Northwest Cherry Research Review

Red Lion, Yakima Center

Tuesday, 11/10/2015

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FINAL PROJECT REPORT

Project Title: Sweet cherry breeding toolbox

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Cooperators: Kate Evans (Washington State University), Jim McFerson (Washington Tree Fruit Research Commission), Amy Iezzoni (Michigan State University)

Total Project Request: Year 1: $5000 **Year 2:** $5000

Other funding sources

Agency Name: NSF DIBBS
Amount awarded: $1.485 M (Jan 2015 – Dec 2017)
Notes: "Tripal Gateway, a Platform for Next-Generation Data Analysis and Sharing." PI: Ficklin (Horticulture). Co-PIs include Main and Jung.

Agency Name: USDA-NIFA NRSP
Amt. requested: $1.99 M (Oct 2014- Sept 2019)
Notes: "Database Resources for Crop Genomics, Genetics and Breeding Research". PI: Dorrie Main, writing team includes Sook Jung, Michael Kahn, Cameron Peace, and Jim McFerson.

Agency Name: USDA-NIFA Specialty Crop Research Initiative
Amount awarded: $2.7 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 Jung, Peace and Oraguzie.

Agency Name: USDA-NIFA Specialty Crop Research Initiative
Amount awarded: $10 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: $2.0 M (Sep 2009 – Aug 2014)
Notes: "Tree Fruit GDR: Translating genomics into advances in horticulture." PI: Main. Co-PIs include Peace and Oraguzie.

Agency Name: WTFRC/OSCC
Amount requested: $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 requested: $13,000 (2014)
Notes: "Consulting for the sweet cherry breeding program." PI: Iezzoni.

Agency Name: WTFRC/OSCC
Amount requested: $7,500 (2014)
Notes: "Consulting for the NW cherry project." PI: Bliss.

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

Agency Name: USDA-NIFA Specialty Crop Research Initiative
Amount awarded: $7.2 M (Sep 2009 – Aug 2014)
Notes: "RosBREED: Enabling marker-assisted breeding in Rosaceae." PI: Iezzoni. Co-PIs include Peace and Oraguzie.

Agency Name: USDA-NIFA Specialty Crop Research Initiative
Amount awarded: $2.0 M (Sep 2009 – Aug 2014)
Notes: "Tree Fruit GDR: Translating genomics into advances in horticulture." PI: Main. Co-PIs include Peace and Oraguzie.

Budget 1

Organization Name: WSU **Contract Administrator:** Carrie Johnston

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Item	2014	2015
Salaries[a]	3600	3600
Benefits[b]	1200	1200
Wages		
Benefits		
Equipment		
Supplies		
Travel[c]	200	200
Plot Fees	0	0
Miscellaneous		
Total	5000	5000

Footnotes: a and b = 5% of Dr. Sook Jung's salary and benefits (at a rate of 25%) for data curation; c = cost of once annual visit of Dr. Dorrie Main to provide face-to-face training on the toolbox to Dr. Oraguzie's group in Prosser.

Originally requested $29,893 in year 1 and $30,928 in year 2.

OBJECTIVES

Overall goal: To maintain and expand the Pacific Northwest Sweet Cherry Breeding ToolBox and continue to enable efficient cherry breeding.

Specific objectives:

1. Enable efficient data management for the Pacific Northwest Sweet Cherry Breeding Program (*years 1 and 2*)
2. Enable selection comparisons through access to data mining tools that utilize up-to-date performance and genotypic data (*years 1 and 2*)
3. Enable efficient parental selection for desired cultivars through access to data analysis tools that utilize up-to-date performance and genotypic data (*more toward start of year 2*)
4. Ensure optimal utilization of the Sweet Cherry Breeding ToolBox through hands-on training to the PNSWBP team (*years 1 and 2*)

Through support provided by other newly funded federal projects (USDA SCRI and NRSP) we will have the resources needed to complete all these objectives, so they remain unchanged.

SIGNIFICANT FINDINGS

1. Curation of publicly useful Prunus trait and marker data in GDR, accessible through the Sweet Cherry Breeding Toolbox of the Pacific North West Sweet Cherry Breeding program (PNWSCBP)

   New data extracted in Year 2 (final year) from the public literature includes 5 genetic maps and 2425 marker loci from 2 publications. We are in the process of checking the curated data from 18 additional publications which will be online by the end of the project end in December 2015. Other relevant data includes Peach Genome v2.0.a1 is available along with the IRSC 9K SNPs anchored to the new assembly.

   New data previously extracted and curated from the public literature in year 1 includes 4 genetic maps, 1 high-resolution mapping of MTL in the peach genome, 1675 marker loci, 108 QTLs/MTLs for 9 traits from 4 publications. It also includes sweet cherry molecular diversity data for self-compatibility from two cultivars and two populations using 3 markers. In total, new data from year 1 have been added for the following traits:

2. Breeding Data: All phenoptype and genotype data for the WTFRC PNWSCBP provided by Dr. Nnadozie and Dr. Peace is being extracted and curated into database templates for upload to the GDR private Sweet Cherry Breeding Toolbox. These complete data for the PNWSCBP will be made available through the secure Toolbox to assigned personnel as decided by the WTFRC and WSU.

3. Editing and uploading capability for the GDR Breeders toolbox is being implemented for the current version of the Cherry Breeders Toolbox. We are upgrading GDR to Drupal 7 and converting the GDR toolboxes to Tripal. To this end we held a Breeders Needs Assessment

Workshop immediately after the National Association of Plant Breeders Annual Meeting in July in Pullman. As part of that meeting, which involved Drs. Iezzoni, Peace, Evans and McFerson, we introduced breeders to Field Book App, an application for use on handheld devices which was designed by Jesse Poland's group at Kansas State University, to enable more efficient collection and upload of wheat field data. We provided breeders with the app, preinstalled on a Samsung handheld device, for them to test in their programs. The breeders are providing us with their recommendations for new or improved functionality as needed for collection of tree fruit and we will work with the developers to provide this to our tree fruit breeders.

Also as part of this Breeders Needs Assessment Workshop we identified more functionality breeders would like in the Breeders Toolbox to provide a more comprehensive solution to managing all aspects of tree fruit breeding. With our other sources of funding we will develop a generic solution, customizable for each breeding program, which we will make available to personnel in the PNWSCBP.

4. As it becomes available we will provide hands-on and audio visual training tutorials for PNWSCBP personnel.

METHODS

1. **Enable efficient data management for the Pacific Northwest Sweet Cherry Breeding Program:** We will curate new germplasm, phenotype and genotype data generated from the PNSCBP. We will also curate the relevant cherry genomics, genetics and breeding data for inclusion in the GDR. This data will be submitted by other *Prunus* researchers and extracted from publications. The collection and curation of data in one integrated database will allow building an efficient system not only for keeping track of the large volume of PNSCBP breeding data, but also for enabling direct utilization of genomics and genetics data worldwide for marker assisted breeding.

2. **Enable selection comparisons through access to data mining tools that utilize up-to-date performance and genotypic data:** We will upload and integrate the PNSCBP data to the GDR so that the current data mining tools can be continuously used for newly updated breeding data. The data mining tools include breeding data search tools by dataset, germplasm names, trait values, alleles and parentage. Breeders can download genotypic and phenotypic data of germplasm that meet the various categories and thresholds that users specified.

3. **Enable efficient parental selection for desired cultivars through access to data analysis tools that utilize up-to-date performance and genotypic data:** In addition to the genotypic and phenotype data, we will integrate the breeding values and DNA-based functional genotype data from available parent pools in PNSCBP. This will allow PNSCBP to use the parental selection tool in GDR. The tool is designed to predict the efficient parent combinations that can produce a target number of seedlings with specific traits thresholds specified by users.

4. **Ensure optimal utilization of the Sweet Cherry Breeding ToolBox through hands-on training to the PNSWBP team:** We will conduct hands-on in-person training on data template completion and use of the toolbox and hold quarterly conference calls to ensure toolbox is kept current with data and functionality.

RESULTS AND DISCUSSION

Publicly available trait and marker data that is relevant to the PNWSCBP continues to be added to GDR and the performance and genotypic data specific to the breeding program will be completed over the next couple of months. We will continue to offer this facility to personnel associated with the PNWSCBP as needed, through the use of easy to use interfaces and tools for the breeding program personnel to be able to upload, edit, browse and compare these data across years and locations. This up to date breeding management system for the PNWSCBP that will help facilitate marker-assisted breeding and more efficient development of new cultivars for PNW sweet cherry growers.

EXECUTIVE SUMMARY

Tree fruit breeding programs generate copious amounts of data. Utilizing this data requires proper management plans and interrogation tools to enable breeders to efficiently mine their data and extract what they need to enable more efficient breeding. Concordant with this is the need to also access all relevant public information such as what's known about traits, markers for these traits, germplasm containing useful traits in the same and related crops. Within the Rosaceae community database, GDR (www.rosaceae.org), a private breeding database for the PNWSCBP exists, connecting the programs private breeding data with all publicly available, quality checked, genomic, genetics and breeding data for Prunus crops. Searchable interfaces allow the data to be searched by trait, trait levels, location, marker, pedigree, germplasm, year, etc and tools enable download of data for upload to analysis programs. The Cross-Assist tool takes this concept one step further. Using component data from the breeding program it outputs the optimal parents to cross and numbers of seedlings needed to generate the desired offspring and eventually new cultivar(s) which meet producer and consumer needs.

The Cherry Toolbox in GDR will continue to be updated with the PNWSCBP breeding data and other public data following the end of this WTFRC award. Funds from this WTFRC project were leveraged to support federal crop database proposals that have generated over $5M in new funds over the next 4 years. Tools to allow direct upload of the data are developed, which enable breeders to be responsible for managing their own data. The Field Book App currently being evaluated for this purpose is looking very promising for this purpose and we are working with its developers to optimize for tree fruit breeding. We will continue to develop the GDR breeding tools into a comprehensive breeding management system that provides secure, one stop access to all the data management and analysis tools that Rosaceae breeders and allied scientists need to more efficiently develop new cultivars.

FINAL PROJECT REPORT

Project Title: New genomic regions controlling production and fruit disorder traits

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Cooperators: Yunyang Zhang, Amit Dhingra, Todd Einhorn, Amy Iezzoni

Other funding sources

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

Agency Name: WTFRC/OSCC
Amount awarded: $141,000 for 3 years from 04/01/2014 to 03/31/2016
Notes: After RosBREED: Developing and deploying new sweet cherry DNA tests led by Dr Peace with Oraguzie as Co-PI

Agency Name: WTFRC/OSCC
Amount awarded: $52,092 for two years from 04/01/2014 to 03/31/2015
Notes: Sweet cherry toolbox project led by Dr Main with Oraguzie as Co-PI

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, Oraguzie, and Main

Total Project Funding: $52, 844

Budget History:

Item	2014	2015
Salaries	**14,585**	**15,168**
Benefits	**6,417**	**6,674**
Wages	**2,800**	**2,800**
Benefits	**272**	**272**
Equipment		
Supplies	**972**	**972**
Travel	**1,000**	**1,000**
Plot Fees		
Miscellaneous		
Total	**26,002**	**26,842**

Footnotes: Salaries and benefits are for 0.42 FTE Research Associate. Wages and benefits are for a temporary assistant working 20 hours/week for 14 weeks at the rate of $10/hr. Supplies include reagents, lab supplies and other consumables.

Objectives: The specific objectives of this study were to:

1. Refine the marker-locus-trait (MLT) associations already determined for PFRF, bacterial canker and powdery mildew incidence.

2. Obtain phenotypic data on cracking incidence and pitting susceptibility and establish MLT associations using genotypic data developed in both RosBREED and the Stem-less cherry projects. Utilize the 'Selah' x 'Cowiche' population for specific trait phenotyping (pitting, PFRF and cracking susceptibility).

Significant findings and achievements

1. We have identified a genomic region on chromosome 2, *PFRF_G2b*, explaining 27.5% of the phenotypic differences in PFRF. This genomic region is common to both 'Selah' x 'Cowiche' population and the 600 sweet cherry accessions used in the RosBREED project. A genetic test is being developed for this locus in the project, CH-14-102 (led by Peace and Oraguzie). In addition, we have also identified genomic regions on chromosomes 3, 5 and 8 specific to 'Selah' x 'Cowiche' population.

2. A genomic region on chromosome 5, *PM_G5*, observed in multiple years associated with foliar powdery mildew (PM) incidence has been identified. This locus explains 40.5% of the differences among individuals in PM incidence and has been targeted for DNA test development in CH-14-102. A study to understand the genetic mechanisms underlying PM fruit infection has been initiated in the current RosBREED project.

3. A genomic region on chromosome 5, *BC_G5*, explaining 15.5% of the phenotypic variation in bacterial canker disease response in the green houses has been identified.

4. The cracking index of ~46 named cultivars in the PNW has been characterized. In addition, the genomic regions associated with cracking index have been identified on chromosomes 1, 2, and 3, across years in the 'Selah' x 'Cowiche' population. The high LOD score for the genomic regions on Chromosome 1 in 2014 (5.4) and chromosome 2 in 2015 (4.6), as well as, the occurrence of both regions over two years suggest that both would be candidates for DNA test development.

5. Approximately 46 PNW cultivars have been characterized for pitting index. We have identified several genomic regions associated with pitting index, however, the genomic region on chromosome 1 which is common across years appears to be a suitable candidate for DNA test development for MAB.

Results and Discussion

Two sets of germplasm were used in these studies. One set consisted of 600 accessions including old cultivars, current commercial cultivars, advanced selections and selected breeding progenies developed in the RosBREED SCRI project, whereas, the other set comprised 110 individuals belonging to the 'Selah' x 'Cowiche' mapping population developed in the Stem-free cherry SCRI project. The genotypic data sets each consisted of a 6K Infinium SNP array.

The objectives of this project as presented in this report focused around phenotypic data collection and integration of both phenotypic and genotypic datasets to identify genomic regions underlying pedicel-fruit retention force (PFRF), bacterial canker (BC) infection, foliar powdery mildew (PM) incidence, cracking index (CI) and pitting index (PI). A pedigree-based analysis in the FlexQTL™ software and interval mapping in MapQTL were used to identify genomic regions in the 600 accessions and in the 'Selah' x 'Cowiche' mapping population, respectively.

Objective 1: Refine the marker-locus-trait (MLT) associations already determined for pedicel-fruit retention force (PFRF), bacterial canker and powdery mildew incidence

a. Pedicel-fruit retention force (PFRF)

Fruit samples from each of 600 accessions were harvested randomly from single tree plots with pedicels attached at physiological maturity determined by color, taste and firmness. Five largest fruits from each individual were selected for PFRF measurements (in grams) in the laboratory using a mechanical force gauge (Imada DPS-11, Northbrook, IL, USA) with a custom fitted polyvinyl chloride attachment for fruit detachment. Data were recorded in 2010, 2011 and 2012. Following data analysis, we identified a genomic region on chromosome 2 (G 2) across years and in the combined data associated with PFRF (Table 1). The percentage of phenotypic variation in PFRF explained by this genomic region in the combined data was 27.5%. Other genomic regions were also observed on G 1 and G 2 in 2011 and one on G 4 in 2012 explaining one third of the phenotypic variation.

Table 1. Marker intervals and additive effect sizes of the PFRF genomic regions detected in the 600 pedigree-linked accessions. Please note that sample size increased with years.

Year	Chr	Locus	Marker interval (cM)	Peak position (cM)	Flanking markers	Posterior Intensity	Additive effect (g)	PVE (%)
2010	2	*PFRF_G2a*	0-16	1	ss490548700 ss490559390	0.04	125.8	3.5
2011	1	*PFRF_G1*	6 – 14	10	ss490545589 ss490546281	0.29	285.5	14.3
	2	*PFRF_G2b*	12 – 23	19	ss490548989 ss490549569	0.16	181.7	11.6
	8	*PFRF_G8a*	1 - 13	11	ss490557717 ss490557851	0.08	218.4	12.7
	8	*PFRF_G8b*	38 - 52	41	ss490551301 ss490558696	0.04	210.0	11.3
2012	2	*PFRF_G2b*	19-31	21	CPSCT038 Ss490547354	0.08	105.3	7.8
	4	*PFRF_G4*	12-15	13	ss490552535 ss490552620	0.76	314.2	33.0
Combined data	2	*PFRF_G2b*	12-14	13	ss490548989 ss490549037	0.59	211.8	27.5

Chr = chromosome; PVE-phenotypic variance

In the ‘Selah’ x ‘Cowiche’ population, 10-15 fruit per individual were screened in 2012, 2103 and 2014, and interval mapping in MapQTL was used to identify genomic regions associated with PFRF (Table 2). The genomic regions on G1, G 2, G 3 and G 5 each were observed across years, while a genomic region on G 8 was observed between years in 2012 and 2013.

Table 2. Genomic regions associated with PFRF in 'Selah' x 'Cowiche' population.

Year	Chromosome	Peak (cM)	LOD
2012	1	85.6	4.23
	1	107.4	4.19
	2	67.9	3.70
	3	15.6	1.72
	5	60.6	1.27
	8	24.5	2.56
2013	1	0.0	2.86
	1	108.3	2.34
	2	62.9	1.15
	3	17.6	1.72
	5	60.6	1.37
	8	20.3	1.76
2014	1	2.6	5.02
	2	67.9	2.85
	5	59.6	3.39
2015	1	2.6	1.48
	2	67.9	1.61
	3	14.6	2.59
	5	59.6	2.23

Note: The gray color indicates that the genomic regions have a LOD score less than 3, but were consistent between years. LOD= Log likelihood ratio.

The genomic region on G 2 was consistent between the 600 accessions and 'Selah' x 'Cowiche' population and this locus has been followed up with genetic test development in the project, CH-14-102. It appears that the genomic regions on G 3, G 5 and G 8 may be specific to 'Selah' x 'Cowiche' population and will be targeted for further DNA test development to select individuals from that population that have low, medium or high PFRF combined with superior fruit quality.

b. Powdery mildew incidence

Powdery mildew infection on sweet cherry leaves was assessed in the field in 2011, 2012, 2013 and 2014 on 600 sweet cherry accessions based on a six point scale where, 0= no infection and 5= severe infection (Chavoshi et al. 2014). Following data analysis in FlexQTL™, we identified a genomic region on G 5 that was consistent across years while another on G1 was observed in 2011 and 2013. A genomic region on G 2 was identified in 2011 and 2014. Genomic regions on G 3 and G 6 were identified in single years (Table 3). Combining all 4 years data, we observed two genomic regions; one on G2, *PM_G2*, explaining 3.7% of the phenotypic variation and another on G5, *PM_G5*, explaining 48.1% of the phenotypic variation. Genetic tests are now being developed for the *PM_G5* region, in the project, CH-14-102, to facilitate marker assisted breeding for foliar PM resistance in sweet cherry.

Although there was no specific funding for PM infection on fruit, we took advantage of the high disease pressure in 2014 to score for PM infection on fruit. However, only 161 accessions which had limited fruit on the tree were assessed in late August, a time when most trees had no fruit or the fruit had dried out. Following analysis, we identified genomic regions on G 4 and G 6 explaining 19.6% and 55.2% of the phenotypic variation, respectively. Due to low disease pressure in 2015, we were unable to take advantage of natural infection in the field to score for fruit PM. With funding from RosBREED, we inoculated a subset of cultivars with the PM pathogen to facilitate developing a standard protocol for fruit infection

for use in screening a larger germplasm set in future for identification of genomic regions for fruit infection.

Table 3. Marker intervals and additive effect sizes of PM genomic regions detected.

Chr	Year	Locus	Marker interval (cM)	Flanking markers	Max Posterior intensity	Additive effect	PVE (%)
1	2011, 2013	*PM_G1*	9.30 – 11.58	ss490545821 - ss490546020	0.19	0.86	30
2	2011, 2014	*PM_G2*	26.04 – 26.98	ss490549912 - ss490550007	0.11	0.85	17-22
3	2014	*PM_G3*	14.02 – 18.10	ss490547825 - ss490551374	0.05	0.53	2
5	2011, 2012, 2013, 2014	*PM_G5*	4.64 – 7.13	ss490553865 - ss490553929	0.34	1.22	37-42
6	2011	*PM_G6*	48.8 – 50.9	ss490555836 - ss490555878	0.10	0.64	4

Chr = chromosome; PVE-phenotypic variance

c. Bacterial canker infection

Bacterial canker screening was conducted in 2013 and 2014 according to Mgbechi-Ezeri (2015). Data analysis based on separate years indicated a positive evidence (BF≥8) for a genomic region on G 5, *BC_G5a*, explaining 17.0% of the phenotypic variation in 2013 while in 2014, there was a decisive evidence for a genomic region on G 5 explaining 15.3% of the variation. Combining both years, there was a decisive evidence for a genomic region on G 5, explaining 15.1% of the variation, and yet another genomic region on G 7, explaining 6.3% of the phenotypic variation.

Please note that these results were obtained from disease response studies following leaf inoculation in the lab with the pathogen, *Pseudomonas syringae* var *syringae*. There was no opportunity to carry out field tests since bacterial canker is considered a quarantined disease in Washington. However, we have identified isolates of other *Pseudomonas* spp from WSU-IAREC Prosser, Roza farm, which we plan to make available for use in field tests in the future.

Table 4. Bacterial canker genomic regions, chromosomes & percentage variance explained by region

Year	Chr	Marker interval	Peak position	Posterior Intensity	Additive effect	PVE (%)	Flanking markers
2013	5	2, 13	9	0.200	0.467	17%	Ss490553738-ss490553963
	7	3, 7	5	0.320	0.387	11%	Ss4905536403-ss490556479
2014	5	(2, 11)	4	0.353	.598	15.3%	Ss490553738-ss490553817
	2	40, 46	43	0.2603	0.407	7.1%	Ss490550529-ss490550691
Combined data	5	2, 13	9	0.282	0.440	15.1%	Ss490553738-ss490553963
	7	4, 7	4	0.274	0.300	6.3%	Ss4905536403-ss490556485

Chr = chromosome; PVE = phenotypic variance

Objective 2: Obtain phenotypic data on cracking incidence and pitting susceptibility and establish MLT associations using genotypic data developed in both RosBREED and the Stem-less cherry projects. Utilize the 'Selah' x 'Cowiche' population for specific trait phenotyping (pitting, and cracking susceptibility)

a. Cracking susceptibility and identification of genomic regions

Fruit were harvested at commercial maturity from ~80 seedlings belonging to 'Selah' x 'Cowiche' mapping population and from ~46 named cultivars in 2014 and 2015. Approximately 10-25 and 60 fruit samples per tree, respectively, at commercial maturity, were selected for phenotyping in the 'Selah' x 'Cowiche' population and from the named cultivars. The 60 fruit sample was subdivided into three groups of 20 fruit representing three replicates in each named cultivar, whereas, only a single replicate of 10-25 fruit per seedling (due to low fruit numbers) was assayed in the 'Selah' x 'Cowiche' population. The diameter of each fruit was recorded as well as the combined weight of fruit in each replicate. The fruit samples were soaked in distilled water and cracking incidence as well as split type was recorded every hour up to 5 hours.

Analysis of variance showed significant differences among genotypes in cracking susceptibility at 1h, 2h, 3h, 4h and 5h following soaking in water ($p<0.0001$)(data not presented). The highest incidence of cracking (CI) was observed at 5h (CI = 0.83 in the named cultivars and 0.97 in 'Selah' x 'Cowiche' population) and the lowest at 1h (CI = 0.35 in the named cultivars and 0.603 in 'Selah' x 'Cowiche' population) ($p<0.05$)(data not presented). There was no significant difference between the incidence at 5h and 4h in both the named cultivars and the 'Selah' x 'Cowiche' population ($p>0.05$). The correlation between years (i.e., 2014 and 2015) was 0.84.

The correlation between fruit size/weight and incidence of cracking was high in the cultivars, with correlation coefficients of 0.45–0.63 ($p<0.01$)(data not presented). However, correlation was generally low and non-significant ($p>0.05$) in 'Selah' x 'Cowiche' population, although the correlation at 1h appeared to be the highest at $r=0.18$, with fruit diameter and $r=0.21$ with fruit weight (data not presented).

Following QTL analysis using interval mapping in MapQTL in 'Selah' x 'Cowiche' population (n=64) in 2014, we identified a genomic region on chromosome 3 (LOD = 5.4) across soaking periods including 1h, 2h, 3h, 4h and 5h, suggesting that only one soaking period may suffice for recording CI (data not presented). Other genomic regions were identified on G 1 (LOD = 2.5), G 2 (LOD = 2.30), G 6 (LOD = 4.2), G 7 (two regions at LOD = 3.7 and 2.6), and G 8 (LOD = 3.3 and 3.40). The FlexQTL™ results combining data sets including 'Selah' x 'Cowiche' population (n=62) and 37 named cultivars on the other hand, identified a major genomic region on G 2 explaining 54% of the phenotypic variation (Table 5). In 2015, the genomic regions identified in the 'Selah' x 'Cowiche' population (n=47) include G 1 (LOD = 3.3), G 2 (LOD = 4.3), G 3 (at LOD = 2.6), G 4 (LOD = 2.3), G 5 (LOD = 2.2, and G 6 (LOD = 2.2). FlexQTL™ analysis combining 'Selah x 'Cowiche' data (n= 45) and data from named cultivars (n= 37) identified a major genomic region on G 4 explaining 34% of the phenotypic variation (Table 5).

A study at INRA, Bordeaux, France (Quero-Garcia et al.2010) conducted in 2006, 2008, 2009, 2010 and 2011 using the 'Regina' x 'Lapins' population, identified genomic regions on G 1, G 3 and G 4 which were weak and variable across years while the genomic region on G 5 was stable across years and explained 15% of the phenotypic variation for CI. The genomic regions on G 1, G 2, and G 3 were common between our study and that of Quero-Garcia et al (2010), although different mapping populations were used. The genomic regions on G 2 and G 3 would appear to be candidates for DNA test development. .

Table 5. Genomic regions for cracking index identified based on MAPQTL and FlexQTL™ analyses.

Year	Chr	LOD	Peak(cM)
S x C family			
2014	1	2.5	0
	2	2.3	40.8
	3	5.4	45.6
	6	4.2	25.9
	7	3.7	27.7
	7	2.6	47.7
	8	3.3	10.3
	8	3.4	33.7
2015			
	1	3.3	92.7
	2	4.3	20.6
	2	4.3	45.6
	3	2.6	15.6
	4	2.3	0
	5	2.2	60
	6	2.2	40
FlexQTL™			
	Chr	Bayes factor	PVE (%)
2014	2	>5	54
2015	4	>5	34

Chr=chromosome; S X C='Selah' x 'Cowiche'

b. Pitting susceptibility and identification of genomic regions

Fruit were harvested at commercial maturity from 'Selah' x 'Cowiche' population (n= 80 and 77 in 2014 and 2015, respectively) and from 46 named cultivars in both years. Approximately 3-25 and 60 fruit samples without blemish per individual, were selected after harvest in the 'Selah' x 'Cowiche' population and in the cultivar subgroup, respectively. The fruit samples from each cultivar were subdivided into three groups of 20 fruit representing three replicates, whereas, only a single replicate of fruit from 'Selah' x 'Cowiche' population was assayed. Each replicate was weighed, transferred into ziplock bags and stored at 4 ºC for 4 hours. Pitting was induced on both sites of the fruit in the 'Selah' x 'Cowiche' population and on one side of the fruit in the named cultivars and fruit were held at 1 °C for 2 weeks. Pitting was rated on a 4 point scale where, 1= no pitting, 2= superficial pitting, 3= medium pitting; pit was deeper and wider and had clearly distinct edges; 4= severe pitting, pit was very deep and edges had sunken into the pulp tissue (Toivonen et al. 2004).

Analysis of variance showed significant differences in pitting index (PI) among cultivars (p<0.0001)(data not presented). Tukey's honest studentized test was used to separate the means at p<0.05. The correlation between years among the named cultivars was r = 0.63, whereas, in the 'Selah' x 'Cowiche' population this was 0.50. 'Heldelfingen' showed the lowest PI (1.70) in 2014, while 'Ulster' was the lowest (1.63) in 2015. By contrast, 'Moreau' recorded the highest PI (3.87 and 3.94 in 2014 and 2015, respectively), while 'Regina' had medium PI (2.1 and 2.06 in 2014 and 2015, respectively) (data not presented). The correlation between PI and fruit weight was low at r= -0.07 in the named cultivars and r= -0.05 in the 'Selah' x 'Cowiche' population (p>0.05). In addition, there was a negative and low correlation (r= -0.21) between firmness and PI in the 'Selah' x 'Cowiche' population (data not presented).

Data analysis based on interval mapping in MapQTL (n=79) using the 2014 data showed genomic regions on G 1 (LOD = 5.7), G 3 (LOD = 4.7), G 4 (LOD =3.5), G 6 (LOD = 2.3) and G 7 (LOD = 2.3)(Table 6). FlexQTL™ analysis with combined data set including named cultivars and 'Selah' x 'Cowiche' population identified genomic regions on G 1 (showing a peak at 34 cM and explaining 5% of the phenotypic variation), G 3 (with a peak at 30 cM explaining 2 % phenotypic variation) and on G 4 (with a peak at 14 cM explaining 4 % phenotypic variation). In 2015, the genomic regions identified in the 'Selah' x 'Cowiche' population (n=77) mapped on G 1 (with a peak at 53.7 cM and a LOD score of 7.7), G 2 (peaking at 51.4 cM with a LOD score of 2.6), and G 8 (LOD = 2.0). The FlexQTL™ analyses (based on n=75 and n=42, in the 'Selah' x 'Cowiche' population and the named cultivar group, respectively identified one genomic region on G 1 (with a peak at 25 cM) explaining 30% of the phenotypic variation.

This is the first study on genetic mechanisms underlying pitting susceptibility in sweet cherry and unfortunately there is no other study to compare and contrast our results with. It appears that the genomic region on G 1 which showed up consistently between years and statistical analyses methods may be a candidate for DNA test development for deployment of MAB strategies for PI. This genomic region was detected in the Selah x Cowiche population in 2014, and in 2015, explained 30% of the phenotypic variation from FlexQTL™ analyses (Table 6).

Table 6. Genomic regions for pitting index identified based on MAPQTL and FlexQTL™ analyses.

Year	Chr	LOD	Peak(cM)
S x C family			
2014	1	5.7	61
	3	4.7	24
	4	3.5	29
	6	2.3	70
	7	2.3	50
2015	1	7.7	54
	2	2.6	51
	8	2.0	25
FlexQTL™			
	Chr	Bayes factor	PVE (%)
2014	1	>5	34
	3	>5	30
	4	>5	14
2015	1	>5	25

Chr=Chromosome; S X C= 'Selah' x 'Cowiche'.

References

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Toivoven P.M.A., Kappel, F, Stan, S., Mckenzie, D-L, Hocking R. 2004. Firmness, respiration and weight loss of 'Bing', 'Lapins' and 'Sweetheart' cherries in relation to fruit maturity and susceptibility to surface pitting. HortScience 39 (5): 1066-1069

Executive Summary

Knowledge of genetic systems controlling the inheritance of horticulturally important traits is important for selection and breeding decisions and for future predictions of the performance of individuals. In this study we have used two population types including a bi-parental mapping population ('Selah' x 'Cowiche') and a pedigree based population comprising ~600 accessions to map and identify the genomic regions associated with traits of importance to the sweet cherry industry including pedicel fruit retention force (PFRF), bacterial canker infection (BC), foliar powdery mildew infection (PM), cracking index (CI) and pitting index (PI). To our knowledge, this is the first study on the identification of genomic regions underlying pedicel fruit retention force, powdery mildew incidence, bacterial canker incidence, and pitting index in sweet cherry. Association analyses using both phenotypic and genotypic datasets were conducted in MAPQTL and FlexQTL™ for the bi-parental population and the pedigree-based germplasm, respectively. A genomic region was identified for pedicel fruit retention force (PFRF), on chromosome 2 (G 2), tagged *PFRF_G2*. This locus was consistent between years and studies, and explained more than one-quarter of the phenotypic variation among individuals for PFRF. In the case of foliar powdery mildew infection, a major locus, *PM_G5*, was identified which explained ~40% of the variation. This genomic region appeared to co-locate with the genomic region, *BC_G5*, associated with bacterial canker disease response in the green houses. The *BC_G5* locus explained ~15% of the variation in disease response for BC. For pitting index, we identified genomic regions on chromosomes 1, 3 and 4, however, the locus on chromosome 1 was consistent between years and study, whereas, other genomic regions were variable between years. Genomic regions were identified for cracking index in 'Selah' x 'Cowiche' population on chromosomes 1, 2, 3, and 6, whereas, only two genomic regions were identified including G 2 and G 4 based on FlexQTL™ analyses using a combined data set including 'Selah' x 'Cowiche' population and the named cultivars. The results for cracking index using 'Lapins' x 'Regina' population by a research group at INRA, France, also identified genomic regions on G 1, G 2 and G 3 in single years but only the genomic region on chromosome 5 was consistent across years. In addition, the profile of ~46 PNW cultivars for both cracking and pitting indices has been established.

Going forward, we suggest developing DNA tests for the genomic region on chromosome 2, *PFRF_G2,* for MAB for low, medium and high pedicel fruit retention force, *PM_G5,* for foliar powdery mildew resistance, *BC_G5,* for green house screening for low bacterial canker disease response, G.2 and G.3 for low cracking index, and for G 1, for low pitting index. The DNA tests (being developed in CH-14-102) will facilitate deployment of MAB for multiple traits in sweet cherry to improve selection efficiency and cost-effectiveness.

FINAL PROJECT REPORT

Project Title: Effect of near-harvest irrigation on fruit quality

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Cooperators: Denny Hayden, Russ LeSage

Total Project Request: **Year 1:** $24,503 **Year 2:** $21,330

Other funding sources

Notes: M.S. student (Nadia Valverdi) in Whiting's lab is supported by a Fulbright scholarship, covering stipend and tuition to work on this project.

Budget 1
Organization Name: WSU **Contract Administrator: Amanda Yager**
Telephone: 509-786-9204 **Email address: ayager@wsu.edu**

Item	2014	2015
Salaries	$3,000	$3,120
Benefits	$1,290	$1,342
Wages	$3,520	$3,661
Benefits	$341	$355
Equipment	$3,000	
Supplies	$3,000	$3,000
Travel	$1,000	$1,000
Miscellaneous		
Plot Fees		
Total	$15,151	$12,478

Footnotes: Salary is one month for technician support + associated benefits; wages are for student timeslip assistance with data collection

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

Item	2014	2015
Salaries		
Benefits		
Wages[1]	$4,810	$4,810
Benefits	$388	$388
Equipment		
Supplies[2]	$1,000	$500
Travel[3]	$850	$850
Miscellaneous[4]	$2,304	$2,304
Plot Fees		
Total	$9,352	$8,852

Footnotes:
[1] Wages are for 370 hours for temporary employee support at $13/hr. Benefit rate is 8.31%.
[2] Supplies include nitrogen gas, rental fee for N gas cylinder; Irrigation tubing and supplies; pvc access tubes for neutron probe installation.
[3] Travel to sites in The Dalles, OR 1,440 miles (240 per week x 6 weeks) at 0.59 per mile.
[4] IrriNet, LLc. neutron probe readings at $4/measurement tube * 48 tubes [i.e., 16 per site x 3 sites] x 3 measurements per week x 4 weeks.

OBJECTIVE:
Improve fruit quality by understanding the role of near-harvest irrigation on key quality traits (firmness, size, soluble solids) and fruit susceptibility to splitting.

SIGNIFICANT FINDINGS:

- Irrigation can be reduced prior to harvest without harming sweet cherry fruit yield or quality
- Withholding irrigation in the weeks prior to harvest does not consistently improve quality nor reduce susceptibility to splitting
- Preharvest termination of irrigation has varied effects on sweet cherry fruit quality, the most consistent response being an increase in soluble solids, and slight decrease in firmness
- Withholding irrigation up to 24, 21 and 15 dbh did not affect yield of mature 'Chelan', 'Lapins' and 'Skeena' trees, respectively
- Withholding irrigation beginning 15 dbh, but not 10- or 5-dbh, led to a significant reduction in fruit growth and final fruit size of Skeena
- Stem water potential is a good indicator of plant water status and responded sensitively and rapidly to irrigation withholding
- A stem water potential value of less than -1.5 MPa one week before harvest resulted in growth limiting conditions for cherry fruit.
- Withholding irrigation water from 'Skeena' trees for up to 9 days before harvest (dbh) had no quantifiable effects on tree yield, fruit size or quality at harvest or after cold storage
- Withholding irrigation for 5 dbh had no effect on fruit growth or quality of 'Sweetheart'/'Gisela6' trees
- Withholding irrigation water from 'Sweetheart' for 10 and 15 days resulted in a significant reduction in fruit size and weight
- Withholding irrigation up to 24 days before harvest (dbh) did not affect splitting susceptibility on 'Chelan' and 'Lapins'
- Sweetheart fruit from 10 and 15 dbh treatments had significantly greater cracking resistance (i.e., ~50% less cracking), compared to control and 5 dbh fruits
- Across cultivars+sites, differences in soil water content among withholding treatments were apparent throughout the 3-foot soil profile measured – tree roots of 'Gisela 6' and Mazzard were clearly extracting water from the 3 ft depth

RESULTS AND DISCUSSION:

This research project has investigated the effects of withholding near-harvest irrigation on fruit quantity and quality, soil water content, and tree physiology for 4 cultivars ('Chelan', 'Lapins', 'Skeena', and 'Sweetheart') over two years and in 3 locations (Brewster, Dufur, and Pasco). The following summary is organized by cultivar/location, and emphasizes results from 2015. Please see our previous report for detailed descriptions of 2014 results.

'Chelan'/Mazzard - Pasco We documented no effect of early termination of irrigation treatments (i.e., 24 and 14 dbh) on stem water potential nor fruit growth rates in the 'Chelan' trial in Pasco (Fig). Stem water potential remained above ca. -0.75 MPa at all sampling dates for 2014 and above ca -1.2 in 2015, irrespective of treatment. Importantly, fruit quality was unaffected by early termination of irrigation (Table 1). In addition, there was no effect of irrigation treatment on yield in either year (Fig. 3).

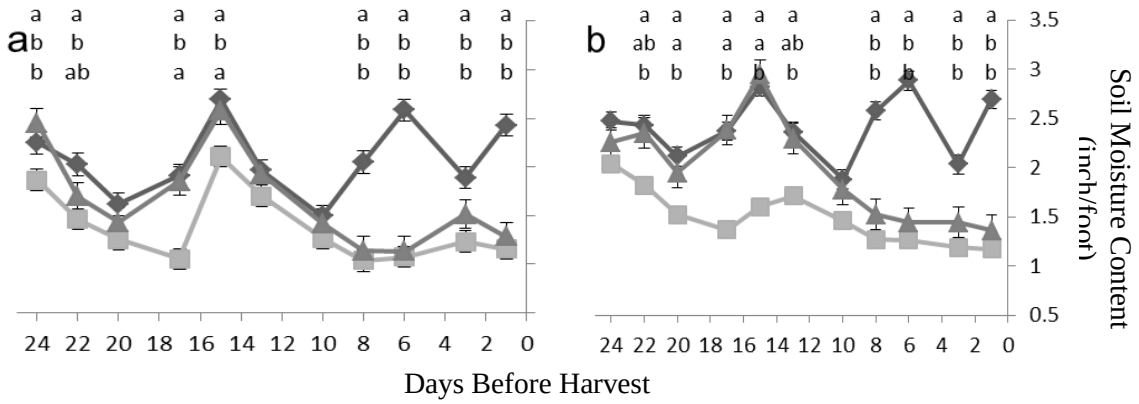

Figure 1. Soil water content at a = 6' depth and b = 12' for 'Lapins'/Mazzard trees on 2015.

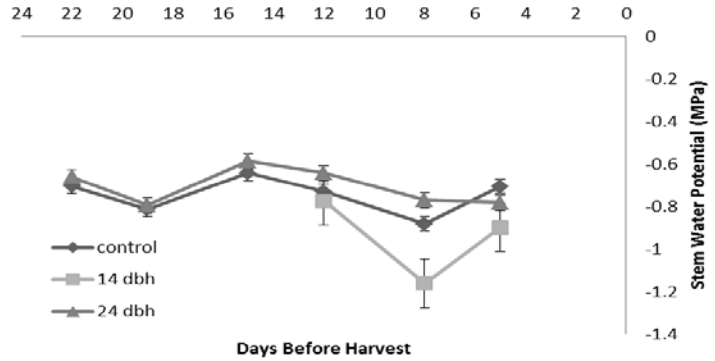

Figure 2. The influence of early termination of irrigation on 'Chelan' fruit for midday xylem stem water potential on 2015. Harvest was on 28 May.

Table 1. The effect of early irrigation termination on 'Chelan' fruit quality. Irrigation was terminated on 4 May (24 dbh) and 14 May (14 dbh), harvest was 28 May. T.A.= titratable acidity; S.S.=soluble solids; PFRF = pedicel-fruit retention force. n=75 for all quality assessments

Treatment	Firmness g/mm	Weight g	Diameter mm	T.A.	S.S. %	Color CTIFL	PFRF g
control	313	10.4	27.8	2.8	16.3	5.07	1.09
14 dbh	319	11.0	28.4	2.8	17.2	5.27	1.08
24 dbh	297	10.6	28.0	2.6	16.7	5.21	1.10
p-value	0.497	0.259	0.344	0.850	0.427	0.647	0.886

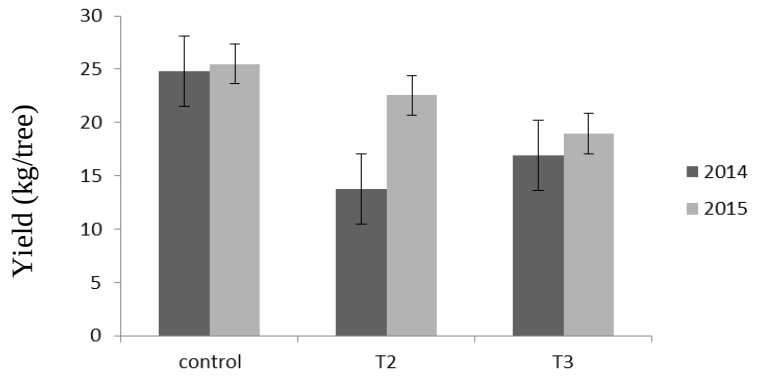

Figure 3. The influence of early termination of irrigation on 'Chelan' fruit yield in 2014 and 2015. T2= 7 dbh and 14 dbh for 2014-2015; T3= 17 dbh and 24 dbh for 2014-2015. Each column is the mean of X trees +/- the standard error.

There was no effect of irrigation treatment on the susceptibility of 'Chelan' fruit to splitting (Fig. 4) when assessed by a bench-top immersion test – this was consistent in both years. 'Chelan' is regarded as a split-resistant cultivar – a contention supported by our research. The cracking index was never greater than 25 in 2014, although in 2015 values peaked about two weeks prior to harvest, between 40 and 50. This is attributed to a heavy rain (1.03 inches) that fell the day we sampled fruit. The dramatic increase in sensitivity to splitting following the rain is likely due to greater turgor in fruit (i.e., less ability to withstand water uptake) and, perhaps, a loss of protection from protective coatings. We sampled additional fruit to assess natural cracking in the field, following the rainfall 14 dbh (>1 inch). The percent of cracked fruit was 14% for control, 17% for 14 dbh and 8.5% for 24 dbh irrigation treatments, differences were not statistical significant.

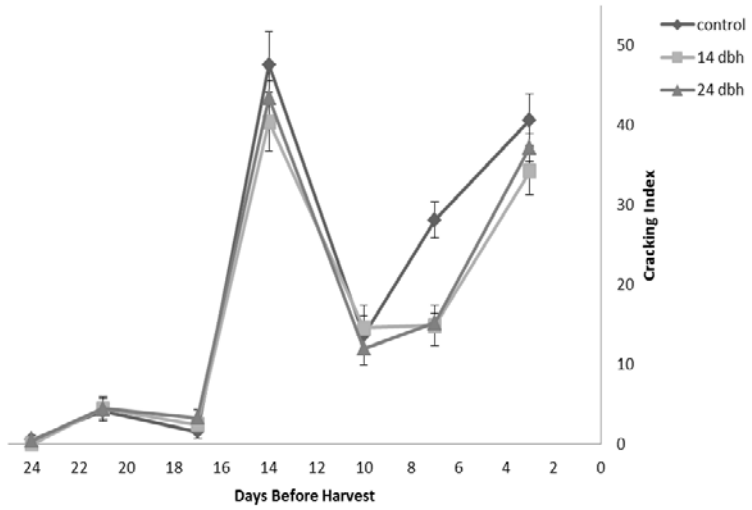

Figure 4. Cracking index (i.e., relative susceptibility) of 'Chelan' fruit to preharvest termination of irrigation treatment on 2015. Harvest was on 28 May.

'Lapins'/Mazzard - Brewster In both years we conducted two separate trials in mature commercial 'Lapins' orchards near Brewster; one being similar to the Pasco 'Chelan' trial in which the full suite of soil and tree testing was conducted regularly (intensive trial); the other being a larger scale trial established in two (2014) or four (2015) contiguous 4 acre blocks in which only fruit quality data were evaluated (extensive trial).

Intensive trial In 2015 we observed a decline in stem water potential in trees receiving less irrigation compared to the regularly-irrigated control, though water potential never fell below ca. -1.2 MPa in 2014 or ca. -1.6 MPa in 2015 from trees unwatered for 16 days. These results suggest that trees were not under significant stress at any point – previous research has suggested that midday stem water

potential needs to drop to below -1.5 to have negative impacts (which it did in 'Sweetheart' in Dufur – see below). The inability to induce significant stress in mature 'Lapins'/Mazzard trees suggests that these trees are accessing water resources deep in the soil profile. Our soil texture analysis revealed a sandy clay loam in the upper 8 inches, transitioning to sandy loam and loam textures. Analyses of soil water content revealed a consistent withdrawal of water from 3 feet deep (our deepest sampling site), further indication that trees were supporting the lack of irrigation with water from soil reserves.

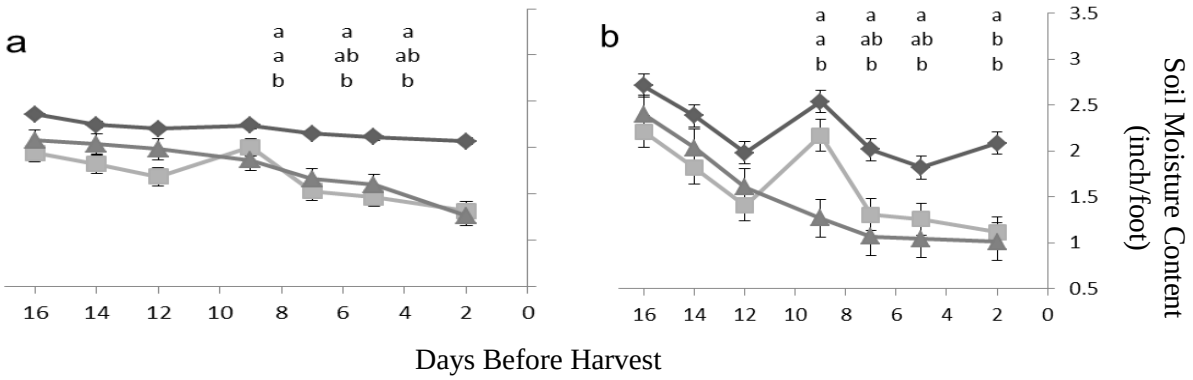

Figure 5. The influence of early termination of irrigation on soil water content at a = 6' depth and b = 12' for 'Lapins' trees on 2015.

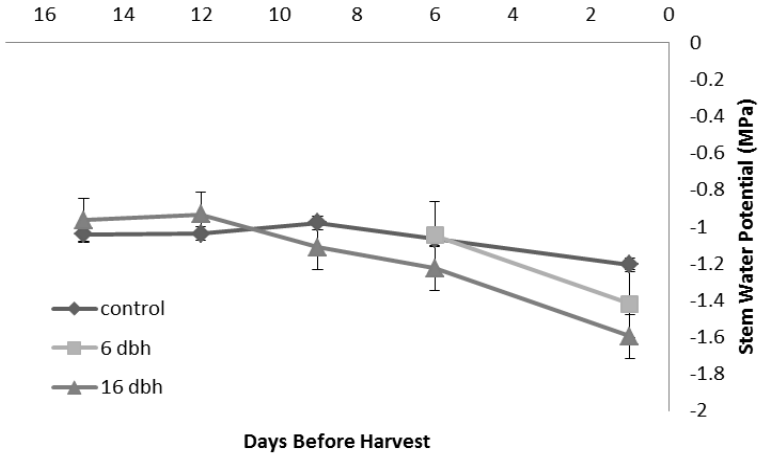

Figure 6. The influence of early termination of irrigation on 'Lapins' fruit for midday xylem stem water potential on 2015. Harvest was on 1 July .

Despite a decline in stem water potential near harvest in deficit irrigation treatments, there was no effect of irrigation treatment on fruit growth (data not shown) nor final diameter (Table 2). Again, this is not unusual for the range of stem water potentials observed across treatments. The only consistent effect on fruit quality attributes across both years was an improvement in fruit soluble solids, which were significantly improved (+10 to 14%) by both early irrigation termination treatments in 2014 and (+14%) for withholding irrigation from 6 dbh in 2015. The increase in soluble solids may be due to fruit dehydration but there was no observable fruit shrivel. An increase of 2% soluble solids is significant and likely sufficient to improve the consumer appeal of the fruit. In 2015 we observed a reduction in fruit firmness of a ca. -6% in response to irrigation being withheld for 16 dbh (Table 2), similar to the results for 'Skeena' in Dufur in 2014. Yield wasn't affected by treatments at any year of study (Fig. 6).

Table 2. The effect of preharvest irrigation termination on 'Lapins' fruit quality and yield. Irrigation was terminated on 15 June (16 dbh) and 25 June (6 dbh), harvest was on 1 July. T.A.=titratable acidity; S.S.=soluble solids; PFRF=pedicel-fruit retention force (n=25) for all the traits.

Treatment	Firmness g/mm	Weight g	PFRF kg	Diameter mm	T.A.	Color CTIFL	S.S. %
control	282 a	9.2	0.7	25.4	2.5	4.8	17.0 a
6 dbh	286 a	9.3	0.7	25.5	2.1	4.6	19.3 b
16 dbh	266 b	9.2	0.7	25.7	2.1	4.9	18.8 ab
p-value	<0.0001	0.832	0.711	0.870	0.534	0.732	0.078

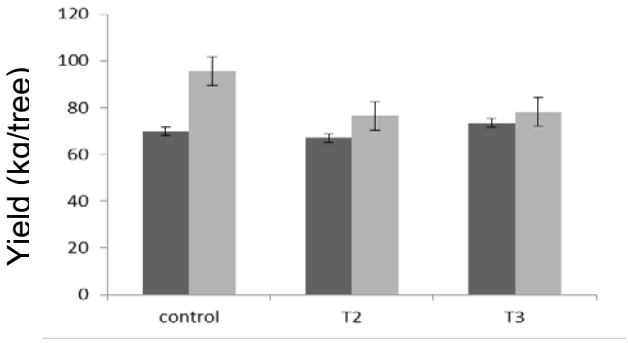

Figure 7. The influence of early termination of irrigation on 'Lapins' fruit yield in 2014 and 2015. T2= 11 dbh and 6 dbh for 2014-2015; T3= 21 dbh and 16 dbh for 2014-2015. Each column is the mean of 9 trees +/- the standard error.

We observed an inconsistent and largely insignificant effect of irrigation treatment on 'Lapins' fruit susceptibility to cracking (Fig. 8). Clearly 'Lapins' is more susceptible to splitting than 'Chelan' – the lowest 'Lapins' cracking index was about the same as the highest cracking index for 'Chelan' in the 2014 trials. Further, the pattern of susceptibility to cracking differed between these two cultivars. For 'Chelan', the index was extremely low, exhibiting nearly complete resistance until the final week before harvest. In 'Lapins' susceptibility increased throughout the final weeks of stage III of fruit development and exhibited a decline at the point of harvest, irrespective of irrigation treatment (Fig.8). In 2015, the trend in fruit susceptibility to splitting was similar to the previous year but fruit the cracking index was consistently about 20 points lower, nearly half of the values in 2014. This supports the conclusion that there is a strong effect of environment on susceptibility to splitting. No irrigation treatment had a significant effect on fruit susceptibility to splitting in 2015.

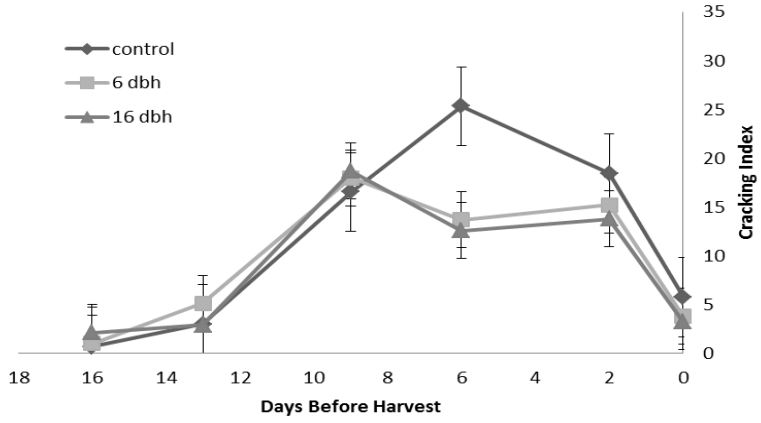

Figure 8. Cracking index (i.e., relative susceptibility) of 'Lapins' fruit to preharvest termination of irrigation treatment on 2015. Harvest was on 28 May.

Extensive trial In 2015, the four irrigation treatments compared were: 1) irrigated every 4-5 days for 5-6 hours (control-lo) 2) irrigating every 2 days for 6 hr sets (control-hi), 3) withholding irrigation from 19 dbh, and 4) withholding irrigation from 9 dbh. We imposed the 19 dbh termination treatment and the over irrigated treatment on 15 June, the 9 dbh termination treatment was imposed on 25 June. Fruit were harvested on 4 July. Fruit from the over irrigated treatments and withhold of 19 dbh shows an increase of (ca. +8 and 6%) in firmness respectively, but it shows a decrease of 11% in soluble solids compared to the control-high treatment.

Table 3. The effect of early irrigation termination on 'Lapins' fruit quality and yield from the large-scale (4 acre) plots. Irrigation was terminated on 15 June (19 dbh), 25 June (9 dbh) . T.A.=titratable acidity; S.S.=soluble solids; PFRF=stem pull force. (n=75).

Treatment	Firmness g/mm	Weight g	Diameter mm	T.A.	S.S. %	Color CTIFL	PFRF kg.
Control-lo	257 b	11.4	27.4	2.2	17.6 a	4.9	0.6
Control-high	277 a	10.5	26.0	2.1	15.9 b	4.7	0.6
9 dbh	268 ab	10.3	26.6	2.1	16.6 ab	4.9	0.6
19 dbh	273 a	9.9	26.6	2.2	17.7 a	4.8	0.6
p-value	0.0070	0.116	0.397	0.620	0.103	0.854	0.121

'Skeena'/'Gisela6' – Dufur High temperatures resulted in much earlier harvest dates than expected (June 24). This was partly due to Skeena's inherent sensitivity to heat stress. Thus, our 9 and 3 dbh treatments were intended to be 15 and 10 dbh, respectively. The 5 dbh treatment had not yet been initiated. Irrespective, our data support that Skeena trees could handle no irrigation for 10 dbh without negative effects on growth or quality, as similarly observed in 2014. Fruit size was not affected by water withholding, therefore we did not evaluate the relative water content and dry matter of fruit. Stem water potential values were close to critical levels (< -1.5 MPa), but not low enough for long enough to reduce fruit growth.

Table 4. Effects of early irrigation withholding for 3 or 9 days before harvest (dbh) on yield and fruit quality attributes of 'Skeena' sweet cherry trees.

Treatment	Yield kg/tree	Fruit diameter mm	Fruit wt. g	FF g/mm	Skin color ctifl	PRF g	SSC %	TA %
Control	34.85	28.35	10.01	339.6	5.7	276 b	18.6	0.88
3-dbh	38.06	27.75	9.45	340.4	5.5	422 a	18.1	0.91
9-dbh	34.76	28.25	9.94	343.4	5.5	356 ab	18.7	0.91
Pr>F	*0.659*	*0.469*	*0.496*	*0.834*	*0.083*	*0.016*	*0.533*	*0.241*

Yield (n=2); fruit diameter and weight (n=400);
fruit firmness (FF) (n=400); skin color (n=100); pedicel retention force (PRF) (n=25);
titratable acidity (TA) and soluble solids content (SSC) (n=2).
Data assigned different letters within columns signifies significant difference at $P < 0.05$ by Fisher's Protected LSD test.

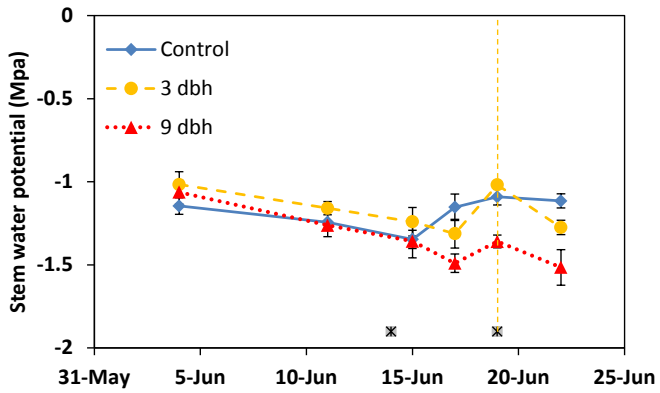

Figure 9. Stem water potential of Skeena trees cut-off from irrigation 3 or 9 days before harvest (dbh), compared to control trees. Asterisks above x-axis signify start date of withholding for each treatment with hashed vertical line. Harvest date was 4-July. For water potential, 4 leaves per replicate were bagged ~1hr prior to measurement. Measurements bracketed solar noon (±1.5 hrs).

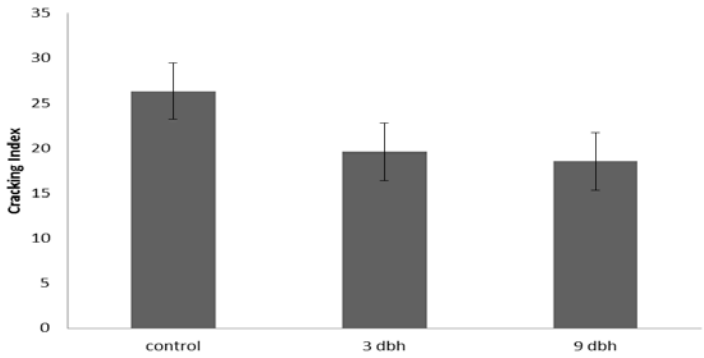

Figure 10. Cracking susceptibility of 'Skeena'/Gisela6 fruit in response to near-harvest deficit irrigation treatments. dbh = days before harvest.

'Sweetheart'/'Gisela6' – Dufur For each withholding treatment, stem water potential declined in synchrony with the irrigation termination date (Fig.11). Within ~1 week of harvest, critical water potential values (i.e., relative to the process of fruit growth) are ≤ -1.5 MPa. These data agree with 2014 results from 15 dbh Skeena. Fruit growth also responded sensitively to water withholding; a ~9% reduction of fruit size was observed (approximately a ½ row size reduction). Photosynthesis of 10 and 15 dbh trees was reduced by 50%, compared to controls and 5 dbh, but only at 2 days before harvest. Dry matter (carbon) of fruit was not reduced by irrigation treatments indicating that growth resources under water stress conditions remained sufficient for growth. In fact, relative water content was reduced in stressed treatments indicating that evaporation at the fruit surface, and/or water flow out of the fruit to organs with lower water potential were responsible for the weight loss. The fact that sugar concentration was higher in these fruit supports that they were slightly dehydrated (ssc represents the percent sugar (predominantly) in solution, therefore processes which cause dehydration increase the ssc). Cracking data support this observation. Less cracking could have been due to changes in the cuticle and/or epidermal layer as a result of water stress, but is more likely associated with the fruit's ability to draw in more water. We would have expected a significant reduction in water content to result in softer fruit, but this was not supported by firmtech data. Interestingly, all treatments of Sweetheart were observed to have 'lost' size in the last few days prior to harvest-presumably due to dehydration. Temperatures were ~100°F several days to 1 week prior to harvest. Despite a reduction in fruit size, all treatments attained an average fruit size at harvest of ~10 row (Table 5). Soil moisture was low in the top 1 foot of soil for 10 and 15dbh trees, but roots were clearly extracting water from lower depths. While soil moisture provides an indication of the stress it is an indirect measure of plant stress. An ample volume of available water in the soil profile (even for the course soils evaluated herein) can compensate for irrigation deficits over a period of 1 week.

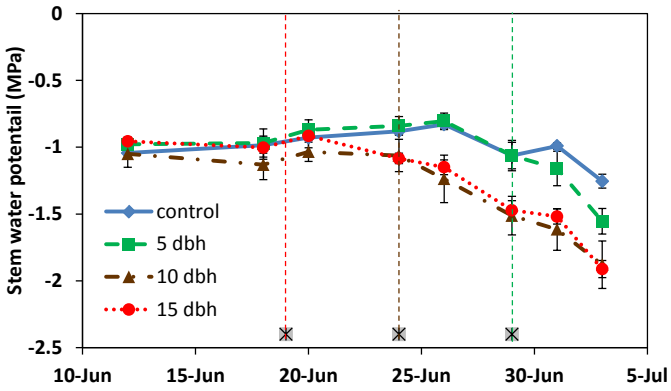

Figure 11. Stem water potential of Sweetheart trees cut-off from irrigation 5, 10 or 15 days before harvest (dbh), compared to control trees. Asterisks above x-axis signify start date of withholding for each treatment with hashed vertical line. Harvest date was 4-July. For water potential, 4 leaves per replicate were bagged ~1hr prior to measurement. Measurements bracketed solar noon (±1.5 hrs).

Table 5. Effects of early irrigation withholding for 5, 10 or 15 days before harvest (dbh) on yield and fruit quality attributes of 'Sweetheart' sweet cherry trees.

Treatment	Yield kg/tree	Fruit diameter mm	Fruit wt. g	DMC g/fruit	RWC %	FF g/mm	Skin color ctifl	PRF g	SSC %	TA %
Control	19.4	27.7 a	9.8 a	2.29	76.98 a	341.8	4.7 a	277	22.1	1.07
5-dbh	20.7	27.4 a	9.5 ab	2.23	77.3 a	343.2	4.4 b	277	21.1	1.08
10-dbh	19.9	26.5 b	9.0 b	2.3	75.03 b	329.6	4.8 a	205	23.6	1.01
15-dbh	19.3	26.9 ab	9.2 b	2.28	75.92 ab	328.3	4.6 a	212	22.4	1.05
Pr>F	*0.948*	*0.042*	*0.024*	*0.851*	*0.031*	*0.587*	*0.008*	*0.109*	*0.098*	*0.195*

Yield (n=2); fruit diameter and weight (n=400); fruit relative water content (RWC) and dry matter content (DMC) (n=60); fruit firmness (FF) (n=400); skin color (n=100); pedicel retention force (PRF) (n=25); titratable acidity (TA) and soluble solids content (SSC) (n=2);
Data assigned different letters within columns signifies signficant difference at $P < 0.05$ by Fisher's Protected LSD test.

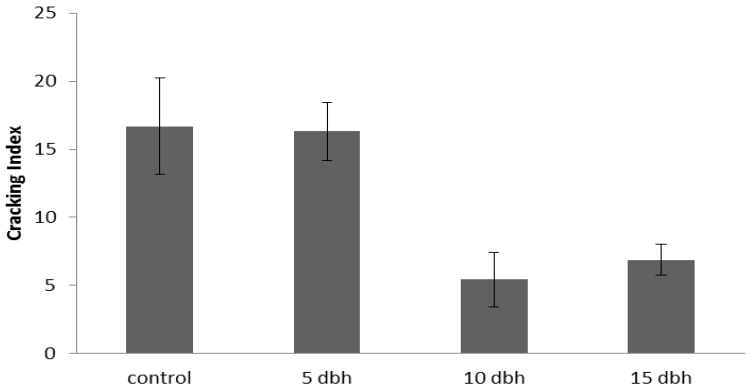

Figure 12. Effect of early irrigation termination on 'Sweetheart' fruit cracking susceptibility. N=100

EXECUTIVE SUMMARY

Effect of near-harvest irrigation on fruit quality

This project has evaluated, over two years, and for four cultivars in three locations (all commercial collaborators), the effects of withholding irrigation prior to harvest on sweet cherry fruit quality and quantity. Combined, our results reveal an apparent resilience of sweet cherry to the reductions in the quantity of water normally applied in the weeks preceding harvest. We found no consistent negative effect of withholding irrigation, up to 3 weeks before harvest. Neither did we discover a consistent effect of pre-harvest irrigation treatments on the fruits' susceptibility to splitting. For example, in 2015, 'Sweetheart' fruit splitting at harvest was 50% lower when trees received no irrigation from 10 or 15 days before harvest (dbh), but these fruit were slightly smaller than fruit from trees receiving 'normal' irrigation.

The greatest risk from withholding irrigation in the weeks prior to harvest appears to be primarily a slight loss in firmness (1 of 5 trials in 2015) or size (1 of 5 trials in 2015). In both cases, midday stem water potential reached levels below -1.5 MPa.

The obvious benefit from withholding irrigation water prior to harvest is clear – the savings of water, particularly important in drought years such as 2015 and predicted for 2016. For example, in 'Skeena', the savings of 3 preharvest irrigation sets (i.e., 9 dbh termination) would have saved that grower an estimated 21,600 gallons per acre with no negative effects on fruit quality. In addition, we have observed fruit quality improvements from withholding near-harvest irrigation: increase in pedicel-fruit retention force (1 of 5 trials in 2015), increase in soluble solids (1 of 5 trials in 2015).

Importantly, midday measurements of stem water potential, using a portable pressure bomb, integrated soil and tree water status, and may be used to predict, and avoid stress. Our results suggest that maintaining midday stem water potential above -1.5 is important for avoiding deleterious stress effects on fruit quality. Utilizing regular assessments of stem water potential to schedule irrigation has been suggested previously and is an area worthy of further investigation.

FINAL PROJECT REPORT

Project Title: Factors affecting the fruit phase of cherry mildew

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Other funding sources: None

Total Project Funding: $ 200,147

Budget History: WSU-IAREC

Item	Year 1: 2013	Year 2: 2014	Year 3: 2015
Salaries	33,504	34,844	36,238
Benefits	17,087	17,770	18,481
Wages	7,075	7,075	7,075
Benefits	667	667	667
Supplies	3,600	1,000	1,000
Travel	1,000	1,000	1,000
Total	62,933	62,286	$64,461

Budget History: OSU-MCARES

Item	Year 1: 2013	Year 2: 2014	Year 3: 2015
Wages	2,686	2,767	2850
Benefits	215	221	228
Supplies	500	500	500
Miscellaneous			
Total	3,401	3,488	3,578

Original objectives

1) Determine the inoculum concentration threshold for infection of cherry fruit at different developmental stages.

2) Determine the effects of temperature and relative humidity (40% - 99%) on infection and spore production (conidia) of *P. clandestina* on infected cherry fruit.

3) Conduct in-depth studies on the temporary susceptibility of several cultivars of cherry fruit to infection by *P. clandestina* in orchard studies.

4) Evaluate quinoxyfen as a key management component of the fruit phase of powdery mildew, overall maintenance of fruit quality, and prevention of postharvest diseases.

5) Investigate the susceptibility of cherry flowers to infection by *P. clandestina* and the potential relationship between blossom and fruit infection.

DISEASE INCIDENCE
Proportion of diseased leaves/ fruit out of the total number of leaves/ fruit observed

DISEASE SEVERITY
Proportion of infected leaf/ fruit surface area

Significant Findings

- Cherry fruit infection is a subtle process. Infection stays unnoticeable during the growing season, making predictions about disease incidence and severity at harvest very difficult.
- Incidence and severity of fruit disease depends on inoculum concentrations. In general, the more spores are deposited on fruit surfaces; the more disease can be expected.
- Interaction between inoculation dates and inoculum concentrations revealed a dependency of disease development and fruit developmental stages (time of spore deposition).
- Neither humidity nor temperature promoted disease on developing cherry fruit at any time before June.
- Fruit transitions from resistant to susceptible sometime in June (BBCH scale 85 to 87). Phenological development or time of year may be important.
- During the resistance phase powdery mildew spores remain quiescent on fruit surfaces
- Ontogenic resistance (disease resistance increases with maturity) is true for leaves but not for fruit
- Quinoxyfen (Quintec™) alone does not reduce powdery mildew on fruit
- Quinoxyfen (Quintec™) in rotation with Fontelis did reduce incidence and severity of foliar and fruit infection. The reduction was not always statistically significant but perhaps economically important

- Quinoxyfen (Quintec™) in rotation with Procure did increase incidence and severity of foliar infection in 2013

- Fungicide rotations (Quintec, Pristine, Fontelis, Topguard, and Procure) did not affect fruit quality or pitting susceptibility

- Pristine® reduced fruit disease (statistically significant) consistently in 2014 and 2015

- Prebloom through fruit set is NOT a critical period for the establishment of powdery mildew infection and does not relate to fruit infection at harvest, unlike the grape: *E. necator* pathosystem.

Results and Discussion

Objective 1

In the orchard studies, differing inoculum concentrations and inoculation dates caused variation in disease incidence and disease severity. Significant variations *(P<0.05)* were observed in mean disease incidence and disease severity among fruits inoculated with different conidia concentrations (Table 1). Generally, disease incidence and disease severity increased with increasing inoculum concentrations. Interaction between inoculation dates and inoculum concentrations revealed dependency of disease development on growth stages of fruits (Fig.1a and b). As age of inoculated fruits increased the percentage of infected fruits and the percentage of fruit area covered by powdery mildew increased. The minimum conidial concentration needed to cause both significant disease incidence and significant disease severity varied depending on the inoculation dates or growth stage of fruits. A minimum inoculum concentration of 500 conidia/ml was needed for significant fruit infection (disease incidence and severity) in fruits inoculated in June and a minimum inoculum concentration of 1000 conidia/ml was needed for significant fruit infection on relatively young fruits inoculated in May (Table 1).
In studies on detached fruit in small environmental chambers, only matured fruits inoculated the end of June showed significant disease development. On those fruits, conidial concentration had significant effects *(P<0.05)* on disease severity (Table 2). Disease severity increased with increasing conidial concentration. The conidial concentration of 5000/ml was minimum for 1% disease severity in mature detached fruits in the lab. Interaction between inoculation date and inoculum concentrations indicated dependency of disease development on growth stages of fruits besides conidial concentrations (Fig. 2).

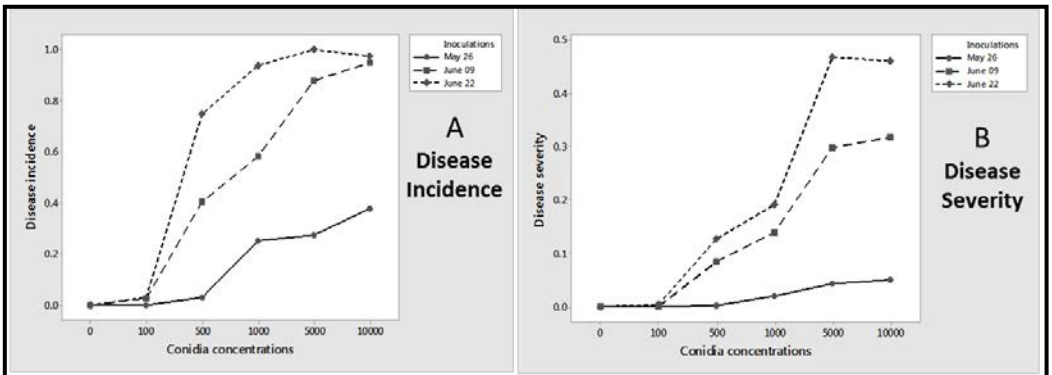

Fig 1 Interaction plot of conidia concentrations (ml^{-1}) and inoculation dates for disease incidence (A) and severity (B) in orchard experiments

Table 1 Powdery mildew disease incidence and severity on cherry fruits caused by inoculation of different suspensions of conidia of *Podosphaera clandestina* on different inoculation dates in orchard experiments - 2015

Inoculation dates	*May 26*		*June 09*		*June 22*	
Conidia concentration	**Powdery Mildew Disease**					
	Incidence	**Severity**	**Incidence**	**Severity**	**Incidence**	**Severity**
10000/ml	0.38 a*	0.050 a	0.95 a	0.32 a	1.00 a	0.46 a
5000/ml	0.27 a	0.044 ab	0.88 a	0.30 a	0.97 a	0.47 a
1000/ml	0.25 a	0.020 bc	0.59 b	0.14 b	0.94 a	0.19 b
500/ml	0.03 b	0.003 c	0.40 b	0.08 c	0.75 b	0.13 c
100/ml	0.00 b	0.00 c	0.02 c	0.0007 d	0.029 c	0.004 d
0/ml	0.00 b	0.00 c	0.00 c	0.00 d	0.00 c	0.00 d

* Results are averages of three replicates. Values for a variable within a column followed by a common letter are not significantly different based on Fisher least significant difference (LSD, *P=0.05*).

Table 2 Powdery mildew disease severity on cherry fruits caused by inoculation of different suspensions of conidia of *Podosphaera clandestina* on different inoculation dates in lab experiments – 2015

Inoculation dates	*June 04*	*June 14*	*June 24*
Conidia concentration	**Powdery Mildew Disease Severity**		
10000/ml	0.000 a*	0.00045 a	0.0300 a
5000/ml	0.000 a	0.0030 a	0.0300 a
1000/ml	0.000 a	0.0010 a	0.0065 b
500/ml	0.000 a	0.000 a	0.0000 b
100/ml	0.000 a	0.000 a	0.0000 b
0/ml	0.000 a	0.000 a	0.0000 b

* Results are averages of three replicates. Values for a variable within a column followed by a common letter are not significantly different based on Fisher least significant difference (LSD, *P=0.05*).

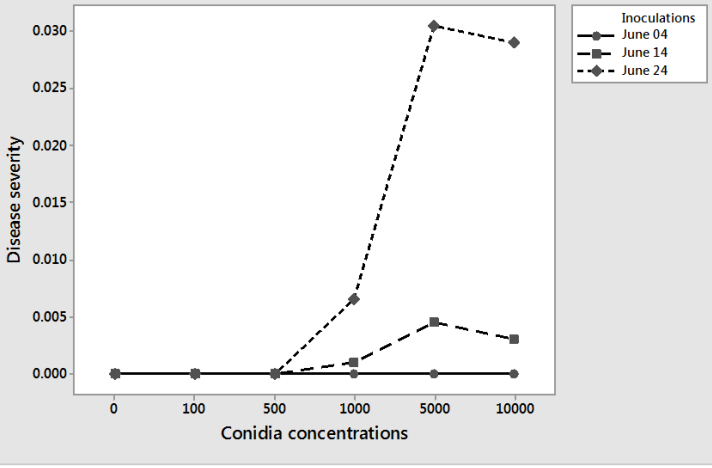

Fig 2 Interaction plot of conidia concentrations (ml^{-1}) and inoculation dates for disease severity in lab experiments.

Objective 2

Cherries (cultivars Bing and Sweetheart) at various developmental stages were used for temperature/ humidity studies. Surface sterilized, detached fruit were inoculated with 5000 spores/ml and incubated at 15^0C (59F), 20^0C (68F), and 25^0C (77F) in small controlled environment chambers (Fig 3). Relative humidity ranged from 10% to 99%. Disease incidence and severity was recorded every 7 days. Four replicates per temperature/humidity combination were assessed and experiments were continuously repeated throughout the season (Fruit set to harvest). No disease could be initiated on cherries picked before the middle of June independent of temperature and humidity regime. The same was true for laboratory fruit inoculation studies discussed in Objective 1. Cherries inoculated 70 days after full bloom showed signs and symptoms of disease in *vitro*. A significant increase in disease incidence was recorded at 25^0C (77F) independent of humidity. In general, disease incidence increased with rising humidity levels. However, the increase was not statistically significant ($P = 0.005$). In general, environmental factors do not seem to play a major role in the initiation of fruit disease. Even under ideal environmental conditions (as determined by our study: 25^0C and above 90% RH) fruit had an 'unexplained' resistance to powdery mildew until the middle of June. This is contrary to observations from leaves or other agricultural commodities (grapes, strawberries, hops) where young tissue is most susceptible to infection and ontogenic resistance develops with increasing maturity levels of the respective host tissue. The inability to produce disease on immature fruit directly reflects the inability of the fungus to produce disease on same aged fruit in the orchard.

Objective 3

Developing cherries were covered with Nitex bags around 4-27 in each years. Applying Nitex mesh covers by the end of April protected the developing cherries from airborne spores. No signs or symptoms were detected in the negative control in 2014 and 2015. In 2013, some disease was observed in some replicates of the negative control caused by an insufficient closure of the bags. Cherry development was not negatively impacted by the Nitex cover. First signs of

Fig 3 Detached fruit assays using small humidity-controlled environment chambers

foliar infection were observed 25 days after full bloom (DAFB) in 2014 and 32 DAFB in 2015. First signs of fruit infection were observed in the middle of June in both years. The time lag between earliest spore deposition and symptom development was 56 days (2014) and 49 days (2015). This is the longest time period of powdery mildew spore quiescence on host tissue ever reported. No consistent and significant relationships between date of inoculation or inoculation type (natural exposure to airborne spores versus spray inoculations with 5000 spores/ml) were observed. This indicates that spores deposited on fruit as early as the onset of disease on leaves will translate to fruit infection at harvest.

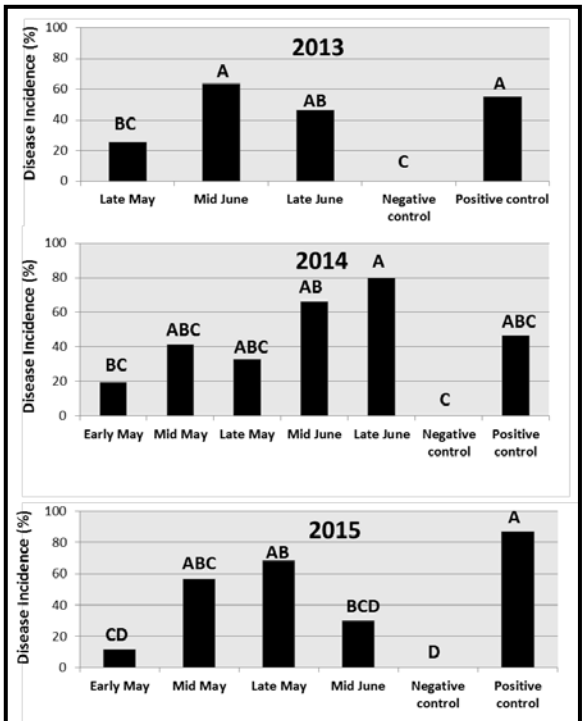

Fig 4 Powdery mildew disease incidence resulting from a two week exposure of fruit to airborne spores. Means with different letters are significantly different (P = 0.05) based on Tukey's HSD test. Data is from cultivar Bing, Prosser, 2013-2015

Objective 4

2013 was a mild powdery mildew year at the WSU experimental orchard, with an average disease incidence of 42% and severity of 2% in the untreated control. No significant effect of fungicide application on leaf disease incidence was found when compared to the untreated control (Table 3). However, a triple application of Quintec reduced disease severity by 15% (Table 3). This was the only significant reduction seen in 2013. In 2014, Procure and Topguard were replaced with Fontelis and Pristine. Additionally, stylet oil was applied in some treatments as a post-harvest control measure. The same fungicides rotations were applied in 2015. In 2014 and 2015, severe powdery mildew outbreaks were observed at the WSU experimental orchard, with an average disease incidence of 85.5 and 90.5 %, respectively. Leaf disease severity peaked at 54 and 32%, respectively, (Table 4 and 5). At harvest, 54% (2014) and 64% (2015) of all inspected fruit (n=400 per treatment) showed signs of infection. Disease severity reached 65% (2014) and 71% (2015) (Table 3). In 2014, one fungicide application (Treatment 4, Fontelis-Quintec rotation) reduced disease incidence significantly by 15.8% and severity by 48%. The same effect was not observed in 2015 (Table 5). However, treatments 2, 8 and 11 did reduce leaf disease severity significantly (P = 0.005) compared to the untreated control in both years (Table 4 and 5). However, none of these treatments reduced the total amount of leaves infected (incidence). Fruit disease was also significantly reduced by Fontelis®-Quintec™ rotations (Treatment 1, 3, and 4) in 2014 but not 2015. On fruit, application of Pristine® (Treatment 10 and 11) significantly reduced (P = 0.005) disease incidence and severity in both years. Pristine also significantly reduced leaf severity but not incidence (Tables 4-6). In this study, Pristine® was the only fungicide able to minimize leaf infection and reduce fruit disease consistently over time. The sole application of Quintec™ (quinoxyfen) is not a key management component for reduction of powdery mildew disease on leaves or fruit. Only in one year and under low disease pressure,

Quintec™ had a significant effect on leaf disease severity (Table 3). Under high disease pressure, disease reduction was only achieved in combination with Fontelis®. Even though not always statistically significant, reduction was great enough to have real life importance.

There were no significant differences in fruit firmness (FF), fruit size, soluble solid content (SSC), and titratable acidity (TA) of 'Bing' fruit at commercial harvest among the 11 treatments (Fig 5). Pitting susceptibility was not affected by the different fungicide treatments. After 2 weeks at 32°F, there were no differences in FF (increased ~20% compared to initial), TA (reduced ~15% compared to initial), fruit color, and stem browning among the fungicide treatments.

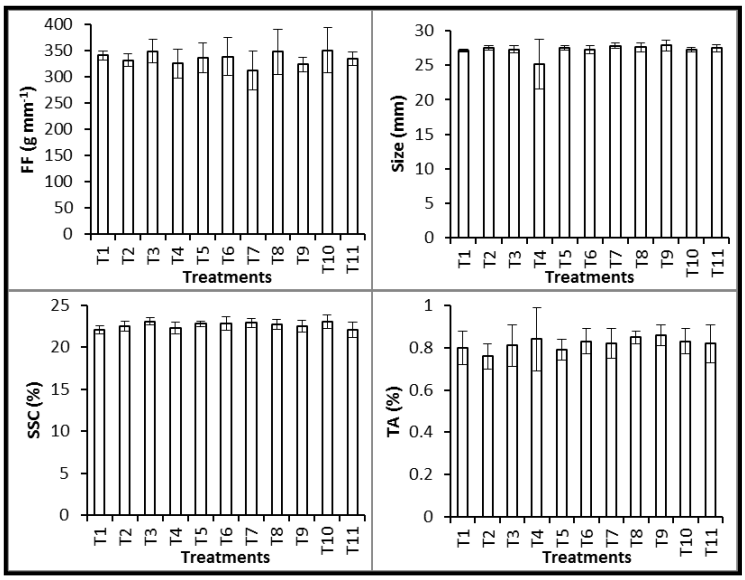

Fig 5 Effect of fungicide treatments (T1-T11) on fruit quality of 'Bing' cherries at harvest in 2015.

Table 3 Effect of fungicide rotations on sweet cherry powdery mildew incidence and severity on **LEAVES** - 2013

2013 TMT #	Pre-harvest Fungicide rotation*	Powdery mildew Incidence (%)**						Powdery mildew Severity (%)**					
		Upper canopy		Lower canopy		Combined		Upper canopy		Lower canopy		Combined	
1	Q-Q-PR	77	AB	35	ABCD	56	AB	2.9	BC	0.4	CD	1.7	BC
2	PR-Q-Q	83	AB	44	ABC	64	AB	2.9	BC	1.1	ABCD	2.0	B
3	PR-PR-Q	87	A	52	AB	70	A	2.6	BC	1.0	ABCD	1.8	BC
4	PR-PR-PR	83	AB	38	ABCD	61	AB	5.8	A	2.0	AB	3.9	A
5	PR-PR-PR	90	A	40	ABCD	65	AB	5.7	A	2.2	A	3.9	A
6	PR-PR-PR	65	ABC	21	BCD	43	BC	3.9	AB	1.2	ABCD	2.5	AB
7	Q-Q-Q	25	D	6	D	16	D	0.5	C	0.1	D	0.3	C
9	T^{14}-Q-T^{14}	46	CD	10	CD	28	CD	2.0	BC	0.2	D	1.1	BC
10	T^{14}- T^{14}- T^{14}	78	AB	35	A	57	A	2.6	BC	0.8	BCD	1.7	BC
11	T^{7}-T^{7}-T^{7}	71	ABC	63	A	67	AB	2.3	BC	1.4	ABC	1.9	BC
8	None	53	BCD	30	ABCD	42	BCD	2.8	BC	1.2	ABCD	2.0	B

*Q = Quintec 250SC, 7 fl oz/A, PR = Procure, 14.5 oz/A, T^{7}= Topguard 1.04SC, 7 fl oz/ A, T^{14} = Topguard 1.04SC 14 fl. Oz / A. Applied to run-off (400 gal/A = 1.5 gal per tree). Fungicide application dates: 5/7/2013, 5/23/2013, 6/6/2013

** Disease evaluation date: 7/21/13. Results are averages of four single tree replicates. Values for a variable within a column followed by a common letter are not significantly different based on Tukey's HSD test (*P=0.05*).

Table 4 Effect of fungicide rotations on sweet cherry powdery mildew incidence and severity on **LEAVES** - 2014

2014 TMT #	Pre-harvest	Post-harvest	Powdery mildew Incidence (%)**						Powdery mildew Severity (%)**					
	Fungicide rotation*	Oil^	Upper canopy^^		Lower canopy^^		Combined^^		Upper canopy^^		Lower canopy^^		Combined^^	
1	Q-Q-F-F	2x	95	A	77	AB	86	AB	65	AB	14	BC	40	AB
2	F-Q-Q-F	2x	100	A	74	AB	87	AB	47	AB	13	C	30	B
3	F-F-Q-Q	2x	100	A	69	AB	84.5	AB	55	AB	18	BC	37	AB
4	F-F-F-Q	2x	89	A	55	B	72	B	40	B	15	B	28	B
5	Q-Q-Q-Q	2x	95	A	66	AB	80.5	AB	44	AB	31	AB	38	AB
6	Q-Q-Q-Q	none	99	A	72	AB	85.5	AB	53	AB	27	ABC	40	AB
8	F-F-F-F	2x	100	A	87	AB	93.5	AB	49	AB	20	BC	35	B
9	F-F-F-F	none	100	A	78	AB	89	AB	53	AB	27	ABC	40	AB
10	P-P-P-P	2x	100	A	71	AB	85.5	AB	60	AB	26	ABC	43	AB
11	P-P-P-P	none	95	A	62	AB	78.5	AB	45	AB	11	C	28	B
7	None	none	95	A	76	A	85.5	A	68	A	40	A	54	A

Table 5 Effect of fungicide rotations on sweet cherry powdery mildew incidence and severity on **LEAVES** - 2015

2015 TMT #	Pre-harvest	Post-harvest	Powdery mildew Incidence (%)**						Powdery mildew Severity (%)**					
	Fungicide rotation*	Oil^	Upper canopy^^		Lower canopy^^		Combined^^		Upper canopy^^		Lower canopy^^		Combined^^	
1	Q-Q-F-F	2x	99	A	80	A	89.5	A	22	C	18	A	20	AB
2	F-Q-Q-F	2x	97	A	65	A	81	A	21	C	10	A	15	B
3	F-F-Q-Q	2x	100	A	65	A	82.5	A	21	C	5	A	13	B
4	F-F-F-Q	2x	100	A	60	A	80	A	39	AB	8	A	23	AB
5	Q-Q-Q-Q	2x	98	A	75	A	86.5	A	29	BC	10	A	19	AB
6	Q-Q-Q-Q	none	100	A	74	A	87	A	32	BC	6	A	19	AB
8	F-F-F-F	2x	100	A	69	A	84.5	A	32	BC	4	A	18	B
9	F-F-F-F	none	100	A	71	A	85.5	A	24	BC	14	A	19	AB
10	P-P-P-P	2x	98	A	85	A	91.5	A	19	C	12	A	16	B
11	P-P-P-P	none	97	A	68	A	82.5	A	22	C	8	A	15	B
7	None	none	100	A	81	A	90.5	A	50	A	14	A	32	A

Table 6 and 7:

*Q = Quintec 250SC, 7 fl oz/A, F = Fontelis, 20 oz/A, P = Pristine, 14.5 oz/A. Applied to run-off (400 gal/A = 1.5 gal per tree). Fungicide application dates: 4-30, 5-14, 5-28, 6-10-2014. Oil application dates: 7-1-14, 7-15-14.

** Disease evaluation date: 7/1/2014. Results are averages of four single tree replicates. Values for a variable within a column followed by a common letter are not significantly different based on Tukey's HSD test (*P=0.05*).

^Post-harvest treatment, 2x = applied twice in a 14 day interval, none = no fungicides or oil were applied.

^^ 25 leaves from 5 branches were evaluated on the upper portion of the tree (upper canopy) and the lower portion of the tree (lower canopy) for a total of 50 leaves per tree (combined)

Table 6 Effect of fungicide rotations on sweet cherry powdery mildew incidence and severity on **FRUIT** – 2014 & 2015

TMT	Pre-harvest	Post-harvest	Powdery mildew FRUIT disease (%)**							
			2014				2015			
	Fungicide rotation*	Oil^	Incidence		Severity		Incidence		Severity	
1	Q-Q-F-F	2x	10	B	10	B	41	AB	41	AB
2	F-Q-Q-F	2x	28	AB	33	AB	35	AB	35	AB
3	F-F-Q-Q	2x	15	B	15	B	38	AB	38	AB
4	F-F-F-Q	2x	12	B	13	B	49	AB	48	AB
5	Q-Q-Q-Q	2x	25	AB	28	AB	34	AB	34	AB
6	Q-Q-Q-Q	none	23	AB	25	AB	37	AB	37	AB
8	F-F-F-F	2x	34	AB	40	AB	45	AB	45	AB
9	F-F-F-F	none	34	AB	40	AB	34	AB	34	AB
10	P-P-P-P	2x	15	B	15	B	23	B	23	B
11	P-P-P-P	none	20	B	20	B	27	B	28	B
7	None	none	54	A	65	A	64	A	71	A

*Q = Quintec 250SC, 7 fl oz/A, F = Fontelis, 20 oz/A, P = Pristine, 14.5 oz/A. Applied to run-off (400 gal/A = 1.5 gal per tree). Fungicide application dates: 4/23/2015, 5/7/2015, 5/21/2015, 6/4/2015; Oil application dates: 6/18/2015, 7/2/2015.
**Disease evaluation date: 7/3/2014, 6/15/2015. Results are averages of four single tree replicates. 100 fruit per rep were evaluated. Values for a variable within a column followed by a common letter are not significantly different based on Tukey's HSD test (*P=0.05*).
^Post-harvest treatment, 2x = applied twice in a 14 day interval, none = no fungicides or oil were applied.

Objective 5

Prebloom through fruit set is a critical period for the establishment of powdery mildew infection in many agricultural commodities (e.g. apple, strawberry, hops, and grapes). Infections during this time can directly dictate severity of infection at harvest. The presence of sweet cherry powdery mildew on cherry flowers and its relation to fruit infection was investigated. Aerobiological studies have shown that the incidence of *P. clandestina* in the orchard air is very low during cherry bloom. Additionally, there is a three to four week gap between average blooming time of cherries (beginning of April, principal growth stage 6) and the onset of disease symptoms on leaves (beginning of May, principle growth stage 7). This is in stark contrast to other powdery mildew susceptible commodities, like apple and grape, where bloom occurs at times when powdery mildew is already established on the host. Flower inoculation experiments and species detection using PCR and *P. clandestina* specific primers were conducted during 2013-2015 to investigate the relationship between bloom and fruit infection. Detached cherry flowers inoculated with powdery mildew conidia did abort quickly after inoculation. The fragile petals turned brown and died within two days post inoculation. No spore germination was observed. In orchard studies, developing cherries protected with Nitex bags (applied at Shuck fall) did not develop any disease symptoms. Cherry flowers collected from experimental and commercial orchards (cultivars Bing, Early Robin, Lapin, and Sweetheart) were subjected to PCR assays using species specific primers. Powdery mildew was not detected in any sample during a three year period but the positive control. The low incidence of airborne conidia in combination with the absence of disease on cherries exposed to airborne spores during bloom, the natural absence of spores on flower petals, and the inability of flower petals to sustain infection strongly indicates that the flowering stage is not relevant to powdery mildew disease at harvest.

Executive Summary

Powdery mildew infection of cherry fruit is best characterized as a slow and invisible process. Developing fruit displays a long period of resistance to infection and the pathogen remains quiescent and viable on fruit surfaces for an extraordinary period of time. These findings are in contrast to findings related to foliar infections. Foliar infections actively progress during the season starting in the beginning of May (Bing cherries in Prosser: 32 days after full bloom in 2015 and 25 days after full bloom in 2014; BBHC scale 73 to 75) and ending with the production of overwintering structures in September and October (principle growth stage 9, BBCH stage 91 to 97). It has been generally assumed that powdery mildew conidia are ephemeral and do not persist without a susceptible host to thrive and proliferate. Sweet cherry powdery mildew persisted under adverse conditions for an 8 week period. However, inoculation concentration studies intimated that a greater number of spores need to be deposited on very young fruit to result in significant disease incidence at harvest. This indicates that not all spores survive the extended periods of quiescence. There is a biochemical line of communication between host and pathogen that still has to be elucidated. It is unknown what transmits resistance to susceptibility in cherries; a development always observed during the month of June (around 60 to 80 days after full bloom in 'Bing'). The inability of *P. clandestina* to thrive on fruit picked before June is clearly not a result of environmental factors. Even under ideal growth conditions, fruit infection could not be initiated *in vitro*. The only times *in vitro* studies resulted in infection was on fruit picked 60 to 80 days after full bloom (BBCH stages 85 to 87). Additionally, environmental conditions in the orchard already favor infection, as can be observed on actively sporulating powdery mildew colonies on leaves. The connection between time and/or crop phenology and disease onset is a key piece in understanding fruit infection and will be invaluable for the breeding program, in particular for breeding of new, powdery mildew resistant late season varieties.

Both the absence of ontogenic resistance (increasing resistance with maturity) in fruit and the fact that cherry bloom is not a critical period for infection *by P. clandestina* is contrary to what is a commonly observed pattern in many other powdery mildew species (apple, grape, hops, strawberry, etc.). Knowledge about critical periods of infection has been proven useful to direct management strategies. To date no critical infection period has been associated with infection of sweet cherry by *P. clandestina*. The continuous application of fungicides will remain crucial for disease management. Quinoxyfen (Quintec™) has become popular due to its novel mode of action. Rotation trials were conducted to elucidate the best application time during the season. Applications of Quintec™ (FRAC group 13) in rotation with Fontelis® (FRAC group 7) reduced incidence and severity of fruit infection, even though the effect was only statically significant in 2014. Pristine® (FRAC group 7 and 11) reduced fruit disease significantly in 2014 and 2015. It did not reduce the total number of leaves infected but reduced the severity of foliar infection. Protecting developing fruit from airborne spores remains a challenge. Most fungicides are protective and not fungicidal and need to be applied to cover the surfaces of both fruit and foliage. If coverage is incomplete, *P. clandestina* will use the untreated space to initiate infections. Since fruit are constantly and rapidly expanding, the protective fungicide layer becomes interrupted quickly leaving the fruit vulnerable to infection. The level and duration of protection of fruit during these rapid periods of expansion should be investigated.

FINAL PROJECT REPORT

Project Title: Maintenance of WSU-IAREC cherry breeding plantings

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Total Project Funding: $150,000 ($125,144.61 actual spending)

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

WTFRC Collaborative expenses:

Item	2015	2015 actual
Wages	8,366	11,703
Benefits	3,256	8,837
Supplies	200	1,500
Travel to plots	2,160	6,240
WTFRC staff	1,500	
Total	**15,482**	**28,280**

Footnotes: Total includes full fruit sampling of WSU-Roza, Pasco, and Wenatchee of selected P1 and selected P2 genotypes; budget table does not include exempt personnel hours.

Budget 2:
Organization Name: WSU Prosser **Contract Administrator:** Carrie Johnson
Telephone: 509 335 4564 **Email address**: carriej@wsu.edu

Item	2015	2015 actual
Salaries[1]	6,588	3,167.66
Benefits	3,030	1,372.57
Wages[2]	32,000	20,549.00
Benefits	3,136	8,000.00
Supplies	809	11,998
Travel	3,000	
Plot fees[3]	9,025	9,025
Plot establishment and maintenance	65,500	
Total	**93,818**	**54,113.61**

[1] Salary and benefits for Assoc in Research, Mojtaba Chavoshi (July 1 – Sept 30) to collect field data and complete labeling..
[2] Wages and benefits for (7) temporary employees @ $10/hr, 40 hrs, for 6 wks, (2) temporary employees @ $10/hr, 40 hrs, for 2 wks, and 1 hourly supervisory employee @ $20/hr, 40 hrs, for 8 wks for remaining data collection and lab analysis.
[3] Land use fee is $475/acre.

Budget 3: Todd Einhorn
Organization Name: OSU-MCAREC **Contract Administrator:** L.J. Koong
Telephone: 541-737-3228 **Email address:** l.j.koong@oregonstate.edu

Item	2015	2015 actual
Salaries[1]	3,666	4,230
Benefits[2]	2,456	2,919
Wages[3]	6,139	12,044
Benefits[4]	347	1,004
Fees and Supplies[5]	8,284	8,284
Travel	0	0
Miscellaneous		
Total	**20,892**	28,481

[1] Salaries are for: 0.083 FTE (1 month) for technician to include planting, irrigation, fertilization, tree training, data collection (bloom, harvest, analyses of fruit quality attributes, vegetative growth, etc.) in selected genotypes of the P2 trial.
[2] Actual OPE rate is 67%.
[3] Wages are for two part-time employees ($13/hr) to assist with tree planting, harvest, data collection and analyses. In addition, 120 hours (1 week for 3 part-time employees via Certified Personnel Services [CPS] at a contracted labor rate of $16.49/hr [$1,979]) are factored into year 1 for installation of the bird netting structure.
[4] Benefits for part-time employees is 8.34%- benefits only apply to the two $13/hr employees, and not for the CPS laborers.
[5] Supplies include materials for bird netting structure over 2 acres [factored into year 1 only]; tree guards/paint; tree 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. Nor all supplies have been purchased yet, but our estimates should be accurate.

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

Item	2015	2016	2017
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Tree propagation[1]**:**			
advanced selections	13,593	13,593	13,593
Parents	677	677	677
Miscellaneous			
Total	**14,270**	**14,270**	**14,270**

[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

Overall project objective:

Apply standard horticultural practices to improve efficiency and productivity in sweet cherry breeding program field plots and maintain the industry's investment in Phase 1 (P1) and Phase 2 (P2) cherry breeding plantings at WSU-IAREC. Conduct focused, systematic phenotypic evaluations of selected germplasm and properly maintain program materials (seeds, plants) in storage or protected facilities.

Specific objectives:

1. Apply standard horticultural practices to all field plots
2. Establish 2015 additions to Phase 1 plantings at the Roza site
3. Expand Phase 2 trials to include 2014-2015 selections at three sites
4. Conduct fruit and foliar evaluations in selected genotypes in P1 and P2 plantings
5. Provide intensive management of seedlings and plant materials in greenhouse

SIGNIFICANT FINDINGS:

1-3. Standard horticultural practices, plant 2015 P1 and P2 at Roza

Under the leadership of Clint Graf (WSU Orchard and Vineyard Manager) and guided by representatives of the WSU Cherry Breeding Program (BPAC) Advisory Committee (Dena Ybarra, Jeff Cleveringa, Dave Allan, Eric Shrum) and WTFRC staff (Tom Auvil, Ines Hanrahan), all horticultural practices proposed were carried out in a timely and efficient manner throughout the season (Table 1). Communication among program staff, WSU perennial crops manager, BPAC, and WTFRC team was a point of emphasis. Blocks were inspected at least weekly to assess overall condition and to ascertain plot-specific needs.

Table 1. Timeline of 2015 crop management activities for WSU-IAREC PNWSCBP field plots.

Activity	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Comments
Pruning/brush	*	*								
PGR (Ethrel)										B48/49 (only to trees less than 3 years old), C50, C51/52 (non-bearing trees only)
Frost protection		*	*	*						
Equipment maintenance	*	*	*	*	*	*	*	*	*	
Herbicide	*	*	*	*	*	*	*			Gramoxone, Indaziflam
Fungicide				*	*	*				A36/37, B48/49, B53, NOT IN OTHER BLOCKS
Insecticide	*	*		*	*	*				
Irrigation		*	*	*	*	*				March through October
Planting			*	*						P2 (B48) and P1
Trellis maintenance			*	*	*	*	*	*	*	
Plant training				*	*	*	*	*		
Rodent control		*	*	*	*	*	*	*	*	
Fruit thinning				*						P2 (B48)
Harvest and evaluations				*	*	*				P2 and P1
Fertilizer		*	*	*						
Boron		*								F50-F73
Urea			*							F1-F49
			*							B48 (only to trees planted before 2013) via dripline
Mowing			*	*	*	*	*	*	*	

Graf distributed a weekly summary of ongoing and planned horticultural and crop protection activities to all participants via e-mail. The project budget as submitted did not include netting early maturing selections to avoid bird damage, thus netting activities by WTFRC employees were an additional expense. Specific horticultural accomplishments for 2015 include:

- P2 trellis system completed
- Site preparation (added 1ft of top soil for P2)
- Planting, training, drip irrigation installation of P1 and P2 at Roza
- Drought related irrigation schedule adjustments and irrigation system modifications, including maintenance and monitoring
- Frost control, pruning/brush clean-up, spray program adjustment to provide maximum plot access, soil tests, nutrient sprays, fertilizer application, weed control, mowing, rodent control, netting

Horticultural improvements were made in the WSU-Roza P2 block beginning in July, 2014. Further improvements were made during 2015. These improvements include standardization of the irrigation systems, improved weed control, improved nutrition, and an aggressive program to manage sage rats and gophers. Basic orchard health has noticeably improved and current horticultural practices will be followed in the future in order to maintain tree health and to enhance tree uniformity.

4. <u>Fruit and foliar evaluations in selected genotypes in P1 and P2 plantings</u>:
P1: Plantings were inspected twice a week starting in mid-May by a team of at least two participants at each time point, including: Dena Ybarra, Tom Auvil, Ines Hanrahan, Jeff Cleveringa, Dave Allan, Sue Watkins. As early maturing selections ripened, netting was erected. Field evaluations targeted market class (early, mid or late), fruit size, and firmness. Postharvest evaluation of fruit from promising selections was conducted at WTFRC (June 4-12) and at WSU-IAREC (after June 12). WTFRC evaluations used standard protocols established by Ines Hanrahan and protocols were distributed to BPAC and WSU on August, 6 and will be available on November 9, 2015 during the BPAC meeting. IAREC evaluations were conducted by WSU breeding program support personnel under the direction of Sue Watkins with input from Ines Hanrahan, Tom Auvil and BPAC.

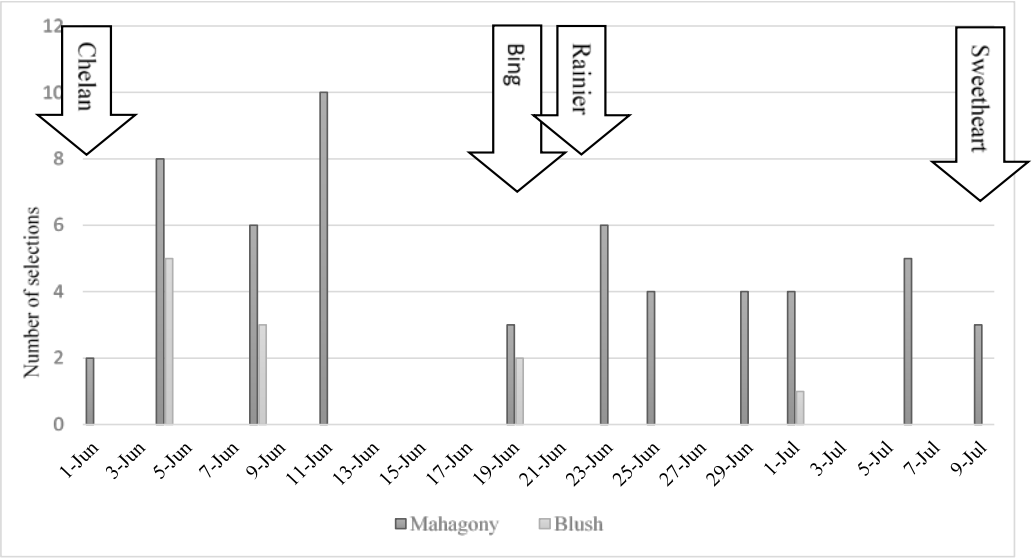


Figure 1: P1 selections selected for laboratory evaluation in 2015

A total of 66 P1 selections (55 mahogany, 11 blush) were harvested for laboratory analysis in 2015. As depicted in Fig. 1, the harvest sequence of selections included:

- Early (Chelan timing): 10 mahogany, 5 blush
- Mid-season (Bing timing): 37 mahogany, 6 blush
- Late (Sweetheart timing): 8 mahogany

No selections were harvested in 2015 that ripened before Chelan or after Sweetheart. Only ten selections met the BPAC minimum quality criteria (>10 row, 300 g/mm^2 firmness) (Table 2). Additional observations were recorded to aid genetic analysis of traits and to fine tune future breeding efforts. Table 2 shows some examples of selections with unusual traits that may be of interest. Data will be provided to Cameron Peace's lab. All P1 selections (1,395 individuals with 32% Sweetheart OP and 102 other parent combinations) were evaluated for mildew infection following a protocol established by Claudia Probst and Ines Hanrahan. Briefly, leaves were rated on a scale of 0-4 (0=zero, 1=mild, 2=moderate, 3=severe infection). In summary, 86 parent combinations had zero leaf infection present in late July. A selection of progeny with the highest number of individual uninfected trees is shown in Table 3. Most notable are progeny of Selah x MIM13, in which all individuals in ten families were completely free of mildew symptoms.

Table 3: Foliar powdery mildew (PM) severity of selected parentage lines in P1

Cross	*# of trees*	*PM score*[1]				*PM severity (%)*			
		0	1	2	3	0	1	2	3
Rainier X Chelan	39	28	3	7	1	71.8	7.7	17.9	2.6
Sweetheart OP	294	19	27	155	93	6.5	9.2	52.7	31.6
12.Sweetheart.OP	155	18	56	63	18	11.6	36.1	40.6	11.6
FR009T033/G6	18	16	1	1	0	88.9	5.6	5.6	0.0
13.8011-2.OP	33	15	18	0	0	45.5	54.5	0.0	0.0
Selah x MIM13	10	10	0	0	0	100.0	0.0	0.0	0.0

[1]PM score performed on leaves in late July; 0=zero, 1=mild, 2=moderate, 3=severe leave symptoms

P2-Washington: Plantings have been established in four locations (Table 5). In addition to the significant heat stress in the 2015 season, robust evaluation was compromised by the absence of, commercial standards for comparison at all sites. All plot maps were revised by WTFRC program staff to correct significant row and tree numbering errors and to make plot interpretation easier. A map for the Roza P2 planting will be provided November 9. Adjustments in this map for 2015 include: we inverted the map to match it to the aerial view, two missing trees were added, 2015 planting was added, all selections were changed to R numbers. In general, there are 5 trees of each advanced selection in the WSU-Roza P2 planting, but some selections are present in lower or higher numbers. The experiment is arranged in a randomized incomplete block design.

All protocols and plot maps will be shared on November 9, 2015 with BPAC and WSU.

WTFRC efforts on 2015 were focused on early selections. Hence, all known early maturing phenotypes and selected industry standards were hand thinned (R2, R25, R7, R6, Chelan, Bing, Rainier, Early Robin). Standard phenotyping protocols were improved or newly developed by Ines Hanrahan and distributed to WSU, OSU and BPAC on August 6. Further modifications will be made based on input from the RosBREED team. A master flow chart for harvest activities and draft protocols to assess heat injury and internal color were developed and distributed to WSU and BPAC

on Aug. 6, 2015. 2015 phenotyping results will be distributed on November 9 (due to space constraints) but firmness and size are summarized in Table 4.

Table 4: Row size and firmness at harvest for P2 selections and selected standards from Roza in 2015.

Selection + harvest date	Row size	Firmness		Selection + harvest date	Row size	Firmness
	(8-13)	(g/mm)			(8-13)	(g/mm)
Early Robin 6/4	9.8	304		**Bing 6/15**	11.1	282
Early Robin 6/8	9.8	277		**Bing 6/25**	11.0	269
Early Robin 6/11	9.6	280		**Chelan 6/1**	11.3	290
Early Robin 6/15	9.7	291		**Lapin 6/29**	11.1	-
R9 6/8	9.7	333		**R2 6/8**	11.0	274
R9 6/11	9.3	299		**R6 6/25**	9.5	278
R9 6/15	9.5	286		**R6 6/29**	10.3	278
R10 6/4	10.5	334		**R8 6/29**	10.1	334
R10 6/8	10.5	282		**R14 6/8**	10.6	253
R10 6/15	10.4	228		**R14 6/11**	9.0	-
R10 6/18	10.3	241		**R14 6/15**	11.2	283
R10 6/25	10.0	255		**R15 6/8**	11.3	269
R10 Thinned 6/15	8.8	177		**R15 6/11**	11.5	242
R10 Thinned 6/25	8.4	266		**R25 6/4**	11.0	337
R16 6/8	10.5	312		**Sweetheart 7/6**	11.3	228
Rainier 6/8	10.6	271				
Rainier 6/11	10.0	270				
Rainier 6/18	10.4	252				

Weekly industry samples were distributed within Washington and Oregon BPAC members (list available upon request).

Key findings for P2 plots in 2015 include:

- Neither Pasco nor Wenatchee P2 plots have standard cultivars included for comparison
- Fruit size for fast track selection R2 was smaller than the BPAC threshold (>10mm). Further horticultural challenges include: fruit maturity one week ahead of Chelan, fruit maturity widespread within the tree, excessive preharvest fruit drop, inconsistent taste
- R25 was the only Chelan timing selection currently in P2. Its fruit was medium size, firm, no doubles, not cracking sensitive, good taste across several color grades, uneven color development, very crunchy
- All mahogany selections previously classified as late-maturing were classified as Bing-Lapins harvest timing; R6 exhibits the best fruit quality characteristics of this maturity group
- Data on three blush selections contradicted available program records. They had been identified as mid/late season or mis-labeled as mahogany. Data collection was compromised since no green fruit thinning was performed
- Wenatchee: 3 of 5 genotypes are Rainier season blush (R5, R7, R11), no late season genotype
- Prosser: inconsistent tree vigor and overset trees (no green fruit thinning performed because 2015 maturation was much earlier than previously observed); compromised fruit quality data for all but R2, R25, Chelan; all other selections evaluated had delayed harvest dates, softer

fruit, reduced size; elevated temperatures at harvest affected consistency of fruit maturation patterns

- Budget deviations (Prosser P1 + 2):
    - 6 additional genotypes assessed in P2 based on observed maturation pattern in 2015 (R8, R9, R10, R14, R15, R16)
    - Bird netting (P1 and P2): 91 trees
    - Opportunistic evaluation based on extreme heat events
        - WTFRC team assessed heat damage
        - 7 genotypes (R10, R6, R16, R8, Bing, Sweetheart, Rainier)

Table 5: P2 and P3 selection distribution and experimental design in 2015

	Location	Replication	Standards	Number of entries
P2	Hood River	YES/NO[Y]	NO	27
	Prosser[z]	YES/NO[y]	YES	27
	Pasco	YES	NO	11
	Wenatchee	YES[Y]	NO	5
P3	Pasco	NO	YES	1
	Hood River	NO	YES	1
	Orondo	NO	YES	1

[Z] R7 not enough fruit for evaluation in 2015

[Y] replication is incomplete or missing for some entries

5. *Management of plan material in greenhouse*:

The WSU program made multiple crosses during 2015. Seed has been scarified and germinated. Seedlings were transplanted to 7" plastic cones containing vermiculate/sunshine mix and incubated in a controlled-environment room at 60°F. When root bound, (November/December) seedlings will be transplanted and moved to the greenhouse.

2015 Outreach Activities by WTFRC team

June 12: Program update and P2 field day

June 21: Program update and discussion of heat damage, North Central Washington Fieldmen's Association

August 6: Season summary by WTFRC staff to WSU and BPAC

December 8: P2 updates and discussion of heat damage at WSTFA (upcoming)

Table 2: Fruit harvest date and fruit quality parameters for P1 selections in the Pacific Northwest Sweet Cherry Breeding Program. WSU Roza, 2015

Orchard Block	Harvest Date	Selection ID	Market class	Weight	SSC	TA	Firmness	Row size	Color	Cracking	Special characteristic
			Mahogany/blush	g	°Brix	%	(g/mm)	(8-13)	(1-7)[1]	(%)[2]	
SELECTIONS MEETING BPAC SELECTION CRITERIA IN 2015											
F	5/28	R19	Mahogany	NA	NA	NA	345	9.9	2.1	73.4	
C	6/8	3-35	Mahogany	10.8	19.3	0.73	313	9.4	4.6	NA	
F	6/18	45-76	Blush	14.0	22.9	0.90	310	8.8[3]	NA	NA	
F	6-19	*Bing*	Mahogany	10.6	21.6	1.11	242	NA	NA	NA	standard
F	6/22	54-19	Blush	12.3	20.6	1.12	305	9	NA	NA	
F	6/23	57-87	Mahogany	12.2	23.9	1.02	301	9.3[3]	4.0	NA	
F	6/23	61-56	Mahogany	12.9	24.6	1.06	368	NA	6.0	NA	
C	6/25	1-78	Mahogany	11.8	25.0	1.09	300	NA	5.3	NA	
F	6/25	39-117	Mahogany	16.5	20.4	0.75	309	NA	5.0	NA	
C	6/29	3-47	Mahogany	9.5	21.0	0.88	338	9.5	6.0	NA	
C	7/1	1-79	Mahogany	10.9	21.0	0.94	324	9.3[3]	4.2	NA	
F	7-9	*Sweetheart*	Mahogany	10.0	17.3	1.12	280	NA	5.1	NA	standard
SELECTIONS WITH ONE OR MORE NOTICABLE FEATURES											
C	6/4	04-72	Mahogany	9.1	20.4	0.81	NA	10.1	3.8	14.9	Speckled fruit
C	6/4	05-13	Mahogany	8.9	17	0.94	302	10.7	3.4	0	No cracking
C	6/4	07-46	Mahogany	10.4	17.8	0.70	252	10.0	3.3	0	No cracking
C	6/4	05-24	Mahogany	8.1	17.8	0.97	344	10.4	4.6	4.9	big, firm
C	6/4	05-100	Blush	10.2	16.3	0.45	249	9.6	NA	10.7	crunchy
C	6/4	08-48	Blush	8.9	17	0.43	270	10.5	NA	8.5	free stone
F	6/4	58-7	Mahogany	NA	NA	NA	260	9.4	6.0	2.0	big, shiny, even color
C	6/8	3-47	Blush (dark)	8.5	16.7	0.80	360	10.4	NA	NA	crunchy, short stems
C	6/8	2-50	Mahogany	15.4	20.6	0.85	218	8.7	4.0	NA	very big, pointed
C	6/8	6-06	Blush	9.0	16.6	0.73	358	10.4	1.5	NA	pointed, firm, very dark
C	6/11	7-70	Mahogany	9.4	20.4	0.78	NA	10.1	4.8	NA	speckled, shiny
C	6/11	4-22	Mahogany	9.5	21.9	0.91	NA	9.9	6.8	NA	very dark and even color
F	6/23	50-104	Mahogany	15.5	21.5	0.93	264	8.8[3]	5.0	NA	huge and attractive

Each selection was picked once (50 fruit sample) from a single tree, cooled within 2 hours and transported to WTFRC in Wenatchee or WSU-IAREC for fruit quality analysis performed on the following day, WTFRC evaluated P1 fruit until June 11, then analysis was performed by WSU staff lead by Sue Watkins (grey highlight) [1] CTIFL color chart, [2] Cracking determined in laboratory as % fruit cracked/50 fruit sample, [3]estimate of row size

Oregon SIGNIFICANT FINDINGS (P2): provided by Todd Einhorn

- We successfully evaluated fruits of 19 genotypes in 2015.
- Fruit of Sweetheart were the latest to harvest (by ~8 d) implying that none of the late-season-mahogany market class selections were sufficiently 'late'. However, 2015 provided a challenging year to base growth and development, given the environmental conditions during dormancy and spring (resulting in relatively early bloom) and the extreme, high temperature events during most of the season.
- The November 2014 freeze event (~3°F minimum temperature at MCAREC) resulted in fairly significant flower mortality, potentially limiting fruit set and tree yields. No selection presently under P2 evaluation, however, appeared to respond differently to the event. One previously discarded selection was observed to be highly sensitive to freeze.
- Fruit from several of the selections were not well-described by their suggested market class (i.e. mahogany cherries were, in fact, blush cherries). All replicates of those genotypes were the same.
- In general, fruit size was small and firmness and pedicel retention force low. Rain events near harvest provided a cracking 'test'; the range of cracking among selections was 0% to 100%. However, the timing of the rain events relative to harvest timing needs to be considered.

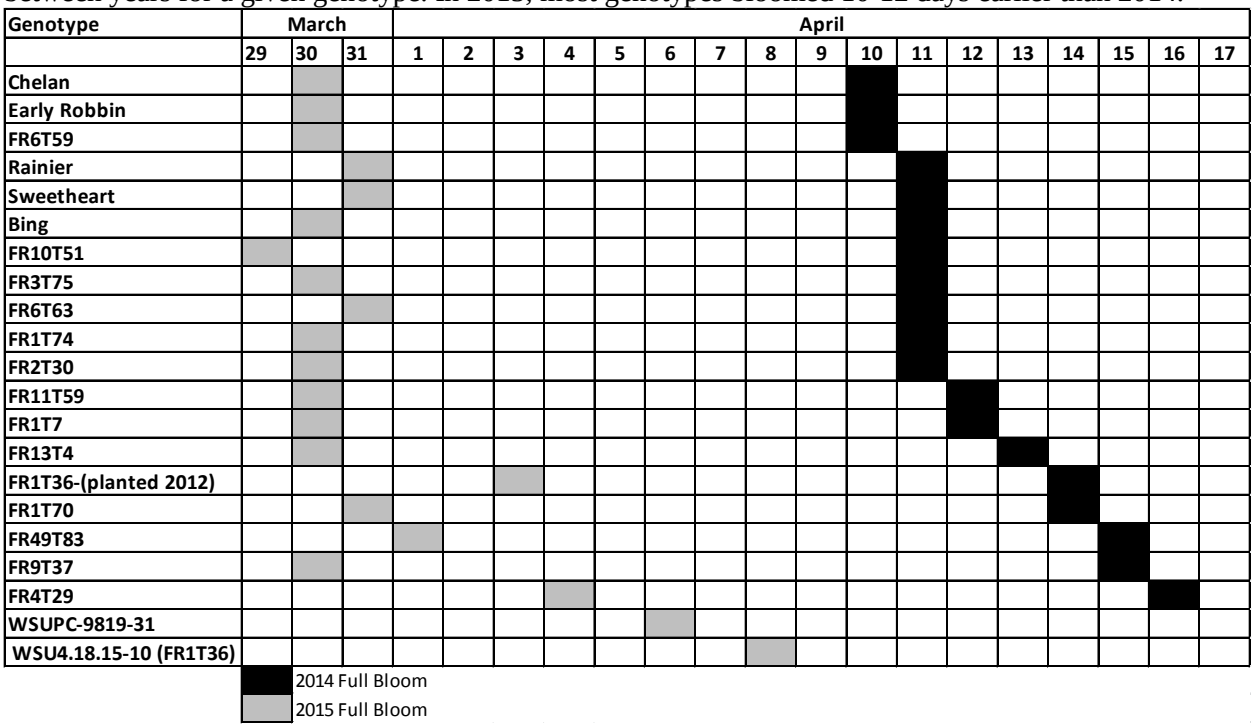
Table 6. 2015 harvest dates, number of replicate trees harvested, market class, yield, and fruit quality attributes for 19 accessions. Market classes were revised to reflect whether fruits were blush or mahogany. The suggested timing (early, mid, late) was not altered. The data show limited number of trees harvested for a few selections, and, importantly, for the 'standards'. This represents a serious issue. High variability in cherry fruit quality (especially in 2015 given the impact of environmental conditions on development) necessitates replication in order for treatment effects (selection) to be observed. Standards need to be represented by equal replication as selections. Shaded data represent values approaching acceptability. For many genotypes, 2015 represented the first fruiting year; partially explaining the low per tree yields. Rainier and 1T36 trees were received in 2 different years (hence the two rows of data for these selections).

Genotype	Harvest (date)	Trees (reps) (no. harvested)	Market class	Yield (lbs/tree)	FF (g/mm)	Fruit size (dia.) mm	Fruit size Row sz	CTIFL (1-7)	Cracking (%)	PRF (g)	SSC (%)	TA (%)
1T5	5-Jun	1	ESM	0.02	364.8	27.4	10	4.6	100	328	19.8	0.99
Early Robin	5-Jun	1	ESB	0.03	415.9	30.3	9	Blush	40	528	16.2	0.56
9T89	5-Jun	1	ESM	0.02	329.5	25.8	10.5	5.3	n.d.	365	18.2	0.68
1T7	10-Jun	1	FT-ESM	0.43	223.1	25.7	10.5	5.6	0	702	16.2	1.03
10T51	15-Jun	4	MSM	1.28	247.6	28.8	9.5	5.4	36.2	513	19.4	1.18
6T59	15-Jun	4	LSM	5.26	289.2	27.4	10	5.5	13.3	568	22.3	1.26
Rainier	15-Jun	4	MSM	26.33	236.5	28.5	9.5	Blush	n.d.	550	19	0.73
Rainier	15-Jun	3	MSM	2.58	214.9	29.9	9.5	Blush	n.d.	544	19.8	0.81
11T59	18-Jun	5	ESB	0.50	270.1	30.4	9.5	Blush	14.4	342	22.1	1.01
1T36	18-Jun	5	MSM	0.79	285.8	27.4	10	4.7	2.8	362	18.4	0.96
6T63	18-Jun	5	LSM	0.76	262	29.5	9.5	5.4	8.2	353	24.5	1.18
13T4	20-Jun	3	LSM	0.24	384.1	30.5	9	5.4	66.7	534	21	0.81
1T74	20-Jun	5	LSM	1.65	309	31.3	9	5.5	12.7	469	20.5	0.98
3T75	20-Jun	4	LSM	0.19	253.3	30.5	9	5.9	1.8	329	23.1	0.95
Bing	20-Jun	1	MSM	1.49	254.9	29.3	9.5	5.9	12.2	360	22.4	0.85
1T36	22-Jun	5	MSM	14.29	312.4	28.2	10	5	2.8	481	n.d.	n.d.
49T83	25-Jun	5	LSB	0.36	377.5	28.6	10	Blush	17.8	300	23.6	0.58
4T29	25-Jun	5	LSB	0.43	360.1	27.4	10	Blush	25.4	184	23.5	0.78
1T70	25-Jun	4	LSB	0.37	279.9	27	10	Blush	10.7	248	23.7	1.08
2T30	29-Jun	4	LSB	0.13	307.7	29.4	9.5	Blush	2.5	333	21.5	0.89
Sweetheart	7-Jul	1	LSM	0.56	218.4	24.2	11	5.3	n.d.	109	25.9	1.02

Table 7. Postharvest storage quality, and in a few cases, pitting susceptibility of those genotypes with ample fruit remaining after harvest evaluations. Fruit was held at 32°F (>95% RH) for 3 weeks prior to evaluation. The number of replicates for each genotype that were evaluated is provided. As stated above, additional replications of standards are required for statistical analyses. Average pit score was a weighted average of the number and size of pits per fruit, where 1= mild insignificant pitting and 4= severe pitting. Percent bruised fruit were characterized by compression bruising (surface of fruit visibly flattened). In many cases, trees were in their first year of production and had insufficient fruit volume to accommodate postharvest analysis and pitting.

Genotype	Trees (reps) (no. evaluated)	Market class	FF (g/mm)	Fruit size (dia.) mm	Fruit size Row sz	CTIFL (1-7)	Cracking (%)	PRF (g)	SSC (%)	TA (%)	Ttl pits/fruit (no.)	Avg. pit score (1-4)	Bruised (%)
10T51	2	MSM	318.6	28.5	9.5	5.8	46	171.1	19.2	1.07	4	3.3	43.3
6T59	4	LSM	348.3	27.6	10	5.9	8.1	175.4	22.8	0.97	3.9	3	17
Rainier	4	MSB	294.4	27.7	10	Blush	2	239.5	18.8	0.65	3.8	3	46
Rainier	1	MSB	254.5	29.8	9	Blush	0	255.7	22.2	0.67	3.2	3.3	56
1T36	5	MSM	298.5	28.3	10	5.1	0	682.8	19.1	0.85	5.4	2.5	22
6T63	2	LSM	323.1	29.8	9	5.4	2.4	670.6	24.5	1.22	5.8	3.2	65
1T74	4	LSM	359.6	31.1	9	5.6	n.d	416.5	20.6	0.92	n.d	n.d	n.d
Bing	1	MSM	288.9	28.7	9.5	5.1	n.d.	205	21.6	0.76	n.d	n.d	n.d

Figure 3. Bloom timing (full bloom) for 2014 and 2015. In general, bloom time was consistent between years for a given genotype. In 2015, most genotypes bloomed 10-12 days earlier than 2014.



FINAL PROJECT REPORT **YEAR**: 2014-15

WTFRC Project Number: CH-14-104

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Cooperators: Shield Bags and Printing Company, Yakima, WA; Stemilt Growers LLC., Wenatchee, WA; Western Sweet Cherry Group, Yakima, WA; Van Doren Sales, Inc., Wenatchee, WA, Tate & Lyle Co., Hoffman Estates, IL; TIC Gums, White Marsh, MD; Pace International LLC, Wapato, WA; AloeCorp Inc., Lyford, TX, Chelan Fruit Co, Chelan, WA, Allan Bros Fruit Co, Naches, WA.

We are very grateful to our co-operators for helping us in many aspects of the execution of this project. Thank you to all the co-operators.

Budget: **Year 1**: $24,567 **Year 2**: $24,932 **Year 3**: N/A

Other funding sources: Partial support from Dr. Ganjyal's new faculty start-up funds to fund the graduate student who worked on this project.

Note: We will be happy to share detailed data that is not included in this report, because of space limitations. In the near future we plan to publish the data in peer reviewed journals that we will share with the commission.

Budget 1:
Organization Name: WSU **Contract Administrator: Carrie Johnston**
Telephone: 509-335-4564 **Email address: carriej@wsu.edu**

Item	2014	2015
Salaries	$13,510	$14,092
Benefits	$2,252	$2,342
Wages	$1,973	$3,553
Benefits	$192	$345
Equipment	$1,500	
Supplies	$3,500	$3,000
Travel	$1,600	$1,600
Miscellaneous		
Plot Fees		
Total	$24,567	$24,932

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.

OBJECTIVES RECAP

1. Test the feasibility of using cold air impingement drying with optimal relative humidity to effectively remove the residual moisture from the cherries before packaging.
2. Screen various edible coatings on to the cherries for their ability to act as a moisture barrier.
3. Develop packaging strategies with desiccant to reduce the moisture available in the packaging environment for cherries.

SIGNIFICANT FINDINGS

Following are the significant findings of the research carried out:

1. The air knives effectively remove excess moisture from the fresh cherries before packing. Removal of excess moisture from cherry surface contributes to the reduction of fruit cracking during refrigerated storage and also improves shelf-life.

2. Air dried cherries showed significant reduction in the cracks during storage studies by more than 60% by the end of 7 weeks compared to other treatments (see Figure 6).

3. The air knife position over the belt and the drain belt type has significant impact on the efficacy of surface moisture removal. The drain belts with larger pore/hole sizes drain the water more effectively.

4. We selected 4 different coatings during the first year (by screening 16 total coatings) and tested them in the final plant in the year 2014. Out of these 4 coatings, we found that gum acacia (gum Arabic) was the best in terms of reducing the fruit splitting and pedicle browning.

5. Coating of cherries with gum acacia (gum Arabic) solutions consistently reduced the number of cracks during multiple trials.

6. The gum acacia concentration of 0.5% and 1.0% were tried in the 2nd year of the project. Both showed similar effectiveness. Although it will be important to test higher concentrations to see if it can increase the effectiveness of coating further.

7. Packaging with desiccant significantly decreased the cracking during storage. We tested one level of desiccant in the package during both the years of testing.

8. Packaging with desiccant and with the holes contributed to significant reduction in the cracks (see Figure 5 & 6).

9. The treatment with “gum Arabic” coating and the air drying together provided the best benefit with significant reduction in the number of cracks during storage and the pedicle browning. The percentage reduction of the cracks and pedicle browning at the end of 7 weeks storage were > 55% compared to other treatments (see Figure 6 and 7).

10. In addition to reduction in the number cracked cherries, the treatments also provided a benefit of reduced pedicle browning.

11. Overall, we have shown that all the three approaches, i) edible coating application, ii) air drying to remove excess moisture/coating solution and iii) packaging with desiccant embedded in the plastic, can help reduce the postharvest cracking of the fruit.

12. The results thus far have taken us into the direction where we believe that there is the potential to help reduce not only the cracks/splits, but also the stem browning.

13. Based on the results obtained so far, we have now proposed a new 3-year project to continue this work on enhancing the edible coating and the packaging and providing a toolbox of solutions that the cherry industry can implement in the future.

METHODS

Materials and Trials

We tested "Chelan", "Skeena" and "Sweet Heart" variety of cherries. During first year trials, the pre-sorted, cleaned and sized fruits were packaged in a bag/box system at packaging houses and transported to the pilot plant at School of Food Science, Washington State University (WSU). The cherries were kindly provided by Western Sweet Cherry Group, Yakima and Stemilt Growers LLC, Wenatchee. Additional to the laboratory trials, during the first year we were able to conduct one plant trial at Stemilt packing facility. During this trial we tested four different coatings and the desiccant packing.

In the second year, a total of three plant trials were conducted at Stemilt Growers LLC in Wenatchee, WA (Trial#1), Chelan Fruit in Chelan, WA (Trial#2) and Allan Bros Inc. in Naches, WA (Trial#3) cherry packing houses. After treatments, cherries were packaged, boxed and brought to WSU for storage and quality evaluation.

We are very grateful to all the co-operators who have supported us significantly during the trials.

Edible coatings

During initial trials (in 2014), a total of 4 different coatings that were prescreened during lab testing, were applied on the cherry fruit surface. In the initial prescreening over 16 different coatings were tested in the laboratory. The 4 coatings were selected based on the ease of application and their ability to dry without leaving any residue in the bags during storage.

Locust Bean Gum, TICA film and Gum Acacia (Gum Arabic) were obtained from TIC Gums (White Marsh, MD) while Aloe Vera coating was obtained from Aloe Corp (Eastern USA). All the coatings were prepared by first dissolving them in warm water at various concentrations as follows Aloe Vera (0.5%), TICA film (0.5%), Gum Arabic (1%) and Locust Bean Gum (0.3%). All coatings were cooled to room temperature before applying to the Cherries. Cherries were coated by dipping them in the solutions followed by blowing air through air-knives for removing the excess coating from the surface. Based on the performance of edible coatings during first year trials, Gum Acacia (1 and 2%) and TICA (0.5 and 1%) solutions were used for coatings of cherries during the second year trials During the 2nd year's work, only gum acacia was found to be more effective and was the only one used for the trials. The solution concentrations of 0.5% and 1% were tried during this year.

The coatings were applied by two different methods, during the plant trials. In the first method (Figure 2a), cherries were dipped in coating solutions in the last section of the packing line (where the

fungicide is usually applied) and then passed on the air knives and the packaging. In the second method (Figure 2b), the coating solution was poured on to the cherries, followed by air drying of coatings and packaging. Both methods of coating application were effective.

Excess surface moisture removal

The surface moisture on the cherries was removed with an air knife system. (Air Control Industries Inc., Maine). The system consisted of two air knives along with an air blower. The system was installed over conveyor belt on the packaging line just before packing. Please see Figure 2, showing the air knife system.

Packaging

Plastic films incorporated with desiccant agent were fabricated by Shield Bag and Printing Company, Yakima. The same plastic film without desiccant was also used as control treatment. Both films were used to prepare bags with and without holes.

A total of 24 holes were punched on each bag distributed as follows: 8 in the bottom and 8 in each face of the bag (front and back). The holes diameter was 8.343 mm (or 0.3284 in). Commercially available bags used for cherry packaging were also used as another control.

The set of different treatment includes; bag without desiccant and without holes, bag without desiccant and with holes, bag with desiccant and without holes, bag with desiccant and with holes, and a standard industry bag.

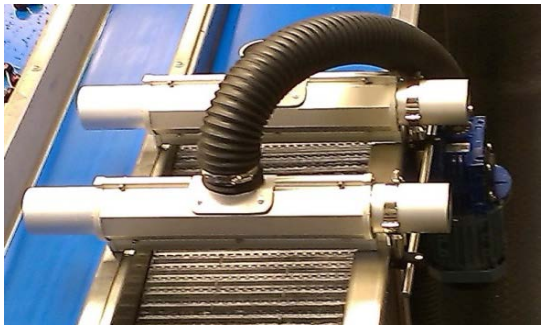
During first year trails, five bags for each treatment were filled with 25 cherries while in the second year trials, packaging bags were completely filled with cherries.

The bags were kept in the cold room maintained at 40-41˚F and relative humidity 86% for 4-6 weeks for storage studies. Based on the findings from first year trials, bags with holes were used in the second year trials.

(a) Dipping method

(b) Water fall method

Figure 1. Methods of edible coating application in the packing house



(a) Air Knife System Installed

(b) Air knife System in Operation

(c) Cherries without Air Drying

(d) Cherries with Air Drying

Figure 2. Air drying system developed for removing the excess moisture in fresh cherries.

Fruit quality determinations

Cherries were analyzed for five quality parameters at regular storage interval. Quality parameters evaluated were including weight loss, color, firmness, ˚Brix, and pH. Weight loss was determined by weighing the samples with digital balance (Startorious, MCL). Color and firmness measurement were performance in a subsample of ten cherries in each treatment. Color change of Chelan and Skeena cherries was measured in the skin with a tristimulus colorimeter (Color spectrophotometer CM-5, Konica Minolta) which provided CIE *L** (lightness), *a** (green to red) and *b** (blue to yellow) values. Fruit firmness was determined by measuring the force required to compress the fruit 2 mm using texture analyzer TA-TX2 equipped with a 3 mm diameter convex probe at a speed of 20 mm min^{-1} (Salato et al., 2013). Every fruit was measured on the equatorial plane and the registered forces were averaged and reported. Then the content on each bag was manually crushed to extract the cherries juice which was used for ˚Brix and pH determination by triplicate in each storage stage. Total soluble solid (TSS) expressed as ˚Brix were determined in each slurries by refractometry with han-held temperature-compensated digital refractometer (Refracto 30GS, Mettler Toledo). The pH values were measured by using a pH meter (Symphony B30PCI). The cherries were also examined visually for cracks and mold growth. Pedicel browning was also observed and expressed as the percentage of fruit with >30% stem surface discoloration.

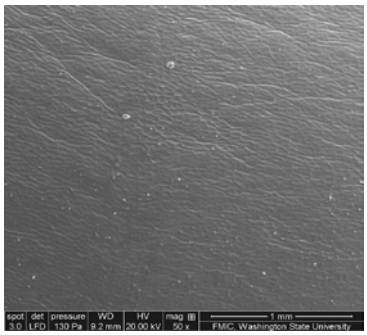
RESULTS AND DISCUSSION

Please refer to Figure 3 for the brief overview of all the testing done during the years 2014 and 2015. In the year one we conducted extensive laboratory testing to arrive at the best 4 coatings and we did conduct one plant trial with all the 4 different coatings. During 2015, we conducted 3 different plant trials. In only the first trial of 2015, we used 2 coatings (gum acacia and TICA film) and only one coating (gum acacia) was used, as it was found to be more effective from all the previous trials.

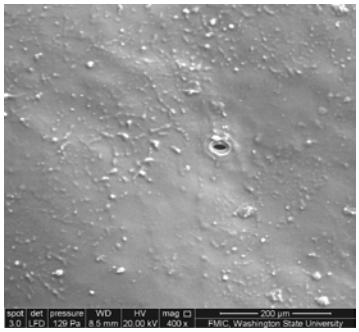
Laboratory trials and Plant trial (summer 2014)

Microscopy studies of the fruit surface

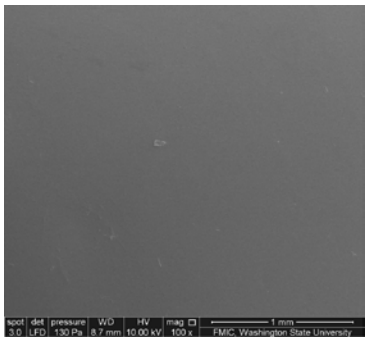
The micrographs obtained using Scanning Electron Microscope (FEI, Model Quanta 200F, FEI Company, U.S.A.) indicated pores of 20 microns in size and the uneven surface (Figure 4a and b). This uneven surface can serve as pooling spots for excess moisture. Our hypothesis is that edible coating can smooth out the fruit surface (Figure 4c and d) and decrease moisture accumulation on the surface even. This should also decrease moisture absorption into the cherries thus contributing to the reduction of cherry cracking.



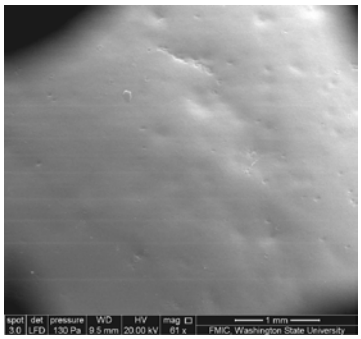
(a) Surface without coating



(b) Surface without coating



(c) Surface with coating (TICA film)



(d) Surface with coating (Gum Acacia)

Figure 4. Microscopy images of fresh cherry surface with and without the coatings

Effect of air drying and edible coatings on fruit quality

The coating of "gum Arabic" solution when stored in bags with both the regular company bags (control bags) and the bags incorporated with desiccant (desiccant bags) reduced the number of cracks in Skeena cherries (Figure 6). The titrable acidity reduced during storage the influence of coatings and air drying was small. The ^{o}Brix of cherries was not influenced by the treatment conditions. The firmness of cherries was reduced with storage time but the "gum acacia" and "aloe

vera" coatings were able to lower the changes in firmness. Both TICA film and "gum acacia" helped reduce the cracks in the cherries significantly. These cherries with edible coatings were all air dried. The change in color was small and consistent will all treatments.

Air drying alone helped reduce the number of cracks (Figure 6). The addition of the coatings helped this more and the effects were more prominent when we looked at the pedicel (stem) browning data (Figure 7). The treatment with the "gum acacia" coating with air drying and stored in the control bag was found to have the least number of brown stems. This suggests that the coating helps to provide a seal on the stems and reduces the rate of moisture loss from the stems to the surroundings.

Effect of packaging on fruit quality

The number of cracks in the cherries was less in desiccant bags compared to the control bags (Figure 5) for Skeena variety in the laboratory trials. The packaging did not show significant added benefit over the coating treatment alone in the plant trials for the Skeena variety (Figure 6). The weight loss in cherries generally increased with storage time. The weight loss in Skeena (5.7%) was higher than Chelan (4.2%) during 35 days of storage. Bags with no perforation had lower losses in the cherry weight. This is because perforation allowed water vapor to escape from the bag.

Incorporation of desiccant in packaging bags significantly reduced the weight loss in Chelan but had less influence in Skeena. The pH and ^{o}Brix of cherries did not change significantly during storage time. The influence of types of packaging on pH and ^{o}Brix was also not significant. The firmness of cherry was not influenced by the packaging conditions. The change in color of cherries was small during storage but packaging conditions did not influence the total color change. The packaging films incorporated with desiccant and having perforation reduced the number of cracks for both types of cherries during and at the end of storage time.

Plant trials (summer 2015)

Effect Surface moisture removal and edible coatings

The "gum Arabic" showed the reduction in the cracks over the storage period of 6 weeks (Figure 8). Figure 8 shows the data of the cracks in cherries from our plat trial #3, when the air knives performed really well. The effectiveness of the air knives was really great during this trial. From this data and from the other trials it was conclusive that the air knives were effective in reducing the cracks, as they removed the excess moisture from the cherry surface before they were packaged in the bags.

The coating (gum Arabic) was effective in reducing the cracks (Figure 6) over the storage period of 6 weeks. From our data from all the plant trials during 2015 this was the trend we observed. In some cases the inclusion of desiccant bags made a difference and in some cases it did not.

The weight loss in coated and uncoated cherries was in the range of 2.6 to 3.5%. Edible coatings and surface moisture removal slightly increased the weight loss in the cherries from trial#1, while cherries from trial #2, weight loss were in the range of 3.6 to 4.9%. Again, surface moisture removal and edible coatings increased the weight loss.

In general, edible coatings significantly reduced the cracking from 7.7% to 3.8% in cherries from trial #1 while in cherries from trial #2 the cracking reduced from 5% to 2.5%. For trial#1 cherries, TICA coating of 0.5% solution was most effective while Gum Arabic coating of 1% was more effective for trial#2 cherries. It is to be noted that the air knives set-up did not perform well during trial#1. Gum Arabic coating of 1% solution was most effective in maintaining the firmness of all the trials.

Packaging

In trial#1, the weight loss in cherries (uncoated and no moisture removal) increased up to 3.6% during five weeks of storage depending on the packaging. It is to be noted that the air knives set-up did not work well during this trial. The weight loss in desiccant incorporated bags (3.6%) was slightly higher than weight loss in company bags (2.6) and control bags (2.4%). However, for the trial #2, there was no significant difference in weight loss of cherries stored in different bags. The weight loss during five weeks of storage was around 3.6%. For trial #1, the % of cracking in desiccant bag (6.4%) was lower than company (7.7%) and control bags (12.3%). For trial #2, the overall cracking was lower than trial #1 and it was in the range of 0.8 to 5% depending upon the bag. Desiccant incorporated bags helped retain fruit firmness for trial#1 while types of bags did not significantly influence the cherry firmness.

Combined effects of treatments

The weight loss in trial#1 cherries packaged with control bags was lowest with TICA coating at 0.5% while for trial#2 cherries TICA coating at 1% with control film and Gum Arabic coating of 1% solution with desiccant bag were resulted in lower weight losses. This suggests that the coating helps reduce the moisture transfer from the cherries to the atmosphere. Gum Arabic coating of 1% with desiccant bag for cherries from trial#1 and TICA coatings at 1% in desiccant bag minimized the fruit cracking. For maximum fruit firmness, Gum Arabic coatings of 1% solution with control bag and no coating with desiccant bag were the best for cherries from trial #1 while for cherries from trial#2, Gum Arabic at 1% with company bags and no coatings with desiccant bags were equally effective in maintaining the firmness. Overall, Gum Arabic coatings of 1% solution and desiccant bags combination were the best for reducing the cracking and maintaining the overall quality of cherries.

Final remarks

All three treatments i.e. surface moisture removal, edible coatings and packaging incorporated with desiccant significantly reduced cherry cracking and pedicle browning. The treatments marginally increased the weight loss while they had positive influence on fruit firmness. All treatments had minimal or no influence on other chemical quality parameters such as pH, ^{o}Brix, and color. The treatments such as surface moisture removal, edible coatings and packaging film incorporated with desiccant significantly reduced the number of cherries with cracks.

SIGNIFICANCE TO THE INDUSTRY & POTENTIAL BENEFITS

Post-harvest cracking/splitting of cherries is one of significant issues to the industry. Any reduction in fruit cracking/splitting and pedicle browning will have direct positive economic impact to the industry. The results from this study are encouraging and suggest that the surface moisture removal, edible coatings and packaging incorporated with desiccant can significantly reduce fruit cracking and pedicle browning.

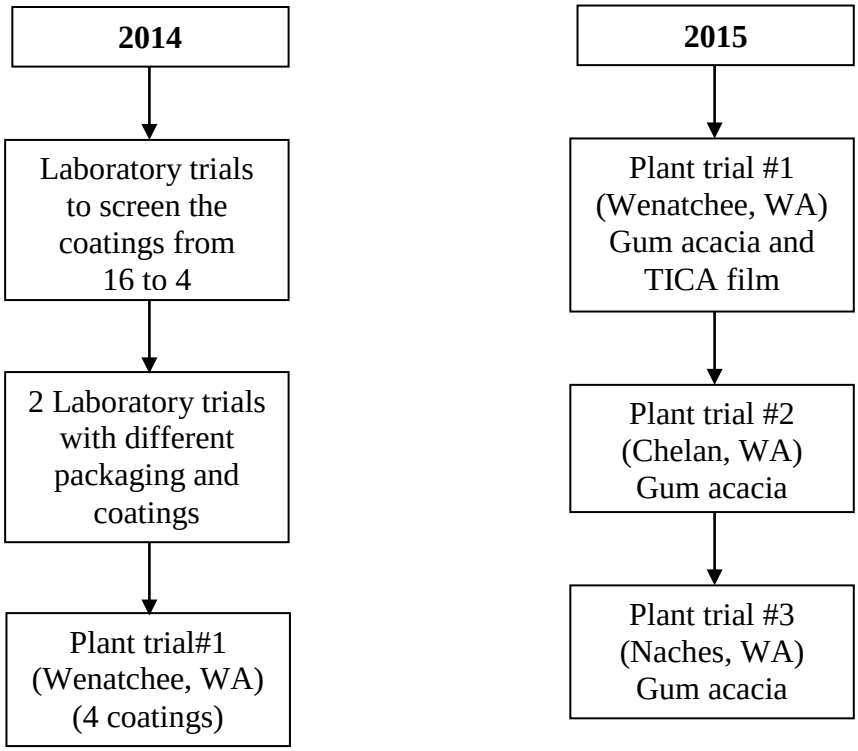
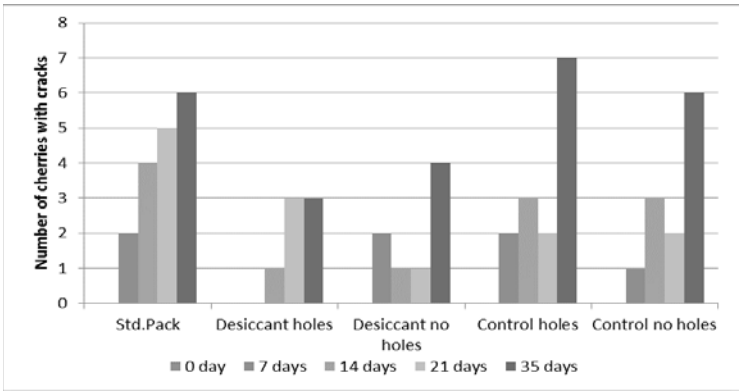
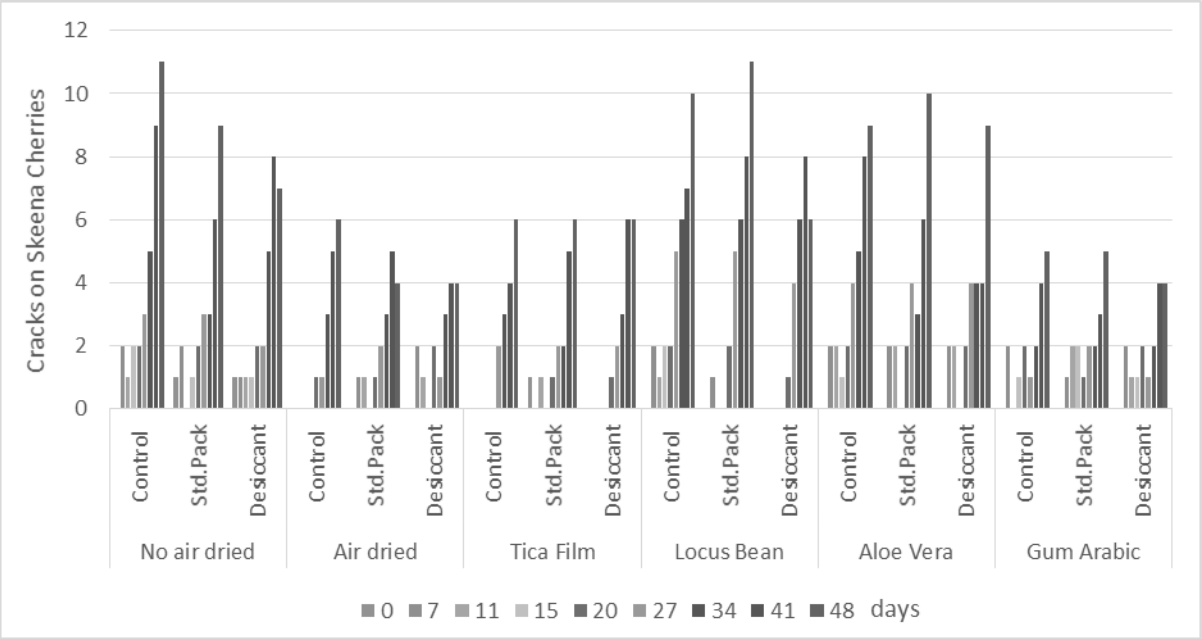


Figure 3: Summary of the trials conducted during the duration of this project (2014 and 2015)



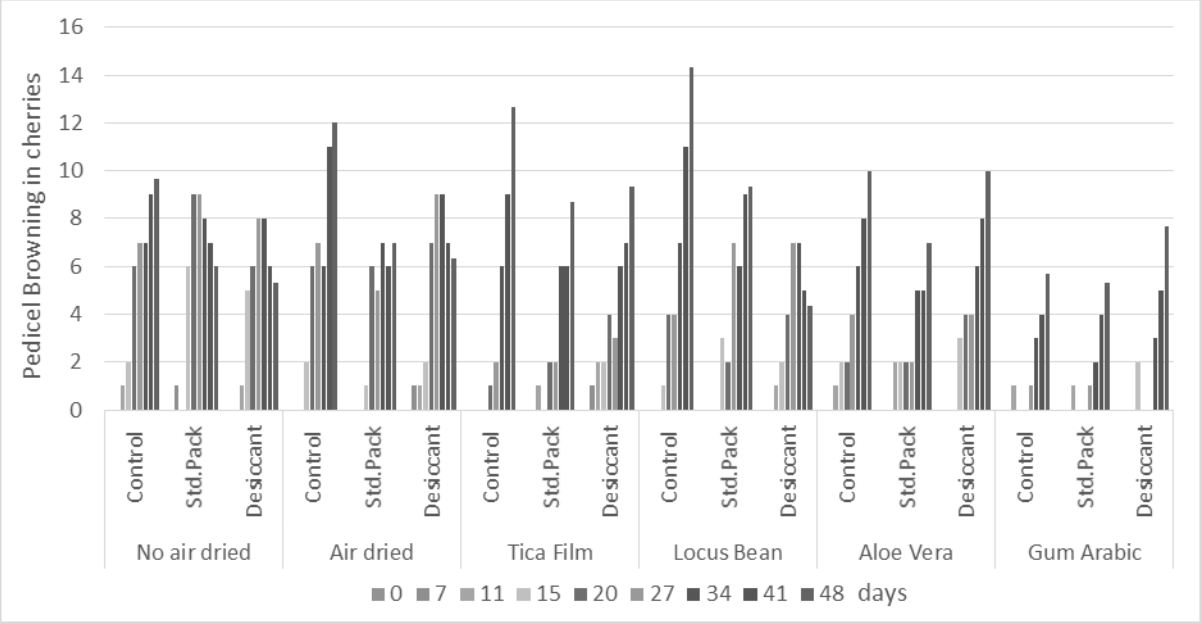
(2014 Trial)

Figure 5. Cracks observed in Skeena Cherries during storage studies (laboratory trials)



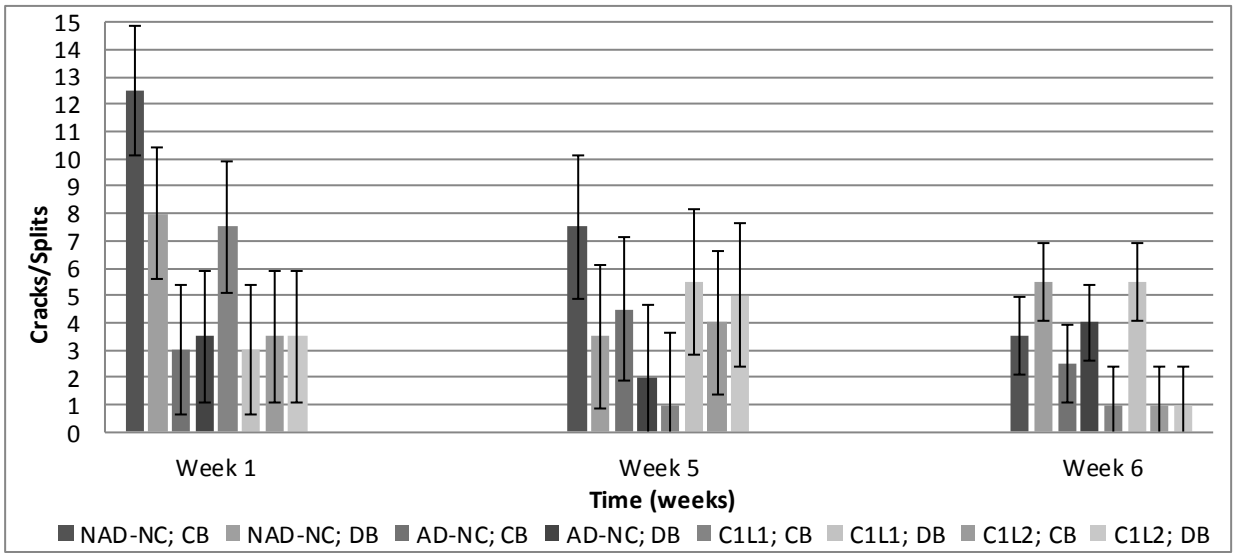
(2014 Trial)

Figure 6. Cracks observed in Skeena Cherries during storage studies (plant trial)



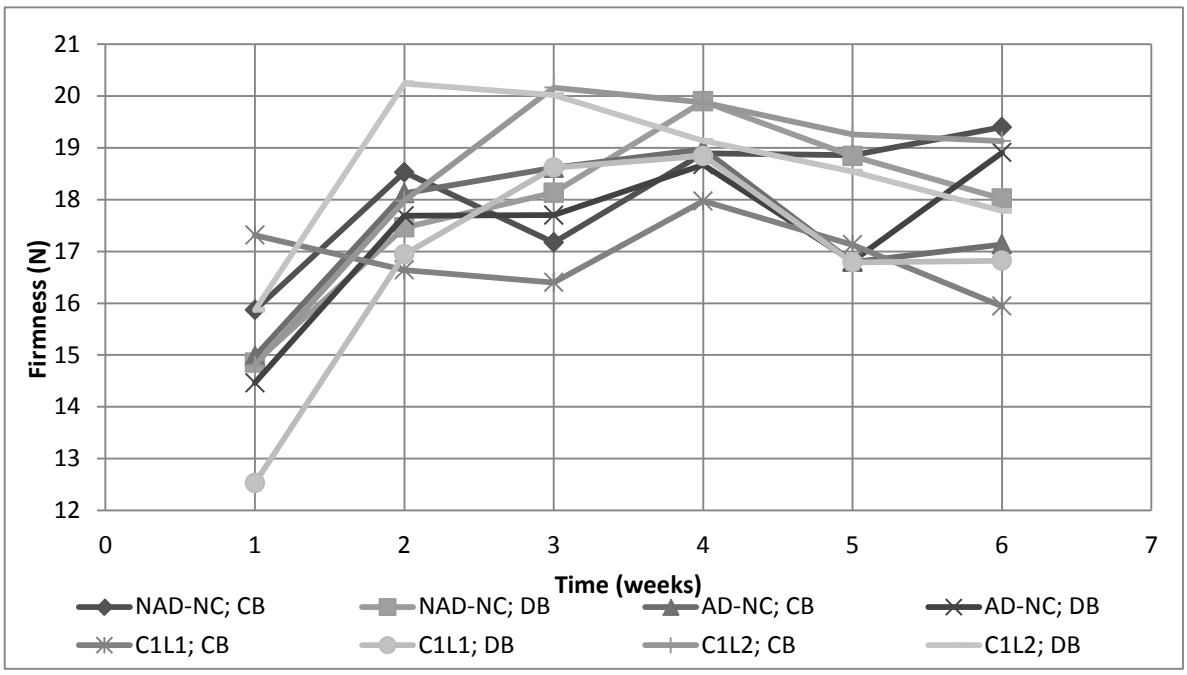
(2014 Trial)

Figure 7. Pedicle browning observed in Skeena Cherries during storage studies (Plant Trials)



(2015 Trial#3)

Figure 8. Cracks observed in Sweetheart Cherries during storage studies (packing house trial #3)
NAD = No Air Drying; AD – Dir Dried; NC = No Coating; C1L1 = Coating 1 (gum acacia) Level 1 (0.5%), C1L2 = Coating 1 (gum acacia) Level 2 (1.0%); CB = Company Bag, DB = Desiccant Bag



(2015 Trial#3)

Figure 9. Firmness of Sweetheart cherries during storage studies (packing house trial #3)
NAD = No Air Drying; AD – Dir Dried; NC = No Coating; C1L1 = Coating 1 (gum acacia) Level 1 (0.5%), C1L2 = Coating 1 (gum acacia) Level 2 (1.0%); CB = Company Bag, DB = Desiccant Bag

CONTINUING PROJECT REPORT **YEAR:** 2 OF 3
WTFRC Project Number: CH-14-102

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

PI:	Cameron Peace	**Co-PI**:	Nnadozie Oraguzie
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City/State/Zip:	Pullman/WA/99164	**City/State/Zip**:	Prosser/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)

Total Project Request: **Year 1**: $40,000 **Year 2**: $42,000 **Year 3**: **$43,000**

Other funding sources

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.

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.

Budget

Organization Name: Washington State University **Contract Administrator:** Carrie Johnston

Telephone: (509) 335 4564 **Email address:** carriej@wsu.edu

Item	**2014**	**2015**	**2016**
Salaries[a]	17,651	18,440	19,265
Benefits	11,242	11,916	12,632
Wages			
Benefits			
Equipment			
Supplies[b]	9,107	9,644	9,103
Travel – within-state	2,000	2,000	2,000
Plot Fees			
Miscellaneous			
Total	40,000	42,000	**43,000**

[a] Half-time support of Paul Sandefur, PhD student and RosBREED double "breeding trainee"; 0.25 FTE Terry Rowland, genetic screening technician of the Washington Tree Fruit Genotyping Lab (WSU, Pullman)

[b] DNA extraction and PCR supplies, minor equipment maintenance, and computing supplies as necessary

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

***Specific objectives - Year 2*:**

1. Ensure DNA tests for market class-defining DNA tests for **fruit color** and **maturity** are available for use in the spring 2015 seedling selection season in combination with existing tests for **self-fertility** and **fruit size & firmness**

2. Complete development of new DNA tests for **powdery mildew** (leaf), **bacterial canker**, and a particular genomic location (on chromosome 5) for **fruit size & firmness** and enable DNA-informed parent selection and seedling selection for these traits in 2016

3. Initiate development of new DNA tests for **cracking**, **pitting**, and **fruit-pedicel abscission**

SIGNIFICANT FINDINGS

- New DNA tests developed for **powdery mildew** (leaf) and **fruit size & firmness** (chromosome 5 source)

- DNA test development initiated for recently uncovered genomic regions influencing **fruit size & firmness** (chromosome 6), and **resistance to powdery mildew** (leaf), **pitting**, and **cracking**

- New DNA information on **fruit color**, **maturity time**, **powdery mildew** (leaf), **bacterial canker**, **fruit-pedicel abscission**, and **fruit size & firmness** available for crossing decisions in spring 2016, in combination with existing DNA information for **self-fertility** ('Stella' and 'Cristobalina' sources), and **fruit size & firmness** (chromosomes 2 source)

- **Fruit color** and **maturity time** DNA tests screened on 3500 seedlings in spring 2015

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

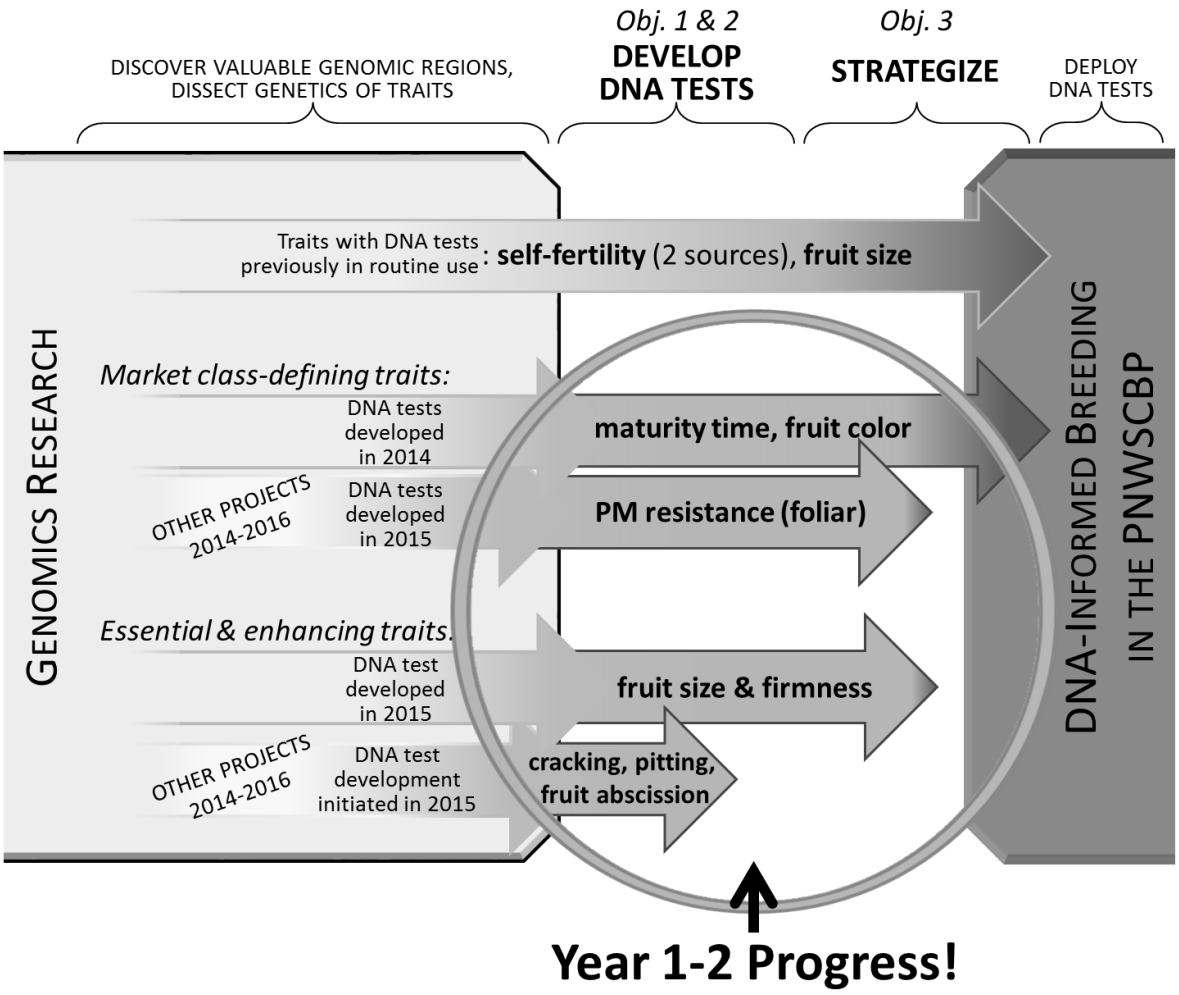

Figure 1: Progress made in Year 2, in the context of the entire project which has and will continue to improve the efficiency, accuracy, creativity, and pace of the Pacific Northwest Sweet Cherry Breeding Program (PNWSCBP) via DNA-informed breeding.

METHODS

DNA test development (Obj. 1 & 2)

DNA test development relies on the knowledge of genomic regions associated with the specific traits targeted by the tests. For this project, such knowledge is based on discovery of regions associated with **fruit maturity time**, **color**, **size**, **firmness**, and **fruit-pedicel abscission** by RosBREED and Stem-free Cherry SCRI project participants and collaborators, and regions associated with sweetness, acidity, and resistance to **powdery mildew**, **bacterial canker**, **cracking**, and **pitting** discovered through the WTFRC-funded project, "New genomic regions controlling production and fruit disorder traits" (Oraguzie and Peace). The trait-specific DNA tests developed will enable efficient functional genotyping of any new parents, elite selections, and seedlings – i.e., beyond the germplasm genotyped in the RosBREED project and especially useful for new families of seedlings. For every DNA test to be developed for a genomic region discovered to be associated with a specific trait, each step (A–E) in the DNA test development process outlined below will be completed.

DNA test development process

A) **Establishment of functional genotype patterns:** Phenotypic data for a specific trait and genotypic data generated during the RosBREED project are combined from which functional genotype patterns are developed. These genotype patterns are "functional" because they are correlated with specific, breeding-relevant trait levels, and form the basis from which DNA tests can be developed.
B) **Functional genotype conversion:** Using Genome Database for Rosaceae tools genomic sequences from the targeted regions are retrieved. For initial testing, five sites in each region are targeted with candidate assays.
C) **Candidate assay checking:** Each candidate assay designed is checked on a small set of individuals that represent functional genotype patterns. Checking includes amplification and visualization of the candidate assay product followed by examination of assay products. This same process is then repeated across a larger set of diverse, breeding relevant individuals (~50) to confirm initial results. If matching results are observed, the candidate assay becomes an official DNA test and is ready for low-throughput genotyping of parent material and conversion to a high-throughput system.
D) **High-throughput conversion:** The successfully developed DNA test is converted to a high-throughput platform (ABI 3730 DNA Analyzer) to maximize resource efficiency. After successful conversion, the DNA test is now named appropriately and is ready for full application.
E) **Effects calculations:** Because only DNA tests with products that directly match the original functional genotype patterns are chosen for application, no additional confirmation of the product-trait level association is required. As additional phenotyped material that was not part of the original association calculations is examined with the new DNA tests, more accurate effect calculations are conducted using standard statistical procedures.

DNA test deployment strategies (Obj. 3)

As each new DNA test is completed, deployment strategies are devised to best integrate into the general breeding scheme. Factors considered include:

- **Timing**: the relative value of deploying DNA tests at various breeding operational stages each year, from crossing through greenhouse-, lath house-, and field-grown seedlings to elite selections.
- **Parent selection**: amount of attention that should be given to developing superior parents carrying desirable alleles for each DNA test so that few or no seedlings need to be culled by each test in future years.
- **Market class**: whether a DNA test is to help achieve a performance level for a trait that defines one of the market classes targeted by the PNWSCBP or whether the performance level is essential or enhancing.
- **Impact on family size**: how much the use of a single DNA test and multiple DNA tests in seedling selection would be expected to reduce the proportion of surviving seedlings.
- **Genetic interactions**: whether the performance levels predicted by the presence of certain alleles for a DNA test can be affected by alleles targeted by a different DNA test.
- **Germplasm sources:** the source(s) and frequency of desirable alleles for each DNA test and whether a DNA test is applicable only for certain lineages.

RESULTS AND DISCUSSION

Obj. 1: DNA test development for maturity time, fruit color, and fruit firmness

Significant progress has been made as of October 2015 toward developing new DNA tests for the valuable traits of **maturity time**, **fruit color**, **fruit size**, and **fruit firmness**. In addition to our

previously obtained DNA test results for **self-fertility** and **fruit size & firmness**, DNA test results for **maturity time** and **fruit color** were used to guide 2015 crossing and seedling selection decisions, with more than 3500 seedlings screened.

A new DNA test for **fruit size & firmness**, "Pav-G5Size-SSR", targeting a newly discovered genomic region (on chromosome 5) is in the final stages of development and will be ready for deployment in spring 2016. By combining Pav-G5Size-SSR with the chromosome 2 DNA test for these traits, a powerful tool has emerged for the routine prediction of genetic potential for these important traits. In addition, a candidate DNA test targeting a third region (on chromosome 6) associated with these traits is undergoing initial checking for technical robustness.

Obj. 2: DNA test development for other traits

Exceptional progress from the WTFRC-funded project, "New genomic regions controlling production and fruit disorder traits" (Oraguzie and Peace) at uncovering new genomic regions associated with valuable traits has led to significant progress being made toward developing new DNA tests for **powdery mildew resistance**, **bacterial canker resistance**, **cracking**, **fruit-pedicel abscission**, and **pitting**. The candidate assays for foliar **powdery mildew resistance** and **fruit-pedicel abscission** designed in Year 1 were developed into predictive DNA tests that are ready for use in parent and seedling selection in spring 2016, with DNA information for these traits already having been used to guide crossing decisions in 2015. After two years of progress, a suite of eight DNA tests targeting traits of importance to the PNW industry is now available (Table 1).

Table 1: DNA tests available for routine use in the PNWSCBP, each of which is best applied for target seedlings to market classes, target essential trait levels, or target enhancing trait levels prior to field-planting.

Trait	DNA test	Market class-defining	Essential attribute-targeting	Enhancing attribute-targeting	Further details
Maturity time	Pav-G4Mat-SSR	Yes			Extra-early under investigation
Fruit color	Pav-R_f-SSR	Yes			Light vs. dark mahogany is also detectable
Abscission	Pav-G2-SSR	Yes	Low abscis. in Mech-M*	High in all others	Current test only covers some genetic effects
PM resistance	Pav-G5PM-SSR		Only in EM*	Especially in MM* & LM*	Fruit PM resistance test needed
Self-fertility	Pav-S4'-indel and EMPAS02b		Self-fertile in all classes		Utilizing two sources reduces genetic bottlenecks
Fruit size & firmness	Pav-G2-SSR and Pav-G5Size-SSR		Weight >10 g, firm >300 g in all classes	Weight >12 g, firm >400 g	Additional genomic region on chromosome 6 targeted for DNA test development

***** "Mech-M" = mahogany fruit suitable for mechanical harvest, "EM" = early-season and mahogany, "MM" = mid-season and mahogany, and "LM" = late-season and mahogany – four of the target market classes of the PNWSCBP

Powdery mildew (leaf) resistance

One of the candidate assays for foliar **powdery mildew resistance** designed in Year 1 was successfully developed into a DNA test, "Pav-G5PM-SSR", and is ready for application in 2016.

Traditionally, costly and error-prone phenotyping is used to evaluate powdery mildew resistance. In U.S. sweet cherry breeding germplasm, such resistance ranges from highly susceptible (e.g., 'Bing') through moderately resistant (e.g., 'Tieton') to resistant (e.g., 'PMR-1', 'Chelan' and 'Mildew Immune Mazzard'). Many sources of resistance are known, but dissection of the targeted genomic region has revealed that the same resistance allele might underlie all sources. In any case, Pav-PM5-SSR is able identify individuals with resistance to powdery mildew from four unique sources. Pav-PM5-SSR is also able to differentiate highly susceptible, susceptible, intermediate, and moderately resistant individuals, all without the need for traditional disease resistance phenotyping.

Bacterial canker resistance, cracking, and pitting

After examining the **bacterial canker resistance**, **fruit cracking**, and **pitting** phenotypic data obtained in the PNWSCBP and arising genomic discoveries for these traits, the need for additional phenotypic data was decided. Once adequate phenotypic data is available, development of DNA tests for these traits will continue. DNA test development for **fruit cracking** targeting a new genomic region discovered by French collaborators was initiated with candidate assays expected to be checked for trait predictiveness and technical robustness, and, if all goes well, ready for routine breeding use in spring 2016.

Fruit-pedicel abscission

A DNA test was developed for **fruit-pedicel abscission** (measured as "pedicel-fruit retention force"). This test uses the same markers as for the long-standing fruit size test for chromosome 2 (Pav-G2-SSR). 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.

Obj. 3: DNA test deployment

With so many DNA tests of different types now in the toolkit of the PNWSCBP (Table 1), sophisticated deployment strategies are needed to best take advantage of the new opportunities. A three-step strategy is proposed (Figure 2).

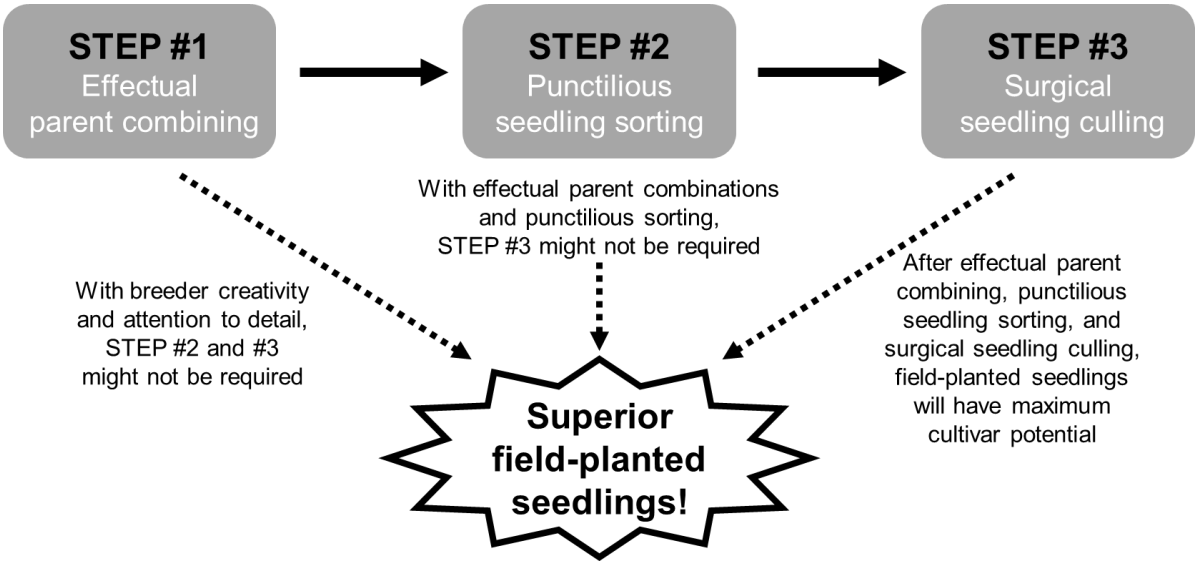

Figure 2: Three-stage strategy proposed for DNA-informed breeding with cultivar-generating families of the PNWSCBP.

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, mid-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*. Such essential attributes typically include **self-fertility**, f**ruit 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. Available DNA tests for **self-fertility** (Pav-S4'-indel) and **fruit size & firmness** (Pav-G2-SSR) and the DNA tests for **powdery mildew resistance**, **fruit-pedicel abscission**, and **fruit size & firmness** recently developed (Table 2) will help to maximize effectual parental combining in spring 2016.

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. In spring 2015, DNA information on maturity time and fruit color for 3500 PNWSCBP seedlings was used to sort seedlings into their appropriate market classes. 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, followed by DNA tests for enhancing attributes as desired. In both cases, the 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 and then enhancing attributes (Figure 2), most seedlings field-planted and evaluated at adulthood for fruit are likely to meet target trait thresholds for each specific market class.

CONTINUING PROJECT REPORT **YEAR: 2 of 3**
PROJECT NUMBER: CH-14-109A-E

Project Title: MSU cherry rootstocks: Pre-commercialization

PI:	Amy Iezzoni	**Co-PI (2):**	Matt Whiting
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City/State/Zip:	Wenatchee, WA 98801	**City/State/Zip**:	The Dalles, OR 97058

Cooperators: Todd Einhorn, Tim Dahle, Stefano Musacchi

Total Project Request: **Year 1:** $50,450 **Year 2:** $53,063[a] **Year 3: $31, 291[b]**

[a] Increased by $4,392 to cover the cost of the extra trees produced due to the high bud take on the MSU rootstocks

[b]Decreased by $3,927 due to the delay of the 2016 planting

Other funding sources: Funds totaling $6,984 to optimize the DNA diagnostic tests for commercial service providers provided from the State of Michigan 21st Century Jobs Fund received through the Michigan Strategic Fund and administered by the Michigan Economic Development Corporation."

WTFRC Collaborative expenses:

Item	2014	2015	2016
Wages	$ 11,000[a]	$ 4,344	$ 4,618[b]
Benefits	$ 3,000	$ 1,175	$ 1,223
Supplies (Trellis, irrigation, fumigation)		$10,850[d]	$4,150[d]
Travel	$ 500[b]	$2,500	$ 4,500
Miscellaneous	$ 1,000[c]	$ 50	$ 100
Total	$ 15,500	$18,919	**$ 14,591**

Footnotes:

[a]Pruning, floral evaluation, harvest and fruit evaluations of the Roza plot.

[b]Travel to participating nurseries, and labor for installing trellis, planting and installing data at the Wenatchee grower plot.

[c]Assist in plot establishment

[d]Plot establishment and planting costs previously in Budget 3 were reallocated to the WTFRC budget upon the decision to use grower locations as opposed to WSU – Sunrise and Roza.

Budget 1 – Amy Iezzoni
Organization Name: Mich. State Univ. **Contract Administrator:** Lorri Busick
Telephone: (517) 353-0391 **Email address:** busick@msu.edu

Item	2014	2015	2016
Salaries (technician)[a]	$ 6,571	$ 4,800	$ 5,468
Benefits[b]	$ 2,829	$ 2,100	$ 2,432
Supplies[c]	$ 600	$ 1,200	$ 1,200
Travel[d]	$ 4,500	$ 4,500	$ 4,500
Liners	$ 1,000		
Trees		$ 17,160[e]	$ 0[f]
Total	$ 15,500	$ 29,760	**$ 13,600**

Footnotes:
[a]Technician will analyze and prepare summary tables and figures of the plot data and conduct the DNA diagnostics.
[b]Benefits calculated at 43.06%, 43.76% and 44.47% for 2014, 2015 and 2016, respectively.
[c]Laboratory supplies for the DNA diagnostics. More DNA tests will be needed in years 2 and 3 as plant materials are increased.
[d]Travel for A. Iezzoni to visit the test plots, liner nurseries and finished tree nurseries.
[e]Increased by $4,392 to cover the cost of the extra trees that resulted due to the high bud take on the MSU rootstocks.
[f] The cost of the trees for the 2016 planting ($3,927) was deleted as the planting is be delayed until 2017

Budget 2 – Matt Whiting
Organization Name: WSU **Contract Administrator:** Amanda Yager
Telephone: (501) 335-7667 **Email address:** ayager@wsu.edu

Item	2014	2015*	2016*
Wages	$ 5,333		
Benefits	$ 517		
Plot fumigation	$ 850		
Supplies	$ 200		
Trellis and irrigation	$ 1,100		
Plot Fees[a]	$ 2,000		
Miscellaneous (tree removal)	$ 1,000		
Total	$ 11,000		

Footnotes:
[a]Standard annual plot fee, Roza Station
*Plot moved to grower site in 2015 and WTFRC will now oversee work at this site. Budget changed to reflect this change.

Budget 3 – Desmond Layne[a]
Organization Name: WSU **Contract Administrator:** Joni Cartwright
Telephone: (501) 335-7667 **Email address:** joni.cartwright@wsu.edu

Item	2014	2015	2016
Plot Fumigation	$ 850		
Trellis posts	$ 1500		
Trellis anchors, wire, clips	$ 600		
Polytube/sprinklers	$ 5000		
Total	$ 7,950[b]		

Footnotes:
[a] Dr. Layne stepped down from this project in Fall 2015 due to a change in his assignment and move to Pullman.
[b]These unspent funds will be used for year 2 and 3. Unused portion will be returned after year 3.

Budget 4 – Lynn Long
Organization Name: OSU **Contract Administrator:** L.J. (Kelvin) Koong
Telephone: (541) 737-4067 **Email address:** l.j.koong@oregonstate.edu

Item	2014	2015	2016
Wages	$ 455	$ 1,700	$ 2,000
Benefits	$ 45	$ 170	$ 200
Supplies			$ 200
Travel		$ 40	$ 40
Plot Fees		$ 1,340[a]	$ 660[c]
Miscellaneous (Stakes)		$ 1,134[b]	
Total	$ 500	$ 4,384	**$ 3,100**

Footnotes:
[a]Plot fees include fumigation, powdery mildew and cherry fruit fly control through the season for the 2015 planting.
[b]Stakes for trees on CASS, CLARE and LAKE
[c]Plot fees include powdery mildew and cherry fruit fly control through the 2016 season.

OBJECTIVES:

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:

- Across all three scions and locations, the tree sizes on the MSU rootstocks are similar to our expectations: Cass and Clare rootstocks produce the smallest trees followed by Lake and then Clinton.
- On average the trees on the MSU rootstocks are significantly smaller than those on the Gisela or Krymsk rootstocks.
- Cass and Clinton can be slower in recovering from transplant stress.
- The five MSU rootstocks perform well in liner and finished tree production; therefore, no nursery barriers to commercialization were identified.
- The MSU rootstock liners being increased for tree production were confirmed to be labeled correctly based on DNA tests.

METHODS BY OBJECTIVE:

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

1.A. 2009 planting of 'Bing' on MSU cherry rootstocks (removal after 2014 season) at WSU – Prosser – Completed

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

The three replicated rootstock trials that were planted in 2015 will continue to be pruned and trained based on the training system used for each plot (Table 1). Data to be collected in 2016 will include tree survival, trunk cross-sectional area, and rootstock suckering. Due to the rootstocks' precocity, at the Mattawa and East Wenatchee sites, flower bud removal will be undertaken along with an additional round of light trunk girdling at green bud stage followed by four applications at weekly intervals of 6Ba + Ga_{4+7} (Promalin, Typey, Perlan) at maximum labeled rates.

1.C. 2016 planting of three small replicated rootstock trials alongside the 2015 trials to evaluate the 5th MSU cherry rootstock

The objective of this trial is to evaluate the fifth cherry rootstock, Crawford, in comparison with the most similar rootstocks, Clinton and Gi5. Crawford was selected for advancement a year later

because of good performance at the Prosser plot with 'Bing' scion. Due to a delay in liner production, this plot will be planted in 2017 instead of 2016.

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

Nine commercial liner and/or finished tree nurseries have virus-certified plant material to produce a limited number of liners of all five MSU rootstocks Cass, Clare, Clinton, Crawford and Lake (Cameron Nursery, Copenhaven Farms, Duarte Nursery, North American Plants, Protree Nursery, Sierra Gold Nursery, Teak Nursery, Willamette Nursery, and Willow Drive Nursery). Collectively these nurseries are using both vegetative and tissue culture procedures to produce liners of the MSU rootstocks. The ease (or difficulty) of liner production at these nurseries will continue to be assessed through visits of A. Iezzoni to these nurseries.

The suitability of the five MSU rootstocks to make finished trees will continue to be assessed using liners and trees produced by Protree Nursery. Liners of the five MSU cherry rootstocks along with the Gi5 control are scheduled to be field planted early in 2016 at this California nursery to enable spring budding. Stand counts and visual observations of liner vigor and suitability for budding will be determined. Extra liners will be budded to enable the assessment of bud take as this is a critical factor affecting nursery profitability, tree cost and availability. Spring budded trees will be ready for planting in 2017. To achieve this objective, A. Iezzoni will visit Protree two times in 2016. The first trip will be made prior to budding to get tissue to enable the verification of liner identity using DNA tests, and the second trip will be to take counts of bud take and assess tree growth.

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

All five MSU cherry rootstocks have been virus certified by the CPCNW-FT and stock plants are being maintained for any future distributions. The main activity for this objective is to insure that the rootstock materials at the collaborating nurseries are true to type. DNA fingerprinting will be done in the Iezzoni laboratory at MSU to verify correct clonal identity of the MSU rootstocks that are being propagated and budded at liner and finished tree nurseries, respectively.

RESULTS AND DISCUSSION:

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

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 1). Of the 22 scion/rootstock combinations needed for the 2015 planting (excluding pollinators), only four combinations had tree numbers less than the 60 needed ('Regina'/Cass, 'Early Robin'/Clare, 'Sweetheart'/Lake, and 'Sweetheart'/Gi5). 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 1).

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

Scion cultivars	Regina, Early Robin, Sweetheart
MSU rootstocks	Cass, Clare, Clinton and Lake
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	All trees were headed to establish a bush 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[a]
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] Wires 2.3 (0.7m) apart vertically

Tree growth and survival: At planting the 'Early Robin' trees on the 7 different rootstocks had approximately the same mean TCSAs (Table 2). However, after the first year of growth the 'Early Robin' trees on Cass and Clare had significantly smaller mean TCSAs compared to the other five rootstocks (Fig. 1). 'Early Robin'/K6 tended to have the highest percentage increase in TCSA which is consistent with K6 being a vigorous rootstock compared to the other six rootstocks (data not presented). For 'Early Robin' the highest tree death was on Cass followed by Gi5 (Table 2).

At planting the 'Regina' trees on the four MSU rootstocks were significantly smaller (measured as TCSA) to the trees on K6, Gi6 and Gi5 (Table 2). This significant difference was still evident at the end of the first growing season with Cass trees significantly smaller than those on Clinton (Fig. 1). The 'Regina' trees on the four MSU rootstocks exhibited on average higher percentage increases in TCSA compared to the 'Regina' trees on Gi5 and Gi6 (data not presented). For 'Regina', the highest tree death was on Cass and Clinton (Table 2).

The 'Sweetheart' trees on Cass, Clare and Lake were significantly smaller than those on Clinton and the three control rootstocks (K5, Gi6 and Gi5) (Fig. 1). The 'Sweetheart' trees on the four MSU rootstocks exhibited percentage increases in TCSA that in general were higher than that exhibited on Gi5 (data not presented). For 'Sweetheart', the highest tree death was on Cass and Clinton followed by Gi6 (Table 2).

In summary, across all three scions and locations, the tree sizes on the MSU rootstocks are similar to our expectations: Cass and Clare rootstocks produce the smallest trees followed by Lake and then Clinton. On average the trees on the MSU rootstocks are significantly smaller than those on the Gisela or Krymsk rootstocks. The poorest tree survival of trees on Cass and Clinton suggests that these trees are 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, as the trees planted in 2015 were very small compared to trees on the other rootstocks.

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

Liners of the MSU cherry rootstocks, Crawford and Clinton, plus the control Gi5, are currently growing at ProTree Nursery. DNA tests of a rootstock sample determined that the liners are labeled correctly (Obj. 3). These rootstocks will be planted at a nursery in the Sacramento area and budded in spring 2016. 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.

Based on the results from the 2015 planting, 60 additional trees of 'Regina'/Cass were added to the 2017 planting.

Table 2. Average TCSA at planting, number and percentage of dead trees for each scion rootstock combination across all three planting locations.

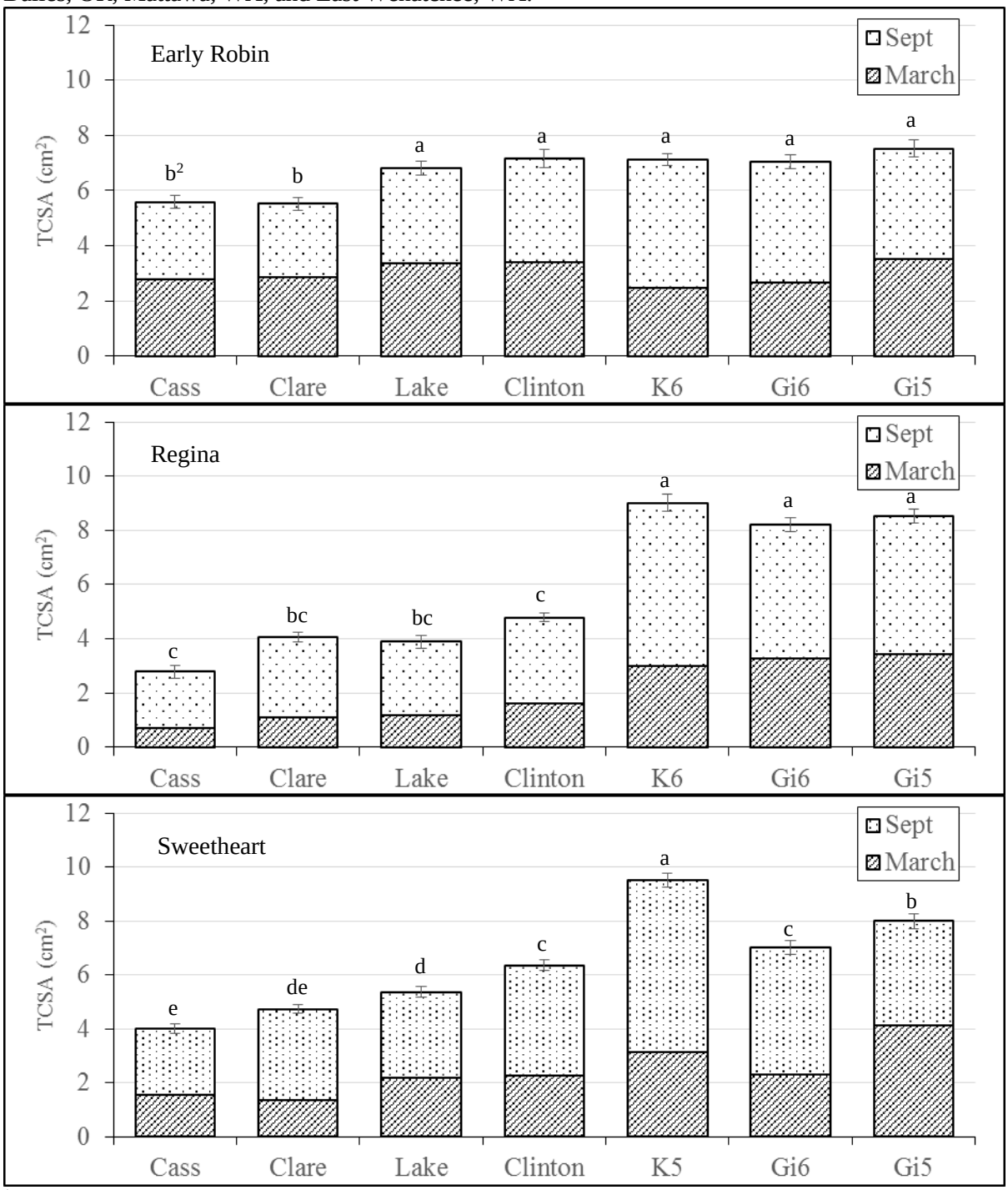
Rootstock	Average TCSA at planting (cm^2)	Number of dead trees (by location 1, 2, and 3)	Percentage of dead trees
Early Robin/Cass	2.7	6 (6, 0, 0)	10%
Early Robin/Clare	2.9	2 (2, 0, 0)	4%
Early Robin/Clinton	3.4	1 (1, 0, 0)	2%
Early Robin/Lake	3.3	3 (2, 0, 1)	5%
Early Robin/Krymsk 6	2.5	2 (2, 0, 0)	3%
Early Robin/Gisela 6	2.6	2 (1, 1, 0)	4%
Early Robin/Gisela 5	3.5	4 (2, 0, 2)	7%
Regina/Cass	0.7	14 (12, 2, NA)	35%
Regina/Clare	1.1	8 (8, 0, 0)	13%
Regina/Clinton	1.6	13 (10, 2, 1)	22%
Regina/Lake	1.2	8 (8, 0, 0)	14%
Regina/Krymsk 6	3.0	3 (3, 0, 0)	5%
Regina/Gisela 6	3.3	1 (1, 0, 0)	2%
Regina/Gisela 5	3.4	1 (1, 0, 0)	2%
Sweetheart/Cass	1.6	4 (4, 0, 0)	7%
Sweetheart/Clare	1.4	2 (2, 0, 0)	3%
Sweetheart/Clinton	2.3	4 (4, 0, 0)	7%
Sweetheart/Lake	2.2	2 (1, 0, 1)	4%
Sweetheart/Krymsk 5	3.1	1 (1, 0, 0)	2%
Sweetheart/Gisela 6	2.3	3 (3, 0, 0)	5%
Sweetheart/Gisela 5	4.1	1 (1, 0, 0)	2%

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: Eight commercial nurseries have all five MSU cherry rootstocks that originated from virus certified materials from the CPCNW-FT. These liner nurseries are gaining experience propagating these rootstocks. To date, liner production appears to be most efficient using tissue culture. Technique to propagate from softwood cuttings is in development as an alternative to tissue culture. Also a layer bed is being attempted. Since the rootstock materials established at the nurseries originated from the 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: Six hundred liners of four of the MSU rootstocks (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%.

Fig. 1. 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, Mattawa, WA, and East Wenatchee, WA.



[1]The lower boxes represents TCSA at planting and the upper boxes represent growth over one season. Bars represent standard error of the means for September TCSA

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

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

All five MSU rootstocks are virus certified and plants are being maintained at the CPCNW-FT. Therefore, the main thrust of this objective was to assure that the genetic identities of the five MSU rootstocks are correct at key points in propagation and distribution. The MSU rootstocks that will be used to make the trees for the 2017 planting were subjected to DNA testing and the rootstock identity was confirmed.

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

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

PI: Todd Einhorn
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Telephone: 541-386-2030 ext.216
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Address: 3005 Experiment Station Dr.
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Co-PI (2): David Gibeaut
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City/State/Zip: Hood River, OR 97031

Co-PI (3): Lynn Long
Organization: OSU-Wasco County Extension
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Email: lynn.long@oregonstate.edu
Address: 400 E. Scenic Drive, Suite 2.278
City/State/Zip: The Dalles, OR 97058

Cooperators: Matthew Whiting

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

Other funding sources: None

Budget 1-Einhorn
Organization Name: OSU-MCAREC **Contract Administrator: Russ Karow**
Telephone: 541 737-4866 **Email address: Russell.Karow@oregonstate.edu**

Item	2015	2016	
Salaries	30,544	31,460	
Benefits	19,276	19,557	
Wages	1,300	1,300	
Benefits	130	130	
Supplies	1,000	1,000	
Travel	850	850	
Total	53,100	**54,297**	

Footnotes: Salaries for 0.66 FTE postdoc (3% is added to year 2); benefits were calculated based on Actuals; wages are for 100 hours part-time summer employee for image analysis of cherry fruit ($13/hr); benefits for part-time (10%); supplies include, some PGRs, Ziploc plastic bags, flagging and lab tape for limb and fruit selection; travel includes 1,500 miles estimated for all sample collections and growth rate analyses at $0.565 per mile.

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

Item	2015	2016	
Wages	4,550	4,550	
Benefits	455	455	
Travel	283	283	
Total	5,288	**5,288**	

Footnotes: Wages are for 350 hours of part-time summer employee for fruit sample collection ($13/hr); benefits for part-time (10%); travel includes 500 miles estimated for all sample collections for fruit set estimates and growth rate analyses at $0.565 per mile.

Objectives:

1) Large-scale pre-bloom PGR trials to enhance size

2) Create a novel temperature-dependent model to predict phenology and fruit development of sweet cherry

3) Accurately reproduce the color and percent of full size cherry for a decision aid tool

Significant Findings:

Objective 1

1) Pre-bloom application of Promalin in a Tri-cities orchard showed little to no enhancement of size in 'Early Robin', 'Chelan', 'Bing' and 'Rainier'
2) Pre-bloom application of Gibberellin containing mixtures at MCAREC showed slight enhancement of size, about 0.5 gram in 'Regina'

Objective2

3) Temperature dependent model of sweet cherry development based on five years of 'Sweetheart' 13 separate trials, one in 2011, three in 2013, five in 2014 and four in 2015
4) Growth response curves prior to bloom were based on experiments comparing explants in controlled environment chambers to trees in the orchard
5) The model incorporates unique growth response curves with dynamic adjustment of temperature min, max and optima to account for changing physiology during the season
6) Modelling begins 1-January
7) Harvest was modelled to within +/- 1.5 days of the average

Objective 3

8) Images of sweet cherry calibrated for size and color
9) Size estimates analyzed by calculation of asymptotic growth
10) Average red in RGB color intensifies rapidly at 90% of full size
11) 99% of full size was attained before the average harvest date

Objective 1- PGRs

Three growers hosted non-crop destruct test trials. Fruit size, set and yield at harvest were measured at one location 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 is 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, justify 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

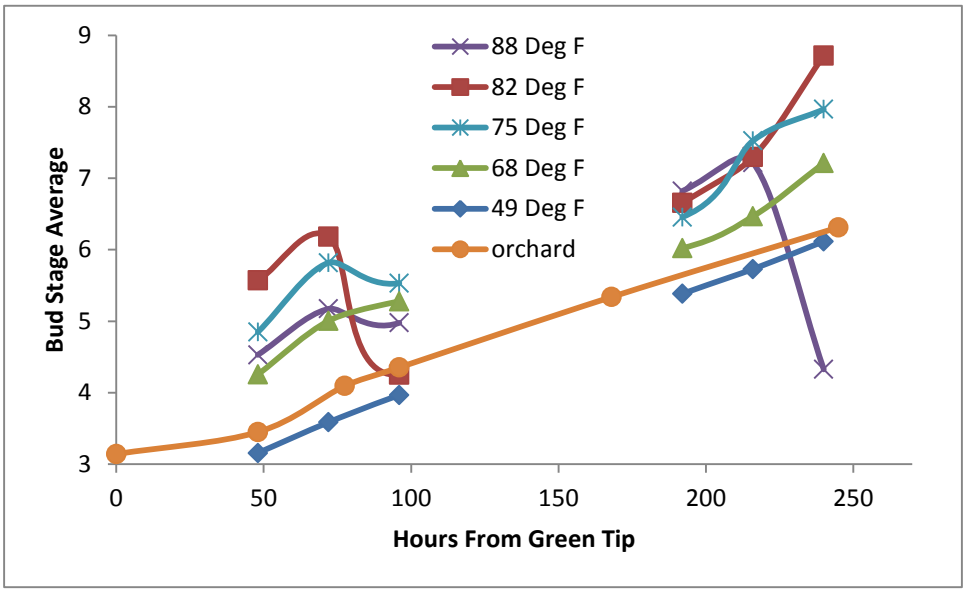
limbs and spurs	Count	Mean	Groups
Control	1280	9.36	a
Novagib, 125 ppm	1045	9.49	ab
Promalin, 125 ppm	1257	9.60	bc
NAA, 250 ppm	591	9.64	bce
Novagib, 62 ppm	1066	9.73	ced
Novagib, 31 ppm	944	9.76	ed
CPPU/Novagib, 20/125 ppm	630	9.92	d

Objective 2- Growth Models

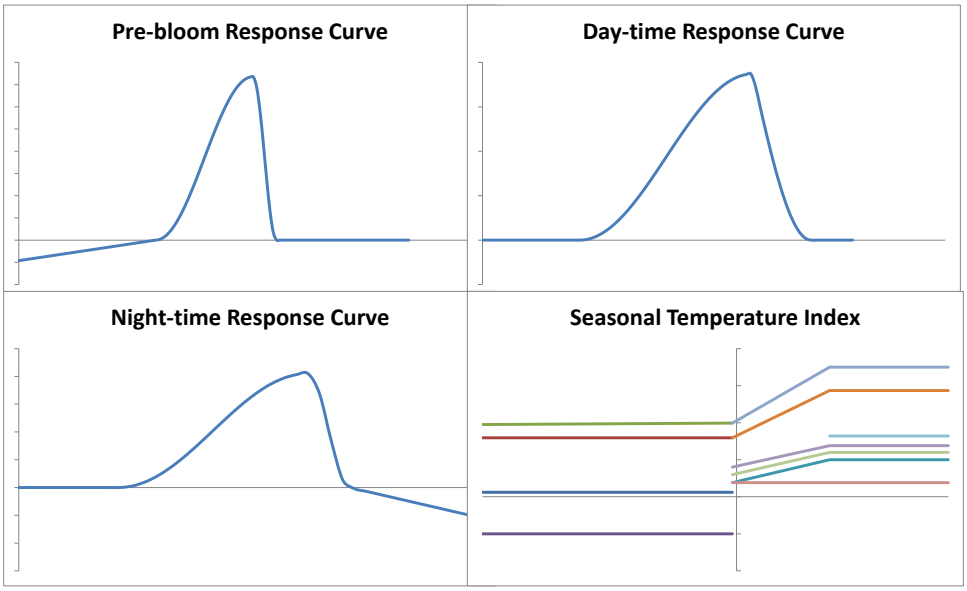
Developmental Response to Temperature is Phenology Dependent

Experimental manipulation of bloom phenology in temperature controlled environment chambers was conducted in two trials of Regina at MCAREC. These were designed similar to augment our previous year of dormancy-break temperature experiments. Seventy-five sections of 2-3 year fruiting wood with good spur development were selected from the orchard and placed in water at five different temperatures up to four days duration. The first trial began with material collected at green tip and analyzed at 48, 72 and 96 hours as well as equivalent material from the orchard. A second trial began

at first white and was analyzed at 24, 48 and 72 hours. The different durations were necessary because the later experimental material was more responsive to temperature. Forcing this material to develop in comparison to the orchard helped establish a sliding scale of temperature indices needed in a temperature dependent model.



Bud stage averages were; 3= green tip, 4=tight cluster, 5=open cluster, 6=first white, 7=balloon, 8=bloom, 9=post bloom. The average phenology in the orchard at the beginning of the first trial was green tip, and the second was first white. Development at a constant 49 Deg F in the controlled environment chambers was close to the natural progression in the orchard. Slightly higher temperatures accelerated development. Upper critical limits of development at the highest temperatures were seen as declines in development. These observations informed the creation of the novel temperature dependent curves shown below.



The developmental response to temperature was described by trigonometric equations describing pre-bloom phenology, day-time photosynthesis and night-time respiration. Seasonal temperature indices describe the increasing base, optimal, and critical temperatures. These equations are

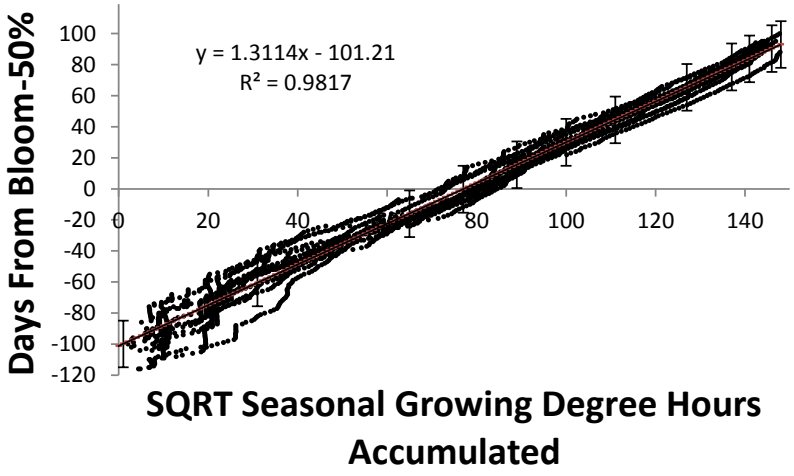
integrated over time and the observed temperatures from IFPnet weather stations as shown below. Negative values were included for pre-bloom acclimation to freezing temperatures and high temperature inhibition of development during maturation due to excessive respiration at high temperature.

GEDAVS

A simulation model with variables for annual and diurnal shifts of temperature indices: Gibeaut and Einhorn Diurnal Annual Variable Simulation (GEDAVS) begins the first of January and describes a classic exponential function of plant growth. Input variables included bloom, mid-growth and harvest dates each with temperature indices. Temperature dependent difference on the predicted average harvest date of Sweetheart over several years and locations was optimized to +/- 1.5 days. The 95% prediction limits fully contained the observations beginning well before bloom. Cultivar differences are also being evaluated. We have previously demonstrated that growth behavior is remarkably similar among varieties. There is no reason to suspect that different temperature indices will be required other than our additional observations of phenology in the coming year.

To date the model was developed from the following data collected over 13 Trials & 4 Years:

- 2011 Skyline Water Tank
- 2013 Anderson, Euwer, Sterr
- 2014 Anderson, Coppers, Euwer, Sterr
- 2015 Cherry Mtn North, Skyline Water Tank , Sweetheart East and West
- Response Accumulation begins 1 January
- Average Predicted +/- 1.5 days
- 95% Prediction Limits +/- 15 days



The square root of growing degree hours accumulated linearizes the data for ease of prediction.

We will run the simulation next season for as many weather stations as possible to further verify the model before general release. Accurate 50%-bloom dates and harvest dates are essential and will be evaluated at multiple sites.

- Grower contribution to bloom- and harvest dates will be sought.

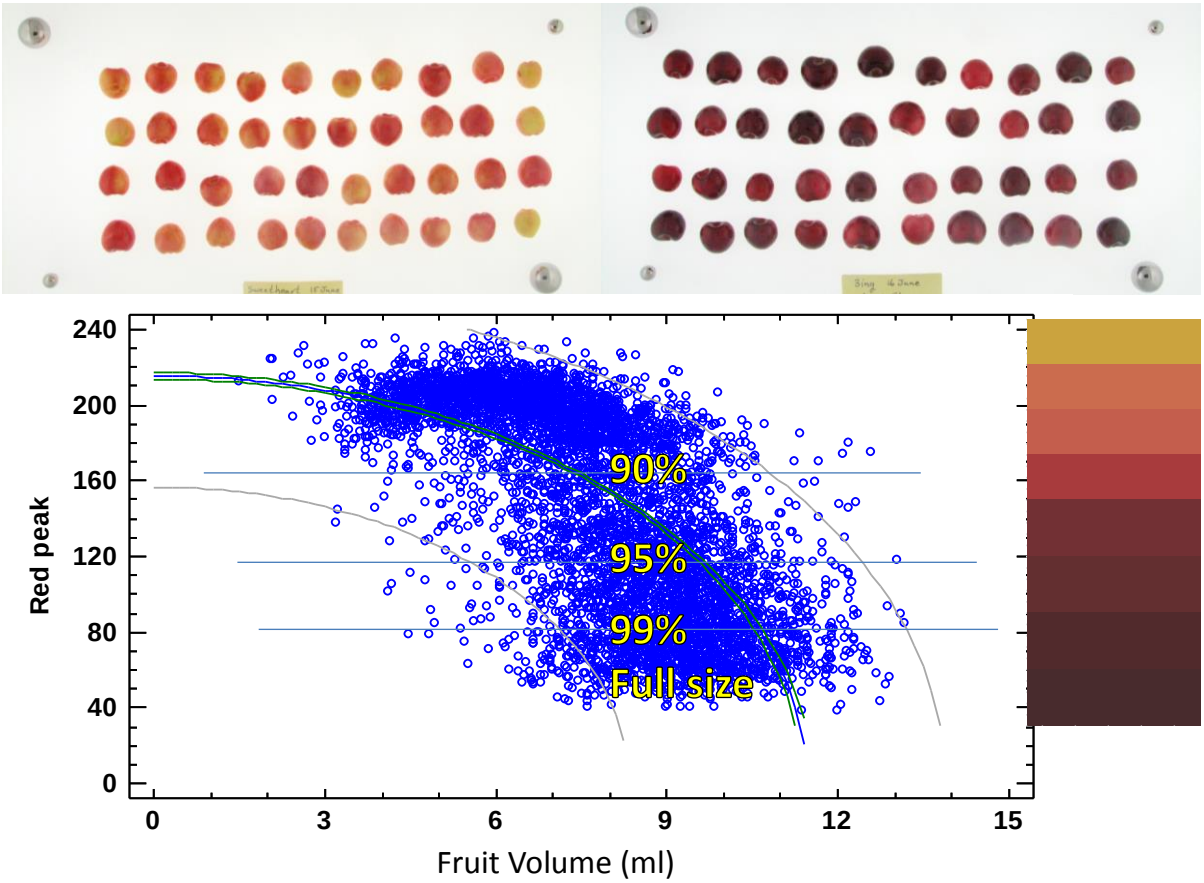
Objective 3- Create a decision aid tool for color and size

The first year of an index of skin color and fruit size for cherry in the PNW focused on Sweetheart, Bing and Regina.

The Sweetheart data (shown below) relates the intensifying red color in RGB color space to growth at 90, 95, 99% and full size. For each variety examined growth was related to color so an accurate assessment of color is also an assessment of how much additional fruit growth can be expected.

Determination of Size and Color

- Spatial Calibration with gauge balls
- White balance with infinity background
- Color balanced fluorescent and LED lighting
- F-stop 5.4, exp 1/800 sec
- 150-300 fruits per cultivar, site, date combinations



Red Value (RGB) vs. Fruit Size during Final Swell provides a relationship between the average red value at 90, 95, and 99% of full- size and shows that color development intensifies rapidly for all sizes of fruit once 90% of fruit growth has been attained.

CONTNUNING PROJECT REPORT **YEAR**: 2 of 3 years

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

PI: Yan Wang
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Co-PI: Todd Einhorn
Organization: MCAREC
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Email: todd.einhorn@oregonstate.edu

Cooperators: Lynn Long, Xingbin Xie

Total Project Request: **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

In this report, shipping quality is defined as fruit firmness (FF), pitting, splitting, flavor, pedicel browning, and decay of sweet cherries after 3 and 5 weeks at 32°F. The goal of this proposed project is to improve shipping quality of the PNW sweet cherry cultivars through pre-harvest calcium (Ca) or salt (NaCl) sprays.

The key objectives are to:

1. Define the relationship between tissue Ca and N contents with cherry shipping quality.
2. Develop Ca spray protocols to improve cherry shipping quality.
3. Determine the effect of NaCl sprays on shipping quality of cherries.
4. Determine the response of fruit growth, fruit size, yield, and return bloom to Ca/NaCl sprays.

SIGNIFICANT FINDINGS

Fruit tissue Ca/N 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 concentration (~300-600ppm in 2014; ~400-800ppm in 2015), but not N concentration (0.9-1.1%).

Ca sprays

2. The optimum Ca spray rate was ~0.1% Ca^{2+}. Higher Ca concentration (i.e., >0.15%) might cause burning or reduce fruit size. Sprays with lower Ca^{2+} (i.e., 0.05%) didn't increase fruit tissue Ca concentration.
3. Multiple applications, such as 6 times at weekly interval from pit-hardening to harvest, were needed to increase fruit tissue Ca content.
4. When sprayed 6 times at weekly interval at the same Ca^{2+} rate of 0.1%, all the Ca sources [$CaCl_2$, $Ca(NO_3)_2$, Ca citrate, $Ca(OH)_2$+organic acid (OA), and Chelate Ca] increased fruit tissue Ca concentration with little difference among the Ca sources.
5. Fruit size and fruit growth rate were not influenced by the Ca sprays.
6. Ca sprays at 0.1% for 6 times tended to increase SSC and TA without affecting coloration.
7. Ca sprays at 0.1% for 6 times increased fruit firmness, reduced pitting, splitting, stem browning, and decay after storage/shipping.
8. None of the Ca treatments applied in 2014 affected 2015 return bloom (buds per spur or flowers per bud) or fruit set of 2 and 3-year-old spur populations.

NaCl sprays

9. NaCl sprays 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 not Cl^-, in 'Lapins' and 'Regina'.
10. NaCl sprays at 60 and 120 ppm increased FF, SSC, and TA in 'Lapins' but not 'Regina' ($p < 0.05$), at harvest and during storage.
11. NaCl sprays at 120ppm but not 60ppm reduced fruit size in both 'Lapins' and 'Regina'. NaCl sprays enhanced fruit color by increasing anthocyanin accumulation in both cultivars.

METHODS

1. Effect of tissue Ca and N contents on shipping quality. Fruit of different cultivars was randomly sampled from different orchards. Ca and N contents and concentrations were determined and fruit quality were recorded at harvest and determined after 3 and 5 weeks of storage.

2. Ca and salt treatments.

Ca Treatments	***% Ca***	***Application timing***
$Ca(NO_3)_2$ (0.4%)	0.10	9x, beginning 1wafb
$Ca(NO_3)_2$ (0.6%)	0.15	9x, beginning 1wafb
$CaCl_2$ (0.25%)	0.10	6x, beginning pit hardening
$CaCl_2$ (0.4%)	0.15	6x, beginning pit hardening
$Ca(NO_3)_2$ (0.4%)	0.10	6x, beginning pit hardening
$Ca(NO_3)_2$ (0.6%)	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
Chelate Ca (Metalosate®)	0.10	6x, beginning pit hardening
Chelate Ca (Metalosate®)	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 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); and, three-year-old spurs (representing spurs that yielded fruit in 2014).

RESULTS

1. Fruit tissue Ca & N concentrations and shipping quality

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

2. Ca sprays in year-2

a. Effects of application rate, frequency and sources on fruit tissue Ca content

Applied 6 times at weekly interval between pit-hardening and harvest, $Ca(NO_3)_2$ at 0.1% Ca increased fruit tissue Ca concentration significantly ($p < 0.05$) in 'Lapins' and 'Skeena' fruit at the time of harvest. $Ca(NO_3)_2$ at 0.05% Ca did not increase fruit tissue Ca content than control fruit. $Ca(NO_3)_2$ at 0.15% Ca did not improve fruit tissue Ca content compared to that at 0.1% Ca. Therefore, no additional benefits were observed at higher $Ca(NO_3)_2$ rate than 0.1% Ca. Increasing spray frequency of $Ca(NO_3)_2$ at 0.1% Ca from 6 to 9 times at weekly interval between full bloom and harvest did not

improve cherry fruit Ca uptake significantly ($p < 0.05$). Ca at 0.1% sprayed twice (pit-hardening + one-week before harvest or two-week + one-week before harvest) did not increase fruit tissue Ca concentration of cherry fruit at harvest compared to control fruit. All the Ca sources tested at rate of 0.1% Ca plus frequency of 6x increased fruit tissue Ca content at similar efficacy (Fig. 1).

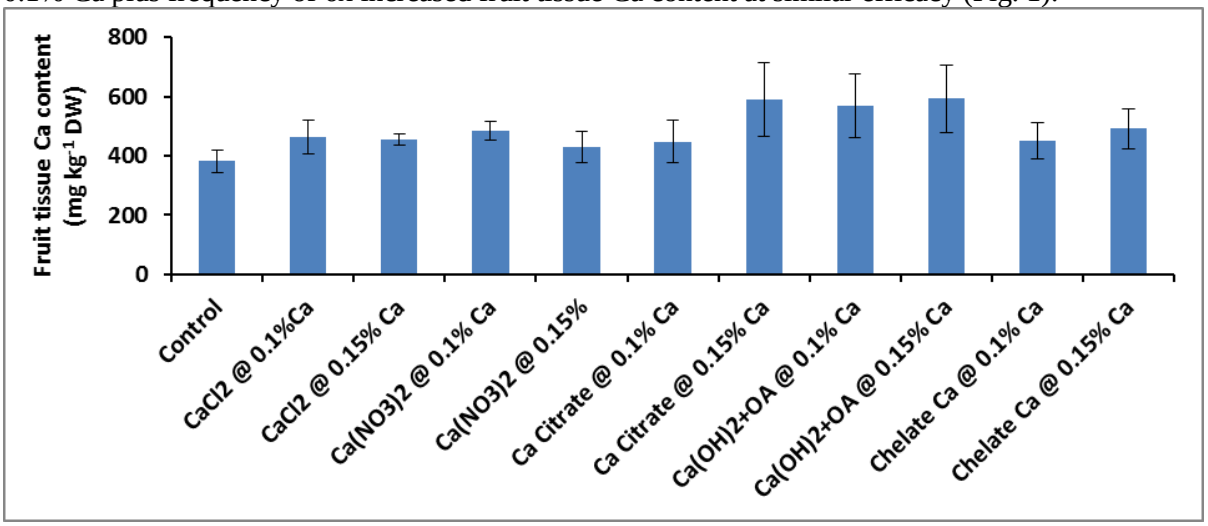

Fig. 1. Effect of Ca sprays on fruit tissue Ca content in 'Lapins' at the time of harvest.

b. Effect of Ca sprays on fruit quality at harvest

All the Ca sources at 0.1% 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. Fruit size and fruit growth rate (fruit growth rate data not shown) were unaffected by the Ca treatments at $p < 0.05$. The fact that fruit growth was adversely impacted by a few Ca treatments in 2014 but not 2015 might be attributed to the relatively light crop load in 2015. Ca sprays tended to increase SSC and TA (Fig. 2).

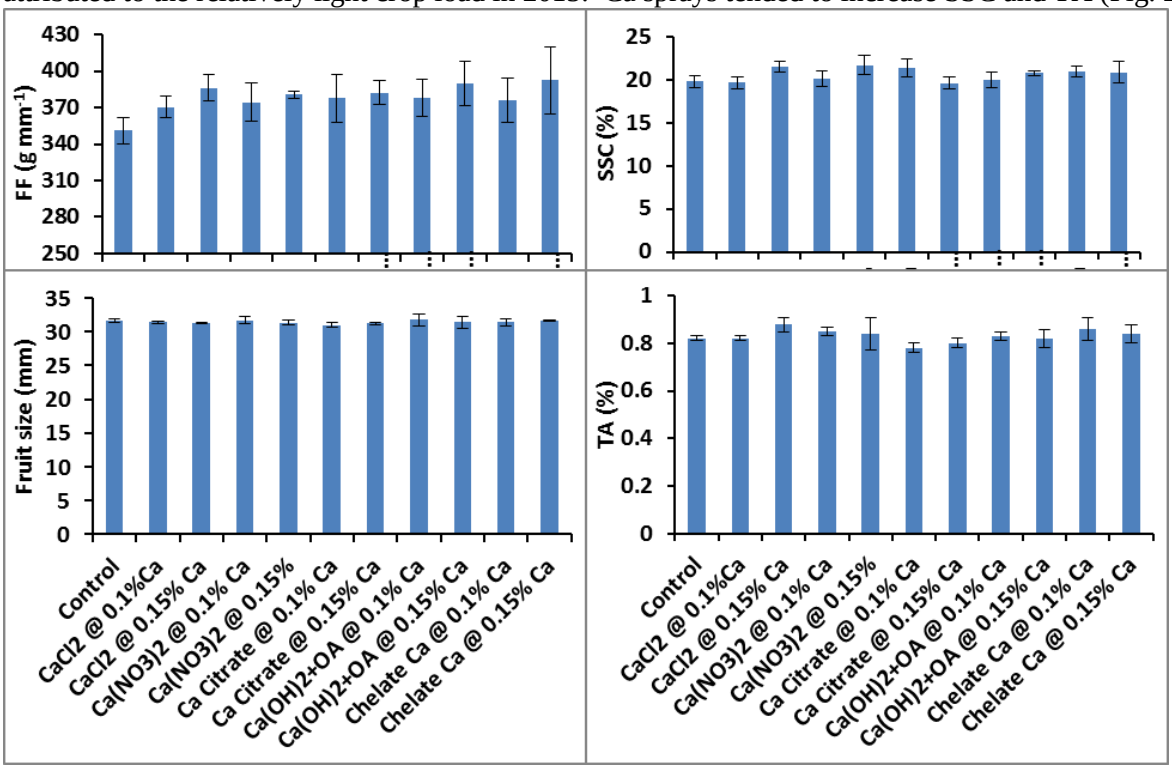

Fig. 2. Effect of Ca sprays on fruit firmness (FF), fruit size, soluble solid content (SSC), and titratable acidity (TA) of 'Lapins' at the time of harvest.

c. Effect of Ca sprays on shipping quality of sweet cherries after three-week of cold storage

For 'Lapins', all the Ca sources at 0.1% Ca for 6x applications reduced pitting, decay, and stem browning incidences and retarded fruit skin darkening during three-week 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 for 6x applications on shipping quality of 'Lapins' and 'Skeena' after three-week 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
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

d. Effect of Ca sprays on horticultural performance of Lapins

Return bloom was not affected by 2014 Ca sprays when 2 and 3-year-old spurs were evaluated. 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).

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

a. Effect of NaCl sprays on fruit tissue Na^+ and Cl^- contents

NaCl sprays increased fruit tissue Na^+ content with a dose response, but did not affect fruit tissue Cl^- contents in 'Lapins' and 'Regina' (Fig. 3).

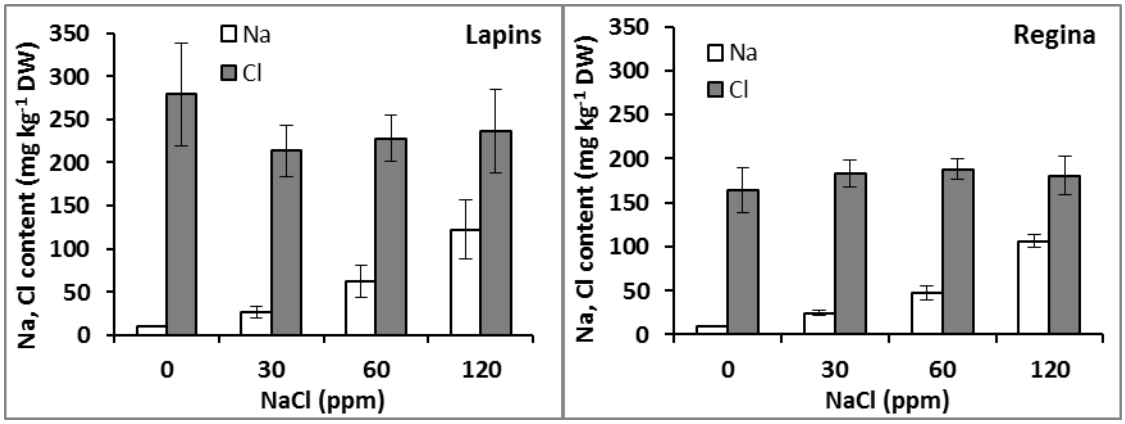

Fig. 3. Effect of NaCl sprays on fruit tissue Na^+ and Cl^- contents in 'Lapins' and 'Skeena'.

b. Effect of NaCl sprays on fruit quality at harvest

NaCl sprays at 30 and 60 ppm did not affect fruit size, but higher rate at 120ppm reduced fruit size in 'Lapins' and 'Regina' (Fig. 4). 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) of 'Lapins' and 'Skeena'.

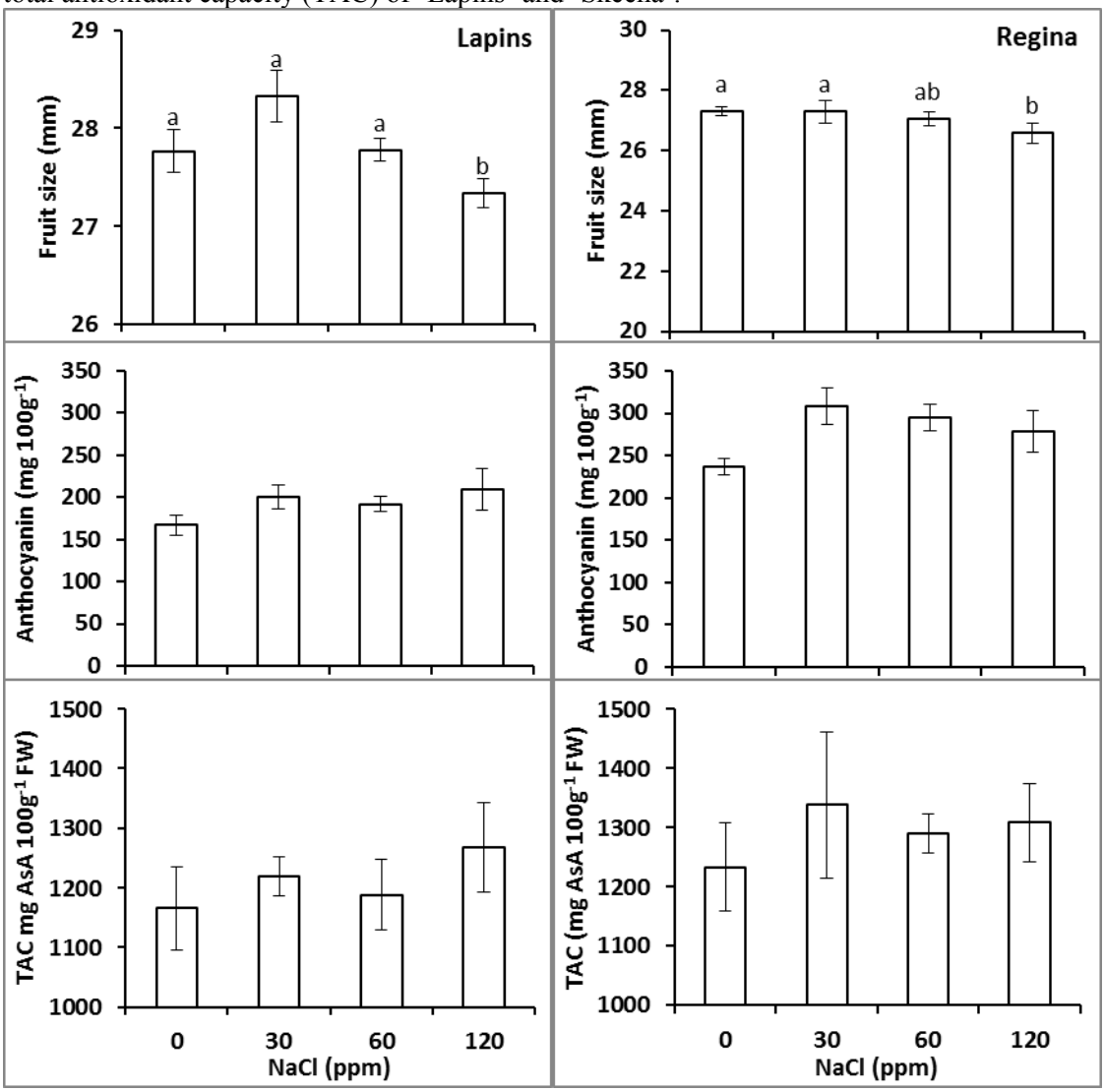

Fig. 4. Effect of NaCl sprays on fruit size, anthocyanin content, and total antioxidant capacity (TAC) of 'Lapins' and 'Skeena'.

c. Effect of NaCl sprays on fruit quality at harvest and during storage

NaCl sprays at 60 and 120 ppm increased FF, SSC, and TA at harvest and during three-week storage in 'Lapins', but not 'Regina' (Fig. 5)

d. Effect of NaCl sprays on stem quality after storage

NaCl sprays at 30, 60, and 120 ppm improved stem quality by increasing green stem incidence in both 'Lapins' and 'Skeena' after three-week storage (Fig. 6). The NaCl sprays maintained higher moisture content in stems of both cultivars after storage (Fig. 6). The natural cuticle wax content in NaCl treated stems was higher with a dose response than the control fruit (data not shown here).

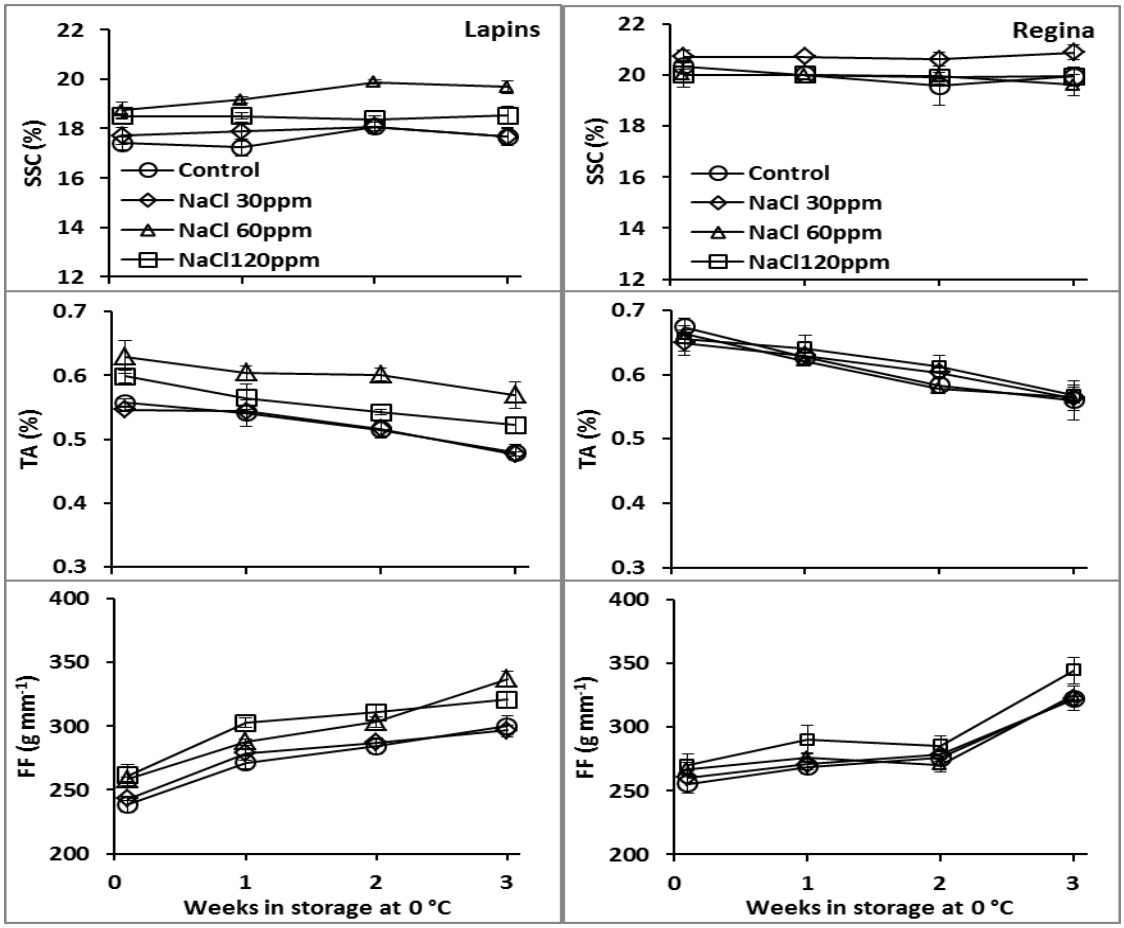

Fig. 5. Effect of NaCl sprays on soluble solid content (SSC), titratable acidity (TA), and fruit firmness (FF) at harvest and during three-week storage at 32°F.

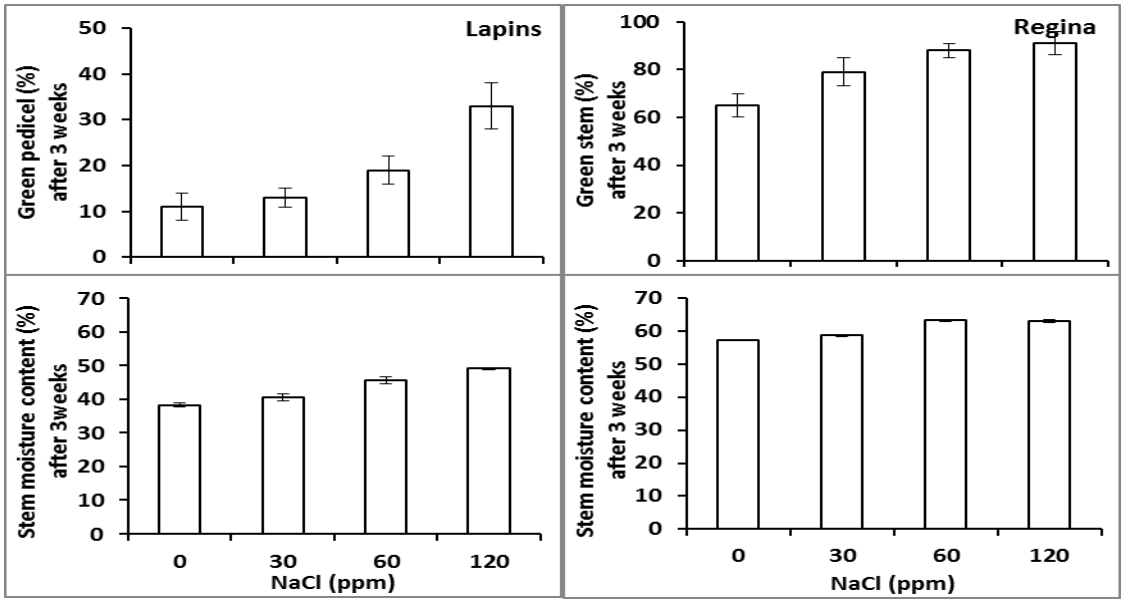

Fig. 6. Effect of NaCl sprays on pedicel quality of 'Lapins' and 'Regina' after three-week storage at 32°F.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number: 15-101

Project Title: PM viability during postharvest handling of cherry fruit

PI: Gary Grove
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City/State/Zip: Prosser, WA, 99350

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: WSU-IAREC **Contract Administrator:** Lisa Bruce Carrie Johnston
Telephone: 509-786-2226 **Email address:** lisa-bruce@wsu.edu carriej@wsu.edu

Item	2015	2016	
Salaries[1]	$ 34,620	$ 36,005	
Benefits	$ 14,887	$ 15,482	
Wages			
Benefits			
Equipment[2]	$ 5000		
Supplies[3]	$ 6500	$ 5000	
Travel[4]	$ 1000	$1000	
Miscellaneous [5]	$ 500	$ 500	
Plot Fees			
Total	$ 62,507	$ 57,987	

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

Objectives

1. Development and validation of a robust viability assay using propidium monoazide (PMA) in conjunction with quantitative PCR to distinguish between viable and non-viable inoculum 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 qPCR assay for detection of *Podosphaera clandestina* has been developed
- Fungal viability is influenced by post-harvest measures
- So far, low levels of spore viability were observed during cold storage, fumigation and immersion in water
- All results are preliminary, and will be re-tested more intensively in 2016

Methods by Objective

Objective 1

A protocol to quantify *P. clandestina* viability has been established. Evaluated parameters included spore retention using polycarbonate membrane filters (0.22 and 0.45 micron pore size), efficacy of vacuum filter apparatus, maximum number of cherries per wash and filtration volume, shaking time and speed to dislodge spores from cherry surfaces, propidium monoazide (PMA) dye concentration (0.5µl to 1µ per 500µl filtrate), and PMA incubation time (10 to 20 minutes).

Step 1: Fruit sampling and washes. Powdery mildew infected cherries were obtained from the experimental orchard at WSU, Prosser. Cherries were sorted into categories relating to degree of infection (0 = no infection, 1 = 1-33% surface area infected, 2= 34-66% surface area infected, 3 = more than 66% infected). Forty arbitrarily chosen cherries per category were submerged in 500ml sterile water (Pyrex bottles) containing 0.001% Tween 20 and immediately placed on a rotary shaker at 300rpm for 5 to 10 minutes. The resulting wash solution was split in two equal parts of 250ml each.

Step 2: Filtration and PMA treatment. Each 250ml was filtered through a polycarbonate membrane (one membrane per filtrate) using vacuum assisted filtration. Filters retained spores contained within the wash solution. The first filter (filter 1) was placed in a bead tube for DNA extraction. The second filter was submerged in a 1.5ml eppendorf tube containing sterile water (500µl), treated with PMA, and incubated in the dark for 10 to 20 minutes. After incubation, tubes were transferred to the PMA-Lite machine to undergo the LED-light exposure for 20 minutes. The light treatment covalently binds PMA to available DNA, such as free DNA and DNA from cells with damaged cell membranes (non-viable cells). After treatment, the filter (filter 2) was transferred to a bead tube for DNA extraction.

Step 3: DNA extraction and qPCR assays. DNA was extracted from filter 1 and filter 2 to quantify the total number of spores (filter 1) and the number of living spores (filter 2). Quantitative real-time PCR (qPCR) assays were performed on a LightCycler 480 system (Roche, Indianapolis, IN) using 96-well plates in a total reaction volume of 20 µl. Each reaction consisted of: 10 µl of DyNAmo HS SYBR Green 2X supermix (New England Biolabs, Ipswich, MA), 1 µl of each primer at 400 nM final concentration, 6 µl of PCR grade water and 2 µl of template DNA solution. QPCR was carried out according to the following protocol: Pre-Incubation at 95°C for 15 min; 45 PCR cycles at 94°C for 10 s, 63°C (primer specific temperature) for 20 s, 72°C for 30 s and 81°C for 3 s with fluorescent data collection; and a cooling period of 10 sec at 40°C. Melting curve analysis of the PCR products was

conducted following each assay to confirm that the fluorescence signal originated from specific PCR products and not from primer-dimers or other artifacts. At least four standards were prepared using a 10-fold dilution series of DNA for each fungal isolate. DNA concentrations used for constructing the standard curve ranged from 1.0 to 15,000 pg for *P. clandestina*. PCR assays were conducted three times and the pooled data was used in regression analyses to derive standard curves. All samples, including standards, positive and negative controls, were tested in duplicates and results were recorded only if no signal was detected from the negative control. DNA samples of negative amplification were tested twice for confirmation. For the interpretation of SYBR®Green qPCR assays, two criteria were taken into consideration: the quantification cycle (Cq) value, and the melting temperature of the amplicon (Tm). The Cq-value represents the cycle at which the PCR amplification reaches the threshold level of the reaction. To be considered as positive, a signal generated in SYBR®Green qPCR analysis should display an (exponential) amplification above the threshold level associated with the specific Tm-value of the amplicon.
Standardized samples containing DNA from a known number of spores (1, 10, 100, 500 and 1000 spores) were subjected to the same qPCR protocol as a reference.

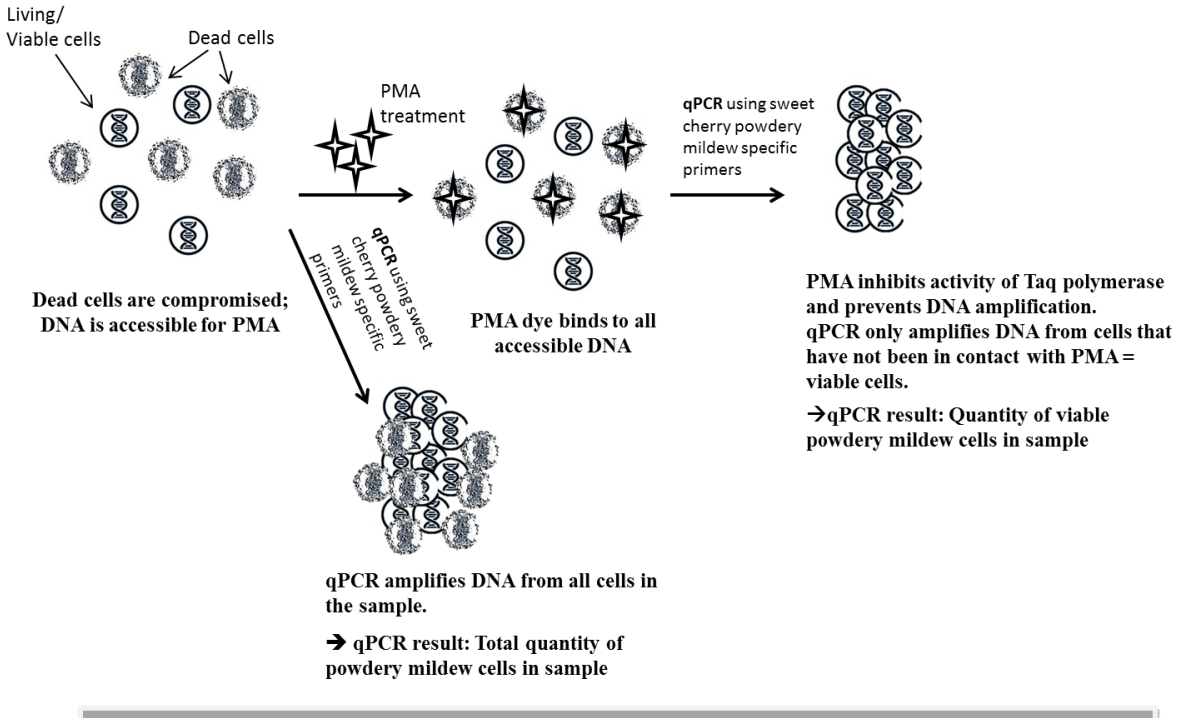

Figure 1 Use of propidium monoazide (PMA) to distinguish between viable and dead cells of sweet cherry powdery mildew

Objective 2

To assess impact of fumigation on powdery mildew viability, diseased fruit (cultivars 'Bing' and 'Sweetheart') were harvested starting in June 2015. Fruit was sorted into classes according to infection: 1 = no visible signs of infection, 2 = 1-33% fruit surface colonized, and 3 $\geq$ 33% colonized. Forty cherries per class and cultivar were selected and subjected to viability testing as described above (number of viable spores before fumigation). Another 6lbs of cherries were arbitrarily picked, packed in 2lbs clamshell containers (3 replicates per class), cooled and immediately transported to Zirkle Fruit in Prosser. Here, the fruit was held at 34F in the dark until fumigation the same night. The fumigated samples were picked up the next morning, transported to the lab and directly subjected to viability testing as described above (number of viable spores after fumigation). Fumigation experiments were conducted four times (June 24, June 29, July 2, July 9, 2015). No more fumigation was conducted at Zirkle Fruit after July 12 2015.

To assess the impact of cold storage on powdery mildew viability, infected cherries were stored at 34F for up to 7 days. A sub-sample (about 40 cherries each) was taken every 24h and total spore numbers and viability was quantified using the qPCR assay. The experiment was repeated three times.

Results & Discussion

Objective 1

Step 1: Fruit washes. To assess the quantity of powdery mildew spores on sweet cherry fruit, spores have to be dislodged from the cherry surface. In general, upon contact with the host surface, spores secrete a liquid exudate that tightly binds them to the fruit surface and protects from being removed by wind and rain. After first attachment, the fungus grows as mycelium and produce feeding structures (haustoria) which are anchored in the epidermal cells of the plant host. Consequently, removing spores/ mycelium from established infection sites requires force. The vigorous shaking at 300rpm of fruit submerged in sterile water (containing Tween20 to increase the even distribution of the spores in the water) removed spores successfully from the fruit surface. Unlike other fungi, water exposure is detrimental for powdery mildew. We tested how water soaking and vigorous shaking for 5, 10, 15 and 20 minutes influences the recovery of viable cells from fruit washes (Figure 2). Viability did decrease exponentially with time; the longer the spores were exposed to water, the fewer viable cells were recovered. Based on this result, a 5 minute time limit for fruit washes was chosen. Cherries were recovered after the washing step and inspected visually and with the dissecting microscope to assess how many spores remained on the fruit surface. No spores were detected on the fruit.

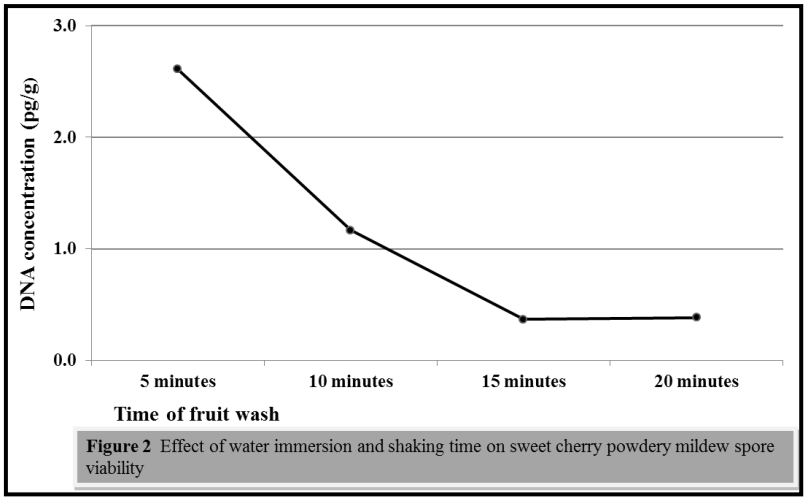

Figure 2 Effect of water immersion and shaking time on sweet cherry powdery mildew spore viability

Step 2: Filtration. The resulting suspension from the fruit wash was split evenly into two parts. Each part was filtered independently through polycarbonate membrane filters (Nucleopore). Optimal filter membrane size is 47 mm in diameter to be able to fit in DNA extraction tubes. Two pore sizes were chosen based on previous studies; 0.22 and 0.45microns. Both pore sizes are sufficiently small enough to retain fungal spores. The tested filtration volume ranged from 100 to 500ml fruit wash

solution per filter. Membrane capacity (all pores become clogged and flow rate approaches 0) for extraction of spores from cherries obtained from tree branches is reached at 100ml (0.22 micron filter) and 200ml (0.45micron filter). Filtration volume is significantly lower for cherries obtained from orchard soil due to larger particles clogging the filter. Since the 0.45 micron filter membrane has the higher filtration capacity, it was chosen over the 0.22 micron membrane.

Step 3: PMA treatment, DNA extraction, and qPCR assays. PMA is a photoactive dye with affinity for DNA (Figure 1). Final dye concentration was 20mM per 500µl sample extract. Ideal incubation time was 10 minutes. No differences in DNA binding was observed with prolonged incubation. Light exposure time was 20 minutes (LED photolysis device). DNA extraction of PMA treated and non-treated filters were conducted with MoBio DNA isolation kits according to manufacturer's instructions. Q-PCR assays for quantification of sweet cherry powdery mildew were established previously. Q-PCR was conducted with species specific primers. Two primer pairs were evaluated, both of which were designed in previous studies. Primer specificity was confirmed with control samples from other powdery mildew isolates (hop, roses, and apple powdery mildew). No amplification was observed for any powdery mildew other than sweet cherry. The qPCR positive and negative controls gave the expected positive and negative results, respectively.

Objective 2

Fumigation. Fumigation has an effect on fungal viability (Table 1, Figure 3). On average, 0.1% of fungal propagules obtained from 80 cherries (cv. Bing) were still viable after fumigation. However, fungal viability before fumigation was low (average = 0.8%) even on class 3 cherries. These results are preliminary. Experiments will be repeated in 2016.

Table 1 Total DNA and % viability *P. clandestina* recovered from orchard sweet cherry samples (n =80) before and after fumigation.

	Before fumigation*		***After fumigation****	
Infection class	**Total DNA(pg/g)**	**% Viable**	**Total DNA(pg/g)**	**% Viable**
1: No visible infection	0.0	0.0	0.2	0.01
2: 1-33% infection	1.6	2.2	0.2	0.3
3: > 34% infection	21.9	0.1	32.2	0.05

* Measurements from two independent samples.

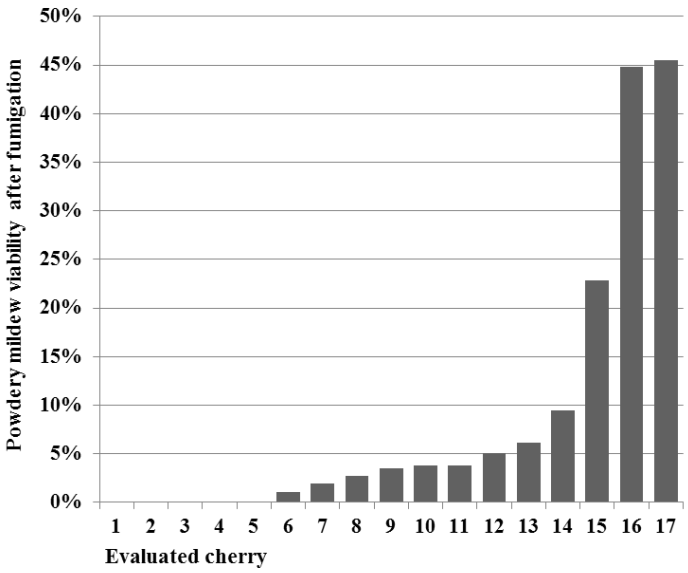

Figure 3 *P. clandestina* viability measured on single cherries directly after fumigation

The variability of the fumigation success is exemplified in Figure 3. Here, fungal viability was measured after fumigation on individual cherries. Viability ranges from 0 to 46% (average = 9% median = 4%). Only 5 out of 17 (29%) had no detectable fungal activity. Placement of the cherry in the container, spore densities, or spore shelter under the pedicel could explain spore protection during fumigation. The effect on few single cherries with highly viable powdery mildew propagules after fumigation on the total viability of the processed bulk sample will be investigated. Viability is a measure of fungal fitness. However, obligate biotrophic fungi need a living host to multiply. Infection of detached cherries, even under ideal environmental conditions, is extremely difficult. In 2016, inoculation experiments will be conducted on fumigated cherries to observe spore germination and multiplication during cold storage and under ideal growth conditions. Preliminary results from a similar study can be found in the next section.

Fungal viability during cold storage. There was no statistically significant decrease of spore viability during cold storage at 34F for up to 3 days (72h). The average viability was 9%. Spore viability dropped to 1.4% after 6 days (144h) in cold storage. The same was found on fumigated cherries. These results are preliminary and warrant further investigation.

Final remarks: Setting up a robust protocol took priority in 2015. Finding and fixing problems (such as observed during filtration steps) are crucial. The protocol is still tested in laboratory experiments. Next season, the protocol will be used to intensively assess fungal survival during post-harvest handling. All steps (e.g. cold storage, fumigation) will be evaluated individually and in sequence. Experiments will be repeated (at least) three times.

2015 WTFRC CHERRY PESTICIDE RESIDUE STUDY

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

400 gallons/acre airblast spray application

TRIAL DETAILS

- Mature 'Bing'/Mazzard multiple leader open vase trees on 10' x 20' spacing near Orondo, WA
- 18 insecticides/acaricides & 11 fungicides applied at or near maximum rates and minimum pre-harvest and re-treatment intervals
- Ground applications made by Rears PakBlast PTO-driven airblast sprayer at 400 gal/acre with Regulaid surfactant
- Measurable rain recorded on 1 day during study: 0.1" 20 days before harvest (DBH)
- Plot split into three equal blocks: 1. Pesticides only 2. Pesticides + 408 oz/acre RainGard (Pace Intl.) at 14 and 7 DBH 3. Pesticides + 128 oz/acre Parka (Cultiva) at 21 and 14 DBH
- Samples submitted overnight to Pacific Agricultural Labs (Portland, OR) for chemical analysis

RESULTS & DISCUSSION

This study simulates a *worst case scenario* for residues of legally applied pesticides with and without protective rain coatings, using very aggressive rates, timings, and spray intervals; the lone exception was buprofezin (Centaur), which was sprayed a week ahead of its labeled preharvest interval to more closely reflect typical industry use patterns. Most materials were applied twice as allowed by product labels, whether or not commercial use patterns would do the same. With that approach, all residues complied comfortably with domestic tolerances; some, however, **exceeded some foreign tolerances,** whether from published MRLs or national default values:

Insectides/acaricides: Assail 70WP, Baythroid XL, Danitol 2.4EC, Perm-Up 3.2EC, Carbaryl 4L, Exirel
Fungicides: TopGuard, Orbit, Topsin 4.5FL, Merivon

These potential violations typically reflect stringent tolerances in certain export markets rather than excessive residues, and cherry producers should routinely monitor changes in posted MRLs (www.nwhort.org) to facilitate compliance with dynamic foreign standards. While fruit from this study were not rinsed prior to analysis, similar studies in 2011 and 2012 found no clear evidence of consistent residue reduction from commercial hydrocooler cycles.

As these studies have routinely demonstrated since 2011, application of pesticides according to label directions consistently produces residues safely below tolerance levels set by the US Evironmental Protection Agency. For the first time, application of the rain protectants RainGard and Parka in 2015 did not preserve elevated pesticide residue levels. Despite this year's results, the long-term trend in these studies has been that fruit treated with rain protectants have carried higher pesticide residues than untreated control fruit. The potential benefits of RainGard and Parka to reduce fruit cracking are substantial, but cherry growers using rain protectants should exercise caution to mitigate potential MRL issues.

Cherries with residues at harvest

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

Measured residue levels vs. MRLs for uniformly applied pesticides on cherry fruit treated with no rain protectant (control), RainGard (408 oz/acre) at 14 and 7 days before harvest, or Parka (128 oz/acre) at 21 and 14 days before harvest. 'Bing'/Mazzard, Orondo, WA. WTFRC 2015.

Common name	Trade name	Application rate[1]	Application timing(s)	Control fruit	RainGard-treated fruit	Parka-treated fruit	US tolerance[2]	Lowest export tolerance[3]
		per acre	*days before harvest*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*
Diazinon	Diazinon 50W	64 oz	21	<0.01	<0.01	<0.01	0.2	0.01 (EU)
Abamectin	Agri-Mek 0.15EC	20 oz	21	<0.01	<0.01	<0.01	0.09	0.01 (EU)
Buprofezin	Centaur	34.5 oz	21	<0.01	<0.01	<0.01	1.9	0.1 (Can)
Zeta-cypermethrin	Mustang MAX	4 oz	21, 14	<0.05	<0.05	<0.05	1	0.1 (Can)
Lambda-cyhalothrin	Warrior II	2.56 oz	21, 14	<0.05	<0.05	<0.05	0.5	0.3 (many)
Imidacloprid	Nuprid 2SC	6.4 oz	21, 7	0.13	0.11	0.12	3	0.5 (many)
Acetamiprid	Assail 70WP	3.4 oz	21, 7	**0.26**	**0.23**	**0.24**	1.2	0.2 (Kor)
Beta-cyfluthrin	Baythroid XL	2.8 oz	21, 7	**0.075**	**0.056**	**0.052**	0.3	0.01 (Tai)
Metconazole	Quash	4 oz	14	0.038	0.035	0.018	0.2	0.2 (many)
Spinosad	Entrust	2.5 oz	14, 7	0.054	0.031	0.035	0.2	0.2 (many)
Spinetoram	Delegate WG	7 oz	14, 7	0.015	0.013	0.013	0.2	0.05 (EU, Kor)
Quinoxyfen	Quintec	7 oz	14, 7	0.055	0.053	0.027	0.7	0.3 (EU)
Flutriafol	TopGuard	14 oz	14, 7	**0.19**	**0.18**	**0.17**	1.5	0.01 (Jap)
Penthiopyrad	Fontelis	20 oz	14, 7	0.17	0.16	0.12	4	1 (Kor)
Flubendiamide	Belt	4 oz	14, 7	0.082	0.073	0.072	1.6	1 (Kor, Tai)
Metrafenone	Vivando	15.4 oz	14, 7	<0.01	<0.01	<0.01	2	0.01 (Tai)
Fenpropathrin	Danitol 2.4EC	21.3 oz	14, 3	**0.43**	**0.46**	**0.27**	5	0.01 (EU)
Permethrin	Perm-Up 3.2EC	8 oz	14, 3	**0.39**	**0.46**	**0.24**	4	0.05 (EU)
Carbaryl	Carbaryl 4L	96 oz	10, 3	**0.99**	**1.2**	**0.71**	10	0.01 (EU)
Cyantraniliprole	Exirel	20.5 oz	10, 3	**0.14**	**0.17**	**0.13**	6	0.05 (Aus)
Propiconazole	Orbit	4 oz	10, 1	**0.29**	**0.25**	**0.21**	4	0.05 (EU)
Thiophanate-methyl*	Topsin 4.5FL	30 oz	10, 1	**0.69**	**0.90**	**0.97**	20	0.3 (EU)
Etoxazole	Zeal	3 oz	7	0.041	0.038	0.041	1	0.2 (Kor)
Spirodiclofen	Envidor 2SC	18 oz	7	0.070	0.063	0.068	1	0.8 (Tai)
Fluxapyroxad	Merivon	6.7 oz	7, 1	**0.39**	**0.35**	**0.38**	3	0.01 (EU)
Pyraclostrobin	Merivon	6.7 oz	7, 1	0.44	0.32	0.28	2.5	0.7 (Can)
Azoxystrobin	Abound	15.5 oz	7, 1	0.19	0.17	0.20	1.5	1 (Tai)
Triflumizole	Procure 480SC	16 oz	7, 1	0.24	0.24	0.16	1.5	1 (Tai)
Trifloxystrobin	Luna Sensation	5.6 oz	7, 1	0.013	0.012	<0.01	2	1 (EU)
Fluopyram	Luna Sensation	5.6 oz	7, 1	0.082	0.067	0.062	0.6	0.6 (Aus)
Bifenazate	Acramite 50WS	16 oz	3	<0.01	<0.01	<0.01	2.5	0.3 (Kor)

[1] All materials were applied by Rears PakBlast sprayer at 400 gal water/acre; pesticides (excluding RainGard & Parka) were applied with 32 oz Regulaid/acre

[2] 7 July 2015. http://www.nwhort.org/CherryMRLs.html, https://www.globalmrl.com

[3] Major export markets for Pacific Northwest cherries; 7 July 2015; tolerances may be based on published MRLs or default values. http://www.nwhort.org/CherryMRLs.html, https://www.globalmrl.com * Reported thiophanate-methyl values reflect sum total of thiophanate-methyl and carbenzadim residue levels

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

WASHINGTON TREE FRUIT RESEARCH COMMISSION

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

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

Item	2014	2015	2016
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 apple mealybug (AMB) and grape mealybug (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

- LChV2 infection is not always correlated with an active MB infestation. The initial infection via insect vectors may have occurred previously, but symptoms become evident only in subsequent years.
- AMB: Control strategies targeting AMB are most effective when sprayed at the delayed dormant timing.
- GMB: Systemic treatments applied at petal fall had the most effect on GMB populations.
- LCVH2: The commercial kit (Reverse Transcription Recombase Polymerase Assay, or RT-RPA) for detecting LChV2 was modified and it now recognizes genetic variants of the virus that were not detected in the previous season.
- WX: A reliable assay system was developed for WX based on the RPA format. This allows more precise identification of the pathogens associated with little cherry disease, a critical factor since that influences appropriate disease management decisions.
- The total production costs for 'Bing' during full production are estimated at $27,665 per acre, and for 'Sweetheart' at $33,342 per acre. The break-even prices are estimated at $1.87/lb for 'Bing' and $1.69/lb for 'Sweetheart'.
- The strategies to manage little cherry disease (tree removal, monitoring, additional mealybug sprays) lead to better financial outcomes compared to doing nothing when there is evidence of LChV2 in the orchard. However, there is a threshold for tree removal; removing trees beyond the threshold results in negative net returns.

METHODS

Obj. 1. *Vector transmission: surface contamination vs. transovarial transmission.* GMB egg masses will be collected from known positive trees in the field. Eggs from either the overwintering or summer generations will be used. Individual egg masses will be divided in half; half will be tested in the egg stage for LChV2 using RT-PCR, and the other half will be allowed to hatch to the crawler stage on uninfected potted cherry trees, and this stage will be tested for LChV2.

Time needed for virus acquisition/transmission. Experiments will be completed using infected and uninfected potted 'Bing' cherry trees in a greenhouse at WSU-TFREC. In January 2016, dormant budwood will be collected from known LChV2 infected trees and will be chip-budded on to healthy trees to establish infection. Virus-free GMB colonies were started from field collections and reared in vented plastic containers on sprouted potatoes. *Acquisition*: Virus-free GMB crawlers will be allowed to feed on infected plant material in the greenhouse for 1, 3, 7, and 9 days, then removed and tested for LChV2. Results will tell us the number of mealybug feeding-days required to acquire virus from an infected plant. *Transmission*: GMBs feeding on infected plant material 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:* During the 2016 growing season, pesticide efficacy tests on GMB will be repeated. For AMB, we plan to examine two organic control strategies using a block of organic apple

trees at WSU Sunrise Research Orchard, which is currently infested with AMB. Treatments will include dormant oil and Neemazad, which will be compared to an untreated check.
Obj. 3. *Field Diagnostic Assay:* (1) Leaves will be collected from trees that are infected with LChV2, WX phytoplasma and/or other viruses. Initially, tests for LChV1 will be performed with virus isolates maintained at the Clean Plant Center Northwest (WSU-IAREC Prosser, WA). Orchard trees infected with WX phytoplasma and other viruses will be sampled and pathogen status determined by PCR and compared with RT-RPA results. (2) Orchard trees that have tested positive for LChV1 by RT-PCR will be identified. Crude leaf extracts will be prepared and frozen for future evaluation. Orchard trees with little cherry disease symptoms will be collected and the crude sap extracts tested by RT-PCR and RT-RPA to obtain an estimate of assay reliability throughout the growing season.
Obj. 4. *Economic impact and decision-making tools (this objective has been completed):* We met in person with five sweet cherry producers representative of North Central Washington, Yakima and the Tri-cities areas between December 2014 and January 2015. The enterprise budgets reflect the current industry practices and average costs to produce the two varieties of sweet cherries.
Analysis of cost scenarios for orchard blocks affected with LChV2: A partial budgeting framework was used to estimate and examine the costs associated with LChV2 due to reduced yield, diminished pack-outs and tree removal; and costs and benefits of strategies to address the disease, i.e., tree removal, monitoring and chemical sprays. We compare and contrast additional costs due to LChV2 with the baseline. Outcomes of the partial budgets will be the net change in profit relative to the baseline and the break-even costs of each scenario. A detailed explanation of all assumptions for the partial budgets is available upon request to Co-PI Gallardo or in the final report.

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

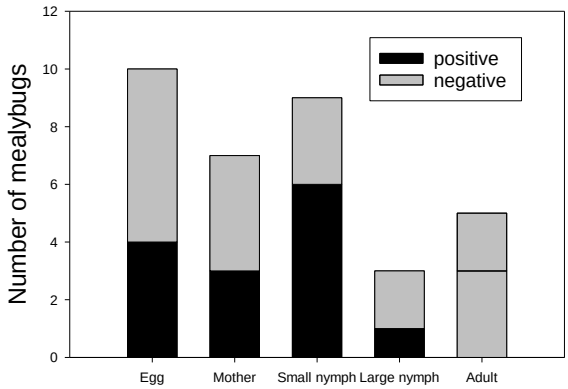

Fig. 1. Number of mealybugs (eggs, mom, small nymph (0.5 – 1.5mm), large nymph (2 – 4mm), and adult) collected from LChV2 positive trees that tested positive and negative for LChV2.

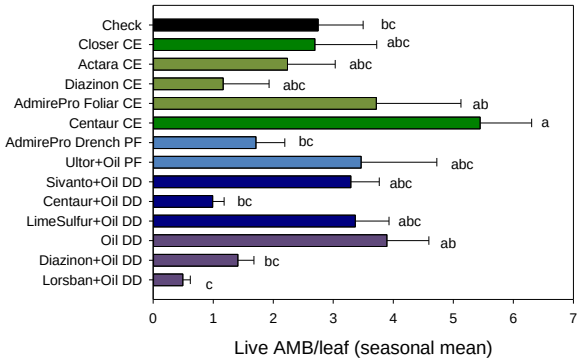

Fig. 2. Seasonal averages of AMB crawlers/leaf (3-Jun – 11-Aug.) from leaves collected from apple trees treated at delayed dormant (DD), petal fall (PF), and crawler emergence (CE).

Obj. 2. *Control methods for AMB:* In 2014 and 2015, AMB was monitored weekly at WSU's Sunrise Orchards, in a conventional apple orchard with a high density of AMB. In 2015, we found that second instar females were present and feeding on woody plant parts near buds as early as 25 Feb, which is almost an entire month earlier than last year's observation of 21 Mar. Emerging males (25-Mar) and hatched crawlers (27 May) were observed about two weeks earlier than last year's timing. This early emergence correlates with the warm temperatures we experienced during the late winter and early spring in North Central Washington. During the 2015 growing season, a field experiment was conducted to determine the effects of chemical insecticides on AMB populations. Treatments were applied, and AMB collection and analysis procedures were the same as in the 2014 experiments. Delayed dormant (DD) treatments (applied 16 Mar) included Lorsban+oil, Diazinon+oil, lime sulfur +oil, Centaur + oil, Sivanto +oil, and oil targeted overwintering females. Lorsban +oil (0.5 ± 0.1 crawlers/leaf), Diazinon +oil (1.4 ± 0.1 crawlers/leaf), and Centaur + oil (1.0 ± 0.2 crawlers/leaf) did show some reduction in AMB population compared to the check (2.8 ± 0.75 crawlers/leaf, Fig. 2). Two systemic compounds, Ultor+oil (foliar) and Admire Pro (soil drench), were applied 14 days after petal fall (PF, 29-Apr) with Admire Pro drench showing some reduction compared to the check. Centaur, Admire Pro (foliar), Diazinon, Actara, and Closer were applied (8-Jun) to target active crawlers on leaves. Trees treated with Diazinon (1.2 ± 0.8 crawlers/leaf) had the lowest crawler numbers of the treatments in this group. The addition of Centaur (insect growth regulator) to the DD treatments was aimed to target molting female nymphs. Emerging during their second instar, females molt at least two times before the adult stage. Centaur showed promising results especially compared to Lorsban, the most effective treatment in 2014 and 2015.

Control methods for GMB: In 2015, GMB was monitored weekly in a commercial 'Bing' orchard, located in E. Wenatchee, with a high density of GMB. The first observations were of newly hatched crawlers leaving egg masses on the bark of trees (17 Mar, Fig.3). Aside from this initial observation, all subsequent MB activity/feeding occurred at the base of fruit and leaf buds and spurs in locations close to the base of the tree/bark. Crawlers became visibly larger over time; however, newly hatched crawlers were observed until 7 May. The first adults were observed on 21-May followed by the first viable eggs and the start of the second generation on 10 Jun. Adults and viable eggs were observed until 29-Jul, but beyond that date nothing was found, and they are assumed to have moved into sheltered places to overwinter as eggs or crawlers. Male GMB were never observed. During the 2015 growing season, a field experiment was conducted to determine the effects of chemical insecticides on GMB. On 8-Jul, 20 spurs were collected from each treated tree, kept cool, and brought to the lab to be examined microscopically. The average number of GMB/spur was calculated for each treatment. Compounds applied at PF included Centaur (14 Apr), and systemic compounds, Ultor +oil, and

Admire Pro drench (29 Apr). Both systemic compounds showed a reduction in GMB populations (0.2 ± 0.05 and 0.1 ± 0.05 GMB/spur, respectively). Of the compounds targeting summer crawlers (15-Jun), Centaur (0.4 ± 0.14) had the lowest number of GMB/spur compared to the check (1.1 ± 0.43, Fig. 4). This experimental site was discovered too late in the season to test compounds at DD. Results show that systemic treatments applied at PF had the most effect on GMB populations. However, soil drenching systemic neonicotinoids is not typical in orchards, and the systemic properties of these types of compounds can have potential harmful consequences for pollinator bees in the following year. This might be a good option for growers who plan to remove trees/orchard due to LChV2 infection, but want to keep the MB from spreading to other trees/orchards before removal.

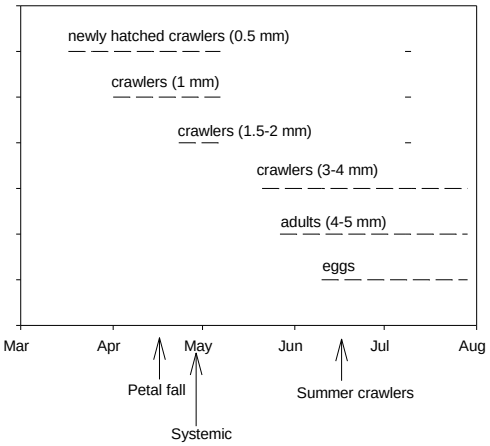

Fig. 3. Phenology of GMB at an E. Wenatchee orchard (2015), and treatment timing.

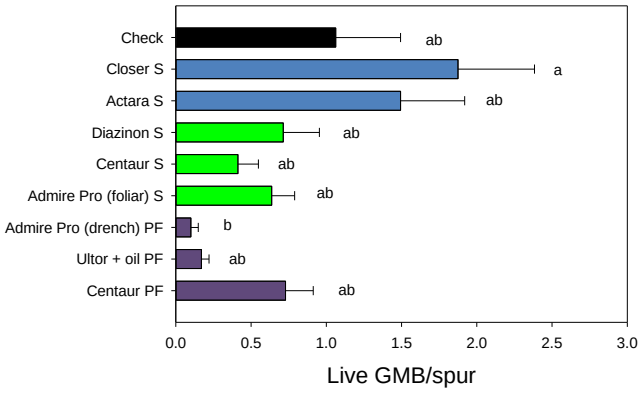

Fig. 4. Seasonal averages of GMB/spur (8-Jun) from spurs collected from cherry trees treated at petal fall (PF), and in the summer (S).

Obj. 3. *Validation of LChV2 field kits:* A diagnostic kit based on RT-RPA technology for LChV2 was made commercially available in the spring of 2014. However, kit performance was subpar for two fundamental reasons: 1) unexpected genetic variability of LChV2; and 2) limited experience with this assay system for the detection of LChV2. The RT-RPA kit was re-tooled using nucleotide sequence information obtained from unique genetic variants of LChV2. Using LChV2 infected trees maintained in the greenhouse of the Clean Plant Center Northwest; a prototype of the re-tooled kit successfully detected the unique LChV2 variant as well as common LChV2 strains. The redesigned test kit still discriminated between LChV2 and the other agents associated with little cherry disease including LChV1 and WX. Field data collected during the 2014 and 2015 growing season highlighted optimal sampling times and sample size, which were incorporated into revised protocols.
Development of an RPA test for LChV1 and WX: The 2014 growing season emphasized the relative importance of WX in Washington orchards; WX accounted for 35% of single infections in little cherry diseased trees. Based on this information, priority was given towards the development of a WX RPA assay. Prior to this study, limited information was known about the specific isolates of WX outside of California. Using the sequence data generated by high throughput sequencing of three isolates of WX from WA, primers and probes were selected from two regions of the genome. In initial PCR tests, both sets of primer pairs detected WX from 26 samples collected in 2014; these samples originated from orchards in Benton, Yakima, Grant and Chelan counties. These results demonstrated the reliability of the primers in detecting wide spectrum of isolates of WX by PCR, and sequence analysis of the PCR products provided evidence of the suitability of the designed probes for WX detection by RPA. However, when crude extracts were analyzed by RPA, one primer pair and probe combination (imp) yielded background reactions from trees not infected with WX. The remaining primer pair and probe combination (idpA) yielded results comparable to PCR (Table 1).

The idpA primer and probe combination was further demonstrated to be specific for WX. Infection by LChV1, LChV2 or bacterial canker did not affect the WX test results. Reliability of PCR and RPA systems to detect WX was monitored through the season. Both assay formats were unreliable in detecting WX during the earliest part of the season (mid-March: full bloom) but gave consistent positive detection a month after full bloom (starting on mid-Apr) (Table 2). Crude sap preparations of leaves from 29 samples gave consistent positive reactions in the WX PCR and RPA assays. Taken together, a reliable RPA assay for WX targeting the idpA region of the pathogen was developed that is suitable for use in crude sap extracts.

Table 1. Detection of WX phytoplasma by PCR and RPA using crude sap preparations.

Sample	PCR		RPA	
	idpA region	imp region	idpA region	imp region
1	++	++	++	++
2	-	-	-	-
3	-	-	-	-
4	-	-	-	+
5	-	-	-	-
6	++	++	++	++
7	++	++	++	++
8	++	++	++	++
9	++	++	++	++
Non WX infected cherry	-	-	-	+
WX positive (purified DNA)	++	++	++	++
water	-	-	-	-

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

Results from the 2014 growing season revealed that less the 10% of the trees displaying little cherry disease symptoms were infected with LChV1, and all of the trees infected with LChV1 were also infected with other little cherry disease agents. Consequently, research on LChV1 was temporarily de-emphasized. Previously, suitable primers and probe were identified that have the best potential for use in RT-RPA for LChV1. An RT-RPA assay based on this data detected LChV1 in both purified RNA preparations and crude sap extracts of 7 LChV1 isolates. Determination of the specificity of the LChV1 RT-RPA kit as well as its reliability for LChV1 detection in field samples throughout the season is currently being pursued.

Obj. 4. *Baseline economic analysis:* The baseline studies consider the production scenarios when the sweet cherry orchard is not infected by LChV2. The studies assumed that a 'Bing' or a 'Sweetheart' orchard starts bearing fruit in the 3rd year and achieve full production in the 6th year; and the pack-out is 80% for both sweet cherry varieties. Given the assumptions, the total production costs for 'Bing' during full production are estimated at $27,665/acre and for 'Sweetheart' at $33,342/acre. Furthermore, the estimated net returns for 'Bing' and 'Sweetheart' are positive. The break-even prices are estimated at $1.87/lb for 'Bing' and $1.69/lb for 'Sweetheart'. Additional details on the baseline economic analysis are available upon request to Co-PI Gallardo.

Analysis of cost scenarios for orchard blocks affected with LChV2: All scenarios assume LChV2 infected orchards have lower estimated profits compared to the baseline. In both 'Bing' and 'Sweetheart', losses are estimated for the scenarios when there are diminished pack-outs and reduced yield. Holding all other factors constant, when gross yield is 20% less than the baseline (scenario 1), losses amount $255/acre for 'Bing'; and while there is a profit of $2,646/acre for 'Sweetheart', it is about 62% lower than the baseline profit. When pack-outs equal 60%, which is 20% less than the baseline (scenario 2), negative net returns amount to $4,505/acre and $2,814/acre respectively for 'Bing' and 'Sweetheart'. These results show evidence that when there is LChV2 in the orchard, doing nothing leads to lower or negative net returns compared to tree removal and monitoring measures. The two management strategies for LChV2 considered in this study (tree removal, and monitoring and additional MB sprays) yield positive net returns. The net returns of the *tree removal* scenario (20% of trees are removed) are lower than the net returns of the *monitoring and additional sprays* scenario by 91% for 'Bing' and 48% for 'Sweetheart". If the impact of LChV2 infection is such that 4.6% of 'Bing' trees or 3.7% of 'Sweetheart' trees need to be removed, then the costs of removal are

equivalent to the additional costs incurred in monitoring and additional sprays. Detailed information on the net returns and the partial budget calculations are available upon request to Co-PI Gallardo.
Sensitivity analysis: We examine the sensitivity of profit by changing the following parameters: (a) crop yield, (b) pack-out, and (c) proportion of trees removed. The net returns for 'Bing' and 'Sweetheart' become negative when their baseline gross yields are reduced by about 18.5% and 32.2%, respectively. The net returns for 'Bing' become negative when pack-out is 71.6%, and for 'Sweetheart' when pack-out is 65.7%. For the tree removal scenario, when 21.4% percent of the 'Bing' trees become infected and are thus removed, the net returns start to become negative. For 'Sweetheart', net returns become negative when 35.7% of the trees are removed.

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

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

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

CONTINUING PROJECT REPORT **YEAR:** 1 of 3
WTFRC Project Number: CH-15-103

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

PI: Dr. Dan Villamor**
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******Dr. Villamor replaces Dr. Eastwell as the PI of this project. Dr. Eastwell is retiring from WSU but will continue to participate in this project as co-PI.

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:** Carrie Johnston
Telephone: (509) 335-4563 **Email address:** carriej@wsu.edu

Item	2015	**2016**	2017
Salaries[1]	$8,143	$8,469	$8,808
Benefits[2]	$3,339	$3,472	$3,611
Supplies[3]	$15,935	$15,689	$15,712
Travel	$0	$0	$0
Miscellaneous	$0	$0	$0
Plot Fees	$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

- Natural infection of luteovirus in sweet cherries in commercial orchards in Washington State was observed. Coincidentally, another new virus belonging to the *Fabavirus* genus present either singly or in combination with the luteovirus was discovered.

- A molecular test for the luteovirus and fabavirus, based on reverse transcription polymerase chain reaction (RT-PCR), was developed. The test is based on virus sequences obtained from trees infected with the viruses.

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.

Prunus avium 'Mazzard' and *P. persica* trees that were used in the first year of the project in the aphid transmission experiment, using black cherry and green peach aphids, will be tested at bi-monthly intervals to check for the presence of the luteovirus. A second attempt to transmit the luteovirus with black cherry and green peach aphid will be performed between both sweet cherry and peach trees.

Since the newly discovered fabavirus has been detected only in sweet cherries, the potential of both black cherry and green peach aphids to transmit this virus will only be investigated in *Prunus avium* 'Mazzard'. Because fabaviruses are known to be non-persistently transmitted (stylet born), aphids will be allowed to acquire the virus (virus acquisition period) from infected potted trees (donor) at different short time intervals before transferring to virus-free sweet cherry trees (recipient) for virus transmission feeding. After a short transmission period (maximum of one day), the aphids will be eliminated and the recipient trees will be retained in a quarantine facility for a minimum of one complete growing season to allow the virus concentration to increase to detectable levels. Tissues from the recipient trees will be sampled at regular intervals and tested for the presence of the fabavirus.

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, P. virginiana, and *Purshia tridendata* that were previously graft-inoculated with bark patches from a luteovirus source tree will be allowed to grow after one dormant cycle, and tested at bi-monthly intervals to check for detectable levels of the virus. A new set of trees of *P. emarginata, P. virginiana,* and *Purshia tridendata* will be obtained and graft inoculated with bark patches from the fabavirus infected tree. Graft-inoculated trees will be retained in a quarantine facility for a minimum of one complete growing season to allow the virus concentration to increase to detectable levels. Tissues from recipient trees will be sampled at regular intervals and tested for the presence of the fabavirus.

Since luteoviruses are not mechanically transmissible, aphid species that successfully transmitted the virus into the woody hosts in objective 1 will be selected and use as vector for virus transmission into herbaceous hosts. Selected weed species commonly found in many sweet cherry orchards namely, clover, dandelion and *Chenopodium* species, will be used in the luteovirus aphid transmission study. On the other hand, fabaviruses are generally sap transmissible. Therefore, herbaceous hosts routinely employed in the indexing of fruit tree viruses at Clean Plant Center Northwest (CPCNW), including *Nicotiana benthamiana, N. occidentalis* 37B, *C. quinoa* and *Cucumis sativus,* will be tested as potential hosts. Attempts to sap transmit the fabavirus to common orchard weeds mentioned above will also be done. Simultaneously, aphid species identified in objective 1 will be used to attempt transmission of the fabavirus to herbaceous hosts.

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

The finding of the luteovirus naturally infecting sweet cherries in Washington as well as the discovery of the fabavirus present either as single or mixed infections with the luteovirus resulted in modification of the experimental design for this objective. Induction of symptoms by single infections of either luteovirus or fabavirus, and dual infections of luteo- and faba- viruses will be investigated in the sweet cherry cultivar ('Bing') grown in either Mazzard or Gisela rootstock. The original observation of symptoms associated with luteovirus was on infected trees of *P. persica*, and based on our recent findings with this host; it takes two years for the stem pitting symptoms to be visible. Thus, it is anticipated that the remaining two years of the project will be sufficient time to evaluate whether these viruses induce this pathology in sweet cherry trees. At the end of the two year incubation period, the trees will be sacrificed, steamed and the loosened bark peeled to reveal any signs of stem pitting.

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

Additional trees (2) infected by the luteovirus will be sent for high throughput sequencing (HTS). To further determine the distribution of the luteovirus in commercial production, a total of 425 leaf samples from sweet cherry orchards from different cherry growing counties of Washington State, namely Chelan, Douglas, Yakima, Benton and Grant, will be collected and tested for the presence of the luteovirus by RT-PCR. For the fabavirus, baseline sequence information of four trees infected by the virus will be sent for HTS. Similarly, the 425 sweet cherry leaf samples will also be tested for the fabavirus by RT-PCR. Representative samples from each county that show positive test results for either viruses will be sent for sequencing to verify the specificity of the test. The sequences generated will also provide information in determining the genetic diversity of luteo- and faba- viruses in Washington State.

RESULTS & DISCUSSION

A new virus of the genus *Fabavirus* was discovered in a sweet cherry tree in an orchard in Grant County, WA. This unexpected result was obtained through high throughput sequencing (HTS). Using the sequence information generated by HTS, a molecular test was developed based on RT-PCR. Evidence of transmissibility was demonstrated by positive results in RT-PCR of *P. avium* 'Mazzard' trees (total of four) graft inoculated with bark patches from the fabavirus-infected tree. Consequently, four out of 12 trees growing in the vicinity of the original fabavirus-infected tree were infected as revealed by RT-PCR. These findings prompted immediate sampling of trees in an orchard nearby, including an orchard from Yakima County. A total of 75 trees were randomly sampled and tested for the presence of the fabavirus as well luteovirus. Of these trees, eight and 32 were found positive for the luteovirus and fabavirus, respectively. Notably, all luteovirus positive trees were also co-infected with the fabavirus. An important observation is that none of samples showed visible leaf symptoms indicative of virus infection but the trees exhibited overall poor growth and slightly reduced fruit size.

The latter could be attributed to virus infection or poor orchard management. These results also show, for the first time, natural infection of luteovirus in sweet cherry trees in commercial production. Together, these findings prompted us to modify the direction of the project by looking at the possibility of symptom induction (stem pitting) by either luteovirus or fabavirus and their interaction on sweet cherry (Bing) grown in either Mazzard or Gisela (6 or 12) rootstocks.

Looking at the transmissibility of the luteovirus on woody hosts in year 1, none of the black and green peach aphid inoculated (*P. persica* and *P. avium* 'Mazzard') and graft transmitted (*P. emarginata, P. virginiana* and *Purshia tridentata*) trees were positive for the virus after testing on two separate occasions (60 and 90 days after inoculation). These trees will be allowed to go dormant and will be tested again for the presence of the viruses in 2016.

Sequence information generated by HTS of two nectarine and one peach trees infected with the luteovirus identified regions in the genome of the virus that are conserved; one region was selected as target for the molecular detection assay by RT-PCR. The molecular test further confirmed the presence of luteovirus in the three HTS analyzed trees but also in different fruit tree accessions received for virus testing at CPCNW plus trees collected in commercial orchards mentioned above. These results show the reliability of the molecular test in detecting luteovirus infection status of fruit trees and will be further evaluated for its ability to specifically detect the virus in large scale testing of samples from commercial orchards in Washington State. Similarly, the molecular test developed for the fabavirus detected the virus in trees sampled from commercial orchards in the state. This molecular assay will be further evaluated for its dependability when subjected to large scale testing of samples from commercial orchards.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number:

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

PI: Peter W. Shearer
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Co-PI: Joanna Chiu
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Telephone: (530) 752-1839
Email: jcchiu@ucdavis.edu

Total Project Request: **Year 1**: $32,058 **Year 2**: $93,397 **Year 3**: **$97,623**

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

Item	2014	2015	2016
Salaries	--	10,485	10,800
Benefits	--	6,763	6,966
Wages	7,280	7498	7,723
Benefits	605	623	642
Supplies	1,000	1,545	1,000
Travel	250	2,000	2,060
Total	$9,135	$28,914	**$29,191**

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.

Budget 2 (Beers)
Organization Name: Washington State University **Contract Administrator:** Joni Cartwright; Carrie Johnston **Telephone:** 509-663-8181 x221; 509-335-4564 **Email address:** joni.cartwright@wsu.edu; carriej@wsu.edu

Item	2014	2015	2016
Wages[1]	7800	8112	8436
Benefits[2]	757	787	818
Supplies[3]	1500	1500	1500
Travel[4]	2966	2966	2966
Total	$13,023	$13,365	**$13,720**

Footnotes:
[1]Wages $13/hr, 40 hrs/week, 15 weeks/year;
[2]benefits 9.7%.
[3]Supplies: traps, drosophila rearing supplies, baits and lures.
[4]Travel to research sites, 350 miles/week, 15 weeks/year, $0.565/mile.

Budget 3 (Van Steenwyk)
Organization Name: University of California Berkeley Contract Administrator: Lynne Hollye **Telephone:** 510-642-5758 **Email address:** Lhollyer@berkeley.edu

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

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

Budget 4 (Chiu and Zalom)
Organization Name: University of California Davis Contract Administrator: Guyla Yoak
Telephone: (530) 752-3794 Email address: gfyoak@ucdavis.edu

Item	2014	2015	2016
Salaries	--	12872	13514
Benefits	--	84	88
Supplies	5,000	6408	6230
Travel	--	--	--
Plot Fees	--	--	--
Miscellaneous		5636	5918
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

OBJECTIVES

1. Design and test traps to capture live SWD adults for insecticide resistance studies (yr 1)
In the first year of the study, methods were developed to collect live adult SWD.

2. Develop ~~discriminating~~ diagnostic doses of insecticides to test susceptibility of SWD populations (yr 1)
Diagnostic doses of insecticides were estimated from dose-response lines for Delegate, Entrust, malathion, Sevin 4F, and Warrior II in 2014. Originally we proposed to use 2X the LC_{95} (twice the amount to kill 95% of test subjects) as a discriminating dose. We changed to a more conservative diagnostic dose of 2X the LC_{99}.

3. Complete development of primers for genetic analyses of SWD alleles that confer resistance (yr 2-3)
We are using next-generation sequencing to identify genetic variation(s) between the *Drosophila suzukii* strains listed in Table 1 as compared with the Genome Strain (reference).

4. Screen SWD from multiple districts in CA, OR and WA for insecticide susceptibility (yr 2-3)
Populations of SWD from western cherry districts are being assayed to determine their susceptibility of target insecticides. The assays include genetic studies and topical applications of discriminating doses to field collected live flies, to be completed in year 3. Two populations each from OR and CA and three from WA were tested in the laboratory for their susceptibility to key insecticides. SWD will be collected and screened from additional cherry growing districts in 2016.

5. Correlate results from discriminating-dose and genetic studies (yr 3)
Results from our genetic analysis will be correlated with bioassay data using robust statistical methods, e.g. Principal Component analysis, that is routinely used to correlate phenotype (insecticide resistance) to genetic variation, to be initiated the in the third year.

SIGNIFICANT FINDINGS

- Traps to capture live SWD for insecticide resistance screening were evaluated in the field and laboratory and at least two trap designs initially appeared promising when baited with commercial SWD lures.
- It is very difficult to capture live SWD for conducting this research.
- A few flies survived in some of the assays when none were expected to. Some of the survivorship is related to a procedural error that has been corrected. Screened populations with survivors are being re-tested.

METHODS

Objective 1. Traps were evaluated in CA and WA for their ability to capture and keep SWD alive. Two trap designs were identified and utilized, hand-assembled traps were used in CA and modified yellow Trappit Dome traps were used in WA and OR (Fig. 1). Traps were deployed overnight or up to two days in the field to capture live adult SWD flies in cherry orchards. Abundance of flies captured in CA was very low using the hand-made trap. The Trappit trap baited with the commercial SWD lure from Scentry effectively captured flies in WA but not in OR or the Dallesport, WA. When working in cherries at MCAREC, we observed drosophila flying just above the ground under cherry trees. We then used a lightweight butterfly net in those sites to capture flies that were in the ground cover under cherry trees at MCAREC and the Dallesport, WA (Fig. 2). At MCAREC, most of the flies captured with the butterfly nets were adult male SWD while at the Dallesport, most of the flies were adult female SWD. Interestingly, besides the extreme differences in sex-ratios, SWD were the most abundant insect captured in the sweep net samples.

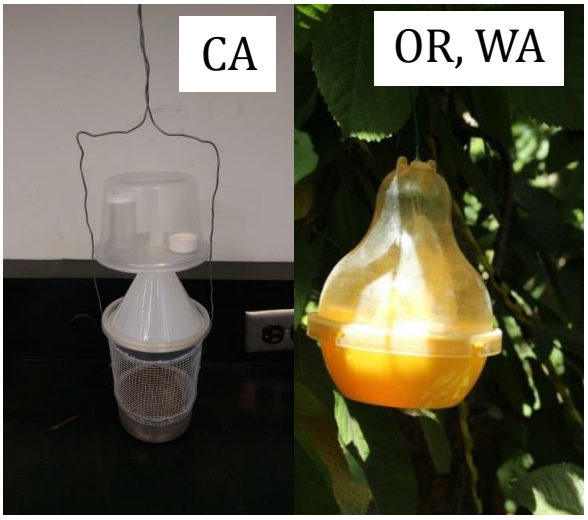

Fig. 1. Traps developed and used in CA and OR/WA.


Fig. 2. Butterfly net for collecting adult SWD.

Captured flies were then identified and both sexes were placed either in screen cages with diet or in vials of diet and allowed to mate and lay eggs. Flies were provided fresh media in which to lay eggs every 3 days or so and ages of adult offspring (F1 flies) were tracked. These flies were then assayed in the laboratory (see Objectives 3 & 4).

Objective 2. Dose-response lines and 2X LC_{95} values were estimated for various insecticides against adult female SWD. This work was conducted in OR and WA in 2014.

Objective 3. For each of the *Drosophila suzukii* strains listed in Table 1, we collected flies for three biological replicates (pooled 15 female flies per replicate) at 4 hours after lights on (L:D = 12 hrs light: 12 hrs dark at 25°C). We extracted total RNA using TriReagent (Sigma, St. Louis, MO) and ensured high RNA quality using the Experion Automated Electrophoresis system (Biorad, Hercules, CA) prior to sequencing library preparation. PolyA mRNA enrichment and paired-end sequencing libraries with an approximate average insert length of around 150 to 200 bp were created using the Illumina Truseq library preparation kit (Illumina, San Diego, CA). Completed libraries (see Table 1) were assessed for integrity using the Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA). To date, we have completed library preparation for six populations, and libraries for two other populations will be completed by the end of October 2015.

Table 1: Strains of *Drosophila suzukii* for genomic analysis to assess molecular basis of insecticide resistance

Strain	Collection Location	Collection Date	Project Status
Genome Strain	Watsonville, CA	2009	Seq. library completed
Clayton-R	Clayton, WA	7/14/2015	Seq. library completed
Clayton-O	Clayton, WA	8/17/2015	Seq. library completed
Brentwood	Brentwood, CA	7/1/2015	Seq. library completed
Tracy	Tracy, CA	9/10/2015	Seq. library completed
Senseney	Senseney, WA	7/2015	Seq. library completed
Spanish Castle	Spanish Castle, WA	7/28/2015	Seq. library in progress
MCAREC-R	MCAREC, OR	7/30/2015	Seq. library in progress
Dallesport	Dallesport, WA	9/3/2015	Seq. library in progress

Upon completion of all library preparations, 12 individual indexed libraries will be subjected to multiplex sequencing in one flow cell lane using Illumina HiSeq3000 platform (100 bp paired-end) to at least 20 million reads per sample. We will perform RNA-Seq read alignment, using TopHat (Trapnell et al. 2012) against the SWD genome (Chiu et al. 2013) to identify SWD transcripts. To detect overexpression of detoxification genes, we will perform transcript abundance estimation and differential expression analysis using the rest of the Tuxedo software suite to assess any differential gene expression between the control genome strain and SWD populations from different geographical regions (Trapnell et al. 2012) Our RNA analysis will also yield single nucleotide polymorphisms (SNPs) in protein coding regions that can confer target-site resistance.

Objective 4. The 2X LC_{99} dose for each insecticide provides the basis for diagnostic testing of SWD populations in the western USA in years 2 and 3 using the following protocol: Female flies, 7-10 days old, were aspirated into vials in groups of 20-25 for a minimum of 100 adult female SWD. Each group of flies were then anesthetized with CO_2 and placed in a new 50-mm diameter Petri dish bottom (Falcon, VWR Catalog #25369-022, Visalia, CA). Pesticides dilutions (1 concentration per material; Table 2) were made by first shaking the container if liquid, then adding the required amount into 1 liter of distilled or de-ionized water. Each mixture was mixed with a magnetic stirrer before being pipetted into the laboratory sprayer (Potter Spray Tower, Rickmansworth, UK). The Petri dish with adults was then placed on the platform of the sprayer and treated with 2 ml of the appropriate insecticide. Product was applied with nozzle pressure of 16 psi, and total deposition of 2.7 mg/cm^2. The flies were then transferred to the post-treatment holding arena, made of a 90-mm diameter x 15-mm deep Petri dish (Catalog No. 25384-302, VWR, Visalia, CA) with 3 vent lugs and 15 ml of *Drosophila* medium. Flies were held at 72 ^oF for 24 hours before being evaluated for survival and mortality. After all runs were completed, all remaining flies were transferred alive to Dr. Chiu, UCD for further testing.

Objective 5. By correlating our genetic data with insecticide bioassays performed on the same fly strains, we aim to determine the genetic basis of insecticide resistance that may be present in these populations. Specifically, we will focus on (1) single nucleotide polymorphisms (SNPs) in protein coding regions that can potentially confer target-site resistance as well as (2) gene expression changes indicative of metabolic upregulation of detoxification enzymes.

RESULTS & DISCUSSION

Collecting enough adult female SWD to start colonies was more difficult than anticipated. In CA, only 11 and 9 female SWD were captured in traps in Brentwood and Tracy, CA, respectively. We originally proposed a minimum of 100 females SWD were needed for genetic diversity. In OR, traps caught very few flies despite adult SWD being present. We switched to using sweep nets to collect flies and caught enough flies after a couple hours using this method at MCAREC (135 female SWD) and Dallesport, WA (125 female SWD). Enough female flies were captured in WA at 3 of 4 sites. The number of female SWD captured in traps in Clayton-Conv., Spanish Castle-Conv., Clayton-Org. and Senseney-ORG. were 164, 69, 145 and 140, respectively.

Eight populations of SWD were screened against 5 insecticides (Table 2). In CA, all flies from the Brentwood site died after being treated with the target insecticides. However, a few flies collected in Tracy did survive diagnostic doses of Delegate, Entrust and Malathion. Three of four populations tested from the WA sites had some flies survive diagnostic doses. One SWD female collected from Clayton (Conv) survived Sevin, 2 SWD collected from an organic site in Clayton survived Delegate, three SWD survived malathion and another four survived Warrior II from the Spanish Castle (Conv) site, two SWD from the Clayton (Org) site survived delegate and all flies survived Entrust. All tests conducted in OR yielded no survivors.

During the assays, we discovered a couple important procedural details that must be adhered to; otherwise, results may be compromised. Simple things like shaking liquid pesticide bottles before dispensing and keeping solutions agitated during the assay procedure enhance results by minimizing settling. It is possible that some of the survivorship that occurred in a few tests was related to pesticide settling. Regardless, we have included a procedure that requires us to retest suspect populations more thoroughly using complete multiple dose-response assays (versus a single dose diagnostic assay). Results from these follow up assays will be compared against our original dose-response line parameters to determine if suspect population are becoming resistant to specific insecticides. Additionally, laboratory bioassay results will be correlated with the genomic analyses for further confirmation.

We predict that our genomic analysis will allow us to identify the genetic basis of resistant populations as determined by insecticide bioassays, and provide additional information regarding more effective management approaches. We also anticipate eventually phasing out topical assays for genomic assays owing to the assumed increase in precision. There is probably more inherent variation in bioassays using live insects than putting insect parts (DNA and such) into a machine. This will be investigated in this grant

Overall, while we have identified a few suspect populations, we are not declaring that SWD has developed resistance to commonly used insecticides. Additional work to more thoroughly assess these populations is being undertaken. More populations will be screened in year 3, 2016.

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Table 2. Pesticides used in diagnostic dose screening

Pesticide	IRAC MOA	Registrant	Lot #	Amt. formulated/ liter	Diag. dose (ppm)[2]	Form. mat./100 gal	Form mat.. i label units
Sevin 4F	1A	Tessenderlo Kerley, Inc.	NK42CX0478	86.1 ml	41,272	1102.06 fl oz	34.4 c
Malathion 57%[1]	1B	Cheminova, Inc.	--	0.87 ml	523.58	11.1 fl oz	0. 4 p
Warrior II 2.08CS	3	Syngenta Crop Protection, LLC	SET3G18011	0.44 ml	109.18	5.61 fl oz	(same
Entrust 2SC	5	Dow AgroSciences, LLC	3CD01160A87	0.92 ml	221.24	11.82 fl oz	(same
Delegate 25WG	5	Dow AgroSciences, LLC	3C06164971	0.377 g	94.35	5.04 oz	(same

[1]Malathion 5EC
[2]2X LC_{99} (twice the amount needed to kill 99% of the population)
[3]Maximum label rate for Sevin 4F is 3qt/A in OR and WA, 4 qt/A in CA

Table 3. Percentage mortality of adult female SWD 24 hr post treatment with diagnostic doses (ppm) of various insecticides

State	Site ID	% Mortality of adult female SWD exposed to diagnostic 2X LC_{99} doses				
		Delegate 25WG (94.35)[1]	Entrust 2SC (221.24)	Malathion 57% (523.58)	Sevin 4F (41,272)	Warrior (109.18
CA	Brentwood (Organic)	100	100	100	100	100
	Tracy (Conv)	91	97	90	100	100
OR	Hood River (Conv) (MCAREC)	100	100	100	100	100
	Dallesport (WA) (Conv)	100	100	100	100	100
WA	Clayton (Conv)	100	100	100	99	100
	Spanish Castle (Conv)	100	100	97	100	96
	Clayton (Organic)	98	100	100	100	100
	Senseney (Organic)	100	100	--	100	100

[1]Concentration of diagnostic dose in ppm.

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

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

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Co-PI(3): Todd Einhorn
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State/Zip: OR 97031

Cooperators: Growers: Stacey Cooper (The Dalles trial) and Alan Weaver (Parkdale trial).

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

Other funding sources
None

Budget 1: Einhorn
Organization Name: OSU-MCAREC **Contract Administrator: R. Karow**
Telephone: 541 737-4866 **Email address: russell.s.karow@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 and Parkdale; [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 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:

- Experiments for this project began fall 2015; results are forthcoming.
- We have designed a model system to evaluate the interaction between cold acclimation and *Pss* infection on freeze injury.
- Thresholds for damage of non-acclimated and acclimated whole-plants were experimentally determined via controlled rate freeze tests. An insulation chamber was constructed to maintain roots above freezing while canopies were exposed to a range of sub-freezing temperatures.
- Growth regulators, ABA and ACC, and lime sulfur applications were applied to Sweetheart trees (10/07/15). Rate of leaf senescence and abscission is currently under evaluation. Pre-treatment samples (spur wood and buds) have been harvested for determination of hardiness and quantification of nutrients. Cold hardiness and nutrient analyses will be performed weekly.

Methods:

Objective 1
Cherry rootstocks were received from North American Plants (NAP) in McMinnville, OR in late August. Plants were roughly 9 inches tall. These plants will be used in a model system to understand the interactions between acclimation and Pss inoculation on hardiness, freeze injury, regrowth and canker development in the spring of 2016. They will also provide key information regarding the sites of entry for Pss cells in damaged and non-damaged tissue. These plants are currently receiving their first set of treatments 1) Naturally acclimated under ambient, outdoor conditions 2) Non-acclimated in a greenhouse (75^{o}F daytime and 60^{o}F nighttime) and 3) artificially induced to acclimate by exposing plants to low night time temperatures within a cold storage unit and moved outdoors in the day. The forced acclimation treatment will accumulate significantly more chill units than plants acclimated under ambient conditions to provide a range of acclimation to test hardiness. Each of these 3 treatments will be divided equally, with one set of plants inoculated with epiphytic populations of *Pss* and the other half not. Based on weekly freeze analyses, we have experimentally determined temperatures of acclimated (i.e., dynamic process since lower temperatures are required with increasing chill unit accumulation) and non-acclimated plants. All plants will be subjected to a slow freeze rate in programmable freeze chambers to elicit five discrete levels of damage; no injury to complete mortality. To ensure that roots, which are markedly less hardy than shoot tissue, do not freeze insulation chambers were fabricated (temperatures did not drop below freezing in the root zones during tests). After freezing, plants will be allowed to enter dormancy under natural conditions.

In spring of 2016, plants will be evaluated for bacterial canker development and shoot emergence and growth rate. To scale up our work, we potted 200 1-year-old grafted nursery trees in spring 2015. Trees have been trained to generate sufficient sample tissue. Trees will be grown throughout 2016 and subjected to a similar study based as described above. Interestingly, 20% of the trees collapsed rapidly due to *Pss* infection (confirmed by OSU-Corvallis). Another 20% collapsed midsummer. Surviving trees were segregated.

Objective 2
Plant tissue collected from the above treatments, in addition to field grown trees located in Corvallis, will be examined using the scanning electron microscope at the Oregon State University Electron Microscopy facility in Corvallis and the light microscope at MCAREC. Cross-sectioned nodes, including buds and petiole scars, will be examined for congregation of *Pss* bacterial cells located in and around natural openings and wounds induced by freezing. Tissue will be preserved in fixative. Tissue from Objective 1 will certainly be used and tissue from Corvallis will be used if correct weather events occur.

Objective 3
In grower collaborator orchards, treatments of elemental (lime sulfur) or commercial plant growth regulators (ABA and ACC) are under evaluation for their ability to induce early defoliation and cold hardiness. The rates of defoliation are presently being examined objectively as the percentage of leaves to senesce and abscise (4 shoots per rep). One site is currently being used to further evaluate potential treatments & timings of the above compounds. Using a differential thermal analysis (DTA) system, acclimation and hardiness can be measured within a relatively high-throughput fashion. Our DTA system was modified from Mills et al. (2006). Briefly, samples are wrapped first in a piece of damp paper towel then aluminum-foil, placed on thermo-electric modules (TEMs) and subjected to a slow rate of freeze of 1°C to 2°C per hour. The TEMs are wired into a data acquisition system (Keithley 2700-DAQ-40) and data are recorded at 5 to 30 second intervals. The exotherm (heat release) associated with the phase change of water from liquid to ice produces a temperature gradient across the TEM resulting in a voltage output. Voltage output is plotted against temperature to facilitate graphic presentation of kill points. Temperature in the freeze chamber is measured via thermocouples placed on reference TEMs without tissue. Up to 30 TEMs can be used for each freeze chamber test and at least ten buds or a single node can be placed on each TEM (i.e., in the case of buds, a few hundred buds are evaluated in each freezer run). DTA runs of flower buds and shoot tissue will each occur twice monthly in alternating weeks. Methods developed by Dr.Einhorn's program will be used. In addition to data generated from the TEMs on the shoot tissue, a color grade will be assigning ratings of stem browning.

Objective 4
Freeze damaged tissue from the inoculated trials will be examined spring 2016 to determine which temperatures were damaging enough to allow *Pss* infection. Protocols are currently being developed to hold tissue in fixative for all treatments to facilitate appropriate evaluations at later dates.

Plan for 2015/2016.
We plan on completing the outlined experiments from above.

CONTINUING PROJECT REPORT **No Cost Extension**

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

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Mike Willet, Northwest Horticultural Council

Other funding sources:
Budget 1
Organization Name: USDA-ARS **Contract Administrator:** Charles W. Myers
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WTFRC Collaborative expenses: None

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

Item	2015	2016	2017
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**	$5,000

***Fruit was not purchased in 2015 and money was not disbursed. It is requested that the funds be carried over to 2016 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 submission date, fall of 2016

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 and NWHC for consideration.

2) Draft a USDA-FAS-TASC grant, with sponsorship from WTFRC (or NWHC), pertaining to the development of postharvest techniques to break MRL-trade barriers for the Northwest sweet cherry and apple industries.(A SPONSOR ORGANIZATION IS CRITICAL, The grant has been drafted and submitted to WTFRC -McFerson- for review and consideration).

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