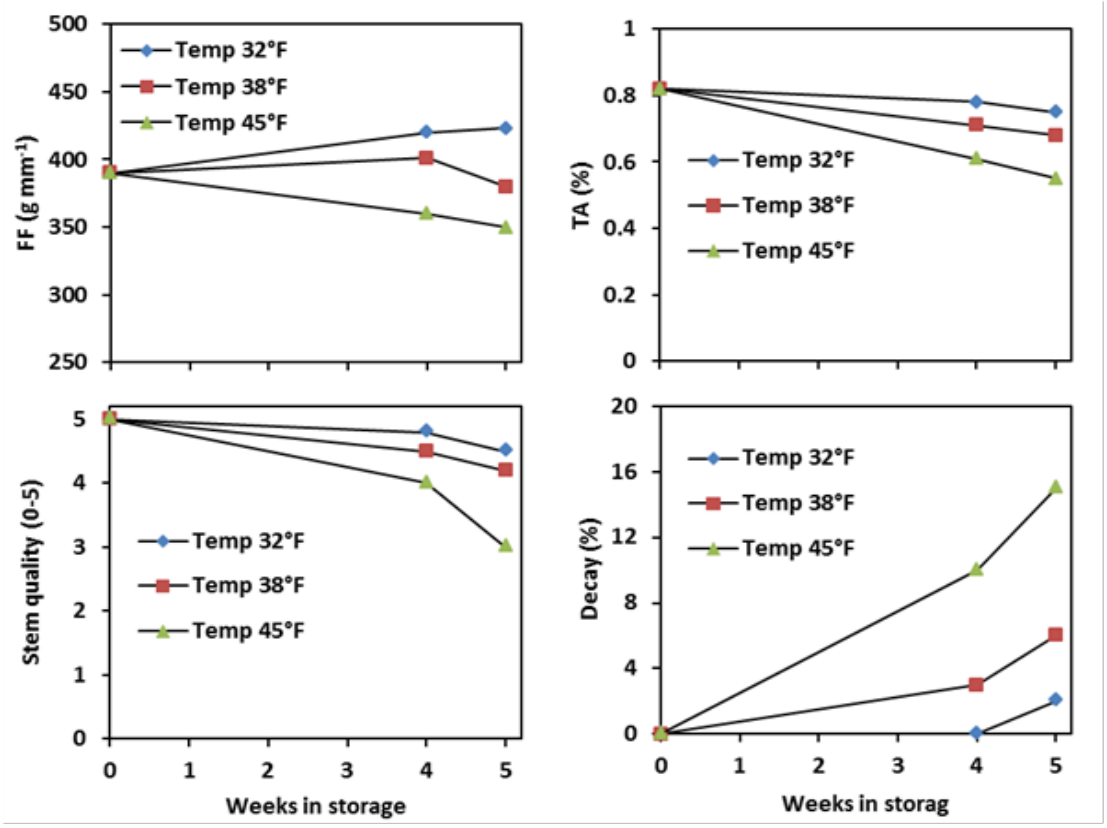



*Fig. 6. Effect of temperatures on fruit quality of 'Lapins' during 5 weeks storage/shipping.*

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

**WTFRC Project Number:** CH-16-103

**Project Title:** Sources of primary cherry powdery mildew inoculum - revisited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**Cooperators**: Ms. Neusa Guerra[3] (WSU-IAREC, Prosser), Washington and Oregon State Growers


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

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

**Other funding sources: None**

**Budget 1**

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

| **Item**[1] | 2016 | **2017** | 2018 |
|---|---|---|---|
| **Salaries** | | | 4620 |
| **Benefits** | | | 1386 |
| **Wages**[4] | 4800 | 4800 | 4800 |
| **Benefits** | 480 | 480 | 480 |
| **Supplies**[5] | 6000 | 6000 | 6000 |
| **Travel**[6] | 2500 | 2500 | 2500 |
| **Total** | 13,780 | **13,780** | 19,786 |

**Footnotes:** [1]Dr.Melba Salazar
[4] Student summer help at $12/ hour (400 hours total)
[5] DNA extraction kits and qPCR supplies, chemicals and supplies for spore germination chambers (Objective 2), Nitex mesh cloth and supplies for inoculations, 'Bing' trees for greenhouse inoculum production
[6] Bi-weekly travels to Oregon and through Washington State to collect cherry fruit at various developmental stages, for flag shoot evaluations and sample delivery OSU-MCAREC

**Budget 2**

**Organization Name:** **OSU-MCAREC** **Contract Administrator:** Russ Karow

**Telephone:** 541-737-4066 **Email address:** Russell.Karow@oregonstate.edu

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

**Footnotes:**

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

**Objectives**

1. Characterize the role of cherry fruit in fungal life and disease cycle
2. Identify cherry volatiles and characterize their impact on spore germination
3. Monitor flag shoot like development and post-harvest fruit management in commercial orchards in WA and OR

**Significant Findings**

The fruit-pedicel zone is the preferred infection court for powdery mildew. Making this zone unavailable for the fungus reduced fruit infection by 100%

So far, cherry flag shoots (infected shoots carrying the primary inoculum of the season) have not been found in orchards. However, they do occur in the greenhouse.

Root shoots (water sprouts) are highly susceptible to powdery mildew and are a major source of secondary inoculum throughout the season.

Orchard floor management strategies varied by grower and generally stopped after harvest. Powdery mildew growth on root shoots was abundant during this period. Post-harvest infection of trees was observed.

Thorough root shoot removal should be considered a standard horticultural practice in sweet cherry orchards.

So far, the importance of asexual survival (seed survival) can be considered minor compared to sexual survival (by means of chasmothecia)

**Methods by Objective**

**Objective 1**

**Abscission zone assays.** Observational evidence points toward fruit abscission zone (the area where the pedicel attaches to the fruit) as the preferred powdery mildew infection court. The experiment was designed to establish the importance of this specific region during initiation of fruit infection. Nitex bags were applied to developing fruit clusters (cvs. Bing and Sweetheart, located in the experimental orchard at WSU in Prosser) on April 26. On June 2, sets of cherry clusters were uncovered and the abscission zone was sealed off with a layer of petroleum jelly. Fruits were left uncovered for natural infection to occur. Positive control clusters were left uncovered but without sealing the abscission zone. Negative control clusters will remain covered all season and will not be exposed to powdery mildew spores. To assess natural disease pressure, clusters of naturally infected cherries were also harvested. All cherries were evaluated for powdery mildew incidence and severity at harvest using a 0-3 severity scale.

**Shoot assays.** Infected cherries (cultivars Bing and Sweetheart) were collected after harvest from the heavily infested experimental orchard at WSU in Prosser. Cherries were arranged on a shallow greenhouse tray, covered with orchard soil and buried just below the soil surface in the experimental cherry orchard. The experiment was repeated using Sweetheart cherries grown at a commercial orchard in The Dalles, OR. These seeds will be recovered in March 2017, individually potted and grown in isolation in a greenhouse.

**Objective 2**

**Cherry volatile identification and spore germination assays.** To identify the role of volatile compounds on spore germination, 30 representative fruit from each of 3 representative trees for each cultivar (Early Robin, Chelan, Bing and Sweetheart) were collected in Washington and Oregon mid-May, late May, early and mid-June 2016. These samples were transported to OSU in Hood River, OR, for quality and volatile analyses under supervision of Dr. Yan Wang.

**Objective 3**

**Early season flag shoot assessment.** An intensive assessment of cherry flag shoots was conducted in commercial orchards in Oregon (Hood River and The Dalles) and Washington (Brewster, Chelan, Pasco, Prosser, Wenatchee, White Salmon, and Zillah) beginning the last week of April 2016 and biweekly thereafter until secondary powdery mildew infection was visible on leaves. One-hundred meter transects were evaluated. The number of infected cherry seedlings growing on the orchard floor was recorded. Early season orchard floor management practices were also recorded and compared among growers to elucidate possible impacts on primary inoculum development.

The first and last week in March, orchards were visited and seeds collected from the orchard floor. Here, the orchard was equally divided into 9 blocks. One block was on average 10 rows wide/ 10 trees long. In each block, one tree was selected randomly and seeds collected in the tree periphery. Seeds were placed in Ziploc bags and transported to the lab in Prosser. At WSU, seeds were stored in moistened vermiculite or soil in Ziploc bags or greenhouse trays to encourage germination. Germinated seeds were planted and grown in isolation to observe occurrence of powdery mildew infections.

**Results and Discussion**

**Abscission zone assays.** Bagging with spore impermeable nylon mesh bags of developing cherry clusters was conducted 14 days before natural disease onset. First signs of foliar powdery mildew were noted on May 10 for both Sweetheart and Bing in the experimental orchard at WSU in Prosser. Bags were removed June2. The same day, petroleum jelly was applied to the abscission zone covering the area around the pedicel. Cherries were left hanging on the tree without a bag and evaluated for disease in the first week of July. As can be seen in Table 1, application of petroleum jelly prevented disease development. It has to be noted that the evaluated Bing fruit already passed maturity. An earlier evaluation was not possible due to the delayed fruit infection in 2016 (no disease at Bing harvest). It still has to be evaluated if the fruit-pedicel zone is the preferred infection court because it is easiest for the spores to land and attach or if secondary factors play a role. Despite the delayed powdery mildew disease onset on fruit, Bing cherries became infected around June 24$^{th}$. Seventy-eight per cent of fruit were infected at the beginning of July. Infection of Sweetheart cherries was even more pronounced with 100% of cherries infected in July. The previously bagged UTC was less infected than the natural control due to a shortened exposure time to the fungus.

Shoot assays. In 2015 we were able to grow infected cherry plants from orchard seedlings. In 2016 we were unsuccessful. However, a severe powdery mildew outbreak occurred in a greenhouse in January 2016 (Figure 1). These plants were all grown from seeds that were stored in Vermiculite (+ Captan) and kept in the cold room until germination was induced.

**Objective 2**

**Cherry volatile identification and spore germination assays.** Results from the volatile analysis are

not available yet. We are still waiting on data for fruit that was send to an outside laboratory for analysis

**Table 1: Powdery mildew (PM) development on cherries treated with petroleum jelly**

| Experiment[1] and cultivar | Rep[2] | Severity scale 0 0 %[3] | 1 1-33% | 2 34-66% | 3 >67% | Total number of cherries | infected cherries | PM % Incidence[4] | Avg. PM Severity[5] |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 10 | 0 | 0 | 0 | 10 | 0 | **0a** | **0a**[8] |
| | 2 | 12 | 0 | 0 | 0 | 12 | 0 | **0a** | **0a** |
| BING | 3 | 13 | 0 | 0 | 0 | 13 | 0 | **0a** | **0a** |
| | UTC[6] | 5 | 5 | 0 | 0 | 10 | 5 | **50b** | **0.5b** |
| 2 | 1 | 11 | 0 | 0 | 0 | 11 | 0 | **0a** | **0a** |
| | 2 | 11 | 0 | 0 | 0 | 11 | 0 | **0a** | **0a** |
| BING | 3 | 5 | 0 | 0 | 0 | 5 | 0 | **0a** | **0a** |
| | UTC | 7 | 4 | 2 | 0 | 13 | 6 | **46b** | **0.6b** |
| BING | Natural[7] | 4 | 11 | 3 | 0 | 18 | 14 | **78b** | **0.9b** |
| 1 | 1 | 6 | 0 | 0 | 0 | 6 | 0 | **0a** | **0a** |
| | 2 | 6 | 0 | 0 | 0 | 6 | 0 | **0a** | **0a** |
| SWEETHEART | 3 | 8 | 0 | 0 | 0 | 8 | 0 | **0a** | **0a** |
| | UTC | 6 | 10 | 10 | 4 | 30 | 24 | **80b** | **1.4b** |
| 2 | 1 | 5 | 0 | 0 | 0 | 5 | 0 | **0a** | **0a** |
| | 2 | 16 | 0 | 0 | 0 | 16 | 0 | **0a** | **0a** |
| SWEETHEART | 3 | 12 | 0 | 0 | 0 | 12 | 0 | **0a** | **0a** |
| | UTC | 0 | 14 | 8 | 0 | 22 | 22 | **100b** | **1.4b** |
| SWEETHEART | Natural | 0 | 1 | 12 | 3 | 16 | 16 | **100b** | **2.1b** |

[1] Single tree experiments. One tree per experiment.
[2] Each rep represents one fruit cluster obtained from a single tree
[3] % fruit surface area affected by powdery mildew
[4] Proportion of fruit diseased.
[5] The number is related to the severity scale. E.g. 2 = on average, the percent of fruit surface affected was between 34-66%.
[6] UTC = Untreated control. Previously bagged.
[7] Natural = Natural disease occurrence on cherries (never bagged).
8 Values for a variable within a column followed by a common letter are not significantly different based on Tukey Kramer test (*P=0.05*)


**Objective 3**

**Early season flag shoot assessment.** As documented in earlier studies (Grove and Boal, 1991) no early season flag shoots were detected in any of the orchards surveyed. One orchard had no powdery mildew disease on any host tissue (root shoots, leaves, fruit) throughout the season. Five orchards developed powdery mildew infection after harvest and 3 orchards developed powdery mildew before harvest. All orchard floors had a vigorous occurrence of root shoots (not seedling shoots). Management strategies ranged from frequent or moderate chemical root shoot removal ('burn-back') to general mowing. Overall, the orchards with frequent root shoot removal had the least amount of powdery mildew disease. Mowing seemed less effective since root shoots located close to the base of the trees were not removed. In all cases, orchard floor management stopped shortly after harvest. Root shoots growing during this period frequently (in 8 out of 9 orchards) were infected with powdery mildew. This infection spread to the trees likely resulting in the production of chasmothecia, one documented (Grove & Boal 1991) mean of perennation. The effect of thorough root shoot removal cannot be questioned and should be part of a standard horticultural program in cherry orchards.

To assess if powdery mildew disease occurs on seedlings grown from orchard seeds, seeds were collected in participating orchards and germinated. The number of seeds found on the orchard floor ranged from 3 to 22 seeds per square foot. As can be seen in Table 2, germination rates ranged from 0 to 1% (average 0.35%). This very low germination rate was also observed in the orchard(s), where most growth is root related. Moreover, powdery mildew was not present in any grown seedlings. Taken all of these observations into account, survival of powdery mildew on seeds and the successful

growth of both the seed and the fungus in the spring may be an event with a very low incidence. In other commodities, e.g. hop, a single occurrence of a flag shoot can start a season wide epidemic. However, the sweet cherry powdery mildew fungus also overwinters through the production of chasmothecia, in contrary to the hop powdery mildew fungus endemic in the Pacific Northwest. As results from 2016 suggest, production of chasmothecia may still be the more successful route for the fungus to withstand the winter. Asexual seed borne overwintering can only be considered important, if flagshoots occur before the burst of chasmothecia, a process linked to environmental conditions (temperature, start of irrigation etc.). So far, we have not found sources of powdery mildew before chasmothecia become activated.

**Table 2: Orchard seed assessment**

| Location | State | Cultivar | No seeds collected | No seeds germinated (%) | No infected |
|---|---|---|---|---|---|
| Brewster | **WA** | **Bing** | **2088** | **0.6** | **0** |
| Brewster | **WA** | **Sweetheart** | **1832** | **0.2** | **0** |
| Chelan | **WA** | **Sweetheart** | **825** | **1.0** | **0** |
| Hood River | **OR** | **Sweetheart** | **2301** | **0.04** | **0** |
| Pasco | **WA** | **Bing** | **1347** | **0.2** | **0** |
| The Dalles | **OR** | **Sweetheart** | **2472** | **0.2** | **0** |
| Wenatchee | **WA** | **Sweetheart** | **1551** | **0.0** | **0** |
| Wenatchee | **WA** | **organic Sweetheart** | **254** | **0.8** | **0** |
| White Salmon | **WA** | **Sweetheart** | **2251** | **0.2** | **0** |
| Zillah | **WA** | **Bing** | **4952** | **0.4** | **0** |

**Literature cited**

Grove GG, Boal RJ. 1991. Overwinter survival of *Podosphaera clandestina* in eastern Washington. Phytopathology. 81:385-391.

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

**WTFRC Project Number:** CH-16-104

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


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**Cooperators**: Oregon State Growers: Stacey Cooper (The Dalles), Washington State Growers: Mark Hanrahan (Zillah, WA); Neusa Guerra (WSU Prosser), Marcia Walters (WSU Wenatchee)


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

**Other funding sources:** None

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

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

**Footnotes:**

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

**Objectives**

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

**Significant Findings**

- **93 fungal genotypes have been collected in Washington State and Oregon**
- **Molecular studies based on these genotypes are conducted during the winter**

**Methods**

**Fungal isolate collection**

Commercial orchards in Oregon (Hood River, The Dalles, and Corvallis) and Washington (Brewster, Chelan, Dallesport, Mattawa, Pasco, Prosser, Quincy, Wenatchee, White Salmon, and Zillah) were visited and scouted for powdery mildew starting in May 2016 until September 2016.

**Fungal isolates**

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

**DNA extractions and Primer development**

DNA extractions were performed using the MoBio UltraClean Microbial DNA isolation kit (MoBio Laboratory Inc., Carlsbad, CA) following the manufactures' protocol.

Species specific primers for multiple genes and the mating-type genes are being developed using a primer software and previous publication of genes in related fungal species. Due to the regional

limitations of the cherry powdery mildew, there are not many published or developed primers for *P. clandestina*.

## Results and Discussion

### Fungal isolate collection

Powdery mildew occurrence varied in the visited commercial orchards. Some orchards had powdery mildew infections by mid-May. In other orchards powdery mildew only occurred post-harvest, after the fungicide sprays had subsided. One orchard was free of powdery mildew in 2016 even though the same orchard had a severe outbreak the year before. Fungal isolates were grown in pure culture to ensure the genotypes are from a single isolate.

Overall, we obtained 38 genotypes from Oregon and 55 genotypes from Washington.

### Molecular studies

The project is based on powdery mildew genotypes. In 2016, these isolates were collected. To achieve most results during the cherry growing season, the molecular aspects (which is mainly lab and computer work) were delayed to the off-season. Molecular studies are part of Objective 1 and 2. We are happy to give a detailed report in 2017.

### Objective 3:

This component is part of Year 2 and 3.

**CONTINUING PROJECT REPORT** **YEAR**: 2 of 3 **(No-Cost Extension)**
**WTFRC Project Number:** CH-15-101

**Project Title:** PM viability during postharvest handling of cherry fruit


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


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

**Other funding sources: None**

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

| **Item** | 2015 | 2016 | **2017** |
|---|---|---|---|
| **Salaries**[1] | $ 34,620 | $ 36,005 | No-cost extension |
| **Benefits** | $ 14,887 | $ 15,482 | |
| **Equipment**[2] | $ 5000 | | |
| **Supplies**[3] | $ 6500 | $ 5000 | |
| **Travel**[4] | $ 1000 | $1000 | |
| **Miscellaneous** [5] | $ 500 | $ 500 | |
| **Total** | $ 62,507 | $ 57,987 | 0 |

**Footnotes:**

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

**Objectives**

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

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

**Significant Findings**

- A viability quantitative PCR has been developed and optimized to determine spore viability of the obligate biotrophic fungus *Podosphaera clandestina* causing sweet cherry powdery mildew
- Post-fumigated spores were not able to initiate disease on susceptible cherry leaves
- Hydrocooling does not reduce spore viability BUT reduces the overall spore load on sweet cherry fruit surfaces
- Fumigation success is increased in cherries which were stored at 35F and submerged in hydrocooling water before fumigation
- The fruit pedicle likely serves as a shelter for spores. Fumigation success was slightly better in cherries without pedicles.

**Methods by Objective**

**Objective 1**

A protocol to quantify *P. clandestina* viability has been established. Evaluated parameters included: 1) the assessment of sample filtration (maximum filtration and sample processing volume, finding the best filter membrane and eluent to recover spores from filter membranes), 2) to optimize the viability qPCR protocol by adjusting dark and light incubation time as well as propidium monoazide (PMA) dye concentration to obtain best qPCR efficiencies, 3) to study PMA cytotoxicity, and 4) to optimize DNA extraction method and qPCR protocol for recovery and detection of *P. clandestina* from laboratory and environmental samples. A detailed description of the methodology will be given in the final report.

**Objective 2**

**Spore viability in hydrocooling water.** The chlorinated water (calcium hypochlorite) in the hydrocooler system has been evaluated for its potential to reduce spore viability. Chlorine is a common disinfectant. It takes an average of 3 min for cherries to go through the hydrocooling process. In this report, we refer to this water source as 'hydrocooling water'. Spore viability was determined for inoculum suspensions made with hydrocooling water. Hydrocooling water was obtained from Zirkle Fruit in Prosser and stored below 47˚F. Viability was determined using the PMA qPCR protocol. Briefly, aliquots (4 aliquots*400µl) of the (hydrocooling water) spore suspension were transferred to a 1.5mL eppendorf tubes after 1 min, 15 min, 30 min, 60 min, 75 min, 90 min, 105 min, 2h, 3h, 4h, 5h and 24h. Two aliquots were treated with PMA (25µM, 10 min dark incubation, 20 min LED light exposure) while two aliquots were left untreated. All four aliquots at each time point were subjected to DNA extraction following the manufacturers' protocol (MoBio Laboratory Inc., Carlsbad, CA) and subjected to qPCR using *P. clandestina* specific primers.

**Fumigation.** All fumigation experiments were conducted at Zirkle Fruit in Prosser, WA. Chamber values conformed to existing requirements for fumigation of sweet cherries prior to export to Australia: 4.5pounds of Methyl Bromide per 1000 cubic feet or 72 gram/$M^3$. Chamber temperature was held at 47- 52 ˚F. Fumigation duration was 2h and 5 min, and conducted over night. Fumigated fruit became available for post-fumigation assessments the following morning. Fumigation experiments were conducted on 6-18, 6-19, 6-23, 6-28, 6-30, 7-1, and 7-12-2016. All fruit were harvested on the day of fumigation from the experimental orchard at WSU (ROZA) in Prosser,WA. Fruit (cv. Bing) undergoing fumigation before 6-19 showed no visible signs of powdery mildew infection. These fruit would have passed powdery mildew control screens by an inspector. Bings harvested on 6-23 had showed first signs of fruit infection, with incidences below10% and less than 10% surface area infected. Fruit (cv. Sweetheart) undergoing fumigation on 6-28, 6-30, 7-1, and 7-12-2016 were visibly infected with powdery mildew. Fruit infection increased from (on average) less than 33% (area fruit surface) infected by late June to more than 66% (area fruit surface) infected in the beginning of July. Nearly 100% of all fruit in July showed signs of infection. The increase of natural infection which is accompanied by an increase of spore viability can also be seen in Figure 4.

**Pre-fumigation activities.** Fruit samples were harvested before 9am and transported to the laboratory. To simulate various post-handling scenarios, different treatments were applied: **1)** Fruit was held in the laboratory at room temperature until fumigation; **2)** Fruit was stored in the cold room at 35˚F until fumigation; **3)** Fruit was submerged for 3 minutes in hydrocooling water (no cold storage), or **4)** Fruit was stored in the cold room and submerged in hydrocooling water before fumigation.
Fumigation of a single layer of cherries was also compared to fumigation of cherries stored in bulk (such as cherries stored in a commercial bin during fumigation). Therefore fruit were either **1)** arranged in a single layer on a standard greenhouse plant propagation tray, with or without pedicles, or **2)** layered in a perforated box (provided by Zirkle Fruit). Each layer in the box was separated by a fine metal mesh. The box contained up to 6 layers and a total of about 600 fruit. Fruit from each layer was evaluated individually to assess how the positioning of the cherries in the box (top, middle, bottom) influences fumigation success. Spore quantity and viability was assessed before (untreated control) and after fumigation.

**Post-fumigation activities**. Fumigated fruit were picked up at the fumigation chamber before 9am, the morning after fumigation. Each tray or box was immediately placed in a plastic bag to prevent any post-fumigation contamination. Cherries were transported to the laboratory and uncovered in a laminar flow hood. Samples (3 reps x 30 cherries per tray or 2 reps x 30 cherries per layer in the box) were removed under sterile conditions and stored in Ziploc bags until processing. All samples were processed the same day using the following protocol: Fumigated fruit samples (30 fruit/ sample/ rep) were placed inside a 1L glass bottle containing ~300mL sterile 0.001% Tween20 water. Tween20 is a mild detergent used to ensure an equal distribution of spores in a liquid. Bottles were vigorously shaken for 10 min on a platform shaker to dislodge the spores from the cherry fruit surface. The resulting spore suspension was filtered through a 12 micron polycarbonate filter membrane using a vacuum filtration apparatus with a tight fitting graduate funnel to ensure all liquid passes through the membrane. At completion of filtration, the filter membrane was aseptically removed from the apparatus and placed into a 2mL eppendorf tube filled with 800µl of 0.1% Tween20 water. Tubes were vortexed for 2 min to release the spores from the membrane to the water. The resulting spore suspension was equally divided into two aliquots (400µl each). One aliquot was treated with PMA (10µl of the 2mM stock solution, 10 min dark incubation, 20 min light incubation) to assess the number of spores with intact cell membranes (= considered viable spores). The other tube received no PMA treatment to assess the total number of spores (control sample). Both tubes were subjected to DNA extraction followed by qPCR. The same process was repeated for all fumigated samples/ reps.

Additionally, the spore suspension from the untreated tube was used in leaf disk assays to assess if (fumigated) spores can initiate disease on susceptible cherry leaves.

**Results & Discussion**

**Objective 1**

**Viability qPCR parameters.** Based on the results of the experiments conducted, the viability qPCR protocol was adjusted for *Podosphaera clandestina*, the causal agent of sweet cherry powdery mildew. Optimized parameters are:

1. **Filter membrane**: Polycarbonate, 12 micron = only particle sizes of 12 µm and above are retained on the membrane. The advantages of using a filter membrane with a larger pore size are: a) a larger volume of liquid (compared to the traditional 0.2 and 0.45 µm filter membrane) can be processed, and b) naturally occurring co-contaminating microbial cells (with a mean size <12 µm), such as bacteria and some post-harvest pathogens (e.g. Penicillium) are not captured on the membrane. Note: powdery mildew spores have a mean length of 21-30 µm and a mean width of 13 to 15 µm.
2. **Filtration capacity**: depends on the turbidity of the filtered liquid. Turbidity is determined by the amount of biotic (e.g. spores) and abiotic contaminants (e.g. dust) on the cherry surface.
3. **Filtration Apparatus used**: 47mm Stainless Steel, 40/35, 300mL graduated funnel, 1L filtering flask (Glassco).
4. **Eluent** used to dislodge spores from filter membrane: 0.1% Tween water (spore recovery rate above 80%).
5. **PMA dye concentration**: 50 µM (equals 10µl of 2mM PMA stock per 400µl of sample suspension).
6. **Dye incubation conditions**: 10 minutes dark incubation, 20 minutes LED light exposure
7. **DNA extraction and qPCR**: following a standardized protocol.
8. **No cytotoxicity was observed**. Spores treated with various concentrations of PMA (10 to 100µM) had similar growth rates on susceptible leaves than spores that had not been in contact with the PMA dye.

**Objective 2**

**Behavior of powdery mildew spores on orchard fruit.** To observe when powdery mildew actively starts growing on sweet cherry fruit, samples were evaluated for the presence of the fungus and the viability of the spores. 2016 has been an unusual year for powdery mildew. Onset of fruit infection was delayed by approximately 2 weeks. There was no visible infection of Bing fruit at harvest (mid-June). Infection became apparent by the end of June but at this point fruit was already decaying. Sweetheart fruit became visibly infected during the last week of June. Infection spread quickly and heavily. The visual onset of infection and the increase of spore activities (increase in quantities and viability) are linked. There was a 168-fold increase in sporulation (expressed as the amount of DNA quantified by qPCR) between June 18 and July 1-2016 (Figure 1).

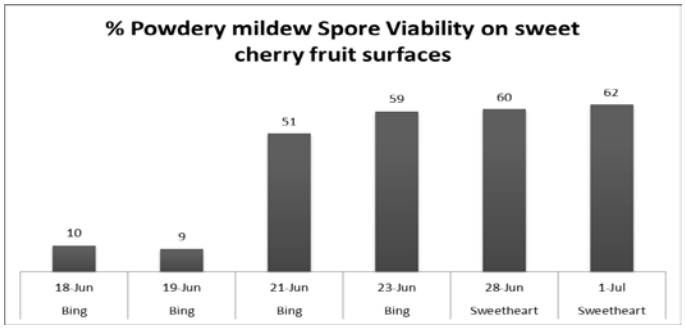


**Figure 1.** Increase of powdery mildew spore viability on sweet cherry fruit surfaces in orchard fruit within 14 days. Only 10% of spores found on sweet cherries were viable on June 18. This number increased to 62% by the beginning of July. This confirms the results of our previous study regarding the prolonged latent period of spores on cherry fruit surfaces. The trigger for the sudden onset of fruit infection is still under investigation.

**Hydrocooling and spore viability.** The average time span for cherries to undergo commercial hydrocooling is about 3 minutes. This study investigated the effect of the chlorine found in the hydrocooling water on powdery mildew spore viability. The results showed that a 24h exposure of powdery mildew spores to hydrocooling water had no significant fungicidal effect. No decrease in viability was noted. However, hydrocooling is a very forceful event during which the fruit is in contact with the chilled water. We replicated the 3 min hydrocooling process in the lab by simply submerging the fruit in chilled hydrocooling water, moving them gently. As a result of this pre-fumigation fruit wash, the spore load on sweet cherry fruit surfaces was drastically reduced. The average reduction was 62.5% (fruit with pedicle) and 81% (fruit without a pedicle). The effect of the hydrocooling water residues, which likely remains on the sweet cherry fruit surface after hydrocooling, on the ability of the fungus to grow and initiate disease on these fruit will be evaluated in 2017.

**Fungicidal effects of methyl bromide fumigation**. Methyl bromide gas is a fumigant used to eliminate post-harvest pests. Methyl bromide is most commonly used in the fumigation of insect infestations and thought to have only a moderate effect on fungi. The effect on powdery mildew survival has not been investigated previously. To assess the reduction in spore viability, the ΔCt is calculated. The Ct (cycle threshold) is defined as the number of cycles required for the fluorescent signal (during qPCR) to cross the threshold of detection. It is a relative measure of the concentration of target DNA in the sample. The lower the Ct level the greater the amount of target DNA (= the greater the amount of spores). The $\Delta C_T$ of a sample is the difference between the Ct-value obtained with PMA treated sample and the Ct-value obtained with the corresponding control sample: $\Delta C_T = C_{T\text{sample w/ PMA}} - C_{T\text{ control sample w/o PMA}}$. $\mathbf{\Delta C_T}$ of **3** is equivalent to a **10-fold reduction** in spore viability ($\mathbf{\Delta C_T}$ of **6 to 9** translates to an approximate **100- to 1000-fold reduction**, etc.). The results of the fumigation experiments are listed in Tables 1 to 4. **Treatment 1):** Fumigation reduced the overall spore viability by approximately 100-fold ($\Delta C_T$ of 6.1 and 5.9 for box and tray, respectively). When looking at the positioning of the cherries in the box, it can be seen that fumigation success in the middle layer (layer 3) was slightly reduced. In this layer, an approximately10-fold reduction ($\Delta C_T$ of 2.6**)** of spore viability was achieved through fumigation. Also, removing the pedicle did not affect fumigation success significantly (Table 1). **Treatment 2):** As noted before, submerging cherries in hydrocooling water reduced the overall spore load on the sweet cherry surfaces. Removing the pedicle before the water treatment likely washed away spores that were sheltered by the pedicle. Fumigation of cherries without the pedicle was slightly more successful in reducing spore viability (about 100-fold decrease) than fumigation of cherries with the pedicle attached to the fruit. This difference was strongest in Bing fruit harvested on 6/23/16. On this day disease incidences were below10% with less than 10% surface area infected by the fungus (Table 2). **Treatment 3):** Only a 10-fold decrease in spore viability was seen in cherries that were stored in the cold room at 35˚F and fumigated in the box. However, spore viability was reduced by at least 100-fold in cold-stored cherries that were fumigated in a single layer (tray). The effect was even more pronounced in cherries without pedicle; here a 1000-fold decrease was achieved (on average) (Table 3). **Treatment 4):** The greatest fumigation success was seen in cherries that were stored in the cold and submerged in hydrocooling water before fumigation. In this scenario, the positioning of the cherries in the box had little influence on fumigation success. For example, the middle layer, which was the least affected by fumigation in the first mentioned scenario, was as successfully fumigated as cherries that made up the top layer. Cherries from these layers had a 10,000-fold decrease in spore viability. Overall, viability of spores on cherry fruit surfaces in the box was decreased by 1000-fold. A similar trend was seen in cherries that were fumigated on trays. Still, removing the pedicle increased the overall fumigation success from 1000-fold to nearly 10,000-fold (Table 4).

| Table 1: No treatment- From harvest to fumigation | | | | | | |
|---|---|---|---|---|---|---|
| **Trial** | **Cultivar** | **Date** | **Position in box** | **Run* 1** | **Run 2** | **Avg Δ $C_T$**** |
| Box - Trial 1 | Bing | 6/18/2016 | Random | 5.2*** | 5.6 | 5.4 |
| Box - Trial 2 | Bing | 6/19/2016 | Top | 5.4 | 6.1 | 5.7 |
| | | | Layer 1 | 6.6 | 11.9 | 9.3 |
| | | | Layer 2 | 5.2 | 3.5 | 4.4 |
| | | | Layer 3 | 4.1 | 1.1 | 2.6 |
| | | | Layer 4 | 5.4 | 7.8 | 6.6 |
| | | | Bottom | 5.8 | 9.8 | 7.8 |
| | | | **Avg Δ $C_T$ /Rep** | **5.4** | **6.7** | **6.1** |
| **Trial** | **Cultivar** | **Date** | **Pedicle status** | **Run* 1** | **Run 2** | **Avg Δ $C_T$** |
| Tray- Trial 1 | Bing | 6/18/2016 | Attached | 4.5 | 4.3 | 4.4 |
| Tray- Trial 2 | Bing | 6/19/2016 | Attached | 8.7 | 7.9 | 8.3 |
| Tray- Trial 3 | Sweetheart | 6/28/2016 | Attached | 3.3 | 3.2 | 3.3 |
| | | | **Avg Δ $C_T$ /Rep** | **6.0** | **5.6** | **5.3** |
| Tray- Trial 2 | Bing | 6/19/2016 | Removed^ | 9.0 | 8.4 | 8.7 |
| Tray- Trial 3 | Sweetheart | 6/28/2016 | Removed | 3.2 | 3.1 | 3.2 |
| | | | **Avg Δ $C_T$ /Rep** | **6.1** | **5.8** | **5.9** |

*Each sample was tested in duplicates in two independent qPCR analyses (Run 1 and Run 2).
** $\Delta C_T = C_{T\text{sample w/ PMA}} - C_{T\text{ control sample w/o PMA}}$: $\Delta C_T = 3$ correlates to a 10-fold reduction, 6 to a100-fold reduction, 9 to a 1000-fold reduction (and so on) in spore viability.
***The reported Δ $C_T$ value is the average of three replicates per sample (per tray or per layer in the box).
^ Pedicle was removed after harvest

Success of fumigation could be determined by the survival of the fungus on a single cherry. While the above mentioned experiments processed a bulk of cherries for each rep and sample, the viability of spores on single cherries was also assessed. These cherries were visibly infected (above 66% fruit surface infection) and fumigated in an open clam shell container in a single layer. Ten fruit were picked at random and subjected to the viability qPCR analyses. The results are presented in Table 5. The average Δ $C_T$ ranged from 4.9 (Cherry 1) to 11.7 (Cherry 2). A trace amount of fungal DNA was still found in cherry 1 but not in any other cherry. Here the reduction in viability was 100%. A similar experiment was conducted on cherry fruit that was fumigated in the box (Table 6). Three random cherries per layer were removed and subjected to viability qPCR. Δ $C_T$ ranged from 4.3 to 10. Puzzlingly, cherries obtained from layer 3 showed the greatest reduction in spore viability. Trace amounts of DNA were detectable in all cherries, meaning a small percentage of spores still had an intact cell membrane and are hence considered viable. Methyl bromide fumigation did not eliminate all viable spores. But (!), leaf disk assays clearly showed that fumigated spores were not able to grow and reproduce on susceptible host material. All conducted post-fumigation leaf disk assays resulted in 0% disease. The reason is unknown but it can be speculated that methyl bromide gas may inactivate these spores by means other than compromising the cell membrane (which is the change that is measured by the viability qPCR). The DNA binding ability of the gas may be one possibility.

| Table 2: Cherries were submerged in hydrocooling water before fumigation | | | | | | |
|---|---|---|---|---|---|---|
| **Trial** | **Cultivar** | **Date** | **Pedicle status** | **Run* 1** | **Run 2** | **Avg Δ $C_T$**** |
| Tray- Trial 1 | Bing | 6/23/2016 | Attached | 2.6*** | 2.5 | 2.5 |
| Tray- Trial 2 | Sweetheart | 6/28/2016 | Attached | 6.4 | 6.3 | 6.3 |
| Tray- Trial 3 | Sweetheart | 6/30/2016 | Attached | 10.2 | 9.3 | 9.8 |
| | | | **Avg Δ $C_T$ /Rep** | **4.5** | **4.4** | **4.4** |
| Tray- Trial 1 | Bing | 6/24/2016 | Removed^ | 7.4 | 7.4 | 7.4 |
| Tray- Trial 2 | Sweetheart | 6/28/2016 | Removed | 4.2 | 4.4 | 4.3 |
| Tray- Trial 3 | Sweetheart | 6/30/2016 | Removed | 10.0 | 9.2 | 9.6 |
| | | | **Avg Δ $C_T$ /Rep** | **5.8** | **5.9** | **5.9** |

| Table 3: Cherries were stored at 35˚F before fumigation | | | | | | |
|---|---|---|---|---|---|---|
| **Trial** | **Cultivar** | **Date** | **Position in box** | **Run* 1** | **Run 2** | **Avg Δ $C_T$** |
| Box - Trial 1 | Bing | 6/23/2016 | Top | 2.3 | 2.6 | 2.5 |
| | | | Layer 1 | 1.5 | 2.1 | 1.8 |
| | | | Layer 2 | 2.7 | 2.7 | 2.7 |
| | | | Bottom | 4.5 | 4.6 | 4.6 |
| | | | **Avg Δ $C_T$ /Rep** | **2.7** | **3.0** | **2.9** |
| **Trial** | **Cultivar** | **Date** | **Pedicle status** | **Run* 1** | **Run 2** | **Avg Δ $C_T$** |
| Tray- Trial 1 | Sweetheart | 6/30/2016 | Attached | 6.9 | 6.5 | 6.7 |
| Tray- Trial 2 | Sweetheart | 7/12/2016 | Attached | 6.1 | 6.2 | 6.2 |
| | | | **Avg Δ $C_T$ /Rep** | **6.5** | **6.4** | **6.4** |
| Tray- Trial 1 | Sweetheart | 6/30/2016 | Removed^ | 4.4 | 4.4 | 4.4 |
| Tray- Trial 2 | Sweetheart | 7/12/2016 | Removed | 14.2 | 14.1 | 14.2 |
| | | | **Avg Δ $C_T$ /Rep** | **9.3** | **9.3** | **9.3** |

*See Table 1 for explanation

| Table 4: Cherries were stored at 35˚F & submerged in hydrocooling water before fumigation | | | | | | |
|---|---|---|---|---|---|---|
| **Trial** | **Cultivar** | **Date** | **Position in box** | **Run* 1** | **Run 2** | **Avg Δ $C_T$**** |
| Box - Trial 1 | Sweetheart | 7/1/2016 | Top | 12.5*** | 14.9 | 13.7 |
| | | | Layer 1 | 9.3 | 8.4 | 8.8 |
| | | | Layer 2 | 6.5 | 5.0 | 5.7 |
| | | | Layer 3 | 11.6 | 11.9 | 11.7 |
| | | | Layer 4 | 8.4 | 8.6 | 8.5 |
| | | | Bottom | 6.6 | 6.8 | 6.7 |
| | | | **Avg Δ $C_T$ - Box** | **9.1** | **9.2** | **9.2** |
| Box - Trial 2 | Sweetheart | 6/30/2016 | Top | 2.4 | 3.8 | 3.1 |
| | | | Layer 1 | 8.9 | 10.2 | 9.6 |
| | | | Bottom | 11.3 | 8.7 | 10.0 |
| | | | Avg Δ $C_T$ - Box | 7.5 | 7.6 | 7.5 |
| **Trial** | **Cultivar** | **Date** | **Pedicle status** | **Run* 1** | **Run 2** | **Avg Δ $C_T$** |
| Tray- Trial 1 | Sweetheart | 6/30/2016 | Attached | 10.2 | 9.3 | 9.7 |
| Tray- Trial 1 | Sweetheart | 6/30/2016 | Removed^ | 10.0 | 9.2 | 9.6 |
| Tray- Trial 2 | Sweetheart | 7/12/2016 | Removed | 13.0 | 13.0 | 13 |
| | | | Avg Δ $C_T$ - Tray | 11.5 | 11.1 | 11.3 |

*see Table 1 for explanation

| Table 5: Spore viability assessment of 10 single cherries fumigated in an open clam shell container | | | | |
|---|---|---|---|---|
| 6-28-16 | **CV. Sweetheart** | **qPCR run 1** | **qPCR run 2** | **Avg Δ $C_T$** |
| | Cherry 1 | 5.1 | 4.6 | **4.9** |
| | Cherry 2 | 12.6 | 10.8 | **11.7** |
| | Cherry 3 | 11.4 | 10.5 | **10.9** |
| | Cherry 4 | 10.0 | 9.3 | **9.7** |
| | Cherry 5 | 9.2 | 5.0 | **7.1** |
| | Cherry 6 | 7.8 | 6.8 | **7.3** |
| | Cherry 7 | 7.7 | 7.5 | **7.6** |
| | Cherry 8 | 7.5 | 7.4 | **7.4** |
| | Cherry 9 | 10.5 | 10.0 | **10.3** |
| | Cherry 10 | 8.2 | 8.7 | **8.4** |

| Table 6: Spore viability of single cherries fumigated in different layers in the box (cv. Sweetheart) | | | | | |
|---|---|---|---|---|---|
| 7-1-16 | **Position in box** | **Cherry 1*** | **Cherry 2** | **Cherry 3** | **Avg Δ $C_T$** |
| | Top | 7.2 | 9.6 | 6.4 | **7.7** |
| | Layer 1 | 1.1 | 8.9 | 2.7 | **4.3** |
| | Layer 2 | 8.4 | 8.7 | 9.4 | **8.8** |
| | Layer 3 | 9.4 | 10.2 | 10.4 | **10.0** |
| | Layer 4 | 6.2 | 9.7 | 8.3 | **8.1** |
| | Bottom | 6.0 | 10.2 | 3.3 | **6.5** |

* 3individual cherries were analyzed for spore viability per layer. The Δ $C_T$ value is the average of the results from 2 qPCR runs.

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

**Project Title:** Cherry Virus Diagnostic Survey of Oregon

**PI**: Jay W. Pscheidt, Extension Plant Pathology Specialist
**Organization**: Oregon State University
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**City/State/Zip**: Corvallis/Oregon/97331-2903
**Cooperators**: Lynn Long (The Dalles), Drew Hubbard (The Dalles and Hood River), Nik Wiman Willamette Valley), Sue Root (Willamette Valley), Clive Kiser (Milton Freewater), Steve Renquist (Roseburg), TBD for the Rogue Valley and Russ and Mary West (3H Ranch) for the Grand Ronde Valley.

**Total Project Request: Year 1:** $8,439 **Year 2: $34,626**

**Other funding sources**
**Agency Name:** Department of Botany and Plant Pathology

**Amt. awarded:** $25,828
**Notes:** Graduate student Lauri Lutes has been accepted and awarded a teaching assistantship at OSU. This covered the first 3 quarters of the 2015-2016 school year but did not include the 4th summer quarter. Tuition (4,050 per quarter) has been subtracted from this amount.

**Agency Name:** OSU Extension Service
**Amt. requested:** $2,000
**Notes:** Annual discretionary statewide travel funds can be used to get to sampling sites.

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

**WTFRC Collaborative expenses: None**

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

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

**Footnotes:**
*Anticipating 2% increase for 2016-2017 school year.

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

**Objectives**

The overall goal is to document which cherry viruses occur in various Oregon geographic regions. This will allow us to assess the threat viruses may (or may not) pose to the Oregon cherry industry (and indirectly to the WA industry). Original objectives included:

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

Guided by our cooperators, we were able to investigate and sample orchards in the Willamette Valley, Hood River, The Dalles and Umpqua (Roseburg) areas. We will continue with Objectives 1-3 in **all regions** next spring with some emphasis on areas we did not get to including the Rouge Valley (Medford), Milton-Freewater, and Grande Ronde Valley (LaGrande) areas. Additional orchard sampling, confirmation and monitoring is needed in The Dalles area.

**Significant Findings**

Viruses new to North America, the USA or the PNW: **None,** so far. (This is a good thing!)

Viruses new to Oregon:

- *Tobacco ringspot virus*
- *Cherry leaf roll virus** (Needs confirmation)
- *Little cherry virus 2**

*Can have significant impact on cherry production.

Viruses new to specific areas in Oregon:

**Willamette Valley**

- *Cherry green ring mottle virus*
- *Cherry mottle leaf virus*
- *Cherry necrotic rusty mottle virus*
- *Cherry twisted leaf-associated virus*
- *Prune dwarf virus*
- *Prunus necrotic ringspot virus*

**Hood River**

- *Prune dwarf virus*
- *Prunus necrotic ringspot virus*
- *Tomato ringspot virus*

**The Dalles**

- *Cherry leaf roll virus*
- *Cherry mottle leaf virus*
- *Cherry necrotic rusty mottle virus*
- *Cherry twisted leaf-associated virus*
- *Little cherry virus 2*
- *Prune dwarf virus*
- *Prunus necrotic ringspot virus*

**Grande Ronde Valley**

- *Cherry mottle leaf virus*
- *Cherry necrotic rusty mottle virus*
- *Cherry rusty mottle virus*
- *Prunus necrotic ringspot virus*
- *Tobacco ringspot virus*

**Milton-Freewater**

- *Cherry rusty mottle virus*
- *Prune dwarf virus*
- *Prunus necrotic ringspot virus*

**Methods**

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

We plan continued visits to cherry growers in all of Oregon's cherry production areas. There will be some emphasis on areas we did not get to last year including the Rouge Valley (Medford), Milton-Freewater, and Grande Ronde Valley (LaGrande) areas. We will continue to use our fieldmen and county agent cooperators as guides to these orchards. Representative growers will be asked about problem trees in their orchards that are likely due to virus. Samples will be collected from these symptomatic trees and nearby trees without symptoms. Samples may include symptomatic leaves, fruit or buds depending on time of year and type of virus suspected. Spring months and near harvest will be critical periods to make visits. Many virus symptoms are best seen either during cool weather in the spring or on fruit at or near harvest. Samples will be either dried rapidly, frozen or RNA extracted, again, depending on the suspected virus.

Based on testing results we may need to resample certain orchards to get fresh samples, confirm the presence of a new virus or monitor for important viruses that were not found. (See results and discussion below.)

**Objective 2**: Determine the most likely cause of these symptomatic cherry trees, virus or not! **Ongoing.**

Samples will be tested for virus via several different methods. Several of the cherry viruses have serological test kits that are available from retail suppliers. Kits will be purchased and utilized in house (generally the cheaper alternative) or samples could be sent for testing to the same supplier. Commercially available serological kits for specific cherry viruses, include: *American plum-line pattern virus, Apple chlorotic leaf spot virus, Apple mosaic virus, Arabis mosaic virus, Cherry leaf roll virus, Cherry rasp leaf virus, Plum pox virus, Prune dwarf virus, Prunus necrotic ringspot virus, Tomato bushy stunt virus, Tobacco ringspot virus,* and *Tomato ringspot virus*.

Virus RNA will be extracted, analyzed with molecular techniques, and compared with virus databases for identification. Published primer sets are available for many of the cherry viruses and some molecular diagnostic services are commercially available for several cherry virus families and genera, including: bromoviridae, closteroviridae, ilarvirus, nepovirus, potyvirus, and tombusvirus.

Public institutions regularly test for some viruses. ODA routinely tests for 5 different viruses including Cherry virus A. The Clean Plant Center Northwest out of Prosser, WA routinely tests for many cherry viruses including: *Little cherry viruses 1* and *2, Apple mosaic virus, Cherry leaf roll virus, Prune dwarf virus, Prunus necrotic ringspot virus,* and *Tomato ringspot virus*.

The objective here is to determine the virus or viruses that may be involved with the symptoms we are shown. For example, there are many biotic and abiotic causes of leaf chlorosis (Putnam 1999). If we do not suspect a virus, then we will continue the diagnostic process to come to the most likely cause. This will include assessment of orchard, tree and/or tissue damage patterns and time development of the damage. Ultimately, some situations may involve sending the correct samples to public laboratories such as OSU's Plant Clinic for a diagnosis of other plant pathogens. Insect and other biotic problems as well as environmental and management problems must all be considered.

Note – We do not want to be presented with every problem that might afflict cherry trees otherwise, we might be at this for decades. Our focus will be cherry viruses and if not, make sure growers are left with a plan to continue the diagnostic process to ultimately figure out the problem.

**Objective 3**: Follow up the diagnosis with management recommendations to each grower/cooperator. **Ongoing.**

Once we know the cause of the problem, we can begin to construct management recommendations for the growers. Virus problems have few after planting management options. Generally, solutions involved leaving the tree alone or tree removal. We can easily develop and deliver fact sheets on each likely problem using the PNW Plant Disease Management Handbook as a guide.

**Objective 4**: Survey historical records for occurrence of cherry viruses in Oregon. **Completed.**

No new activity needed on this objective.

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

See tables below. These will be updated as we generate new information.

## Results and Discussion

### Historical Information

Cherry samples sent into the Oregon State University (OSU) Plant Clinic from 1956 – present for diagnosis of virus problems were compared with published literature (Table 1). Several unpublished virus reports were discovered as well as more accurately knowing where in Oregon these viruses occur (Table 2). *Tobacco ringspot virus* was a new report for Oregon on cherry from the Grand Ronde Valley.

We also inquired about Oregon samples sent to WA laboratories, specifically the Eastwell lab, for virus diagnosis. They confirmed the occurrence of *Cherry green ring mottle virus* both in the Willamette Valley and also Hood River. The important viruses *Cherry leaf roll virus* and *Little cherry virus* 2 were not found by this lab in any samples known to have come from Oregon. Additional specific locations of various viruses can be found in Table 2.

One of our cooperating fieldmen (stationed in Washington) had collected samples for an Oregon grower in The Dalles. These samples were sent to the Eastwell lab for virus diagnosis in 2015. The trees were positive for *Little cherry virus* 2 and were removed from the orchard. We verified tree removal and collected samples from symptomless surrounding trees. All sampled trees were negative for *Little cherry virus* 2. This is the first known report of this virus in Oregon. Continued monitoring in 2017 is recommended.

The OSU Herbarium *Prunus sp.* collection (oldest sample dated 1882) was visually inspected for commonly associated viral symptoms, such as mosaics, ringspots, line patterns or enations. Of the 373 *Prunus sp.* vouchers surveyed in the OSU Herbarium, one, *Prunus emarginata* (bitter cherry) from Douglas County, OR, displayed possible leaf mosaic symptoms. Trips to this location did not

reveal any specific viral symptoms on live *P. emarginata.* Samples collected from many trees in this area were tested for common viruses but all were negative.

**Sample Collections**

Guided by our cooperators, we were able to investigate and sample orchards in the Willamette Valley, Hood River, The Dalles and Umpqua (Roseburg) areas. Because Little Cherry Disease is such a concern, we were guided to several orchards with "small cherries". None of these orchards had symptoms where we suspected Little Cherry Disease and none were positive for *Little cherry virus* 2 via RPA testing (Agdia, Elkhart, IN). Each had other problems contributing to "small cherries" or tree decline including frost, lack of water, bacterial canker, gophers, possible crown gall and root stock fruiting.

Virus-induced cherry decline from *Cherry leaf roll virus* was also a concern but no one was specifically interested in this disease. Several orchards sampled did have some symptoms consistent with this disease. All but one orchard were negative for *Cherry leaf roll virus.* Diagnosis of these problems included: lack of water, bacterial canker, gophers, possible crown gall and Phytophthora root rot. The one positive tree from The Dalles had declined greatly since 2012 to a point that it had a thin, sparse canopy and short internodes resulting in rosetting, mottled and chlorotic leaves, and some dieback of limbs. It was also positive for PDV and PNRSV. Additional samples are needed to confirm and verify this virus identity as well as additional samples from this and surrounding orchards.

Several orchards had trees with enation symptoms on the leaves. These unusual symptoms can be due to different combinations of viruses including *Cherry rasp leaf virus*, *Tomato ringspot virus* and *Cherry leaf roll virus* co-infecting with *Prune dwarf virus*. Although testing of all samples is not complete, *Tomato ringspot virus* was detected in Hood River for the first time.

Many samples were positive for the common, pollen-transmitted viruses P*rune dwarf virus* and *Prunus necrotic ringspot virus.* Due to the easy mode of transmission and age of trees we expected to find these viruses in all geographic locations. Although there were no specific reports of any viruses from the Roseburg area and the few commercial growers were unconcerned about viruses, we easily found trees with symptoms, which tested positive for these viruses.

Paired sampling has been helpful to identify likely viral culprits for symptoms observed. Many paired samples were positive for certain viruses on symptomatic trees while at the same time negative on non-symptomatic, nearby trees. Of concern are paired samples positive for certain viruses on both trees. This could mean that the asymptomatic tree has gone through a shock reaction and has recovered. But it could also mean we have not found the virus responsible for the symptoms. Continued testing for other viruses is necessary to determine the likely cause.

**Significance to the industry**

The occurrence of *Little cherry virus* 2 in The Dalles is concerning but with high alert among everyone in the industry the problem was not only identified early but also eradicated (we hope). Avoiding festering, spreading situations is best for the entire cherry industry.

If the occurrence of *Cherry leaf roll virus* is confirmed, we may have to focus more on tree removal and rootstock selection to limit the spread of this virus. The extent of this viral disease in The

Dalles is unknown. Continued sampling and testing will help give us an idea. Virus-induced cherry decline can significantly shorten the productive life of an orchard and we may have to be more alert (as an industry) for this particular disease.

Knowing that nematode-transmitted virus (such as *Tomato ringspot virus*) have been found in several areas indicated we need to strongly recommend nematode sampling prior to planting especially if new orchards are not scheduled to be fumigated. This can help avoid fields with these vectors in the future.

We know that planting certified virus-free material is the best way to avoid many of these viruses. We still find situations where growers are using untested field stock to propagate new orchards. This will simply perpetuate several yield limiting, tree killing virus problems well into the future.

Table 1. Viruses in Oregon by geographic region based on reports in the literature.

| Name of Pathogen | Presence in Oregon | Region | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | High Rainfall | | Arid | | | | | | |
| | | Willamette Valley | Coastal | Hood River | The Dalles | Umpqua | Rouge | M-F | Grande Ronde | Other |
| *American plum-line pattern virus* | ? (Yes) | ? (+) | | | | | | | | |
| *Apple chlorotic leaf spot virus* | ? | | | | + (EW) | | | | | |
| *Apple mosaic virus* | ? (+) | (+) | | | | | | | | |
| *Arabis mosaic virus* | ? | | | | | | | | | |
| *Cherry green ring mottle virus* | Yes | ? (+) | | | | | | | | |
| *Cherry leaf roll virus* | ? | ? (walnut) | | | | | | | | |
| *Cherry mottle leaf virus* | Yes | ? | | | ? | | | ? | ? | |
| *Cherry necrotic rusty mottle virus* | Yes | | | | | | | | | |
| *Cherry rasp leaf virus* | Yes | ? | | + (apple) | | | | | ? | |
| *Cherry rosette virus* | ? (No) | ? | | | | | | | | |
| *Cherry rusty mottle virus* | Yes | + | | | + | | | | | |
| *Cherry twisted leaf-associated virus* | Yes | | | | + | | | | | |
| *Cherry virus A* | Yes | + | | | | | | | | |
| *Hop stunt viroid* | No (?) | ? (hop) | | | | | | | | |
| *Little cherry virus-1* | Yes | + | | | | | | | | |
| *Little cherry virus-2* | ? | | | | | | | | | |
| *Myrobalam latent ringspot virus* | No | | | | | | | | | |
| *Peach latent mosaic viroid* | No | | | | | | | | | |
| *Petunia asteroid mosaic virus* | No | | | | | | | | | |
| *Plum bark necrosis stem pitting-associated virus* | No | | | | | | | | | |
| *Plum pox virus* | No | | | | | | | | | |
| *Prune dwarf virus* | Yes | ? (+) | | | + (EW) | | | | | |
| *Prunus necrotic ringspot virus* | Yes | ? | | | + (EW) | | | ? | ? | |
| *Stocky prune virus* | No | | | | | | | | | |
| *Tobacco ringspot virus* | ? | | | | | | | | | |
| *Tomato ringspot virus* | Yes | + | | | | | | | | |
| X-Disease | Yes | | | | + | | + | + | | + |

Table 2. Viruses in Oregon by geographic region based on reports in the literature and survey work. (Results part of Lauri Lutes' thesis.)

| Name of Pathogen | Presence in Oregon | Region | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | High Rainfall | | Arid | | | | | | |
| | | Willamette Valley | Coastal | Hood River | The Dalles | Umpqua | Rouge | M-F | Grande Ronde | Other |
| *American plum-line pattern virus* | ? (Yes) | ? (+) | | | | | | | | |
| *Apple chlorotic leaf spot virus* | ? | | | | + | | | | | |
| *Apple mosaic virus* | ? (+) | (+) | | | | | | | | |
| *Arabis mosaic virus* | ? | | | | | | | | | |
| *Cherry green ring mottle virus* | Yes | + | | + | | | | | | |
| *Cherry leaf roll virus* | ? (yes) | ? (walnut) | | | ? (+) | | | | | |
| *Cherry mottle leaf virus* | Yes | + | | + | + | | | ? | + | |
| *Cherry necrotic rusty mottle virus* | Yes | + | | + | + | | | | + | |
| *Cherry rasp leaf virus* | Yes | ? | | + (apple) | | | | | ? | |
| *Cherry rosette virus* | ? (No) | ? | | | | | | | | |
| *Cherry rusty mottle virus* | Yes | + | | + | + | | | + | + | |
| *Cherry twisted leaf-associated virus* | Yes | + | | | + | | | | | |
| *Cherry virus A* | Yes | + | | | | | | | | |
| *Hop stunt viroid* | No (?) | ? (hop) | | | | | | | | |
| *Little cherry virus-1* | Yes | + | | | | | | | | |
| *Little cherry virus-2* | Yes | | | | + | | | | | |
| *Myrobalam latent ringspot virus* | No | | | | | | | | | |
| *Peach latent mosaic viroid* | No | | | | | | | | | |
| *Petunia asteroid mosaic virus* | No | | | | | | | | | |
| *Plum bark necrosis stem pitting-associated virus* | No | | | | | | | | | |
| *Plum pox virus* | No | | | | | | | | | |
| *Prune dwarf virus* | Yes | + | | + | + | + | | + | | |
| *Prunus necrotic ringspot virus* | Yes | + | + | + | + | + | | + | + | |
| *Stocky prune virus* | No | | | | | | | | | |
| *Tobacco ringspot virus* | Yes | | | | | | | | + | |
| *Tomato ringspot virus* | Yes | + | | + | | | | | | |
| X-Disease | Yes | | | | + | | + | + | | + |

**CONTINUING PROJECT REPORT** **YEAR**: 2 of 3
**WTFRC Project Number:** CH-15-103

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


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**Total Project Request:** $83,178 Year 1: $27,417 Year 2: $27,630 **Year 3: $28,131**

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

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

| **Item** | 2014 | 2015 | **2016** |
|---|---|---|---|
| **Salaries** | $8,143 | $8,469 | $8,808 |
| **Benefits** | $3,339 | $3,472 | $3,611 |
| **Wages** | $0 | $0 | $0 |
| **Benefits** | $0 | $0 | $0 |
| **Equipment** | $0 | $0 | $0 |
| **Supplies** | $15,935 | $15,689 | $15,712 |
| **Travel** | $0 | $0 | $0 |
| **Plot Fees** | $0 | $0 | $0 |
| **Miscellaneous** | $0 | $0 | $0 |
| **Total** | $27,417 | $27,630 | **$28,131** |

**Footnotes:**
1. 0.20 full time equivalents of a Post-Doctoral Research Associate.
2. Benefits calculated at standard Washington State rates.
3. Expenses over 3 year project period:

| | |
|---|---|
| Acquisition and retention of 84 trees | $16,647 |
| Herbaceous assays (3 species, 20 plants each) | $516 |
| Sample extraction and RT-PCR assays (180) | $6,173 |
| Deep sequence analysis of 10 isolates | $6,000 |
| Aphid colony establishment and maintenance (3) | $3,000 |
| Assay development primers, probes and enzymes | $15,000 |

**OBJECTIVES**:

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

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

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

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

**SIGNIFICANT FINDINGS**

- Virus occurrence within Washington orchards: The luteovirus (named Nectarine stem pitting-associated virus, NSPaV) occurred only in 1.8% of the trees tested (8 out of 450), whereas the fabavirus (named Prunus virus F, PrVF) was detected in 11.7% of the trees tested for the virus (51 out of 450).

- Graft transmission: *P. emarginata* (bitter cherry) and *P. virginiana* (choke cherry) are likely to be not hosts of either NSPaV or PrVF; the graft inoculated trees will be tested for again for further confirmation and other *Prunus* spp. will be evaluated as possible hosts of these viruses.

- Aphid transmission: both black cherry and green peach aphids did not successfully transmit either virus to corresponding *Prunus* hosts (sweet cherry and peach for NSPaV, and sweet cherry for PrVF); the influence of virus concentration in host tissues on aphid transmission, and the efficiency/competency of the two aphid species to transmit either virus will be investigated.

**METHODS**

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

The lack of positive transmission of NSPaV onto *Prunus avium* 'Mazzard' and *P. persica*, and PrVF onto *Prunus avium* 'Mazzard' trees using either black cherry or green peach aphids indicate that either these aphid species are not the right vectors of NSPaV or PrVF or optimum transmission for each virus needs to be worked out. The second possibility will be further investigated.

Aphid transmission studies will be done at different time points during the growth of the source tree for each virus. In this case, virus concentration in the source trees (using samples from the tissue that will be used for aphid feeding) will be measured by quantitative RT-PCR whenever transmission experiment is done; this will provide information on whether aphid transmission is possible even at low virus concentration. Additionally, the concentration of the NSPaV and PrVF in the aphid vectors after virus acquisition will also be measured by quantitative RT-PCR. Tissues from the recipient trees will be sampled at regular intervals and tested for the presence of either virus.

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

*P. emarginata* (bitter cherry) and *P. virginiana* (choke cherry) trees that were graft-inoculated with either bark patches from NSPaV (this year and last years' trees) or PrVF (this years' trees) will be further allowed to grow after one dormant cycle, and tested to check for detectable levels of the corresponding virus. On the other hand, *Purshia tridentata* (antelope bitterbrush) plants (three plants) that were graft inoculated during the previous year (2015) with bark patches from NSPaV positive tree did not survive during the 2016 growing season. These *Purshia* plants were taken within borders of a sweet cherry orchard but establishing these plants in the greenhouse was a challenge, hence, no inoculations for *Purshia* were made during the 2016 growing season. For the following year (2017), established source plants for *Purshia* will be used and graft inoculations will be done for both viruses.

Preliminary graft inoculation studies did not result in positive transmission of either NSPaV or PrVF in both bitter- and chokecherry, indicating that these viruses may have limited host range within *Prunus* spp. Hence, different *Prunus* spp. will be tested as possible hosts of these viruses. Within *Prunus* spp., we have previously shown that NSPaV is graft transmissible to *P. avium* and *P. tomentosa* (Nanking cherry) whereas PrVF could be graft transmitted to *P. avium* and the interspecific hybrid rootstock 'Krymsk6' (*P. cerasus x (P. cerasus x P. maackii*). In addition, PrVF was detected on 'Gisela' rootstocks with parentage consisting of combinations of either *P. cerasus* 'Schattenmorelle', *P. canescens*, or *P. avium*. In this regard, the ornamental cherry *P. serrulata* 'Kwanzan', plum (*P. domestica*), the interspecific hybrid rootstocks 'Krymsk5' (*P. fruticosa x P. lannesiana*), 'Krymsk6', 'Gisela6' (*P. cerasus x P. canescens*) and the 'Mahaleb' rootstock (*P. mahaleb*) will be tested as possible hosts of NSPaV. For PrVF, 'Kwanzan', the interspecific hybrid rootstocks 'Krymsk5' and 'Gisela6', plum, nectarine/peach (*Prunus persica*) and the 'Mahaleb' rootstock (*P. mahaleb*). Graft transmission of both viruses onto apricot (*P. armeniaca*) will be used.

Since successful aphid transmission of either virus onto *Prunus* hosts have yet to be demonstrated, transmission experiments of both viruses onto herbaceous hosts will be done if a competent aphid vector is identified.

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

Potted trials to determine the impact of PrVF and NSPaV on sweet cherry was initiated. Single and dual infections of each of these viruses were investigated in the sweet cherry cultivar 'Bing' grown in either 'Mazzard' or 'Gisela6' rootstock (six trees per treatment). The inoculated trees were kept in the greenhouse. The trees will be allowed to grow and visual observation of symptoms (leaf symptoms, and other growth abnormalities, if present) will be recorded. By the end of next year's growing season (2017), two trees (per host/virus combination) will be initially sacrificed, steamed and the loosened bark peeled to reveal any signs of stem pitting. If stem pitting symptoms are present, remaining trees will also be sacrificed and examined as described, otherwise, the trees will be allowed to grow for additional year to allow more time for possible symptom expression.

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

Sequence information generated by high throughput sequencing (HTS) of additional NSPaV and PrVF isolates (two trees for each virus) will be used. This, along with near full genome sequences of either virus from known positive samples from the Washington virus survey that will be generated, will be used to identify conserved regions within the genome of either virus. The conserved regions will be targeted to design improved primers for RT-PCR detection of each virus. Representative samples, consisting of all positive and selected negative samples, from the Washington survey will be re-tested with the newer primer and results will be compared with the current primers for each virus. In addition, a quantitative RT-PCR will be developed for each virus.

## RESULTS & DISCUSSION

The newly discovered fabavirus and luteovirus in *Prunus* spp. were named Prunus virus F (PrVF) and Nectarine stem pitting-associated virus (NSPaV), respectively. PrVF was originally detected from a sweet cherry tree in Grant County, WA whereas NSPaV was detected from a symptomatic nectarine (stem pitting symptoms in the wood cylinder above the graft union - in the scion part of the tree). NSPaV was further shown to be graft transmissible to sweet cherry (*P. avium*). Based on these findings, we immediately sampled sweet cherry trees from orchards in Yakima and Grant counties (total number of 75 samples) to determine the occurrence of these viruses within these regions. We expanded our survey for these two viruses to include additional sweet cherry growing counties within Washington State (additional 375 samples). NSPaV occurred only in eight out of 450 samples tested (1.8% occurrence, Table 1); these positive trees were detected only in Yakima and Grant county sampled orchards. PrVF, on the other hand, was detected more abundantly in 51 out of the 450 samples (11%

occurrence, Table 1); PrVF positive samples originated from orchards in Yakima, Grant and Chelan counties. Notably, all NSPaV positive trees were also co-infected with PrVF. While these results show natural infection of NSPaV in commercial orchards, all positive trees did not show any visible leaf symptoms but the trees exhibited overall poor growth and slightly reduced fruit size. The latter could be attributed to virus infection or poor orchard management. In order to determine if these two viruses will induce disease, sweet cherry were graft inoculated with either virus, and combination of both viruses ('Bing' on 'Mazzard' and 'Bing' on 'Gisela6' at six trees per virus-rootstock combination); results will be presented on the following year and if necessary, symptomatology (if there is any) will be observed for additional year of growth.

Table 1. Occurrence of Nectarine stem-pitting associated virus (NSPaV) and Prunus virus F (PrVF) within cherry production regions in Washington State.

| **County** | **Number of NSPaV positive samples[1]** | **Number of PrVF positive samples[1]** | **Total number of samples** |
|---|---|---|---|
| Benton | 0 | 0 | 75 |
| Douglas | 0 | 0 | 25 |
| Grant | 8 | 21 | 63 |
| Chelan | 0 | 13 | 175 |
| Yakima | 0 | 17 | 112 |
| **Total** | **8 (1.8)** | **51 (11.3)** | **450** |

[1] Numbers in parenthesis indicate percentage occurrence.

Graft inoculations of either NSPaV or PrVF onto *P. emarginata* (bitter cherry) and *P. virginiana* (choke cherry) did not result in positive detection of either virus by RT-PCR; six trees of each virus/host combination were used. In addition, trees of the same species that were graft inoculated with NSPaV during the 2015 growing season yielded negative results for this virus (note: since PrVF was not in the original proposal, no graft inoculated trees for this virus was done during the 2015 growing season). These results could indicate that NSPaV and PrVF are not graft transmissible to these *Prunus* spp.; however, for a more comprehensive evaluation, all graft inoculated trees of *P. emarginata* and *P. virginiania* will tested for the presence of corresponding virus after additional year of growth. On the other hand, it is possible that both viruses have a limited host range within the *Prunus* genus. In this regard, we will test other *Prunus* spp. as possible hosts for this virus; specifically, the semi-dwarfing cherry rootstocks that are interspecific hybrids of different *Prunus* spp. will be included.

Insect transmission of either virus was initiated using black cherry or green peach aphids. Prior to aphid feeding, virus source trees for either NSPaV or PrVF were tested for the presence of corresponding virus by RT-PCR to ensure that virus was present in detectable amounts in host tissues. Both black cherry and green peach aphids were not able to transmit NSPaV to either sweet cherry (*P. avium* 'Mazzard') or peach (*P. persica* 'Boone County') using two aphid feeding periods, 24hr and 72hr virus acquisition phase (six trees/aphid species/virus acquisition time were used in this experiment). Similarly, positive transmission of PrVF using either aphid species onto sweet cherry was not obtained (six trees/aphid species) using short aphid feeding period; maximum of five minutes was employed for PrVF since fabaviruses (to which PrVF belongs) are not only known to be non-persistently transmitted and previous studies have shown that efficiency of virus transmission of fabaviruses decreases significantly with greater than five minutes acquisition feeding. In addition, since PrVF has only been

confined to sweet cherry, peach/nectarine trees were not included in transmission studies. These results suggest that either species of aphids may not be the appropriate aphid vector species of either NSPaV or PrVF, or it is also that these two viruses are not vectored by aphids. For 2017, we intend to conduct a more thorough investigation on aphid transmission by looking at two factors: (1) the influence of virus concentration in host tissues on aphid transmission and (2) efficiency/competency of the two aphid species to transmit either virus. In addition, all trees that were used in aphid transmission experiment will be tested again for the corresponding virus next year.

Finally, two additional NSPaV trees were sent for high throughput sequencing (HTS); the near full genome sequence of NSPaV from these two trees are currently being completed. Similarly, two additional PrVF positive trees were sent for HTS and near full genome sequences for each PrVF isolate are available. These sequences will be used to identify conserve regions of each virus that will be used to improve the current RT-PCR assay for each virus.

**CONTINUING PROJECT REPORT** **YEAR**: 1 of 3
**WTFRC Project Number:** CH-16-102

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

**PI**: Elizabeth H. Beers
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**Total Project Request:** Year 1: $85,424 **Year 2: $57,932** Year 3: $60,064

**Other funding sources**

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

**WTFRC Collaborative Expenses: None**

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

| Item | 2016 | **2017** | 2018 |
|---|---|---|---|
| **Salaries**[1] | 16,042 | 32,085 | 33,368 |
| **Benefits**[2] | 6,192 | 12,385 | 12,880 |
| **Wages**[3] | 4,922 | 8,364 | 8,699 |
| **Benefits**[4] | 118 | 448 | 467 |
| **Equipment** | 0 | 0 | 0 |
| **Supplies**[5] | 5,000 | 1,000 | 1,000 |
| **Travel**[6] | 1,150 | 1,150 | 1,150 |
| **Miscellaneous** | 0 | 0 | 0 |
| **Plot Fees**[7] | 2,500 | 2,500 | 2,500 |
| **Total** | $35,924 | **$57,932** | $60,064 |

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

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

| **Item** | 2016 | **2017** | 2018 |
|---|---|---|---|
| **Salaries**[1] | 0 | 0 | 0 |
| **Benefits**[2] | 0 | 0 | 0 |
| **Wages** | 31,320 | 0 | 0 |
| **Benefits** | 10,187 | 0 | 0 |
| **Equipment** | 0 | 0 | 0 |
| **Supplies**[3] | 3,411 | 0 | 0 |
| **Travel**[4] | 1,582 | 0 | 0 |
| **Plot Fees** | 3,000 | 0 | 0 |
| **Miscellaneous** | 0 | 0 | 0 |
| **Total** | 49,500 | **0** | 0 |

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

**Objectives**:

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

**Significant Findings:**

- Entrust, Delegate, Exirel and Warrior provided control of SWD in a field trial
- Several compounds, including oil, provided oviposition deterrence
- Dimilin residues appear to either sterilize female SWD or kill larvae in fruit
- Attracticide (GF-102) gave comparable control to airblast under low pest pressure

**Methods**

1. *Control with pesticides*. Previous experience has shown that it is difficult to conduct in-field assessments of SWD efficacy because infestation levels are often inconsistent, which makes efficacy testing challenging; despite this, field tests will be the first priority. However, they will be supplemented with field-lab assays where leaves and fruit are treated in the field and then samples are brought back to the laboratory to be exposed to adult SWD.

*Field trials*. Pesticide trials will be conducted in a research orchard to avoid exposing grower to the risk of crop damage. Pesticides tested will be from a pool of materials with evidence of good efficacy and unregistered candidate materials as space allows. Treatments will be replicated four times in a randomized complete block design. Each plot will consist of a single tree, with 2-3 buffer trees in-row, and 1 untreated buffer row between replicates. A composite pre-treatment sample will establish the level of SWD infestation (if any) of fruit in the block existing before the treatments are applied. Pesticides will be applied with an airblast sprayer calibrated to deliver 100 gpa beginning at the period of fruit susceptibility (straw color) and re-applied at the labeled re-treatment interval until shortly before harvest. Each treatment will be composed of multiple applications of the same insecticide. Samples taken from buffer trees weekly will help establish when natural infestation occurs. A harvest sample will be taken from the treatment trees at a time corresponding to commercial ripeness in terms of firmness, brix, and color, and commercial harvest of the same variety in nearby blocks. The harvest sample will consist of 300-500 fruit/tree (depending on crop load). A subsample of 50 fruit will be inspected for SWD damage immediately after removal from the field. The subsample, along with the remainder of the harvest sample, will be placed in lots of 50 fruit in clear plastic clamshells, and held for 16 days at 21°C to allow development of larvae and emergence of adults. Clamshells will have an absorbent compound (Terra-Sorb) place in the bottom to absorb excess juice generated by fruit decomposition, which can drown emerging adult flies. Clamshells will be check periodically for emerging adults until emergence is complete.

*Field-lab experiments*. If no opportunity for a field experiment presents itself, a field-lab bioassay will be conducted to determine efficacy and longevity of various insecticides against SWD. Candidate insecticides will be applied to bearing cherry trees, spraying as many trees as necessary to produce the appropriate amount of fruit for 5-6 replications and 4-5 post-treatment timings. The insecticides will be applied with airblast sprayers calibrated to deliver 100 gpa. Leaves and fruit will be collected at 1, 3, 7 and 10 days after treatment then brought back to the laboratory for testing. We will place the leaves and fruit in plastic arenas, add adult flies from laboratory colonies and then evaluate mortality at 24 and 48 h. We will also count the number of egg punctures per fruit to assess impacts on oviposition. The fruit will then be held at room temperature to determine if the eggs produce adult flies.

2. *Post-infestation sanitation.* Laboratory studies will be used in concert with field studies to determine which products effectively kill larvae in fruit after an infestation has occurred. While prevention of infestation is the primary goal, if fruit become infested despite control measures, a secondary goal is to prevent further infestation, which could affect neighboring blocks or later-maturing cultivars. Additionally, a simple means of post-harvest sanitation is needed for dropped fruit, as well as fruit that remain hanging in the tree.

The laboratory studies will use untreated, un-infested fruit from a research block, which will be artificially infested using a laboratory colony. Three stages of SWD will be produced for assay: eggs, small larvae (instars 1-2) and large larvae (instars 2-3). After a pre-treatment count of the number of SWD in each fruit, the fruit will be suspended from the lid of a plastic container, and treated with candidate compounds using a laboratory aerosol sprayer. Each bioassay arena will contain five fruit (Fig. 1). The compounds tested will include imidacloprid (Pasada), lambda-cyhalothrin (Warrior II), cyantraniliprole (Exirel), spinosad (Entrust 2SC) and spinetoram (Delegate). All bioassays will include a standard (dimethoate) and an untreated control. Each treatment will be replicated six times, with a replicate consisting of a bioassay arena.

**Fig. 1.** SWD bioassay arena with 5 fruit.

After treatment with pesticides, the lid will be replaced on plastic container, and the SWD reared to the adult stage in a controlled temperature room (6-12 days depending on the stage at time of infestation). The total number of SWD adults produced from the 5 infested fruits will be the measure of efficacy.

The field studies will use a naturally occurring infestation of SWD, or if not available, an infestation created by caging SWD from a laboratory colony on a tree. Prior to treatment, a fruit sample will be taken to determine the stage distribution of SWD in the fruit. This can be controlled to an extent with a cage study, but greater variability will likely occur in a natural infestation. Single tree replicates will be sprayed using an airblast sprayer. A fruit sample (200-400 fruit/tree) will be collected one week after treatment, and incubated to rear SWD in the fruit. The fruit sampled will be divided into lots of 50 to ensure accurate assessment of the number of adults/fruit. As with the laboratory studies, the number of adults emerging successfully from the fruit will be the measure of pesticide efficacy.

3. *Trap-based thresholds*. Previous recommendations for control of SWD have been extremely conservative due to uncertainty on the relationship of trap capture and risk of infestation. To develop an IPM program, this relationship must be established. In order to accomplish this, we will test four regimes to determine their relative success in controlling SWD. We will use the Scentry trap and lure system, as this has provided evidence of higher/earlier capture than other systems. The first regime is a prophylactic spray program, where fruit are sprayed on a weekly basis from the time they reach the straw stage of maturity through harvest. The current threshold of 1 fly/trap will be tested as an action threshold for pesticide treatment. Two other action thresholds (3 and 10 flies/trap) will also be tested.

To provide robust data for these action thresholds, they will be tested over multiple years and sites. Cooperators will be solicited for blocks of ca. 5 or more acres in size. The blocks will be divided into four sections, one for each treatment regime and treatments randomized within blocks. Traps will be placed in the center and two borders of each block, and checked weekly for male and female SWD starting after shuck fall. The prophylactic program will be sprayed at regular intervals (7-10 days) regardless of trap captures. The trigger for the initial spray in the three threshold treatments will be 1,

3, and 10 flies/trap, respectively. All subsequent sprays will target both SWD and WCFF. The sequence of pesticides used for control will be developed in conjunction with cooperators, and tailored to re-treatment and preharvest intervals, using multiple modes of action (pyrethroids, spinosyns, carbamates or organophosphates) for resistance management purposes.

The success of the treatments will be evaluated by the level of fruit infestation. A small fruit sample will be taken just prior to each spray to determine if infestation is occurring. A larger fruit sample (5 lb/plot) will be taken as close to commercial harvest as possible. This fruit sample will be divided into two lots to determine infestation. For the first lot, a subsample will be examined for evidence of infestation, and all fruit will be incubated in lots of 50 to determine infestation as evidenced by adult emergence. The second lot will be crushed using the standard procedure used by the WSDA Inspection Service, and the number of larvae counted. The success of the treatment strategy will be the degree of infestation as determined by these two measures.

**Results and Discussion**

**Obj. 1. Chemical control to prevent infestation**

Columbia View orchard experienced a high level of infestation in a 2015 test and was considered a good candidate for a field trial. The 2015 test of a single application of each material on 5 June provided moderate to good control of further SWD development (Table 1); only Admire Pro appeared to less effective than the other materials tested, although the mean emerged flies was only significantly higher than Warrior. The 2016 test had a similar treatment list, but no damage occurred in any treatment, including the checks.

**Table 1.** Field trial of insecticides against SWD, CV-14, 2015

| | | Fruit harvested 18 June; emergence evaluated 28 June | | | | | |
|---|---|---|---|---|---|---|---|
| Treatment | n | Emerged flies | SEM | | Adults/fruit | SEM | |
| 1.Entrust | 4 | 25.00 | 12.36 | bc | 0.06 | 0.03 | bc |
| 2.Delegate | 4 | 21.00 | 10.78 | bc | 0.05 | 0.03 | bc |
| 3.Exirel | 4 | 28.00 | 11.45 | bc | 0.07 | 0.03 | bc |
| 4.Warrior | 4 | 14.50 | 5.52 | c | 0.04 | 0.01 | c |
| 5.AdmirePro | 4 | 85.75 | 32.47 | ab | 0.21 | 0.08 | ab |
| 6.Check | 4 | 125.25 | 37.02 | a | 0.31 | 0.09 | a |

*Lab trials.* Two lab trials were conducted to examine different approaches to SWD control prior to infestation. The first examined oviposition deterrence by various compounds. Uninfested fruit were suspended from the lids of plastic containers, and then sprayed with a laboratory aerosol mister. Cherry leaves were placed in the bottom of the container to provide humidity. The treated fruit were exposed to 5 female SWD for a 24 h period. Mortality was assessed, and the numbers of ovipositions counted. Flies were removed, and the fruit incubated for 16 days to determine emergence.

Interesting, repellent materials appeared to cause female SWD to lay eggs on the surface of the fruit (external ovipositions) as opposed to the normal insertion of the egg into the fruit flesh. Because there is limited evidence that some of the external ovipositions can survive and develop, we analyzed the total number. All compounds tested reduced total oviposition relative to the check in terms of emerged adults or adults/live female. Only the 10% rate of methyl anthranilate caused appreciable mortality.

**Table 2.** Oviposition deterrence of SWD by various compounds

| Treatment | Internal ovipositions | External ovipositions | Total ovipositions | Dead females (of 5 orig) | Emerged adults | Emerged adults/ live female |
|---|---|---|---|---|---|---|
| Butyl Anthranilate 1% | 5.40 | 1.20 | 6.60 c | 1.00 ab | 2.40 b | 0.71 b |
| Methyl Anthranilate 1% | 9.40 | 1.40 | 10.80 bc | 0.20 ab | 5.00 b | 1.13 b |
| Methyl Anthranilate 5% | 17.00 | 6.20 | 23.20 b | 0.40 ab | 8.20 b | 1.85 b |
| Methyl Anthranilate 10% | 7.00 | 1.20 | 8.20 c | 1.40 a | 5.00 b | 1.34 b |
| Oil 1% | 2.60 | 0.60 | 3.20 c | 0.20 ab | 2.00 b | 0.44 b |
| Check | 47.00 | 0.00 | 47.00 a | 0.00 b | 19.00 a | 3.80 a |

The second trial tested different routes of exposure of two IGR insecticides, Rimon and Dimilin. IGRs typically cause little direct mortality, but can have significant sublethal effects. We tested topical, residual, and *per os* (ingestion) by female flies in plastic arenas. The arenas were constructed as described above, but the pesticides applied at different points to achieve the route of exposure.

All treatments caused a moderate level of mortality (16-40%) with the highest levels tending to be in the Rimon treatments (Table 3). Rimon (topical, residual) had a much stronger oviposition deterrent effect than Dimilin, significantly reducing the number ovipositions/live female. The most interesting effect was Dimilin/residual exposure treatment, in which no adults were produced. This suggests that this material either induces sterility in female SWD (in that the number of ovipositions was unaffected) or kills developing larvae in fruit (both leaves and fruit were treated).

**Table 3.** Oviposition deterrence of SWD by Rimon and Dimilin.

| Treatment | n | % Mortality | Ovipositions/ fruit | Emerged flies | Ovipositions/ live female | Adults/ oviposition |
|---|---|---|---|---|---|---|
| 1.Dimilin/Topical | 5 | 36 ab | 6.16 a | 7.60 a | 1.89 a | 0.94 a |
| 2.Rimon/Topical | 5 | 40 a | 0.92 c | 1.20 bc | 0.23 c | 0.71 a |
| 3.Dimilin/Residual | 5 | 16 bc | 7.32 a | 0.00 c | 1.69 a | 0.00 a |
| 4.Rimon/Residual | 5 | 32 ab | 1.40 bc | 1.20 c | 0.44 bc | 0.56 a |
| 5.Dimilin/*Per Os* | 5 | 24 abc | 3.96 abc | 1.20 bc | 0.90 abc | 0.45 a |
| 6.Rimon/*Per Os* | 5 | 40 a | 5.68 ab | 2.00 abc | 1.47 ab | 0.43 a |
| 7.Check | 5 | 8 c | 4.56 abc | 3.80 ab | 0.92 abc | 1.12 a |

We also tested an attracticidal method (applied with a small ATV-mounted sprayer) in comparison to the standard airblast spray technique. The attracticide (GF-120) has proven very successful and IPM-friendly for control of WCFF, but has provided only suppression of SWD. We scaled up the previous mesocosm (1.5 x 1.5 ft cages with excised cherry branches and fruit clusters) to cages with mature, field-grown trees in the Sunrise 4 'Sweetheart' block. We compared spinosad as an attracticide (GF-120) to spinosad as a foliar spray (Entrust 2SC). To improve the control with GF-120, we increased the rate (4.8x maximum label rate of 20 fl oz/acre) and distributed the bait droplets more evenly throughout the canopy (3 nozzles/side vs 1). The Entrust was applied at

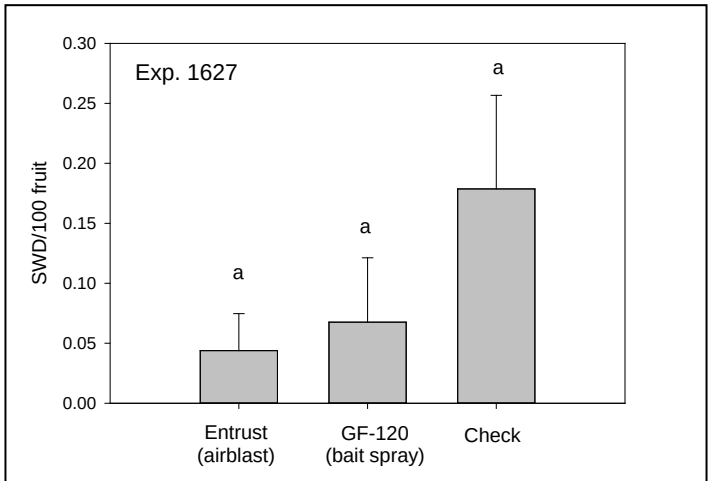


**Fig. 2.** Control of SWD by an attracticide vs airblast spray.

100 gpa with a two-tank plot airblast sprayer (Rears Pak-Blast, Eugene, OR). One week prior to harvest, the pesticides were applied, and adult flies from a laboratory colony (50 males, 50 females) were released into the cages.

Fruit damage was extremely low in the checks ($\bar{x}$=0.0017 SWD/fruit) in comparison to previous experiments (Fig. 2). In previous experiments with similar fly release numbers, we found 0.2 to 0.6 SWD/fruit. While there was a trend for control with both of the spinosad applications, neither was significantly different than the check.

**Obj. 2. Test chemical control products to determine ability to kill early stages of SWD in fruit (years 1-3)**

Several laboratory bioassays were conducted to determine the ability of pesticides to prevent fly emergence from infested fruit. Fruit were collected from an un-infested orchard, and checked for signs of ovipositions or damage; any such fruit were discarded. Fruit were placed in bioassay arenas suspended from the lids of plastic bowls, and exposed to female flies for 16 h. The resulting eggs were incubated for 6 days in order to produce 2 or 3rd instar larvae, and then sprayed with an aerosol mister.

Only Dimethoate reduced the number of flies emerged relative to the check, although Bexar, Warrior and Danitol also suppressed emergence (Table 4). Only BeLeaf appeared to have no effect on developing larvae or subsequent emergence.

**Table 4.** Effect of various insecticides on 2nd-3rd instar larvae in cherry fruit

| Treatment | n | Ovipositions/fruit | | Emerged flies | | Adults emerged/ oviposition puncture | |
|---|---|---|---|---|---|---|---|
| Warrior | 5 | 44.60 | a | 1.40 | bc | 0.04 | b |
| Danitol | 5 | 37.60 | a | 2.20 | abc | 0.10 | ab |
| Dimethoate | 5 | 37.20 | a | 0.00 | c | 0.00 | b |
| BeLeaf | 5 | 38.00 | a | 8.20 | a | 0.19 | a |
| Bexar | 5 | 37.20 | a | 2.20 | abc | 0.05 | b |
| Check | 5 | 36.80 | a | 7.40 | ab | 0.17 | a |

A second bioassay was conducted with an expanded treatment list. All methods were the same except that fruit were exposed to flies for 24 h. The number of adults/oviposition was reduced relative to the check in all treatments except Grandevo, with Delegate providing the numerically greatest suppression (Table 5).

**Table 5.** Effect of various insecticides on 2nd-3rd instar larvae in cherry fruit

| Treatment | n | Ovipositions/fruit | | Emerged flies | | Adults/oviposition | |
|---|---|---|---|---|---|---|---|
| Delegate | 5 | 18.4 | a | 0.2 | e | 0.01 | c |
| Endigo | 5 | 8.2 | b | 0.4 | e | 0.05 | c |
| Entrust | 5 | 16.2 | a | 0.8 | de | 0.05 | c |
| Success | 5 | 17.6 | a | 1.0 | de | 0.06 | c |
| Warrior | 5 | 15.8 | a | 2.0 | cde | 0.15 | bc |
| Exirel | 5 | 16.6 | a | 4.4 | bcd | 0.27 | b |
| Pasada | 5 | 17.0 | a | 5.0 | bc | 0.28 | b |
| Grandevo | 5 | 17.0 | a | 9.8 | a | 0.58 | a |
| Check | 5 | 15.4 | a | 8.0 | ab | 0.50 | a |

**Obj. 3. Test provisional spray thresholds to determine initial and subsequent spray timing in commercial orchards (years 2-3).**

Because of the high pest pressure and record levels of fruit damage in 2015, growers were reluctant risk crop loss in 2016, and no willing cooperators were found to test thresholds. It is ironic that 2016

pressure was very low based on trap captures, packinghouse finds of SWD, and damage in the check plots in WSU research orchards. We continued work on optimizing trapping systems in preparation for threshold tests in 2017.

The 2016 experiments continue work from previous years examining efficacy and selectivity of both traps and lures for SWD. Having a sensitive and reliable monitoring tool for SWD is key to the development of usable thresholds, and their successful implementation.

The Scentry lure continues to be the most attractive lure of the commercial synthetic lures or user-fabricated baits (Table 6). The AlphaScents lure was the next most attractive at these low densities. ACV, Dros'Attract, Suzukii Trap performed poorly at the densities during this time of the year, with the two Trécé lures intermediate. However, the most attractive lures to SWD were also the most attractive to other *Drosophila*, making counting laborious.

**Table 6.** Efficacy and selectivity of SWD lures and baits (PBJ trap body) at low densities (5-26 July, 2016)

| Treatment* | Total SWD | % Female SWD | Other *Drosophila* | % SWD of all *Drosophila* |
|---|---|---|---|---|
| 1.ACV | 0.20 cde | 100.00 a | 14.85 c | 4.45 d |
| 2.Scentry | 2.58 a | 77.89 a | 191.90 a | 1.11 d |
| 3.AlphaScents | 0.85 b | 79.63 a | 70.95 b | 1.10 d |
| 4.Dros'Attract | 0.05e | 50.00 a | 0.65 e | 13.10 bc |
| 5.Suzukii Trap | 0.18 de | 100.00 a | 0.65 e | 25.42 b |
| 6.Trécé High Specificity | 0.40 cd | 88.10 a | 0.45 e | 58.33 a |
| 7.Trécé Broad Spectrum | 0.45 c | 68.57 a | 6.13 d | 6.38 c |

* Trts 1, 4 and 5 are liquid baits; trts 2, 3, 6 and 7 are sachets

The PBJ trap captured significantly more SWD than all other traps, despite the similarity in design to the Scentry and Trécé traps, followed by Trappit Dome and Yellow and White AlphaScents sticky cards (Table 7). Although red is attractive to SWD, the AlphaScents red card caught the fewest SWD of any of the traps tested. The PBJ trap also caught the highest numbers of other *Drosophila*; the yellow sticky card caught the lowest numbers, and as a result, had the highest % SWD. The percentage female SWD was moderate to high. The three sticky cards had more males as in previous tests, an advantage for traps where females are difficult to discriminate from other *Drosophila*. Overall, the AlphaScents yellow and white sticky cards appear to be a good compromise between attractiveness and ease of use.

**Table 7.** Efficacy and selectivity of SWD traps (Scentry lure) at low densities (5-26 July, 2016)

| Treatment* | Total SWD | % Female SWD | Other *Drosophila* | % SWD of all *Drosophila* |
|---|---|---|---|---|
| 1.PBJ | 1.80 a | 78.00 abc | 90.20 a | 2.45 c |
| 2.Scentry | 0.53 cd | 51.04 bcd | 47.68 d | 1.13 d |
| 3.Trécé 2016 | 0.13 e | 100.00 a | 55.53 c | 0.25 e |
| 4.Trappit Dome | 0.93 b | 76.78 ab | 62.53 b | 1.31 cd |
| 5.AlphaScents Yellow | 0.80 bc | 41.27 d | 6.88 g | 9.83 a |
| 6.AlphaScents White | 1.03 b | 46.30 cd | 16.18 e | 4.83 b |
| 7.AlphaScents Red | 0.25 de | 45.00 bcd | 10.25 f | 1.84 c |

* Trts 1-4 are liquid traps; trts 5-7 are sticky cards

**CONTINUING PROJECT REPORT** **YEAR**: 3 of 3 (No Cost Extension)
**WTFRC Project Number: CH-14-106**

**Project Title**: Insecticide resistance of spotted wing drosophila in sweet cherry


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

**Other funding sources: None**

**WTFRC Collaborative Expenses: None**

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

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

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

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

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

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

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

| Item | 2014 | 2015 | 2016 |
|---|---|---|---|
| **Salaries** | 0 | 12,872 | 13,514 |
| **Benefits** | 0 | 84 | 88 |
| **Wages** | 0 | 0 | 0 |
| **Benefits** | 0 | 0 | 0 |
| **Equipment** | 0 | 0 | 0 |
| **Supplies** | 5,000 | 6,408 | 6,230 |
| **Travel** | 0 | 0 | 0 |
| **Plot Fees** | 0 | 0 | 0 |
| **Miscellaneous** | 0 | 5,636 | 5,918 |
| **Total** | $5,000 | $25,000 | $25,750 |

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

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

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

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

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

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

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

***Obj. 2. Develop discriminating doses of insecticides to test susceptibility of SWD populations (yr 1)***
Baseline susceptibility information using a probit bioassay will be generated for candidate insecticides using an SWD population collected in OR in 2009, just after the detection of SWD. Insecticides screened will include Malathion, Sevin, Delegate, Entrust and Warrior. For each insecticide, a minimum of five concentrations will be evaluated which will provide responses between 25 and 95% mortality in addition to two doses that yield 100% mortality. Water will be used as a control. For each concentration there will be a minimum of 40 adult female SWD. Flies will be treated using a Potter Spray Tower, and mortality will be assessed 24 h post-treatment. The probit bioassays will be analyzed using PoloPlus program, and the diagnostic dose calculated as 2x the $LC_{99}$.

***Obj. 3. Complete development of primers for genetic analyses of SWD alleles that confer resistance (yr 1)***
In order to monitor the presence and frequency of mutations that confer target site resistance in *D. suzukii*, populations will be collected from different regions of the Western U.S. PCR-based assays and primers will be developed to amplify genomic regions that are associated with development of resistance. Research in this proposal will focus on: (i) *ace*, which encodes acetylcholinesterase and is a target for organophosphates and carbamates; (ii) *nAC-hR Dα6*, which encodes a subunit of the nicotinic acetylcholine receptor and is proposed as a target for spinosad/spinosyns; and (iii) *para*, which encodes a voltage-gated sodium channel that is a target for pyrethroids. The *D. suzukii* genome has recently been sequenced and annotated a high quality reference gene set, which will greatly facilitate primer design. Genomic DNA will be isolated from individual flies that are collected from the field populations and stored in 95% EtOH. PCR using primer sets that amplify regions covering potential target site mutations will be performed using Accuprime Taq DNA polymerase (Life Technologies, Grand Island, NY) for high fidelity. Resulting PCR products will be purified using PCR purification kits (Qiagen, Valencia, CA) and subsequently submitted for DNA

sequencing at the UC Davis Sequencing Core Facility. Results will be analyzed using sequence alignment packages, e.g., CLC sequence workbench, to determine the presence and allele frequency of nucleotide polymorphisms that might confer insecticide resistance.

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

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

| CA | OR | WA |
|---|---|---|
| N. San Joaquin Coastal | The Dalles | Okanogan Cty |
| | Hood River | Chelan/Douglas |
| | Willamette Valley | Col. Basin |
| | Milton-Freewater | Tri-Cities |

Shaded regions are those from which populations have been collected and screened.

***5. Correlate results from discriminating-dose and genetic studies (yr 2-3)***
We will correlate our genomic data with insecticide bioassays performed on the corresponding fly strains in comparison to the genomic baseline SWD strain.

**Results and Discussion.**

**Obj. 1. Design and test traps to capture live SWD adults for insecticide resistance studies (yr 1)**
A number of trap designs were tested for live capture of SWD. Trap design focused on 1) attracting flies into the trap body; 2) preventing escape; and 3) keeping flies alive until retrieved. Some of the custom-fabricated prototypes were large, and labor-intensive/expensive to produce. The primary difficulty, however, was simply low SWD densities in the orchard. To overcome this, a larger number of traps (up to 100/block) was deployed, and flies were collected over a 1-3 day period. A second technique was found to be a practical means of collecting flies, viz., sweep netting beneath the trees. Where populations were adequate, sufficient flies could be collected in a few hours. The majority of the colonies were started with more than the target number of founding females (100), but a few fell short of this mark (Table 2).

**Table 2.** SWD population information for diagnostic dose screening

| Year | state | Region | Orchard | Mgmt. | Coll. date | No. founding females |
|---|---|---|---|---|---|---|
| 2014 | WA | Orondo | AU | Conv | 10/4/2014 | 199 |
| 2014 | WA | Brewster | GL | Conv | 11/4/2014 | 95 |
| 2014 | WA | Royal City | RZ | Conv | 11/10/2014 | 237 |
| 2014 | WA | Malaga | SN | Conv | 9/16/2014 | 138 |
| 2014 | WA | Stemilt Hill | SH | Org | 10/27/2014 | 113 |
| 2014 | WA | Rock Island | SC | Conv | 10/2/2014 | 106 |
| 2015 | WA | Orondo | CC | Conv | 7/14/2015 | 164 |
| 2015 | WA | Orondo | CO | Org | 8/17/2015 | 145 |
| 2015 | WA | Malaga | SE | Org | 7/20/2015 | 140 |
| 2015 | WA | Rock Island | SC | Conv | 7/28/2015 | 69 |
| 2015 | OR | Hood River | MC | Conv | 7/31/2015 | 135 |
| 2015 | OR | Dallesport | DP | Conv | 9/4/2015 | 125 |
| 2015 | CA | Brentwood | BW | Org | | 11 |
| 2015 | CA | Tracy | TC | Conv | | 9 |
| 2016 | OR | Dayton | ST | Org | 7/14/2016 | 94 |
| 2016 | WA | Brewster | HA | Conv | 9/2/2016 | 68 |
| 2016 | WA | Prosser | OB | Conv | 9/9/2016 | 561 |
| 2016 | CA | Brentwood | BW | Org | | 30 |
| 2016 | CA | Tracy | TC | Conv | | 18 |

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

Discriminating doses (2x the $LC_{99}$) were developed for five pesticides: (Delegate (94.35 mg AI/liter), Entrust (221.24), Sevin (41,272), Malathion (523.58), and Warrior II (109.18), using a standard probit bioassay (Fig. 1). The reference colony used was named 'OSU', which was collected from a blueberry field in the Willamette valley in 2009, shortly after the first detection of SWD in the Pacific Northwest. This colony has been in continuous culture from 2009 until probit bioassays were conducted in 2014-2015.

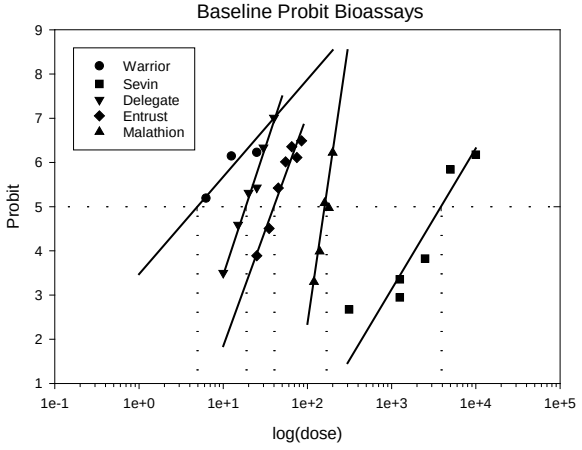


**Fig. 1**. Baseline probit bioassays for five candidate

**Obj. 3. Complete development of primers for genetic analyses of SWD alleles that confer resistance (yr1)**

A total of 12 populations of SWD have been sequenced to date. We have focused the identification of differentially expressed genes (DEG) to those that are known to be involved in conferring metabolic insecticide resistance, e.g., metabolic detoxification (glutathione-S-transferase [GST], cytochrome

P450, and esterase) and reduced cuticle penetrance. Results of these analyses are presented in Objective 5. Bioinformatic analysis is in progress to yield single nucleotide variants (SNVs) in protein coding regions that can confer target-site resistance.

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

To date, 19 populations have been screened against the candidate pesticides (Table 3). For the Washington populations, there were no survivors in the 2014 screenings. Unlike the 2015-16 data, these populations had been in culture 4-6 months versus the 4-7 weeks for the later collections. While the 2014 population screening did not conform to the protocol (collection close to harvest, screening of $F_1$ females), they represent an initial proof of concept for the diagnostic dose procedure. In 2015, however, there were 3 instances of survivorship in the initial screenings; only one population (CY/Org – Delegate) also had a survivor in the repeat screening. A full probit line was run on this population, and while the $LC_{50}$ was slightly lower than the original OSU line (12.6 vs 18.7), the $LC_{99}$ was slightly higher (59.4 vs 47.2).

**Table 3.** Percentage mortality in diagnostic dose screening of five candidate SWD insecticides

| State | Year | Orchard | Regime | Delegate 5.04 oz | Entrust 11.82 fl oz | Malathion 6.99 fl oz | Sevin 34.4 qt | Warrior 5.61 fl oz |
|---|---|---|---|---|---|---|---|---|
| WA | 2014 | AU | Conv | 100 | 100 | 100 | 100 | 100 |
| | | GL | Conv | 100 | 100 | 100 | 100 | 100 |
| | | RZ | Conv | 100 | 100 | 100 | 100 | 100 |
| | | SN | Conv | 100 | 100 | 100 | 100 | 100 |
| | | SH | Org | 100 | 100 | 100 | 100 | 100 |
| | | WB | Conv | 100 | 100 | 100 | 100 | 100 |
| | 2015 | CY | Conv | 100 | 100 | 100 | 100 | 100 |
| | | SC | Conv | 100 | 100 | 97 | 100 | 96 |
| | | SC rep | Conv | | | 100 | | 100 |
| | | CY | Org | 89 | 100 | 100 | 100 | 100 |
| | | CY rep | Org | 99 | | | | |
| | | SN | Org | 100 | 100 | 100 | 100 | 100 |
| | | DP | Conv | 100 | 100 | 100 | 100 | 100 |
| | 2016 | HA | Conv | 100 | 100 | | | |
| | | OB | Conv | 100 | 100 | 100 | 100 | 100 |
| CA | 2015 | BW | Org | 100 | 100 | 100 | 100 | 100 |
| | | TC | Conv | 91 | 97 | 90 | 100 | 100 |
| | 2016 | BW | Org | 100 | 98 | | | |
| | | TC | Conv | | | | | |
| | | GL | Conv | 100 | 100 | 100 | | |
| | | GN | | | 100 | | | |
| OR | 2015 | HR | Conv | 100 | 100 | 100 | 100 | 100 |
| | 2016 | ST | Org | 100 | 100 | 100 | 100 | 100 |

Cells highlighted in yellow had 1 or more survivors. The designation ‘rep’ indicated a screening that was repeated due to survivors.

**Obj.5. Correlate results from discriminating-dose and genetic studies (yr 2-3)**
The goal of this objective is to correlate our genomic data with insecticide bioassays performed on the corresponding fly strains in comparison to the genomic baseline SWD strain, Specifically, we will focus on (1) gene expression changes indicative of metabolic upregulation of detoxification enzymes or genes known to be involved in reducing cuticle penetrance of insecticides; as well as (2) single nucleotide variants (SNVs) in protein coding regions that can potentially confer target-site resistance. Whereas the bioinformatic analysis for SNVs is in progress and is expected to be completed by the end of 2016, we have completed the differential gene expression analysis to identify genes that are up- and down-regulated in the various populations of SWD as compared to the SWD genome strain (Tables 4, 5).

**Table 4.** Number of Up- and Down-regulated genes in SWD populations as compared to the genome strain.

| Strain | Collection Location | Collection Date | Up-regulated | Down-regulated |
|---|---|---|---|---|
| BT | Brentwood, CA | 7/1/2015 | 867 | 1374 |
| TC | Tracy, CA | 9/10/2015 | 690 | 1696 |
| CY | Bray's Landing, WA | 7/14/2015 | 743 | 1331 |
| CYO | Bray's Landing, WA | 8/17/2015 | 767 | 1754 |
| SN | Malaga, WA | 7/20/2015 | 776 | 1726 |
| SC | Rock Island, WA | 7/28/2015 | 544 | 1552 |
| DPt | Dallesport, WA | 9/3/2015 | 1392 | 2264 |
| HR | Hood River, OR | 7/30/2015 | 1063 | 2008 |

**Table 5.** Differential expressions of selected metabolic detoxification genes. Values are $\log_2$(fold_change) compared to the SWD Genome Strain, and only shown if they are significant, i.e., value of +1 = 2-fold increase.

| Strain | Cyp12a4 | Cyp12b2 | Cyp12c1 | Cyp12d1-d | Cyp18a1 | Cyp28c1 | Cyp28d1 | Cyp301a1 | Cyp304a1 |
|---|---|---|---|---|---|---|---|---|---|
| BT | | | | | | | | 0.54 | |
| CY | -0.94 | | -0.60 | -0.74 | | | -0.78 | | 2.76 |
| CYO | -0.55 | -0.96 | -0.57 | | | -1.03 | -0.79 | | 2.25 |
| DP | -0.65 | -1.12 | -0.77 | -0.89 | -0.60 | -1.38 | -1.21 | | |
| HR | -0.45 | -0.91 | -0.58 | -0.51 | | -1.11 | -0.68 | | |
| SN | | | | | | -1.59 | -0.76 | | 2.37 |
| SC | | -0.94 | | | | | | 1.08 | 2.00 |
| TC | -0.46 | -0.85 | -0.73 | | | -1.28 | -0.55 | | 2.78 |

| Strain | Cyp305a1 | Cyp308a1 | Cyp309a2 | Cyp311a1 | Cyp312a1 | Cyp4ac1 | Cyp4ad1 | Cyp4d1 | Cyp4d14 |
|---|---|---|---|---|---|---|---|---|---|
| BT | -0.60 | | -0.80 | -0.64 | | | | -0.75 | 2.10 |
| CY | | | -0.89 | -1.50 | | -0.73 | | -0.70 | 1.45 |
| CYO | -0.63 | | -0.95 | -1.50 | | -0.91 | | -0.59 | 1.74 |
| DP | -1.08 | | -1.48 | -1.65 | | -1.21 | | -0.85 | 1.38 |
| HR | -0.96 | 2.17 | -0.94 | -1.47 | | -0.93 | | -0.57 | 1.94 |
| SN | | 1.64 | | | | | 1.16 | -0.75 | 1.65 |
| SC | | | -0.45 | -1.26 | -3.33 | -0.75 | | -0.96 | 1.61 |
| TC | | | -0.43 | -1.09 | -3.34 | -0.56 | | | 1.81 |

| Strain | Cyp4d20 | Cyp4d8 | Cyp4g15 | Cyp4p1 | Cyp4p2 | Cyp4s3 | Cyp6a13 | Cyp6a14 | Cyp6a20 |
|---|---|---|---|---|---|---|---|---|---|
| BT | -0.66 | -1.82 | 0.77 | | | -0.50 | | 0.58 | |
| CY | -1.39 | -2.05 | | -0.56 | | | | | |
| CYO | -0.97 | -1.28 | | -0.49 | | -0.95 | -0.68 | | |
| DP | -0.88 | -2.21 | | -0.85 | -0.57 | -1.54 | -0.80 | | -0.62 |
| HR | -0.66 | -2.04 | 0.72 | -0.64 | | -1.71 | | | |
| SN | -1.33 | -1.17 | 0.64 | | | -0.71 | | | |
| SC | -0.81 | | 1.01 | | | -0.75 | | | |
| TC | -1.23 | -1.95 | 0.61 | | | -0.53 | | | |

| Strain | Cyp6a22 | Cyp6a23 | Cyp6d4 | Cyp6d5 | Cyp6w1 | Cyp9b2 | Cyp9c1 | Cyp9h1 | Est-6 |
|---|---|---|---|---|---|---|---|---|---|
| BT | -1.12 | | 1.12 | | 0.72 | -0.61 | | | -0.55 |
| CY | -0.94 | | 1.09 | | | | -1.17 | | |
| CYO | | | 0.75 | 0.67 | | | | | -0.79 |
| DP | -0.55 | -0.57 | 0.69 | | | -0.77 | -1.27 | | -1.00 |
| HR | | | 0.76 | | | -0.87 | | -1.09 | -0.80 |
| SN | -0.64 | | 0.73 | 0.72 | | | | | -0.54 |
| SC | -0.64 | | 0.78 | | 0.59 | -0.65 | | | |
| TC | -0.74 | | 0.83 | | | | | | -0.92 |

| Strain | Est-Q | GstZ2 | α-Est1 | α-Est2 | α-Est3 | α-Est8 |
|---|---|---|---|---|---|---|
| BT | -0.93 | | | | | |
| CY | -1.57 | | | | | |
| CYO | -1.44 | | | | | |
| DP | -2.13 | -0.78 | -0.85 | -1.44 | -0.71 | -0.77 |
| HR | -1.33 | -0.82 | | | -0.43 | |
| SN | -2.45 | | | | | |
| SC | -1.00 | | | | | |
| TC | -1.46 | | | | | |

Among all the metabolic detoxification genes, there is only one that shows changes in gene expression that occur in slightly resistant/tolerant populations, as shown in our bioassays. Cyp312a1 is a cytochrome P450 gene whose expression level is reduced by roughly 10-fold in the Spanish Castle and Tracy populations. We performed the same analysis for genes that are involved in regulating cuticle penetrance of insecticides to identify any correlation between SWD populations that are more tolerant to insecticides, and identified 3 genes that are differentially expressed (CCAP-R, Cam, l(3)mbn) (Table not shown).

Additional bioinformatics analysis on this data set is in progress to identify single site mutations (SNVs) that are known to confer insecticide resistance. It would be particularly interesting if we find SNVs in insecticide target genes in the three SWD populations that appear to be slightly resistant/tolerant to insecticides in our bioassays.

**CONTINUING PROJECT REPORT** **YEAR**: 3 of 3 (No Cost Extension)
**WTFRC Project Number: CH-14-110**

**Project Title**: Developing a management strategy for little cherry disease


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


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

**Other funding sources**

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

**WTFRC Collaborative Expenses: None**

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

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

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

**OBJECTIVES**

**Obj. 1.** Determine mechanisms of Little Cherry Virus 2 (LChV2) transmission via insect vectors (apple and grape mealybug [AMB and GMB]).

**Obj. 2.** Determine control methods for AMB and GMB in conventional and organic cherries.

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

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

**SIGNIFICANT FINDINGS**

- A parasitoid wasp, *Anagyrus schoenherri*, has been collected and identified for the first time in WA State from WSU TFREC Sunrise orchard. It could play a major role in controlling apple mealybug, a known vector of LChV2.
- Foliar applications of organic compounds, Aza-Direct and M-Pede, provided no control of GMB crawlers in an infested organic orchard.
- Systemic compounds applied via soil drench, Aza-Direct and Admire Pro, as well as an insect growth regulator, Centaur, reduced GMB nymph and adult numbers on potted trees in a greenhouse experiment.
- For the economic analysis of 'Bing' and 'Sweetheart' operations, the lowest estimated profits are with the do-nothing scenario and the highest LChV2 rate of spread. The highest estimated profits are when all possible management tactics are exercised (monitor, test trees, spray for insect vectors, remove infected trees) and the lowest rate of spread. The cost of controlling spread is offset by higher returns.
- The RPA kit for detecting WX phytoplasma was modified and it now recognizes isolates of the bacteria that were not detected in the previous season.

**METHODS**

**Obj. 1**.*Time needed for virus acquisition/transmission*. *Acquisition*: In order to determine the time needed for a GMB to acquire LChV2, mealybugs were allowed to feed on infected potted cherry trees in the WSU TFEC greenhouse (see full methods below) for 24 hours and 144 hours. During this upcoming year, RNA extraction and PCR will be completed to determine presence or absence of LChV2 infection of those MB samples. Based on these results we will determine if GMB need more or less time to acquire a detectable amount of virus. *Transmission*: GMBs feeding on infected plant material for an appropriate time period (determined via acquisition experiment), will be transferred to virus-free trees and allowed to feed for 1, 3, 7, and 9 days before removal. Trees will be tested for LChV2 after 30 days (when virus becomes detectable). Results will tell us the number of MB feeding days required to transmit the virus to a healthy tree.

**Obj. 2.** *Vector Control:* We have tested candidate insecticides in field and lab/greenhouse tests for both of the known vectors of LChV2, AMB and GMB. Field trials were applied either by airblast or backpack sprayer; lab/greenhouse tests were applied with a backpack sprayer. Pre- and post-treatment counts of mealybugs were performed to assess the effectiveness of the various treatments.

**Obj. 3.** The WX RPA detection system was re-designed to accommodate detection of a few isolates of the bacteria. A newer version of the kit is available; however, a thorough validation is needed in order to ensure its reliability. A lack of LChV1 positive samples identified during the 2016 growing season hindered progress on the validation of the LChV1 RPA assay system.

*Sub-objective 1*: Evaluate the reliability of the retooled WX RPA assay on field samples. *Proposed Activities:* Testing of the retooled WX RPA assay against purified DNA preparations from WX positive

samples during the 2015 growing season. In addition, the WX RPA system will also be used on future WX PCR positive samples (2017 growing season) received at CPCNW. As a result of these activities, we anticipate a set of assay kits will be available to identify all three of the known pathogens associated with little cherry disease.

*Sub-objective 2*: Determine the specificity and reliability of the LChV1 RT-RPA kit. *Proposed Activities:* Testing of LChV1 RT-RPA kit against RNA and crude sap preparations from trees infected with LChV2 and WX phytoplasma as well as trees infected with other sweet cherry viruses commonly found in Washington. Identify LChV1-infected trees in Washington orchards by RT-PCR and compare with results from the LChV1 RT-RPA assay on crude sap preparations from LChV1 infected trees, as revealed by RT-PCR. Tests will be performed throughout the growing season to determine appropriate sampling protocols for LChV1. Results will verify the specificity of the RPA assay systems for the targeted pathogens. This will provide a measure of the reliability and confidence in the assay results. A set of assay kits will be available that will identify all three of the known pathogens associated with little cherry disease.

**Obj. 4.** ***Analysis of cost scenarios for orchard blocks affected with LChV2***

Using average production costs of 'Bing' and 'Sweetheart' cherries derived from interviewing producers, we developed scenarios with two management responses to little cherry. The first was a 'do nothing' response – the grower is unaware he/she has little cherry in the orchard, and no mitigation or control measure are taken. The second scenario assumes that the grower takes all possible measures to control the disease, including vector control, monitoring and testing, and tree removal. Because the rate of spread is unknown, we calculate costs and returns for three rates of spread in each scenario and cultivar over the 25-year life of an orchard. Our scenarios further assume that the orchard is free of infection when planted, but is under constant threat of re-infection from surrounding blocks.

**Scenario 1:** Do nothing. There are no added costs for control of LChV2. Each infected tree will be 100% symptomatic the year where fruit appears in the tree (3rd year). We assume that the grower picks all fruit regardless of size and color, and sends it to the packinghouse. At the packinghouse this fruit will be culled (return of $0.20/lb), whereas packed fruit returns $2.10/lb. The rate of infection increases linearly in years 1 to 10, with a maximum constant rate during years 11 to 25, and is assumed at 1%, 3%, and 5% per year.

**Scenario 2:** All possible measures to control LChV2 are taken, including monitoring for disease symptoms, testing symptomatic trees, removing positive for LChV2 trees, and spraying for mealybugs. Infected trees are detected in June of a given year (when fruit are near harvest) and removed in October of that year. The rate of spread is low in the first 2 years (no fruit to assess symptoms) and constant at 0.5%, or a minimum of 1 new infected tree/year. In years 3-25, three rates of spread are evaluated (0.5%, 1%, and 2% per year), remaining constant for next 23 years of orchard life.

**RESULTS & DISCUSSION**

**Obj. 1.** *Time needed for virus acquisition/transmission.* Small potted 'Bing' trees on Mazzard rootstocks were maintained in the WSU-TFREC greenhouse. During January 2016, dormant budwood was collected from a 'Bing' tree in an orchard in Rock Island, WA, known to be positive for LChV2. Budwood was cleft-grafted onto the base of greenhouse plants in an attempt to infect them with LChV2. The trees were maintained in the greenhouse and leaf samples were tested in July 2016 to determine LChV2 infection status. Plants testing positive for LChV2 (4) were infested with GMB nymphs and crawlers, from a laboratory colony maintained on potatoes. Mealybugs were allowed to settle for 24 hours and feed for an additional 24 and 144 hours before a portion of the mealybugs from each tree were removed, collected into sterile centrifuge tubes, and placed in storage

(-80 ºC freezer) for later extraction and analysis. We are still in the process of completing RNA extraction and PCR to determine presence or absence of LChV2 infection in GMB samples.

**Obj. 2.** *Control methods for AMB:* In 2014, 2015, and 2016, AMB was monitored weekly at WSU's Sunrise Orchards, in a conventional apple orchard with a high density of AMB. In 2016, we found that second instar females had emerged and were feeding on woody plant parts near buds as early as 9 March, followed by the emergence of mature winged males on 4 April. Females continued to feed and grow through late April, then proceeded to lay eggs (Fig. 1). The first ovisacs were observed on 20 April; however most were parasitized. A parasitized female AMB creating an ovisac will produce little or no eggs, resulting in very few crawlers hatching and feeding. The parasitized AMB's body is used as a nursery for developing parasitoids and is referred to as a mummy. In 2015, these parasitoid wasps were identified as *Anagyrus schoenherri* (Westwood 1837) by Dr. Serguei Triapitsyn (UC Riverside). This the first record of this species in North America.

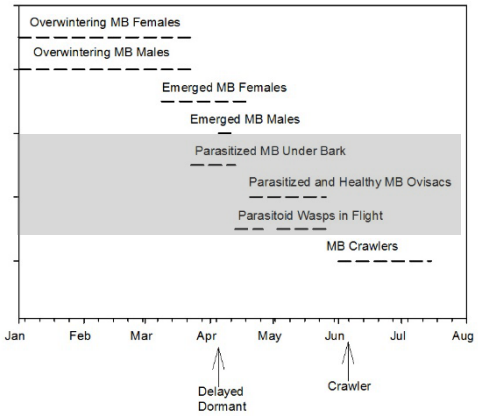


Fig. 1: Apple mealybug phenology observed in an infested apple orchard at WSU Sunrise Orchards in 2016. Parasitoid wasp, *A. schoenherri*, was observed within AMB mummies as well as in flight (shaded area). Delayed dormant sprays were applied to target emerged females, while crawler sprays targeted emerging crawlers.

In 2016, the first observation of parasitism occurred in nymphs under bark on 23 March. On 13 April, parasitoid wasps were seen flying, mating, and injecting eggs into mature female AMB. A few female mealybugs beginning to make ovisacs were collected from the field on 20 April, and when examined microscopically, parasitoid larvae were found inside. All collected ovisacs on 27 April and 3 May were also parasitized. Collections on 18 May revealed that most of the parasitoids had emerged, leaving only empty mummies behind. Parasitoid wasps then inject eggs into newly hatched crawlers. Yellow sticky cards were used to monitor the flight of *A. schoenherri* adults. Yellow cards/traps were collected and replaced on a weekly or biweekly basis, starting on 21 April and ending on June 2. Numbers of *A. schoenherri* on sticky cards peaked between 3 May and 26 May, with over 100 individuals on each card. Very few *A. schoenherri* were captured during other times.

In 2016 a field experiment was conducted to determine the effects of chemical insecticides the AMB population at the conventional apple orchard described above. Treatments were applied, and AMB collection and analysis procedures were the same as previous years. Delayed dormant treatments (applied 4 April) included Lorsban+oil, Closer + Regulaid (surfactant), Centaur + Regulaid, Azara + oil, and Aza-Direct + oil, targeted overwintering females. Emerging crawlers were targeted by treatment applications (6 June) of Closer + Regulaid, Centaur + Regulaid, Actara, Diazinin, and Azera. Leaf samples were collected (1 June) prior to applications targeting crawlers to determine their presence on leaves. Additional treatments (#2 and 3), one of Closer and one of Centaur, were applied at delayed dormant,

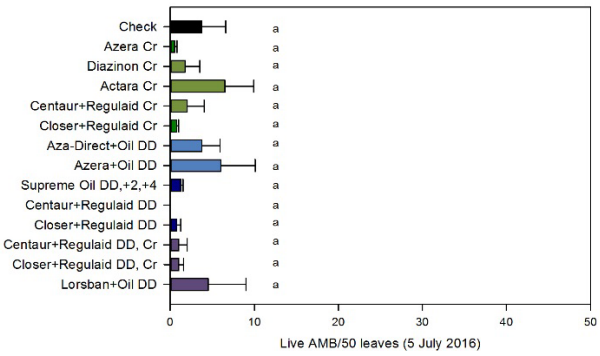


Fig. 2: Effects of compounds applied at delayed dormant (DD) and at crawler emergence (Cr) on AMB numbers in an infested apple orchard at WSU Sunrise Orchards.

but also again at crawler emergence. Finally, repeated applications of Supreme oil were applied on 1 April, 21 April, and on 6 May (Fig. 2).

On 5 July, 50 leaves were collected from each treatment tree, to determine the effects of the applied treatments. Average numbers of crawlers/50 leaves was determined to be similar for all treatments including the check (Fig. 2). The average number of crawlers/leaf for the check treatment was 15.2 in 2014, 2.75 in 2015, and < 1 crawler/leaf in 2016. Observed parasitism jumped from affecting approximately 20% of egg laying females in 2014, to 70-80% in 2015, and to almost 100% in 2016. A reduced crawler population resulting from extreme parasitism can likely be attributed for the lack of significant differences in average crawler numbers.

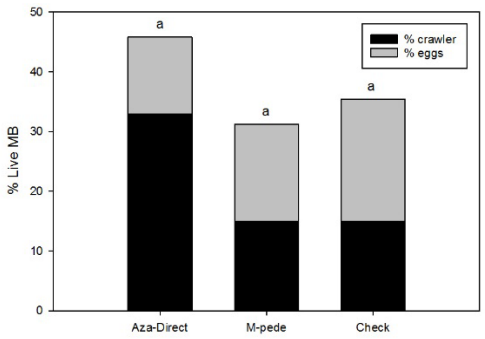


Fig. 3: Percent live GMB crawlers and viable eggs in egg masses treated with organic compounds, Aza-direct and M-pede, in an infested plum orchard in Rock Island, WA.

*Organic control of GMB:* In 2016, a field experiment was conducted to determine the effects of organic compounds on a heavy infestation of GMB in an organic plum orchard located in Rock Island, WA. Treatment trees (12) were chosen based on the presence of 3 viable colonies/egg masses on each. A hand lens was used to determine egg mass viability, and viable masses were labeled A, B, and C using a laminated label and a push pin (pushed into the tree). Pesticides were applied with a backpack sprayer to the point of drip on 14 July with label rates of Aza-Direct and M-Pede, and an untreated check (4 replicates/ treatment). Five days after treatment (19 July), labeled egg masses were removed from trees using forceps, and percentage of live and dead crawlers/eggs was determined using a microscope. The average percentage live crawlers, eggs, and total live (crawlers + eggs) was similar for all treatment groups (Fig. 3). These results suggest that organic compounds provided no control of GMB crawlers in this experiment. Eggs and crawlers in the nest are protected by waxy filamentous secretions of the ovisac, making them extremely difficult to reach with insecticides. In this experiment, egg masses were removed from trees for analysis when a percentage of eggs had hatched, and hatched crawlers were either crawling around in or leaving the egg mass. The fate of the mobile, unprotected, newly-hatch crawlers is unknown, since we only looked at crawlers within the nest. We may have had a different result if we were able to effectively sample these mobile crawlers. Predation and parasitism played a major role in reducing this GMB population, as we observed a number of syrphid predators and a parasitoid wasp in many egg masses.

*Systemic control of GMB:* An experiment was conducted on 1-tr-old, potted 'Bing' trees on Mazzard root stock, in the WSU-TFREC greenhouse, to determine the effects of foliar- and drench-systemic insecticides on GMB. Mealybugs from colonies were transferred to greenhouse trees and given 7 days to become established, then treated with Admire Pro and Aza-Direct as soil drenches. Ultor, a foliar-systemic compound and Centaur, an insect growth regular, were both applied to the point of drip with a backpack sprayer, using an equivalent insecticide concentration of 100 gpa. Counts of GMB crawlers (0.1-0.5 mm in length), nymphs (0.6-2 mm in length), and adults (>2mm in length) were completed 1 day before; and 19, 28, 35, and 56 days after treatments were applied. The average number of crawlers, nymphs, adults, and total GMB was calculated for each treatment for each sampling date. Statistical analysis was done by using the difference between the pre- and the post-treatment means for a given date. Average crawler differences were similar for all treatments and all dates. Nymph numbers were reduced to zero on Centaur-treated plants at 19 days post-treatment and nearly to zero on trees treated with Admire Pro and Aza-Direct at 28 days (Fig. 4). Admire Pro, Aza-Direct, and Centaur reduced adult numbers to zero 19 days after application (Fig. 5). Ultor did not significantly reduce GMB numbers for any life stage.

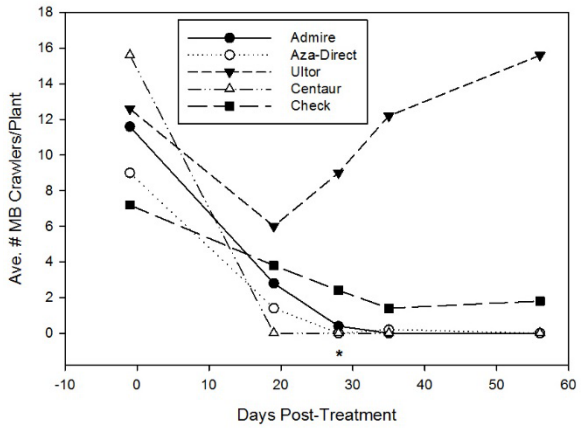


Fig. 4: Effects of foliar- and drench-systemic compounds and an insect growth regulator on the average number of GMB crawlers/plant over time. *significant reduction in ave. crawlers/plant compared to check.

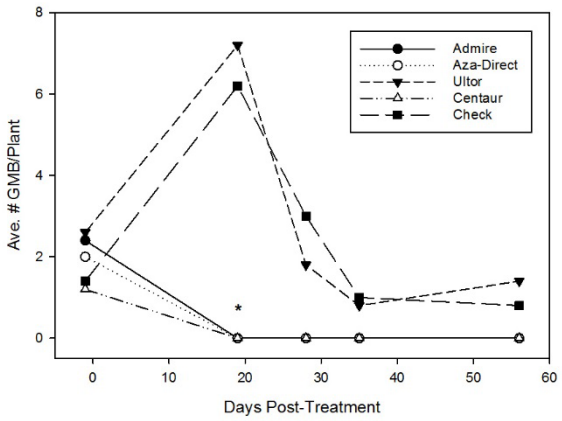


Fig. 5: Effects of foliar- and drench-systemic compounds and an insect growth regulator on the average number of GMB adults/plant over time. *significant reduction in ave. adults/plant compared to check.

**Obj. 3.** An RPA assay system for WX phytoplasma was developed; however, three WX PCR positive samples during the 2016 growing season were not detected by the kit. Examination of nucleotide sequence from two regions of the WX genome did not reveal genetic variation of these three samples with other isolates of WX phytoplasma. Several attempts to accommodate detection of these three WX PCR positive samples by the previous WX RPA assay system (i.e., changing primer and probe concentrations) were also not successful, prompting re-designing of the WX RPA assay system. A newer version is available that detects the three WX PCR positive samples. Crude leaf extracts from 23 WX PCR positive samples were then tested by the new version of WX RPA assay system; all 23 samples were positive. We examined the spatial variation in WX in infected trees, which is known to be unevenly distributed. Symptoms are most apparent when fruit are nearing harvest, and in late summer-early fall when leaves appear yellow to orange, referred to as 'bronzing'. Leaf samples from symptomatic and non-symptomatic branches from seven WX infected trees were tested by both PCR and RPA. All symptomatic branches tested positive for WX by both PCR and RPA but only one non-symptomatic branch was positive (Table 1). Overall, these results showcase the comparable sensitivity of RPA with PCR and further highlight the necessity of uniform sampling in order to get reliable detection of WX. The newer version of the WX RPA assay system needs to be tested extensively to ensure its reliability in detecting field isolates of the pathogen; this activity will be pursued during the 2017 growing season.

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

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

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

**Obj. 4.** For both 'Bing' and 'Sweetheart' operations (Figs. 6 and 7, respectively), the rate of spread of LChV2 and management of infected trees are both critical when estimating the impact of LChV2 on the orchard profits. The lowest estimated profits are with the do-nothing scenario (no monitoring,

testing, spraying for vectors or tree removal) and the highest LChV2 rate of spread (assumed at 5% per year, constant for the 25 years of orchard life). In this case, negative profits are observed by year 7 of production. The highest estimated profits are with the management scenario (monitor, test trees, spray for insect vectors, remove infected trees) and the lowest rate of spread (assumed at 0.5% per year, constant for the 25 years of orchard life). In this case, the orchard yields a positive profit during the whole of its productive life (years3-25).

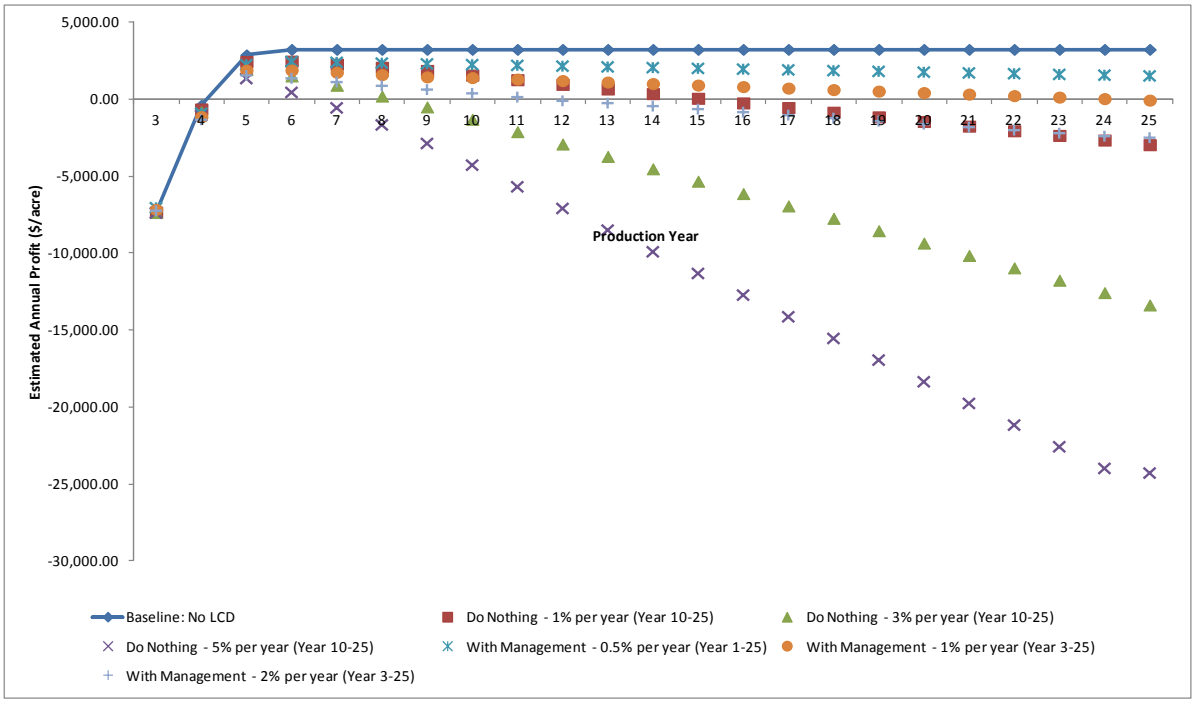


Fig. 6: Comparison of estimated annual profits under different LCD scenarios for BING cherries

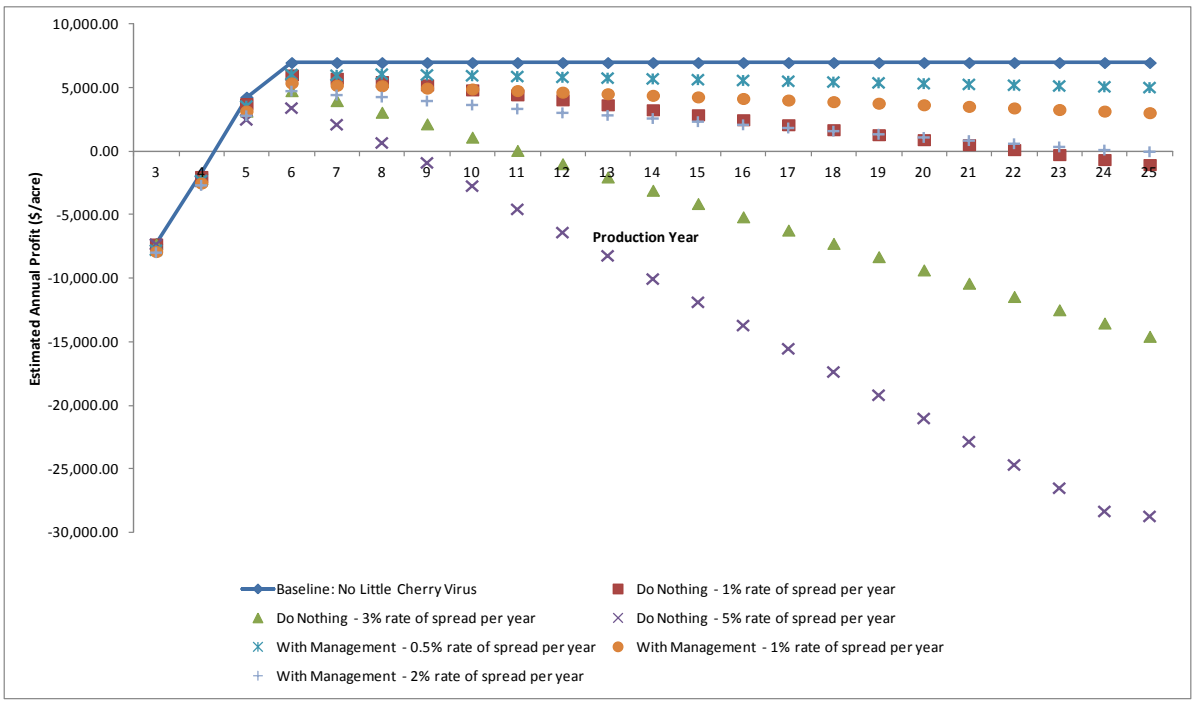


Fig 7: Comparison of estimated annual profits under different LCD scenarios for SWEETHEART cherries

**CONTINUING PROJECT REPORT** **YEAR: 1 of 3**
**WTFRC Project Number: CH-16-105**

**Project Title**: The Hunt for leafhopper vectors of western X in Washington cherries


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**Cooperators:** Washington cherry growers, Stemilt Growers

**Total Project Request:** Year 1: $33,740 **Year 2: $34,603** Year 3: $35,499

**Other funding sources**

**Agency Name:** National Clean Plant Network – Fruit Trees
**Amt. Requested/Awarded:** NCPN-FT pays land rental fees and maintenance costs of the virus research block where field experiments are conducted, sources of plant material for research and plant maintenance. The estimated cost associated with this project is $22,300 and is a portion of a larger NCPN grant to WSU-Prosser.
**Notes:** WSU is including this information on other funding available for the support of similar research undertaken by the faculty member proposing this research. These resources are listed to identify other support granted for this research and are not included as a commitment of cost-share by the institution.

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

| **Item** | 2016 | **2017** | 2018 |
|---|---|---|---|
| **Salaries**[1] | $15,601 | $16,226 | $16,875 |
| **Benefits**[2] | $5,939 | $6,177 | $6,424 |
| **Wages** | $0 | $0 | $0 |
| **Benefits** | $0 | $0 | $0 |
| **Equipment** | $0 | $0 | $0 |
| **Supplies**[3] | $12,200 | $12,200 | $12,200 |
| **Travel** | $0 | $0 | $0 |
| **Miscellaneous** | $0 | $0 | $0 |
| **Plot Fees** | $0 | $0 | $0 |
| **Total** | $33,740 | **$34,603** | $35,499 |

**Footnotes:**
**1. 0.20 and 0.10 FTE of Research Associates Ferguson and Villamor, respectively.**
**2. Benefits calculated at standard Washington State rates.**
**3. Supplies include partial funding of:**
**Fuel to travel to research sites**
**Field sampling supplies**
**Sample extraction and PCR assays**
**Leafhopper colony establishment and maintenance**
**Acquisition and retention of inoculated plants over two years**

## OBJECTIVES

1. **Conduct survey of leafhoppers in Western X affected orchards.**
   The 2016 leafhopper survey in cherry-producing regions yielded informative data in 3 out of 4 of the counties sampled. We plan to conduct a similar study in 2017, beginning in March and concluding in October. Very few leafhoppers were captured in our Chelan County sites; we plan to locate different sites in that county for the survey in 2017. We anticipate that a couple of our orchard sites in Grant County will be taken out before the 2017 sampling season begins. If that happens, we plan to seek out other sites to sample. We plan to continue molecular testing of leafhoppers to determine which species have the potential to transmit Western X phytoplasma.
2. **Conduct survey of host plants for leafhoppers and/or WX phytoplasma in affected orchards.**
   During the months of June, July, and August, potential host plants for leafhoppers will be collected from field sites and propagated in the greenhouse. Data from sticky card sampling in ecosystems outside of the orchard will also provide information on potential host plants.
3. **Examine the capability of selected leafhopper species to transmit WX phytoplasma.** A colony of *Colladonus reductus* was newly established during the late summer of this year. We anticipate that with expansion of this colony by the spring of 2017, we will be able to conduct our initial transmission studies. We also plan to initiate a colony of *C. geminatus* in early summer of 2017.

## SIGNIFICANT FINDINGS

- **Two leafhopper vector species, *Colladonus geminatus* and *C. reductus*, were the most abundant species found on sticky cards in cherry tree foliage and in habitats outside of the orchards.**
- **Data from sticky cards placed in extra-orchard habitats (e.g., sageland, roadside weeds, field border) provided new information on alternative habitat/host plants and on seasonal patterns of movement of certain leafhopper vectors.**
- **A laboratory colony of *C. reductus* was established on celery seedlings. We plan to use these leafhoppers for transmission studies.**
- **From the molecular diagnostic testing results of 218 *C. geminatus* and 168 *C. reductus* DNA samples, we confirmed that both species can carry the Western X phytoplasma. We noted that WX-positive samples for these two species were primarily found in the orchard during two periods of time—early May and late July/early Aug.**

## METHODS

**1. Conduct survey of leafhoppers in Western X affected orchards.**
In 2017, we will conduct leafhopper sampling from March to October in Western X affected cherry orchards to determine species composition and peak activity periods. Yellow sticky cards (4" X 10") will be placed in the tree canopy to catch leafhoppers in cherry foliage, and sweep nets will be used to sample the orchard floor vegetation. Sticky cards will be placed at a height of 5-6 feet. We will use the same orchard sites we used this past summer. If blocks have been removed, we will locate additional blocks for sampling. We plan to sample additional alternative habitat areas near orchards. We plan to sample for leafhoppers in Benton, Grant, and Yakima Counties. As part of a collaboration with Stemilt Growers in Wenatchee, sampling for leafhoppers is also planned in Chelan County cherry blocks.

Leafhoppers will be identified, counted, and preserved in ethanol until analysis. Leafhoppers separated by species will be subjected to DNA extractions. DNA samples will be tested for WX using our recently developed detection assay involving PCR that specifically detects WX phytoplasma.

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

At the same sampling sites described in Objective 1 Methods, we will continue to characterize the plant species on the orchard floor and in neighboring ecosystems from spring to fall. We will attempt to propagate select weedy or woody host species in the greenhouse to determine their suitability as hosts for leafhopper and/or WX phytoplasma.

**4. Examine the capability of selected leafhopper species to transmit WX phytoplasma.**

During the second year, we will continue to develop, propagate, and maintain infective sources of WX phytoplasma in greenhouse culture. An infective source is needed to conduct the transmission experiments. We will continue to maintain the *C. reductus* colony on celery which was initiated this past summer from field-collected specimens. We plan to initiate a colony of *C. geminatus* on celery similarly. Assessment of vector competency of these species using clean laboratory-reared leafhoppers will involve at least one of these approaches: (1) acquisition of WX from infected field trees, (2) transmission from infected woody host to clean herbaceous recipient (celery), (3) transmission from infected herbaceous host to clean herbaceous recipient, and (4) transmission from infected cherry or peach host to clean recipients (cherry or peach seedlings). Potentially infected celery, cherry, and peach plants will be maintained in the greenhouse and observed for symptom development; leaf samples will be collected when appropriate to test for WX infection.

## RESULTS & DISCUSSION

**1. Conduct survey of leafhoppers in Western X affected orchards.**

The 2016 leafhopper survey in cherry-producing regions yielded informative data in Yakima, Grant, and Benton County orchard sites but not in the four blocks sampled in Chelan County (Wenatchee, Malaga). For Chelan County, very few leafhoppers were found on the sticky cards in the cherry foliage and very few were found in the habitats outside of the orchards. Later in the season, we learned that these orchards had been subjected to a number of insecticide sprays pre- and post-harvest.

While a few dozen leafhopper taxa were present in sticky card and sweep net samples, our focus was on potential WX vector species. The sticky card data yielded information on the leafhoppers present in the foliage of the cherry trees as well as the foliage of plant species in extra-orchard habitats (alfalfa, sageland shrubs, broadleaf weeds). The two most abundant species in sticky card samples were *Colladonus reductus* and *C. geminatus.* We expect that these two species have the greatest potential to vector WX to sweet cherry trees. While *C geminatus* is known to be a vector of WX in Washington State (Wolfe et al. 1951), little is known of the vector status of *C. reductus*. We found cherry leafhopper, *Fieberiella florii,* which is a significant vector of WX in California sweet cherries (Purcell et al. 1987), in two locations: in the weedy field border of the Granger block and in the roadside weeds outside of the Benton City block. Cherry leafhopper is a difficult leafhopper to capture as it is nocturnal and is not attracted to yellow sticky cards. A blacklight trap was deployed on several occasions in the Granger orchard but this species did not show up in light trap catches either. For all sites, we are still collecting samples through late October.

In the Granger block (Yakima County), *C. reductus* and *geminatus* remained more abundant in the alfalfa field across the road than in the cherry foliage throughout the season (Figs. 1 and 2). *C.*

*reductus* and *geminatus* showed a peak of activity in alfalfa in early June, and both species appeared to be on the rise in the orchard in early August.

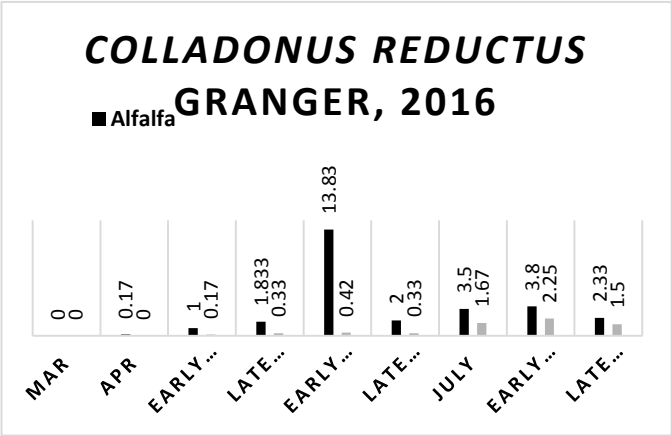


**Fig. 1.** *Colladonus reductus* abundance in orchard and extra-orchard habitat (alfalfa field), Granger, Yakima County, WA.

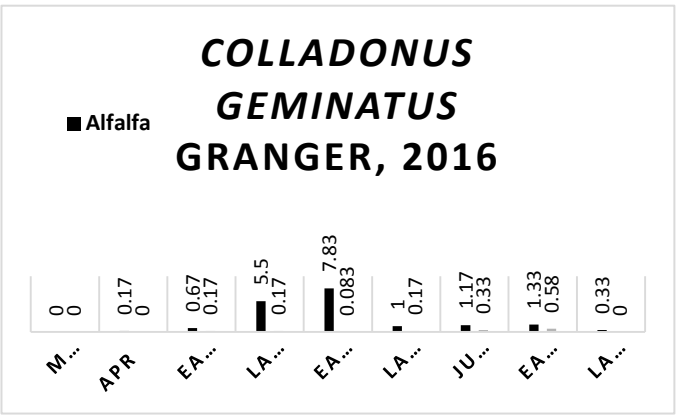


**Fig. 2.** *Colladonus geminatus* abundance in orchard and extra-orchard habitat (alfalfa field), Granger, Yakima County, WA.

All of the Grant County blocks had sageland within 200 feet of the edge of the orchard. A different pattern of incidence was seen for two of the blocks. In the Mattawa1 block, incidence of *C. reductus* was greater in the orchard than in the sageland, while incidence of *C. geminatus* was greater in the sageland than in the orchard (Figs. 3 and 4). During the springtime in the Mattawa4 cherry block, *C. reductus* appeared to occur with similar frequency in both orchard and sageland habitats, while incidence of *C. geminatus* was greater in the sageland than in the orchard (Figs. 5 and 6).

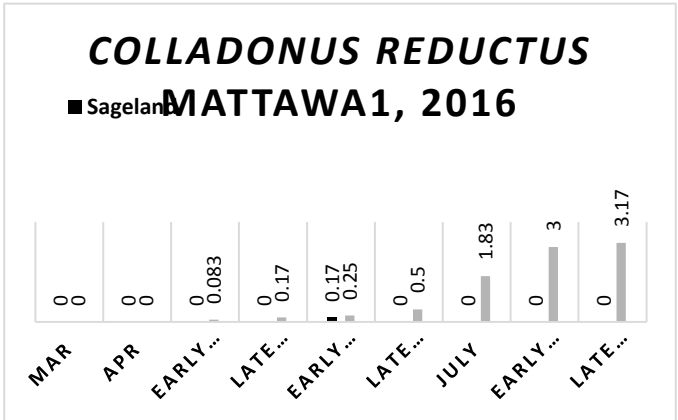


**Fig. 3.** *Colladonus reductus* abundance in orchard and extra-orchard habitat (sageland) in Mattawa, Grant County, WA.

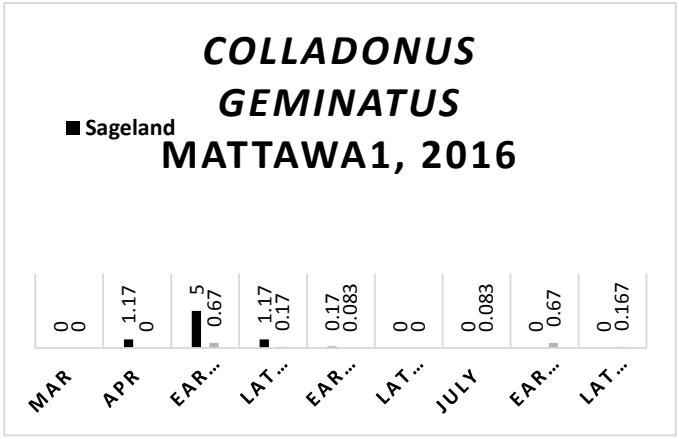


**Fig. 4.** *Colladonus geminatus* abundance in orchard and extra-orchard habitat (sageland) in Mattawa, Grant County, WA.

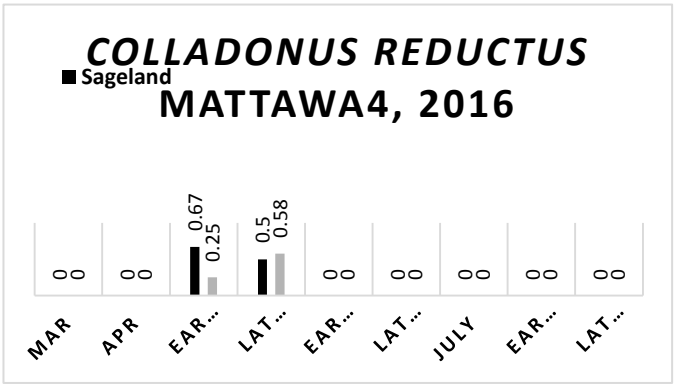


**Fig. 5.** *Colladonus reductus* abundance in a second orchard block and extra-orchard habitat (sageland) in Mattawa, Grant County, WA.

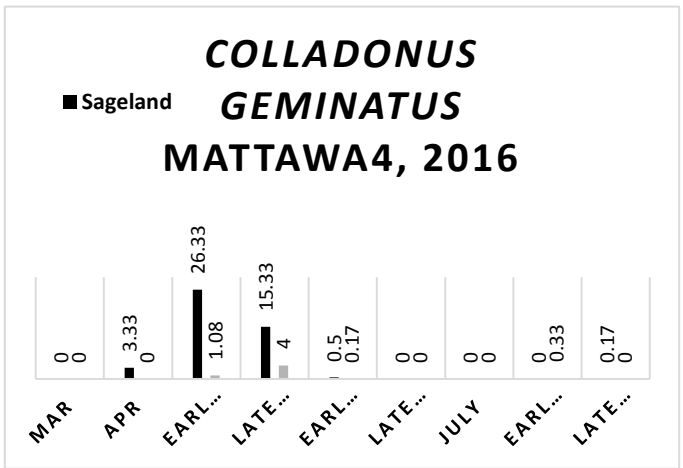


**Fig. 6.** *Colladonus geminatus* abundance in a second site with orchard and extra-orchard habitat (sageland) in Mattawa, Grant County, WA.

**From the molecular diagnostic testing results of 218 *C. geminatus* DNA samples and 168 *C. reductus* samples, we confirmed that both species can carry the Western X phytoplasma. From the sticky cards that have been processed so far, we noted that WX-positive samples for these two species collected in orchards were primarily found during two periods of time—early May and late July/early Aug (Table 1).**

**Interestingly, during late April and early May, most of the *C. geminatus* and *reductus* collected in extra-orchard habitats were positive for WX. In addition, percentage of positive samples was greater in extra-orchard habitats compared with those collected from within the orchard block. This suggests that springtime reservoirs of WX are found outside of the orchard. The upsurge of positive orchard leafhopper samples in late season may be explained by the simultaneous increase in WX titer in the cherry trees at that time of the year (**Suslow and Purcell 1982; Uyemoto and Luhn 2006**). During late season, leafhopper abundance was comparatively low in the extra-orchard habitats, although WX-positive specimens continued to be found.**

**Table 1. Incidence of Western X in leafhoppers collected in cherry orchard and extra-orchard habitats, 2016. Extra-orchard habitats included alfalfa, sageland plants, and roadside herbaceous weeds.**

| *Colladonus geminatus* | | | | | | |
|---|---|---|---|---|---|---|
| | Cherry orchard | | | Extra-orchard habitat | | |
| Period | # samples | # positive | % | # samples | # positive | % |
| Late April | 0 | -- | -- | 28 | 22 | 79 |
| Early May | 22 | 5 | 23 | 59 | 37 | 63 |
| Late May | 34 | 1 | 4 | 21 | 1 | 5 |
| Early June | 6 | 0 | 0 | 8 | 0 | 0 |
| Late June | 2 | 0 | 0 | 4 | 0 | 0 |
| Early July | 5 | 1 | 20 | 1 | 0 | 0 |
| Late July | 8 | 1 | 13 | 5 | 2 | 40 |
| Early Aug* | 13 | 5 | 38 | 2 | 1 | 50 |
| | | | | | | |
| *Colladonus reductus* | | | | | | |
| Late April | 0 | -- | -- | 1 | 1 | 100 |
| Early May | 9 | 3 | 33 | 9 | 7 | 78 |
| Late May | 15 | 0 | 0 | 9 | 0 | 0 |
| Early June | 5 | 0 | 0 | 11 | 0 | 0 |
| Late June | 6 | 0 | 0 | 11 | 0 | 0 |
| Early July | 7 | 2 | 29 | 4 | 0 | 0 |
| Late July | 38 | 16 | 42 | 6 | 4 | 67 |
| Early Aug* | 33 | 19 | 58 | 4 | 4 | 100 |

*Sample processing is not complete. Sticky card sampling continued into October 2016 but these samples have not yet been processed.

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

We collected and identified 39 potential host plant specimens, and maintained selected species in the greenhouse for host plant studies. Unfortunately, the numbers of leafhoppers required for these studies were not available. However, data from sticky cards hung in the foliage of plants in extra-orchard habitats provided host plant information. Based on these data, two species have been identified as host plants for *C. reductus* and *geminatus*. They are: alfalfa (*Medicago sativa,* at the Granger site) and antelope bush (*Purshia tridentata*, in the sageland across from the Mattawa4 block).

**3. Examine the capability of selected leafhopper species to transmit WX phytoplasma.**

**A laboratory colony of *C. reductus* was established on celery seedlings. We plan to use these leafhoppers for transmission studies in 2017.**

**Practical application:** This information may be used by growers to improve the timing of both chemical and cultural leafhopper management practices, which in turn will improve management of Little cherry disease. Our data show that there are two main time periods of WX-positive leafhopper activity in the orchard. To adopt a more targeted approach to leafhopper management, chemical controls could be implemented especially during those time periods.

**Citations:**


Purcell, A.H., Uyemoto, J.K., Van Steenwyk, R.A., Schreader, W.R., Suslow, K.G., and Kirkpatrick, B. 1987. Buckskin disease of cherry. Calif. Agric. 41: 26-27.

Suslow, K.G. and Purcell, A.H. 1982. Seasonal transmission of X-disease agent from cherry by leafhopper *Colladonus montanus*. Plant Disease 66: 28-30.

Uyemoto, J.K. and Luhn, C.F. 2006. In-season variations in transmission of cherry X-phytoplasma and implication in certification programs. J. Plant Pathol. 88: 317-320.

Wolfe, H.R., Anthon, E.W., Kaloostian, G.H., and Jones, L.S. 1951. Leafhopper transmission of western X-disease. J. Econ. Entomol. 44: 616-619.

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

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


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

**Other funding sources: None**

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

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

**Footnotes: [1]Salary is for graduate student (D. Hubbard) at 0.25FTE and postdoc at 0.2FTE. A 3% increase is factored into years 2 and 3; [2] Benefits are based on a graduate student static cost and the actuals of a postdoc rate; [3]supplies include lab consumables, nursery stock & supplies and several chest freezers and rates for microscopy lab use at OSU-Corvallis; [4]travel is for # trips to Corvallis at 0.565 cents per mile and travel to research plots in The**

**Dalles; [5]shipping and nutrient analysis (factor $25/ship date for shipping fees and $11/sample x # of samples per date); [6]greenhouse space at 0.21 cents/sqft/mo and cold room space at 0.94 cents/sqft/mo**

## Objectives:

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

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

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

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

## Significant Findings 2015:

**Objective 1**

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

**Objective 3**

- All defoliation treatments were efficient at abscising leaves, though only on a single sampling date was an increase in acclimation observed.

**Objective 4**

- **Leaves, buds and spur tissue were sampled weekly for nutrient content.**

**Objective 1**

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

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

| | Acclimation level | | |
|---|---|---|---|
| | Non-Acclimated | Artificially Acclimated | Naturally Acclimated |
| UTC | --- | --- | --- |
| Temp 1 | -2º | -4º | -4º |
| Temp 2 | -6º | -8º | -8º |
| Temp 3 | -8º | -15º | -13º |

| | | | |
|---|---|---|---|
| Temp 4 | -12º | -17º | -15º |

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

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

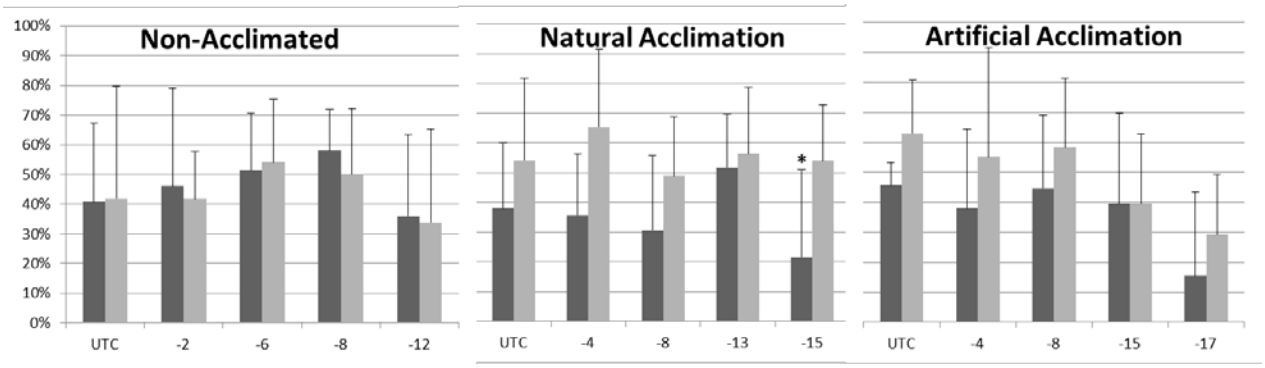


Figure 1. Percentage of relative regrowth of Gisela 6 rootstock of 3 levels of acclimation after being subjected to differing injurious temperatures. Dark bars represent inoculated treatments, while light bars are non-inoculated. Lines are top of bars are ± one standard deviation. * represents significance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

In addition to the model system described above we also planned to determine acclimation and disease incidence on larger containerized trees that we have maintained since 2015. These were intended to 'scale up' experiments in order to better reflect orchard trees. However, due to a deer infestation at MCAREC, these trees were devastated by grazing and developed insufficient new growth to accommodate 2016 freeze tests. These trees were regrown this season and have been repurposed to assess at the hardiness of stem tissue as affected by different rates and timings of ABA. Since multiple applications of lower rates of ABA have previously been shown to increase hardiness in prior studies (Guak and Fuchigami 2001). Scaled up experiments using similar methods to the small plants will likely be carried out in 2017 once enough tissue has developed.

2016: For fall 2016 experiments we purchased Mazzard rootstocks, rather than Gisela, from North American Plants (McMinnville, OR) in early September. These plants have not completed their growth for the season, and are presently being hardened off. Several artificial freeze tests are planned

for this season to evaluate the role of acclimation and non-acclimation on freeze injury with and without inoculation. Modifications to the inoculation procedure will also be tested this year. The freeze procedure will remain in place with additional temperature mapping of the freeze chamber to account of variability within the unit. Heating equipment in the Greenhouse has been restored to working order and will be monitored to ensure that the environment remains controlled.

**Objective 2**

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

**Objective 3**

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

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

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

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

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

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

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

2016: Beginning on 6 October, initial samples were taken with applications of ABA & Lime Sulfur subsequently following. Contrary to 2015's trial, floral peaks could be found on the initial DTA assays. This occurrence of peaks so much earlier than 2015 did not allow the testing of floral buds to confirm they are expiring in the mass ice nucleation mentioned prior. Further analysis of the orchard temperatures prior to sampling is needed and comparisons to last year's weather data will be included. Defoliation evaluations are now being made twice weekly and DTA of floral buds and stem tissue are being made weekly in order to tighten the point of separation if one truly exists.

**Objective 4**

2015: Tissues were dissected into leaf, bud & spur as sampling occurred. These tissues have yet to have just been sent for analysis. A decision to refrain from sending tissue once all was collected was made by myself as I was unaware of the status of my project with my primary advisor leaving the university and what would happen to the working group and myself within the college.

2016: As stated above, all tissue is being dissected into separate parts and being dried in ovens to eliminate excess water. Upon completion of acclimation & dormancy, these tissues will follow the 2015 samples to the lab for analysis.

**Literature Cited:**

Guak, S., Fuchigami, L. 2001. Effects of Applied ABA on growth cessation, bud dormancy, cold acclimation, leaf senescence and N mobilization in apple nursery plants. J. Hortic. Sci. Biotech. 76:459-464.