FINAL PROJECT REPORT 2014

Project Title: Consulting to the WTFRC and OSCC for cherry improvement

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Cooperators: Jim McFerson, Cameron Peace, Nnadozie Oraguzie, Amy Iezzoni

Total Project Funding: $7,500

Budget History:

Item	2014
Salaries	
Benefits	
Wages	
Benefits	
Equipment	
Supplies	
Travel	$4,000
Plot Fees	
Miscellaneous	3,500
Total	$7,500

ORIGINAL OBJECTIVES:

1. Provide analysis and critique of technical aspects of proposals and reports for competitive funding of research and development related to cherry improvement.

2. Provide ideas and analysis of approaches and methods to facilitate adoption of new sweet cherry cultivars by clientele groups in the sweet cherry production and delivery pipeline.

3. Facilitate adoption and use of technology and materials from research projects worldwide to support sweet cherry improvement.

4. Interact with WSU, OSU and ARS scientists and PNW growers on scientific matters related to cherry improvement for the region.

ACTIVITIES AND ACCOMPLISHMENTS:

- Provided expertise and analyses to WTFRC and OSCC
    - Reviewed and critiqued research proposals and reports to the Boards
    - Critiqued proposals from cherry team members to WTFRC and competitive grants programs.
    - Visited California nurseries (Duarte and ProTree) with Amy Iezzoni

- Participated in the NW Cherry Research Review Nov. 12 & 13, 2013 in Wenatchee, WA
    - Presented assessment of and discussed proposals
    - Participated in Cherry GGB workshop prior to the Cherry Research Review

- Attended the Plant and Animal Genome Conference (Fruit and Nut Crop Workshop) and other sessions, January 11, 2014, San Diego, CA.

- Facilitated interaction among breeders and scientists.
    - Participated in discussions with members of the guiding committee about and reviewed drafts of the new RosBREED2 proposal.
    - Reviewed and discussed cherry research data and information with PNW and other researchers.
    - Provided information about graduate education for future plant breeders and plant breeding capacity needed in fruit and nut crop breeding.

- Alerted cherry team members to key references for breeding and genetics of sweet cherry.

- Submitted invoices for expenditures on a quarterly basis.

RESULTS & DISCUSSION:

I provided science-based reviews of proposals and reports for which I have expertise to the commissions. In addition to those submitted for support from the commissions, researchers continue to submit good proposals that are competitive and are being funded at a level commensurate with other public institutions. The grant proposals I reviewed have innovative ideas and approaches that I believe contribute to continued competitiveness and opportunities for funding that will benefit the PNW cherry industry. Amy and I visited the Duarte and ProTree nurseries in California in February

to see their operations for the production of cherry (and other) rootstocks using tissue culture. Both are producing large numbers of uniform, high quality plant materials for use in producing compound cultivars for growers.

The cherry GGB workshop organized by Dr. Oraguzie and held prior to the Research review was a good forum for presentation of research findings and information by PNW scientists. The morning session is primarily for discussion of scientific issues among the scientists, while the afternoon session is for a breeding program update for commission advisory committee members. Overall the cherry breeding program benefits from collaborative interaction with other supporting scientists in the PNW (WSU, OSU, ARS, WTFRC and others) who devote significant resources and effort to issues and opportunities impacting sweet cherry improvement and the tree fruit industries. Collaboration and exchange of ideas among members of the cherry improvement team promotes synergy and minimizes redundancy and duplicated effort.

The federally-funded RosBREED project was successfully completed and most of the activities, milestones and goals achieved. Scientists associated with the PNW Cherry Improvement have played key roles in the success of RosBREED and the functional Genome Database for Rosaceae (GDR) which is critical for success of DNA-facilitated breeding, genetics and genomics. The PNW cherry breeding program stands to be a major beneficiary of tools and materials for DNA-informed breeding when it is well integrated into a targeted breeding program for cultivar development. Without these projects it would not have been possible to utilize the extensive tools and information that are changing all areas of crop improvement. The availability of these collaborative, community wide projects have been very important for implementing and will have a continuing impact on the ultimate success of the cherry breeding program, measured by development and release of outstanding new cultivars for the cherry industry. The WTFRC and OSCC have been key supporters of these initiatives. It is a win-win situation wherein provision of matching funds leverages several times more federal funding for programs important to continuing profitability for the PNW cherry industry. This is the only public sweet cherry breeding program in the U.S. taking advantages of these key resources.

For the PNW cherry breeding program, DNA-based information and technology are critical plant breeding capacity elements for success and efficiency. Diagnostic marker-locus-trait (M-L-T) associations are becoming available for a growing number of important traits, e.g., incompatibility/ fertility alleles, fruit maturity date, fruit size, firmness, color, flavor components, etc. Others showing promise include flowering time, cracking, stem retention force. Using tools from the breeder tool box, informed decisions can be made for parental choice and production of the most efficient crosses for segregating traits. Molecular genotyping provides the opportunity for early generation, marker assisted selection of preferred genotypes, genetic verification of selected phenotypes, and genetic fingerprinting of elite selections for intellectual property protection.

Continuing collaborations among breeders and other scientists include; new sources of genetic variability for important fruit traits (Iezzoni), data base management and breeder toolbox (Main), development of marker-locus-trait associations and genotyping of breeding materials (Peace), genome sequencing and Fast-trac breeding (Dhingra), testing and commercial evaluation of elite selections (Einhorn, Long, Whiting, Commission scientists). Linkages to foreign scientists through personal interactions and various international meetings support a robust global Rosaceae community. Interaction among these (and other) programs is critical to continued support and success.

A new project, "'RosBREED: Combining disease resistance with horticultural quality in new rosaceous cultivars' has been recommended for funding by the USDA-NIFA Specialty Crop Research Initiative. The $10 million grant means five more years of research and innovation in rosaceous crop

breeding programs across the country." Amy, Jim and Cameron were key members of the steering committee developing this project, with contributions from many others. I had the opportunity to participate during development and will serve as a member of the Scientific Advisory Committee. It will be a critical resource for the PNW cherry breeding program.

Because of continuing restrictive research and development budgets in all sectors – private, state and federal - it is important to prioritize cherry improvement activities in order to maximize return on investment for all programs. Advancement of promising new selections into pre-commercial testing and evaluation is progressing as anticipated. Collaborative efforts among the breeding team, growers in Washington and Oregon, and WTFRC personnel will ensure effective evaluation and that the selections meet industry needs and opportunities to expand profitability. New elite selections will be identified each year in the breeding cycle. Therefore, it is important to have a strategy to utilize phenotypic and molecular marker information along with grower evaluations and feedback from various stage trials to decide whether to either discard/discontinue selections or introduce and release them as new commercial cultivars.

Along with the research and breeding studies, these projects provide opportunities to train and prepare the next generation of breeders and genetic support scientists at W.S.U. Grad students and post doctoral researchers often have key roles in the programs. I continue to work with faculty to review curriculum and program components of plant breeder education and training.

EXECUTIVE SUMMARY
Title: Consulting to the WTFRC and OSCC for Cherry Improvement
PI: Fredrick A. Bliss

WTFRC and OSCC Funding: $7,500

After a decade of consulting to the WTFRC and OSCC for cherry improvement, I am not submitting a funding request for the coming year. The breeding, genetics and genomics programs have made excellent progress and have reached a level of maturity and productivity such that there have been minimal requests for my input and expertise the past year. The information, tools and materials provided through the initial RosBREED program and that will continue from RosBREED2 were possible in part because of the exceptional support and collaboration from the PNW industries, WSU and the ARS-USDA. This is a very strong and productive private-public partnership that you are rightly proud of and should continue to support with clear direction and commitment. I have enjoyed the opportunity to work with all of you and surely have gotten as much or more than I have given. I will remain engaged with fruit breeding through participation in RosBREED2 and other interactions in the public and private sectors. I expect to see a continuing stream of exciting new cherry cultivars and rootstocks moving into commercial use in the PNW and fruit of unprecedented quality in the market place.

My objectives were to: 1) Provide analysis and critique of technical aspects of proposals and reports for competitive funding of research and development related to cherry improvement; 2) provide ideas and analysis of approaches and methods to facilitate adoption of new sweet cherry cultivars by clientele groups in the sweet cherry production and delivery pipeline; 3) facilitate adoption and use of technology and materials from research projects worldwide to support sweet cherry improvement; and 4) Interact with WSU, OSU and ARS scientists and PNW growers on scientific matters related to cherry improvement for the region.

These objectives were met through telephone calls, electronic communication, and participation in various meetings. Activities included: 1) reviewing and critiquing research proposals from cherry team members and other scientists as requested; 2) participating in the Cherry Research Review and the GGB workshop prior to the annual research meeting; 3) working with the steering committee to develop a new RosBREED project proposal that has recently been approved, 4) facilitating interaction among breeders and scientists; 5) alerting cherry team to key references and ideas for breeding and genetics of sweet cherry, and 6) working on education and curriculum for breeders.

My role was to provide information and feedback to Jim McFerson and Board members about progress toward objectives and to support the breeder and researchers working on this project. I worked with researchers, cooperators and members of the industry to provide expertise and knowledge about fruit breeding. I provided insight, guidance and ideas for identifying and applying appropriate technology to facilitate efficient cultivar development. I accompanied Amy on a visit to the Duarte and ProTree nurseries in California in February to see their operations for the production of cherry (and other) rootstocks using tissue culture. I evaluated research proposals when requested.

The PNW cherry breeding program can be a major beneficiary of tools and materials for DNA-informed breeding when it is well integrated into a targeted breeding program for cultivar development. This is the only public sweet cherry breeding program in the U.S. taking advantage of these valuable resources. Interaction among university, ARS-USDA and commission internal programs provides for continued support and success. The advancement of promising new selections into pre-commercial testing and evaluation is gaining momentum. Collaborative efforts among the breeding team, growers in Washington and Oregon, and WTFRC personnel are required to ensure effective evaluation and that the selections meet industry needs and opportunities to expand profitability.

FINAL REPORT **YEAR: 2 of 2**

Project Title: Early season estimation of fruit set and size potential

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Cooperators: Matthew Whiting

Total project Funding: $120,874

Other funding sources: None

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

Item	2013	2014
Salaries	28,784	29,648
Benefits	18,064	18,604
Wages	3520	3520
Benefits	352	352
Equipment		
Supplies	2310	1960
Travel	1000	1000
Miscellaneous		
Plot Fees		
Total	54,030	55,084

Footnotes: Salaries for 0.75 FTE postdoc (3% is added to year 2); benefits were calculated based on Actuals; wages are for 300 hours part-time summer employee for image analysis of cherry fruit ($11/hr); benefits for part-time (10%); supplies include fixative, PGRs, tubes for storage of fruit in fixative, bee exclusion netting (only factored into year 1), Ziploc plastic bags, flagging and lab tape for limb and fruit selection; travel includes 1,700 miles estimated for all sample collections and growth rate analyses at $0.55 per mile.

Budget 2- Long
Organization Name: OSU-MCAREC **Contract Administrator: L.J. Koong**
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Item	2013	2014
Salaries		
Benefits		
Wages	4800	4800
Benefits	480	480
Equipment		
Supplies	200	200
Travel	400	400
Plot Fees		
Miscellaneous		
Total	5880	5880

Footnotes: Wages are for 2.5 months of part-time summer employee for fruit sample collection ($12/hr); benefits for part-time (10%); supplies include Ziploc bags, flagging, and lab tape and dry ice for transport; travel includes 740 miles estimated for all sample collections for fruit set estimates and growth rate analyses at $0.55 per mile.

Objectives:

1) Develop sampling and measurement protocols at the tree, row and orchard scale for Rainier, Bing, Chelan, and Sweetheart. Define the number of fruitlets required for precise crop estimates
2) Analyze growth rates of unfertilized and fertilized fruit of Rainier, Bing, Chelan, and Sweetheart to strengthen our model
3) Develop models of fruit growth that incorporate calendar date and growing degree units so they may be broadly applicable to the cherry growing regions of the PNW
4) Time whole-tree PGR applications with early-season growth of cherry and determine their effect on fruit set, yield, harvestable fruit size, and fruit quality

Significant Findings:

1) The dry weight of 2000 to 3000 ovaries sampled randomly was sufficient for crop estimates by 18 days after bloom
2) Ovary length to width ratios improved detection of potential fruit versus developmentally failed fruit
3) Crop estimates based on fruit from 30 spurs per sampling date, when combined with ovary shape, provided estimates of fruit set by 20 days after bloom
4) Sweetheart grown in three locations with differing seasonal temperature indicated the Base Temperature for accumulation of Degree Days (43°F) is inappropriate and should be lowered
5) Pre-bloom ovary growth was significantly and positively related to temperature
6) The calendar day order for beginning of the Sweetheart season at five locations was The Dalles (BA, SK, JH), Hood River and Parkdale
7) 40°F was sufficient, and 50°F was near the upper limit of a growth response in the green tip phase
8) 70°F produced a large growth effect during the open cluster and first white phases
9) Flowers that bloom early, with respect to average bloom date, produce larger fruit at harvest
10) Pre-bloom (~first white) application of Promalin or cytokinin alone (CPPU) increased fruit size between 7% and 14% when sampled around pit hardening. Promalin significantly increased stem length and leaf area indicating absorption

Results:

Fruit Growth. Our first goal was to complete a growth analysis from dormancy to bloom. An essential component of these growth analyses was the segregation of fertilized fruit from non-fertilized fruit, *prior to their abscission*. These two populations cannot be statistically differentiated within the first 18-20 days from bloom based on their growth rates (Fig. 1).

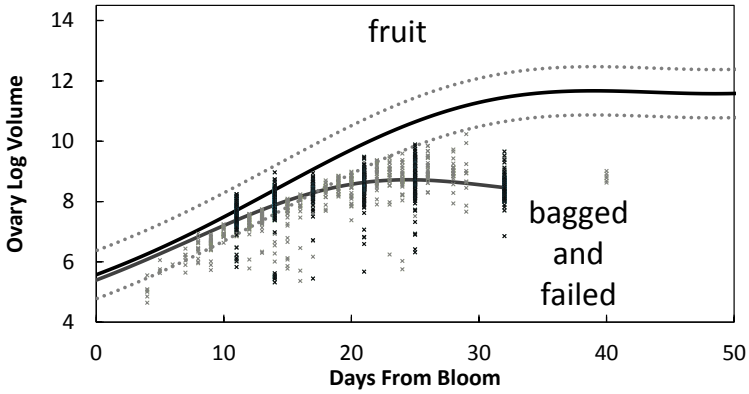

Figure 1. Ovary growth from bloom of a population of fruit comprising both fertilized fruits and fruits destined to abscise compared to non-fertilized fruits developing in bee-exclusion bags.

We then eliminated all fruit that were destined to drop through statistical procedures, of cluster and discriminate analysis, in order to only describe the growth of harvestable fruit of Chelan, Bing, and Sweetheart. Surprisingly, relative growth rates (and timing) defining growth of early developmental stages (First swelling through Stage II) did not differ among these three cultivars (Table 1).

Table 1. Days from bloom of growth phase transitions determined from the minima, maxima and up or down inflexion points of relative growth rate (RGR) curves (not shown).

Variety	**Growth phase**							
	FS,SG	SG,GT	GT,OC	I	I,II	II,III	III	Maturation
	Direction of relative growth rate curve							
	minimum	down	up	maximum	up	minimum	maximum	asymptote*
	Days from bloom							
'Sweetheart'	-39	-31	-17	11	29	44	60	75,79,88
'Bing'	-37	-29	-14	12	30	45	64	70,72,77
'Chelan'	-38	-29	-14	15	30	43	56	59,61,65

First swelling (FS), side green (SG), green tip (GT), open cluster (OC).
**Days from bloom of the additional 90, 95 and 99% increase in phase III volume as determined by logistic functions.*

Based on these similarities, we then developed sampling protocols that provide a good representation of fruit set and variability in fruit size. We attempted moderate (300) and large (3,000) fruit sampling protocols.

Fruit set. Set was determined in two ways. Recounting fruits per flower on flagged limbs at weekly intervals during the season yielded good results but was difficult (see last year's continuing report). A more random sampling proved to be more informative. Sampling at random for dry weight measurements was good but required a lot of sample (>>1000; Fig. 2). A convenient unit to base fruit set on is the spur. Spurs can be sampled as random units throughout the orchard and based on pre-determined average bud and flower numbers per spur (Table 2), the fruit remaining on a spur represents the percentage of fruit set (Table 3). In comparison to limb sampling, sampling entire spurs captured much of the variability and was possibly more accurate; this is attributed to each spur representing flowers at various stages of development so sampling by single spurs from many trees is more likely to represent the orchard as a whole.

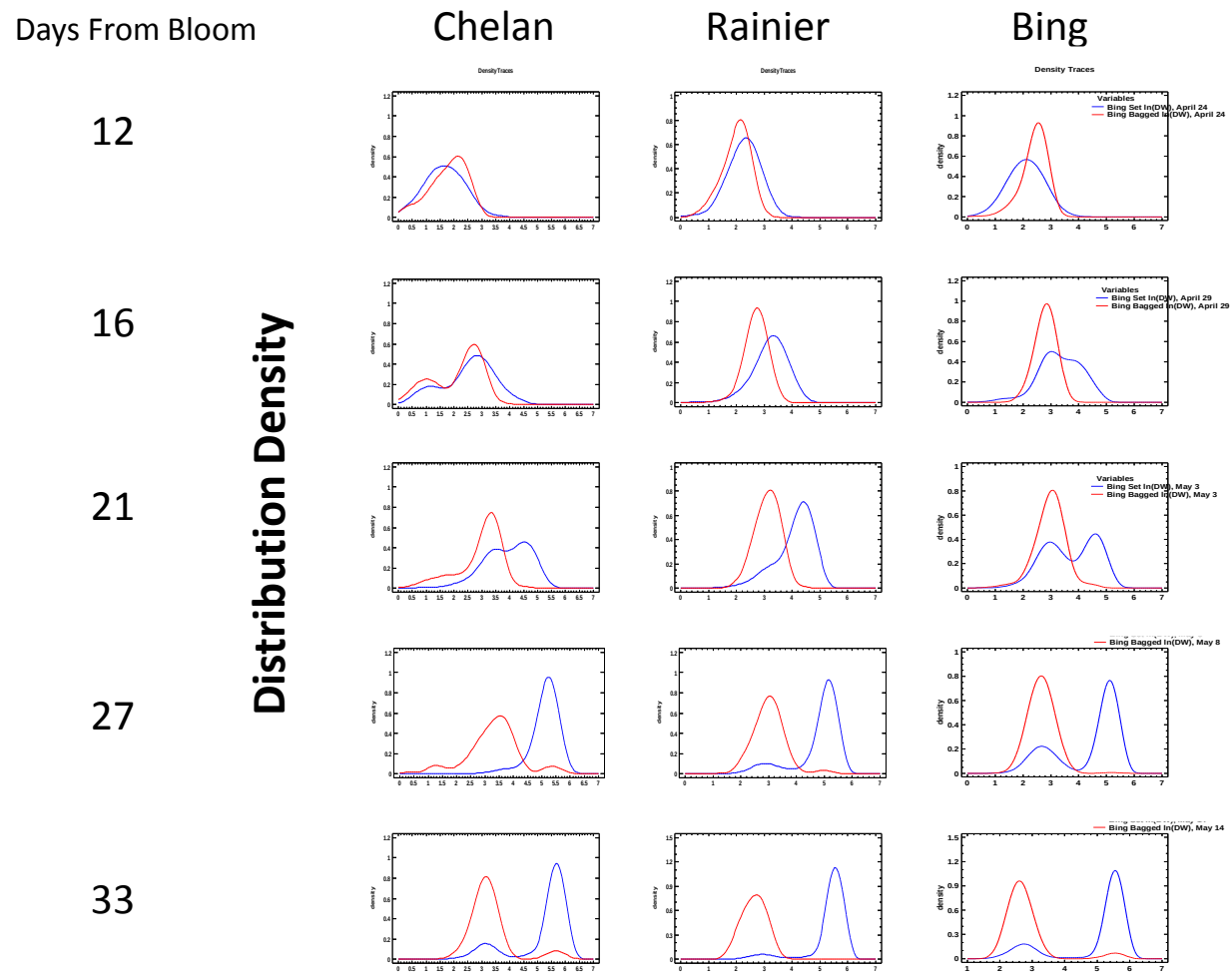

Figure 2. Dry weight gave a sufficient early estimate of fruit set, 16 to 21 DFB, but only if >1000 ovaries were measured. Populations of fruit form two distinct curves- the curve to the left of each graph is for bagged, non-fertilized fruit. The curve to the right is from a random sampling (it is comprised of both fertilized and unfertilized fruit, as can be seen by the bi-modal distribution beginning ~16 to 20 DFB).

Table 2. Spur data used for the fruit set and growth analysis of Sweetheart across multiple sites. This baseline data were best taken before bud break.

	The Dalles			Hood River	Parkdale
	BA	SK	JH	HR	PD
	Average of 30 spurs				
Flower per bud	2.87	3.06	3.48	3.06	3.69
Bud per spur	4.42	4.37	4.44	5.12	3.61
Potential Flowers per spur	12.6	13.3	15.4	15.5	12.8

Location, which includes biological variability attributed to tree age, rootstock, etc., affected flower and bud number.

To reduce sample size we developed a better sampling protocol. In addition, a more sensitive, discriminant measure of ovaries was conducted by integrating shape and volume estimates from

digital images (data not shown). A fruit set estimate was reliably detected about 15 DFB from 200-300 fruit collected from sampling 30 spurs on separate trees (Table 3).

Table 3. Fruit set of Sweetheart determined from spur sampling and photographic analysis.

	The Dalles			Hood River	Parkdale
	BA	SK	JH	HR	PD
			% fruit set		
10 to 19 DFB	47	52	68	37	35
Harvest	41	46	56	37	42

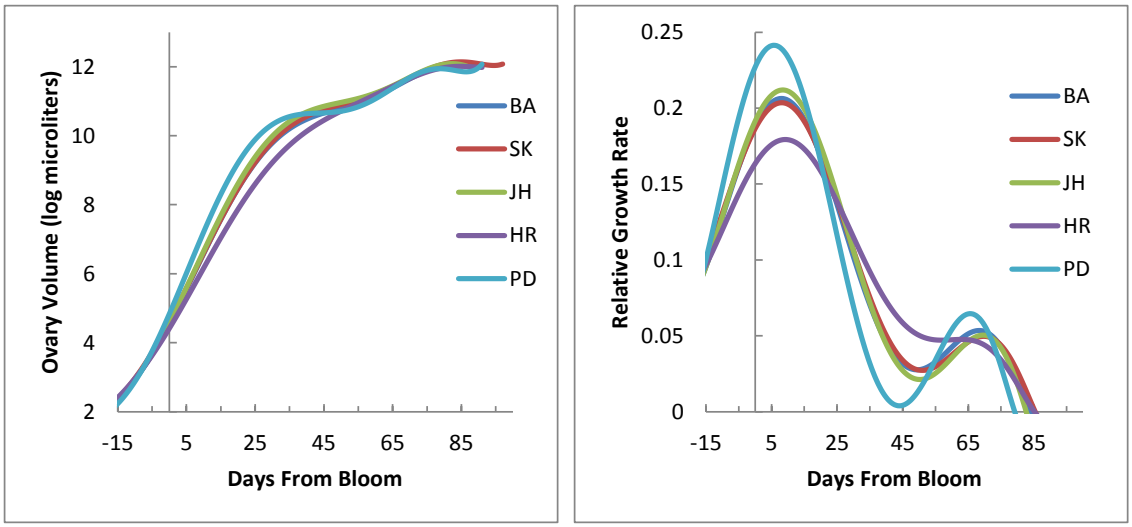

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Figure 3. Growth curves of Sweetheart were derived from spur sampling twice weekly at five locations. Left: growth in volume expressed in logarithmic form. Right: Relative growth rates. The similarity of the minima, maxima and inflection points (data not shown but see Table 1) on relative growth rate curves from 2014 (Fig. 3, right panel), and those of the previous year (provided in 2013 continuing report) show synchrony in development despite varied environments.

Sources of variability in growth and fruit size. Bloom dates have always presented a question mark with no uniformly agreed upon protocol for its determination. And this is surprising considering how important bloom date can be in determining fruit size. Given that cherries are typically harvested in one pass, bloom that is significantly behind the curve (as we have previously demonstrated) do not catch up and will be smaller at harvest. The most straight forward way to approach this question is to count blooms as they open (Fig. 4). As expected, a range in bloom progression and timing was observed at different sites. A consistent ranking of size on given dates was not found between sites; however, after pit hardening (45 DFB) fruit from sites in The Dalles were larger than Hood River and Parkdale where protracted bloom periods were observed (Fig. 4).

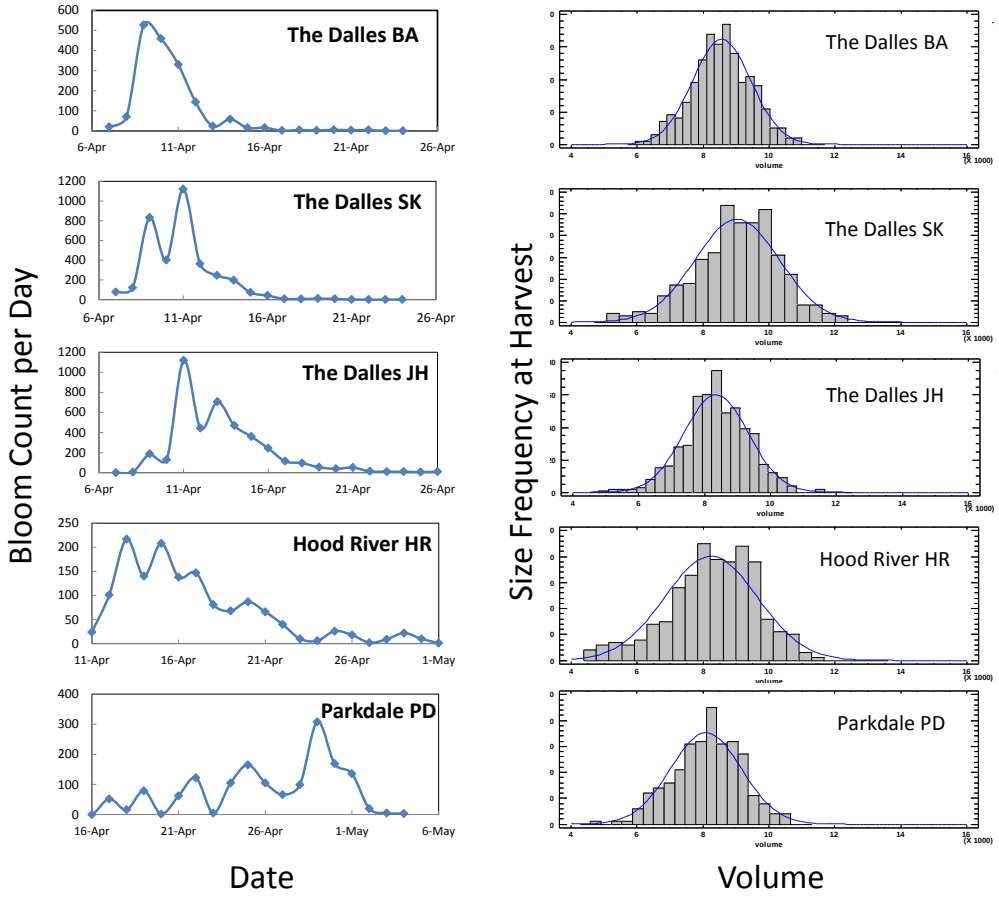

Figure 4. Bloom progression of Sweetheart at 5 sites may offer insights into fruit volume differences at harvest. Left panel: Blooms were removed and counted on the day they opened from portions of 15 limbs (of separate trees). Right panel: The narrowest distribution in fruit size was from site BA (10% variation) and the broadest was HR (17% variation) mirroring the bloom progression. PD had fewer large fruit than may be expected (poor pollination of early bloom) explaining the smaller size, but narrow distribution.

We've settled on an approximate 50% bloom to begin our fruit growth and set calculations, but this choice is debatable (HR) or delayed blooming (PD) could have a large effect on the variation of fruit size, and possibly detrimental to overall size if the early bloom was left unfertilized. Additionally, the prolonged bloom would have affected the fruit vs. failure determination adding to the variation. HR and PD were smaller and had long duration of bloom.

An experiment with Regina also tested the importance of bloom date and its relationship to final fruit size. In 2014, 250 flowers were tagged each day as they opened from the beginning to the end of the bloom period. At harvest the fruit were recovered to record the fruit size. As we have previously shown, early flowers yield the largest fruit (Fig. 5). Interestingly, fruit set of this orchard was quite low indicating that even under ample carbon supply, potential fruit size (of later blooming ovaries) cannot be made up.

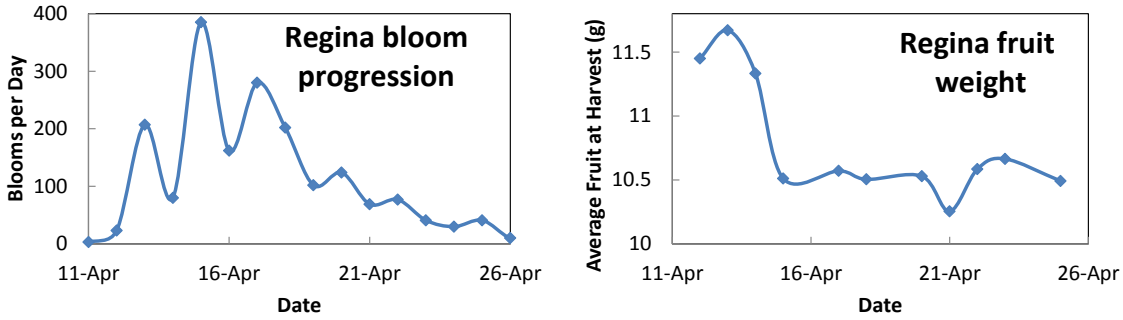

Figure 5. Relationship of bloom date to fruit size. The first three dates of bloom resulted in significantly larger fruit.

Growth models.

Temperature affects the progression of bloom and the growth of ovaries. We experimentally manipulated temperature prior to bloom in order to determine temperature optima for ovary growth. This is a necessary step toward model development. For these experiments, Bing and Regina whole limbs were harvested and placed in temperature controlled growth chambers. These two cultivars were selected based on their different developmental timelines in early spring. As low as 40°F was sufficient for growth effects approximately 22 DFB (i.e., in the green tip phase; Fig. 6). Near 50°F was probably the upper limit for growth but did appear to have a marked influence on Regina ovary growth.

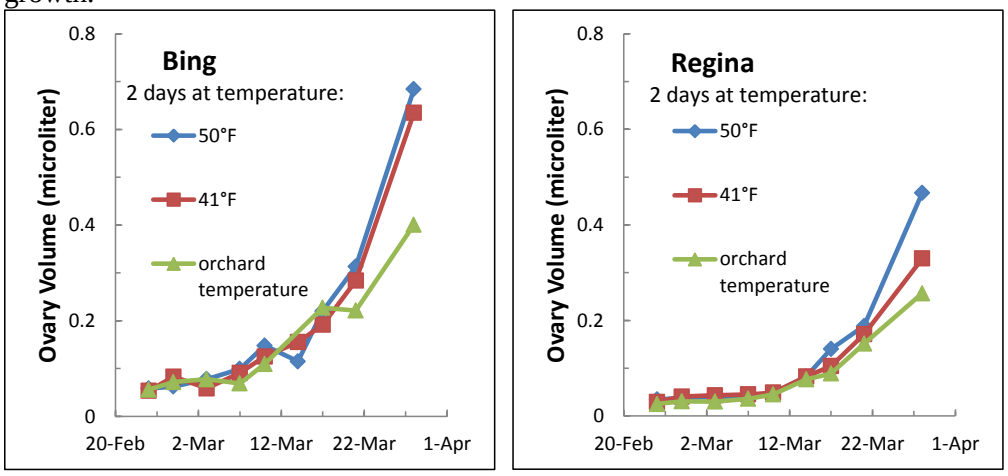

Figure 6. Growth of Bing and Regina ovaries between dormancy and green tip as affected by temperature.

However, for advanced stages of bud break, temperatures of 70°F produced a significant growth effect approx. 9 DFB when buds were in the open cluster to first white phases. These responses need to be expanded upon (see Einhorn New Proposal) in a systematic manner to determine how temperature optima for growth change with development. This is absolutely essential to the development of an accurate growth model.

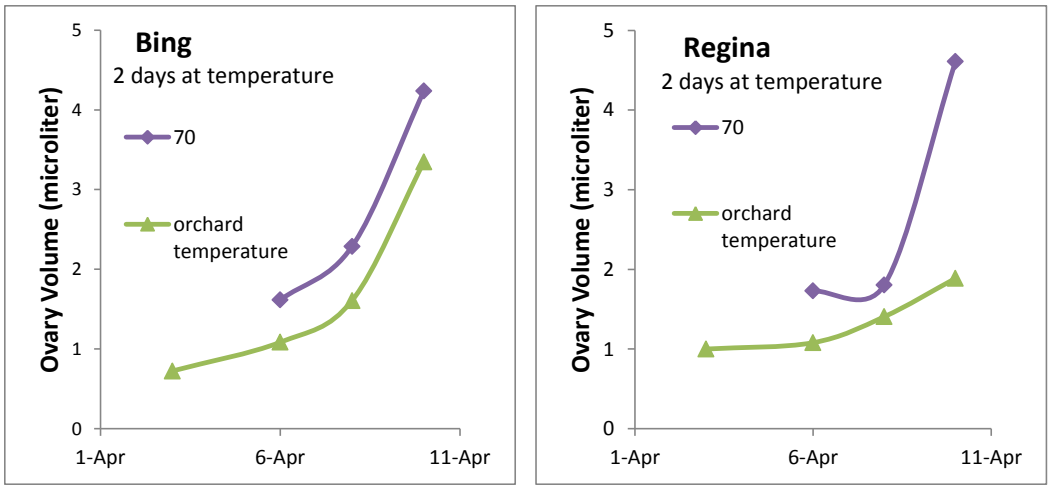

Figure 7. Growth of Bing and Regina ovaries in advanced stages of bud-break.

Adjusting model indices in step with the season

In addition to experimentally determining the optima of the growth response, temperature indices can be manipulated to explain the seasonality of growth, especially post-bloom. We created a spreadsheet with inputs for temperature data from the IFPnet, sunrise and sunset data from the Naval Observatory, and of course growth measurements. The spread sheet uses easily adjusted temperature indices for asymmetric curves of the growth response to temperature. The temperature response we observed in the pre-bloom phases (Fig 6 and 7) matches well with the empirical choice of temperature indices we used in our new model (Table 4).

Table 4. Adjusting temperature indices can result in a more accurate model.

Location, Year	Calendar Days From Bloom	Linear Degree Hour Model	Our NEW Adjusted Degree Model
The Dalles, 2013	91 (April 14)	20820	6129
Hood River, 2013	**92 (April 21)**	**27085**	**6077**
Parkdale, 2013	97 (April 27)	25252	6119
The Dalles2, 2011	**95 (May 2)**	**27026**	**6150**
Average Coefficient of Variation	94 3% (+/- 3 days)	25046 12% (+/- 11 days)	6119 0.5% (<1 day)

This model changes indices for day/night, and seasonal progression. Day and night indices are changed to account for photosynthesis and respiration, while indices are also adjusted seasonally to account for phenology and year to year variation.

PGRs

Stem growth is complete by pit-hardening; in nearly every case pre-bloom applications of solutions containing GA were highly effective in elongating stems (comparable results were observed, but not quantified, for leaf area- a process similarly completed in a relatively short time span). These data provided evidence of uptake and translocation when applied at first white; a possibly prohibitive time given the relatively limited supply of absorptive green tissue present. Fruit growth, however, appeared to be more greatly affected by cytokinins. Packout data (~2,000 fruit per treatment) of Sweetheart revealed a significant size improvement for the prebloom (-7 dfb) CPPU application producing 72% 9.5 row and larger fruit compared to 59% for the control.

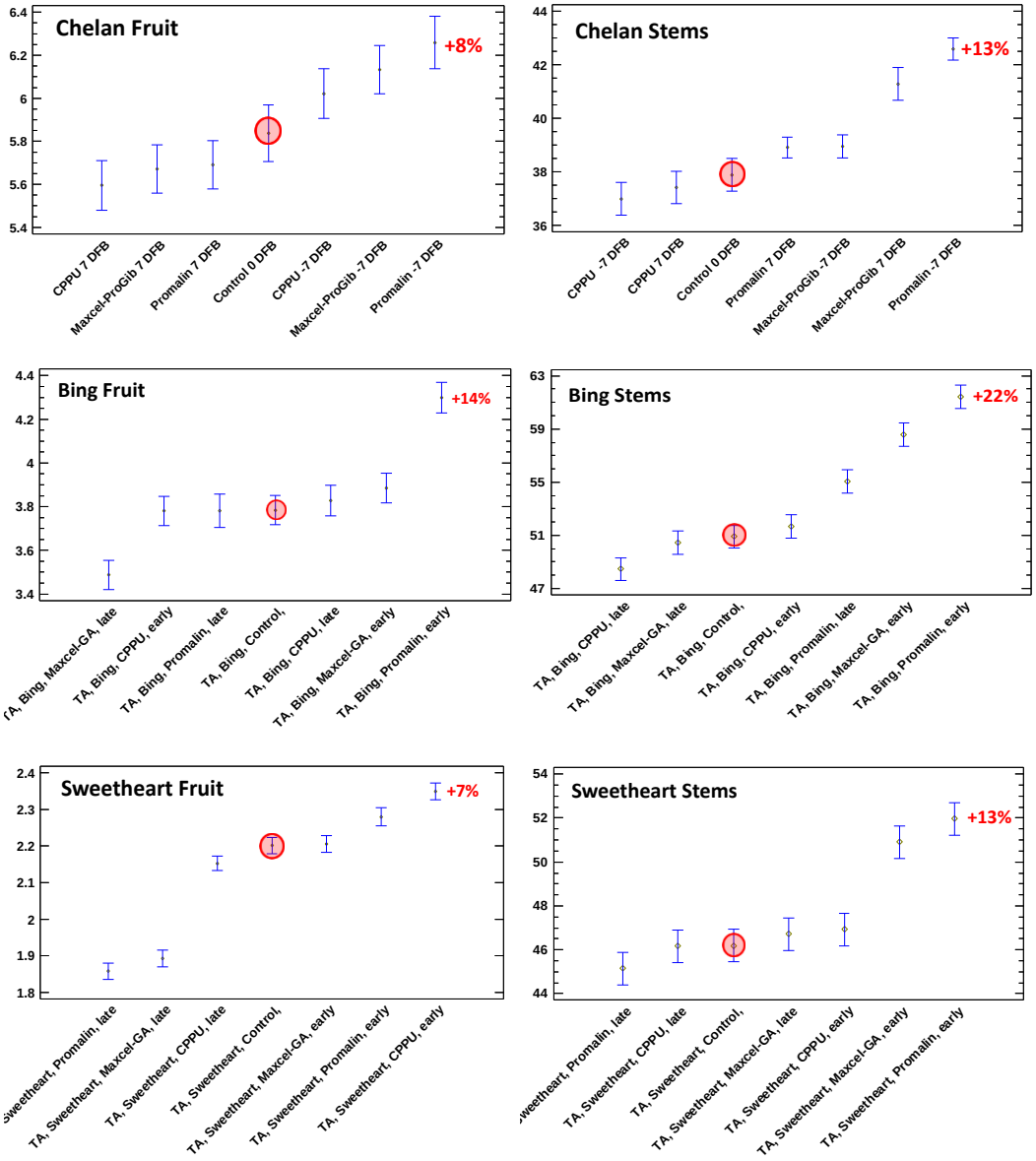

Figure 8. Pre-harvest sampling of Chelan, Bing, and Sweetheart fruit from a commercial orchard in WA. Fruit size data are grams (n=250); stem length data are mm (n=250). Treatments on the x-axis are ascending with respect to the data and are therefore not consistently ordered across graphs. On each graph, controls are circled for comparisons and treatments showing the greatest percent increase relative to the control are indicated. Late applications were performed at ~7 days after bloom; early applications were made between open cluster and first white (-5 to -7 days from bloom).

These data aligned with our pre-season measurements, which were taken prior to pit hardening (in the case of Sweetheart). For Bing and Chelan, however, no significant differences at harvest were quantifiable- a perplexing outcome given a visibly noticeable size improvement in rows treated with early Promalin. Our pre-harvest sampling of individual fruit of Chelan, for example, was taken ~2.5 weeks prior to harvest. Chelan cropload (and yield) was exceptionally high, and could have increased the demand for carbon during the last few weeks of stage III growth, thus limiting the growth potential established early by CPPU and Promalin, relative to controls. The greater leaf area, produced by Promalin in particular, would have likely augmented carbon available to supply fruit. More work is needed on early-season PGRs before programs can be recommended.

Executive summary

Growers can use these guidelines for assessing their orchard:

- Sample one spur from at least 30 trees for a good size and set estimate
- Count bloom progression from one limb portion from 15 trees to set bloom date accurately
- 40°F is sufficient to enhance ovary growth at green tip phase
- 70°F at open cluster to first white advances growth considerably
- Good crop estimates can be made 20 days after bloom
- Pre-bloom PGR applications increased fruit size, stem length and/or leaf area
- Effort to set early bloom should be made; these flowers produce big fruit

Further work is proposed because:

- Maturation could be better qualified with photographic analysis of color
- A more descriptive model of growing degree units can, and needs, to be done
- Early season cytokinin sprays to enhance fruit size appear promising

CONTINUING PROJECT REPORT **YEAR:** Year 1 of 2
WTFRC Project Number: TR-13-105A

Project Title: Improving tools for early detection of brown marmorated stink bug

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Co-PI: Ashot Khrimian
Organization: USDA-ARS
Telephone: 301-504-6138
Email: Ashot.Khrimian@ars.usda.gov
Address: 103800 Baltimore Blvd.
City/State/Zip: Beltsville/MD/20705

Cooperators: Todd Murray, Skamania County Extension

Budget: **Year 1**: $110,927 **Year 2: $39,863**

Other funding sources

Agency Name: USDA SCRI awarded to Washington State University, Brunner
Amt. awarded: $67,693 over three years (2012-2014)
Notes: This SCRI grant provides funds to Washington State University to assess distribution of BMSB in WA and to evaluate pheromone technology. Some of the funding (≈ $40,000) from the WSU portion of the SCRI BMSB budget will be used to support the activities proposed here.

Agency Name: USDA SCRI awarded to Oregon State University, Shearer
Amt. awarded: $146,995 over three years (2012-2014)
Notes: This SCRI grant provides funds to Oregon State University to develop management strategies for BMSB across several crops. The funds requested here are not provided in the SCRI funding.

Agency Name: USDA SCRI awarded to USDA-ARS, Leskey
Amt. awarded: $559,072 over three years (2012-2014)

WTFRC Collaborative expenses: *None*

Budget 1:
Organization: WSU-TFREC **Contract Administrator:** Carrie Johnston; Joni Cartwright
Telephone: 509-335-4564; 663-8181 X221 **Email:** carriej@wsu.edu; joni.cartwright@wsu.edu

Item	2013	2014
Salaries	14,080	**0**
Benefits	5,562	**0**
Wages[1] (temporary labor)	11,520	**7,200**
Benefits [1]	1,118	**698**
Supplies[2]	500	**1,000**
Travel[3]	5,032	**1,680**
Total	37,812	**10,578**[4]

Footnotes:
[1] Temporary labor – (1FTE, $15/h, 12 weeks); benefits at 9.7%.
[2] Includes pheromone, traps and monitoring supplies.
[3] 3.000 miles @ $.56 per mile.
[4] *Justification for changed budget* – due to a delayed start to the project we missed the spring activity for BMSB so were not able to accomplish all the tasks identified for year one, therefore, we had carry over funding that extends into year two so are asking for less funding.

Budget 2:
Organization: Oregon State Univ. **Contract Administrator:** Kelvin Koong
Telephone: 541-737-3228 **Email:** L.J.Koong@oregonstate.edu

Item	2013	2014
Wages[1] (temporary labor)	11,250	**7,830**
Benefits (10%) [1]	1,125	**4,385**
Equipment	9,800	**0**
Supplies[2]	3,000	**5,500**
Travel[3]	1,000	**1,000**
Total	26,175	**18,715**[4]

Footnotes:
[1] Temporary labor (1 FTE, $15/h, 3mo); benefits at 56%.
[2] Includes supplies for analyzing plant chemicals plus monitoring supplies.
[3] Within state travel.
[4] *Justification for changed budget* – the budget for OSU is slightly higher ($5,000) than was anticipated in the year two budget in the original grant. These extra funds are to cover work conducted by Elizabeth Tomasino for assistance with GC-Mass Spec analysis of host plants in OR and WA.

Budget 3:
Organization: USDA-ARS-NAA **Contract Administrator:** Ingrid Charlton
Telephone: (215) 233-6554 **Email:** ingrid.charlton@ars.usda.gov; naagrants@ars.usda.gov

Item	2013	2014
Salaries	20,822	**0**
Benefits (35%)	7,288	**0**
Wages[1]	13,565	**7,009**
Benefits (8%) [1]	1,085	**561**
Supplies[2]	4,180	**2,000**
Travel[3]	0	**1,000**
Total	46,940	**10,570**[4]

Footnotes:
[1] Temporary labor (0.5 FTE, 6 months); benefits at 8%.
[2] Includes construction and shipping of traps to WA and OR.
[3] Travel to WA to coordinate and consult on project activities.
[4] *Justification for changed budget* – the budget for USDA is significantly reduced as there was a significant amount of carry over funding from the year one budget. Funds that are requested are for additional traps and BSMB pheromone.

OBJECTIVES:

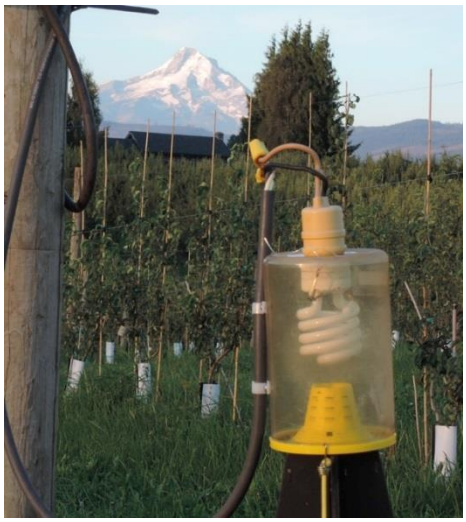
1. Compare a new BMSB light trap with standard pheromone-baited pyramid trap.
2. Determine the pheromone release rate that optimizes attraction to and capture of BMSB in traps.
3. Determine host-plant odors (kairomones) that enhance attraction/capture of BMSB in pheromone-baited and/or light traps.

SIGNIFICANT FINDINGS

1. BMSB were detected in site considered to have low populations and in three sites considered to have medium populations. At three of the four positive catch sites, BMSB were captured in pheromone-baited traps.
2. The release rate of the USDA#20 pheromone was no different when placed in polyethylene packets of different thicknesses and pheromone appeared to be gone in just a few days.
3. The release rate of USDA#20 pheromone from a commercial lure was low and lures appeared to be depleted after about one week.
4. The release rate of MDT pheromone from a commercial lure was higher than that of the USDA#20 lure and lures were releasing after 21 days.
5. Two compounds derived from English holly, which were also present in tree of heaven, (hexyl formate (hex) and cis-3-hexen-1-ol (cis3)) showed positive response by BMSB in Y-tube bioassays.
6. When hex and cis3 were placed alone in polyethylene lures there was no capture of BMSB.
7. When hex and cis3 were combined with aggregation pheromones (USDA#20 and MDT) there was some increase in BMSB capture.

METHODS

Light and pheromone traps provided by the USDA-ARS were set up in nine locations, five in WA and four in OR. A series of two light (see at right) and two pheromone-baited traps were established at each location starting in mid- to late-August and were monitored through October. Traps were checked approximately weekly and any BMSB captured were counted and sexed. Two lures were used in traps, the USDA #20 (a crude formulation which has performed the same the #10 lure) and a lure containing the pheromone of *Plautia stali*, methyl (2*E*,4*E*,6*Z*)-decatrienoate (MDT). Lures were changed every two to three weeks.



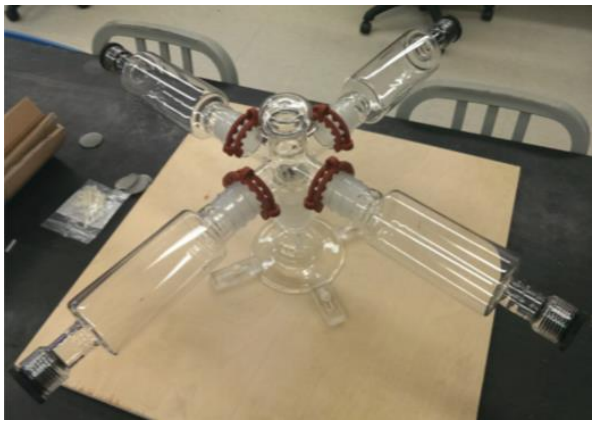
The USDA provide BMSB #20 pheromone that was placed in polyethylene packets of different thicknesses along with a cotton wick which was then heat-sealed The release of BMSB pheromone from these sealed polyethylene packets was assessed in a fume hood by measuring weight loss over time. We also assessed the release rate from commercial BMSB lures, one containing the USDA #20 pheromone and one containing the MDT pheromone. Release rate was again determined in the laboratory by following weight loss over time. Based on results of these tests we did not make additional lures containing the USDA #20 pheromone but will obtain more #20 pheromone in 2014 to evaluate the impact of release rate on BMSB capture in the spring and again in the summer. We will also evaluate the release rate of the MDT pheromone sealed in polyethylene packets in the laboratory prior to the 2014 season and make experimental lures to test in the field comparing BMSB capture results with the USDA #20 lure and with commercially available BMSB lures.

Initial studies on the response of BMSB to plant volatiles were conducted at OSU. The focus was on two host plants that consistently have BMSB populations early in their colonization of an area, English holly (*Ilex aquifolium* L.) and tree of heaven (*Ailanthus altissima* (Mill.)).

Extraction and analysis of plant volatiles. Approximately 1 lb. of ripe (red) holly berries was collected from three different trees in Corvallis, OR. After mixing, the berries were frozen with liquid N. A mortar and pestle was used to crush the berries into a fine frozen powder. Approximately one gram of material was added to six scintillation vials and was placed in the Shimadzu gas chromatography mass spectrophotometer. Analysis returned many compounds, but the primary volatiles and those that also occurred in the well-known host plant for BMSB, *Ailanthus* were selected for the volatile attraction bioassay. We also referenced The Pherobase (http://www.pherobase.com/) for potential behavioral activity of volatile compounds. The initial candidate compounds were: hexyl formate (hex), 1-octanol (oct), cis-3-hexen-1-ol (cis3), 1-heptanol (hept), 2-phenylethanol (phen). All chemicals were purchased from Sigma Chemical.

Preparation of lures. Membrane lures consisted of 1.5 in 2 mil poly tubing that was impulse sealed at one end. A 1 in. cotton pad was placed in the bag, 1 ml of the volatile compound was added, and the other end of the bag was sealed. For control lures, 1 ml of water was added instead of volatile compound.

Olfactometer bioassay. Stink bugs were collected from host plants in the Willamette Valley. Stink bugs were kept in cages in the laboratory and used in the bioassays within three days of collection. Individual stink bugs were only used in a bioassay one time. Subjects were held individually in 2oz cups prior to the bioassay. A cardboard box was designed to hold a 5cm diameter (2 in) glass Y-tube to minimize visual stimuli. Each side of the Y-tube was attached to a filter flask with a lure. One lure was the blank or control (water), and the other contained a volatile compound. The airflow into the flask was humidified with a water bubbler and air speed was maintained at approximately 0.3 m/s by a carbon-filtered regulator. Subjects were released into the bottom of the tube and were given 10 min to respond. A positive response meant that the BMSB entered the chamber connected to the flask containing the volatile odor, and a negative response meant that the insect went to the control side. If the bug did not move or leave the bottom of Y-tube, it was considered to be a non-responder. After three trials, the Y-tube was disconnected and washed first with soap and water and then rinsed in acetone. Once dry, the positive and control sides of the Y-tube were reversed from their previous configuration.



RESULTS

Light and Pheromone Trapping. Light and pheromone traps were set up in nine locations, five in WA and four in OR. A series of two light and two pheromone-baited traps were established at each location (Fig 1). Two locations were in Hood River, OR and two were in the Willamette Valley (Aurora and Talent, OR). There were three other locations in southwestern WA, one in Vancouver, one in Pringle and one in Underwood. The other two locations in WA were in the Yakima area near to where BMSB was detected in 2012, Wiley, WA and the Apple Tree Golf Course. Since our objective was to determine what traps might be best at detecting low levels of BMSB we focused on areas were we anticipated housed low to moderate populations. The anticipated BMSB population at each location based on previous detections was rated by the project participants and is shown in Table 1. The Vancouver location was at the WSU extension center on 78th street, which was known to have BMSB but not in high numbers. Traps were placed in an open field but near to habitats that would likely house BMSB. This location was not in the epicenter of BMSB in the area but it, along with the Aurora, OR site, was thought to be the most likely location to easily capture BMSB adults. Most other locations were considered to have low to very low BMSB populations. The low BMSB sites were known to have existing populations in the area but not necessarily at the location where traps were placed. The very low BMSB sites were where only one or two bugs had been previously detected.

Fig. 1. Arrangement of light and pheromone traps at one location.

Five of the nine sites provided no data, thus either the BMSB populations were very low and begin below the detection level of the monitoring system used or there were not sufficient BMSB within the attractive range of the monitoring system. The one site that was considered to have a low population but where a few BMSB were detected, Underwood, set a pattern noted in other sites where only the pheromone-baited traps captured bugs. The sites that were considered medium for BMSB did result in captures of BMSB, but primarily in pheromone-baited traps. Even in the one site that was considered between medium and low for BMSB populations, 88% of the bugs captured were in the pheromone-baited traps. (See Table 1)

Table 1. The location, estimated population level and captures in pheromone and light traps in 2013.

Location	BMSB Population	Pheromone trap	Light trap
Aurora, OR	Medium-low	216	29
Vancouver, WA	Medium	18	0
Prindle, WA	Medium	13	0
Underwood, WA	Low	4	0
Hood River 1	Low	0	0
Hood River 2	Low	0	0
Talent, OR	Very Low	0	0
Yakima 1	Very Low	0	0
Yakima 2	Very Low	0	0

Observation of BMSB at some of these sites suggests that the light traps might bring the adults into the area at night. However, because BMSB activity slows when temperatures drop below 65 ºF, it is possible that the lights attract the insects, then the rapidly dropping temperature interferes with BMSB's ability to enter the traps. In this case, BMSB respond to the pheromone-baited traps as temperatures warm up the following day causing the BMSB to become attracted to and captured in the pheromone trap. This would make the light traps appear less effective than pheromone traps.

Plans for 2014 include obtaining some additional light traps so we can set up more locations and test the premise that the light traps are attracting BMSB from surrounding areas at night and that then these bugs move to and are captured in pheromone-baited traps. We may also combine light traps with pheromone attractants to assess the synergy between two attractant sources. It seems clear that the light traps alone are not as attractive, or effective at capturing BMSB, as pheromone-baited traps.

Optimized pheromone release. USDA #20 pheromone was placed in polyethylene packets, which were sealed and then weighed. Packets were then placed in a fume hood and weighed after 2, 5 and 8 days. The release rate from all packets was essentially the same on day 2, about 6 mg per day (Fig. 2). Release rate then declined sharply between day 2 and day 5 with an average of only about 0.3 mg release from each packet per day regardless of the thickness of the packet. Between day 5 and day 8 almost no change in weight (release of pheromone) could be detected. We did not have enough #20 pheromone to repeat this study so moved to an evaluation of commercial lures.

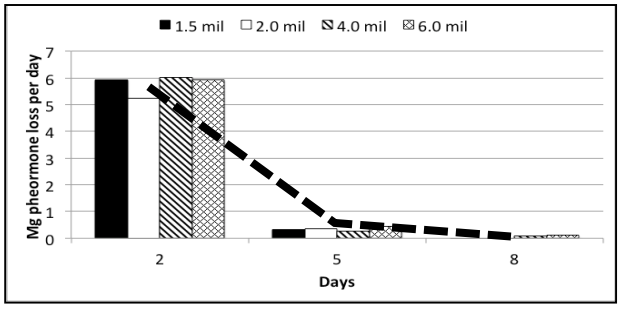


Fig. 2. Average weight loss (mg) of polyethylene packets over eight days when placed in fume hood in the laboratory.

USDA #20 lures were provided by the West Virginia laboratory for use in field monitoring associated with the light trapping study (Obj. #1). ChemTica International provided *Plautia stali* (MDT) lures were for use in the light trap study. Both of these lures, 10 of each, were weighed and then placed in a fume hood and weight loss recorded. These weight loss studies were not run concurrently but data are shown as the average weight loss per day for each lure in Fig. 3. The average weight loss in the #20 lures on day 4 was about 0.8 mg per day but the weight loss declined sharply and after day 7 was only about 0.1 mg or less through day 25. While the amount of weight loss was less than from the polyethylene packets the pattern of weight loss was similar (Fig. 2). Weight loss from the MDT lures averaged almost 3.5 mg per day between day 0 and day 3. Weight loss declined to about 1.5 mg per day between day 3 and day 10 and then declined only slightly between day 10 and day 21, 1.3 mg per day. Assuming weight loss from the polyethylene packet and commercially available lures represented the loss of stink bug pheromone it is concerning that the #20 BMSB pheromone seems to be released very quickly or it is not being released at all or at very low levels after only a few days. Additional weight loss studies in

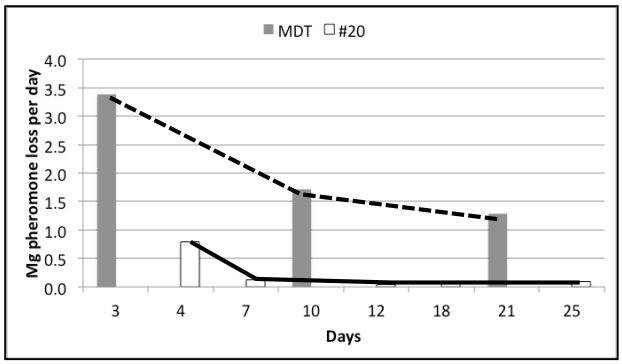


Fig 3. Average weight loss (mg) of commercially provided USDA #20 and MDT lures over twenty-five or twenty-one days, respectively when placed in fume hood in the laboratory.

laboratory are ongoing and will be reported at the technology committee to compliment these results.

Olfactometer bioassay. The initial studies evaluating different host plant volatiles were conducted in the spring. Two compounds, cis3 and hex showed greatest activity in Y-tube bioassays (Fig. 4). Future studies will focus on the use of adults of a known age either by collecting nymphs from the field and rearing them to adult or by using adults from laboratory colonies.

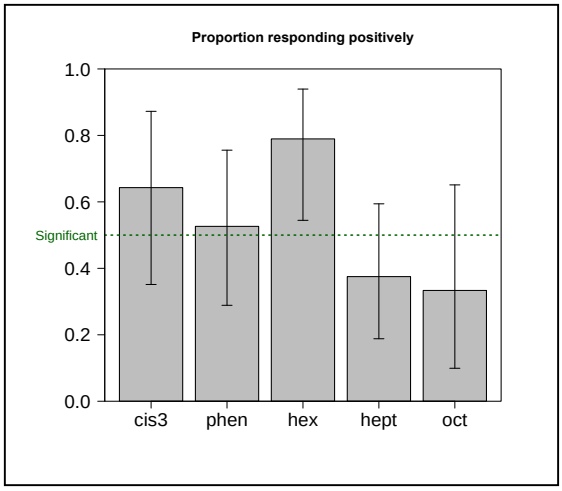


Fig. 4. Proportion response of BMSB in Y-tube bioassay to compounds derived from host plants.

Preliminary field evaluation. The hypothesis that host plant volatiles will provide enhanced capture of BMSB in the spring when the pheromone and synergist are marginally attractive could not be tested as olfactometer tests on candidate volatile compounds were being conducted during spring. However, toward the end of the season (9/12-10/23) some volatiles lures were deployed to the field to determine if they had any biological activity. Unfortunately, this was during the time when BMSB pheromone and synergist (MDT) are highly attractive so it was difficult to compare host plant volatiles with pheromone. Volatile lures were combined with synergist and sometimes with pheromone to examine potential synergy. Lures were placed into Rescue ® stink bug traps and hung in hazelnut trees in an abandoned orchard near Tualatin, OR. This orchard was located as a site of high BMSB activity in 2012 and permission was secured to conduct research. Traps were maintained at 50 ft spacing and were rotated weekly. Lures were changed every two weeks. Although the pheromone + synergist was the most effective lure tested, the candidate volatile lures cis3 and hex enhanced trap capture when used in combination with MDT compared to MDT alone or un-baited controls (UBC) (Fig. 5). The compounds cis3 and hex were not attractive on their own at this time of year. These data need to be considered as very preliminary, and compounds need to be tested prior to the onset of BMSB aggregation behavior in the spring when they leave overwintering sites.

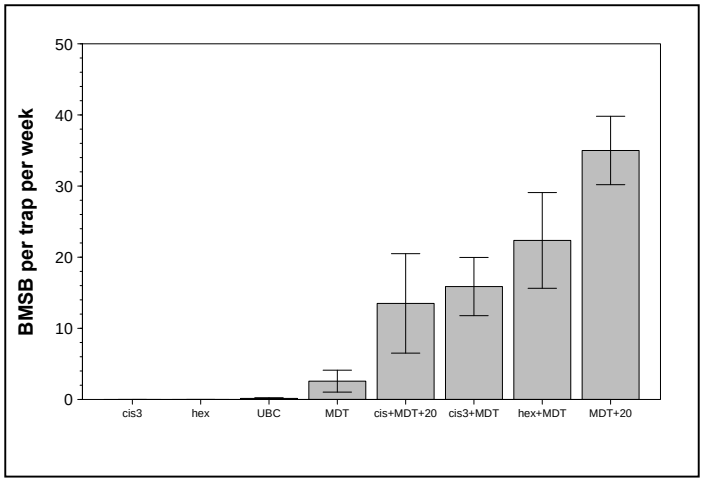


Fig. 5. Average capture of BMSB in traps baited with lures containing candidate plant volatile compounds, pheromones or combinations of volatiles and pheromones.

Plans for 2014. We have requested additional light traps from the USDA so we can conduct studies in more locations than in 2013. We will be evaluating the relative attraction of light versus pheromone-baited traps in the spring and summer. We will also evaluate test the hypothesis that light traps are attracting BMSB from a distance to the general area of the light traps but that bugs enter pheromone-baited traps in close proximity the following day. We will also assess the value of

combining light and pheromone in the same trap to optimize attraction and capture.

Additional release rate studies of the USDA #20 and MDT pheromones will be conducted with commercial lures and from lures made from polyethylene packets of different thicknesses. Once a pattern of release rate has been established we will place lures with known different release rates in pyramid traps in areas where there are known BMSB populations. BMSB captured in traps baited with different lures will be recorded in the spring and summer to determine those that capture the most bugs.

Host plants already identified as attractive will be evaluated in the spring when BMSB adults are coming out of overwintering quarters. It is possible that the attraction to these sources differs throughout the season, which could mean that volatiles are different or the bug's attraction to them changes. Additional assessments of volatiles associated with different host plants will be conducted and bioassays on candidate compounds will be conducted using the new bioassay apparatus.

Budget for 2014. Because there were some delays in getting this project going in 2013 we have significantly reduced the budget request since there are carryover funds in accounts from two institutions that will be used for 2014 studies. Those funds that are requested are the best estimate of additional costs needed to complete the objectives of the project.

FINAL PROJECT REPORT

Project Title: Identification of chemical lure for spotted wing drosophila

PI:	Peter Landolt	**Co-PI (2)**:	Helmuth Rogg
Organization:	USDA, ARS 5230 Konnowac Pass Rd. Wapato, WWA 98951	**Organization**:	Oregon Dept. Agric. 635 Capitol Street NE Salem, OR 97301
Telephone:	(509) 454 6570	**Telephone**:	(503) 986-4662
Email:	peter.landolt@ars.usda.gov	**Email**:	hrogg@oda.state.or.us

Other funding sources

Agency Name: NIFA, NE RIPM Program
Amount awarded: $24,000
Notes: Grant awarded to CT Ag. Exp. Station with subcontract to USDA, ARS Wapato

Total Project Funding: $101,000

Budget History:

Item	Year 1:	Year 2:	Year 3:
Salaries			
Benefits			
Wages	20,300	20,300	20,300
Benefits	7,700	7,700	7,700
Equipment			
Supplies	5,500	4,500	2,500
Travel	1,500	1,500	1,500
Plot Fees			
Miscellaneous			
Total	35,000	34,000	32,000

RECAP OF ORIGINAL OBJECTIVES:

The overall objective of the project was to develop a reliable detection system for cherry growers, which would allow them to react to a spotted wing drosophila infestation early and at low population densities. Technical objectives were to:

1. Isolate and identify attractants from wine and vinegar.
2. Determine an optimized combination of attractive chemicals.
3. Develop a controlled release dispenser for use as a lure in a trap.

SIGNIFICANT FINDINGS

1. A blend of four chemicals from wine and vinegar were found to be strongly attractive to female and male adult SWD. These four chemicals are acetic acid, ethanol, acetoin, and methionol.
2. The same blend of four chemicals is much more selective to SWD, and attracts much fewer non-target insects.
3. In a comparison between the chemical lure and food bait materials used by pest managers, the chemical lure was more effective in attracting the first flies in the spring in Hood River.
4. A sachet system of dispensing the chemicals was worked out to provide a controlled release dispenser to place in a trap.
5. Information on the chemical attractant and means of dispensing the attractant was shared with five companies, and two of those companies (Scentry and Trece) are marketing the attractant. We maintain a dialogue with two other interested companies (Sterling and AgBio).

RESULTS AND DISCUSSION

<u>Fly responses to antennal-active volatiles of wine and vinegar</u>

Thirteen wine and vinegar chemicals, in addition to ethanol and acetic acid, consistently elicited fly antennal responses determined with an electro antennal detector system (EAD) Table 1). A combination of all 13 of these chemicals tested in the field was not as attractive as the starting material of wine and vinegar, and indeed was quite weak. This problem was anticipated, because the GC-EAD assay determines chemicals detected (smelled) by the antennae, and does not indicate chemical attractiveness. There is potential for chemicals that are "smelled" by the fly to be attractive, repellent, or neutral.

A laboratory assay was developed and used to test 13 EAD-active chemicals for attractiveness and repellency to SWD when added to acetic acid a mixture of and ethanol. Results of this series of assays indicated that seven chemicals reduced the fly response to acetic acid and ethanol, while the six other chemicals either improved the attraction response or were neutral (Table 1).

Table 1. Chemicals from wine and vinegar headspace analysis that elicited electro antennal responses from spotted wing drosophila, and fly response in laboratory bioassay when added to acetic acid and ethanol.

Chemical	In Wine headspace	In Vinegar headspace	Bioassay Response
Ethyl acetate	X	X	repellant
Acetoin	X	X	attractive
Ethyl butyrate	X		repellent
Ethyl lactate	X	X	repellent
Hexanol	X		repellent
Isoamyl acetate	X	X	repellent
2-methylbutyl acetate	X	X	repellent
Grape butyrate	X	X	neutral
Methionol	X		neutral
Isoamyl lactate	X		neutral
Ethyl sorbate	X		repellent
2-phenyethanol	X	X	neutral
Diethyl succinate	X		neutral

Fly responses to subsets of the antennal-active volatiles of wine and vinegar.

A second generation chemical blend, consisting of 6 chemicals plus acetic acid and ethanol, was field- tested as a bait for a trap. This blend was improved over the previous blend but was still significantly less attractive than wine plus vinegar.

A series of field tests showed that only three of thirteen EAD-active wine and vinegar chemicals significantly improved fly captures in traps when added to acetic acid plus ethanol. A third generation chemical blend then was comprised of acetic acid, ethanol, and those three chemicals that were EAD-active and also co-attractive in field test. This third generation blend was equal in attractiveness to SWD compared to wine plus vinegar in field tests (Figure 1).

These results provided a basic set of chemicals to use to optimize a chemical lure for use in trapping and detecting SWD. Our previous work showed that the combination of wine and vinegar is much more attractive to the fly than either wine or vinegar. During the duration of this project, that combination of materials has been the strongest food type bait for SWD. Our combination of the four chemicals was initially as attractive as that best bait. This result provides a clear opportunity to develop and use a lure that is powerful in luring both sexes, can be formulated to provide even attractiveness for long periods of time, and can be used in a dry trap or a wet trap. All of the active chemicals are commercially available and are relatively inexpensive.

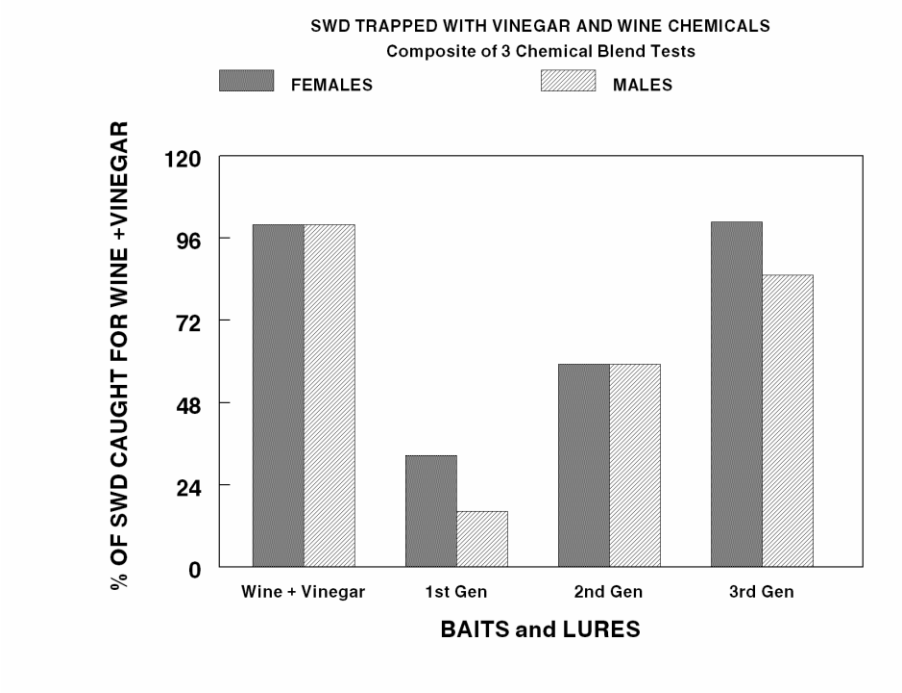


Figure 1. The attractiveness of the three blends of chemicals that were field-tested is shown in this graph, in relation to the attractiveness of wine plus vinegar. The first generation blend was the combination of EAD-active chemicals. The second generation blend was the EAD active chemicals minus the chemicals that were repellent in a laboratory assay. The third generation blend was limited to those chemicals that were co-attractive when tested individually in the field.

2013. Dispenser development. We expected that evaluations of chemical release rates and ratios would show further improvements in the attractiveness of the blend of chemicals. In a series of trapping experiments, increases in the release rates of acetoin and greater doses of acetic acid and ethanol resulted in increases in numbers of SWD captured. Thus, a new dispensing system (sachet lure) with acetoin and methionol released from sachets and acetic acid and ethanol released from the trap drowning solution yielded further improvement in the SWD trap catches. This dispensing system was then compared with the previous lure system that involved dispensing acetoin and methionol from vials, and acetic acid and ethanol from the trap drowning solution. We found that the use of the new sachet lure was significantly more attractive to SWD than the previous system or the wine plus vinegar mixture that was the basis for the research project.

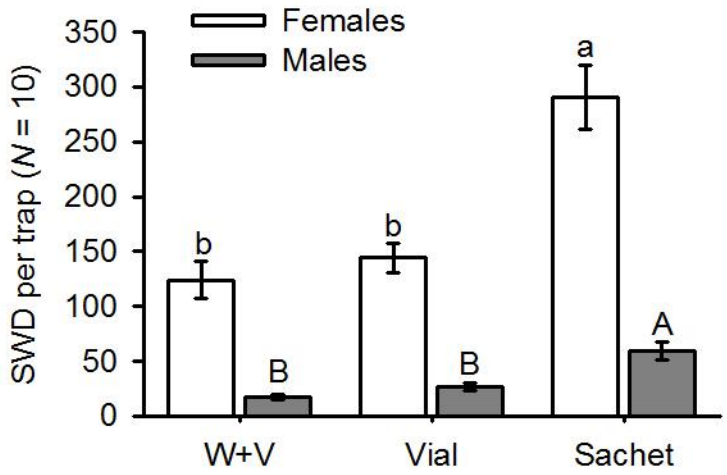


Figure 2.

This graph shows SWD flies captured in traps baited with the combination of wine and vinegar (W + V), with acetoin and methionol in a vial and ethanol and acetic acid in the drowning solution (vial), and with acetoin and methionol in two individual sachets and ethanol and acetic acid in the drowning solution (sachet).

Assessment of non-target insect responses. Numbers of the two cutworm moths (spotted cutworm and olive dart) and two armyworms (bertha and true armyworms) were much fewer in traps baited with the SWD chemical lure, compared to the wine/vinegar mixture. Similar results were seen for the two yellow jacket wasp species that were abundant in test 3, and the false stable fly which was abundant in test 2. Numbers of the little house fly were numerically but not statistically less with the chemical lure. These decreases in non-target insects in traps baited with the chemical lure compared to the wine/vinegar mixture (Table 2) are of great potential importance to the costs of detection and monitoring programs. Large numbers of non-target insects in traps greatly increases the time it takes to sort, identify, and tally the spotted wing drosophila in the trap. Also, large numbers of these insects can interfere with the effectiveness of the effectiveness of the drowning solution to retain SWD that enter the trap.

Additionally, numbers of non-target species of *Drosophila* flies were generally reduced in traps with the chemical lure, compared to the wine/vinegar mixture (Table 3). We include here only the data for the species commonly encountered. Again, we suggest that these reductions in non-target catches in SWD traps should reduce the costs of trap checking and trap maintenance. Note that it takes considerable skill to sort and identify SWD from a strong mixture of related Drosophila flies. Reducing the numbers of non-target Drosophila in traps should make trap checking and SWD detection and monitoring considerable easier.

Table 2. Mean numbers of insects per trap per week, for traps baited with a mixture of wine and vinegar, and traps baited with the SWD chemical lure.

	Wine + Vinegar	4-component lure
Test 1.		
Spotted cutworm moth	10.7 ± 1.0a	0.3 ± 0.2b
Bertha armyworm moth	6.7 ± 1.1a	0.0 ± 0.0b
Test 2.		
False stable fly	431.6 ± 97.5a	82.3 ± 36.3b
Little house fly	8.3 ± 3.6a	5.0 ± 2.4a
Test 3		
German yellow jacket	19.0 ± 2.1a	5.0 ± 1.2b
Western yellow jacket	22.9 ± 5.5a	5.2 ± 1.3b
Test 4		
Olive dart moth	21.7 ± 1.9a	1.9 ± 0.6b
True armyworm moth	1.2 ± 0.4a	0.0 ± 0.0b

Means within a row followed by a different letter are significantly different by a paired T-test, at $P < 0.05$.

Table 3. Mean numbers of different species of *Drosophila* flies per trap per week, for traps baited with a mixture of wine and vinegar, and traps baited with the SWD chemical lure. Tests were conducted in the lower Yakima Valley and near Salem, Oregon.

	Wine + Vinegar	4-component lure
Test 1. WA		
D. suzukii (SD)	92.9 ± 10.0a	104.0 ± 7.3a
D. immigrans	15.0 ± 3.0b	5.0 ± 2.9a
D. melanogaster group	1015.0 ± 142.9b	64.5 ± 18.5a
D. obscura	625.1 ± 91.6b	146.4 ± 26.2a
Test 2 OR		
D. suzukii (SD)	1489.8 ± 422.4a	1751.2 ± 457.9a
D. immigrans	3.2 ± 1.9a	3.1 ± 1.5a
D. melanogaster group	39.1 ± 9.1b	15.0 ± 3.4a
D. obscura	88.5 ± 33.2a	72.9 ± 23.0a

Early season detection of SWD with a chemical lure. A key advantage of a strong lure is the ability to detect either populations of the fly, or their first movement, so as to determine appropriate pest management needs. Working with Peter Shearer at OSU Hood River, a comparison of traps and baits was set up to look at early season catches of SWD in cherry orchards. In that test, traps baited with the chemical lure (Cha lure) caught more SWD than traps baited with apple cider vinegar (ACV) or traps baited with yeast/sugar bait, in early season cherry orchards in Hood River.

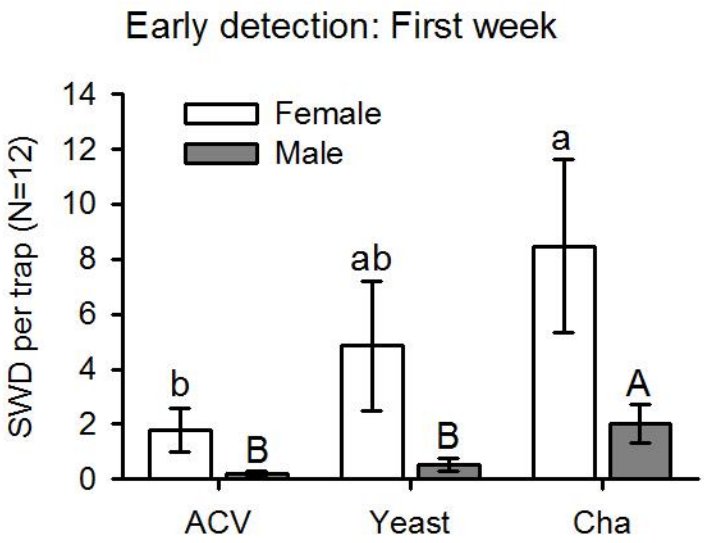


Figure 3. Mean numbers of SWD flies captured per trap per week, for traps baited with apple cider vinegar (ACV), a yeast/sugar formulation (yeast) and the SWD chemical lure (Cha).

Technology transfer.

An important outcome of a successful research project is the availability and use of the information or technology produced. To this end, information on the results of the research has been shared with multiple companies that manufacture and market lures and traps for the detection and monitoring of insect pests. These companies are AgBio, ChemTiki, the Hungarian Academy of Science, Scentry, Sterling and Trece. At this time, Scentry and Trece are producing and marketing lures based on the results of our studies.

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EXECUTIVE SUMMARY

For project "Identification of chemical lure for spotted wing drosophila".

Significant Outcomes.

1. This work determined that the combination of four chemicals (ethanol, acetic acid, acetoin, and methionol) can constitute a strong attractant for the spotted wing drosophila fly.
2. Methods were worked out for optimum release of acetoin and methionol from plastic sachets, and optimum amounts of acetic acid and ethanol in water, for making a strong trap bait.
3. It was determined that the chemical lure is as strong or stronger than the starting material of wine and vinegar, which was shown to be a superior food type bait.
4. It was determined that the chemical lure is much less attractive than wine and vinegar to other types of insects (called non-target insects) that interfere with SWD detection and monitoring efforts.

Summary of Findings

These results provide a chemical lure for use in detecting and monitoring SWD. At this time, this combination of chemicals is as attractive as our best food type bait. This result provides a clear opportunity to develop and use a lure that is powerful in luring both sexes, can be formulated to provide attractiveness for long periods of time, and can be used in a dry trap or a wet trap. All of the active chemicals are commercially available and are relatively inexpensive. Two companies are producing and marketing lures based on this work, and we are working with them to assist their efforts and to make the chemical lure available for further study by other researchers and for detection and monitoring efforts.

We expected that isolation of the volatile chemicals from wine and vinegar that attract SWD would also lead to a lure that is less attractive to non-target insects, which would reduce labor and trap maintenance effort when trapping SWD. Our experimental results support this expectation, although the chemical lure is still attractive to other types of insects and it is not a species specific lure.

This work is conducted with the purpose of providing a powerful chemical lure for reliable early season detection of SWD for cherry orchards, information that is needed for making sound pest management decisions. We anticipate that the approach and strategy taken here also will provide a trap/lure combination that is easier to use and more consistent in its attractiveness compared to current monitoring methods in use. The direct practical impact will be to reduce crop damage and losses due to undetected SWD populations, and also to reduce costs of pest control incurred when a fly population is not present

Future Directions.

Work is ongoing at a number of other institutions to develop applications of the lure and to incorporate it into IPM systems. We anticipate further technology developments, such as the incorporation of the chemistry into sprayable baits, and attract-and-kill technologies.

FINAL PROJECT REPORT

Project Title: Spotted Wing Drosophila management on sweet cherry

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Cooperators: Tim Smith, WSU Chelan County Extension; Doug Walsh, WSU-Prosser; Peter Shearer, OSU-MCAREC

Other funding sources

Agency Name: **WSDA Specialty Crop Block Grant**
Amount awarded: **$**170,241, 2 years, 10/1/2011 through 12/31/2013
Notes: Previous SWD project used as match for SCBG; Co-PIs Beers & Yee

Agency Name: FAS-TASC
Amt. requested/awarded: $72,096 for year 1 (Beers, Walsh; includes indirect costs).
Notes: Grantees are California Grape and Tree Fruit League and the Northwest Horticultural Council; Beers & Walsh are Washington PIs. (Funding is yearly, with a planned 3-year term).

Agency Name: USDA-SCRI
Amt. requested/awarded: ca. $20,000/year, 5 years.
Notes: Walton et al.; amount above is portion to E. Beers via WSU subcontract.

Total Project Funding: $150,000

Budget History:

Item	2012	2013	2014
Salaries	12,000	12,480	12,979
Benefits	4,829	5,023	5,224
Wages	15,925	15,925	15,925
Benefits	12,199	12,199	12,199
Equipment	0	0	0
Supplies	2,395	1,722	1,022
Travel	2,652	2,652	2,652
Plot Fees	0	0	0
Miscellaneous	0	0	0
Total	50,000	50,000	50,000

Objectives

1. Provide a crop protection alert system to cherry/stone fruit producers and seasonal phenology information through a regional SWD trapping program.
2. Determine timing of cherry fruit susceptibility in the field.
3. Test standard trap types for capture efficiency of SWD (in collaboration with SCRI-SWD regional group).
4. Test pesticide efficacy for control of SWD in cherries in laboratory, field-laboratory, and field settings.

Significant Findings

- SWD population densities followed the same general pattern each year (low in winter-mid-summer, increasing in late summer/fall), but the absolute numbers varied widely. In some years, SWD were captured during each month.
- The years with earliest first capture profiles tended to have the highest densities overall.
- Green cherries are not susceptible to attack by SWD, but all subsequent stages are. This indicates a potential control period essentially the same as Western cherry fruit fly. Only a late first capture of SWD should delay control measures for this pest.
- Red and yellow were found to be attractive colors for SWD, and should be incorporated into future trap designs. Other design features may depend on the type of lure being used (wet bait versus synthetic).
- The spinosyns (Entrust, Delegate) are generally the most active and long residual materials for control of SWD; Warrior and Diazinon are intermediate, while Sevin and Malathion have a very short residual.

Results and Discussion

Regional Trapping Alert system. The website http://www.tfrec.wsu.edu/pages/swd has served as the portal to information on activity of SWD in eastern Washington throughout the three years of the project (2012-2014), as well as in the previous project (2011). The database consists of trap captures in 200-300 traps distributed from the Canadian border to the Oregon border, checked weekly by the Beers, Walsh, and Yee programs, and volunteer fieldmen from throughout the state. The majority of the traps are in cherry orchards, but other known or potential sources were also trapped. The essential feature of this is a table listing the first capture of SWD in ca. 17 growing districts (Fig. 1), coupled with advice that if fruit are susceptible and flies are active, control measures should be taken; control recommendations were also posted on the website. The first capture in each region was sent to an email list developed by Tim Smith; visitors to the website could subscribe to the list from the website. The website also allowed users to graph individual or groups of traps, and provided links to other information on SWD.

Region	Traps deployed	Flies first recorded
Oroville/Tonasket	Yes	May 28th
Okanogan/Omak	Yes	May 30th
Brewster/Pateros	Yes	May 23rd
Chelan/Manson	Yes	May 30th
Orondo–Beebe Bridge	Yes	May 17th
Entiat	Yes	May 17th
Wenatchee/E Wenatchee/ Wenatchee River Valley	Yes	May 16th
Stemilt Hill/Wenatchee Heights	Yes	May 23rd
Rock Island/Malaga	Yes	May 30th
Quincy/Moses Lake	Yes	May 17th
Royal City	Yes	May 23rd
Othello	Yes	May 24th
Mattawa/Desert Aire	Yes	May 22nd
Yakima/Tieton	Yes	May 28th
Union Gap/Zillah	Yes	June 7th
Sunnyside/Prosser	Yes	Feb 7
Tri–Cities	Yes	May 24th

Fig. 1. Regional trapping alert system – first SWD capture by region (2013)

SWD phenology. The pattern of SWD phenology has remained relatively constant throughout the five years of trapping (Fig. 2), but the absolute numbers have varied widely from one year to the next. While part of the variation can be ascribed to changes in traps and lures with differing capture efficiencies, it is likely that the biological differences are real. The pattern that we have seen is low densities (or trap activity) in the winter months through mid-summer, with capture levels rising in mid-August, and peaking in the fall (October-November). The lateness of the high capture rates is limited by freezing temperatures. In 2013, captures were recorded in every month of the year. The extremely high populations in 2013 and 2014 may reflect better establishment of this pest coupled with moderate winters. The extremely low captures in 2011 followed a severe freeze event in late November of 2010, and seems the most likely explanation of the low numbers. What is clear is that SWD is not limited in terms of distribution by the climate of eastern Washington (either by high or low temperatures), but fluctuations in density may be a result of unusually high or low temperatures.

Dates of first capture also varied from year to year, as well as among regions. Although only 5 years of data have been collected so far, there appears to be a correlation between earliness of capture and overall higher seasonal captures. Two extremes are represented by the 2011 year (late first capture, low seasonal means) and 2013 (early first capture, high seasonal means).

Cherry fruit susceptibility. Three years of experiments have demonstrated that if flies are active, fruit that is straw or blush in color is susceptible to attack by ovipositing females. This principle was demonstrated with four cultivars (Sweetheart, Bing, Lapins, and Rainier) irrespective of maturation period. Two approaches were used to ensure consistent results: a lab bioassay (cherries brought in weekly and challenged with adult female SWD) (Fig. 3), and caged tree trials, where flies from a laboratory colony were released into a cage for a one-week period throughout the maturation period (green fruit through post-harvest). Ovipositions and successful adult emergence were recorded from the fruit, and corresponding data were taken to characterize their maturity (firmness, size, color, brix, titratable acidity, pH).

Trap style. One of the requests of the Advisory Panel of the SCRI-SWD project was to develop a standard trap for SWD. A large collaborative effort was devoted to this objective, all using apple cider vinegar (ACV) as the bait. Some of the basic principles were established in the first study (Lee et al., 2012), which correlated increasing trap captures with larger entry areas, and possibly broader surface area (Haviland) from which the odor of the bait could diffuse. However, the bait volume was

not held constant among the different traps. A second regional effort portioned out the effect of trap color from other design features (Lee et al., 2013). However, the early trap tests were comparisons of mostly custom-fabricated traps whose designs arose out of convenience or necessity, rather than a rigorous investigation of the underlying principles. We conducted two experiments that looked at specific factors (e.g., bait volume, bait surface area, and distance from the surface to the entry point) while holding other parameters constant. In the first test, we found that increasing bait volume and surface area increased trap captures; in the second, bait volume increased trap captures, but the effect of surface area was inconsistent.

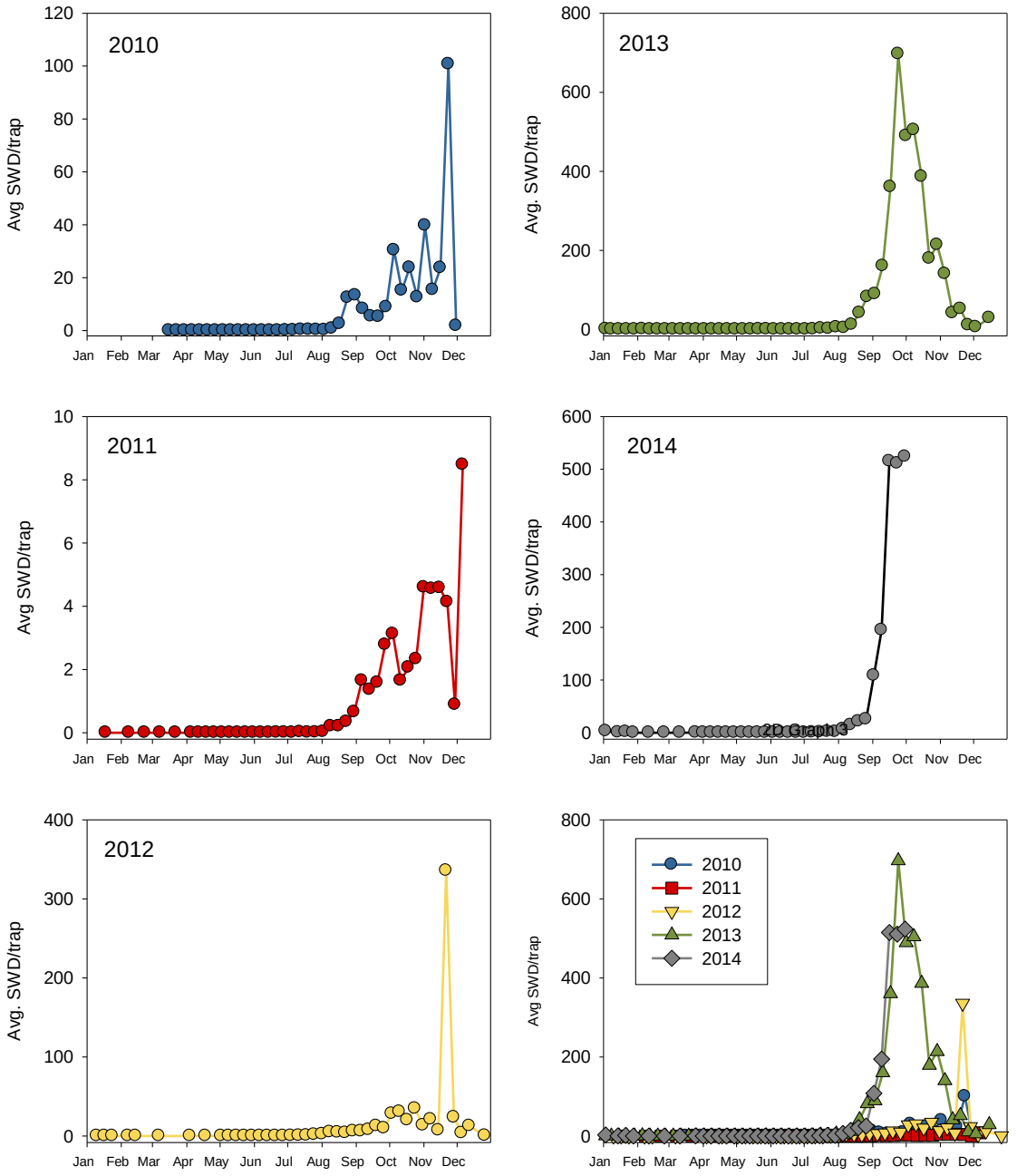

Fig. 2. Phenology of SWD in eastern Washington, 2010-2014.

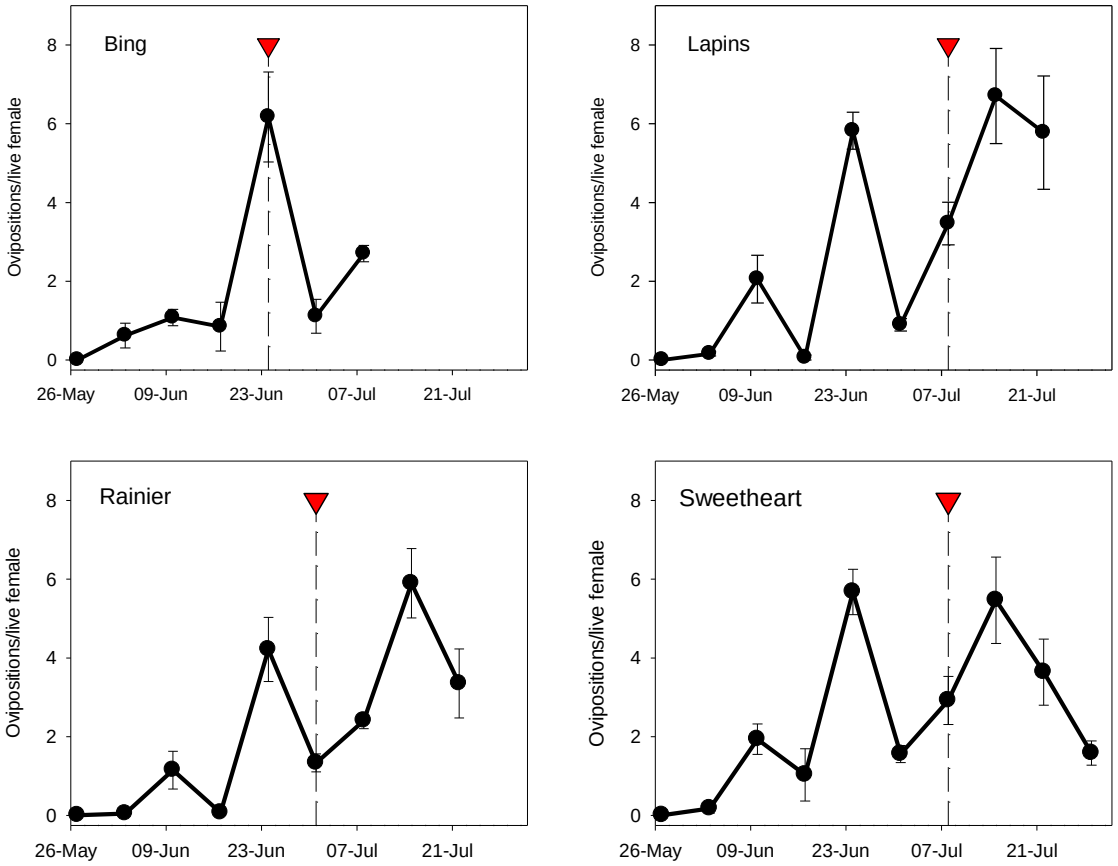

Fig. 3. Susceptibility of four cultivars of cherry to SWD, 2014.

Bait. Although ACV was considered a standard and effective bait, a number of other compounds were tested. These included a yeast-sugar-water mixture, various types of wine-vinegar mixtures (one including molasses), and a commercial bait product made from corn steep liquor (Monterey Ag Bait). Other regions had great success with the yeast bait, however, tests in Washington indicated its seasonal capture was lower. This may be due to poorer performance at low temperatures relative to vinegar or wine-vinegar mixtures, which is typically the time when the greatest number of flies are available. At warmer temperatures (those prevailing during the cherry fruit maturity period), it out-performed ACV in some tests, but not others.

A shift in approach occurred with experiments designed to determine which of the volatile components of the wine-vinegar mixture were biologically active. This was determined through iterative testing by electroanntenagram. Four components were found to be key to attraction (Cha and Landolt, 2013), allowing the synthesis of a dry lure. Two companies (Trécé and Scentry) produced synthetic lures in 2013 and 2014, respectively, and were field tested under eastern Washington conditions. In 2013, the Trécé lure (with or without ACV as the drowning fluid) captured more flies in June and July than the yeast bait, a wine-vinegar bait, or ACV alone (Fig. 4). In 2014 (June-mid-August), the Scentry lure caught ca. 17x more flies than ACV, and ca 3x more than the Trécé lure with water or ACV as the drowning fluid (Fig. 5).

However, a more appropriate measure of the efficiency of the trap is not just the total number of flies caught, but how early the capture occurs. Early comments on the ACV trap were that fruit were already infested by the time the ACV trap caught flies. We examined sensitivity (earliness of capture) of the new lures in 2013 in seven replicate orchards. In all replicates, one of the new Trécé lures caught the first SWD, in advance of the ACV trap by 7-28 days. The Scentry lure was added to the test in 2014, and deployed in 10 replicate orchards. One of the new lures caught the first fly in 6 out of 10 replicates, preceding capture in the ACV trap by 13-35 days. In the remaining four replicates, a new lure captured the same week as the ACV. Thus, the new lures are often more sensitive, and never less sensitive, than the ACV trap. The two dry lures hold considerable promise to provide the needed sensitivity to develop an action threshold for treatments on an individual block basis, replacing the regional capture system.

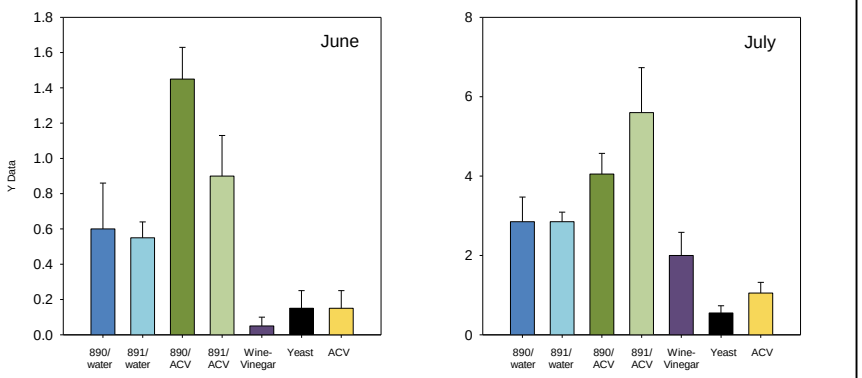

Fig. 4. Mean weekly captures of SWD by different baits or lures, 2013

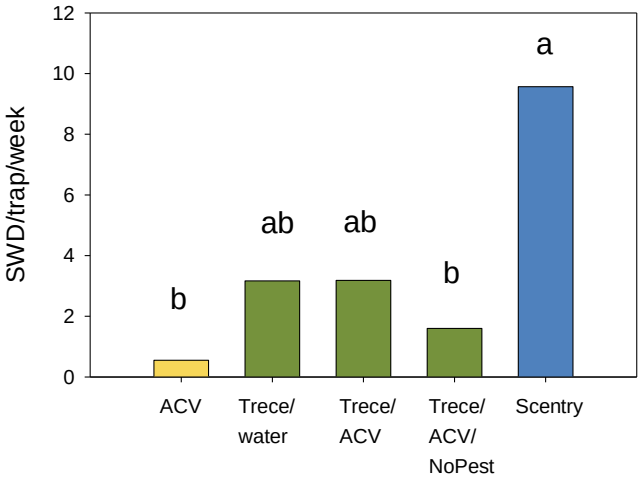

Fig. 5. Mean weekly captures of SWD with five baits or lures, 2014

Pesticide Efficacy. The efficacy of candidate insecticides for SWD control was tested over the three-year span of the project. Early laboratory screening efforts by small fruit researchers (Bruck et al., 2011).and cherry researchers (Van Steenwyk et al., 2012; van Steenwyk and Novotny, 2011) were used to select the most likely products for use in eastern Washington cherry production. Because the research orchard failed to develop a natural infestation during the course of the study, a field-lab bioassay approach was used to ensure consistent evaluation of efficacy and protection. Pesticides were applied in the field (Sunrise blk 4 'Sweetheart' cherries) using an airblast sprayer at 100 gpa, and fruit and leaves collected at intervals post-spray to determine residual control. The residues were challenged with lab-reared flies in an arena that included treated leaves and fruit from the field, and we measured adult mortality, oviposition, and successful emergence of adults from the ovipositions. In addition, fruit maturity measurements were made in conjunction with the bioassays to link the susceptibility to attack with maturity.

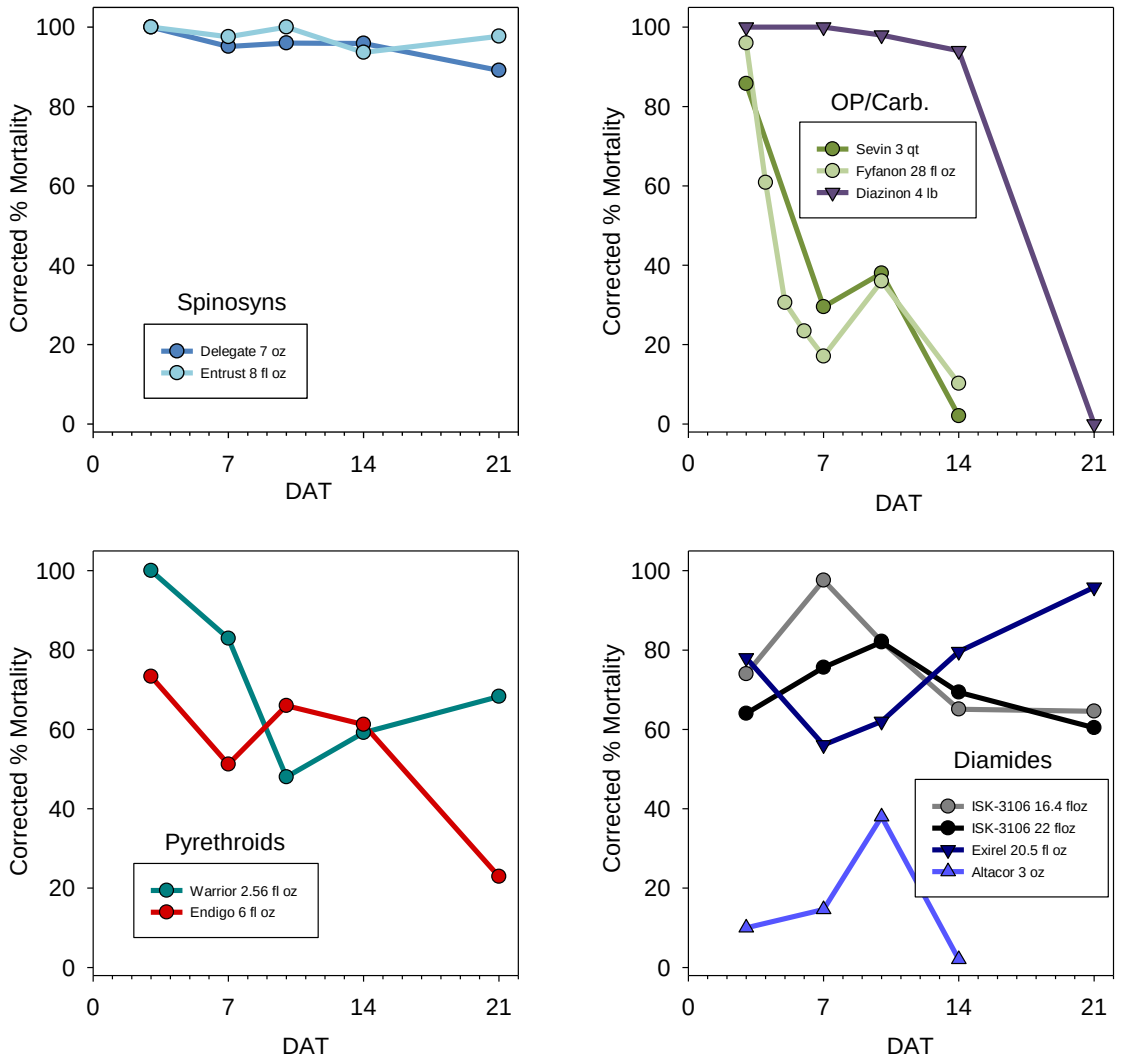

Fig. 6.. Residual control of adult SWD by single applications of various pesticides, 2014.

In 2012, treatments consisted of programs of insecticides (Warrior and Entrust) based on their respective re-treatment and preharvest intervals. Bioassays were timed for the presumed weakest point in coverage, the day before next application. Fly mortality in the Warrior treatment dropped off rapidly after the single 14 days before harvest (DBH) application. Protection from oviposition by females was high initially, but decreased to ca. 40% reduction compared to the check by harvest. Unsurpisingly, the 3-spray program of Entrust (either the 80W or 2SC) provided more consistent levels of mortality and fruit protection over the 17-day period.

A preliminary study in late summer of 2012 indicated that using a higher gallonage might be helpful in extending the period of coverage. This was tested during the preharvest period of 2013 using Warrior at different application volumes (400 and 100 gpa airblast, and handgun, estimated 187 gpa). However, all three treatments provided excellent control through 10 DAT, and did not differ statistically from one another.

A second study in 2013 examined the length of residual control of various pesticides following a single application. While single applications are unlikely in commercial settings, this approach helps evaluate individual products, so that growers can build programs with appropriate coverage, taking PHI into consideration. Fyfanon caused high levels of mortality through 4 DAT; Sevin and Diazinon through 10 DAT, and Entrust and Delegate through 14 DAT. Fruit protection dropped off much more quickly. Rimon+Warrior provided high levels of mortality through 21 DAT, but the effect of Rimon on oviposition and emergence needs to be re-examined.

A similar study was performed in 2014, but with an expanded range of treatments. All were applied on 23 June, about 2 weeks before commercial harvest. A pattern of differences among different groups of insecticides was evident (Fig. 6). The spinosyns (Delegate and Entrust 2SC) provided high levels of mortality though 21 DAT, and were the longest-residual materials tested. The pyrethroids were intermediate; while there is still significant mortality at 21 DAT, the rate of decline was steeper. There was quite a bit of variability in the OP/carbamate insecticides tested; Diazinon provided good mortality through 14 DAT, but declined sharply thereafter, while Sevin and Fyfanon (whose PHIs are much shorter) provided mortality for only a few DAT. Three of the diamide treatments (two rates of a numbered compound, ISK 3106, or cyclaniliprole, and Exirel/cyantraniliprole) provided higher levels of mortality throughout the test period, and while never as high as the spinosyns, it remained relatively steady. Altacor (chlorantraniliprole) was relatively weak against SWD in terms of acute toxicity.

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Executive Summary

This three-year project focused on key management issues for a new, invasive pest of sweet cherries in eastern Washington, spotted wing drosophila (SWD). Four objectives were addressed in the course of the project: 1) Provide a crop protection alert system to cherry/stone fruit producers and seasonal phenology information through a regional SWD trapping program; 2) Determine timing of cherry fruit susceptibility in the field; 3) Test standard trap types for capture efficiency of SWD (in collaboration with SCRI-SWD regional group); 4) Test pesticide efficacy for control of SWD in cherries in laboratory, field-laboratory, and field settings.

The first objective was met by creating a regional alert system for SWD, which was posted on a dedicated website http://www.tfrec.wsu.edu/pages/swd. The core information for the website was a network of SWD traps located from the Canadian border to the Oregon border. Traps were checked weekly, and results uploaded within 24 h of retrieval. Trap retrieval and counting SWD were performed jointly by the Beers, Walsh, and Yee programs, and volunteer fieldmen. A table on the front page of the website informed visitors of which cherry growing regions in eastern Washington had caught at least one adult SWD, and the date of that capture. Recommendations for control (also posted on the website) advised that control measures should begin if 1) fruit were susceptible (see Obj. 2) and the first fly had been detected in the region. In addition, an email list developed by Tim Smith (with the option to subscribe posted on the website) sent notification for each new region with a first capture, and other news of note on SWD activity in the state.

The trap network also served to establish the phenology of SWD in our region, which was unknown at the time of invasion (2010). Five years of data show a similar seasonal pattern (low in winter through mid-summer, rising in late summer and peaking in fall). However, the relative numbers captured (even allowing for year to year differences in traps with different efficiencies) varied widely among the 5 years. Severe winter temperatures are the most likely explanation for some, but not all, of this variation, and needs further exploration.

Significant progress has been made on fabricating a more efficient trap. With the wet-bait (apple cider vinegar trap), higher bait volumes, and to a lesser extent, greater surface area, correspond to higher capture rates. The development of synthetic lures from Trécé and Scentry, and commercial traps (Biobest, Contech) hold promise for greater sensitivity and ease of use. The issue of sensitivity (earliness of capture) may allow traps to be used for an action threshold on an individual block basis, rather than spraying all cherry orchards in a region based on a single fly.

Candidate pesticides have been screened for length of residual control and fruit protection against SWD. The spinosyns (Delegate, Entrust) provide a high level of activity, and the longest residual control of the compounds tested. The pyrethroids (Warrior, Endigo) also provide a high level of initial control, but residual control was more variable. Diazinon was similar to Warrior in that it provided at least 2 weeks of control. The other older materials (Sevin and Fyfanon, or ULV malathion) have short PHIs, and correspondingly short lengths of residual control (<1 week). The diamides are variable, with little mortality caused by Altacor, and higher levels by Exirel and a currently unregistered compound, cyclaniliprole.

FINAL PROJECT REPORT **YEAR: 2 of 2**
WTFRC Project Number: CH-13-104

Project Title: Novel postharvest fumigation of sweet cherries for fruit fly pests

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Cooperators: P. Landolt & W. Yee, USDA-ARS, Wapato, WA

Total Project Funding: Year 1: $34,000 **Year 2:** $14,000

Other funding sources: None

Budget history:

Item	2013	2014
Salaries (60% GS-5)	23,950	5,500
Benefits (included above)		
Wages		
Benefits		
Equipment		
Supplies	8,550	5,500++++
Travel	1,500	
Miscellaneous (shipping)		3,000
Plot Fees		
Total	34,000	14,000

Footnotes: Supplies include 1-pallet of fruit, rearing supplies and costs related to fumigation
++++++ if more fruit quality evaluations are wanted, more fruit will be needed

Original objectives:

Specific objectives - Year 2 (2013)

This project is planned in 3 phases as indicated below. Each phase will have its own objective and these objectives will feed those of the following phase.

Phase I. Establish and maintain a colony of SWD in Parlier, CA with the throughput necessary to routinely conduct fumigation studies.

Timeline: Already accomplished.

Phase II. Determine the mortality of phosphine as well as several key phosphine mixtures to eggs, larvae, pupae and adults of SWD in $1ft^3$ chambers at 35 °F. Report dose-mortality regressions with statistical validity (Probit v. 2007 software) to establish most tolerant SWD life stage.

Timeline: April-May

Phase III. Optimize phosphine and its mixtures to control the most tolerant SWD lifestage as quickly as possible at 35 °F. With intent of decreasing stand-alone fumigation requirements, the effect of hydro-cooling on SWD mortality will be evaluated and integrated with fumigation data to be reflective of mortality expected from entire "packing system".

Timeline: Nov-Feb 2012, May-June

Phase IV. Perform a confirmatory treatment at the dose derived from Phase II in 9 $1ft^3$ chambers at 35 °F with 30,000 SWD specimens (most tolerant stage) while fruit is packed in wooden bins. To ensure adequate exposure for complete mortality, gas concentrations will be measured throughout load over the course of the fumigation. Sorption and box effects will be quantitatively analyzed and reported.

Timeline: May-June

Phase V. Document phytotoxicity (Dr. Obenland) that occurs from exposure to phosphine and its mixtures at dosages that are efficacious toward the most tolerant stage of the SWD. Three key export varieties (recommended by industry) will be investigated.

Timeline: Concurrent with Phase IV

Phase VI. Quantify residues in cherries that result from exposure to phosphine and its mixtures at dosages that are efficacious for killing the most tolerant stage of the SWD.

Timeline: Concurrent with Phase IV & V.

Specific Objectives Year 2 (2014)

Repeat Year 1 (2013) objectives with another species of fruit fly pest, such as the Western cherry fruit fly and the brown marmorated stinkbug (BMSB) maintained in the Contained Research Facility at the University of California at Davis.

Significant findings:

- Phosphine fumigation at cold-storage temp will control SWD in 36 to 48 h
- Phosphine fumigation at cold-storage temp will control BMSB in 36 to 48 h
- Fumigate max. chamber loads of packed and palletized cherries
- Maintaining a threshold concentration 750 to 1500 ppmv phosphine is required
- Residues and worker exposure with phosphine are favorable (relative to MB)
- Fruit quality evaluations following phosphine fumigation look promising
- Phosphine fumigation of fresh fruit gaining "international traction"

Results & Discussion:

Materials and Methods.

Insects

SWD pupae were obtained from the laboratory colonies of Drs. Arytom Kopp (University of California at Davis) and Robert Van Steenwyk (University of California at Berkeley; both colonies originated from wild specimens captured in cherry orchards of coastal California USA. SWD pupae were also obtained from a laboratory colony of Dr. Jana Lee (USDA-ARS), which originated from wild specimens captured in raspberry fields of Marion County, Oregon USA. Pupae from these three sources were integrated into a single colony that was maintained in several (6-8 ct.) nylon mesh enclosures (Bug Dorm-2®, BioQuip Products, Rancho Dominguez, CA, US) housed in an 22.65-m^3 incubation unit (24-27 °C, 80% RH, 16:8 [L:D] h) at the USDA-ARS-SJVASC (Parlier, California USA). Approximately twice a year, SWD adults were captured in raspberry fields located in the Salinas Valley of California and introduced into the SJVASC colony along with new pupae from each of the original sources. Plastic vials (20-dram) containing saturated aqueous solutions of sucrose were capped with cotton wicks to serve as a food and water source for adults. As described in Walse et al. (2012), larvae were reared on standard cornmeal-(dextrose or sucrose)-agar-yeast medium layered to ($\bar{x} \pm s$, AVE. ± STDEV) 4.0 ± 0.6 mm on the bottom of 8.7 ± 0.1-cm diameter Petri dishes, which also served as ovipositional substrate. Formalin ® (2 mL), a fungistat, was added to each 4-L batch of diet. Four diet-containing Petri dishes were placed in each enclosure, replaced after 2-d ovipositional periods, and transferred to a separate communal rearing enclosure for the duration of development. When adults began to emerge from a particular dish, it was transferred back into a community of reproductively-active adults maintained at ~ 2000 individuals per enclosure.

BMSB (*Halyomorpha halys*) eggs were obtained from the laboratory colony of Dr. Tracey Leskey (USDA-ARS-Kearneysville, WV) that originated from wild specimens captured in small fruit and orchard crops in West Virginia, USA. Upon receipt of the BMSB eggs in Oct. 2012 at the Contained Research Facility at UC Davis (Davis, CA), a BSL-III agricultural quarantine facility, all specimens were transferred to an environmentally-controlled chamber set at 26 °C, 65% RH and a 16:8 diurnal light cycle until a strong colony of mixed life stages could be established. A second shipment of eggs was received from Dr. T. Leskey in March 2013 to supplement the CA colony. All non-egg life stages are reared concurrently inside 0.34-m^3 aluminum mesh cages on live bush bean and cowpea plants, supplemented with organic raw almonds, pumpkin seeds, sunflower seeds, and walnuts. Cages are maintained inside a greenhouse at 26 °C, 65% RH and a 16:8 diurnal light cycle. Eggs are collected every 48 h from rearing cages and allowed to hatch on green bean plants in an isolated cage to prevent egg predation. 1st instar nymphs are then transferred via a #4 (3/32") round brush back into the rearing cages 4 d after hatching to complete their life cycle and to maintain reproductive populations at ~500/cage.

Fruit infestation

To simulate a naturally occurring infestation scenario for SWD, ovipositional/diet substrate was removed from an enclosure and replaced with stainless-steel trays (30 × 30 × 2 cm) that were filled with a monolayer of fresh sweet cherries. The stainless-steel trays containing infested sweet cherries were removed after ovipositional periods that varied by test type, infested cherries were transferred to a pull-string cloth bag (~25 per bag) (8" x 12"; ULine, Waukegan IL), and used in laboratory-scale exploratory fumigations or buried throughout the load of commercial fruit bins in confirmatory-scale fumigations. Alternatively, cloth bags were not fumigated and held as untreated controls to estimate the number of individuals treated during a respective fumigation.

All seven life stages (eggs, 1^{st} – 5^{th} instar nymphs, and adults) of BMSB were evaluated in the exploratory fumigations. Egg samples were cut from oviposition substrate (usually a dried bean leaf) and counted under a dissecting microscope under low (10x) power. Nymphs (1^{st} – 5^{th} instars) were randomly collected from the rearing cages described above. Adults were aspirated from the walls and top of the rearing cages. Samples from each life stage were then isolated in vials with mesh-screen covered openings on the top, bottom and sides (2 locations) and placed into cloth bags (8" x 12"; ULine, Waukegan IL). Several pumpkin seeds and a wetted cotton wick were placed in to the cages to serve as food and water sources, respectively. Cloth bags containing vials of the life stages to be treated, were placed inside the environmental room, housing the fumigation chambers, for tempering.

For the exploratory fumigations, SWD-infested fruit were incubated for 0, 48, or 96 h to yield ages, respectively, of 0- to 48-h, 48- to 96-h, and 96- to 120-h old specimens at the start of a 12-h pre-fumigation period of temperature equilibration (i.e., tempering). Infestations and subsequent incubations were synchronized so that all BMSB life stages and all SWD ages concurrently entered the tempering period that preceded fumigation. For the confirmatory fumigations, infestation and incubation was planned to yield 2^{nd} and 3^{rd} instar BMSB as well as 12- to 60- h old SWD (age at fumigation), only the most PH3-tolerant forms (*vide infra*) .

Exploratory fumigations

Exploratory fumigations were performed in modified Labonco® 28.32-L vacuum chambers housed in a walk-in environmental incubator with programmable temperature and humidity (USDA, 2009). A series of experiments was conducted determine the treatment duration, ranging from 6 to 72 h, required to control ages comprising the egg through pupal life stages of SWD as well as all life stages of BMSB with phosphine (PH3) at headspace concentrations $\geq$ 1.5 mgL^{-1} (1000ppmv) at 1.4 ± 0.5°C ($\bar{x} \pm s$). Chambers loaded with SWD-infested fruit, fruit infested with control specimens of SWD, BMSB cages (treated and untreated control specimens), source-gas cylinders, and gas-tight syringes were tempered for at least 12 h to treatment temperatures prior to fumigation. Chamber temperature was confirmed prior to fumigation by a HOBO data logger (HOBOware version 2.7). Chamber lids were then clamp-sealed in preparation for treatment. SWD and PTB were fumigated concomitantly in a chamber.

A pressure of approximately 70 mmHg was established in each chamber. Gas-tight super-syringes (Hamilton ® 500, 1000, or 1500 mL) were filled with a volume of fumigant from a cylinder of 1.6 % (v/v) PH3 balanced with nitrogen (Cytec Canada, Inc., Niagara Falls, Ontario, Canada) to achieve the requisite applied dose of ~ 1.65 mgL^{-1} (1100ppmv) as predetermined in preliminary calibration studies. A syringe was fitted to a LuerLok ® sampling valve, which was subsequently opened so that fumigant was steadily drawn into the chamber. The syringe was then removed and normal atmospheric pressure was established in each chamber before the valve was closed; this marked the beginning of the exposure period. Gas samples (40 mL) were taken temporally at standard intervals from the chamber headspace

through a LuerLok® valve using a B-D® 100 mL gas-tight syringe and quantitatively analyzed for PH3 with GC-PFPD. Fumigant concentrations were measured and exposures, expressed as concentration × time product ("CT"s) calculated by the method of Monro (1969), were tracked.

Following the final sampling to determine headspace concentration, chamber valves were opened to atmosphere, a 1-h aeration period was initiated, and chamber lids were then opened. Treated and non-treated SWD-infested cherries were retrieved from the bags, transferred in pairs into a stainless-steel mesh ball cage (5.1-cm diameter), and the mesh cage was placed back into the respective bag. SWD-infested cherries as well as caged-BMSB were transferred into a rearing incubator at 27.0 ± 1.0 °C and 80 ± 2% RH ($\bar{x} \pm s$), and placed into treatment-respective 0.03-m^3 nylon-mesh rearing cubicle.

Confirmatory export fumigations

To simulate a commercial scenario, fumigations were conducted using 241.9-L steel chambers housed in a walk-in environmental incubator with programmable temperature and humidity (USDA, 2010). On the same day that they were packaged for export, either Bing or Coral variety sweet cherries were obtained from commercial wholesale sources in California or Washington State. Uninfested fruit were removed from cartons and replaced with an equivalent volume of cloth bags containing SWD-infested cherries or caged-BMSB. Liners, if present, were not sealed but folded over atop the cherries. The chamber was first loaded with four 0.5 ft^3 sand bags each wrapped in plastic packaging that displaced ~84.9 L total of chamber volume. Chambers were loaded with six cartons (~8 kg/carton, fruit size 12 row, 43.2 l x 33.0 w x 14.0 h cm carton size, 19.9 L volume). The chamber load was estimated as a fractional percentage, 76.4 ± 0.8% ($\bar{x} \pm s$), of the volume occupied by the load relative to the chamber volume (i.e., $V_L\ (V_{chamber})^{-1}$ x 100) (Monro, 1969).

Chambers loaded with test specimens and uninfested fruit as well as control specimens were acclimated to fumigation temperature of ~1.4 °C (~34.3°F) for 12 h prior to treatment (i.e., tempered) within a temperature-controlled storage unit (USDA, 2009). Fruit pulp temperature was confirmed prior to fumigation by each of three probes (YSI scanning tele-thermometer) that recorded the respective pulp temperature in three uninfested fruit distributed at different locations within the load of the fruit undergoing treatment. Temperature probes were then removed and chamber lids clamp-sealed in preparation for treatment. The chamber ventilation valve was opened and chambers were filled with a volume of fumigant from a cylinder of 1.6 % (v/v) PH3 balanced with nitrogen (Cytec Canada, Inc., Niagara Falls, Ontario, Canada) to achieve the requisite dose as predetermined in preliminary calibration studies. The valve was then closed which marked the beginning of the exposure period. Gas samples (40 mL) were taken from the chamber headspace through a LuerLok® valve using a B-D® 100 mL gas-tight syringe and quantitatively analyzed for PH3 with GC-PFPD at standard intervals corresponding to 5 (initial), 60, 480, 1440 (1-d end), or 2880 (2-d end) min. Fumigant exposures were expressed as concentration × time cross products, "CTs", and calculated by the method of Monro (1969).

After completion of the exposure, chamber valves were opened to atmosphere and vacuum was pulled to aerate the chamber until headspace concentration of the fumigant was below the mandated ventilation requirements of 0.3 ppm (0.45μg/L) phosphine. Chamber lids were opened and the treated and non-treated specimens were collected, SWD were transferred to mesh cages as described above, and all specimens were placed into separate 0.03-m^3 nylon-mesh rearing cubicles maintained in an incubator at 27.0 ± 1.0 °C and 80 ± 2% RH ($\bar{x} \pm s$) as described above. Noninfested fruit was retrieved and used for residue determination and fruit quality evaluation. Samples of noninfested fumigated fruit (75 g each), selected from 3 different locations within the load, were placed into a cooler filled with dry ice

within 5 minutes of the end of aeration and were used to estimate initial residue levels. The remaining noninfested fumigated fruit transferred into cold storage at 1.1 ± 0.6 °C ($\bar{x} \pm s$) (~34.0°F) and temporally retrieved from storage and used for residue determination(s)(*methods and results available upon request*).

Mortality evaluation

SWD mortality was assessed at 1-d intervals post-fumigation for 21 d; rearing cubicles were examined and live adult specimens were tallied and discarded before cubicles were resealed for further incubation and evaluation. The cumulative number of adults, which emerged from each piece of fruit designated as an untreated control for paired fumigation trials, was counted. An average ($\bar{x}$) emergence from each infested fruit left untreated was calculated along with a standard deviation ($\pm s$). The number of SWD specimens ($n \pm s$) that were treated was estimated by multiplying the number of infested fruit treated in each trial by the average emergence from each fruit that was infested and untreated ($\bar{x} \pm s$). The total number of specimens that were treated across all exploratory- or confirmatory-trials was estimated by summing the number from each respective trial and propagating the respective standard deviation.

Two days following treatment, BMSB specimens were retrieved from all cages for evaluation. Egg mortality was diagnosed visually by discoloration, while survivability of other life stages was diagnosed by locomotion or by prodding-induced motion. Post-embryonic BMSB were categorized as moribund if the survivability was inconclusive. Moribund BMSB were placed inside a labeled plastic snap-cap cage with food and water source as described above prior to further incubation until an additional evaluation the following day. Mortality of control BMSB specimens was included as a natural response in the efficacy modeling for exploratory fumigations. For the confirmatory trials, Abbott's method (Finney, 1971) was used to estimate the percentage mortality of BMSB used in Probit calculations. The total number of specimens that were treated for each exploratory- or confirmatory-trial was estimated by summing the numbers treated, while the total number of specimens treated across exploratory- or confirmatory-trials was estimated by summing the numbers from each respective trial.

Rearing and incubation conditions of 27.0 ± 1.0 °C, 80 ± 2% RH, and 16:8 [L:D] h photoperiod were fixed to maintain a consistent progression of development between trials and controls; resulting mortality in control specimens was assumed to be equal to that in fumigation trials. Insects were more likely to survive and there was greater certainty in diagnosing survivorship after the treatment if incubated under conditions described above rather than if refrigerated post-fumigation at < 5 °C under simulated commercial transport conditions, which confound the effect of a fumigation event on mortality. To be detailed in a forthcoming publication on the effect of refrigeration on SWD and BMSB development, for both species we generally observed increases in the mortality of all life stages, the length of the developmental periods of each life stage, and heterogeneity in the times required to complete development within each life stage.

Chemicals and Chemical analysis

A 300-lb cylinder of 1.6 % (v/v) PH3 balanced with nitrogen was obtained from Cytec Canada, Inc. (Niagara Falls, Ontario, Canada) and used as the source for gas chromatography calibrations as well as fumigations.

PH3 levels in headspace of fumigation chambers were measured using gas chromatography; retention time (PH3, $t_r = 3.2 \pm 0.2$min) was used for chemical verification and the integral of peak area, referenced relative to liner least-squares analysis of a concentration – detector response curve, was used to

determine concentration. Detector response and retention indices were determined each day in calibration studies by diluting known volumes of gases into volumetric gas vessels. PH3 analyses were with a Varian 3800 and splitless injection (140 °C) using a gas sampling port with a 10 μL-sample loop, a Teflon column (L = 2 m, OD = 2 mm) packed with Porpak N (80/100 mesh) held at 130 °C for 10 min, and a PFPD detector (13 mL/min H_2, 20 mL/min air, and 10.0 mL/min N_2 make-up) at 250 °C that received only 10% of the 15 ml He/min column flow.

Fruit quality. The effects of fumigation on fruit quality were quantified by methods reported in Obenland et al. (2011) and Mitcham et al (2003) by evaluating characteristics of non–fumigated cherries relative to those fumigated in confirmatory SWD fumigations with 1000 ppm PH3 and treatment durations of either 24 or 48 h. Quality parameters were evaluated after storage for 2 days at 1.1 ± 0.6 °C ($\bar{x} \pm s$) (~34.0°F) plus 16 hours at 22.2 ± 0.6 °C ($\bar{x} \pm s$) (~72.0°F) to simulate air shipment and marketing. Surface browning, stem browning, pitting, cracking, shrivel, decay and overall acceptability were subjectively evaluated as listed in Table 1. Ratings that would likely be unacceptable to a consumer are indicated. Ratings are presented as calculated indices or in terms of acceptability. Skin color was evaluated using a Minolta colorimeter by measuring the same spot on the skin of 10 fruit for each replication before treatment and after storage and expressed in the L*C*h scale as amount of color difference (poststorage - pretreatment). Acidity was determined from the juice of 5 pooled fruit for each replication by titration with NaOH. Soluble solids were measured from the same juice using a digital refractometer as in Obenland et al. (2005). Firmness (g-1mm deflection) was measured with a Bioworks Firm Tech 2 instrument.

Executive summary:

A new postharvest treatment option to control SWD and BMS has been developed for those Western US sweet cherry growers/packers. Packed-boxes need to be fumigated at cold-storage temperature for 48 h. A report can be drafted ad presented to Industry (and thereafter APHIS) for consideration. Currently, market options include those countries willing to fumigate with phosphine on arrival (e.g., chile). ARS is working with industry and USEPA to gain registration for PH3 so that fumigations can be done at the packinghouse.

PH3 chamber fumigations at 1.4 ± 0.5 ºC ($\bar{x} \pm s$)(~34.5°F) were evaluated for postharvest control of spotted wing drosophila, *Drosophila suzukii*, and brown marmorated stink bug (BMSB) in fresh sweet cherry exports from Western USA. The most PH3-tolerant age of SWD (0 – to 48-h old, ~95% eggs) as well as life stage of BMSB (2^{nd} & 3^{rd} instars) was established via a series of exploratory fumigations. In confirmatory fumigations, which simulated the commercial scenario, complete mortality of 35,265 ± 1,006 (*n* ± *SE*) SWD eggs as well as 5,149 BMSB, was achieved with an applied dose of 1000 ppm, a load factor of ~ 76.4%, and a treatment time of 48 h at 1.4 ± 0.5 ºC ($\bar{x} \pm s$)(~34.5°F).

Sorption, off-gassing (i.e., depuration), and residue data were obtained. Results can be used by industry in the context of quantifying fumigant inputs to ingestion exposure and worker inhalation exposure that are respectively derived from the consumption of fruit residues and off-gassing of palletized fruit in cold-storage. Relative to methyl bromide, ~10-fold less mass of phosphine is sorbed by palletized loads of fruit during fumigation, phosphine respectively off-gasses ~15-fold faster from loads in cold-storage, and ~15-fold shorter amount of time is required for phosphine residues in sweet cherries to meet USEPA food tolerances.

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

Project Title: Extending storage/shipping life and assuring good arrival of sweet cherry

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Total Project Request: **Year 1:** $26,375 **Year 2:** $26,913 **Year 3:** $24,466

Other funding sources: None

WTFRC Collaborative expenses: None

Budget 1: Yan Wang
Organization Name: OSU-MCAREC **Contract Administrator:** L.J. Koong
Telephone: 541-737-4066 **Email address:** l.j.koong@oregonstate.edu

Item	2012	2013	2014
Salaries		10,384[1]	10,696[7]
Benefits		1,848[2]	1,903[7]
Wages	9,600	5,312[3]	5,471[7]
Benefits	8,275	1,222[4]	1,259[7]
Equipment			
Supplies	8,000	7,647[5]	4,637
Travel	500	500[6]	500
Miscellaneous			
Total	26,375	26,913	24,466

Footnotes:
[1]Postdoctoral Research Associate (Dr. Xingbin Xie): 550hr at $18.88/hr.
[2]OPE: $3.36/hr.
[3]Wages: 390hr for a Biological Science Tech. at $13.62/hr.
[4]OPE: 23% of the wage.
[5]Supplies: fruit, Ca and Cl analysis, GC-MS volatile analysis, gases (helium, nitrogen, hydrogen, standard gases), gas tank rental, chemicals, and MCAREC cold room use fee.
[6]Travel to grower's fields
[7]3% increase

OBJECTIVES

The goal of this project was to minimize pitting, postharvest splitting, acid loss, dull color, and stem browning, therefore improve shipping quality of the PNW and California sweet cherries.

The key objectives were to:

1. ***Modified atmosphere packaging (MAP):*** Determine the optimum MAP parameters (O_2, CO_2) and efficacy of the major commercial MAP liners and consumer packaging for improving shipping quality of the PNW and California cultivars.

2. ***Calcium (Ca):*** Study the mechanism and practical postharvest Ca treatment to minimize pitting and splitting of PNW sweet cherries.

3. Evaluate ***edible coatings and GRAS compounds*** on shipping quality of PNW sweet cherries.

SIGNIFICANT FINDINGS

1. Respiration physiology influenced by $O_2$and CO_2, temperature, and cultivars

- At shipping temperatures, respiration rates of the major PNW and California cultivars were affected very little by reduced O_2 from 21 to 10%, but declined significantly from 10 to 5%.
- Estimated fermentation induction points were about1-4% O_2 for the major cultivars depending on temperatures.
- CO_2 at 0-15% did not affect respiration rates of 'Bing', 'Sweetheart', and 'Coral'.
- 'Skeena' had a higher RQ (respiration quotient) and respiration Q_{10} than other cultivars. Therefore, 'Skeena' is more susceptible to anaerobic injury.
- 'Skeena' fruit stressed by heat had a higher respiration rate and are more susceptible to anaerobic injury.

2. MAP Technologies

- It was found that the major commercial MAP liners (7) had extremely varied equilibrium O_2 (i.e., 1-15%) and CO_2 (i.e., 5-13%) concentrations for the major PNW and California cultivars at simulated commercial shipping conditions.
- ***O_2 concentration affected flavor.*** MAP liners with equilibrium O_2 5-8% at 32 °F reduced respiration rate and therefore maintained titratable acidity (TA) and flavor of the major cultivars after 4-6 weeks of cold storage. MAP liners with O_2 > 10% did not maintain flavor. MAP liners with O_2 < 5% may cause anaerobic fermentation during commercial storage/shipping.
- ***CO_2 concentration affected fruit color darkening.*** MAP liners with equilibrium CO_2 10-15% maintained the shiny fruit color at simulated storage/shipping conditions. MAP liners with CO_2 < 8% had little beneficial effect on maintaining fruit shiny color.
- ***'Regina', 'Skeena', and 'Lapins'*** produced a bitter taste after 3-6 weeks storage/shipping. MAP liners with O_2 at 5-8% prevented or reduced bitter taste development.
- ***'Skeena'*** is more susceptible to anaerobic fermentation at fluctuated temperatures, therefore, needs MAP liners with relatively higher gas permeability (i.e., O_2 8-10%) to avoid anaerobic injury in commercial storage/shipping.
- ***Consumer packaging.*** Zipper-lock bags and clamshells with perforation ratio of 0.5% (3mm diameter) maintained cherry pedicel healthier than the commercial ones (perforation at 2-5%, 8mm diameter), without generating extra condensation or fermentation after a simulated storage/shipping/marketing period.

3. Postharvest Ca application in hydro-cooling water

- Pitting susceptibility was found to be correlated negatively with fruit tissue Ca content.
- Splitting potential was correlated with fruit tissue Ca content and pectin chemistry.
- Adding Ca (0.2-0.5%) in hydro-cooling water (32 °F) efficiently increased fruit tissue Ca content in 5 min.
- The enhanced Ca concentration increased fruit firmness (FF) and retarded fruit senescence, therefore, reduced pitting susceptibility, maintained TA and Vc, and reduced postharvest splitting and decay of 'Bing', 'Skeena', 'Lapins' and 'Sweetheart'.
- EDTA (a chelator of divalent cation) or low pH (i.e., <4) depleted Ca from fruit and increase splitting of cherry fruit.
- Ca application rate and temperature gradient between fruit and solution were the key factors determining efficacy of the Ca treatments.
- Higher Ca rates (1.0-2.0%) damaged cherry stems.
- Cherry fruit didn't take up Cl.

4. Edible coatings and GRAS compounds

- Semperfresh™ at appropriate rates (i.e., 0.5% a.i.) reduced moisture loss, maintained stem quality, and reduced pitting expression of cherries packed in clamshells. Semperfresh™ at its label rate of 1.0% a.i. increased pitting expression of 'Sweetheart'.
- Postharvest applications of salicylic acid (SA) and oxalic acid (OA) tended to reduce respiration rate and maintain higher TA during storage.
- There may be little benefit at commercial level from postharvest applications of Chitosan, Sodium alginate, Jasmonic acid (JA), Methyl Jasmonate (MeJA), ethanol, GA_3, and Homobrassinolide (HBR) on PNW sweet cherries.

METHODS

1. Respiration physiology

Cherry samples of ~500g of 'Bing', 'Skeena', 'Regina', 'Lapins', 'Sweetheart', and 'Coral' were placed in hermetically sealed glass containers (960mL) equipped with 2 rubber sampling ports at 32 and 68°F. Headspace O_2 and CO_2 concentrations were periodically monitored by an O_2/CO_2 analyzer. Respiration rates based on O_2 consumption and CO_2 production, fermentation induction point, and respiration quotient (RQ) were plotted with O_2 and CO_2 concentrations.

2. MAP Trials

The major commercial MAP liners (ViewFresh, Xtend, LifeSpan, Breatheway, Primpro, PEAKfresh, FreshLOK) with distinct technologies were obtained from the manufactures. Fruit of different cultivars were either obtained from packinghouses shortly after packing or harvested directly from the field and then packed into different MAP liners after pre-cooling. The concentrations of O_2 and CO_2 in MAP liners were determined every day in the first week then every 3-5 days until at the end of the tests. At 2, 4, and 6 weeks, 50 fruit were randomly selected from each box for determinations of respiration, FF, color, anthocyanin, SSC, TA, Vc, ethanol, and volatile-aroma compounds (GC-MS) immediately after cold storage and plus 2 days at 68°F. Fifty fruit were randomly selected for evaluations of pitting, splitting, stem quality, decay, and sensory evaluation. Experimental units were boxes and there were three replications per treatment at each evaluation period. The experimental design was completely randomized.

3. Postharvest Ca Application in hydro-cooling water

Ca solutions at 0, 0.2, 0.5, 1.0, and 2.0% were cooled to 32 °F before treatments. Fruit harvested at commercial maturity from MCAREC with fruit pulp temperature 70-80 °F were immediately hydro-cooled in the cold Ca solutions for 5 min to simulate the commercial hydro-cooling procedures. Fruit tissue Ca and Cl content (ICP-AES and Lachat Quikchem autoanalyzer methods, respectively),

shipping quality (pitting, splitting), eating quality, nutracuetical values, and biochemical changes were evaluated after 2, 4, and 6 weeks of cold storage.

4. Postharvest Applications of edible coatings and GRAS Compounds

Semperfresh™, Chitosan, Sodium alginate, Salicylic acid (SA), Oxalic acid (OA), Jasmonic acid (JA), Methyl Jasmonate (MeJA), ethanol, GA_3, Homobrassinolide (HBR, a brassinosteriod) are applied postharvest on certain PNW cultivars.

RESULTS AND DISCUSSION

1. Respiration Dynamic

While respiration rate of cherry fruit was inhibited linearly by reduced O_2 concentration from 21% to 3-4% at 68 °F, at 32 °F it was affected very little from 21% to ~10% but declined significantly from ~10% to ~1% for 'Bing', 'Sweetheart', and 'Coral' (Fig. 1). Estimated fermentation induction points determined by a specific increased RQ were ~1% and 3-4% O_2 for all cultivars at 32 and 68 °F, respectively. As a consequence, the gas permeability of MAP has to be modified to reduce O_2 between 10-5% at 32 °F within the package to inhibit cherry fruit respiration activity to maintain fruit quality (flavor) without anaerobic fermentation during commercial storage/shipping.

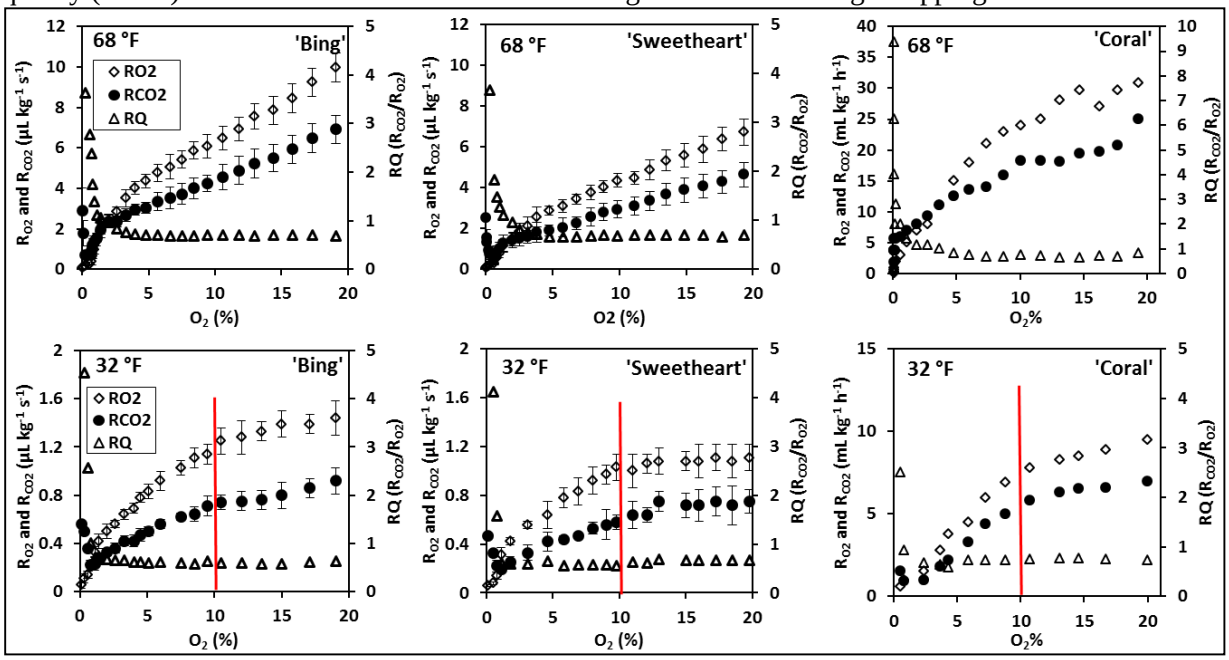

Fig. 1. Respiration dynamics of sweet cherries affected by O_2, temperature, and cultivars.

'Skeena' has a higher RQ at elevated temperatures and therefore is more sensitive to anaerobic injury due to temperature fluctuations during shipping (Fig. 2). MAP liners with equilibrium 8-10% O_2 at 32 °F may be suitable for 'Skeena' at commercial shipping. Q_{10} was determined to be 3.5, 3.3, 3.1, and 3.0 at temperatures from 32 to 50 °F for 'Skeena', 'Lapins', 'Regina' and ''Sweetheart', respectively. 'Skeena' fruit stressed by heat in the field had higher respiration rates, a shorter shelf-life, and were more susceptible to anaerobic injury (Data not shown). Heat stressed Skeena could show pitting on the trees.

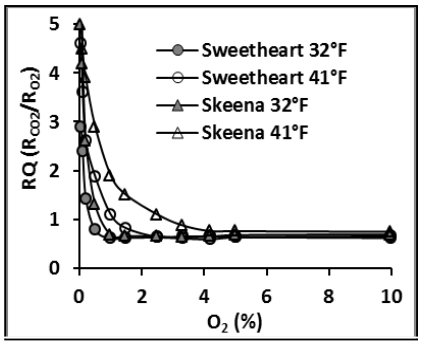

Fig. 2. RQ of Sweetheart and Skeena.

2. MAP Technologies

1) Gas permeability of different MAP liners.

The seven commercial MAP liners used in sweet cherry industry generated extremely varied equilibrium O_2 and CO_2 concentrations for different cultivars at recommended shipping temperatures (Fig. 3). O_2 ranged from 1-15% and CO_2 ranged from 5 to 15% for 'Bing', 'Lapins', 'Skeena', 'Regina', 'Sweetheart', and 'Coral'.

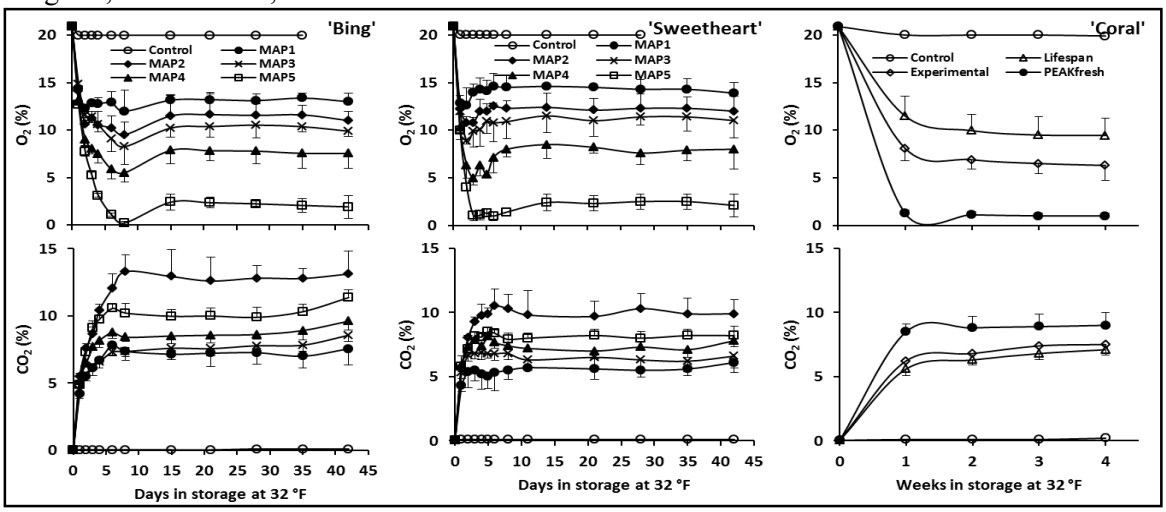

Fig. 3. O_2 and CO_2 contents in different MAP liners for 'Bing', 'Sweetheart', and 'Coral' at 32°F.

2) Effect of elevated temperatures on O_2 and CO_2 in MAP liners and anaerobic fermentation.

Elevated transit temperatures from 32 to 41 °F reduced O_2 significantly (Fig. 4) but did not change CO_2 much in MAP liners. The equilibrium O_2 in MAP4 and MAP5 were reduced from ~6% and 2% at 32 °F to ~3.5% and 0.5% at 41 °F for Sweetheart and Skeena, respectively (Fig. 4). At 36 °F, the equilibrium O_2 was 4.5% and 1% in MAP4 and MAP5 during 2 weeks of cold storage and there was no significant accumulation of ethanol in 'Sweetheart' and Skeena after 2 weeks of cold storage (data not shown). At 41 °F, ethanol was accumulated significantly in 'Sweetheart' packed in MAP5 and Skeena packed in MAP4 and MAP5 (Fig. 4). Fermentation flavor was detected in the fruit with significant ethanol accumulation. In conclusion, MAP with appropriate gas permeability (i.e., 5-8% O_2 for most of the cultivars and 8-10% O_2 for Skeena) are suitable for commercial application to maintain flavor without damaging the fruit through fermentation, even if temperature fluctuations, common in commercial storage/shipping, do occur.

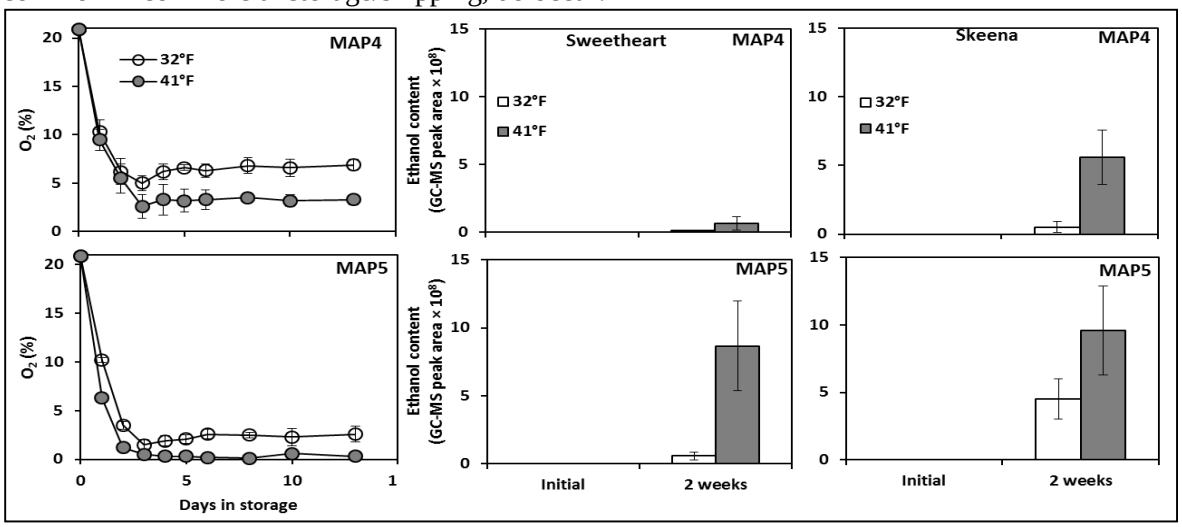

Fig. 4. Effect of elevated temperature on O_2 in MAP and ethanol accumulation in cherry fruit.

3) Efficacies of different MAP liners on maintaining fruit shipping quality. While all the MAP liners maintained higher FF and reduced decay, only the MAP liners with lower O_2 permeability (i.e., equilibrated at 5-8% O_2) reduced fruit respiration rate and maintained TA and flavor compared to the standard macro-perforated PE liners after 4-6 weeks of cold storage. In contrast, MAP liners that equilibrated with atmospheres of 10-15% O_2 had little effect on inhibiting respiration rate and TA loss, MAP with 1-2% O_2 enhanced ethanol accumulation and fermentation flavor during cold storage (Fig. 5).

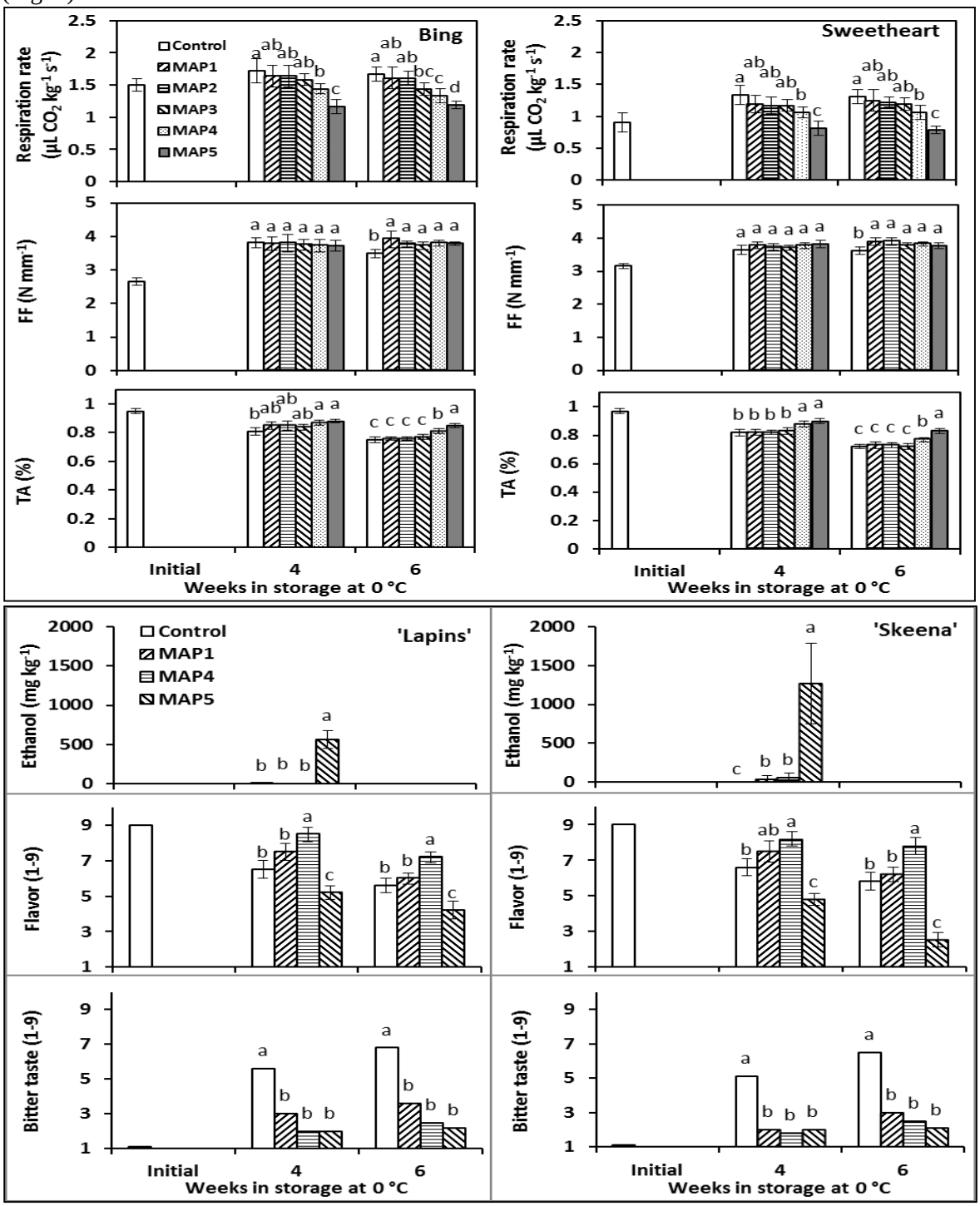

Fig. 5. Effect of MAP on cherry fruit quality during storage.
Cherry fruit skin darkening during storage gave the fruit a dull and over-ripe appearance that affected consumer preference. Fruit skin darkening during storage was reflected by reduced L* and increased anthocyanin accumulation. Higher CO_2 concentrations (10-15%) in MAP retarded anthocyanin accumulation and fruit skin color darkening significantly. In contrast, CO_2 < 8% had much less effect on retarding anthocyanin synthesis and maintaining the luster skin color of cherry fruit after cold storage/shipping (Fig. 6).

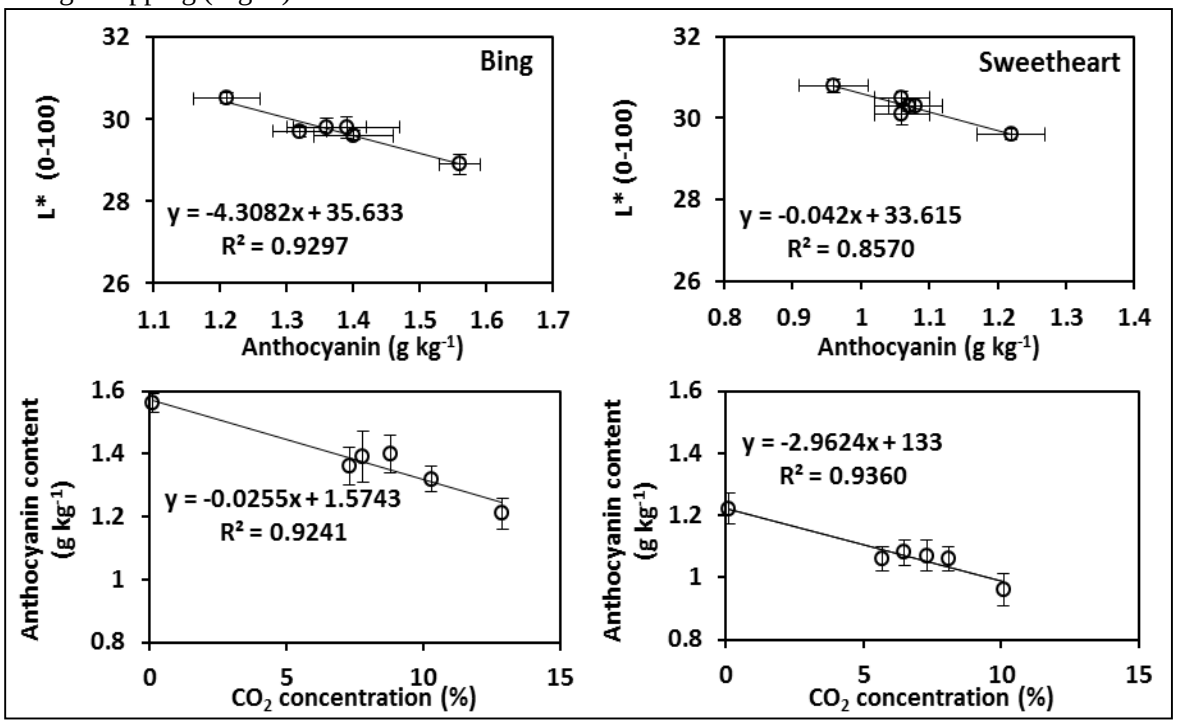

Fig. 6. The relationship of cherry fruit skin darkening with anthocyanin and CO_2 concentration in MAP during storage at 32 °F.

4) Consumer packaging. The perforation ratios of commercial zipper-lock bags or clamshells were ranged from 2-5%. The RH within zipper-lock bags with perforation of 2% were 96%, 93%, and 91% at environment temperatures of 32°F (RH 88%), 50°F (RH 75%), and 68°F (RH 65%), respectively. The RH within zipper-lock bags with perforation of 0.5% were 99%, 98%, and 96% at 32°F, 50°F, and 68°F, respectively. RH within the bags with perforation of 0.05% was close to 100% at each of the temperatures tested (Fig. 7). Stem moisture losses of Chelan and Lapins were higher in bags with 2% perforation than 0.5% and 0.05% at each of the simulated marketing stages. Stem visual quality was higher in bags with perforation at 0.5% than at 2% after 1 week at 32°F + 2 days at 50°F + 2 days at 68°F. Bags with perforation at 0.05% had higher condensation and higher decay incidence (data not shown).

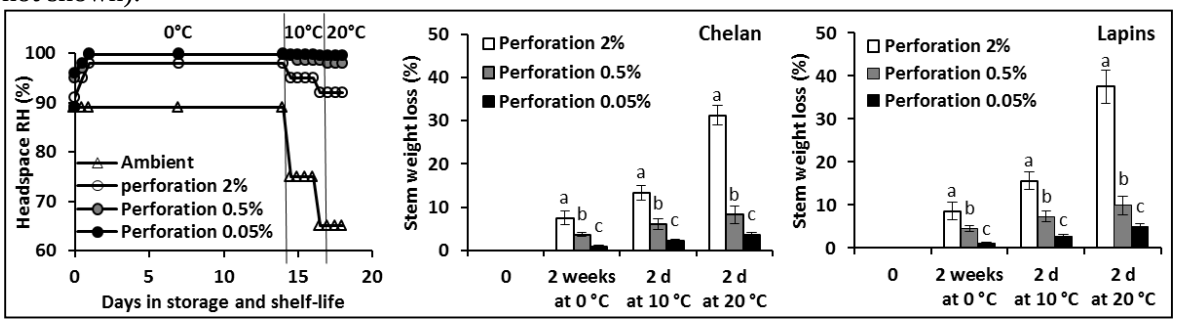

Fig. 7. Effect of perforation ratio in zip-lock bag or clamshell on RH and cherry stem weight loss.

3. Postharvest Ca Application in Hydro-Cooling Water

1) Increasing fruit tissue Ca content. Cherry fruit absorbed Ca with increasing Ca concentration from 0.2 to 2.0% in cold water (0 °C) for 5 min (simulating commercial hydro-cooling), but did not take up Cl (Fig. 8). Extending the exposure time from 5 to 30 min increased tissue Ca content of both cultivars at each Ca rate numerically but not at a statistically significant level.

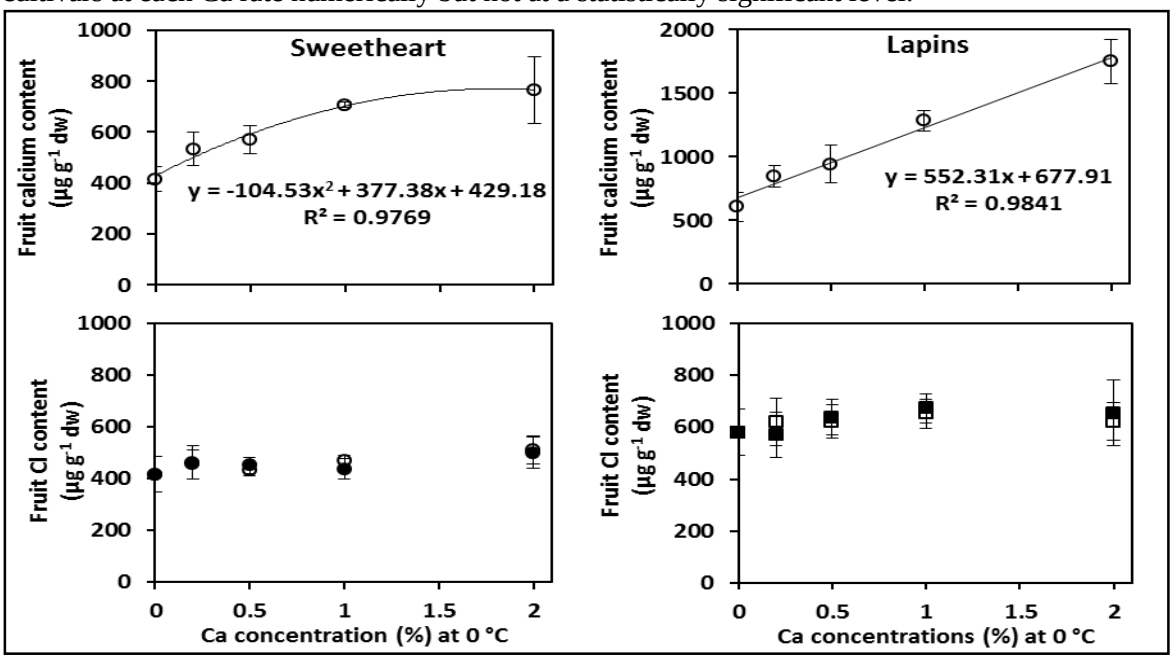

Fig. 8. Tissue Ca and Cl uptakes by cherry fruit **as** affected by $CaCl_2$ in cold water at 0 °C.

2) Retarding senescence, increasing firmness, and reducing pitting. The increase of fruit tissue Ca content was accompanied by reductions in respiration rate, ascorbic acid (AsA) degradation, and membrane lipid peroxidation (Fig. 9). The Ca treatments enhanced total phenolics content and total antioxidant capacity, and resulted in increases in fruit firmness and pitting resistance (Fig. 10) and decreases in TA loss and decay (data not shown) of both cultivars. Pedicel browning was inhibited by Ca at 0.2% and 0.5%, but increased by higher rates at 1.0% and 2.0% (Fig. 11), possibly via modifying membrane lipid peroxidation.

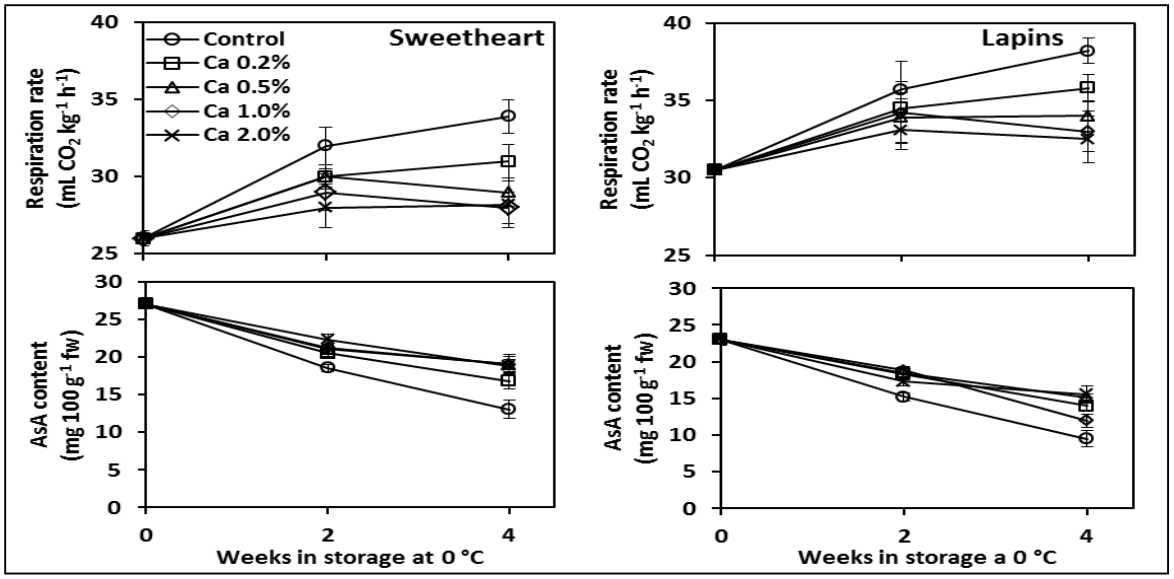

Fig. 9. Effect of Ca in hydro-cooling water on cherry respiration rate and AsA degradation.

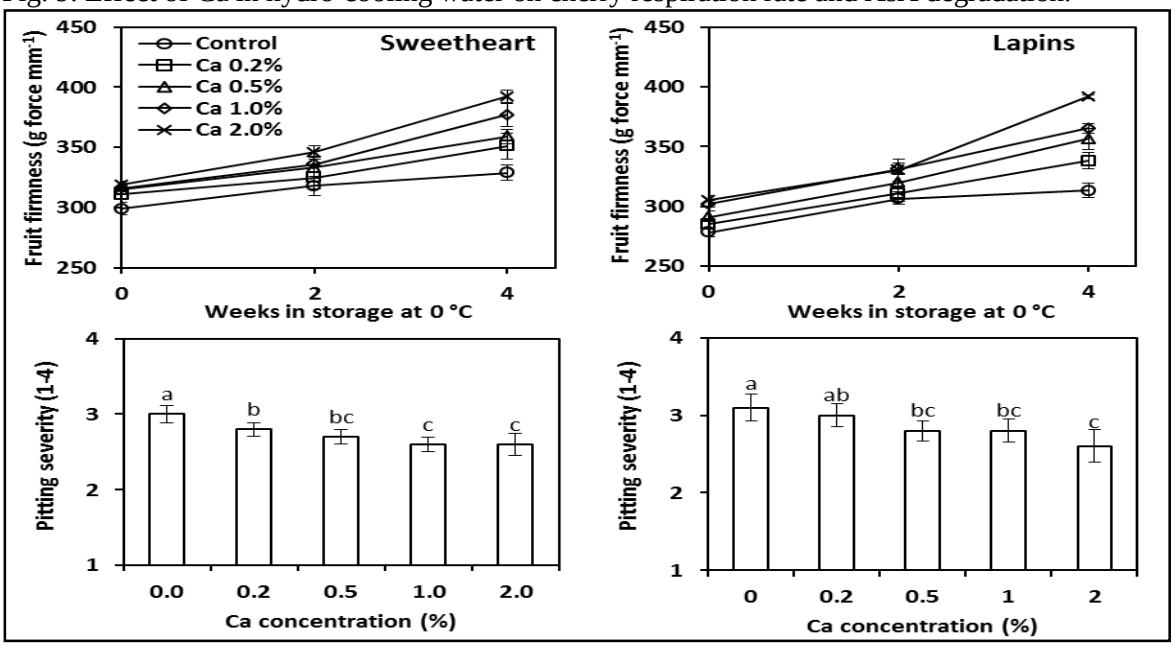

Fig. 10. Effect of Ca in hydro-cooling water on cherry fruit firmness and pitting susceptibility.

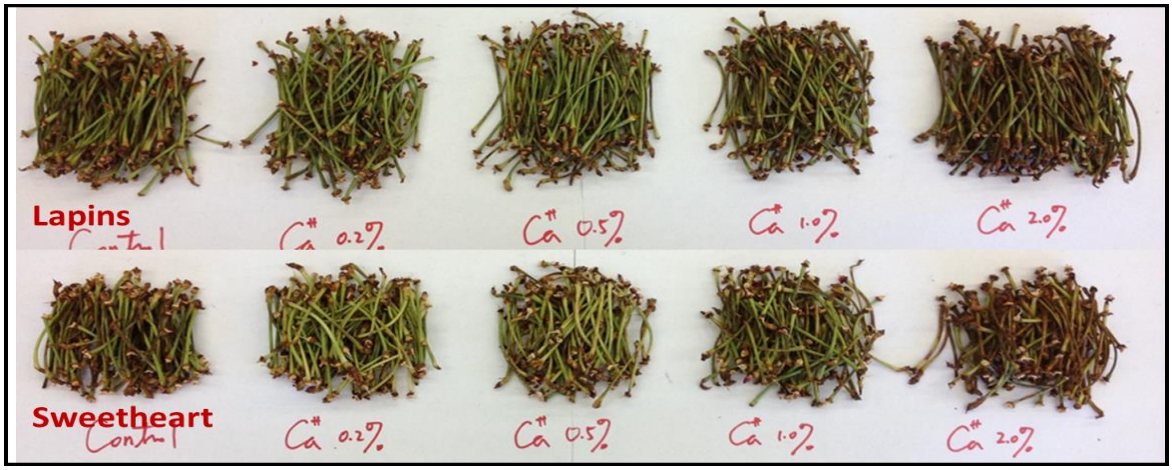

Fig. 11. Effect of Ca in hydro-cooling water on cherry stem quality after 2 weeks of storage.

3) Reducing splitting of Skeena and Bing. The enhanced tissue Ca content reduced splitting potential of the splitting-susceptible cultivars (i.e., Skeena) by decreasing fruit soluble pectin release and increasing the splitting threshold. In contrast, depleting Ca from fruit tissue by EDTA or low pH increased soluble pectin release and splitting potential (Fig. 12).

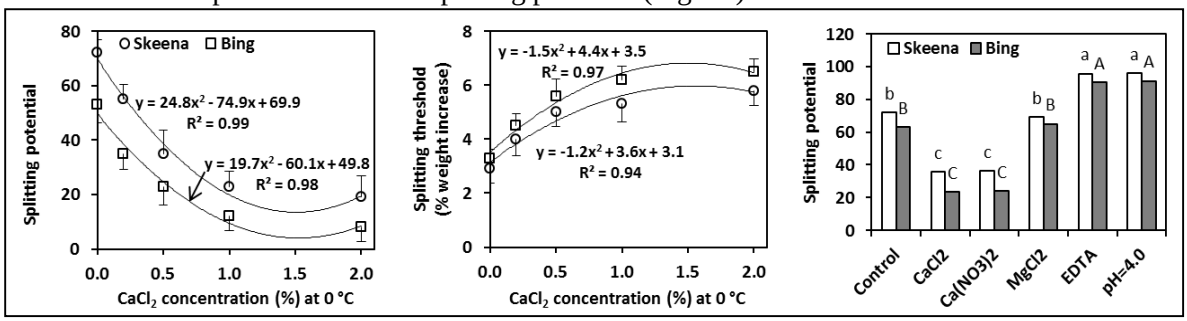

Fig. 12. Effect of Ca in hydro-cooling water on splitting potential of Skeena and Bing cherries.

4. Postharvest Treatments with GRAS Compounds and edible coatings

1) SA, OA, JA, MeJA, ethanol, HBR,

Postharvest applications of SA and OA tended to reduce respiration rate and maintain TA of PNW cultivars packed in clamshells during storage (Fig. 13). It was reported that both SA and OA enhanced total antioxidant capacity (TAC) in 'Cristilina' and 'Prime Giant' cultivars (Valero et al., 2011), however, they do not seem to affect TAC of PNW cultivars during cold storage (Fig. 13). Postharvest treatment with JA, MeJA, ethanol, and HBR had little effect on shipping quality of 'Lapins' and 'Skeena' at commercial level (data nor shown).

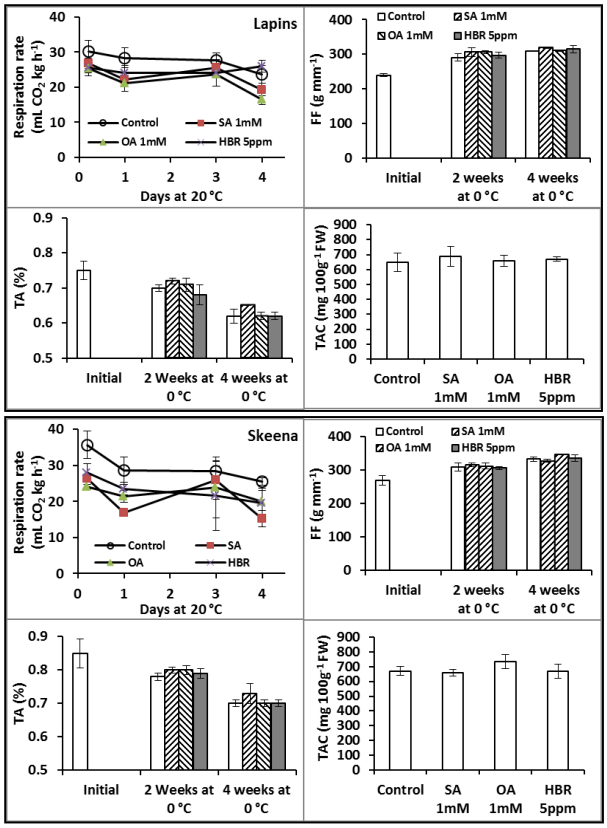

Fig. 13. Effect of SA, OA, and HBR on respiration rates, TA, FF, and total antioxidant capacity (TAC) of 'Lapins' and 'Skeena'.

2) Semperfresh™, GA_3, sodium alginate, chitosan

Semperfresh™ at 0.5% a.i. reduced moisture loss and maintained green stem of 'Chelan' and 'Lapins' packed in clamshells at simulated marketing conditions (Fig. 14). GA_3 at 100ppm did not affect shipping quality of 'Chelan' and 'Lapins'. Semperfresh™ reduced pitting of 'sweetheart' at application rate of 0.5% a.i., but increased pitting at its label rate of 1.0% a.i.(Fig. 15). Pitting formation seems to be associated with moisture loss and localized O_2 deficiency. Chitosan and alginate had little effect on shipping quality of 'Chelan' and 'Lapins' (data not shown).

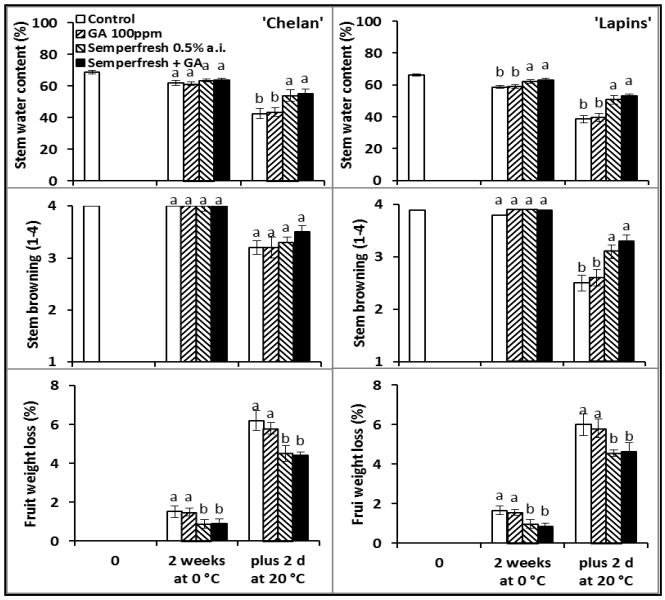

Fig. 14. Effect of Semperfresh™ and GA_3 on shipping quality of Chelan and Lapins at simulated marketing conditions.

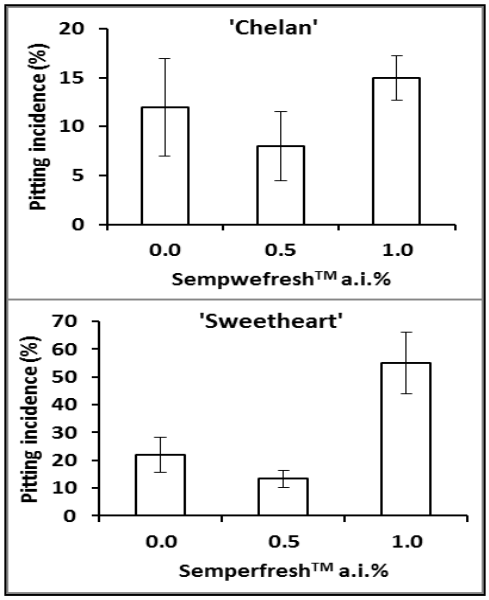

Fig. 15. Effect of Semperfresh™ on pitting incidences of Chelan and Sweetheart after 2 weeks of cold storage.

EXCUTIVE SUMMARY

Project title: Extending storage/shipping life and assuring good arrival of sweet cherry

Due to a high respiratory activity, minimal reserve carbohydrate, and high susceptibility to mechanical damage and water internalization injury, sweet cherries are highly perishable and have a shelf life of only about 2 weeks under cold chain management. Their shelf life is often shortened due to loss of flavor, darkening of fruit skin color, pitting, splitting, pedicel browning, and decay development. Choosing the MAP liners with right gas permeability and postharvest Ca treatment are found to improve shipping quality of sweet cherries.

Modified atmosphere packaging (MAP).

Understanding respiration dynamics influenced by $O_2$and CO_2, temperature, and cultivars is an essential knowledge for reducing respiration rate and extending storage/shipping life of cherries. We found that while respiration rate of PNW and California cultivars was inhibited linearly by reduced O_2 concentration from 21% to 3-4% at 20 °C, it was affected very little from 21% to ~10% but declined significantly from ~10% to ~1% at 0 °C. Estimated fermentation induction points were ~1 - ~4% O_2 for PNW and California cultivars depending on temperature. CO_2 between 0-15% did not affect respiration rate, but inhibited fruit skin darkening by retarding anthocyanin accumulation.

The commercially available MAP box liners for sweet cherries were found to have extremely varied gas permeability (i.e., 1-15% O_2 + 5-15% CO_2). While all the MAP liners maintained higher fruit firmness, greener stem, and reduced decay, only the MAP liner with 5-8% O_2 maintained higher TA and better flavor by reducing respiration rate. The MAP liners with 10-15% CO_2 maintained shiner skin color. The MAP liners with 1-2% O_2 increased fruit ethanol accumulation and therefore anaerobic flavor after storage/shipping. Most of the PNW and California cherry cultivars packed in the MAP liners with 5-8% O_2 did not accumulate ethanol at temperature fluctuation between 32-41 °F. Skeena is more susceptible to anaerobic injury and should be packed in MAP liners with 8-10% O_2.

Implementing Ca in hydro-cooling water (5 min)

Calcium (Ca^{2+}) plays an extremely important role in the fruit for cell wall structure and strength, plasma membrane structure and integrity, and cellular signaling responses. However, fruit are often deficient in Ca due to its low mobility in plants. Enhancing Ca content can be extremely beneficial in reducing disorders and maintaining quality of fruit during storage. We found that cherry fruit tissue Ca content increased significantly as Ca rate increased from 0.2% to 2.0% at 0 °C for 5 min. The increase of fruit tissue Ca content was accompanied by reductions in respiration rate, ascorbic acid degradation, and membrane lipid peroxidation, which enhanced total phenolics content and total antioxidant capacity, and resulted in increases in fruit firmness and pitting resistance and decreases in titratable acidity loss and decay. The enhanced tissue Ca content also reduced cherry fruit splitting potential by decreasing fruit soluble pectin release and increasing the splitting threshold. In contrast, depleting Ca from fruit tissue by EDTA or low pH increased soluble pectin release and splitting potential. Pedicel browning was inhibited by Ca at 0.2-0.5%, but increased by higher rates at 1.0-2.0%, possibly via modifying membrane lipid peroxidation.

Edible coatings and GRAS compounds

Some benefits on cherry fruit quality from applications of edible coatings and GRAS compounds are reported in literatures. We did not find significant improvement at commercial level on shipping quality of PNW cultivars by postharvest applications of SA, OA, JA, MeJA, ethanol, HBR, GA_3, sodium alginate, chitosan in our conditions. Semperfresh™ helps reducing stem browning, but the rate at 1.0% a.i. may increase pitting expression for certain cultivars.

CONTINUING REPORT **PROPOSED DURATION**: 1 of 2 Years
WTFRC Project Number: CH-14-100

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

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Cooperators: Yunyang Zhang, Amit Dhingra, Umesh Rosyara, Todd Einhorn, Amy Iezzoni, BPAC

Total Project Request: **Year 1:** 26,002 **Year 2: 26,842**

Other funding sources

Agency Name: WTFRC/OSCC
Amount awarded: S442, 847 for 3 years from 04/01/2012 to 03/31/2014
Notes: PNW Sweet cherry breeding and genetics program

Agency Name: WTFRC/OSCC
Amount requested: $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 requested: $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

Budget

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

Item	2014	**2015**
Salaries	14,585	**15,168**
Benefits	6,417	**6,674**
Wages	2,800	**2,800**
Benefits	272	**272**
Supplies	928	**928**
Travel (in-state)	1,000	**1,000**
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 pedicel-fruit retention force (PFRF), bacterial canker and powdery mildew incidence (**Oraguzie, Peace, Zhao**).

2. Obtain phenotypic data on cracking 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' (S x C) population for specific trait phenotyping (pitting, PFRF and cracking susceptibility) (**Oraguzie, Peace, Zhao, Hanrahan, Wang**).

SIGNIFICANT FINDINGS AND ACHIEVEMENTS

1. We have identified a genomic region on cherry chromosome 2, *PFRF_G2b*, explaining ~30% of the phenotypic variation for PFRF. This genomic region is common to both the S x C population and the 600 sweet cherry accessions. A genetic test is being developed for this locus in the project, CH-14-102. In addition, we also identified genomic regions on chromosomes 1, 3, 5 and 8 specific to S x C population.
2. A genomic region on chromosome 5, *PM_G5a*, observed in multiple years explained 40% of the phenotypic variation for foliar powdery mildew incidence. This locus is now the target of DNA test development in CH-14-102. In addition, genomic regions for fruit infection have been preliminarily identified on chromosomes 4 and 6.
3. A genomic region on chromosome 5 explaining 15% of the variation in bacterial canker susceptibility have been identified and submitted to CH-14-102, for genetic test development.
4. Following preliminary analysis we identified a genomic region on chromosome 3 for cracking susceptibility explaining 15% of the phenotypic variation. Minor genomic regions were also identified on chromosomes 1, 2, 6 and 8.
5. Genomic regions underlying pitting susceptibility have been identified on chromosomes 1 and 3 using two data sets. We are planning to validate these genomic regions before MAB strategies are developed.

Results and Discussion

Two sets of germplasm were used in the studies. One set consisted of 600 accessions including old cultivars, current commercial cultivars, advanced selections and selected seedling populations developed in the RosBREED SCRI project, whereas the other set comprised 110 individuals in single tree plots belonging to the Selah x Cowiche (S x C) mapping population developed in the Stem-free cherry SCRI project. The genotypic data sets were also in these projects. The objectives of the project 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, 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 S x C mapping population, respectively.

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. The five largest fruit 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 across years and in the combined data set associated with this trait (Table 1). The percentage of phenotypic variation in PFRF explained by this genomic region in the combined data was 27%. Other genomic regions were observed on chromosomes 1 and 2 in 2011 and one on chromosome 4 in 2012 explaining one third of the phenotypic variation.

Table 1. The genomic regions for PFRF identified per year and in the combined data in the 600 pedigree-linked accessions.

Year	Chromosome	Locus
2010	2	*PFRF_G2a*
2011	1	*PFRF_G1*
	2	*PFRF_G2b*
	8	*PFRF_G8a*
	8	*PFRF_G8b*
2012	2	*PFRF_G2b*
	4	*PFRF_G4*
Combined data	2	*PFRF_G2b*

In the S x C population, 10-15 fruit per individual were screened in 2012, 2103 and 2014, and interval mapping in MapQTL was used to identify genomic regions (Table 2). Chromosomes 1, 2, 3 and 5 each had a genomic region which was observed across years, while a genomic region on chromosome 8 was observed between years in 2012 and 2013. The combined data showed genomic regions on chromosomes 2, 3, 5 and 8.

Table 2. The chromosomes that harbor genomic regions identified for PFRF per year and in the combined dataset in the S x C population.

Year	Chromosome
2012	1
	2
	3
	5
	8
2013	1
	2
	3
	5
	8
2014	1
	2
	5
Combined data	2
	3
	5
	8

The genomic region on chromosome 2 is consistent between the 600 accessions and the S x C population and this is being followed up with genetic test development in the project, CH-14-102. It appears that the genomic regions on chromosomes 3, 5 and 8 may be specific to S x C population and these will be targeted for further development to select individuals from this 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 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, we identified a genomic region on chromosome 5 that is consistent across years including 2011, 2012, 2013 and 2014. Genomic regions on chromosomes 2, 3, 5, 6 and 7 were also identified in individual years (Table 3). Combining all 4 years data, we observed two genomic regions; one on chromosome 1, *PM_G1*, explaining 5% of the phenotypic variation and another on chromosome 5, *PM_G5a*, explaining 40% of the phenotypic variation. Genetic tests are now being developed for the *PM_G5a* locus, in the project, CH-14-102, to facilitate MAB strategy development 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 fruit PM susceptibility. 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 chromosomes 4 and 6 explaining 20% and 55% of the phenotypic variation, respectively. These results however, are preliminary and will require dedicated funding and high disease pressure in future years to validate these genomic regions to be able to design marker-assisted breeding (MAB) strategies for fruit infection.

Table 3. Genomic regions identified for foliar PM infection.

Year	Chromosome	Genomic region Name
2011	2	*PM_G2*
	5	*PM_G5a*
	5	*PM_G5b*
	6	*PM_G6a*
2012	5	*PM_G5a*
	6	*PM_G6b*
	7	*PM_G7*
2013	3	*PM_G3*
	5	*PM_G5a*
2014	3	*PM_G3*
	5	*PM_G5a*
Combined data	1	*PM_G1*
	5	*PM_G5a*

c. Bacterial canker infection

Bacterial canker screening was conducted in 2014 according to Mgbechi-Ezeri et al. (2014). Data analysis in separate years (including 2013 and 2014) indicated a positive evidence for a genomic region on chromosome 5, *BC_G5*, explaining 17% of the phenotypic variation in 2013 while in 2014, there was a decisive evidence for a genomic region on chromosome 5 explaining 15% of the variation (Table 4). Combining both years, there was a decisive evidence for a genomic region on chromosome 5, explaining 15% of the variation, and yet another genomic region on chromosome 7, explaining 6% of the phenotypic variation. Genetic tests are now being developed in the project, CH-14-102, based on the genomic region on chromosome 5 to facilitate incorporation of bacterial canker resistance into high quality parents via MAB.

Table 4: The genomic regions identified for bacterial canker infection in different years and with combined data.

Year	Chromosome	Genomic region
2013	5	*BC_G5*
	7	*BC_G7*
2014	5	*BC_G5*
	2	*BC_G2*
Combined data	5	*BC_G5*
	7	*BC_G7*

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

a. Cracking susceptibility

Fruit were harvested at commercial maturity from 110 seedlings belonging to S x C mapping population and from 46 named cultivars. Approximately 10-25 and 60 fruit samples per tree, respectively, at commercial maturity, were selected for phenotyping in the S x C 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 S x C 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 the number of cracked fruit as well as split type was recorded every hour up to 5 hours. A cracking index (CI) was developed using the following formula:

$$CI\ (\%) = \frac{\sum(Nc \times F)}{100} \times 100,$$

where, Nc and F are the number of cracked fruit in each hour and weighting factors, respectively. We also recorded natural rain-induced cracking in the S x C population, but the correlation between natural cracking in the field and induced cracking in the lab was low (r = 0.11, p>0.05).

Data analysis based on interval mapping in MapQTL in the S x C population showed a major genomic region on chromosome 3. Two other genomic regions were also identified on chromosomes 6 and 8. These results are corroborated by FlexQTL results based on combined data sets including S x C population and the 46 cultivars which identified a genomic region on chromosome 3 which explained 15% of the phenotypic variation. Minor genomic regions were

also identified on chromosomes 1, 2 and 8 with the combined data. A similar study on sweet cherry cracking incidence conducted at INRA, Bordeaux, France (Quero-Garcia et al. 2010) for five years including 2006, 2008, 2009, 2010 and 2011, using a 'Regina' x 'Lapins' population, identified genomic regions on chromosomes 1, 3, and 4 which were weak and variable across years while the genomic region identified on chromosome 5 was stable across years and explained 15% of the variation for cracking incidence in the 'Regina' x 'Lapins' population. We plan to collect more data in 2015 to confirm the stability of the genomic regions particularly for the genomic region on chromosome 3, before genetic tests are developed to facilitate developing MAB strategies for cracking resistance and/or tolerance.

b. Pitting susceptibility

Fruit were harvested at commercial maturity from the S x C population and from 46 named cultivars. Approximately 7-25 and 60 fruit samples without blemish per individual, were selected after harvest in the S x C 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 the S x C population was assayed. Each replicate was weighed, transferred into ziplock bags and stored at 4 ºC for 4 hours. Thereafter, pitting was induced on both sites of the fruit (using a modified device developed by Toivonen et al. (2004) in the S x C population and on one side of the fruit in the named cultivars. 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 'Hedelfingen' showed the lowest PI (1.7) whereas 'Moreau' and 'Cashmere' recorded the highest PI (~3.9) while 'Regina' had medium PI (2.1) . The correlation between PI and fruit weight was low at r= -0.07 in the named cultivars and r= -0.05 in the S x C population (p>0.05). In addition, there was a negative and low correlation (r= -0.21) between firmness and PI in the S x C population.

Interval mapping in MapQTL showed genomic regions on chromosomes 1, 3 and 4, while FlexQTL analysis with combined data including named cultivars and the S x C population identified two genomic regions on chromosome 1 and chromosome 3. The percentage of variation explained by these genomic regions ranged from 1.1 to 3.2, suggesting that they are minor genomic regions. More data is required which we plan to collect in 2015 to confirm these genomic regions to facilitate developing MAB strategies for low pitting incidence.

References

Chavoshi M., Watkins C., Oraguzie B., Zhao Y., Iezzoni A. Oraguzie N. 2014. Phenotyping protocol for sweet cherries (*Prunus avium* L.) to facilitate an understanding of trait inheritance. Journal of American Pomological society 68(3): 125-134.

Mgbechi-Ezeri, J., K. Johnson, and N. Oraguzie. 2014. Effect of inoculum concentration, isolates and leaf age on bacteria canker disease development in sweet cherry (*Prunus avium* L.) cultivars. 7th International Cherry Symposium. Acta Hort. (In press).

Quero-Garcia, J., Campoy, J.A., Joly, J., Tauzin, Y., Rosyara, U., Iezzoni, A., Dirlewanger, E. 2012. QTL detection for fruit weight, firmness and cracking tolerance in sweet cherry. PAG, January 14-18 2012, San Diego, California, USA

Toivonen 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.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2 years
WTFRC Project Number: CH-14-101

Project Title: Sweet cherry breeding toolbox

PI:	Dorrie Main	**Co-PI (2):**	Nnadozie Oraguzie
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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
Telephone: 509 335-4564 **Email address:** carriej@wsu.edu

Item	2014	2015
Salaries[a]	3600	3600
Benefits[b]	1200	1200
Travel[c]	200	200
Plot Fees	0	0
Total	5000	**5000**

Footnotes: a and b = 5% of Dr. Sook Jungs 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. Oraguzies 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 from the public literature 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 have been added for the following traits:
   - Bloom date
   - Chilling requirement
   - Ecodormancy release date
   - Heat requirement for ecodormancy release
   - Heat requirement for blooming
   - Period of time between endodormancy and ecodormancy release
   - Period of time between endodormancy release and blooming
   - Ripening time
   - Root-knot nematode resistance

2. Breeding Data: Performance data for the PNWSCBP is currently being collated into the data templates by Dr. Oraguzies team for upload to the Sweet Cherry Breeding Toolbox. Associated information on genotype data is being provided by Dr. Cameron Peaces team. Both will be made available in the toolbox by the end of the year. Face-to-face training of the Toolbox is planned for January, so the new data can be demonstrated in the hands on training.

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 tfor 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 has been added to GDR and the performance and genotypic data specific to the breeding program will be added over the next couple of months. This will continue to provide an 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.

CONTINUING PROJECT REPORT **YEAR: 1 (2014)**
PROJECT NUMBER: CH-14-109A-E

Project Title: MSU cherry rootstocks: Pre-commercialization

PI: Amy Iezzoni
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Cooperators: Todd Einhorn, Tim Dahle, Stefano Musacchi

Total Project Request: **Year 1:** $50,450 **Year 2: $53,063**[a] **Year 3:** $35, 218
[a] Increased by $4,392 to cover the cost of the extra trees produced due to the high bud take on the MSU rootstocks

Other funding sources: None

WTFRC Collaborative expenses:

Item	2014	2015	2016
Wages	$ 11,000[a]	$ 3,159[b]	$ 3,241[b]
Benefits	$ 3,000	$ 1,060	$ 1,090
Supplies (Trellis, irrigation, fumigation)		$4,900[d]	$500[d]
Travel	$ 500[b]	$ 500	$ 500
Miscellaneous	$ 1,000[c]	$ 50	$ 100
Total	$ 15,500	**$ 9,669**	$ 5,431

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 a grower location as opposed to WSU – Sunrise.

Budget 1 – Amy Iezzoni
Organization Name: Mich. State Univ. **Contract Administrator:** Lorri Busick
Telephone: (517) 355-5191 **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
Wages			
Benefits			
Equipment			
Supplies[c]	$ 600	$ 1,200	$ 1,200
Travel[d]	$ 4,500	$ 4,500	$ 4,500
Liners	$ 1,000		
Trees		$ 17,160[e]	$ 3,927
Total	$ 15,500	**$ 29,760**	$ 17,527

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.

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	$ 1,185	$ 1,377
Benefits	$ 517	$ 115	$ 133
Plot fumigation	$ 850	$ 850	
Supplies	$ 200	$ 100	$ 100
Travel			
Trellis and irrigation	$ 1,100	$ 5,000	$ 3,550
Plot Fees[a]	$ 2,000	$ 2,000	$ 4,000
Miscellaneous (tree removal)	$ 1,000		
Total	$ 11,000	**$ 9,250**	$ 9,160

Footnotes:
[a]Standard annual plot fee, Roza Station

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

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

Footnotes:
[a] 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
Salaries			
Benefits			
Wages	$ 455	$ 1,700	$ 2,000
Benefits	$ 45	$ 170	$ 200
Equipment			
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 fumigation for the 2016 planting and 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 (removal 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:

- Five MSU cherry rootstocks produce dwarf precocious sweet cherry trees with 'Bing' scion based on evaluation of the trees planted at the WSU-Roza Station in spring 2009. These five rootstocks, which are named after Michigan counties, are CLINTON, CASS, CLARE, LAKE and CRAWFORD. The trees produced are significantly smaller than 'Gisela®6' (Gi6) but of similar size to 'Gisela® 5' (Gi5) measured as trunk cross-sectional area (TCSA), except CLARE which produced trees significantly smaller than Gi5.
- 'Bing' fruit maturity date differed among the seven rootstocks tested at the Prosser plot with CASS, CLARE and LAKE ripening ahead of CLINTON, CRAWFORD, Gi5 and Gi6.
- In 2014, all five of the MSU candidate rootstocks had yield efficiencies (kg fruit/cm^2) that were higher than that of Gi5 and Gi6. However, the fruit size for CRAWFORD was significantly less than that for Gi5 due to the high crop load on CRAWFORD compared to Gi5 and insufficient thinning of CRAWFORD. These results suggest that producing large fruit is possible on the MSU rootstocks given the proper training system and crop load adjustments. The proposed plantings for 2015 and 2016 will address these management systems.
- Virus certification of all five MSU rootstocks was completed by the CPCNW-FT.
- To date, liner production of the MSU cherry rootstocks is most efficient using tissue culture as opposed to softwood cuttings.
- Production of 'Regina', 'Early Robin' and 'Sweetheart' trees on the four MSU rootstocks that were available for 2015 planting was successful due to high bud take.
- DNA tests confirmed that the MSU rootstock identities of the trees that will be planted in spring 2015 are correct.

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'

- *Plot locations and cooperators*: Three plots with the same plant materials will be planted in 2015. The plots will be in Prosser, Wash. (WSU - Roza Station) under the direction of Matt Whiting, in Wenatchee, Wash. (grower location to be determined) under the direction of Tom Auvil, Des Layne (collaborator - Stefano Musacchi), and in The Dalles, Ore. (grower – Tim Dahle) under the direction of Lynn Long (collaborator – Todd Einhorn). Assistance with these plots will be provided by Tom Auvil (WTFRC). Plots will be fumigated prior to planting.
- *Plant materials*: Each plot will include 7 rootstock genotypes: the MSU rootstocks (CASS, CLARE, CLINTON and LAKE), Gi5, Gi6 and either Krymsk 5 or 6 depending upon the scion. Three scions will be included at all three sites: 'Regina', 'Early Robin' and 'Sweetheart'. For 'Regina' and 'Early Robin' the Krymsk rootstock will be Krymsk 6 while for 'Sweetheart' the Krymsk rootstock will be Krymsk 5. 'Sam'/Gi5 and 'Chelan'/Gi5 will be included as pollinators for 'Regina' and 'Early Robin', respectively.
- *Training systems*: Each of the three plots will use a different training system. The training systems for the plots in The Dalles, Prosser, and Wenatchee will be VCL, UFO, and SSA, respectively. Specific plot design and training system details are as follows:
    - VCL (The Dalles): Between row spacing will be 15 ft (4.57 m). In-row spacing will vary depending on the rootstock as follows: Gi6 and Krymsk (8 ft × 16 ft = 340 t/ac)(2.44 m × 4.88 m = 840 t/ha); Gi5 and CLINTON (6 ft × 16 ft = 454 t/ac)(1.83 m × 4.88 m = 1120 t/ha), and CASS/LAKE/CLARE (4 ft × 16 ft = 681 t/ac)(1.22 m × 4.88 m = 1680 t/ha). Stakes will be used to support the trees on CASS, CLARE and LAKE.
    - UFO (Prosser): Between-row spacing will be 9 feet. In-row spacing will vary, depending on the expected vigor control of the rootstocks. Gisela®6 and Krymsk will be planted 6 feet apart (6 ft × 9 ft. = 806 t/ac)(1.83m × 2.74m = 1994 t/ha); Gi5 and CLINTON (4 ft × 9 ft = 1210 t/ac)(1.22m × 2.74m = 2992 t/ha); CASS/LAKE/CLARE (3.5 ft × 9 ft = 1383 t/ac)(1.07m × 2.74m = 3411 t/ha). Trees will require a 4-wire trellis structure with the first wire at 20 in and 3 additional wires spaced at 25-in intervals [i.e., top wire at 8 ft (2.44m)]. Final canopy height will be 10 ft (3.05m).
    - SSA (Wenatchee): Between row spacing will be 10 ft (3.05 m). In-row spacing will vary depending on the rootstock as follows: Gi6 and Krymsk (2.3 ft × 10 ft = 1894 t/ac)(0.7 m × 3.05 m = 4684 t/ha); Gi5 and CLINTON (1.6 ft × 10 ft = 2723 t/ac)(0.5 m × 3.05 m = 6557 t/ha), and CASS/LAKE/CLARE (1.3 ft × 10 ft = 3351 t/ac)(0.4 m × 3.05 m = 8197 t/ha). Trees will be grown on a 3-wire trellis where the wires are spaced 2.3 ft (0.7 m) apart vertically.
- *Replication*: Each rootstock scion combination will be represented by 20 trees per location.
- *Data to be collected*: Data collected in 2015 and 2016 will include plant survival and trunk cross-sectional area. Data to be collected in 2016 will also include suckering. Due to the rootstocks' precocity, it is possible that there will be a small harvestable crop in 2016. If so, the fruit numbers will be recorded and fruit size (weight and row size) will be determined.
- *Potential problems/limitations*: There is always the concern of over cropping with highly precocious and abundantly flowering rootstocks. However, with prior knowledge of the rootstock's potential for promoting high crop loads, training, pruning and thinning practices can be put into place to avoid over cropping.

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

The objective of the 2016 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 than the other four MSU rootstocks due to what at first appeared to be graft incompatibility with 'Hedelfingen' scion at the original plot at MSU's Clarksville Station. However, because of good

performance at the Prosser plot, and no evidence of graft incompatibility with 'Bing' scion, CRAWFORD was advanced in 2012. Because of this delay, CRAWORD liners were not available to make trees for the proposed 2015 plantings (Obj. 1.B.).

These trials will be planted at the same three locations as the 2015 plantings. There will be 3 rootstocks and 2 scions at each site. The three rootstocks will be CLINTON, CRAWFORD, and Gi5 as control. The scions will be 'Regina' and 'Sweetheart' so that the results are comparable with those obtained from the 2015 planting. Each rootstock scion combination will be represented by 20 trees per location resulting in a total of 120 trees per location. The number of pollinators will be determined based on the proximity of the 2016 planting to the 2015 planting. Data collected in 2016 will include plant survival and trunk cross-sectional area.

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

Seven commercial liner nurseries have virus-certified plant material to produce a limited number of liners of CASS, CLARE, CLINTON and LAKE (Cameron Nursery, Copenhaven Farms, Duarte Nursery, North American Plants, Protree Nursery, Teak Nursery, and Willamette Nursery). Three of these liner nurseries (Duarte Nursery, North American Plants, and Protree Nursery) received budwood of CRAWFORD in Sept/Oct 2013 and are propagating limited quantities of CRAWFORD. The remaining four nurseries will receive CRAWFORD budwood in February 2015. Sierra Gold Nursery will also receive all five MSU rootstocks in 2015. 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 2015 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 2016. To achieve this objective, A. Iezzoni will visit Protree two times in 2015. 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.

Virus certification has been achieved for all five MSU rootstocks. Therefore, the main thrust of this objective will be to assure that the genetic identities of the five MSU rootstocks are correct at key points in propagation and distribution. 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. All five MSU rootstock selections can be differentiated with a combination of three DNA markers. Plant materials for DNA extraction will either be collected by A. Iezzoni during nursery visits (Obj. 2) or will be sent to the Iezzoni lab from the collaborating nurseries.

RESULTS AND DISCUSSION:

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

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

This year was the last year of evaluation for the plot at WSU – Prosser Roza Station as it was removed at the end of the growing season. This plot was planted in 2009 with trees spaced at 8 ft × 15 ft in five-tree replicates and were trained to a multiple leader architecture. All five MSU rootstocks produced trees significantly smaller than Gi6 but similar size to Gi5 measured as trunk cross-sectional area (TCSA), except CLARE which produced trees significantly smaller than Gi5 (Fig. 1). Despite their small sizes, CLARE and LAKE trees appeared to have more roots than Gi5, CASS and CRAWFORD trees (Fig. 2). Root number and size was assessed by digging up one tree per each rootstock combination with the exception of CLARE, for which two trees were evaluated.

'Bing' fruit harvest date differed among the seven rootstocks tested at the Prosser plot with CASS, CLARE and LAKE, ripening ahead of CLINTON, CRAWFORD, Gi5 and Gi6 (Table 1). The four day spread in harvest dates would likely have been more pronounced in a cooler June as the temperatures at Prosser were an average of 5F above normal and June 19 had a maximum temperature of 85.7 F. In 2014, all five of the MSU candidate rootstocks had yield efficiencies (kg fruit/cm^2) that were higher than that of Gi5 and Gi6. However, fruit size for CRAWFORD was significantly less than that for Gi5. This was due to the high crop load on CRAWFORD compared to Gi5, where fruit thinning was not sufficient. These results suggest that producing large fruit is possible on the MSU rootstocks given the appropriate intensive training systems and crop load adjustments required for these dwarf precocious rootstocks. The series of proposed plantings for 2015 (Obj. 1B) and 2016 (Obj. 1C) will address these management 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'.

For the 2015 planting, CASS, CLARE, CLINTON and LAKE liners were budded with three scions: 'Early Robin', 'Regina' and 'Sweetheart'. Due to reduced MSU liner growth resulting from the late spring planting date, the MSU rootstocks were not budded in the fall of 2013 at two of the nurseries, instead they were budded in spring 2014. Of the 22 scion/rootstock combinations needed for the 2015 planting (excluding pollinators), only four combinations had tree numbers less than the 60 needed for all three plots ('Regina'/CASS, 'Early Robin'/CLARE, 'Sweetheart'/LAKE, and 'Sweetheart'/Gi5).

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

Liners of the five MSU cherry rootstocks have been increased at ProTree Nursery along with the Gi5 control. The goal is to spring bud for a target planting date of 2016; however, this will depend on the size of the liners. These plant materials will provide the trees needed for the 2016 plantings along with an assessment of bud take for these scion/rootstock combinations.

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 either have the five MSU cherry rootstocks or will have them all by spring 2015. All nurseries receive 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; however, the cost effectiveness of tissue culture versus softwood cuttings has not yet been determined. Since the rootstock materials established at the nurseries

originated from the virus-certified and genetically verified plant material at the CPCNW-FT, liners from these plant materials could be commercialized if a decision is made to release one or more of the MSU cherry rootstocks.
Finished tree nursery performance: Liners of four of the MSU rootstocks (CASS, CLARE, CLINTON and LAKE) were planted at three Washington nurseries in spring 2013. Because of the late delivery/planting time, only liners at one nursery were of sufficient size to bud in fall 2013. The other two nurseries budded in spring 2014. The scions used were those for the 2015 plantings: 'Regina', 'Early Robin' and 'Sweetheart'. The stand of CLINTON was better than that of CASS, CLARE and LAKE across all nurseries. However, once the liners were established, they all had acceptable nursery characteristics, including sufficient apical dominance and minimal branching. For those liners that were of sufficient size to bud, the bud take was over 90% for all four MSU rootstocks across all three scion cultivars. An informal assessment of bud take suggested that the bud take on the MSU rootstocks was higher than that on Gi5 and Gi6. The extra tree numbers obtained with the MSU rootstocks resulted in significantly more trees needing to be purchased than originally budgeted. This additional tree cost was added to the 2015 budget request (see Budget 1).

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

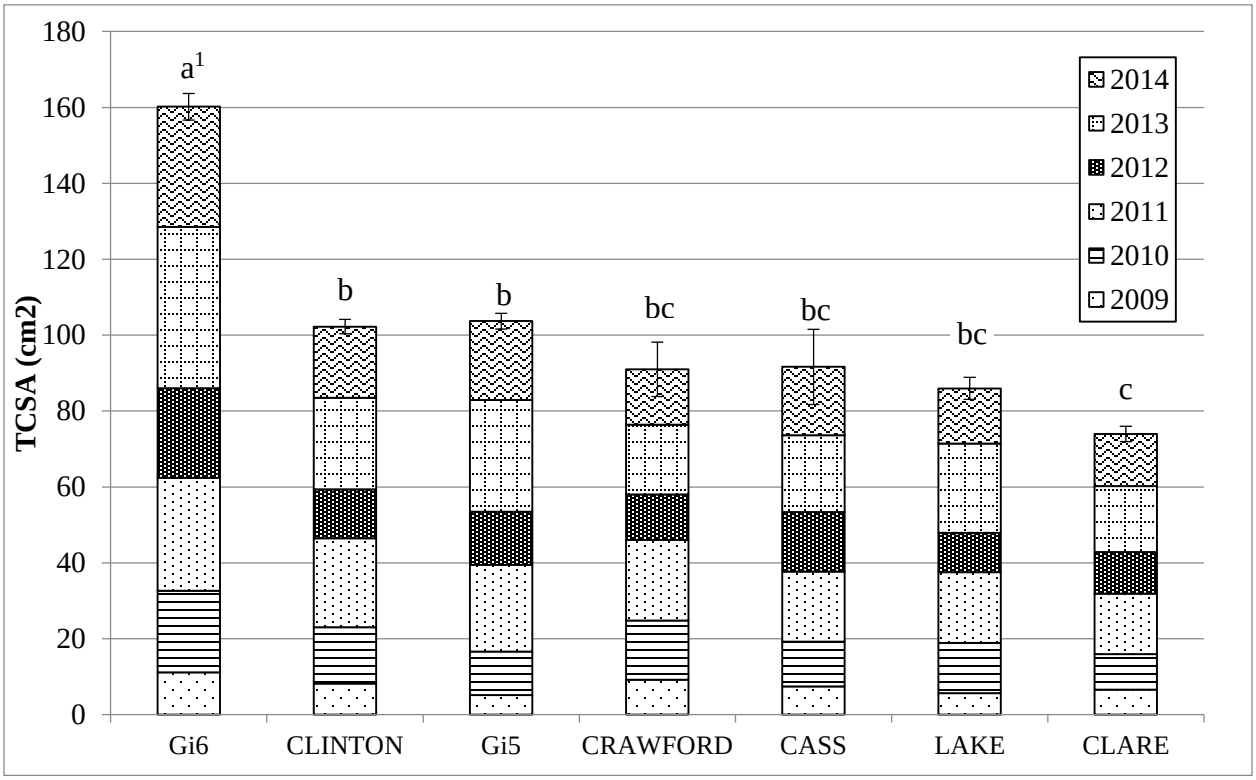
The virus certification of CRAWFORD was completed at the CNCNW-FT and preparations have been made to send budwood in winter/spring 2015 to the nursery collaborators who have not previously received this selection. The certification of CRAWFORD completes the virus certification of the five MSU cherry rootstocks. Three markers are available to distinguish all five MSU rootstocks, Gi5 and Gi6. Two of the markers were used previously and a third marker was newly designed from the peach genome sequence. The MSU rootstocks that were used to make the trees for the 2015 planting were subjected to DNA testing to confirm rootstock identify. Three plants per rootstock clone were sampled from each of the three finished tree nurseries. These DNA diagnostic tests have confirmed that the MSU cherry rootstocks are labeled correctly. All the participating nurseries have been informed that MSU will perform DNA diagnostics on the MSU rootstocks at no cost to the nursery if there are any identity concerns. The goal of this strategy is to avoid any delays and financial losses at the nurseries that would be associated with a plant material mix-up.

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

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

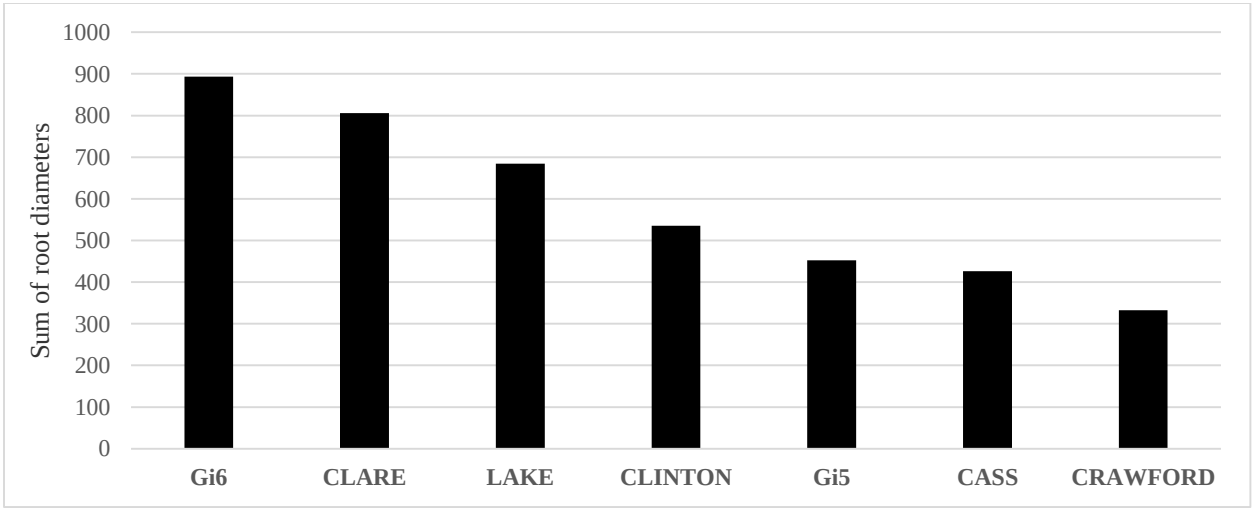
[1]Means that are significantly different ($P < 0.05$) are denoted by different letters.

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



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

Fig 2. Cumulative root diameters[1] (mm) for 'Bing' trees grafted on 5 MSU rootstock candidates, Gi5, and Gi6 for trees planted in 2009 at WSU - Prosser Roza Experiment Station. Data was taken in August of 2014. Roots were cut at 30 cm from the base of the tree and the diameters of roots that were larger than 5mm were recorded.



[1]Statistical analyses are not possible because trees were represented by only one measurement.

CONTINUING PROJECT REPORT **YEAR:** 1 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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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 + $7,500 (2014)
Notes: "Consulting for the sweet cherry breeding program; NW cherry project." PIs: Iezzoni/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, Oraguzie, and Main.

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: $2.0 M (Sep 2009 – Aug 2014)
Notes: "Tree Fruit GDR: Translating genomics into advances in horticulture." PI: Main. Co-PIs include Peace and Oraguzie.

Budget

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

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

Item	2014	2015	2015
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:

5. 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.

6. Develop new DNA tests for **pitting** and **cracking incidence**, **fruit 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.

7. Ensure appropriate use of new DNA tests by devising and trialing strategies for their routine deployment within the context of existing tests and ongoing PNWSCBP operations.

***Specific objectives - Year 1*:**

1. Complete first marker-assisted seedling selection for **fruit color** and **maturity time** in combination with existing tests for fruit size and firmness.

2. Complete development of new DNA tests for **fruit firmness** and **fruit size** and conduct marker-assisted parent selection for both traits.

3. Initiate development of new DNA tests for **resistance to powdery mildew** (leaf) and **bacterial canker**, **pedicel fruit retention force (PFRF)**, **cracking**, and **pitting**.

SIGNIFICANT FINDINGS

- **Fruit color** DNA test developed and ready for 2015 marker-assisted seedling selection

- **Fruit color** DNA test screened on parent material to help guide 2015 crossing decisions

- **Maturity time** DNA test developed and undergoing final preparations for 2015 use

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

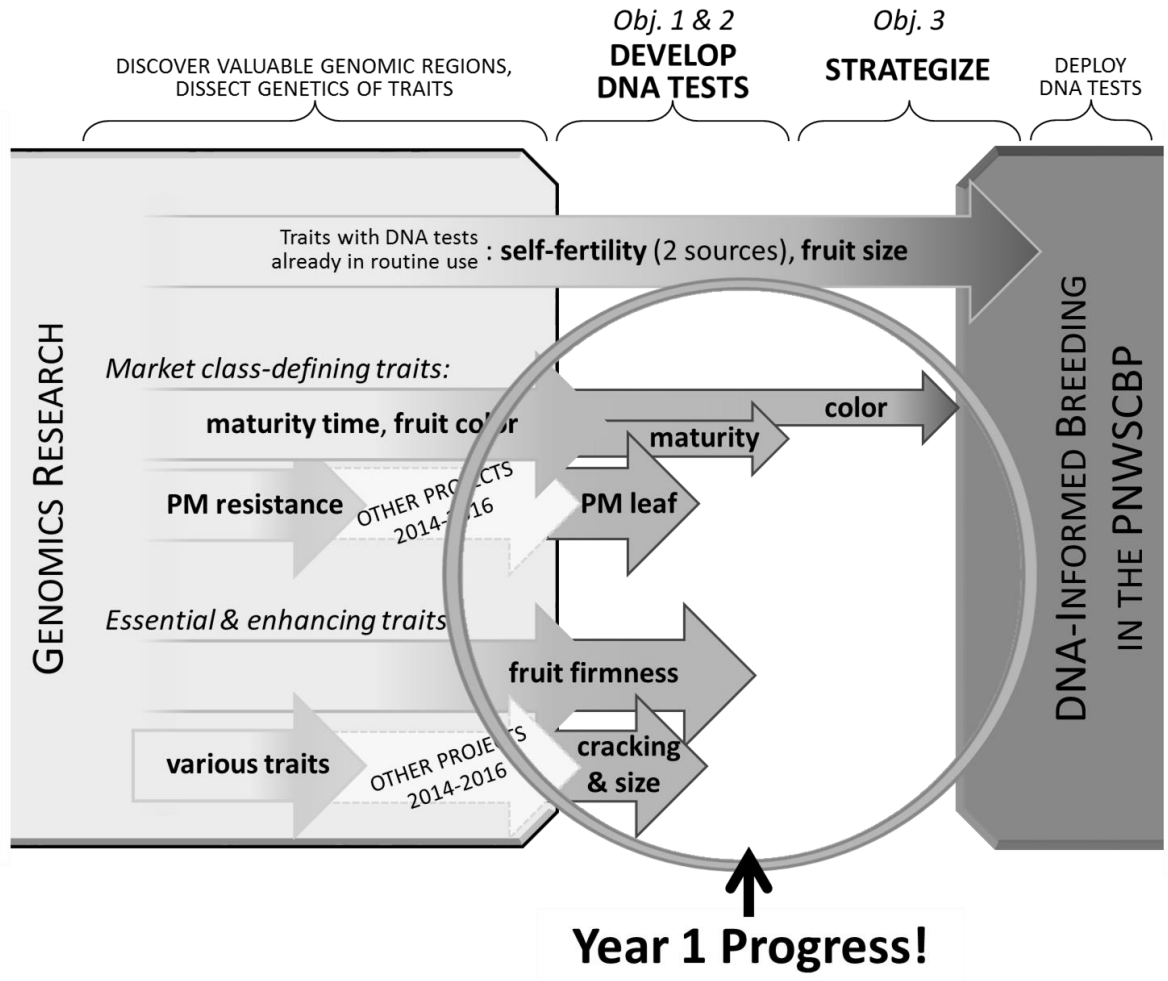

Figure 1: Progress made in Year 1, in the context of the entire project which has and will continue to improve the efficiency, accuracy, creativity, and pace of the Pacific Northwest Sweet Cherry Breeding Program (PNWSCBP) via DNA-informed breeding.

METHODS

DNA test development (Obj. 1 & 2) – Years 1-3

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 the discovery of regions associated with fruit color, maturity time, firmness, fruit size, and fruit 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 they form the basis from which DNA tests can be developed.

B) **Functional genotype conversion:** Using the Genome Database for Rosaceae's Peach GBrowse tool genomic sequences from the targeted regions are retrieved. For initial testing, five sites in each region are targeted with candidate DNA tests designed using the software tools of BatchPrimer3 v1.0 and Primer3Plus.

C) **Candidate DNA test analysis:** Each candidate test designed is screened on a small set of individuals that represent functional genotype patterns. Screening includes amplification and visualization of the candidate test product using standardized lab procedures followed by examination of test products to choose those candidate tests with products that match the functional genotype patterns. 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 DNA test 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) by ordering the primer pairs with the appropriate fluorescent chemistries. If required, the primers are re-designed to increase or decrease the product size to facilitate the combination of existing DNA tests – 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 more phenotyped material that was not part of the original association calculations is screened with the new DNA tests, more precise effect calculations are conducted using standard statistical procedures.

DNA test deployment strategies (Obj. 3) – Years 2-3

As each new test is completed deployment strategies that consider how the trait performance level that each DNA test helps achieve fits within the general breeding scheme and how it fits with other available DNA tests are developed. Many of the factors considered include:

- 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.
- how much the use of a DNA test in seedling selection would be expected to reduce the proportion of surviving seedlings.
- how much the use of multiple DNA tests in seedling selection in a given family would be expected to reduce the proportion of surviving seedlings.
- 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.
- the source(s) and frequency of desirable alleles for each DNA test and whether a DNA test is applicable only for certain lineages.
- 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.
- how much attention 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.

RESULTS AND DISCUSSION

Obj. 1: DNA test development for maturity time, flesh and skin color, and fruit firmness
Significant progress has been made as of Nov 2014 toward developing new DNA tests for the valuable traits of **maturity time, fruit color,** and **fruit firmness**. In addition to self-fertility and fruit size, DNA test results for fruit firmness were used to guide 2014 crossing and seedling selection decisions. The tests for maturity time and fruit color were not completed for 2014 screening but will both be applied for seedling selection beginning February 2015.

Maturity time
Several candidate DNA tests for maturity time were screened. These tests are currently in the final stages of the development process. One of the candidate DNA tests differentiates *late-season* individuals from *mid-season* and *early-season* individuals (Figure 2) and can be deployed immediately. Because the ability to differentiate additional maturity times is important, more candidate tests are being screened to discern five maturity alleles: extra early, early, mid, late, and very late. The various combinations possible among these five alleles would then be used to predict maturity time of any individual. We expect the improved maturity time DNA test to be ready for use in 2015 crossing and seedling selection.

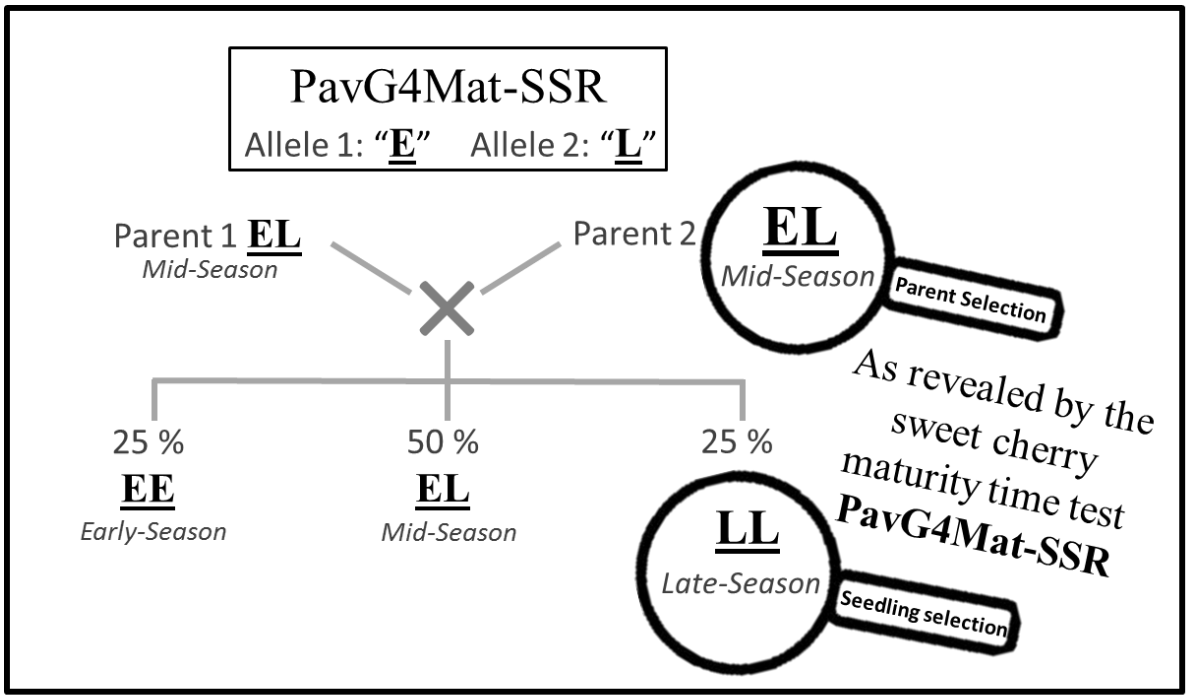

Figure 2: The PavG4Mat-SSR DNA test reveals two contrasting genetic factors (alleles) that predict maturity time allowing selection of cross combinations that are guaranteed to produce a proportion of seedlings with the desired trait level and for selecting at the seedling stage only those offspring with the desired trait level.

Fruit color

A DNA test for sweet cherry fruit color ("PavMYB10-SSR") was developed and tested across ~200 diverse individuals of importance to the PNWSCBP. PavMYB10-SSR is now ready for deployment – especially for guiding crossing decisions. This DNA test clearly differentiates *blush* types from *mahogany* types (Figure 3). Crossing efficiency can be improved by using PavMYB10-SSR to determine the alleles possessed by each parent. For example, if mahogany parents that are carriers for the recessive blush allele are crossed with other carriers or with blush types, some blush-type seedlings are predicted to result. If only mahogany types are desired in a family, at least one parent must be "pure" for mahogany – possess two mahogany alleles. Furthermore, RosBREED results indicated that "pure" mahogany types tend to be slightly darker than those mahogany types carrying a blush allele.

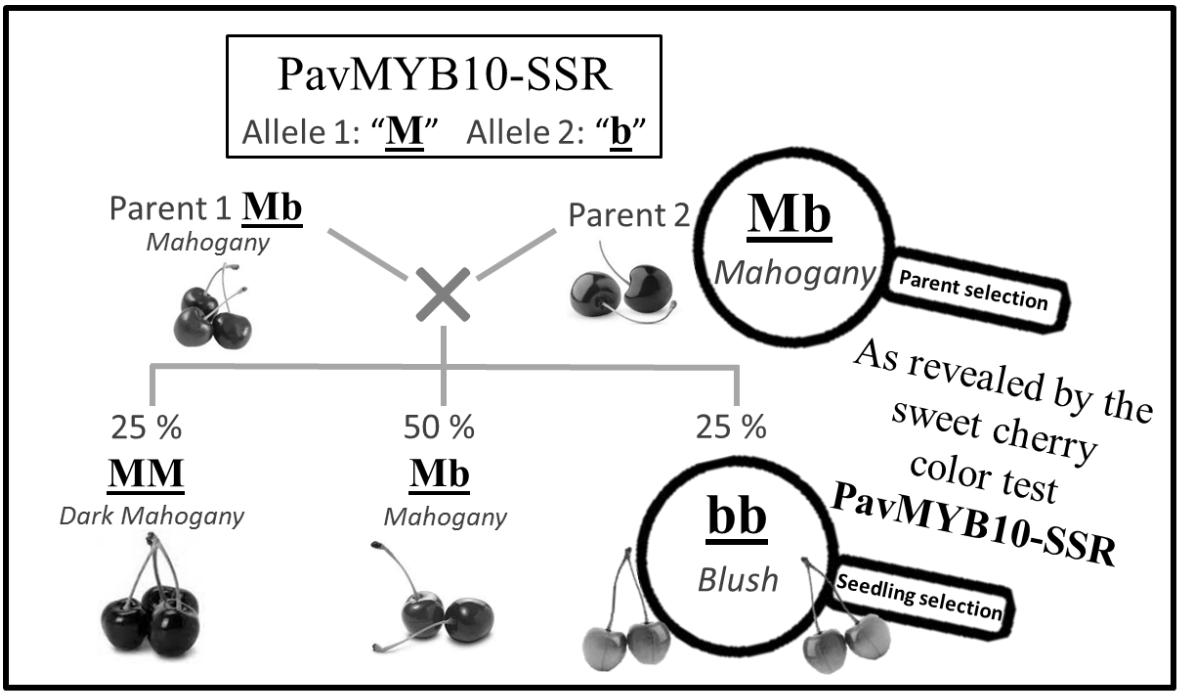

Figure 3: The PavMYB10-SSR DNA test reveals two contrasting genetic factors (alleles) that predict fruit color allowing selection of cross combinations that are guaranteed to produce a proportion of seedlings with the desired trait level and for selecting at the seedling stage only those offspring with the desired trait level.

Fruit firmness

Certain alleles of the CPSCT038-BPPCT034 fruit size DNA test already used in the PNWSCBP are associated with increased levels of fruit firmness. Recently, two new genomic regions associated with firmness were reported by French collaborators. Candidate DNA tests were designed to target this new region and screening will continue in late 2014. The various DNA tests will then be combined for refined predictions of cherry fruit firmness with use expected in 2015 crossing and seedling selection.

Obj. 2: DNA test development for other traits
Exceptional progress in 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 **fruit size, powdery mildew resistance, bacterial canker resistance, cracking resistance, fruit abscission, pitting, sweetness,** and **acidity**. Exceeding expectations, candidate DNA tests for fruit size and resistance to powdery mildew (leaf), bacterial canker, and cracking have been developed with at least one expected to be ready for use in 2015 seedling screening. In addition, DNA tests for the remaining traits described have been designed and are awaiting screening.

Fruit size
For the past five years, a DNA test for fruit size has been routinely deployed in the PNWSCBP to support crossing and seedling selection decisions. Recently, two new genomic regions associated with fruit size were reported by French collaborators. Candidate DNA tests were designed to target these new regions, and screening will continue in late 2014 anticipating use in 2015. The combination of a new DNA test for fruit size with the CPSCT038-BPPCT034 test already used in the breeding program should significantly enhance our ability to predict fruit size.

Powdery mildew (leaf) resistance, bacterial canker resistance, and cracking incidence
Candidate DNA tests were designed based on new knowledge of genomic regions associated with resistance to powdery mildew (leaf), bacterial canker, and cracking recently discovered through the WTFRC-funded project, "New genomic regions controlling production and fruit disorder traits" (Oraguzie and Peace). Screening will continue in late 2014.

Powdery mildew (fruit) resistance, fruit abscission, pitting incidence, sweetness, and acidity
Genomic regions associated with powdery mildew (fruit) resistance, fruit abscission, pitting incidence, sweetness, and acidity were recently discovered through the WTFRC-funded project, "New genomic regions controlling production and fruit disorder traits" (Oraguzie and Peace). DNA tests are currently being developed for these genomic regions.

Obj. 3: DNA test deployment
Deployment strategies for the DNA tests of **self-compatibility**, **fruit size**, **firmness**, and **fruit color** have been developed and are ready for application in 2015 seedling selection. DNA information on prospective parents in the PNWSCBP for maturity time and fruit color, in addition to fruit size and firmness, self-fertility, and cross-compatibility, was used to guide 2014 crossing decisions. The DNA tests being developed in Objectives 1 and 2 will be deployed for seedling selection in the families resulting from these targeted crosses, which will result in a double deployment of DNA information thereby maximizing the genetic potential (new cultivar potential) of the offspring produced. This double deployment is expected to improve the overall efficiency and impact of the PNWSCBP. The bulk of the deployment strategizing will take place in Years 2 and 3 of the project as more DNA tests become available, at which point the trait levels targeted, proportion of superior seedlings resulting, frequency of desirable allele combinations, and other deployment factors will be thoroughly examined. The success of 2014 seedling DNA testing is currently being evaluated and will guide the specific deployment decisions made in 2015.

CONTNUNING PROJECT REPORT **YEAR**: 1 of 3 years

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

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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:** L.J. Koong
Telephone: 541-737-4066 **Email address:** l.j.koong@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 (Dr. Xingbin Xie): 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:** L.J. Koong
Telephone: 541-737-4066 **Email address:** l.j.koong@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

The goal of this proposed project is to improve shipping quality (pitting, splitting, acid loss, pedicel browning) of 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 and cherry shipping quality.
2. Develop pre-harvest Ca spray protocols to improve cherry shipping quality.
3. Determine the effect of NaCl spray on cuticle development and shipping quality of cherries.
4. Determine the response of fruit growth, fruit size, yield, and return bloom to Ca/NaCl sprays.

SIGNIFICANT FINDINGS

1. Cherry shipping quality is related to fruit tissue Ca concentration, but not N concentration.
2. Cherry fruit Ca total content per fruit increased but concentration decreased during growth.
3. Ca spray frequency was optimized at 6 times weekly from pit-hardening to 1-week before harvest for enhancing fruit tissue Ca concentration and fruit quality.
4. Ca spray rate was optimized at ~0.1% (0.07-0.15%) Ca for enhancing fruit tissue Ca concentration and fruit quality.
5. No additional benefits were observed at higher rate or frequency regarding fruit tissue Ca concentration and fruit quality.
6. Sprayed 6 times weekly, "Cal-8" at 0.2% Ca and "Chelate Ca" at 0.05% were more efficient than $CaCl_2$ (0.2-0.4%) at 0.07-0.15% Ca, $Ca(NO_3)_2$ (0.4-0.6%) at 0.1-0.15% Ca, and "6% CALCIUM" at 0.07% Ca for enhancing fruit tissue Ca concentration and fruit quality.
7. Beginning two to three weeks prior to harvest, fruit growth was negatively affected by $CaCl_2$ (0.2% and 0.4%, applied 6x) and Ca citrate (applied 6x or 9x), but ***not*** in other Ca treatments.
8. Enhanced fruit tissue Ca concentration improved shipping quality in terms of reducing respiration rate, pitting, decay, pedicel browning, skin darkening, and losses of FF, and TA after 3 weeks of cold storage.
9. Frequent applications are more important than exact spray timing. No selected stage of fruit growth is more important than another for Ca sprays.
10. NaCl sprays increased fruit TA at harvest and after 3 weeks of storage and reduced stem browning after 3 weeks of storage (results will be reported next year)

METHODS

1. Ca and salt treatments.

Ca Treatments	% Ca	Application timing
$CaCl_2$ (0.2%)	0.07	9x, beginning 1wafb
$CaCl_2$ (0.2%)	0.07	2x, pit hardening + 1wbh
$CaCl_2$ (0.4%)	0.15	2x, pit hardening + 1wbh
$CaCl_2$ (0.2%)	0.07	2x, 1 and 2 wbh
$CaCl_2$ (0.4%)	0.15	2x, 1 and 2 wbh
$CaCl_2$ (0.2%)	0.07	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
"6% Calcium" (Ca citrate)	0.07 (upper label rate)	6x, beginning pit hardening
"Cal-8"	0.20 (within label rate)	6x, beginning pit hardening
"Chelate Ca"	0.05 (upper label rate)	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).

2. 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 firmness and pitting incidence were recorded.

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 will be evaluated in years 2 and 3 by counting returning flowers and fruit on the same limbs.

RESULTS

1. Effect of fruit tissue Ca & N concentrations on shipping quality of PNW sweet cherries

Fruit with varied tissue Ca concentration from different orchards had different fruit firmness (FF) and pitting incidence for both Sweetheart and Skeena. A trend exits that the higher Ca concentration, the higher FF and the less pitting susceptibility. Fruit tissue N concentration was between 0.9 – 1.1% and was not correlated with fruit FF and pitting susceptibility for both cultivars (data not shown).

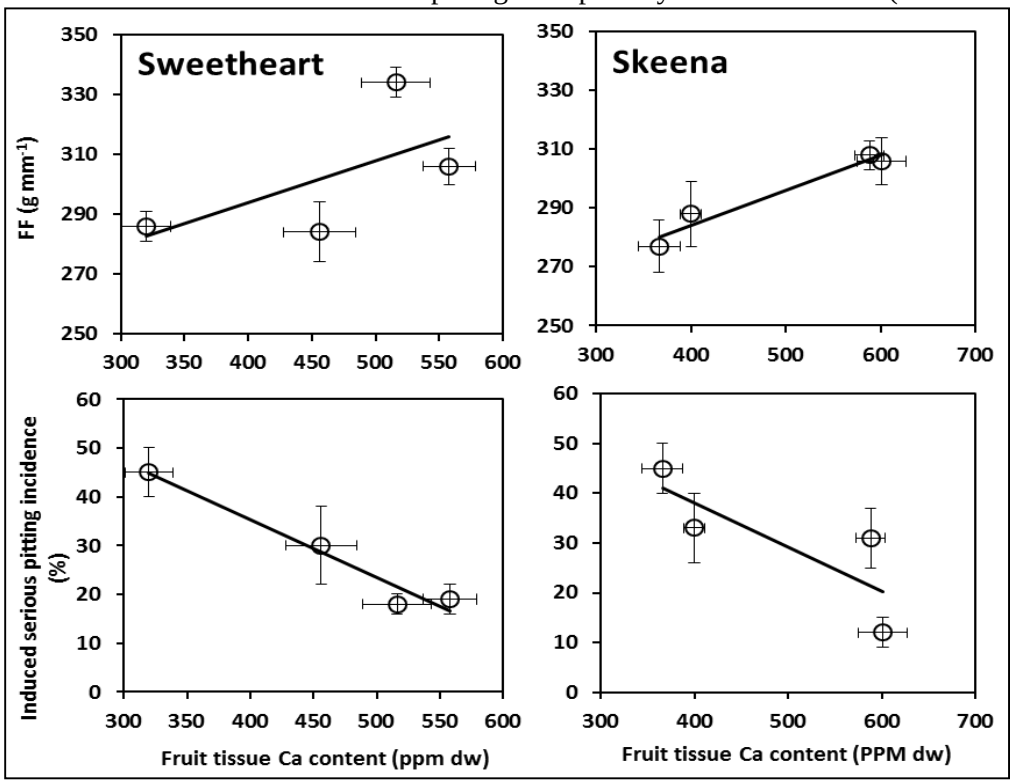


Fig. 1. The effect of fruit tissue Ca concentration on fruit firmness (FF) and pitting incidence of Sweetheart and Skeena from 4 different orchards after 2 weeks of cold storage.

2. Ca and N total contents and concentrations in cherry fruit during development

During fruit development, while the total Ca and N contents in each fruit were increased (data not shown), their concentrations were decreased in a quadratic polynomial manner (Fig. 2).

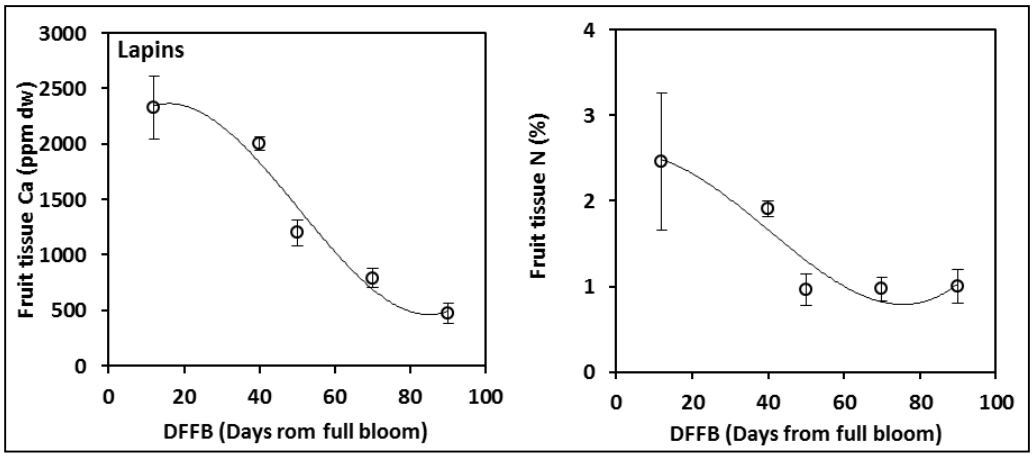


Fig. 2. Change of Ca and N concentrations in Lapins during development.

3. Effect of Ca application rate on cherry fruit ca concentration

Applied 6 times weekly between pit-hardening and 1-week before harvesting, $CaCl_2$ at 0.2% (Ca≈0.07%) increased Lapins cherry fruit Ca concentration significantly ($p < 0.05$) from 436ppm (control) to 615ppm at the time of harvest. Compared to 0.2%, $CaCl_2$ at 0.4% (Ca≈0.15%) increased fruit tissue Ca concentration numerically but not at a statistically significant level ($p < 0.05$). Therefore, no additional benefits were observed at higher $CaCl_2$ rate than 0.2% (Ca≈0.07%) when spraying 6 times between pit-hardening and 1-week before harvesting (Fig. 3).

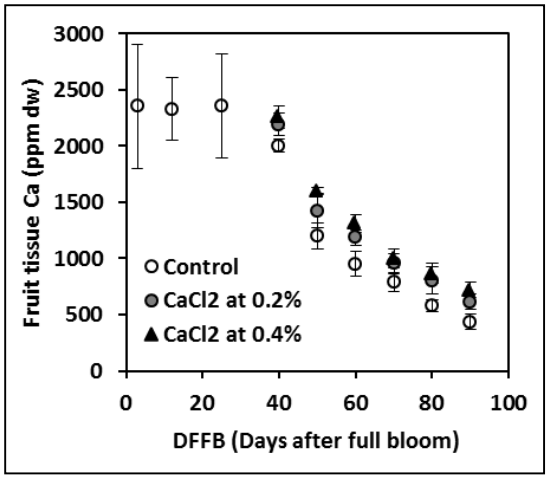


Fig. 3. Effect of Ca application rate on fruit Ca concentration of Lapins sweet cherry (The trend was similar between $CaCl_2$ and $Ca(NO_3)_2$ and only the data of $CaCl_2$ were presented here).

4. Effect of frequency and timing of $CaCl_2$ spray on cherry fruit Ca concentration

Increasing spray frequency of Ca at 0.07% from 6 to 9 times weekly between full bloom and 1-week before harvesting did not improve cherry fruit Ca uptake significantly ($p < 0.05$) (Fig. 4A&B). Ca at 0.07% sprayed twice (pit-hardening + one week before harvesting or two-week + one-week before harvesting) did not increase Ca concentration of cherry fruit at harvest compared to control (Fig. 4C&D).

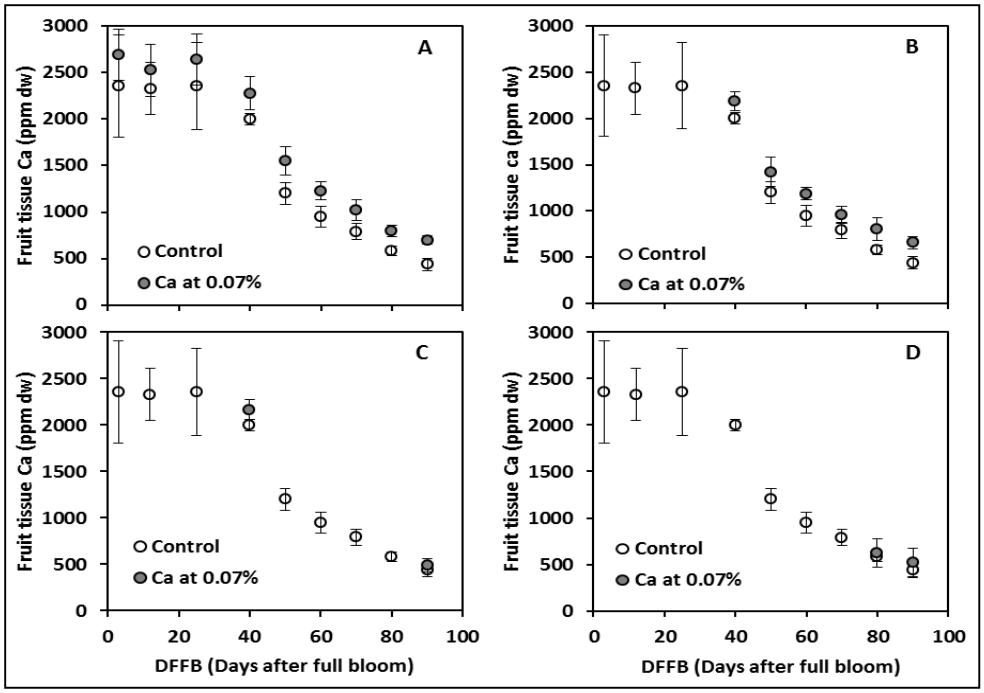


Fig. 4. Effect of frequency and timing of $CaCl_2$ sprays on Ca concentration in Lapins cherry fruit.

5. Effect of Ca sources on cherry fruit Ca uptake

Sprayed 6 times weekly between pit-hardening and 1-week before harvest, $CaCl_2$ (0.2-0.4%) at 0.07%-0.15% Ca, $Ca(NO_3)_2$ (0.4-0.6%) at 0.1-0.15% Ca, "6% CALCIUM" at 0.07% Ca (its maximum label rate), "Cal-8" at 0.2% Ca (within its label rate), and "Chelate Ca" at 0.05% Ca (its maximum label rate) increased fruit tissue Ca concentration significantly ($p < 0.05$), except $Ca(NO_3)_2$ (0.4%) at 0.1% Ca, compared to control (Fig. 5).

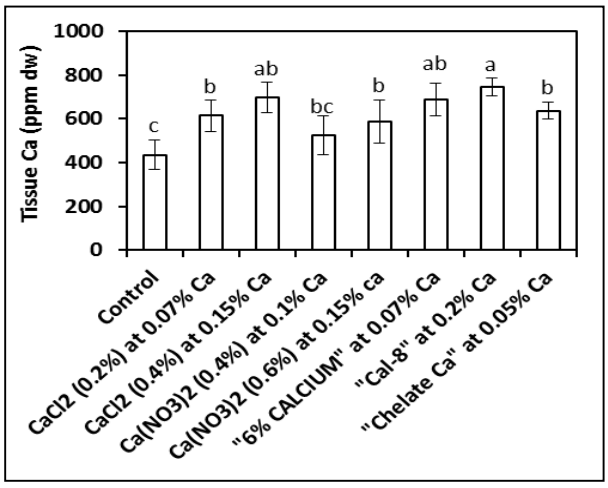


Fig. 5. Effect of Ca sources on Ca concentration in Lapins cherry fruit.

6. Effect of Ca sources and application rate on cherry fruit growth

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

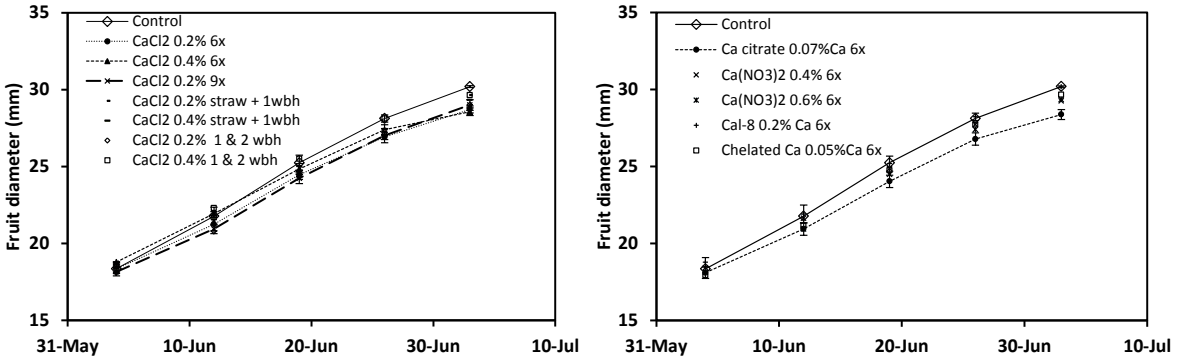


Figure 6. Fruit growth of 'Lapins' sweet cherry as affected by Ca source, concentration and application frequency. All data are means of 3 replicates (n=15). Given the large number of treatments, the data were split between 2 graphs for clarity of presentation; control fruit are shown in each graph for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

7. Effect of Ca sprays on cherry fruit maturity and quality at harvest

Ca sprays did not affect fruit maturity based on fruit color (Fig. 7C). All Ca sprays increased FF ($p < 0.05$), except $Ca(NO_3)_2$ (0.6%) at 0.15% Ca, compared to control (Fig. 5B). Fruit treated with "Cal-8" at 0.2% Ca and "Chelate Ca" at 0.05% Ca had the highest FF. Compared to control, only "Cal-8" at 0.2% Ca and "Chelate Ca" at 0.05% Ca treatments reduced fruit respiration rates (Fig. 5C). $CaCl_2$ (0.4%) at 0.15% Ca, $Ca(NO_3)_2$ (0.6%) at 0.15% Ca, "Cal-8" at 0.2% Ca, and "Chelate Ca" at 0.05% Ca increased TA ($p < 0.05$). $CaCl_2$ and $Ca(NO_3)_2$ tended to increase SSC ($p < 0.05$) (Fig. 5E) and $CaCl_2$ reduce fruit size (Fig. 5F), probably due to the "salt stress".

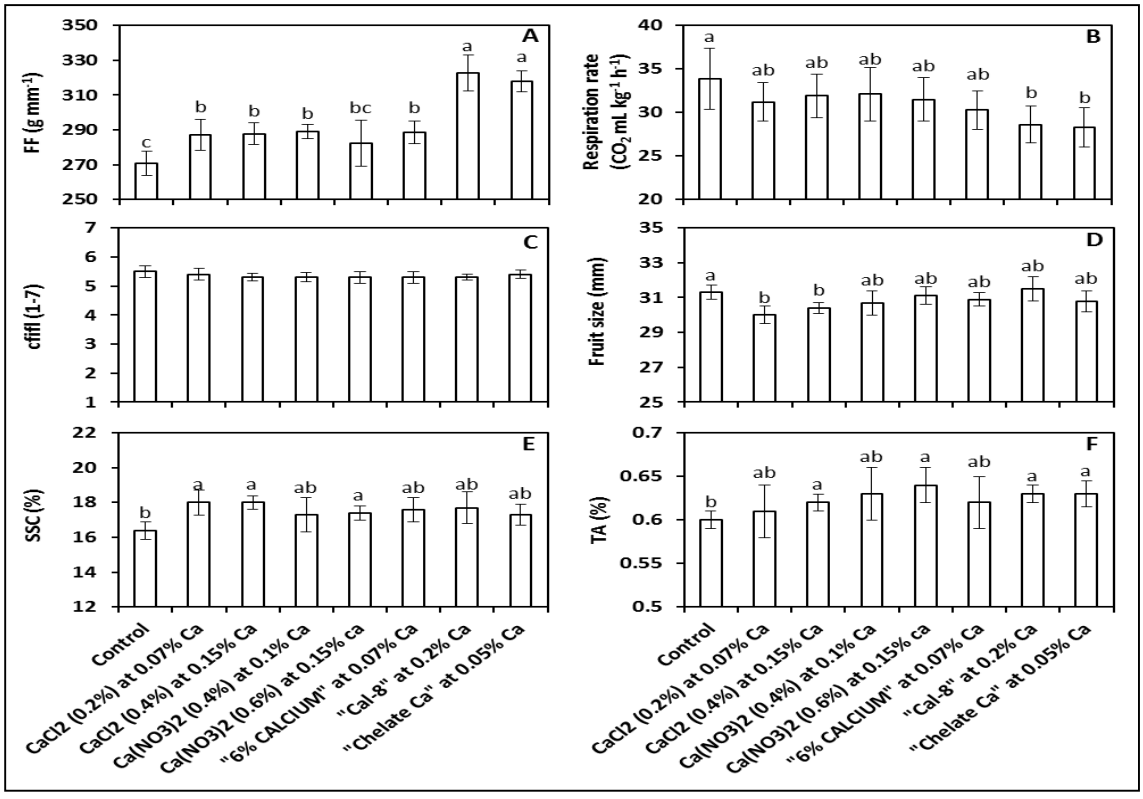
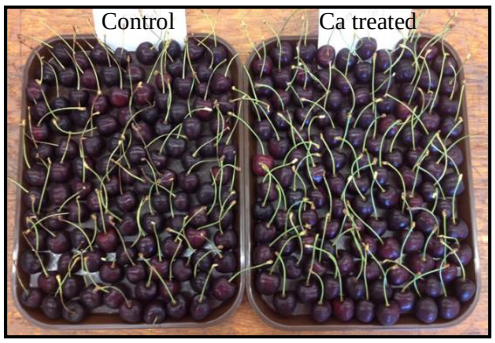


Fig. 7. Effect of Ca sprays on Lapins fruit maturity and quality at harvest.

8. Effect of Ca sprays on shipping quality of sweet cherries after 3 weeks of cold storage
The Ca sprays reduced pitting and decay incidences, retarded fruit skin darkening and TA degradation, maintained higher FF and SSC, and decreased pedicel browning incidence of Lapins after 3 weeks of storage at 32 °F (Table 1). "Cal-8" at 2% Ca and "Chelate Ca at 0.05% Ca were more effective than $CaCl_2$ at 0.15% Ca and $Ca(NO_3)_2$ at 0.15% Ca on improving shipping quality of Lapins.

Table 1. Effect of Ca sprays on shipping quality of Lapins and Skeena after 3 weeks of storage at 0 °C. Different letters indicate significant differences between treatments according to Fisher's protected LSD test at $p < 0.05$.

	Natural pitting (%)	Decay (%)	Pedicel browning (%)	Fruit skin darkening (L*)	Fruit firmness (g mm^{-1})	SSC (%)	TA (%)
				Lapins			
Control	13.5a	8.7a	22.3a	29.9b	288c	16.5b	0.48c
$CaCl_2$ (0.4%) at 0.15% Ca	11.0ab	3.6b	15.5b	29.0b	296b	17.8a	0.52b
$Ca(NO_3)_2$ (0.6%) at 0.15% Ca	10.6ab	4.0b	13.9b	30.3b	291b	17.6a	0.53b
"6% CALCIUM" at 0.07% Ca	9.8b	2.8b	11.7b	30.6b	303b	17.5a	0.55ab
"Cal-8" at 0.2% Ca	8.9b	1.6b	6.5c	32.1a	318a	17.3ab	0.56a
"Chelate Ca" at 0.05% Ca	7.7b	2.1b	9.9c	31.8a	322a	17.2ab	0.57a
				Skeena			
Control	5.6a	5.5a	5.5a	29.0a	335a	18.8a	0.58a
"6% CALCIUM" at 0.07% Ca	4.9a	2.2b	2.2b	29.4a	327a	18.5a	0.61a



CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number: CH-14-104

Project Title: Strategies to reduce postharvest cracking and splitting of cherries

PI: Girish Ganjyal
Organization: WSU
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Email: girish.ganjyal@wsu.edu

Co-PI: Shyam Sablani
Organization: WSU
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Co-PI: Yan Wang
Organization: OSU
Telephone: 541-386-2030 (ext 214)
Email: yan.wang@oregonstate.edu

Cooperators: Shield Bags and Printing Company, Yakima, WA; Glade Brosi at 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.

Budget: **Year 1**: $24,567 Y**ear 2**: **$24,932**

Other funding sources: None

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: The travel amount request is exclusively for project related travel expenses. The travel amount requested above includes the allocation of $600/year for Dr. Yan Wang to travel for project related work. Budget is requested to cover salaries and wages for the students and technicians working on the project, to conduct experiments as described in this proposal. A small percentage of money is requested for purchasing laboratory supplies and small equipment for the experiments. Travel funds are also requested to visit our co-operators in Yakima and Wenatchee for project work and for co-PI Yan Wang to visit WSU for supervising experimental work.

OBJECTIVES

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.

Goals and progress thus far for the 1st year:

1. Thoroughly review the Cherry packing process (Completed).
   - We visited over 6 different Cherry packing lines in Yakima and Wenatchee areas. Some of them were visited more than once. Had discussions with various packers, farmers and the council members. Thanks to all for their inputs. This has been of tremendous help.

2. Design and develop an air knife system for efficient removal of the moisture from the Cherries before packing (Completed).
   - We spent significant amount of time to design a low cost system to remove excess moisture from the surface of the Cherries.
   - We now have a system in place with two air knives and a blower that we can install in any of the packing facilities.
   - We have successfully tested this system in one packing facility. Please see Figure 1 in the report below.

3. Understand the surface cracks of the Cherries and the potential for the moisture migration into the Cherries (Completed).
   - We studied the surface of the Cherries under the microscopes to understand the surface structures. Please see Figure 2.

4. Screen numerous edible coating systems and narrow down to less than 5 coatings for thorough testing (Completed).
   - We screened over 12 different edible coatings to see if they can be applied to fresh Cherries without a lot of packing process changes.
   - From these we selected 4 different coatings and tested them in the final plant trial this year.
   - One of them, "Gum Arabic" coating, showed the best results thus far.

5. Develop packaging systems for managing the moisture in the package during storage (Completed).
   - We worked with one of our co-operators and obtained one package with one level of desiccant. Further we tested the packages with and without the holes.

Note:

- We do not have any deviation in our proposed objectives. All the works streams are going well.
- We are on track and in fact ahead of the schedule for completing the proposed work. We have been very lucky to have excellent industry collaborators. We thank all the co-operators and collaborators for their solid support.
- We plan to conduct thorough plant trials during next year. Thus we openly request all your support in these plant trials.
- Please contact us, if you are willing to provide access to your packing lines for the plant trials during next year.

SIGNIFICANT FINDGINS

Following are the significant findings we had for the first year of the work:

1. The air drying system, effectively removes excess moisture from the fresh Cherries before packing.
2. Air drying of the Cherries to remove excess moisture contributes to the overall quality of the Cherries and has the potential to increase the shelf life of the Cherries.
3. 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 4).
4. Packaging with desiccant and with the holes contributed to significant reduction in the cracks. (see Figure 3 & 4).
5. Even though we saw reduction in cracking of the Cherries for the above mentioned treatments, there was a clear benefit in avoiding the pedicle browning.
6. 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 4 and 5).

METHODS

Materials

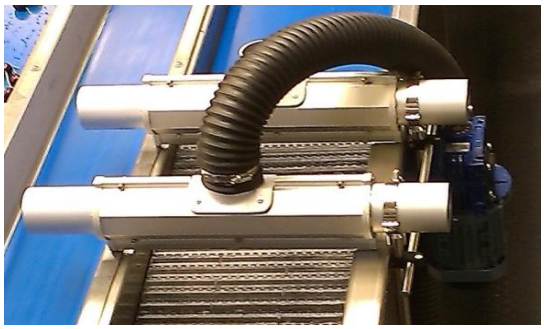
The sweet cherries tested were "Chelan" and "Skeena". The cherries were kindly provided by Western Sweet Cherry Group, Yakima, WA and Stemilt Growers LLC at Wenatchee, WA, USA. In the packing house, the cherries were pre-sorted, cleaned, sized and manually-sorted. The sorted fruits were packaged in a bag/box system and transported to the pilot plant at School of Food Science, Washington State University.

Packaging

Plastic films incorporated with desiccant agent were fabricated by our cooperator Shield Bag and Printing Company (Wenatchee, WA). The same plastic film without desiccant was also used as 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 hole diameter was 8.343 mm or 0.3284 in. Commercially available bags used for cherry packaging were also tested in this study. 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. Five bags for each treatment were filled with 25 cherries and kept in the cold room maintained at 39˚F and relative humidity 86% for 6 weeks for storage studies.

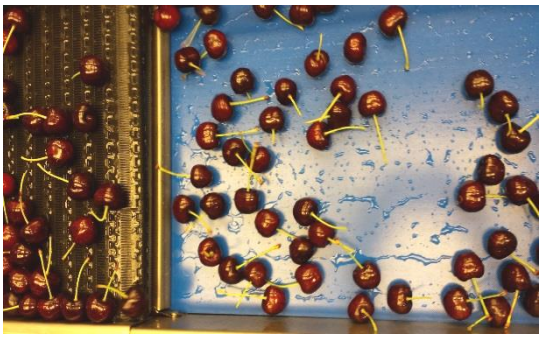
Air Drying of moisture on cherry surface

The surface moisture on the cherries was removed by drying with an air knife system. (Air Control Industries Inc., Maine). The system consisted of two air knifes along with a blower and a fan. The system tested at one of the packing houses in Wenatchee, WA on one of the conveyor belts moving at a speed of 50m/min where the cherries were air dried just before packing.



(a) Air Knife System Installed

(b) Air knife System in Operation



(c) Cherries without Air Drying

(d) Cherries with Air Drying

Figure 1. Air Drying System developed for drying the excess moisture in fresh pack Cherries

Edible coatings

A total of 4 different coatings that were prescreened for lab testing, were applied on the cherry fruit surface. Locust Bean Gum, TICA film and Prehydrated 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%), Preheated Gum Arabic (1%) and Locust Bean Gum (0.3%). All coatings were cooled before applying to the Cherries. Cherries were coated by dipping them in the solutions for 1-2 min followed by air-drying at a speed of 50m/min on the conveyor belt as described in the previous section.

Fruit quality determinations

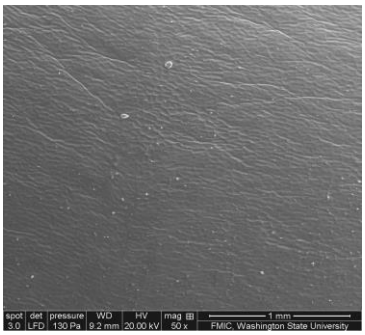
Cherries were analyzed for five quality parameters at regular storage interval. Quality parameters evaluated were including weight loss, color, firmness, ˚Brix, and pH. Weight loss was determined by weighing the samples with digital balance (Startorious, MCL). Color and firmness measurement were performance 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.

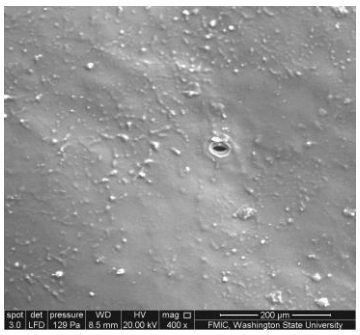
RESULTS AND DISCUSSION

Microscopy studies of the fruit surface

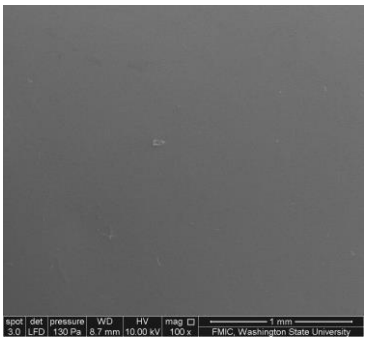
Fresh Cherries we observed under the Scanning Electron Microscope (FEI, Model Quanta 200F, FEI Company, U.S.A.) at WSU. Figure 2 below shows the selected images of the Cherry skin with and without the selected coatings. From the figure we can clearly see that there are pores (for transpiration) of about 20 microns in size and the surface is uneven (undulated). This uneven surface can serve as pooling spots for excess moisture. Our theory is to have the coating on the surface enough that we can make the surface even and not have any chance for the moisture to stay on the surface. This should aid in reducing the moisture absorption into the Cherries.



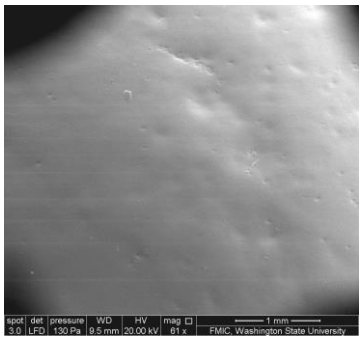
(a) Surface without coating



(b) Surface without coating



(c) Surface with coating (TICA film)



(d) Surface with coating (Gum Arabic)

Figure 2. Microscopy images of fresh Cherry surface with and without the coatings

Effect of packaging on fruit quality

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. The 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 ºBrix of cherries did not change significantly during storage

time. The influence of types of packaging on pH and ºBrix 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 significantly reduced the number of cracks for both types of cherries during and at the end of storage time.**

Effect of air drying and edible coatings on fruit quality

The coating of Gum Arabic solution when stored in perforated bags incorporated with desiccant reduced the weight loss in Skeena cherries. The titrable acidity reduced during storage the influence of coatings and air drying was small. The ºBrix of cherries was not influenced by the treatment conditions. The firmness of cherries was reduced with storage time but the Gum Arabic and Aloe Vera coatings were able to lower the changes in firmness. **Both TICA film and Gum Arabic 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.

Final remarks and future plans

Overall we observed that the **Cherry fruit quality parameters such as pH, ºBrix and firmness were not influenced by treatments.** The statistical analysis still needs to be done on this data. Although there was a significant reduction in weight loss of cherries in desiccant packaging, Aloe Vera, Locust Bean and Gum Arabic coated samples. This can be attributed to the natural physiological changes in the Cherries. The change in cherry color during storage was small and consistent with all treatments. The treatments such as air drying of surface moisture and packaging film incorporated with desiccant significantly reduced the number of cherries with cracks. **The preliminary results obtained from the first year of the work are encouraging. We have also established all the protocols for testing. All the treatments showing influence on cherry cracking will be studied thoroughly during the next season. A thorough statistical analysis of the data will be conducted to make definite conclusions during the next year.**

SIGNIFICANCE TO THE INDUSTRY & POTENTIAL BENEFITS

Post-harvest cracking/splitting of Cherries is one of significant issues to the industry. If we can increase the shelf life of the Cherries by reducing the cracking/splitting and pedicle browning, there will be direct positive economic benefits to the industry. The preliminary results from our first year of the work are encouraging and needs to be investigated thoroughly during next year.

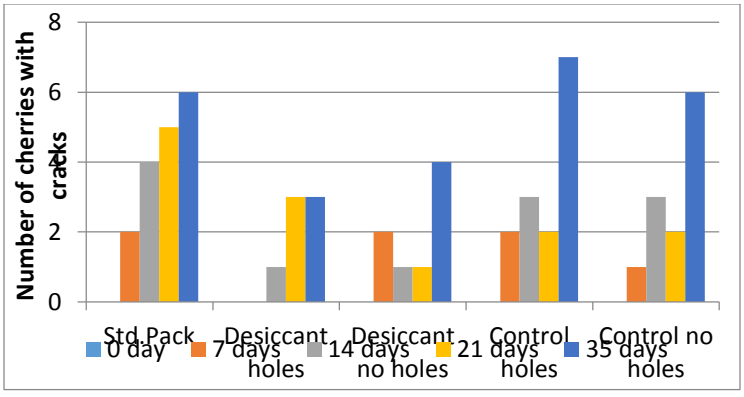


Figure 3. Cracks observed in Skeena Cherries during storage studies (Lab Trials)

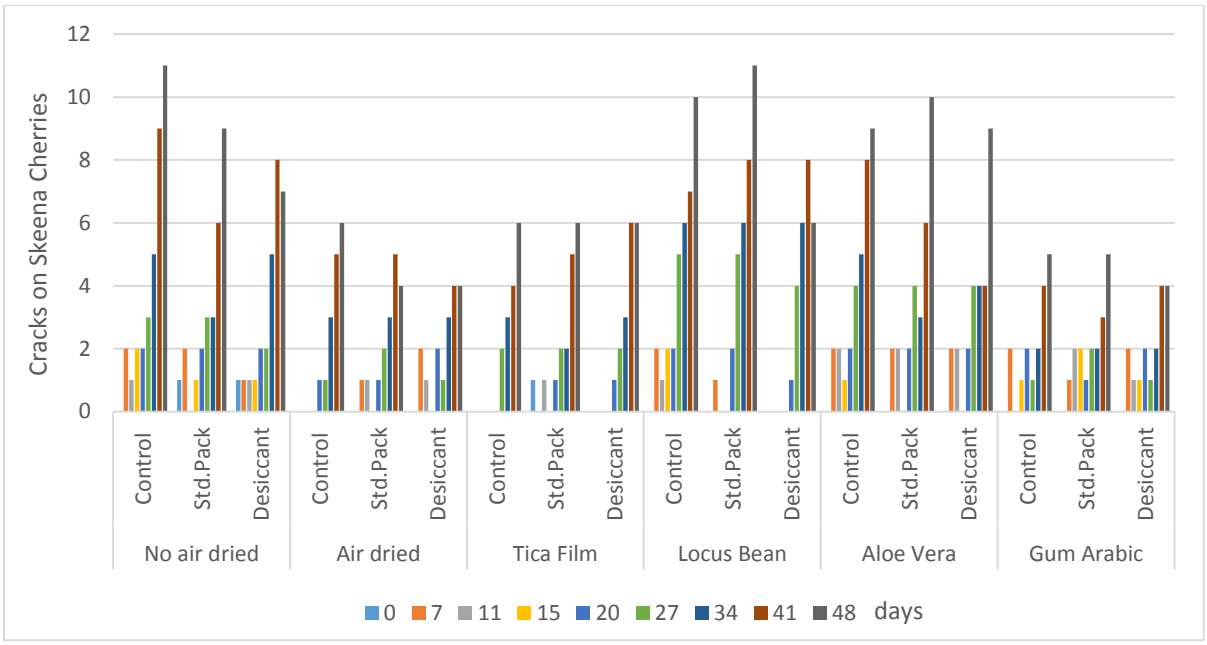


Figure 4. Cracks observed in Skeena Cherries during storage studies (Plant Trials)

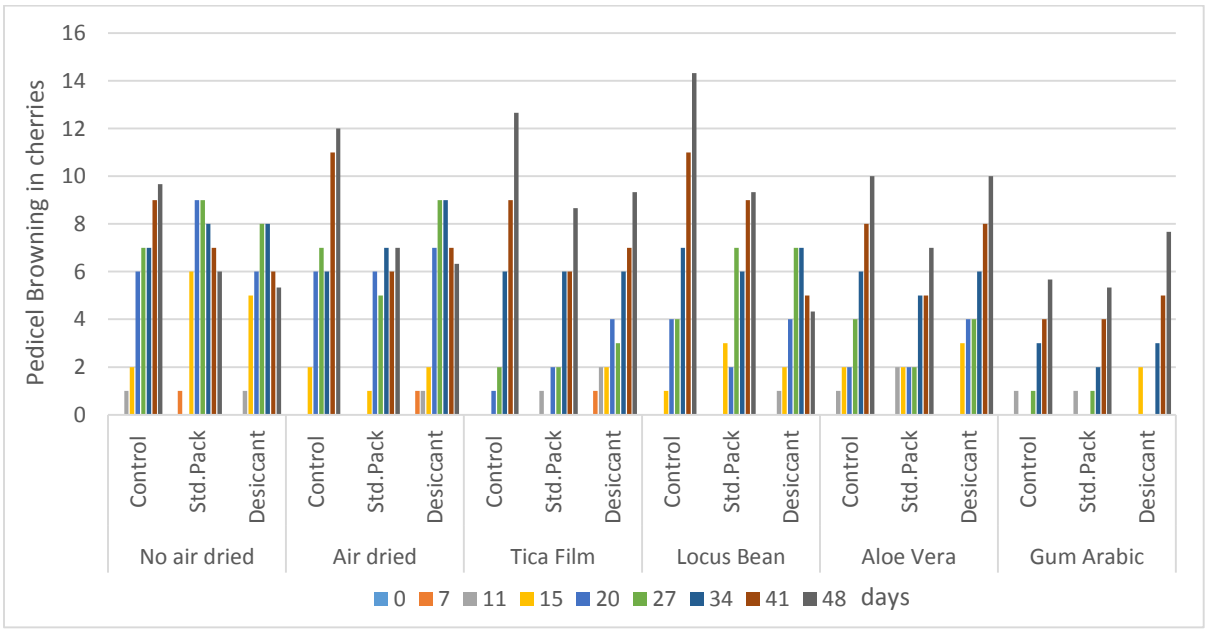


Figure 5. Pedicle browning observed in Skeena Cherries during storage studies (Plant Trials)

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number: CH-14-105A

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

PI: Matthew Whiting
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Co-PI(3): Todd Einhorn
Organization: OSU-MCAREC
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Address: 3005 Expt Station Drive
City/State/Zip: Hood River OR 97031

Cooperators: Denny Hayden, Russ LeSage

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

Other funding sources

Notes: A 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:

- Preharvest termination of irrigation has varied effects on sweet cherry fruit quality and yield
- Withholding irrigation up to 21 days before harvest (dbh) may improve fruits' resistance to splitting without affecting quality
- Withholding irrigation water from up to 21 and 15 dbh did not affect yield of mature 'Lapins' and 'Skeena' trees, respectively.
- Early termination of irrigation in a mature 'Chelan' orchard reduced yield
- 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. Other fruit quality attributes (firmness, SS, titratable acidity, post-harvest cracking, fruit color, and pedicel retention force) were not significantly affected by irrigation withholding, irrespective of the number of days imposed.
- Stem water potential is a good indicator of plant water status and responded sensitively and rapidly to irrigation withholding.
- Differences in soil water content among withholding treatments were only apparent in the top 15 cm of soil. Soil water content of controls was only slightly higher than withholding treatments.

Methods:

This research project was carried out in 3 commercial orchards (2 WA, 1 OR).

Site 1: Pasco, WA. Drip irrigated 12-year-old 'Chelan'/Mazzard.
Site 2: Brewster, WA. Microsprinkler irrigated 14-year-old 'Lapin'/Mazzard.
Site 3: Dufur, OR. Drip irrigated, 10-year-old 'Skeena'/'Gi6'

In sites 1 & 2 we imposed two early termination treatments that were compared to the growers' standard irrigation practices leading up to harvest. Irrigation was withheld from either 18 or 7 days before harvest to impose the deficit treatments. All treatments were replicated 3 times with 3 sample trees per rep (i.e., 9 trees per treatment). In Pasco, treatments were imposed to entire rows; in Brewster, treatments were imposed to 3-row sets with sampling from the middle row. In addition, we conducted a large-scale experiment in Brewster in which a 4-acre plot was subjected to an early termination of irrigation and compared with fruit from a contiguous 4-acre plot that received irrigation regularly up to harvest.

Soil moisture content was assessed using a neutron probe every 2-3 days at 6-inch increments to a 3-foot depth between the onset of the treatment and harvest. One access tube per replicate was installed about a month before the initiation of treatments.

At harvest, fruit yield per tree was determined in the field. Fruit samples (25-50 fruit) were collected from each tree and transported to the fruit quality lab in Prosser. Each sample was analyzed for fruit color, weight, pedicel-fruit retention force, firmness, row-size, soluble solids and titratable acidity. Fruit susceptibility to splitting was determined using an artificial cracking test in the lab every three days between the onset of treatments and harvest.

Site 3 has three treatments compared to a control (irrigated several times per week at~ 60% of actual ET); irrigation terminated 5 days before harvest (dbh), irrigation terminated 10 dbh, and irrigation terminated 15 dbh. All treatments were replicated 4 times. Each replicate comprised 4 trees (the two center trees were used for measurements, the two outer trees were treated as guard trees). At the start date for each withholding treatment, irrigation was bypassed around treatment tree reps via blank poly-tubing controlled by a series of ball valves so that irrigation could be delivered to other treatment and non-treatment trees within rows.

Soil Moisture. Soil moisture content was measured two to three times per week at 6-inch increments to a depth of 3 feet using a neutron probe. One access tube per replicate was installed in early spring between the two center trees of each 4-tree replicate ~6 inches inside of the drip tube.

Results and Discussion:

Site 1 ('Chelan'/Mazzard) We documented no effect of early termination of irrigation treatments (i.e., 18 and 7 dbh) on stem water potential nor fruit growth rates in the 'Chelan' trial in Pasco (data not shown). Stem water potential remained above ca. -0.75 MPa at all sampling dates, irrespective of treatment. Fruit quality was unaffected by early termination of irrigation (Table 1), yet inexplicably, yield per tree was reduced, being ca. 45% and 32% lower than control from the 7 and 18 dbh early termination treatments, respectively (Table 1). This is confounding given the lack of apparent stress on the trees and lack of yield affect in other trial sites (see below). We intend to monitor fruit drop in 2015 to determine whether this played a role in 2014.

Table 1. The effect of early irrigation termination on 'Chelan' fruit quality and yield. Irrigation was terminated on 16 May (18 dbh) and 26 May (7 dbh), harvest was on 2 June.

Treatment	Firmness g/mm	Weight (g)	Size (mm)	T.A. (%)	S.S. (%)	Yield (kg/tree)	Color CTIFL	PFRF (kg)
Control	359	10.5	27.7	1.76	17.3	24.84	4.2	1.17
7 dbh	323	10.7	27.8	1.80	17.5	13.79	4.2	1.21
18 dbh	360	10.4	27.8	1.55	17.8	16.89	4.2	1.21
p-value	0.115	0.749	0.961	0.251	0.45	0.007	0.942	0.707

T.A.= titratable acidity; S.S.=soluble solids; PFRF = pedicel-fruit retention force. n=25 for all quality assessments; n=9 for yield.

There was no effect of irrigation treatment on 'Chelan' fruit susceptibility to cracking, as assessed by a benchtop immersion test (Fig. 1). 'Chelan' is one of the most split-resistant cultivars – this was underscored from our testing as the cracking index was never greater than 25 (contrast with Lapins below).

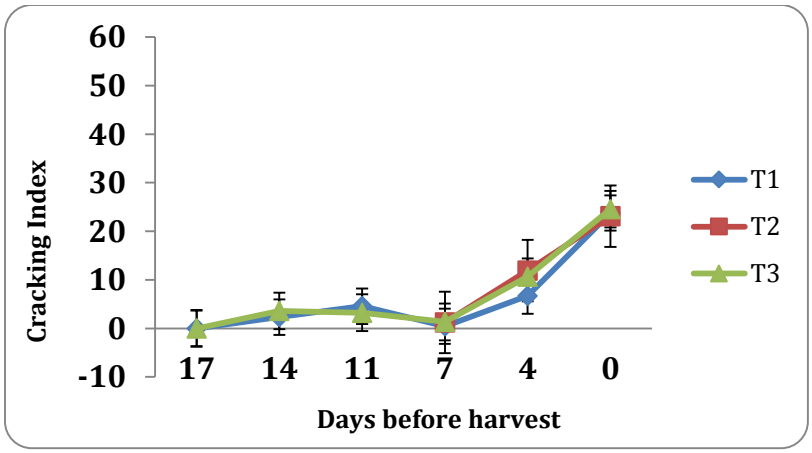

Figure 1. The effect of early irrigation termination treatments on cracking index of 'Chelan' fruit. T1=Control; T2=irrigation withheld 7 dbh; T3=withheld 18 dbh. Each point is the mean +/- SEM of 180 fruit.

Site 2 ('Lapins'/Mazzard) There were two separate trials conducted in the commercial 'Lapins' blocks near Brewster. One was setup similar to the Pasco 'Chelan' trial in which the full suite of soil and tree testing was conducted regularly; the other, large-scale trial was established in two contiguous 4 acre blocks (i.e., treated vs. control) in which only fruit quality data were evaluated. In the smaller scale trial we observed a significant decline in stem water potential from the irrigation treatments compared to the regularly-irrigated control (Fig. 2), though water potential levels never fell below ca. -1.2 MPa. This occurred however only within about 1 week of harvest, when both early termination treatments caused a similar (ca. -0.42 MPa) decline in stem water potential.

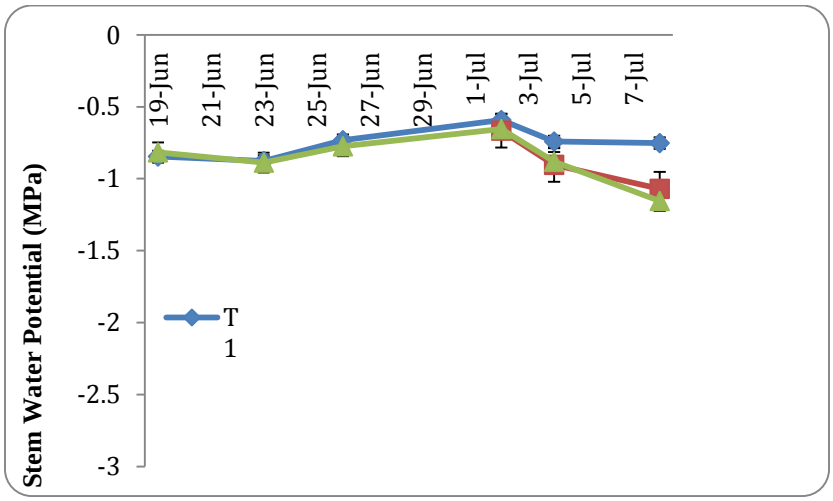

Figure 2. Effect of irrigation treatments (T1=control, T2=withheld 11 dbh, T3=withheld 21 dbh) on stem water potential of 'Lapins'. Harvest was 10 July.

Despite differences in stem water potential near harvest, there was no effect of irrigation treatment on fruit growth (data not shown) nor final diameter (Table 2). This is not unusual given the marginal decline in stem water potential from early termination treatments. With the exception of fruit soluble solids, which were significantly improved (+10 – 14%) by both early termination of irrigation treatments, there was no effect of treatment on fruit quality or yield (Table 2). The increase in soluble solids may be due to fruit dehydration but there was no observable fruit shrivel. In general, fruit of other species increase soluble solids in response to water stress though this phenomenon was not common across the 3 sites in the current study. The increase of 2% soluble solids is significant and likely sufficient to improve the consumer appeal of the fruit.

Table 2. The effect of early irrigation termination on 'Lapins' fruit quality and yield. Irrigation was terminated on 19 June (21 dbh) and 29 June (11 dbh), harvest was on 10 July.

Treatment	Firmness g/mm	Weight (g)	Size (mm)	T.A. (%)	S.S. (%)	Yield (kg/tree)	Color CTIFL	PFRF (kg)
Control	310	11.1	27.6	1.23	17.3	69.68	4.8	0.81
11 dbh	300	11.8	28.6	1.21	19.7	67.30	4.8	0.84
21 dbh	295	12.0	28.6	1.20	19.1	73.34	5.1	0.86
p-value	0.101	0.184	0.250	0.783	0.003	0.531	0.365	0.712

T.A.=titratable acidity; S.S.=soluble solids; PFRF=pedicel-fruit retention force (n=25) for all the traits.

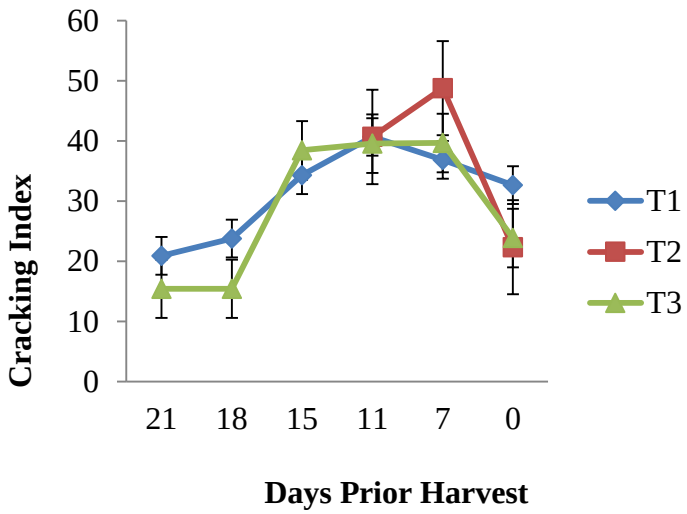

Figure 3. The effect of early irrigation termination treatments on cracking index of 'Lapins' fruit. T1=Control; T2=irrigation withheld 11 dbh; T3=withheld 21 dbh. Each point is the mean +/- SEM of 180 fruit.

We observed an inconsistent and largely insignificant effect of irrigation treatment on the fruit susceptibility to cracking (Fig. 3). 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'. 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, particularly for the two early termination treatments (Fig. 3). Remarkably, cracking index dropped by more than 50% in the 11 dbh termination treatment, from 49 to 22. A similar but less dramatic drop occurred with the 21 dbh treatment where the cracking index dropped from 39 to 24 between 7 days before harvest and harvest.

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 29 June (13 dbh & 17 dbh), harvest was on 12 July (fruit on canopy exterior) and 16 July (fruit from canopy interior).

Treatment	Firmness g/mm	Weight (g)	Size (mm)	T.A. (%)	S.S. (%)	Color CTIFL	PFRF (kg)
First harvest							
Control	286	12.8	29.1	1.08	19.6	5.0	0.76
13 dbh	276	11.9	28.6	1.08	20.7	4.9	0.65
p-value	0.317	0.026	0.341	0.935	0.600	0.824	0.112
Second harvest							
Control	320	12.5	29.5	1.17	17.7	5.1	0.63
17 dbh	279	12.2	28.8	1.30	19.9	5.2	0.60
p-value	0.003	0.488	0.569	0.020	0.100	0.687	0.614

T.A.=titratable acidity; S.S.=soluble solids; PFRF=pedicel-fruit retention force. (n=25)

In the large-scale 'Lapins' trial we imposed the irrigation termination treatment on 29 June. Fruit were harvested in two passes through this orchard, first on 12 July, picking mostly fruit on the exterior and upper regions of the canopy, and, second, on 16 July to harvest remaining fruit. Fruit from trees that were un-irrigated for 13 days were about 8% smaller than those from regularly-irrigated trees. No other fruit quality attribute was affected by irrigation treatment (Table 3). In contrast, the fruit from the second harvest were similar size and weight but the trees un-irrigated for 17 days this time yielded fruit that were 13% softer and had 11% greater acidity than control fruit.

Site 3 ('Skeena'/'Gi6'). For each withholding treatment, stem water potential declined in synchrony with the irrigation termination date (Fig. 4). Within ~2 weeks of harvest, critical water potential values (i.e., relative to the process of fruit growth) are likely ≤ -1.75 MPa. By harvest, leaves of both the 10- and 15-dbh treatments were noticeably wilted. Fruit growth also responded sensitively to water withholding (Fig. 5); the differences among treatments being quite small and likely reflective of differences in fruit water status *and not* direct effects of water potential on cell growth. However, measurements of the relative water content and dry matter of fruit did not confirm this assertion. Compared to control fruits, fruit volume of the 15 dbh withholding treatment was ~6% reduced at harvest (Fig. 5). Average fruit size (weight or diameter) at harvest confirmed our growth data (Table 4). In general, overall fruit size was excellent (~9 row). The fact that fruit growth isn't reduced for 10 and 5 dbh treatments is supported by previous detailed growth analyses of cherry fruit by PI Einhorn (Einhorn and Gibeaut, WTFRC/OSCC 2014 Final Report), which show only minimal accumulation of dry matter during this time span. Moreover, this would especially be the case under most commercial conditions, since over-irrigation ensures a sufficient supply of available water in the soil profile to compensate for deficits in irrigation over a period of several days to weeks, depending on soil type and rooting depth. In the 'Skeena' orchard, seasonal irrigation below daily ET provided us with a site that would respond rapidly to irrigation termination (i.e., only ~2 inches of water per foot of soil to a 3 ft. depth). Soil moisture monitoring was not as sensitive a technique as direct measures of plant water status (i.e., fruit growth and water potential). In fact, soil moisture content only differed among treatments in the top 15 cm (~6 inches) of soil, possibly due to high frequency/low volume irrigation events limiting the depth of the saturated wetting front to < 12 inches (data not shown). With increasing depth, withholding treatments could not be differentiated and were only slightly lower than controls.

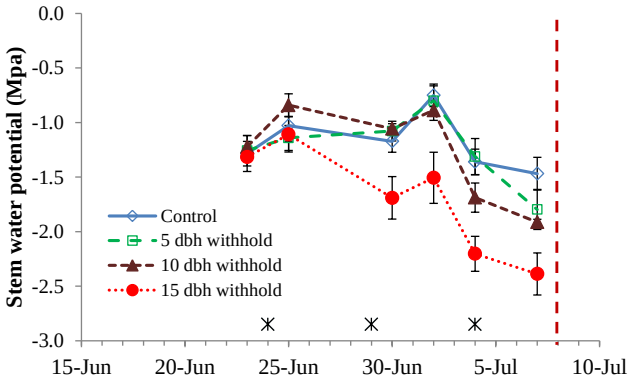

Figure 4. Stem water potential of 'Skeena' trees cut-off from irrigation 5, 10 or 15 days before harvest (dbh), compared to control trees. Asterisks above x-axis signify start date of withholding for each treatment. Hashed vertical line indicates harvest date (8-July). Four leaves per replicate were bagged ~1hr prior to measurement. Measurements bracketed solar noon (±1.5 hrs).

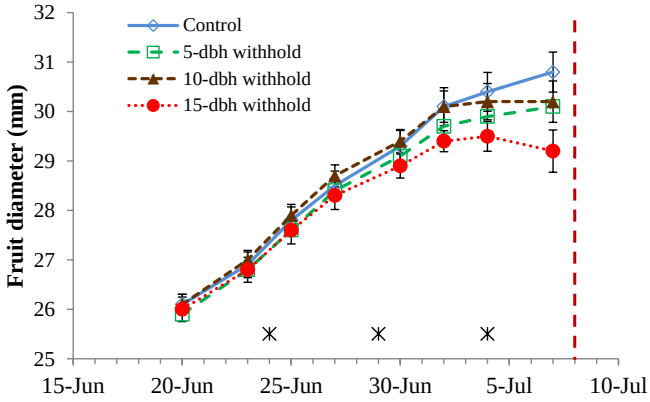

Figure 5. Fruit growth of 'Skeena' as affected by irrigation withholding 5-, 10- or 15-days before harvest (dbh). Asterisks above x-axis signify start date of withholding for each treatment. Hashed vertical line indicates harvest date (8-July). Tape was applied to pedicels of 20 fruits per replicate on 19-June. Fruit were measured nondestructively using a digital caliper.

Table 4. Effect of withholding irrigation for 5, 10, or 15 days before harvest (dbh) on 'Skeena' fruit quality.

Treatment	FF	FF +21dPH	Fruit diameter	Fruit wt.	Skin color	Yield	PRF	SS	TA	RWC	DMC	Percent cracking			
	g/mm	g/mm	mm	g	ctifl	lb/tree	g	%	%	%	g/fruit	1 hr	2 hr	3 hr	4 hr
Control	315.7	403.1 a	30.2 a	12.2 a	5.3	78	338.6	21.2	0.68	78.9	2.6	0.5	16	14	30.5
5-dbh	302.5	385 ab	29.6 a	11.7 a	5.3	81.3	332.9	21.5	0.68	78.1	2.6	0	8	15	23.1
10-dbh	300.1	379.3 ab	29.8 a	11.5 ab	5.2	84.2	405.1	21	0.66	77.7	2.5	1	8	9.5	18.5
15-dbh	300	363.8 b	28.4 b	10.8 b	5.5	72.8	449	22.3	0.68	77.1	2.4	0.5	5.5	7	13.5

TA, titratable acidity (n=1); PRF, pedicel retention force (n=25); RWC, relative water content of fruit and DMC, dry matter content of fruit (n=1 [100 fruit composite sample]). SS, soluble solids (n=1); FF, fruit firmness (n=100); Fruit diameter and fruit weight (n=100); cracking index (n=25); skin color (n=50).

Some fruit (typically in the exterior of canopies) of the 15 dbh were noticeably shriveled; however, we could not empirically determine quality degradation at harvest from samples of fruit taken from whole canopies (i.e., FF, SS, TA, etc.). A slight increase in SS of the 15 dbh treatment would appear to support fruit dehydration (i.e., passive accumulation of sugars), but the differences were not significant. 'Skeena' is sensitive to preharvest conditions, warm temperatures in particular; this cultivar may also be more sensitive to water stress since in none of the other experiments did we observe fruit shrivel, although stem water potential was markedly lower than recorded in other trials. Firmness, following 3 weeks of cold storage, increased for all treatments as is commonly observed. Interestingly, the increase was significantly less for 15 dbh fruit compared to controls. Rate of cracking was monitored over a 4-hour period and was not affected by treatments; however, 3 applications of protective anti-cracking films were applied over the 2-week prior to harvest (due to forecasted rain events) likely confounding the assay's results.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: CH-14-106

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

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

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

Other funding sources: None

Budget 1
Organization Name: OSU MCAREC **Contract Administrator:** L.J. Koong
Telephone: 541-737-4066 **Email address:** l.j.koong@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
Equipment	--	--	--
Supplies	1,000	1,545	1,000
Travel	250	2,000	2,060
Miscellaneous	--	--	--
Plot Fees	--	--	--
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
Organization Name: **Contract Administrator:**
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
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
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
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. This methodology will be utilized to capture adults for use in discriminating dose and target site and metabolic resistance screening in years 2 and 3.

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

Diagnostic doses of insecticides have been estimated for Delegate, Entrust, malathion, Sevin 5F, and Warrior II.

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

In order to monitor the presence and frequency of mutations that confer target site resistance in *D. suzukii* populations will be collected from different regions of Western U.S. and PCR-based assays and primers to amplify genomic regions will be developed that are associated with development of resistance. This objective has just started and will be finished during the 2015 cherry season.

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

Populations from western cherry districts will be assayed to determine their susceptibility of target insecticides. The assays will include genetic studies and topical applications of discriminating doses to field collected live flies, to be initiated the next two years.

5. Correlate results from discriminating-dose and genetic studies (yr 2-3)

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 next two years.

SIGNIFICANT FINDINGS

- Traps for capturing live SWD for screening for insecticide resistance were evaluated in the field and laboratory and at least two trap designs appear promising when baited with commercial SWD lures.
- Dose-response lines and LC_{50} and LC_{95} values have been estimated for various insecticides against adult female SWD. These mortality-dependent doses will provide the basis for diagnostic testing of SWD populations in the western USA in years 2 and 3.

METHODS

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

In CA, all live traps consisted of a standard monitoring trap (white opaque plastic 1 qt container (Consolidated Plastics Company, Inc., Stow, OH) with a $1/8^{th}$ inch screen mesh side panel and was filled with 4 oz liquid bait. The bait consisted of a 3:2 Apple Cider Vinegar (ACV): Merlot Mixture. The ACV was 4% acidity (Amerifoods Trading Co., Los Angeles, CA) and the Merlot was 12 % ABV (Peter Vella Vineyards, Modesto, CA). All treatments contained 4 ml Pure and Clear soap per gallon (Colgate-Palmolive Company, New York, NY) with wire mesh in place to prevent contact between adult flies and the liquid bait. In live traps, the lids were replaced by an inverted plastic funnel, the stem of which opened into a 540 ml deli container (Fabri-Kal, Plastics Place, Kalamazoo, MI 49001) containing a food and water source for the captured flies. Laboratory experiments were conducted with each replicate consisting of one of each trap type (see table 2) contained within a Bug Dorm 2 Rearing cage (BioQuip, Rancho Dominguez CA 90220). All the lures (Trece SWD A & B lures) were secured via a paperclip in accordance with manufacturer's directions, and a food and water source was placed in each rearing cage.

In the retention study, 25 SWD were placed in the upper chamber of the live trap and the number of adult remaining in the upper chamber recorded after 48 hrs. In the attraction study, 100 SWD were placed in each Bug Dorm 2 Rearing Cage and the number of adults in the upper chamber of the live trap were determined after 24 hrs.

In the field study, traps were placed in the field on Dec. 17 and removed on Jan. 3. Adult flies were removed from the upper trap chamber in the live traps every 5-6 days. All bait was replaced and the traps rotated at each collection date. All SWD were sexed and all other drosophila were counted, but not sexed, under magnification in the laboratory at UCB.

In WA, various designs of live traps were tested in the fall of 2013, varying attributes such as total volume, the number of chambers, bait type and placement, and the size and orientation of the entry area. In one first test, apple cider vinegar (ACV) was used either as a liquid bait, or absorbed into a gel to help minimize drowning of flies and spills. It was also tested alone or in combination with the Trécé synthetic lure. One series added whole fruit (raspberries) as a supplementary attractant. While these tests give a rough indication of the number of females that might be trapped, they were done at different locations with (presumably) different ambient populations, and as such, cannot be rigorously compared. However, it appeared that 1-3 females/trap-day was the best that could be expected from the live traps, even during a period of typically high fly activity levels in standard traps.

A series of laboratory tests was done during the winter of 2013-2014 to better understand the capture efficiency of various traps designs. The traps were tested in pairs in a rectangular cage in a growth room, with the candidate trap at one end, and a fly release platform at the other. All traps tested except the 2-chamber trap were very efficient in this bioassay, recapturing 70-84% of the flies released. However, there was little discrimination among designs (with the exception of the 2-chamber trap), so this experiment was discontinued.

These tests continued in late summer of 2014, using only the synthetic Trece lure. Three trap styles were tested (the Quadra-Shrek, Trappit Dome, and Van Steenwyk 2-chamber). Observations indicated that there might be more Drosophila close to ground level, so the effect of trap height was also tested. This was correct in a sense; higher numbers were caught closer to the ground of total Drosophila, but not SWD, which were higher in the standard (1.5 m above ground) height. In these tests, the Quadra-Shrek and Trappit Dome caught similar numbers of SWD females (the target species/sex), while the 2-chamber trap caught few or no SWD. One surprising development was the highly skewed sex ratio in the live trap; only 20% of the total SWD captured were females. This is problematic in that only females can produce the F_1 generation.

Two full-scale field tests of the Quadra-Shrek trap in a commercial orchard were conducted in the fall. The first was in mid-September 2014 (1-day deployment), however, out of 45 traps, only 66 of the 207 SWD were females, or 1.46 females/trap/day. The problem of the highly skewed sex ratio persisted, in that only 31% of the SWD caught were females. A similar test (same trap, 24 total, different commercial orchard) was deployed for a 2-day period in late September-early October. While the females/trap/day were similar (1.3), 2.6 were caught/trap for the 2-day deployment. In this orchard, the sex ratio was close to 50:50. These results leave lingering questions concerning the temporal effects of trap efficiency, but clearly to be certain to capture a large number of females, a large number of traps will be necessary, even during periods of high trap activity (late summer and fall).

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

Diagnostic doses of insecticides have been estimated for malathion, Delegate, Entrust, Sevin 5F, and Warrior II. Assays were conducted for each of the listed insecticides. For a particular insecticide, 45-100 female per dose SWD were treated with serial dilutions of insecticide using a Potter Spray Tower, a device that precisely delivers small amounts of solution to test insects.

RESULTS & DISCUSSION

The success of live traps to produce SWD for diagnostic dose screening is dependent on several factors: 1) the ambient population in the orchard; 2) the attractiveness and retention ability of

the trap; 3) the fecundity of the wild females captured, and 4) the sex ratio of the progeny (only females will be used for testing). These factors were explored in a series of experiments in 2013/2014. The target number of females for bioassay (100/insecticide tested) could be generated in a number of ways. One principle of this testing was that the collection of the populations should be as close as possible to period of attack by SWD (May-August, depending on the region). However, it is understood that in two of the regions (eastern Washington and the Mid-Columbia), the highest densities, and thus the optimal time for collection, is usually later in the summer and fall (mid-Aug through October).

In the laboratory retention study, traps with SWD lures placed in only the upper chamber of the trap retained more flies than those traps with lures in both the upper and lower chambers combined, only in the lower chambers or when no lures were used (Table 1). The largest difference was when SWD lures were placed only in the upper chamber of the trap as compared to when lures were placed only in the lower chamber. It appears the lures in the lower chamber attracted the flies out of the upper chamber. Thus if SWD lures are to be used they should be placed only in the upper chamber.

Table 1. Retention of 25 SWD by various live trap types in laboratory dome cage over 48 hrs

	Mean total SWD		
Trap type	Live	Dead	Total
Live trap with upper lure	16.0	6.0	22.0
Live trap with lower lure	9.0	2.0	11.0
Live trap with upper and lower lure	11.7	8.3	20.0
Live trap with no lure	15.7	2.7	18.3

Table 2. Attraction of live trap types in laboratory dome cage over 24 hours

	Mean total SWD		
Trap type	Live	Dead	Total
Live trap with upper lure	3.7	1.0	4.7
Live trap with lower lure	0.7	0.0	0.7
Live trap with upper and lower lure	1.0	0.3	1.3
Live trap with no lure	3.0	1.0	4.0

In the laboratory capture study, traps with lures placed in the upper chamber of the trap captured more flies than those traps with lures in both the upper and lower chambers combined, only in the lower chambers or when no lures were deployed (Table 2). Again, the largest difference was when SWD lures were placed only in the upper chamber of the trap as compared to when lures were placed only in the lower chamber. Thus again if SWD lures are to be used they should be placed only in the upper chamber.

Initial tests indicated that testing field-collected females directly with a discriminating dose was likely not feasible. Given a 50:50 sex ratio, about 840 individuals for a give site would need to be trapped. The alternative, using F_1 progeny from field-collected adults, was chosen as the more appropriate model. This approach also has the advantage of using individuals of known age and nutritional status in the bioassays, eliminating a potential source of variation. In addition, the individuals used were not directly exposed to any of the pesticides used (another source of variation).

Table 3. Capture of adult SWD in various traps in the lab.

Treatment (Trap Type)	N	Mean flies captured (males+females)	SEM		% recapture (of 50 flies)
1.Biobest	5	37.80	1.96	a	76%
2.4-powder funnel	5	35.20	1.66	a	70%
1.Biobest	5	36.80	1.02	a	74%
3.Standard PBJ	5	36.00	1.26	a	72%
1.Biobest	5	42.20	1.46	a	84%
4.4 small-funnels	5	41.60	3.06	a	83%
1.Biobest	1	42.00	.		84%
5.Caroline trap	1	0.00	.		0%
1.Biobest	5	37.40	2.29	a	75%
6.3-vent Eppendorf	5	39.00	1.18	a	78%

In order to produce a cohort of similarly aged individuals, they must be produced from a short oviposition period, generally 24-48 h. One way to overcome the effects of low fecundity is to replicate over time; thus, if there are 5 replicates with 20 females each (the target being 100/insecticide), this greatly reduces the need for a large number of females available simultaneously. The same females from the original collection can be moved to new medium sequentially to produce the required numbers for bioassay. Replication over time increases the labor in transferring females to new medium and mixing the concentrations/spraying, but represents a reasonable trade-off in the time spent collecting from the field.

A discriminating dose is simply a dose of toxicant that would be expected to kill 95% of individuals of a susceptible population (LC_{95}) although, some researchers have used discriminating doses from LC_{75} to $LC_{99.}$ To determine the $LD_{95,}$ a dose-mortality bioassay was conducted on a large number of susceptible laboratory reared individuals. Probit analysis of the dose-mortality bioassays data generated the LC_{50} and LC_{95}values (Table 4). In years 2 and 3, suspected resistant field populations will be tested using a LC_{95} dose of the toxicant.

Table 4. LC_{50} and LC95 values (± 95% Fiducial limits) of insecticides screened against female SWD.

Insecticide	LC_{50} ppm	95% F.L.	LC_{95} ppm	95% F.L.
Delegate	19	17-20	36	32-42
Entrust	40	36-44	82	72-100
malathion	137	128-148	210	184-271
Sevin 5F	3703	1,799-5,202	17,080	13,893-24,640
Warrior II	1.2	1-1.5	3.6	3-5

Efforts for years 2 and 3 will involve generating molecular markers for insecticide resistance and screening numerous SWD populations against various target insecticides using discriminating doses in western USA cherry growing districts. Results from the molecular and diagnostic assays will be correlated to determine the accuracy of the molecular assays with the hypothesis being that these newer methodologies will provide reliable information regarding insecticide resistance monitoring that is faster than traditional bioassay methods.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: CH14-10

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
Supplies[5]	15,756	15,590	14,580
Travel[6]	5,066	5,066	5,325
Plot Fees	0	0	0
Total	63,479	**65,020**	62,743

Footnotes: [1]Salaries are for post-doctoral scientists (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 LChV2 transmission via insect vectors (apple and grape mealybug [AMB and GMB]). Our first objective aims to better understand the vector/disease relationship. Little is known regarding mechanisms of LChV2 transmission within and between orchards via grape and apple mealybug. In 2014, we located LChV2 infected trees, and extracted mealybugs of different life stages to determine corresponding rates of infection. So far, we have detected LChV2 in field-collected crawlers, but the analysis of other stages is still underway (see results); the difficulty with the diagnostic kit delayed this work somewhat. In 2015-16, we plan to continue to develop basic information such as: which species and/or stage of mealybug is more efficient at passing virus to healthy trees or acquiring virus from an infected tree; how much time and how many vectors are necessary to successfully pass virus to an uninfected tree; and can mealybug eggs that hitch a ride on non-plant material (bins, tractors) and orchard prunings, transmit virus to healthy trees. Results from these studies will help us to better understand how LChV2 is spreading between trees and orchards, improve management efforts, and ultimately slow the spread of LChV2.

Obj. 2. Determine control methods for AMB and GMB in conventional and organic cherries. Our second objective is to explore the efficacy of various pesticides for the control of apple and/or grape mealybug. In 2014, we examined delayed dormant, foliar and soil-applied systemic, and contact-foliar sprays for control of apple mealybug (AMB). In 2015, we will look at repeated applications of dormant and neem oil on mealybug populations for organic control.

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). Our third objective is to validate a low cost test for LChV2 that can be performed with minimal specialized equipment. Results from 2014 revealed the presence of genetic variants of LChV2 in WA State; these genetic differences dramatically reduced sensitivity of the assay system under investigation. Using this information (see results), modifications are being made to the detection kit to improve detection of LChV2, including the newly revealed genotypes. Before the issue with the diagnostic kit/genetic variants was discovered, several user groups were trained on the use of the kit. The experience gained from using this technology in the 2014 season will be applied during the 2015 season. Modifications in protocol were delineated in an effort to increase sensitivity. The redesigned assay system and protocols will be validated during the 2015 growing season, and training will be available on request.

Another goal for this objective is to develop field diagnostic tests that will differentiate LChV2 from two other pathogens that occur in sweet cherries and cause little cherry disease (LCD), namely LChV1 and Western X phytoplasma (WX). The contributions of LChV1 and particularly WX to LCD in WA State became more evident during testing in the 2014 season. Sequence information was developed for isolates of LChV1 and WX detected in 2014. This information was used to design primers and probes for detection purposes. The primers and probes for detection of LChV1 and WX will be evaluated during the 2015 growing season.

An accurate assessment of the contribution of LChV1, LChV2 and WX to LCD is critical for the development of management plans by affected growers.

Obj. 4. Assess the economic impact of LChV2 given its effects on crop yield, crop quality, and tree death. The post-harvest period (fall) was targeted as the most convenient time for growers to participate in focus groups, and a list of names was developed. Despite numerous contacts, participants willing to share production cost information have not been identified. In 2015, we will assess the impacts of LChV2 by using a partial budgeting procedure and assessment of three yield-loss scenarios (see Methods).

SIGNIFICANT FINDINGS

- Control strategies for AMB, including a delayed dormant spray in combination with a spray targeting the crawler stage provided the best results.
- Loresban+oil sprayed at delayed dormant and Delegate sprayed to target AMB crawlers resulted in significantly lower crawler numbers when compare to control.
- A new genetic variant of LChV2 was discovered in Washington orchards. This genetic variability contributes to reduced sensitivity of the assay systems.
- WX has been found to be an important pathogen associated with LCD in Grant and Chelan counties. It was previously primarily associated with LCD in Yakima County.

METHODS

Obj. 1. *Vector transmission:* Mealybugs will be collected from virus-free trees in the field during 2015 growing season, and transferred to infected and uninfected, small, potted 'Bing' cherry trees grown in a greenhouse at WSU TFREC. Ten mealybugs will be removed from infected trees at 1, 3, 7, and 9 days, and will be subject to virus verification procedures. At least five mealybugs feeding on virus-free trees will be tested for virus as a check. These results will tell us the number of mealybug feeding days required, on an infected plant, to acquire virus. The remaining mealybugs will be transferred to 20 virus-free cherry trees and will be allowed to feed for 1, 3, 7, and 9 days, before removal (five trees per group). Trees will be verified for LChV2 after 30 days (when virus becomes detectable). These results will tell us the number of mealybug feeding days required to inoculate a healthy tree with virus. These experiments will be completed using crawlers and only other life stages verified to acquire virus, as determined in 2014 experiments. Experiments will occur as mealybugs become available in the field. In 2016 we will revise these methods to determine if mealybug density can affect the likelihood of uninfected healthy trees acquiring virus by examining the time to inoculation of a virus-free seedling when infected mealybugs are allowed to feed in high and low numbers.

Obj. 2. *Vector Control:* During the 2015 growing season we plan to examine the use of two organic control strategies for the management of apple mealybug in a block of organic apple trees at WSU Sunrise Research Orchard, which is currently infested with apple mealybug. Treatments will include dormant oil and Neemazad, which will be compared to an untreated check. Experimental setup, monitoring, and sampling will occur as described in the proposal.

Obj. 3. *Field Diagnostic Assay:* A new genotype of the LChV2 virus was discovered in WA orchards during 2014. The molecular assay has been redesigned to compensate for this genetic variation. Also, testing parameters were further refined during the 2014 season and this information will be incorporated into operating procedures during the 2015 season to validate the reliability of the assay system.

Sequence information obtained in 2014 from Washington State pathogen isolates, in conjunction with other available sequence information, was used to design reagents for detection of WX and LChV1; these components of the assay system are being synthesized. In 2015, these will be incorporated into detection assays for WX and LChV1, and evaluated in the laboratory setting using known reference isolates of the pathogens. Once basic operating protocols have been established, testing of grower samples will be implemented late in the 2015 growing season to further validate the assay system. During the course of the evaluation, results from the new molecular assay system will be compared to those from established polymerase chain reaction assay systems, in terms of both sensitivity and reliability.

Obj. 4. *Economic impact and decision-making tools:* We will develop a baseline enterprise budget for production of 'Bing' sweet cherries not infected with LChV2, and three partial budgets that take into account the three scenarios mentioned above for LChV2-infected trees (assume losses due to

lower yields per acre, assume losses due to diminished pack-outs and removal of infected trees). To develop the budgets, we will gather information on production costs by organizing focus group meetings with at least ten sweet cherry producers who have experience in dealing with LCD, to be held during the Spring-Summer of 2015. The purpose of the focus group will be to achieve a consensus among producers and to generate a representative cost of production study for "Bing" cherries. The group session will take place on a date and venue agreed upon by the participants. We will follow this group session with in-person visits to growers' sites.

RESULTS & DISCUSSION

Objective 1: Mechanisms of LChV2 transmission via insect vectors

During the 2014 growing season, we addressed LChV2 acquisition for various stages of apple and grape mealybug from infected trees in the field. Mealybug nymphs and eggs were collected from trees that tested positive for LChV2 as well as trees that tested negative, but are located in orchards with a history of LChV2 infection. Molecular analysis of plant material was used to determine/verify virus infection. Grape mealybug eggs, nymphs and adults were collected from four LChV2 positive trees in two orchards, and nine LChV2 negative trees in three orchards. Apple mealybug eggs and nymphs were collected from one tree testing positive for LChV2. Mealybugs/mealybug eggs were also collected from trees other than cherries to act as negative controls for molecular analysis. Mealybugs were extracted from plant material and placed in sterile micro-centrifuge tubes, and stored at -80º C until analysis could be performed. A small group of samples from a virus positive tree were analyzed. It included seven samples of newly hatched grape mealybug (GMB) crawlers (≈ 0.5mm), containing 10 crawlers/sample, and one sample containing two late instar crawlers (≈ 1.5mm). Molecular analysis (RT-PCR) of virus extract showed the virus to be present in all eight samples tested. However, when we tested those same extracts using the LChV2 diagnostic kits from Agdia (RT-RPA), 50% of the samples showed a negative result (Table 1). We plan to use molecular analysis for all the remaining samples, and because it is more time consuming than the diagnostic kits, we are still in the process of analysis (see objective 3).

Table 1: Detection of LChV2 in extracts from GMB nymphs, using RT-PCR and RT-RPA (diagnostic kit)

GMB sample #	RT-PCR	RT-RPA
1	+	-
2*	++	++
3	++	-
4	+	-
5	++	+
6	++	++
7	+	-
8	++	++

Reaction: ++, strong positive, +, weak positive, -, negative
* Sample # 2 contained 2 late instar crawlers. All other samples contained 10 newly hatched crawlers.

Objective 2: Control methods for apple mealybug

Apple mealybug (AMB), the documented vector of LChV2, was only recently recorded for the first time on sweet cherry in Washington; therefore, little is known about its phenology in this region. In 2014, AMB was monitored weekly at WSU's Sunrise Orchards, in a conventional apple orchard with a high density of AMB (Fig. 1). We found that second to third instar females are present and feeding on woody plant parts near buds as early as 21 March, while immature males were still in overwintering structures. Nymphs of both sexes overwinter in individual structures underneath bark. Females continued to feed under loose bark

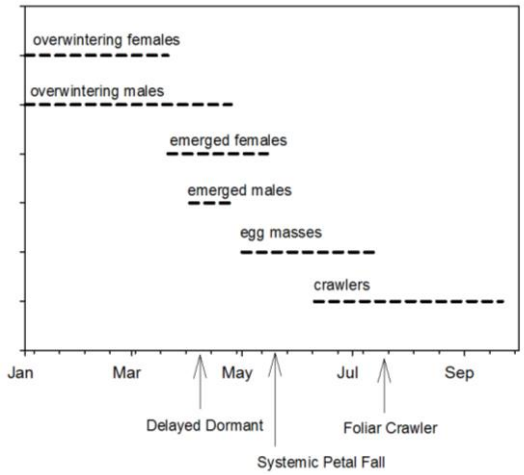

Fig. 1. Phenology of apple mealybug (AMB) at Sunrise Orchards (2014) and chemical timing of delayed dormant sprays; systemic treatments, applied 14 days after petal fall; and foliar sprays targeting crawlers on leaves.

and grow rapidly throughout the spring (21 March – 24 April), then became active, dispersing to more exposed parts of the tree (cracks in the bark, old pruning scars, and at the ends of twigs), before settling to lay eggs. Emerged males were observed as early as 2 April from bark removed from infested trees. Males emerged gradually over time and were observed mating starting on 9 April, however, some could still be found in overwintering structures a week later. Crawlers emerged gradually from egg masses staring around10 June through 16 July, and remain feeding on the underside of leaves though the fall.

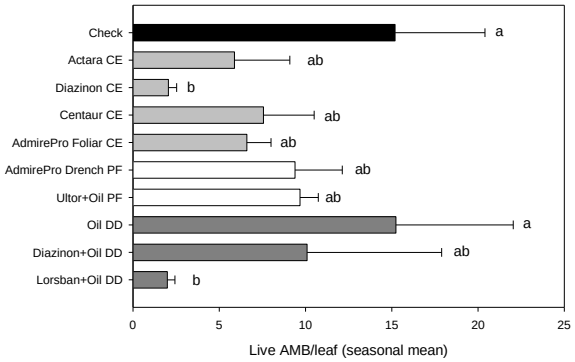

Fig. 2. Seasonal averages of AMB crawlers/leaf (17 June – 22 Sept.) from leaves collected from apple trees treated with delayed dormant (DD), petal fall (PF), and crawler emergence (CE) spray timings.

During the 2014 growing season, a field experiment was conducted in the aforementioned AMB infested orchard, to determine the effects of chemical insecticides on AMB populations. Treatments were applied using a speed sprayer to groups of three trees per replicate (4). Twenty leaves per tree were collected weekly, from 17 June – 5 Aug, and then every other week, from 5 Aug. – 22 Sept. The average number of crawlers/leaf was calculated for each treatment for each sampling date, and then those means were averaged again for the entire season. Delayed dormant treatments included Lorsban+oil, Diazinon+oil, and oil targeted overwintering females. Lorsban +oil (2.0 ± 0.4 crawlers/leaf) was the only compound that showed a significant lower crawler population compared to the check (15.2 ± 5.2 crawlers/leaf) (Fig. 2). Two systemic compounds, Ultor+oil (foliar) and Admire Pro (soil drench), were applied 14 days after petal fall, however season crawler averages were similar to the control. Finally, we applied Admire Pro (foliar), Centaur, Diazinon, and Actara to target active crawlers on leaves. Trees treated with Diazinon (2.1 ± 0.5 crawlers/leaf) had significantly lower crawler numbers than the control trees (15.2 ± 5.2 crawlers/leaf), while all other treatments targeting crawlers had similar seasonal crawler averages compared to the control.

Pinning down the phenology of the AMB in Washington is important for determining the timing, application method, and active ingredient of successful chemical control strategies. Mealybugs tend to live in protected areas of trees, such as bark cracks and crevices, and the undersides of leaves, and the eggs are protected by waxy filamentous secretions of the ovisac, making them extremely difficult to reach with insecticides. We know that newly hatched crawlers are the most susceptible stage, but delayed dormant sprays targeting females emerging from overwintering places can also be a successful management strategy. The timing of delayed dormant sprays allows control of females before they have a chance to lay eggs. A management plan that includes a delayed dormant spray in combination with a spray targeting the crawler stage is likely to provide the best results.

Objective 3: Validation of Little cherry virus 2 (LChV2) field kits

A diagnostic kit for LChV2, based on the reverse transcription recombinase-polymerase amplification (RT-RPA) technology, was made available during this year's growing season. During May to June of the 2014 growing season, samples from symptomatic trees showing little cherry disease symptoms (e.g., small fruits) were tested for the presence of LChV2 by RT-RPA assays. However, many samples from symptomatic trees did not give positive results in the RT-RPA assay format. Initially, these apparent discrepancies were investigated on 13 symptomatic samples. Reverse transcription polymerase chain reaction (RT-PCR) confirmed that each was infected with LChV2. When crude

plant sap extracts (the method being employed in the RT-RPA kit) from these samples were tested, only 7 of the 13 samples yielded positive results. When RT-RPA was repeated using purified RNA, 12 out of 13 samples yielded positive reactions for LChV2. The RNA purification process concentrates the RNA relative to crude extracts and removes many enzyme inhibitors from the sample. Although RNA purification greatly enhanced sensitivity of LChV2 detection by RT-RPA, this approach adds significantly to the cost of the assay and requires more specialized equipment that would not be amenable for use in field offices. These results suggested that the sensitivity of RT-RPA relative to RT-PCR is resulting in inaccurate test results.

Different parameters were investigated to increase sensitivity of the RT-RPA assay. Increasing the reaction incubation time from 15 minutes to 30 minutes increased signal strength and the results easier to interpret, but it did not increase the sensitivity of the assay in terms of the number of positive samples detected by RT-RPA. Increasing the incubation time to 45 minutes resulted in a marked decrease in sensitivity. Increasing the sample volume per reaction from 1 µl to 5 µl per reaction greatly inhibited RT-RPA reactions, and positive reactions were lost for all but the strongest positives.

Previous studies had suggested that detectable LChV2 might be increased by storing samples at 39°F and/or by collecting samples later in the growing season. Storing samples for 10 days did not increase sensitivity of the RT-RPA assay system. Testing symptomatic trees by RT-RPA using crude sap preparations during the latter part of the growing season (beginning early July) provided much more accurate virus detection without the need to purify RNA (Table 2). This improved detection during the latter part of the season is presumably due to decreased carbohydrate inhibitors present in crude sap and the increase in concentration of LChV2 in infected tissue.

Table 2. Detection of Little cherry virus 2 (LChV2) by RT-RPA using crude sap preparations at different times of the growing season

Sample	RT-PCR	RT-RPA	
		Jun-14	Jul-14
1-1	+	-	-
1-2	-	-	-
1-3	++	++	++
1-4	++	-	++
1-5	++	-	++
1-6	++	-	+
1-7	++	-	++
1-8	++	-	++
1-9	++	-	+
1-10	++	-	++
1-11	++	+	++
non LChV2 infected	-	-	-
		Jun-14	Aug-14
2-1	++	-	++
2-2	++	-	++
2-3	++	-	++
2-4	+	-	-
2-5	++	-	++
2-6	++	-	++
2-7	++	-	++
2-8	+	-	-
2-9	++	-	++
2-10	++	-	++
2-11	++	n/a	-
2-12	++	n/a	-
2-13	++	n/a	++
2-14	++	n/a	++
2-15	++	n/a	++
2-16	++	n/a	++
2-17	++	n/a	++
2-18	++	n/a	-
2-19	++	n/a	++
2-20	++	n/a	-
2-21	++	n/a	++
non LChV2 infected	-	-	-

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

As previously indicated in this project, two other pathogens, namely LChV1 and Western X phytoplasma (WX) are associated with LCD. In summary, a total of 145 samples from symptomatic

trees showing small fruit were tested by RT-PCR for the presence of these two other pathogens along with LChV2. Results revealed 89 samples infected with LChV2, and 65 with WX and 14 with LChV1. Samples that contained only a single infectious agent were 71 for LChV2, 51 for WX and none for LChV1 (Table 3). It was previously presumed that WX is more confined along the southern regions of WA State (e.g. Yakima and Grant counties) whereas LChV2 is predominantly located in the northern part (e.g. Chelan and Douglas counties). The results of the 2014 testing reveal a dramatic increase in WX in the northern regions and LChV2 in the southern sweet cherry growing regions of the state. Specifically, the high percentage of samples infected only with WX is one of the reasons behind the seemingly failed detection of LChV2 in symptomatic samples; many negative samples were singly infected with WX and not LChV2.

Table 3. Summary of Little cherry virus 1 (LChV1), Little cherry virus 2 (LChV2) and Western X phytoplasma (WX) RT-PCR test

Pathogens	Number of positives per county				Total
	Chelan	Yakima	Grant	Benton	
LChV1	0	0	0	0	0
LChV2	38	23	0	10	71
WX	29	6	15	1	51
LChV1/LChV2	1	7	0	0	8
LChV1/WX	4	0	0	0	4
LChV2/WX	6	1	0	2	9
LChV1/LChV2/WX	2	0	0	0	2
Total					**145**

Even after the efficacy RT-RPA detection method was improved and the presence of other pathogens were accounted for, one symptomatic sample still remained negative for LChV2 in RT-RPA and even when using purified RNA preparation. The presence of LChV2, however, was confirmed by RT-PCR.

The possibility of a different genetic variant of LChV2 was examined by testing additional symptomatic samples by both RT-PCR and RT-RPA. Four symptomatic trees tested negative by RT-RPA but positive by RT-PCR. A unique genotypic variant of LChV2 was revealed from the nucleotide sequences of the RT-PCR amplicons from these four trees. A detailed examination of the nucleotide sequences showed that the currently employed probe in the RT-RPA kit would detect this variant of LChV2 with reduced sensitivity. A new probe was designed and is being evaluated to correct this situation.

Development of an RPA test for LChV1 and WX phytoplasma:
Since there was very limited existing sequence information available describing the genome of LChV1, the development of an RT-RPA assay for LChV1 was initiated by determining full to near full genome sequences of ten different LChV1 isolates by deep sequencing and identifying conserved regions therein. Using this information along with sequences available in public databases, target regions in the LChV1 genome were identified for potential use in an RT-RPA assay. A six DNA primer pairs were evaluated. Preliminary tests using the real time platform of RT-RPA with crude extracts from three LChV1 isolates yielded promising results. Currently, the lateral flow platform of RT-RPA, which is more suitable for field use, is being evaluated, along with selection of the best primers and probes. Similarly, two isolate of WX identified in WA State were subjected to deep sequencing leading to the design of four primers and corresponding probes from two regions of the WX genome. Selection of the best primers and probes for use in the RT-RPA assay is currently being pursued.

CONTINUING PROJECT REPORT **YEAR**: 2 out of 3
WTFRC Project Number: CH-13-106A

Project Title: Factors affecting the fruit phase of cherry mildew

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[1] research lead on fruit quality aspects of objectives 4 and 5

Total Project Request: **Year 1**: $66,334[1] **Year 2**: $67,822[1] ***Year 3**: **$68,039[1]**

Other funding sources: None

Budget 1
Organization Name: WSU-IAREC **Contract Administrator:** Amy Hill
Telephone: 509-786-2226 **Email address:** amy.hill@wsu.edu

Item	**2013[1]**	**2014[1,]**	**2015[1,*]**
Salaries	33,504[2]	34,836	36,238
Benefits	17,087	16,373	18,481
Wages	7,075	7,076	7,075
Benefits	667	686	667
Supplies	3,600[3]	3,000	1,000
Travel[4]	1,000[4]	1,000[5]	1,000[5]
Total	$62,933	$62,971[6]	**$64,461[6]**

Footnotes: *Progress-dependent
[1]WSU-IAREC and WSU Plant Pathology are partnering to provide a PhD student who has been assigned to the project
[2]associate in research (Maurisio Garcia)
[3]reagents (anhydrous glycerol) and supplies, Black Tartarian trees, Nitex cloth (8 um, 48 Pyrex #74 glass crystallization jars ($1600; 2013 only))
[4]travel to WSU-IAREC Roza farm in 2013 to collect cherry fruit, flowers, and shoots; travel to WSU-TFREC and OSU-MCAREC to establish plots related to objective 4
[5]industry wide travel to collect cherry fruit (various levels of maturity) 2014-15 pending the need for a wider window of fruit availability; travel related to fungicide trials described in objective 4
[6]partial funding for objective 4 will be requested from the Washington State Commission on Pesticide Registration in 2014 and 2015

Budget 2: OSU-MCAREC
Organization Name: Agricultural Research Foundation **Contract Administrator:** L.J. Koong
Telephone: 541-737-4066 **Email address:** l.j.koong@oregonstate.edu

Item	2013	2014	2015
Salaries			
Benefits			
Wages	2,686[1]	4,086	2850
Benefits	215[2]	940	228
Equipment			
Supplies	500[3]	500	500
Travel			
Miscellaneous			
Total	3,401	5,526	**3,578**

Footnotes:
[1]Wages: 200hr for a Biological Science Tech. at $13.43/hr. 3% increase is factored into Year 2 and 3.
[2]OPE: 8% of the wage.
[3]Supplies: cold rooms, buying gases (helium, nitrogen, hydrogen, air, and standard gases), gas tank rental, and chemicals.

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 (60% - 95%) 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.

SIGNIFICANT FINDINGS

- Fruit inoculated 25 days after full bloom became infected at harvest
- 25 days after full bloom was the first onset of natural disease on leaves
- The conidial concentration to cause significant disease at harvest is dependent on developmental age of fruit (higher concentrations are needed to cause infection on immature fruit)
- Spores can remain latent (=time from initial spore deposition to onset of disease) for 56 days
- Not all spores remain viable throughout the latent period. Disease incidence on fruit exposed to early season inoculum was significantly less than on those exposed to inoculum later in fruit development.
- Disease development occurred only at full fruit maturity and coincided with formation of chasmothecia on foliage.
- Immature fruit is not susceptible to infection and does not develop signs or symptoms
- 90% relative humidity and 25^0C/77F are ideal conditions for fruit infection
- Sequential spray applications of Fontelis and Quintec significantly reduce disease incidence (foliar) and severity (fruit), fruit quality was not affected by fungicide regime.

METHODS

Inoculum production: Immature, disease-free foliage was used for inoculum production under controlled conditions. About ten trees were planted every 7-10 days beginning in mid-March and inoculated by brushing or spraying spores (conidia) of mass isolates of *P. clandestina* onto immature foliage. Trees were grown in a greenhouse at 64F – 72F. To harvest inoculum, infested, immature leaves were placed in sterile distilled water and mixed using a vortex. Inoculum concentrations were adjusted to specified levels using additional sterile distilled water and a haemocytometer.

Disease assessment: Disease on leaves and fruit was assessed visually based on absence/ presence (incidence) and percentage leaf area infected (severity). Fruit were sorted into one of four categories based on the amount of the fruit surface colonized: 0 = no infection, 1 = 1 – 33% colonized, 2 = 34 – 66% colonized, and 3 $\geq$ 67% colonized.

Orchard studies: Two experimental orchards at WSU in Prosser, WA were used for fruit studies. One orchard did not receive any fungicide treatment during the season. The other orchard was used for fungicide timing studies. Four single tree replicates were randomly selected for study objective 1 and 3. Fruit clusters were covered with Nitex cloth bags 18 days after full bloom (DAFB) to avoid natural powdery mildew infection. First signs and symptoms of natural disease onset were observed on May 5th, 2014 (24 DAFB, on leaves) and on July 1st, 2014 (80 DAFB on fruit). Fruits were harvested on July 1st (cv. Bing) and 15th (cv. Sweetheart). Fruit and leaves were evaluated for disease incidence and severity.

For inoculation studies (Objective 1), inoculum suspension [0 conidia/ml (negative control), 100 conidia/ml, 500 conidia/ml, 1000 conidia/ml, 5000 conidia/ml and 10,000 conidia/ml] were applied to developing fruit clusters at three different dates starting approximately 45 days after full bloom and every two weeks thereafter. Nitex bags were removed and reapplied within 30 minutes. For temporal susceptibility studies (Objective 3), fruit were inoculated with 5000 spores/ml or were exposed to natural inoculum for a two week period starting 37 DAFB, and every two weeks thereafter. At each treatment date, Nitex bags were removed and reapplied within 30 minutes (inoculation) or after two weeks (natural exposure). For Fungicide timing studies (Objective 4), sequential applications of Quintec were applied at different stages of fruit development starting at 19 DAFB (fruit set). Fontelis or Pristine was applied at all other stages in each treatment regimen. Fungicide trials were conducted in a WSU orchard under completely randomized conditions. Disease incidence and severity on leaves and fruit were evaluated from July 1st to July 5th, 2014. Fruit for quality analyses were delivered to Hood River were delivered to within 6h.

Lab studies: Detached fruit and shoots (cvs. Sweetheart and Bing) were harvested from trees by severing the fruit pedicel with a razor blade. Fruit were immediately transported to the lab and surface sterilized with10% bleach water (20 min) followed by a triple rinse with sterile water. Fruit were air-dried under sterile conditions and pedicles immersed in 2- 5ml Eppendorf tubes containing sterile distilled water. In accordance with results from 2013, only fruit at growth stage 3 (approx. 45 DAFB, cell expansion) were used.

For inoculation studies (Objective 1), fruits were inoculated using various conidia concentrations as describe above and incubated (22^0C/ 71.5F, 90% relative humidity) inside small humidity controlled chambers. Disease severity was recorded based on visual signs 28 days after inoculation. For temperature and relative humidity studies (Objective 2), six fruit per rep (4 reps for each treatment combination) were inoculated with 5000 spores/ml and incubated at (15^0C/59F, 20^0C/68F and 25^0C/77F at various humidity levels) inside small humidity controlled chambers. Disease severity was recorded every 7 days. Experiments were repeated 5 times. For Objective 5, cherry flowers were collected from WSU orchards and at a commercial orchard in Zillah, WA. Flowers were kept on sterile water agar, point inoculated with 20ul of a freshly prepared spore suspension (5000 spores/ml) and incubated at 20^0C/68F in an environmental growth chamber with a 16 h light period. Experiments were repeated three times. Disease development was assessed every two days. At the commercial site, four tree branches were randomly selected (three trees per cv., cvs. Early Robin, Bing, Rainier, Lapins and Sweetheart) and transported to the lab. Blossoms and leaves were stored separately at-80^0C/-112F for DNA extraction.

Data analysis: Analysis of effects of inoculum concentrations on disease incidence and disease severity were performed by analysis of variance general linear model procedure (Minitab 17, Minitab Inc. 2014 and JMP®, Version 11, SAS Institute Inc., 1989-2007). Means were separated with Fisher's least significant difference (LSD, *P=0.05*) and Tukey's honest significant difference test (HSD, *P=0.05*).

RESULTS AND DISCUSSION

Objective 1: In the orchard studies, significant variations *(P<0.05)* were observed for mean disease incidence and severity among fruits inoculated with different conidia concentrations (Table 1). Disease incidence and severity increased with increasing inoculum concentrations. Interaction between inoculation dates and inoculum concentrations showed dependency of disease development on growth stages of fruits (Fig.1). A minimum inoculum concentration of 500 conidia/ml was needed for significant fruit infection at all fruit growth stages examined in this study. The minimum conidial concentration needed to cause disease in more than 50% fruits varied depending on the inoculation dates. A concentration of 500 conidia/ml resulted in average 76% disease incidence when fruits were inoculated on June 26 (76 DAFB) and it resulted in average 26% fruit infection when fruits were inoculated on May 29 (48 DAFB). The minimum conidial concentration needed to cause significant differences in disease severity also varied depending on the growth stages of fruits. It ranged from 500/ml for mature fruits to 5000/ml for relatively young fruits (Table 1).

Table 1 Mean disease incidence and severity on cherry fruits caused by inoculation of various conidial suspensions of *Podosphaera clandestina* throughout the growing season.

Inoculation dates	*May 29*		*June 12*		*June 26*	
Conidia concentration	Disease incidence	Disease severity	Disease incidence	Disease severity	Disease incidence	Disease severity
10000/ml	0.85 A*	0.18 A	0.89 A	0.24 AB	1.00 A	0.46 A
5000/ml	0.82 A	0.13 A	0.95 A	0.30 A	0.94 AB	0.39 AB
1000/ml	0.45 B	0.03 B	0.88 A	0.14 BC	0.93 AB	0.29 BC
500/ml	0.26 BC	0.03 B	0.65 B	0.10 CD	0.76 B	0.18 C
100/ml	0.11 CD	0.01 B	0.13 C	0.01 D	0.10 C	0.01 D
0/ml	0.00 D	0.00 B	0.00 C	0.00 D	0.00 C	0.00 D

* Results are averages of four replicates. Values for a variable within a column followed by a common letter are not significantly different based on Fisher's LSD test (*P=0.05*)

Only mature fruits inoculated on July 3 showed significant disease development in laboratory studies and on those fruits, conidial concentration had significant effects *(P<0.05)* on disease severity (Table 1). Disease severity increased with increasing conidial concentration. The conidial concentration of 1000/ml was minimum for 1% disease severity in mature detached fruits in lab studies. Interaction between inoculation date and inoculum concentrations was also noteworthy indicating dependence of disease development on developmental stage of fruit (Fig. 2).

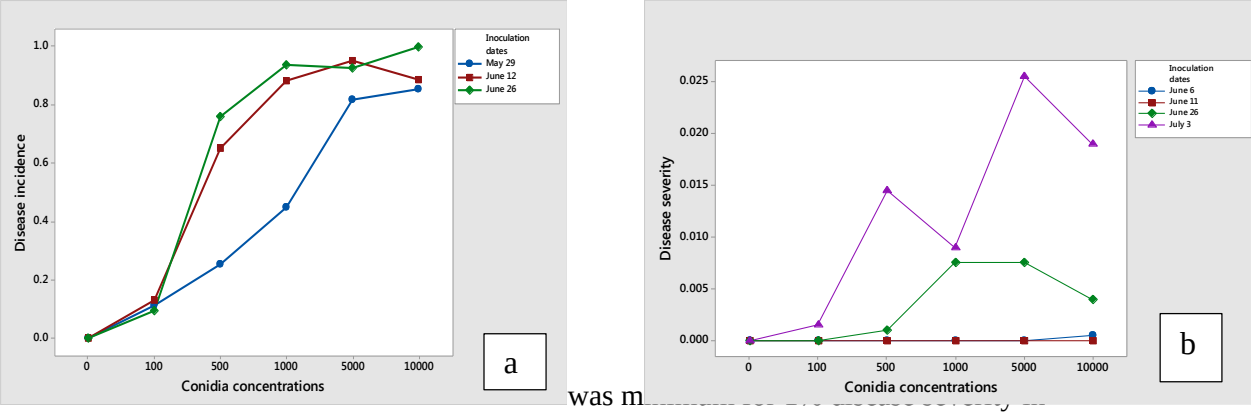

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Figure 1 Interaction plot of conidia concentrations (ml^{-1}) and inoculation dates for disease incidence (a) and severity (b) in orchard experiments.

Table 2 Powdery mildew disease severity on cherry fruits caused by inoculation with varying conidial concentrations of *Podosphaera clandestina* in laboratory experiments.

Inoculation dates	*June 6*	*June 11*	*June 26*	*July 3*
Conidia concentration	Mean disease severity	Mean disease severity	Mean disease severity	Mean disease severity
10000/ml	0.001 A	0.000 A	0.008 A	0.026 A
5000/ml	0.000 A	0.000 A	0.008 A	0.019 A
1000/ml	0.000 A	0.000 A	0.004 A	0.015 AB
500/ml	0.000 A	0.000 A	0.001A	0.009 B
100/ml	0.000 A	0.000 A	0.000 A	0.002 BC
0/ml	0.000 A	0.000 A	0.000 A	0.000 C

*Values for a variable within a column followed by a common letter are not significantly different based on Fisher's LSD test (*P=0.05*)

Objective 2:

Fruit only became infected at full maturity. Fruit infection and the initial detection of chasmothecia on leaves coincided in 2014. Fruit held at high RH and temperature had higher levels of infection than fruit at any other temperature/humidity combination. To quantify these results, spore counts are currently being assessed but (in general) results are consistent with observations made in Objective 1.

Objective 3:

In all cases, conidia deposition on fruit led to disease at harvest. The earliest inoculation was coincident with the first observed onset of disease on leaves, around 25 DAFB.

Significant variations *(P<0.05)* were observed for mean disease incidence/severity and date among fruit naturally exposed to powdery mildew inoculum on cv. Sweetheart (Table 3a) and cv. Bing (Table 3b). Disease incidence and severity of fruit exposed to airborne conidia as early as 37 days after full bloom (pit hardening) was statistically similar to fruit exposed two weeks before harvest and the positive control. The application of Nitex mesh covers at fruit set (18 days after full bloom) prevented natural infection through airborne conidia throughout the growing season. No diseased fruit were found

in the negative control (=fruit always covered). In 2013 and 2014, an extensive latent period (=time from initial infection to disease onset) on fruit surfaces has been observed. *Podosphaera clandestina* conidia have the ability to survive for extended period of times (56 days in 2014, 40 days in 2013) on fruit surfaces before causing disease.

In 2014, no significant differences were observed for inoculated fruit trials. Still, all inoculated fruit displayed various degrees of disease at harvest. This indicates that successful inoculations remained viable for an extensive period of time.

Table 3a Disease incidence and severity on mature 'Sweetheart' fruit as a result of artificial inoculation or natural exposure to airborne conidia

Inoculation/Exposure date*	Mean Powdery mildew disease - 7/15/14							
	Inoculated				**Natural exposure**			
	Incidence (%)		Severity		Incidence (%)		Severity	
5/5/2014	n/a		n/a		10.1	AB	0.1	A
5/18/2014	22.2	AB*	0.3	AB	38.8	ABC	0.4	AB
6/2/2014	54.0	AB	1.0	AB	95.0	D	1.2	B
6/16/2014	33.2	AB	0.4	AB	76.4	CD	1.1	B
6/30/2014	22.4	AB	0.3	AB	62.7	ACD	0.9	AB
Negative control, Always covered	0.0	A	0.0	A	0.0	B	0.0	A
Positive control, Never covered	64.5	B	1.1	B	64.5	CD	1.1	B

Table 3b Disease incidence and severity on mature 'Bing' fruit as a result of inoculation or natural exposure to airborne conidia

Inoculation/Exposure date	Mean Powdery mildew disease - 7/15/14							
	Inoculated*				**Natural exposure****			
	Incidence (%)		Severity		Incidence (%)		Severity	
5/5/2014	n/a		n/a		19	AB	0.2	AB
5/18/2014	32	A*	0.7	A	41	AB	0.5	AB
6/2/2014	64	A	0.9	A	33	AB	0.4	AB
6/16/2014	31	A	0.4	A	66	AB	0.8	AB
6/30/2014	33	A	0.4	A	80	A	1.1	A
Negative control, Always covered	0	A	0.0	A	0	B	0.0	B
Positive control, Never covered	58	A	0.7	A	46	AB	0.7	AB

* Fruit were spray inoculated with 5000 spores/ml and covered with Nitex bags 30 minutes after treatment.

** Fruits were exposed to natural, airborne inoculum for two weeks and subsequently covered with Nitex bags.

***Results are averages of four 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*)

Objective 4:

2014 was a severe mildew year with 93.5% of leaves, and 44% of cherries infected at harvest on July 1st 2014. In contrast, in 2013 only 30% of leaves were infected in the untreated control. In 2014, Procure and Topguard were replaced with Fontelis and Pristine. Also, disease was evaluated on both mature fruit and leaves. No significant effect of fungicide application and disease incidence and severity on

leaves was found when compared to the untreated control (T7) or among each treatment (Table 4). In contrast to infection of leaves, a significant decrease in disease incidence and severity on mature fruit was achieved by Treatments 1, 3, 4, 10 and 11. Here fungicide application reduced disease incidence from 44% (T11) to 80% (T1) when compared to the untreated control.
Fruit quality analyses concluded that 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. Pitting susceptibility was not affected by the different fungicide treatments. After 2 weeks at 32°F, there were no differences in FF (increased ~20%), TA (reduced ~15%), fruit color, and stem browning among the fungicide treatments.

Objective 5:
Cherry flowers inoculated with powdery mildew conidia did abort quickly after inoculation. The fragile petals turned brown and died within two days post inoculation. Cherries that were covered with Nitex bags did not develop any disease symptoms. The cover was applied after shuck fall and no signs of fungal infection were visible on orchard leaves. The first onset of natural disease in the orchard happened around May 5, 2014 (25 DAFB). The absence of airborne conidia in combination with observations and results from the other objectives leads to the conclusion that the flowering stage is not connected to disease at harvest. However, we are still analyzing frozen cherry flowers for the presence of powdery mildew spores via DNA extraction using primers specific for cherry powdery mildew.