2014 NW Cherry Research Review
Confluence Technology Center, Wenatchee

Tuesday, November 12, 2013

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FINAL PROJECT REPORT

Project Title: SWD control

PI:	Peter W. Shearer, Ph.D.	**Co-PI**:	Preston H. Brown
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Cooperators: Orchard View Farms, Inc.
Various anonymous orchardists
Peter Landolt, Dong Cha. USDA-ARS, Wapato, WA

Total Project Request: Year 1: $23,201

Budget History:

Item	2013
Salaries	
Benefits	
Wages	19,500
Benefits	1,701
Equipment	
Supplies	900
Travel	1,100
Miscellaneous	
Total	**$23,201**

Footnotes:
3.25 hourly temp. help for 3 months: $12.50/hr
Other payroll expenses for hourly temp. help: 8.6% + $2.43/mo
Mileage: weekly travel to The Dalles and Hood River: $0.555/mile

OBJECTIVES

1. ***Determine efficacy of insecticides against spotted wing drosophila in large plot trials in sweet cherry.***
   This replicated study compared efficacy of Sevin, Delegate, and Warrior II / Danitol for SWD control in sweet cherries.
2. ***Evaluate intensive sampling and monitoring for SWD to predict risk of infestation.***
   This trapping and fruit monitoring study evaluated 3 attractants, including the new Cha-Landolt lure developed at the USDA-ARS lab in Wapato, and two traps for monitoring SWD in sweet cherry orchards in the Mid-Columbia.

SIGNIFICANT FINDINGS

Objective 1. Determine efficacy of insecticides against spotted wing drosophila in large plot trials in sweet cherry.

Results from a large plot replicated study of grower-applied insecticides demonstrated that Delegate (7 oz/A), Sevin XLR (3 qt/A) and the pyrethroids Warrior II and Danitol (2.56 oz and 11 oz/A, respectively) provide protection against SWD. This is the first large plot SWD efficacy study conducted in the Mid-Columbia and results indicate that high label rates of Sevin XLR will control SWD as will the other products and rates listed above.

Objective 2. Evaluate intensive sampling and monitoring for SWD to predict risk of infestation.

The four-component Cha-Landolt SWD attractant, developed at the USDA-ARS facility in Wapato, WA, was the most effective bait tested in 12 Mid-Columbia cherry orchards. Traps baited with this attractant captured more SWD than traps baited with either apple cider vinegar (ACV) or yeast+sugar+water (YSW). Traps baited with YSW caught more SWD than ACV baited traps.

Significantly more flies were captured in orchards at the earliest ripening location (Dallesport) than in orchards at other locations. SWD were captured in these earliest ripening orchards when the fruit was mostly green.

RESULTS AND DISCUSSION

Objective 1. Determine efficacy of insecticides against spotted wing drosophila in large plot trials in sweet cherry.

The purpose of this study was to assess efficacy of two individual products, Sevin XLR and Delegate, and a class of products, pyrethroids, which have different PHIs (Warrior II and Danitol). The products were applied 3 times to the study sites except Warrior II was applied twice followed by Danitol shortly before harvest. The intent was to determine if these products protect cherries from SWD infestation. Results from this study can be used to develop rotational use patterns based on other target insects and insecticide resistance programs. This study was conducted in 12 blocks of Sweetheart cherry located in Dufur, OR. Blocks were approximately 1-2 acres in size. Treatments were randomly assigned to 4 blocks per treatment. The grower applied the treatments 3 times with an airblast sprayer calibrated to deliver 100 GPA (Table 1).

We placed red cup traps baited with yeast+sugar+water in each of the blocks to monitor for SWD. Female SWD were detected in the study sites but there were no differences in abundance between treatments (Table 2).

Table 1. Insecticides applied to replicated large plots and application dates.

Material[1]	Rate/acre[2]	Application Dates		
Sevin XLR	3 qt	4 July	14 July	20 July
Delegate	7 oz	4 July	14 July	20 July
Warrior	2.56 oz	4 July	14 July	--
Danitol	11 oz	--	--	20 July

[1]All applications included Silwet at 2.56 oz/A.
[2]Applied in 100 gpa.

Table 2. Average number of female SWD captured per week in yeast-baited traps.

	Average (±SEM) number of adult female SWD per trap[1]		
	Date		
Treatment	16 July	22 July	29 July
Sevin XLR	0.5±0.3ns[2]	1.8±0.3ns	0.8±0.5ns
Delegate	0.3±0.3	1.3±0.8	2.0±0.0
Warrior/Danitol	0.5±0.3	1.0±1.0	1.0±0.7

[1]Pre-treatment levels of SWD were monitored using two traps baited with yeast, sugar and water, 1June-8 July. No SWD were detected during that period.
[2]ns = means within a column are not significantly different.

Two fruit samples were collected to assess SWD infestation and fruit color. Four hundred fruit were collected from each of the 4 blocks per treatment (20 fruit from 20 trees in each of 4 blocks per treatment, n=1600 fruit per treatment per sample date). Thirty fruit per plot (n=120 fruit per treatment) were assessed for fruit color using the CTIFL color scale. All sampled fruit had color ranging from pink to various stages of red indicating the fruit was susceptible to attack by SWD (Fig. 1). Fifty fruit per plot (n=200/treatment) were examined for SWD eggs under a stereo-microscope. No eggs or oviposition sites were observed in any of the sampled fruit (Table 3). All fruit was then returned to its lot, aged for 5 days and then run through a cherry crusher on a block-by-block basis. Crushed fruit was then placed in brown sugar + water (15 brix) to asses for internal larvae. No SWD or Western cherry fruit fly larvae were found (Table 4).

Table 3. Number of SWD eggs observed in fruit, n=200 fruit per treatment per date.

	Average (±SEM) number of SWD eggs observed per fruit	
	Date	
Treatment	16 July	22 July
Sevin XLR	0.0±0.0	0.0±0.0
Delegate	0.0±0.0	0.0±0.0
Warrior/Danitol	0.0±0.0	0.0±0.0

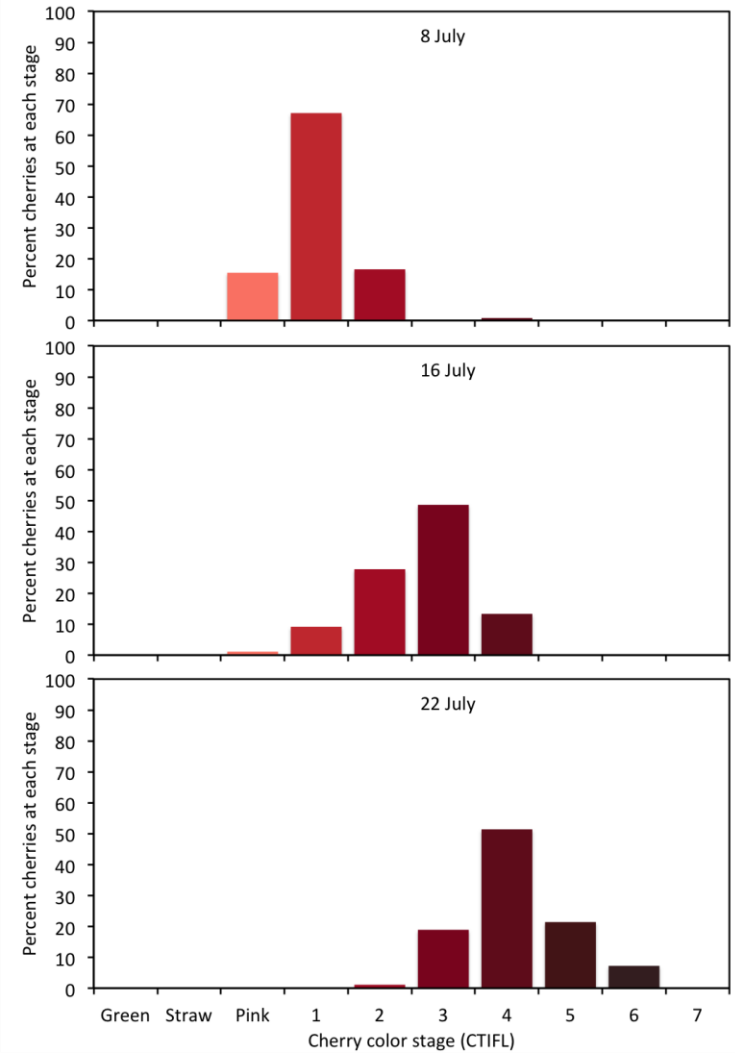

Figure 1. Stage distribution of Sweetheart fruit color during the study period.

Table 4. Number of SWD larvae recovered from fruit, n=1600 fruit per treatment per date.

	Average (±SEM) number of SWD larvae observed per fruit	
	Date	
Treatment	16 July	22 July
Sevin XLR	0.0±0.0	0.0±0.0
Delegate	0.0±0.0	0.0±0.0
Warrior/Danitol	0.0±0.0	0.0±0.0

The information from this study demonstrates that these insecticides are effective against SWD when applied at the rates provided above. It is important for PCAs and growers to understand that sequential applications were made so the products could be assessed individually without the confusing aspects associated with testing programs that contain multiple insecticides. Sequential applications of the same insecticide or class of insecticide can lead to faster development of insecticide resistance. Sequential applications of Sevin XRL caused leaf phytotoxicity that became

more apparent with each application, thus, this product should be used with care if applied more than once per season.

Objective 2. Evaluate intensive sampling and monitoring for SWD to predict risk of infestation. This study was conducted in 12 sweet cherry orchards (three orchards in each of four locations in the Mid-Columbia district). Orchards were located in Dallesport, WA, The Dalles, Hood River and Parkdale, OR. At each site, 6 commercially available yellow Trappit dome traps and 6-16 oz red cups with lids (Solo) traps with 2-0.4 X 2" screen-covered entrances cut into the sides of the cups near the top (Fig. 2) were deployed, half in the border cherry row along the western edge, the other half in the interior of the orchard. The red Solo cup trap was chosen for this study because it was a superior trap when compared with the clear deli trap in previous studies. The Trappit dome trap was used per Dr. Peter Landolt request. This will allow him to compare these results with his previous efforts.

Traps were baited with one of the following attractants: apple cider vinegar (ACV), yeast+sugar+water (YSW), or the experimental Cha-Landolt (CHA) 4-component lure (USDA-ARS, Wapato, WA). There were two bait X trap combinations per site with one of each combination placed in the border row and interior of the orchard, respectively. The ACV, YSW and liquid component of the CHA lure were changed weekly. The remaining two components of the CHA lure were changed every two weeks.

Figure 2. Example of red cup (left) and Trappit dome traps (right) used in study.

Fruit samples were collected weekly to assess SWD infestation and fruit color. Fruit were collected from each orchard (20 fruit from 10 border and 10 interior trees per orchard, n=400 fruit per sample date). Thirty fruit per plot (n=120 fruit per treatment) were assessed for fruit color using the CTIFL color scale. All fruit was then returned to its lot, aged for 5 days and then run through a cherry crusher on a block-by-block basis. Crushed fruit was then placed in brown sugar + water (15 brix) to asses for internal larvae. One SWD larvae was found during the last sample date in a Parkdale orchard. Sampling fruit and traps continued until a particular block was harvested.

Traps were placed in orchards when the majority of the fruit were green and/or straw colored. When the seasonal abundance of female SWD captured was averaged within a location and then compared with the other locations, more flies were captured in Dallesport, WA orchards than any other location while the fewest flies were captured in the later ripening areas of The Dalles (Fig. 3). This was this was opposite of what we saw in previous years. Usually we capture low levels of flies early and more flies later in the season.

The Cha-Landolt lure captured more adult SWD than the apple cider vinegar or yeast+sugar+water baited traps (Fig. 4) (Table 5).

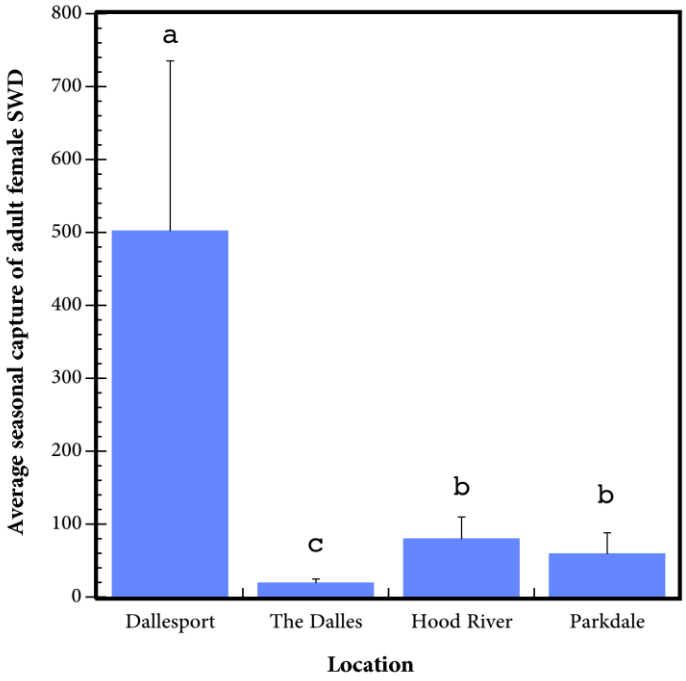

Figure 3. Seasonal abundance of SWD captured in baited traps through harvest. Three orchards were sampled in each location. (ANOVA, Tukey, $F_{3, 63} = 42.7$, $P<0.0001$).

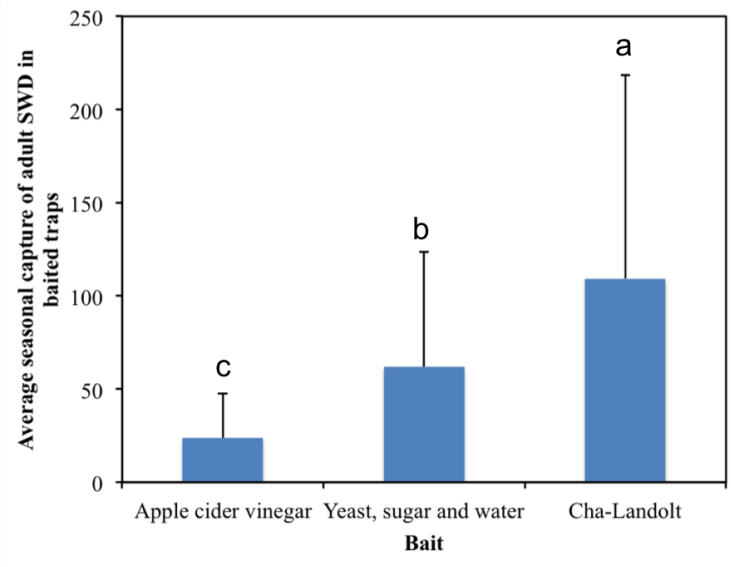

Figure 4. Average seasonal capture of adult SWD in baited traps from 12 Mid-Columbia sweet cherry orchards. (ANOVA, Tukey, $F_{2, 63} = 22.81$, $P<0.0001$).

Table 5. Effect of attractant on average seasonal capture of adult female SWD in baited traps within a location.

	Average (±SEM) seasonal capture of adult female SWD in baited traps		
	Attractant		
Location[1]	Apple cider vinegar (ACV)	Yeast, sugar and water (YSW)	Cha-Landolt (CHA)
Dallesport, WA	62.3±40.4	158.0±78.1	282.7±114.9
The Dalles, OR	2.7±0.3	6.0±1.7	11.0±3.6
Hood River, OR	10.3±5.9	32.3±6.8	38.0±16.8
Parkdale, OR	7.0±2.3	18.7±9.2	34.3±17.3

[1]n=3 orchards per location.

The Trappit dome trap captured more SWD than the red Solo trap (Fig. 5) (Table 6). The price of the Trappit dome trap was about $9 but it is durable and reusable. The red cup trap was hand-made, sometimes broke and occasional dried out.

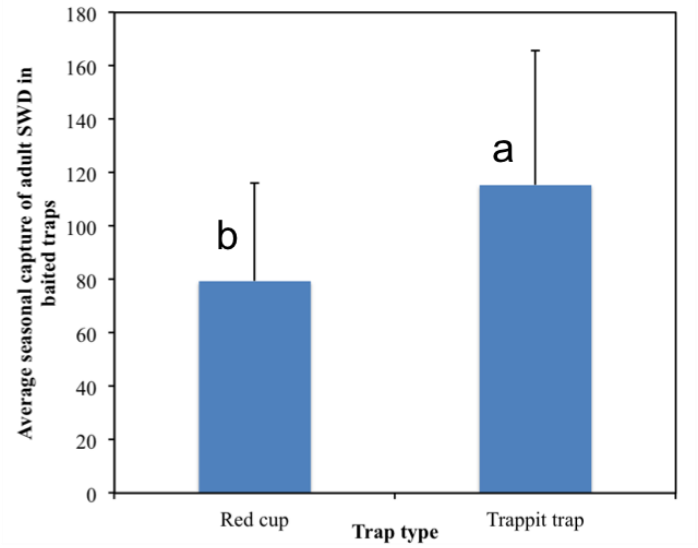

Figure 3. Average seasonal capture of SWD in the two trap types. (ANOVA, Tukey, $F_{1, 63} = 6.09$, $P=0.016$).

Table 6. Effect of trap type on average seasonal capture of adult female SWD within a location.

	Average (±SEM) seasonal capture of adult female SWD in baited traps	
	Trap Type	
Location[1]	Red cup	Trappit dome trap
Dallesport, WA	208.0±101.4	295.0±131.2
The Dalles, OR	8.3±3.5	11.3±1.8
Hood River, OR	35.3±14.9	45.3±14.2
Parkdale, OR	21.0±11.0	39.0±17.5

[1]n=3 orchards per location.

EXECUTIVE SUMMARY

Project Title: SWD Control

A large plot replicated efficacy study conducted in a commercial orchard demonstrated that Sevin XLR, Delegate and the pyrethroids Warrior II and Danitol protected fruit from SWD infestation despite SWD being present in this orchard.

A SWD attractant developed in Dr. Peter Landolt's lab with funds from the WTFRC was tested as a SWD lure in 12 Mid-Columbia cherry orchards. It was very effective in capturing adult SWD in traps when compared with two other standard baits, apple cider vinegar and yeast+sugar+water.

Yeast+sugar+water was more effective than apple cider vinegar in attracting adult SWD to traps. However, it is not pleasant to work with.

A commercially purchased dome trap (Trappit dome trap, Great Lakes IPM, Vestaburg, MI) captured more SWD than a hand-made red cup trap.

Considerably more adult SWD were captured during the growing season in Dallesport, the earliest ripening cherry district in the Mid-Columbia.

FINAL PROJECT REPORT

Project Title: Improving fruit set in Regina

PI:	Lynn Long.	**Co-PI**:	Matt Whiting
Organization:	OSU - Wasco County Cooperative Extension	**Organization**:	WSU
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Email:	lynn.long@oregonstate.edu	**Email**:	mdwhiting@wsu.edu

Total Project Request: Year 1: $8,000

Budget History: No budget itemization given

Note: This project was funded out of cycle through OSCC only.

Information submitted:
Nice results this year at the highest rate of 1.5 pouches per acre - increasing fruit set of Tieton by about 50% and Regina by about 63%.

Tieton 2 locations, 10% FB timing

Treatment	Fruit set
0.5AVG	10.68B
1AVG	16.28B
1.5AVG	23.92A
Control	15.79B

Regina 2 locations, 10% FB timing

Treatment	Fruit set
0.5AVG	24.73AB
1AVG	21.63B
1.5AVG	29.01A
Control	17.84B

FINAL PROJECT REPORT

Project Title: Investigating post-bloom thinning

PI: Matthew Whiting
Organization: WSU-IAREC
Telephone: 5097869260
Email: mdwhiting@wsu.edu
Address: 24106 N. Bunn Road
City/State/Zip: Prosser, WA 99350

Cooperators: Bryan Peebles, Harold Schell, Chelan Fresh; Allyson Leonhard and Lu Zhang, Washington State University

Total Project Request: **Year 1:** $48,483 **Year 2:** $50,144

Other funding sources: None

Budget 1
Organization Name: WSU **Contract Administrator: Mary Lou Bricker**
Telephone: 5093357667 **Email address: mdesros@wsu.edu**

Item	2012	2013	
Salaries	28,732	29,954	
Benefits	5,420	5,653	
Wages	9,198	9,386	
Benefits	883	901	
Equipment			
Supplies			
Travel	2,000	2,000	
Miscellaneous[1]	2,250	2,250	
Total	48,483	50,144	

Footnotes: Salaries are for ¼ time technician, Allyson Leonhard and for Ph.D. student Lu Zhang. Wages are for temporary timeslip assistance at $10/hour. Travel is for transport to field plots with collaborators. [1]For orchard maintenance at WSU-Roza farm ($4,500/acre @ 0.5 acre).

OBJECTIVE: To develop pragmatic, cost-effective post-bloom thinning strategies

SIGNIFICANT FINDINGS:

- Ethephon applications are effective at reducing fruit set in sweet cherry post-bloom (as great as 90% reduction)
- Thinning efficacy is largely rate-dependent
- Timing of application is important – greater thinning efficacy was observed with earlier applications
- Fruit quality improvements were inconsistent, irrespective of quality parameter
- Fruit soluble solids were improved consistently from thinning – size was not always improved, despite significant reductions in crop load
- There was no relationship between fruit set and fruit quality parameters

METHODS

The need for post-bloom thinning tools is clear – one cannot assess fruit set until well after flowering. Currently, the only reliable means of post-bloom thinning in sweet cherry is manual fruit removal, an expensive operation. We propose to develop a post-bloom thinning strategy focusing on Ethephon because it showed promise in our previous work on 'Sweetheart', and 'Rainier'. Ethephon will be compared to hand thinning. There are two key elements that need to be determined – the best time for application and the rate-response.

I – TIMING OF APPLICATION

Treatments:

- unthinned control (water sprayed)
- hand thinning to about 30 fruit per foot
- Ethephon at 200 ppm

Timing of application:

- shuck fall
- shuck fall + 1 week
- shuck fall + 2 weeks
- shuck fall + 3 weeks

Methods:

Applications will be made using a pressurized spray gun or commercial airblast sprayer to 'Sweetheart', 'Rainier', and 'Skeena' trees that exhibit heavy fruit set. Two experiments will be conducted for each cultivar – one in a commercial orchard and one at the WSU-Roza experimental orchards. In addition, we will work opportunistically with additional growers interested in evaluating post-bloom thinning strategies by providing suggestions for protocols and helping with data collection on efficacy. On each application date, treatments will be made to entire trees, with 6 whole-tree replications. Hand thinning will be accomplished by manually removing fruit from throughout entire trees with a goal of leaving ca. 30 fruit per foot (preliminary work shows this is a reasonable target to balance fruit number with quality). Depending on the orchard, we will use either a completely randomized design or a randomized complete block design, with at least 2 border trees between adjacent treatments. We will require 96 trees in each orchard (4 treatments x 4 timings x 6 reps). Key environmental conditions (e.g., wind speed, temperature, humidity) during and following application will be monitored using AgWeatherNet stations in the vicinity.

Within a day of application, we will flag two limbs in every tree and count fruitlet density (fruitlets/limb cross-sectional area and length), measuring limb caliper as well. In addition, we will

measure fruit diameter on 30 fruit per limb to record fruitlet size at the time of treatment – this will facilitate comparisons among cultivars with respect to timing). We will record the time required to hand thin and 'rake' thin each replicate tree. In addition, we will collect thinned fruit and measure fruit size and weight to see whether the population of thinned fruitlets differs significantly from the remaining unthinned fruitlets. A photo journal will be collected as well to visually document application timings and crop densities. At commercial fruit maturity we will make fruit counts to the same limbs and assess thinning efficacy as % fruitlet removal. Fruit subsamples (minimum 100 fruit per replication) will be collected and analyzed for quality attributes including color, weight, diameter, firmness, and surface damage.

Scope of work:
3 cultivars (Rainier, Skeena, Sweetheart)
2 sites for each cultivar (1 commercial orchard + WSU Roza farm)
16 'treatments' (4 timings and 4 treatments)
6 replicates

II – RATE OF ETHEPHON

Treatments:

- unthinned control (water sprayed)
- Ethephon at 100 ppm
- Ethephon at 200 ppm
- Ethephon at 300 ppm

Methods:

These experiments will be conducted as described above with respect to applications, experimental design, data collection, and analyses. Again, we will make applications to Rainier, Skeena, and Sweetheart in 2 locations (a commercial orchard + the WSU Roza farm), identifying commercial orchards once fruit density can be determined. The treatments will be made at shuck fall + 1 week by pressurized spray gun or commercial airblast sprayer. We will require 24 trees for these experiments (4 treatments x 6 reps).

In the second year, we will repeat post-bloom thinning experiments and generate outreach material describing the results from our post-bloom thinning trials. These may include videos (describing benefits of post-bloom thinning), presentations at winter meetings, and written reports for the Good Fruit Grower.

RESULTS

Fruit set

In 2013 we conducted 5 distinct thinning trials, 4 with commercial growers and 1 at the WSU-Roza farm. In 2013 we included abscisic acid (ABA) in addition to the Ethephon treatments from 2012. The following will highlight the results from 3 of those trials – they are representative of the overall response.

In a 'Sweetheart' trial in the Yakima valley natural fruit set was about 80% of available flowers and fruit density was about 35 fruit/foot. Average fruit weight from untreated control limbs was 8.8 g (about 10.5 row). Ethephon treatment reduced fruit set proportional to rate, but only at the earliest application timing (FIG 1). Ethephon applied on the 6th of May (i.e., shortly after shuck fall, 11.9 mm mean fruit diameter) reduced fruit set by 4, 19, and 73% compared to the control at 100, 200, and 300 ppm, respectively. ABA was generally ineffective as a post-bloom thinner in this trial; in fact, later applications of ABA at 500 ppm improved fruit set by roughly 10-14%. Similarly, later

applications of Ethephon were ineffective as thinners and, in some cases, increased fruit set by up to 17% (Ethephon at 300 ppm applied 20 May).

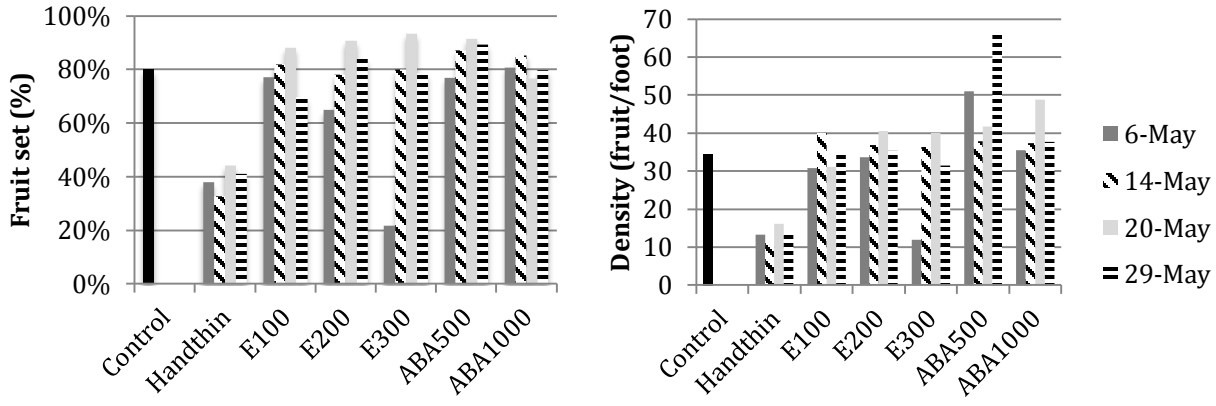

Figure 1. Thinning efficacy of Ethephon or ABA applied to 'Sweetheart'.

Hand-thinning treatments consistently reduced crop load, to about 12 fruit/foot. These thinning treatments did not improve fruit weight/size however (FIG). This suggests that fruit were not source-limited during growth and development; therefore, thinning was unnecessary. Fruit size from the first applications of Ethephon on 6 May (i.e., those treatments that did provide thinning) was improved 6% by 100 ppm, unaffected by 200 ppm, and reduced 25% by 300 ppm despite that treatment reducing fruit set 73%. Interestingly, similar to results in 2012 with different cultivars, several Ethephon treatments improved fruit quality without providing any thinning. The greatest improvements to fruit size/weight were from 100 ppm Ethephon at the first and second timings (+6 and 11%, respectively) and 300 ppm Ethephon on the second application date (+7%).

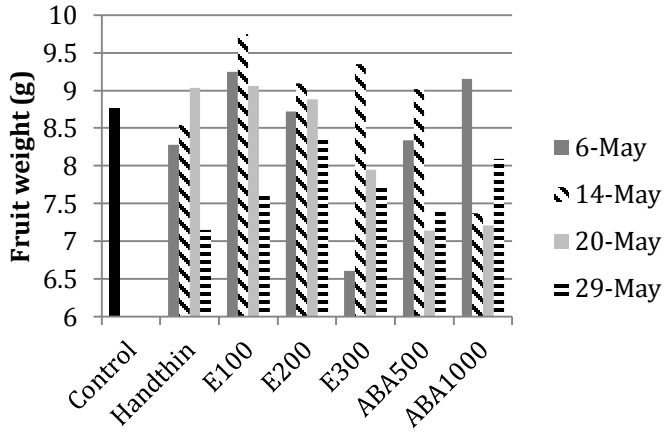

Figure 2. Fruit weight of 'Sweetheart' following application of thinners.

In a 'Lapins' trial in 2013 natural fruit set was high, about 90%, and fruit density was 46 per foot. Average fruit weight from untreated, control limbs was about 9.2 g (peaking on 10 row). Ethephon treatment at 100 ppm was ineffective as a thinner, average fruit set across all four application timings was about 91%. Ethephon at 200 ppm was effective for thinning but only on the first two application dates (5 and 14 May); later applications did not affect fruit set. Fruit set was reduced by Ethephon at 200 ppm to 45% on both the first two application dates. At 300 ppm Ethephon was an effective post-bloom thinner only on the first application timing when this treatment reduced fruit set to 20% (i.e., about 20% of control). Later applications of Ethephon at 300 ppm did not effectively thin fruit. ABA at 1000 ppm reduced fruit set by about 29% compared to unthinned control on the earliest timing, but was ineffective with later applications. ABA at 500 ppm was not an effective thinning agent at any timing. Ethephon applied at 100 ppm on the later two timings improved fruit set by 6%.

Fruit density was reduced by hand-thinning treatments consistently, to about 42% of the unthinned limbs (46 fruit/foot vs. 20 fruit/foot). The hand-thinning improved fruit size/weight by about 25%. Fruit weight was 11.5 g from all hand-thinned timings combined compared to 9.2 g in unthinned limbs (FIG). Interestingly fruit size/weight was improved by nearly every treatment,

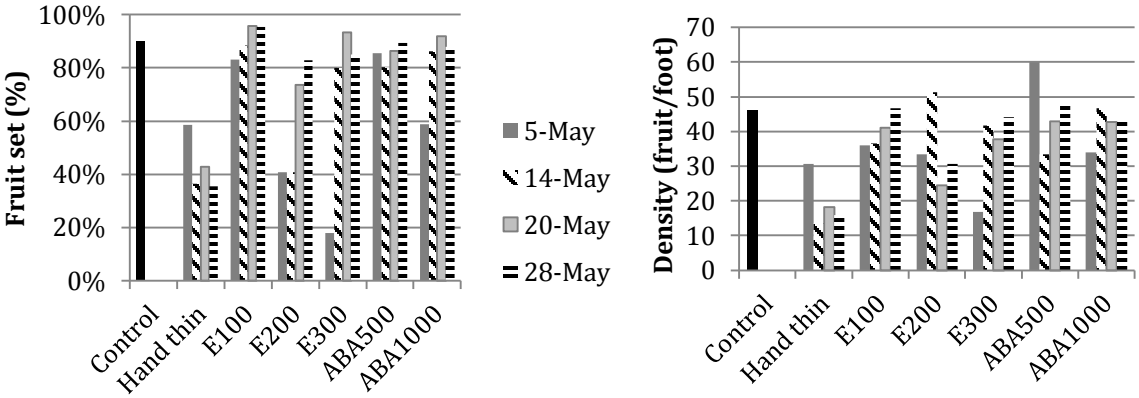

Figure 3. Thinning efficacy of Ethephon or ABA applied to 'Lapins'.

despite the inability of most treatments to thin the fruit. The greatest improvements in fruit weight were in response to Ethephon at 100 ppm applied on the first two dates – these treatments led to improvements in fruit weight of 30 and 33%, respectively. This is similar to previous results from 2012 in which improvements in fruit quality were not associated with reductions in fruit set. The lack of relationship between fruit density and fruit weight suggests across all treatments and timings suggests that the PGR treatments are altering limb/tree source-sink relationships.

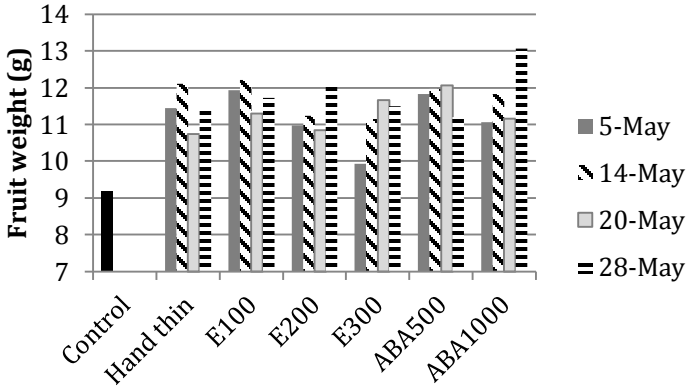

Figure 4. Fruit weight of 'Lapins' following application of thinners.

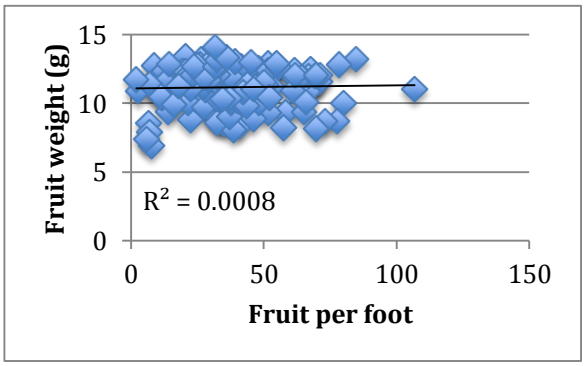

Figure 5. Relationship between fruit density (fruit/ft) and individual fruit weight in 'Lapins'.

Executive Summary

Ethephon showed potential to thin fruit after bloom. The thinning response was proportional to rate and earlier applications were more effective than late applications. ABA showed little potential as a post-bloom thinning agent for sweet cherry in the application timings we studied. We documented an interesting disconnect between thinning and fruit quality improvements – treatments that reduced fruit density did not always improve fruit quality, and, in many cases, Ethephon treatments of 100 or 200 ppm improved fruit quality without reducing fruit density. This is deserving of further study. It is recommended to conduct further trials with Ethephon at 100-200 ppm within 2 weeks after shuck fall.

Abbreviated summary of results from 2012:

Ethephon applications reduced fruit set significantly in every cultivar tested (data not shown). In Skeena, fruit set in untreated control was ca. 66%. Hand thinning treatments reduced final fruit by about half (fruit set = 31% overall), irrespective of timing of thinning (Figure 1). In comparison, mean fruit set across all timings was 68%, 50%, and 33% in response to treatment with 100 ppm, 200 ppm, and 300 ppm Ethephon, respectively. Therefore, 100 ppm was ineffective, and 300 ppm closely matched the hand thinning targets. Timing of Ethephon application was important – thinning efficacy was greatest on the first application and declined with each of the next two application dates (Figure 2). Expressed as a % of control fruit set, 300 ppm was effective at thinning on each application date, whereas 100 ppm was effective only on the first application date, and 200 ppm was effective only on the first two application dates (Fig. 2). These results suggest that there is a positive relationship between Ethephon rate and thinning efficacy, and that at higher rates, efficacy is greatest at early stages of fruit development.

In Sweetheart, fruit set of untreated limbs was similar to Skeena at about 66% (Figure 3). Hand thinning treatments reduced fruit set by about 65%, to 27% across all timings. In comparison, mean fruit set across all timings was 73%, 59%, and 34% in response to treatment with 100 ppm, 200 ppm, and 300 ppm Ethephon, respectively (each very similar to final fruit set in Skeena). Therefore, 100 ppm was ineffective, and 300 ppm most closely matched the hand thinning targets. Timing of Ethephon application was important – thinning efficacy was greatest on the first application and declined with each of the next two application dates (Figure 2). Expressed as a % of control fruit set, 300 ppm was effective at thinning on the first three application dates, whereas 100 ppm was effective only on the first application date, and 200 ppm was effective only on the first two application dates (Fig. 4). These results suggest support our conclusion with Skeena that there is a positive relationship between Ethephon rate and thinning efficacy, and that at higher rates, efficacy is greatest at early stages of fruit development. Interestingly, Ethephon applied at 100 ppm and 200 ppm on the later application dates led to subtle improvements in final fruit set, with both treatments yielding about 40% more fruit than untreated control when applied on 8-June.

FINAL PROJECT REPORT

Project Title: Support for a full time technician

PI: Nnadozie Oraguzie
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Address 2: Dept of Horticulture
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State/Zip: WA, 99350

Cooperators: Dena Ybarra, Dave Allan, Jeff Cleveringa, Ines Hanrahan, Tom Auvil, Todd Einhorn, Lynn Long, Amy Iezzoni, Fred Bliss

Other funding sources

Agency Name: USDA-CSREES Specialty Crops Research Initiative
Amt. requested/awarded: $3.4M plus equal matching Sept 2009-Aug 2013
Notes: A total systems approach to developing stem-free sweet cherry production, processing, and marketing system. PI: Whiting. Co-PI includes Oraguzie

Agency Name: USDA-CSREES Specialty Crops Research Initiative
Amt. requested/awarded: $2.1M plus equal matching Sept 2009-Aug 2013
Notes: Tfr-GDR: Tree fruit genome resource database with Dorrie Main as PI and Oraguzie as Co-PIs.

Agency Name: USDA-CSREES Specialty Crops Research Initiative
Amount awarded: $7.2 mil plus equal matching, Sep 2009 – Aug 2013
Notes: "RosBREED: Enabling marker-assisted breeding in Rosaceae". PI: Iezzoni. Co-PI includes Oraguzie

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

Total Project Funding: $58,648. However, this project was funded in 2013 at $28,749 but in 2014 it will merge with the Breeding project, CH-12-107, according to WTFRC board's recommendation and the second year's funds will be rolled over into the Breeding project.

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

Item	2013		
Salaries	18,633		
Benefits	10,062		
Wages			
Benefits			
Equipment			
Supplies			
Travel	54		
Plot Fees			
Miscellaneous			
Total	**28,749**		

Justification
One of the objectives of the WSU sweet cherry scion breeding project funded by the Washington Tree Fruit Research Commission and the Oregon Sweet Cherry Commission (WTFRC # CH-12-107) was to establish and implement a written protocol for best nursery and field management that will ensure optimal tree growth for trait selection. The breeding program has two phases, P1 and P2, while Phase 3 will be operational in two years with the planting of a fast-tracked advanced selection, FR001T007, in grower co-operator trials. Currently, there are two technicians in the program assisting the breeder in the day-to-day management of breeding operations. One FTE (Seyed Chavosi) is funded through WSU-ARC while the other (Sue Watkins) is funded from the sweet cherry breeding project, WTFRC # CH-12-107 (0.5 FTE) and another project 'Support for a full time technician,' WTFRC # CH-10-110 (0.5 FTE). WTFRC # CH-10-110 comes to an end at the end of March, 2013. This proposal seeks funds to support the 0.5 FTE that will expire next year to provide continuity in the program. The overall goal of this position is to ensure efficient horticultural manipulation of trees in the lath-house and field, field plot management, coordination of fruit sampling during harvest and tree planting and propagation, as well as bar coding to maintain tree identity in the field.

Objectives:

- To acquire support for a full time technician to ensure that healthy, vigorous seedlings of adequate size and precocity are produced and managed in all phases of the breeding program using best horticultural practices.

SIGNIFICANT FINDINGS AND ACCOMPLISHMENTS

- The PNWSCBP personnel are now in charge of tree pruning and training in the program with guidance from key BPAC members. The Central leader system is now the architecture of choice and older plantings in Phase 2 have been pruned and trained to conform to this architecture.

- Pruning and training procedures and improvements on seed germination, seedling establishment in the green house and field, cultural practices, in particular, promotion of early flowering and fruit sampling and evaluation techniques have been integrated in the 'Best Management Practice' document.

- Every tree in the Breeding blocks have been mapped and assigned a unique bar-code to facilitate identification. Harvested fruit samples are also barcoded and lab fruit analysis is automated to minimize error in data recording and analyses.

- In line with our policy of renovation and recycling of orchard blocks, we have pulled out over 1000 trees from the seedling blocks as well as 7 flawed advanced selections from Phase 2 blocks. New seedling plantings are located in warmer blocks to minimize the impact of frost damage on flowering.

- The Breeding personnel constructed 30 tree cages for bird control and also installed a Bird Gard unit that emits distress calls for 8 different bird species. This is additional to bird netting that already exists in the seedling blocks.

- Approximately 2000 trees on Gisela 6® including breeding parents, other cultivars and F_1 progenies were planted in the mother blocks this spring. These trees have been carefully

chosen for traits of interest for use in controlled pollination and for genetic studies. This will ensure that we achieve the target seed number for different market classes following controlled crosses. Flowering is often erratic on own rooted trees and pollination usually results in poor fruit set.

- The Breeding Team hired 12 temporary assistants in spring months and 14 in summer to assist with the enormous task of hand-pollinations and fruit picking/evaluation, respectively.

- Three key industry members including Dena Ybarra, Dave Allan and Jeff Cleveringa worked with the PNWSCBP leader once a week for 2 months, taste-testing fruit from the seedling block and the advanced selection block. This industry input was key to identifying a total of 16 seedlings for advancement to Phase 2 in 2012 and 2013, one early advanced selection belonging to the ESM class to fast-track to Phase 3 in 2012, and 10 flawed advanced selection to pull out of Phase 2 in 2012 and 2013.

RESULTS AND DISCUSSION

a. Tree pruning and training

The Breeding staff had several meetings with Dave Allan, Dena Ybarra, Jeff Cleveringa, Lynn Long, Matt Whiting and Tom Auvil in the winter, of 2012/13, and with Dave Allan, Dena Ybarra, Jeff Cleveringa and some Oregon BPAC members, in the summer of 2013, to decide on a uniform, standardized pruning and training system which will align with the goals and vision of the PNWSCBP, especially with regards to Phase 2 of the program. In addition, the system must be simple, maximize early yields and easy to be communicated to anyone involved in the breeding program, including orchard workers. The consensus was to adopt a '*central leader*' architecture going forward with new plantings and to re-direct existing trees to conform to this architecture even if it means losing a fruiting year. The pruning workshop in the December of 2012 in Oregon organized by Lynn Long was also very helpful in taking on the pruning responsibility by the Breeding staff. We performed summer pruning after harvest to ensure that trees have time to harden off for winter. This procedure opens up a tight canopy to allow fruiting buds to intercept more light. The 'sucker woods' removed should also provide more light to the lower branches and will help to improve fruit set the following year. Summer pruning also helps to reduce the risk of bacterial canker infection. The plan in the coming years is to manage the existing large trees in the seedling block (rows 1-55) suffering from overzealous pruning in the previous years to save as much fruit-bearing wood as possible while opening up the canopy to allow light to penetrate to lower branches. Removal of vigorous growth and blind-wood and leaving behind weaker branches will encourage fruit production in the next year.

The written protocol for tree pruning and training has been incorporated in the updated 'Best Practice Management' handbook which you can obtain from the PNWSCBP staff.

b. Renovation of Seedling Blocks

Approximately 500 trees in the seedling block deemed of no value to the breeding program were removed in November 2012 by Trepanier Excavating, Inc. of Yakima. Selected trees were removed by bull-dozer, leaving valuable trees intact. An additional 400 trees were removed in August 2013 by chainsaw. Stumps were left in place and painted to discourage vegetative growth from roots. This exercise leaves behind only the mother trees of advanced selections already in Phase 2 as well as trees used for genetics/genomics studies out of 1300 trees originally planted in 2006/7/. In 2014, many trees planted in 2008 will be pulled out following fruit evaluations in summer.

c. Implementation of Sample Tracking System

Each tree in the seedling blocks is tagged with a unique plastic bar-code ribbon identifying the location of the tree. During harvest, the barcode is scanned and reproduced as a stick-on label which is fixed to the collection bag. Upon entering the fruit evaluation laboratory, each sample label is immediately logged into a universal data collection file via bar code scan, to provide a record of collection which includes the tree location and date. A reproduced label accompanies each individual fruit sample as it rotates through the evaluation stations, minimizing and/or eliminating hand-written errors. Evaluation results are entered into the data collection file by electronically scanning the unique label, which automatically locates the correct line (sample location/collection date) for data entry, eliminating mis-match errors.

d. Collection of Leaf Samples for Marker Assisted Seedling Selection

Leaf samples are collected from nascent seedlings still in the yellow cone-tainers in the growth room. Earlier collection and analysis limits transplantation of seedlings to those showing genetic potential. Seedlings are identified only by parentage until leaf samples are taken, at which time each sampled individual is collared with a barcoded tag identifying year of seed harvest, seed parents, stratification bag number (for cross reference, lending additional assurance for accurate heritage assignment) and individual seedling number. The barcodes are scanned directly into a spreadsheet matrix duplicating the configuration of the leaf collection plates so no handwritten collection sheets are generated. Spreadsheets for each collection plate are then sent in electronic format to the lab performing the DNA tests, again eliminating hand-written error.

e. Greenhouse Seedling Transplanting

Seedlings showing genetic promise are transplanted to smaller pots (2 gallons) at transplant time, saving money on potting soil, labor and greenhouse space. In the past, all seedlings (both favorable and inferior determined by genetic tests) were transplanted into 5 or 10 gallon pots before genetic tests are performed

f. Bird Control

In addition to the bird netting in the seedling block (Phase 1), individual trees particularly, early ripening genotypes, in both phases of the breeding program were enclosed in portable bird cages constructed by the Breeding staff. These cages were moved around and placed over mid-season or late genotypes as the season progressed to reduce the risk of bird damage. Further, a Bird-gard unit emitting eight different distress calls was mounted at the seedling block for additional control.

EXECUTIVE SUMMMARY

Renovation and recycling of seedling blocks are ongoing in the PNWSCBP. To date, we have pulled out ~1000 trees and plan to remove more in future. Because the current location of the seedling block is too cold and prone to frost damage, new seedling plantings have been re-located to warmer blocks to minimize the impact of frost damage during hand pollinations. We are also exploring alternative pollination strategies in combination with frost control measures to enhance pollination and fruit set. In addition, the use of propagated trees for controlled hybridization is poised to boost flower numbers and fruit set. New tree plantings in Phases 1 and 2 of the program conform to the central leader architecture adopted this year while trees planted previously were pruned and trained to adapt to this architecture. Summer pruning was introduced in the seedling blocks to open up the canopy of large trees for more light interception and to reduce the risk of bacterial canker. Unique bar codes have been assigned to each tree and bar-coding was also used to track fruit from the field to the lab to minimize error in data recording. Marker assisted breeding (MAB) has been used routinely since 2010 for parent selection, to establish genetic identity, re-assign/confirm parentage and to cull inferior seedlings before field planting. Genetic tests and culling of inferior seedlings are often performed prior to field planting. However, this was carried out this year while seedlings were ~2 months old in the growth room resulting in more cost effective seedling development prior to field planting. We are using a multi-pronged approach combining bird netting and a device that emits bird distress calls for bird control. We appreciate the partnership between PNWSCBP and the industry and hope that this will continue to ensure that new high quality cultivars with high consumer appeal are released in an efficient and timely manner to provide competitive advantage to the PNW industries.

FINAL PROJECT REPORT

Project Title: Establishment and testing of MSU sweet cherry rootstocks

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City:	East Lansing	**City**:	Prosser
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Co-PI(3):	James Susaimuthu	**Co-PD(4):**	Tom Auvil
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Address 2:	24106 N. Bunn Rd	**Address 2:**	
City:	Prosser	**City:**	Wenatchee
State/Zip:	WA 99350	**State/Zip:**	WA 98801

Other funding sources: None

Total Project Funding: $114,302

Budget History:

WTFRC

Item	2011	2012	2013
Salaries[1]	$9,000	$9,270	$9,550
Benefits[1]	$2,880	$2,966	$3,056
Crew Wages & Benefits[1]	$1,022	$1,533	$2,555
Equipment			
Supplies			
Travel			
Plot Fees			
Miscellaneous			
Total	$ 12,902	$ 13,769	$ 15,161

Footnotes:

[1]This represents an allocation of time of WTFRC salaried and hourly employees to help with the activities associated with the test plots in Wash. and Ore.

Budget 1: Amy Iezzoni
Organization Name: Mich. State Univ. **Contract Administrator:** Lorri Busick
Telephone: (517) 355-5191 x 1363 **Email address:** busick@msu.edu

Item	2011	2012	2013
Salaries[1]	$5,650	$5,820	$5,995
Benefits[1]	$2,395	$2,506	$2,622
Wages[2]	$500	$500	$500
Benefits[2]	$38	$38	$38
Equipment			
Supplies	$500	$500	$500
Travel	$1,000	$1,000	$1,000
Misc.			
Plot cost	$1,000	$1,000	$1,000
Total	$11,083	$11,364	**$11,655**

Footnotes:
[1]Partial salary support for project technician Audrey Sebolt (fringe rates 42.38% 2001, 43.05% 2012, 43.73% 2013).
[2]Funding for an undergraduate student helper (fringe rate 7.65%).

Budget 2: James Susaimuthu
Organization Name: National Clean Plant Network **Contract Administrator:** James Susaimuthu
Telephone: (509) 786-9251 **Email address:** james.susaimuthu@wsu.edu

Item	2011	2012	2013
Virus testing	$ 10,800	$ 0	$ 0
Total	$ 10,800	$ 0	$ 0

Footnotes: Virus testing of the 9 MSU rootstock candidates @ $1,200 selection.

Budget 3: Matt Whiting
Organization Name: WSU - Prosser **Contract Administrator:** Mary Lou Bricker
Telephone: (509) 335-7667 **Email address:** mdeseros@wsu.edu

Item	2011	2012	2013
Salaries[1]	$2,550	$2,652	$2,758
Benefits	$1,250	$1,299	$1,351
Wages	$3,500	3,500	$3,500
Benefits	$336	$336	$336
Equipment			
Supplies	$200	$200	$200
Travel	$200	$200	$200
Plot charges[2]	$1,000	$1000	$1000
Miscellaneous			
Total	$9,036	$9,187	**$9,345**

Footnotes:
[1]One month technician salary for oversight of orchard, plant measurements, yield and quality assessments and data management
[2]Charges for irrigation and maintenance of the orchard (pesticides, fertilizers, mowing).

OBJECTIVES:

Overall project objective: Identify dwarfing precocious rootstocks that increase the profitability of sweet cherry production in the PNW through the establishment and evaluation of trees in test plots.

Specific objectives

1. Determine if the nine MSU rootstock candidates originally planted at MSU's Clarksville Horticultural Experimental Station continue to show commercial promise.
2. Evaluate the influence of nine candidate rootstocks on 'Bing' fruit quality and productivity in the experimental plot at WSU - Prosser (trees were planted in spring of 2009).
3. Test the five MSU candidate rootstocks at the Clean Plant Center Northwest – Fruit Trees (CPCN-FT) for viruses and other infectious agents to provide a source of commercial propagation material.
4. Conduct DNA fingerprinting to assure that the genetic identity of the rootstock selections is correct.

SIGNIFICANT FINDINGS:

- Five MSU cherry rootstocks were identified that produced 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 that are named after Michigan counties are CLINTON, CASS, CLARE, LAKE and CRAWFORD. All five MSU rootstocks produced trees of similar size to 'Gisela® 5' (Gi5) measured as trunk cross-sectional area (TCSA), except CLARE which produced trees significantly smaller than Gi5.
- In the third leaf (2011), 'Bing' on all five MSU rootstocks exhibited more flowering spurs than Gi5 and 'Gisela® 6' (Gi6). The MSU rootstocks also induced high flower densities on 'Bing' in the fourth and fifth leaf, 2012 and 2013, respectively.
- In 2012, all five of the MSU candidate rootstocks had yield efficiencies (kg fruit/cm^2) that were not significantly different from that of Gi5. However, in 2013, three MSU rootstocks, CLARE, CLINTON and CRAWFORD, exhibited significantly higher yield efficiencies compared to Gi5.
- Mean fruit size for 'Bing' fruit from all five MSU rootstocks and Gi5 and Gi6 were not significantly different suggesting that producing large fruit is possible on the MSU rootstocks given the proper training system and crop load adjustments.
- Four of the MSU rootstocks were virus-certified by the CPCN-FT (CLARE, CASS, CLINTON, and LAKE). The fifth rootstock, CRAWFORD, is anticipated to be certified in August 2014.
- These five rootstocks were established at commercial liner nurseries for limited propagation trials and the generation of liners for future trials. All plant material originated from the stock plants at the CPCN-FT. To date, liner production appears to be most efficient using tissue culture as opposed to softwood cuttings.
- DNA diagnostic tests confirmed that the identities of the MSU rootstocks at the CPCN-FT and the identities of the liners generated for the next series of experimental trials are correct.

RESULTS and DISCUSSION:

Performance of the MSU candidate rootstocks:

In 2009, a test plot of nine MSU rootstocks with 'Bing' scion was planted at WSU- Prosser Roza Station with (Gi5) and (Gi6) included as controls. The trees were spaced at 8 ft × 15 ft in five-tree replicates and were trained to a multiple leader architecture. Pruning was done annually to achieve three main leaders, with heading and thinning cuts to maintain balanced cropping. Based on

performance at this plot, five MSU selections (CASS, CLARE, CLINTON, CRAWFORD, and LAKE) named after Michigan counties to avoid potential confusion with the use of numbers as names, were chosen for future testing. Therefore the data presented in this final report will only include the five promising MSU selections compared to the controls (Gi5 and Gi6).

Tree size: All five of the MSU cherry rootstocks produced 'Bing' trees that were significantly smaller than Gi6 based on trunk cross sectional area (TCSA, cm^2)(Fig. 1). TCSA for four of the MSU rootstocks were similar to that for Gi5 while the TCSA for CLARE was significantly smaller than that for Gi5. These differences were consistent for all three years of this project (2011-2013).

Bloom: All five MSU rootstocks induced early and abundant flowering of 'Bing' in 2011, 2012 and 2013. For example, in the third leaf (2011), three of the MSU candidate rootstocks had significantly more flowering spurs than 'Bing' on Gi5 or Gi6 (data not shown). On average trees on LAKE, CASS, and CLINTON had 79, 74, and 54 flowering spurs/tree compared to 33 and 29 spurs per tree for Gi6 and Gi5, respectively. One MSU rootstock, CLARE had on average 34 flowering spurs/tree which was similar to Gi5 and Gi6.

In the fourth leaf (2012), 'Bing' grafted on five of the MSU candidate rootstocks had higher average numbers of flowers per node compared to Gi6 (data not shown) with CRAWFORD, CLINTON and LAKE having an average of over four flowers per node compared to 2.4 flowers per node for 'Bing' on Gi6.

In the fifth leaf (2013), LAKE, CLARE and CRAWFORD had significantly more flowers per leader cross-sectional area compared to Gi6 (Fig. 2.A). All five MSU rootstocks had flower numbers per leader cross-sectional area that were not statistically different from each other or from Gi5.

The average number of flowers per spur on 'Bing' trees was not significantly different in 2013 for all seven rootstocks evaluated (Fig. 2.B.). In 2011, the only significant difference was a higher number of flowers per spur on CLINTON compared to CLARE. In 2012, the only significant difference was a higher number of flowers per spur on CRAWFORD compared to Gi6.

Fruiting: Unfortunately, due to a spring freeze and subsequent flower death in spring 2011, yield and fruit quality data could not be obtained. Therefore, the fruit data presented is for harvests in 2012 and 2013. In 2012 and 2013, the numbers of flowers per node on the five MSU rootstocks, Gi5 and Gi6 were excessive and would have resulted in small fruit size if left unthinned. Therefore, in 2012, the fruit were thinned to 50% when they were pea-sized. Fruit were also thinned in 2013 based on achieving standard crop loads for each selection.

Cumulative 'Bing' tree yields per tree for 2012 and 2013 ranged from ~ 12 kg (26 lbs) for CASS to ~ 18 kg (39 lbs) for CLINTON and CRAWFORD (Fig. 3). 'Bing' yields on CLINTON were consistently higher than Gi5, CLARE, and CASS. Despite the yield differences, 'Bing' mean fruit weights and row sizes for the different rootstocks were not significantly different in both years (Table 1). In 2012, all five of the MSU rootstocks had yield efficiencies (kg fruit/cm^2) that were not significantly different from that of Gi5 (Table 1). However, in 2013, three MSU rootstocks, CLARE, CLINTON and CRAWFORD, exhibited significantly higher yield efficiencies compared to Gi5.

Evaluations of harvest and post-harvest fruit quality did not identify any consistent fruit quality problems that could be attributed to the MSU rootstocks and not associated with the differing crop loads and harvest maturities. For example, the significant firmness differences seen may reflect differences in crop load maturity reflected as fruit skin color, Brix and percent acidity (Tables 2 and 3). For example, LAKE was harvested earlier that the other selections in 2013 and the data suggests that 'Bing' fruit from CASS and CLARE may have been over mature at the time of harvest. In 2013, 'Bing' fruit firmness, Brix, storage acidity, cracked fruit, and skin shine for the MSU rootstocks were not significantly different from that of the Gi5 and Gi6 trees (Tables 2, 3 and 4).

In 2012, average tree yields and gross returns were highest with the use of CLINTON and CRAWFORD rootstocks (Table 5). In 2013, CLINTON had the highest tree yield. However, the

more dwarfing rootstocks, CASS, CLARE and LAKE, may produce high per acre yields if planted at increased densities compared to CLINTON and CRAWFORD.

Several observations are relevant for considering the use of these rootstocks and for designing future plantings. In general, 'Bing' fruit maturity for the MSU rootstocks and Gi5 was more uniform than that produced on Gi6, presumably due to the better light penetration. All MSU rootstocks produced at least some fruit in the 9 row category indicating that producing large fruit is possible given the proper training system and crop load. Harvest timing also appeared to differ based on rootstocks with fruit on LAKE, CASS, and CLARE exhibiting an earlier harvest maturity. However, the biggest influence on fruit quality was crop load indicating the importance of using appropriate intensive training systems for these dwarf precocious rootstocks.

Generation of virus-certified rootstock budwood for the MSU cherry rootstocks.

Based on the abundant floral display exhibited in the 3rd leaf (2011) for LAKE, CASS, CLARE and CLINTON in comparison to Gi5 and Gi6, these four rootstocks were selected for virus certification and future propagation trials. CRAWFORD was not initially chosen for further testing as it showed symptoms of graft incompatibility with 'Hedelfingen' scion in the original plot at MSU's Clarksville Experiment Station. However, as CRAWFORD performed well in the Prosser plot with 'Bing' scion, and showed no signs of graft incompatibility, it was selected for further testing the following year, 2012.

Four of the MSU cherry rootstocks were virus certified by the CPCN-FT (CASS, CLARE, CLINTON, and LAKE). The fifth rootstock, CRAWFORD, was "provisionally released" meaning that one more year of testing needs to be conducted prior to full certification.

Distribution of rootstock budwood for pilot propagation trials and limited liner production.

Distribution of the MSU candidate rootstocks to liner nurseries was accelerated to provide a mechanism for generating liners for future trials, give the nurseries an opportunity to gain experience propagating these rootstocks, and begin to establish stock plants in case of commercialization. CLINTON, CLARE, and LAKE were distributed to liner nurseries in September 2011 followed by CASS in 2012 and 2013.

- Cameron Nursery, Eltopia, Wash. (Todd Cameron)
- Copenhaven Farms, Gaston, Ore. (Christopher Dolby)
- Duarte Nursery, Hughson, Calif. (John Duarte)
- North American Plants, Lafayette, Ore. (Yongjian Chang)
- Protree Nurseries, Brentwood, Calif. (Richard Chavez)
- Helios Nursery (DBA Teak Nursery) Orondo, Wash. (Tye Fleming & Todd Erickson)
- Willamette Nursery, Canby, Ore. (Devin Cooper)

All seven nurseries were able to establish these rootstocks. Collectively, the nurseries are using a range of propagation techniques that include: softwood cuttings, tissue culture and even stool beds. To date, propagation has been most successful using tissue culture.

CRAWFORD was provisionally released to liner nurseries for establishment in tissue culture with full release anticipated in August 2014. Budwood of CRAWFORD was sent to North American Plants in September 2013 and Duarte Nursery and Protree Nursery in October 2013.

In 2013, liners of CASS, CLARE, CLINTON and LAKE were propagated by North American Plants and shipped to three finished tree nurseries to make trees for the next series of rootstock trials (see Proposal for 2014-2016).

The liner nurseries that have the MSU cherry rootstocks are gaining experience propagating these rootstocks. To date, liner production appears to be most efficient using tissue culture. Since the rootstock materials they have established originated from the virus-certified and genetically verified plant material at the CPCN-FT, liners from these plant materials could be commercialized if a decision is made to release one or more of the MSU sweet cherry rootstocks.

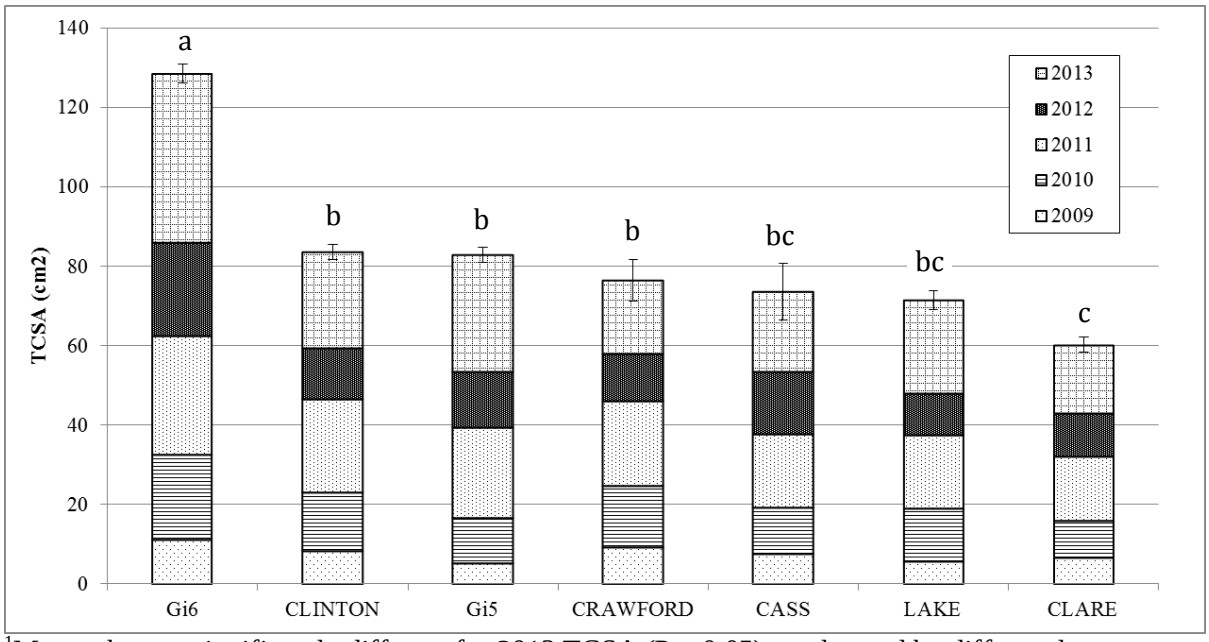
Genetic-verified plant materials.

DNA diagnostic tests were done in the Iezzoni lab at MSU to determine if the identities of the five MSU cherry rootstocks at the CPCN-FT are correct. The rootstocks were screened using four different molecular markers. The genetic tests determined that the identities of the MSU cherry rootstocks at the CPCN-FT are correct.

In preparation for the next series of rootstock trials, North American Plants generated 1000 liners each of CLARE, CLINTON and CASS and 600 liners of LAKE. Three plants of each of these selections were sent to MSU and their identities were verified by DNA tests.

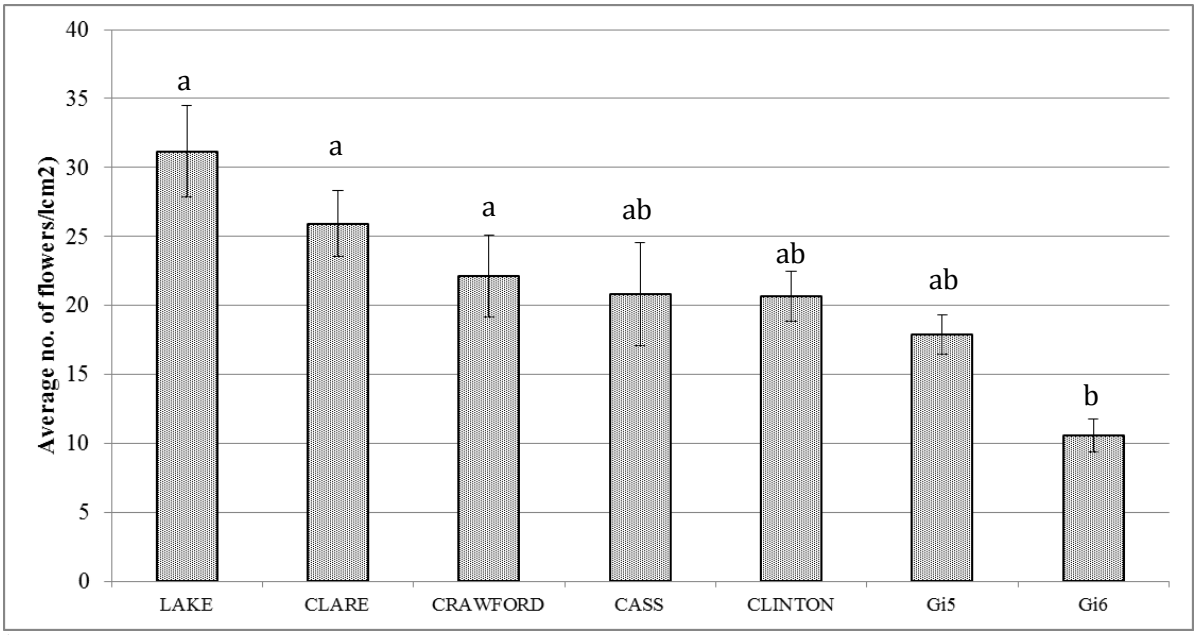
These DNA diagnostic tests have confirmed that the MSU cherry rootstocks are labeled correctly, thereby avoiding any delays and financial losses at the nurseries that would be associated with a plant material mix-up.

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 Roza Experiment Station. Boxes represent growth over one season. TCSA measurements were taken on the following dates: March 16, 2010; October 13, 2010; September 28, 2011; July 9, 2012; and July 23, 2013. Bars represent standard error of the means for 2013 TCSA.[1]



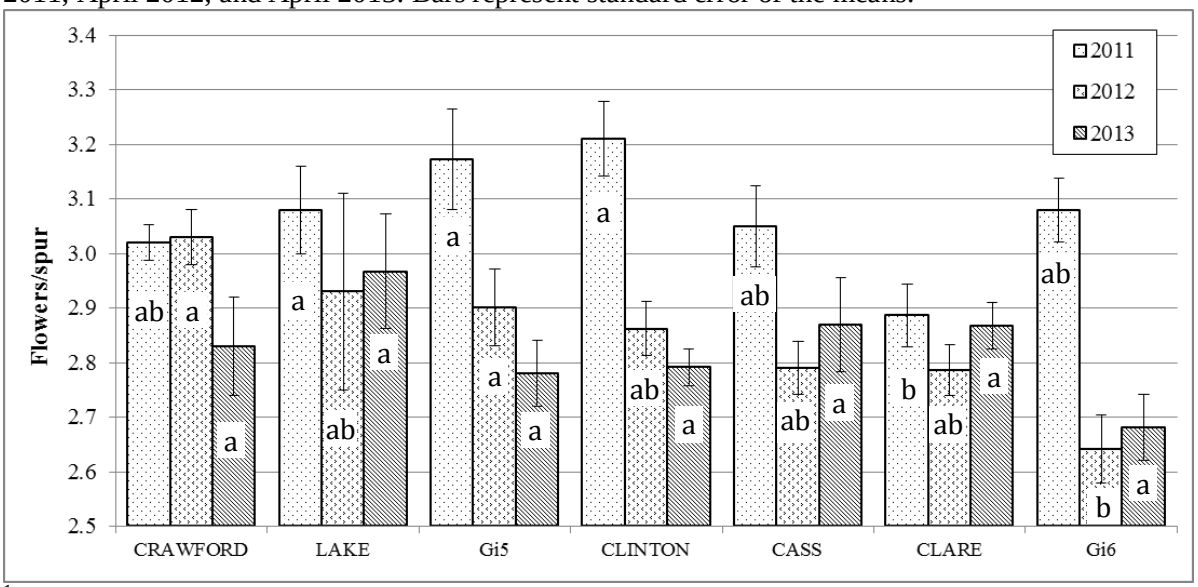
[1]Means that are significantly different for 2013 TCSA ($P < 0.05$) are denoted by different letters.

Fig. 2.A. Average number of flowers per leader cross-sectional area[1] on 'Bing' trees grafted on 5 MSU rootstocks, Gi5, and Gi6 for trees planted in 2009 at WSU - Prosser Roza Experiment Station. Data was taken in April 2013. The values were calculated from two scaffolds per tree using the following equation: average number of flowers ÷ leader cross-sectional area. Bars represent standard error of the means.[2]



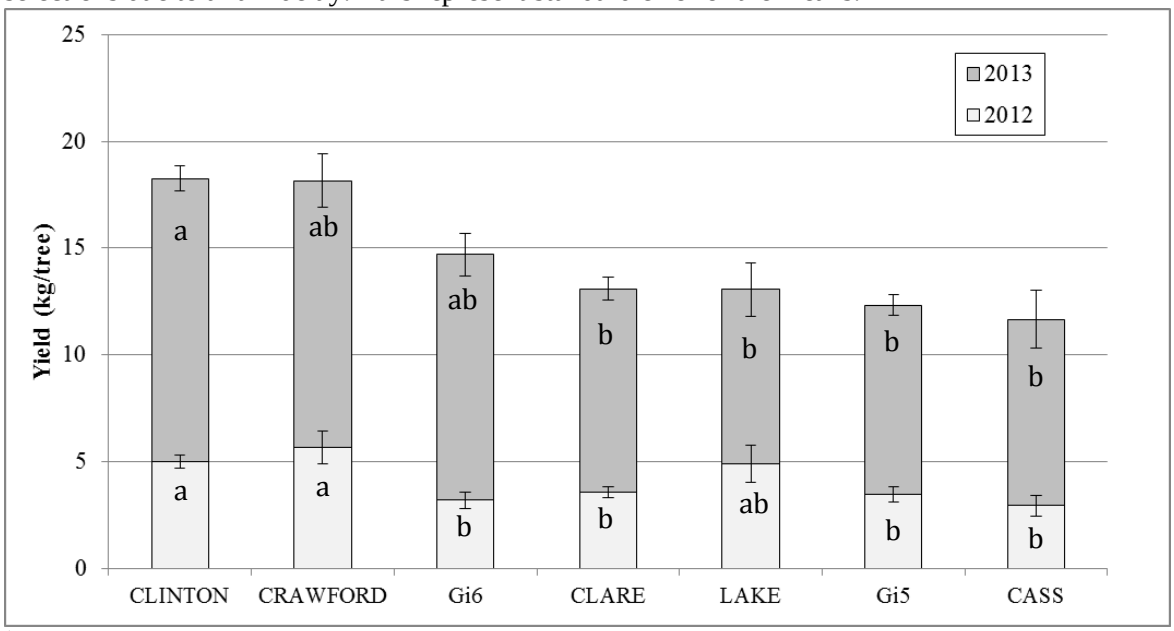
[1]A 30 inch (0.75 meter) segment on two leaders per tree was evaluated.
[2]Means that are significantly different ($P < 0.05$) are denoted by different letters.

Fig. 2.B. Average number of flowers per spur on 'Bing' trees grafted on 5 MSU rootstocks, Gi5, and Gi6 for trees planted in 2009 at WSU-Prosser Roza Experiment Station. Data was recorded in May 2011, April 2012, and April 2013. Bars represent standard error of the means.[1]



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

Fig. 3. Cumulative tree yields (kg) for 2012 and 2013 of 'Bing' trees grafted on 5 MSU rootstocks, Gi5, and Gi6 for trees planted in 2009 at the WSU - Prosser Roza Experiment Station. Fruit were harvested in 2012 on June 28 and in 2013 on June 18 for LAKE and on June 26 for the remaining selections due to a rain delay. Bars represent standard error of the means.[1,2,3]



[1]Pea-sized fruit were thinned by 50% in 2012. In 2013, fruit were thinned based on achieving standard crop loads for each selection.
[2]Means that are significantly different ($P < 0.05$) are denoted by different letters.
[3]Refer to Table 5 for total tree yields in pounds.

Table 1. Fruit weight, mean row size and yield efficiency for 'Bing' grown on five MSU rootstocks, Gi5 and Gi6. Fruit were harvested in 2012 on June 28 and in 2013 on June 18 for LAKE and on June 26 for the remaining selections due to a rain delay.[1]

Rootstock selection	2012 Fruit weight (g)	2013 Fruit weight (g)	2012 Mean row size	2013 Mean row size	2012 Yield efficiency (kg/cm^2)	2013 Yield efficiency (kg/cm^2)
Gi5	10.2 a[2]	11.1 a	9.8 a	9.6 a	0.066 ab	0.107 b
Gi6	9.6 a	10.4 a	9.9 a	9.8 a	0.037 b	0.091 b
CASS	10.3 a	10.7 a	9.7 a	9.8 a	0.059 ab	0.120 ab
CLARE	9.9 a	10.3 a	9.9 a	9.8 a	0.086 a	0.160 a
CLINTON	10.1 a	10.5 a	9.8 a	10.0 a	0.086 a	0.161 a
CRAWFORD	9.5 a	9.3 a	10.0 a	10.2 a	0.099 a	0.173 a
LAKE	9.0 a	9.6 a	10.1 a	10.0 a	0.106 a	0.118 ab

[1]Pea-sized fruit were thinned by 50% in 2012. In 2013, fruit were thinned based on achieving standard crop loads for each selection.
[2]Means that are significantly different ($P < 0.05$) are denoted by different letters.

Table 2. Fresh and post-harvest values for fruit firmness (g/mm^2) and acidity for 'Bing' on five MSU rootstocks, Gi5 and Gi6. Fruit were harvested in 2012 on June 28 and in 2013 on June 18 for LAKE and June 26 for the remaining selections due to a rain delay. Storage acidity and firmness in 2013 was measured from fruit stored at 33°F for 4 days. [1]

Rootstock selection	2012 Firmness (g/mm^2)	2013 Firmness (g/mm^2)	2012 Storage firmness (g/mm^2)	2013 Storage firmness (g/mm^2)	2013 Acidity (%)[2]	2013 Storage acidity (%)[2]
Gi 5	269 ab[3]	235 ab	369 a	261 a	0.86% ab	0.82% ab
Gi 6	262 abc	214 ab	360 a	227 b	0.83% abc	0.81% ab
CASS	231 d	228 ab	332 b	261 a	0.85% ab	0.81% ab
CLARE	252 c	222 ab	357 a	250 a	0.83 abc	0.81% ab
CLINTON	238 d	200 b	333 b	216 b	0.77% bc	0.72% b
CRAWFORD	253 bc	224 ab	312 b	212 b	0.72% c	0.72% b
LAKE	277 a	248 a	311 b	255 a	0.90% a	0.86% a

[1]Pea-sized fruit were thinned by 50% in 2012. In 2013, fruit were thinned based on achieving standard crop loads for each selection.
[2]Data not shown for 2012 because statistical analyses were not possible due to lack of replicated data for CASS and LAKE.
[3]Means that are significantly different ($P < 0.05$) are denoted by different letters.

Table 3. Fruit skin color, Brix and percentage of fruit cracked for 'Bing' grown on five MSU rootstocks, Gi5 and Gi6. Fruit were harvested in 2013 on June 18 for LAKE and on June 26 for the remaining selections due to a rain delay[1,2].

Rootstock selection	2013 Fruit skin color	2013 Brix (%)	2013 Fruit cracked (%)
Gi5	6.3 ab[3]	20.4 ab	38% a
Gi6	6.3 ab	19.6 ab	34% a
CASS	6.8 a	22.0 a	44% a
CLARE	6.8 ab	20.9 ab	38% a
CLINTON	6.6 ab	19.5 ab	40% a
CRAWFORD	5.9 b	18.6 b	44% a
LAKE	4.9 c	19.2 ab	25% a

[1]Pea-sized fruit were thinned by 50% in 2012. In 2013, fruit were thinned based on achieving standard crop loads for each selection.
[2]Data not shown for 2012 because statistical analyses were not possible due to lack of replicated date for CASS and LAKE.
[3]Means that are significantly different ($P < 0.05$) are denoted by different letters.

Table 4. Post-harvest mean values for stem browning, fruit cracking, skin shine, and skin pitting for 'Bing' grown on five MSU rootstocks, Gi5 and Gi6. Fruit were harvested in 2012 on June 28 and in 2013 on June 18 for LAKE and on June 26 for the remaining selections due to a rain delay. Measurements were taken from fruit stored at 33°F for 14 days.[1,2]

Rootstock selection	2013 Stem browning rating[3]	2013Fruit cracked (%)	2013 Fruit 100% skin shine (%)[5]	2013 Skin pitting (%)
Gi5	3.15 ab[4]	41% a	79% ab	7% a
Gi6	3.19 a	36% a	71% ab	6% a
CASS	3.07 ab	44% a	72% ab	21% b
CLARE	2.98 ab	40% a	62% b	12% ab
CLINTON	3.10 ab	39% a	74% ab	13% ab
CRAWFORD	3.17 a	39% a	76% ab	6% a
LAKE	2.41 b	28% a	94% a	8% a

[1]Pea-sized fruit were thinned by 50% in 2012. In 2013, fruit were thinned based on achieving standard crop loads for each selection.
[2]Data not shown for 2012 because statistical analyses were not possible due to lack of replicated data for CASS and LAKE.
[3]Stem browning was rated on a scale of 1-4 with 1=0-25%, 2=26-50%, 3=51-75%, and 4=76-100%
[4]Means that are significantly different ($P < 0.05$) are denoted by different letters.
[5]Skin shine data not available for 2012.

Table 5. Gross returns in 2012 and 2013 for 'Bing' trees grafted on 5 MSU rootstock candidates, Gi5, and Gi6 for trees planted in 2009 at WSU - Prosser Roza Experiment Station.[1]

Rootstock selection	2012 Average Tree Yield (lb)	2013 Average Tree Yield (lb)	2012 Gross Return	2013 Gross Return	Cumulative Yield (lb)	Cumulative Gross Return
Gi5	7.63 b[2]	19.51 b	$16.38	$59.03	27.14	$75.41
Gi6	7.05 b	25.27 ab	$14.21	$75.94	32.32	$90.15
CASS	6.49 b	19.18 b	$14.59	$57.99	25.67	$72.58
CLARE	7.87 b	20.95 b	$16.41	$62.92	28.82	$79.33
CLINTON	11.06 a	29.10 a	$23.14	$85.51	40.16	$108.65
CRAWFORD	12.48 a	27.46 ab	$25.27	$75.21	39.94	$100.48
LAKE	10.82 ab	17.92 b	$20.37	$52.26	28.74	$72.63

[1]Calculated by summing the price per pound for each row size. The returns for each row size was calculated by multiplying the average tree yield (lb) x percent fruit for that row size category x row size price = 2012 Gross Returns: Row size values used are as follows: Row Size 9= $2.50/lb, Row Size 9.5 = $2.50/lb, Row Size 10 = $1.80/lb, Row Size 10.5 = $1.80/lb, Row Size 11 = $1.50/lb, Row Sizes 11.5-13 = $1.20/lb. 2013 Gross Returns: Row size values used are as follows: Row Sizes 8, 8.5, 9, 9.5= $3.08/lb, Row Size 10 = $3.20/lb, Row Size 10.5 = $2.50/lb, Row Size 11 = $2.16/lb, Row Sizes 11.5-13 = $1.76/lb.
[2]Means that are significantly different ($P < 0.05$) are denoted by different letters.

Project Title: Establishment and testing of MSU sweet cherry rootstocks

Executive Summary: Five MSU sweet cherry rootstocks were identified that induce precocious abundant flowering and significantly reduce tree size compared to Gi6. All five MSU rootstocks produced trees of similar size to Gi5 except CLARE which produced trees significantly smaller than Gi5. In 2012, all five of the MSU candidate rootstocks had yield efficiencies (kg fruit/cm^2) that were not significantly different from that of Gi5. However, in 2013, three MSU rootstocks, CLARE, CLINTON and CRAWFORD, exhibited significantly higher yield efficiencies compared to Gi5. 'Bing' fruit size on the MSU rootstocks was not significantly different from that on Gi5 and Gi6 suggesting that premium fruit can be produced on these rootstocks given the proper training system and crop load adjustments.

Based on these observations, the five MSU cherry rootstocks were advanced to provide plant materials for future trials and potentially commercialization. Four of the MSU rootstocks were virus-certified by the Clean Plant Center Northwest - Fruit Trees (CPCN-FT) (CLARE, CASS, CLINTON, and LAKE). The fifth rootstock, CRAWFORD, is anticipated to be certified in August 2014. These five rootstocks were established at commercial liner nurseries for limited propagation trials and the generation of liners for future trials. To date, liner production appears to be most efficient using tissue culture as opposed to softwood cuttings. DNA diagnostic tests confirmed that the identities of the MSU rootstocks at the CPCN-FT and the identities of the liners generated for the next series of experimental trials are correct.

NEW PROJECT PROPOSAL **PROPOSED DURATION**: 3 years

Project Title: MSU cherry rootstocks: pre-commercialization

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Cooperators: Todd Einhorn, Tim Dahle, Stefano Musacchi, WSU-TFREC

Total Project Request: **Year 1: $50,450** **Year 2:** $48,671 **Year 3:** $35, 218

Other funding sources: None

WTFRC Collaborative expenses:

Item	2014	2015	2016
Salaries			
Benefits			
Wages	$ 11,000[a]	$ 1,100[b]	$ 1,100[b]
Benefits	$ 3,000	$ 300	$ 300
Supplies			
Travel	$ 500[b]	$ 500	$ 500
Miscellaneous	$ 1,000[c]	$ 50	$ 100
Total	**$ 15,500**	$ 1,950	$ 2,000

Footnotes:
[a]Pruning, floral evaluation, harvest and fruit evaluations of the Roza plot.
[b]Travel to participating nurseries
[c]Assist in plot establishment

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		$ 12,768	$ 3,927
Total	**$ 15,500**	$ 25,368	$ 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.

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		$ 2,059[c]	$ 2,141
Benefits		$ 760	$ 790
Plot Fees		$ 500[d]	$ 500
Plot Fumigation	$ 850[b]	$ 850	
Trellis posts[a]	$ 1500	$ 750	
Trellis anchors, wire, clips[a]	$ 600	$ 300	
Polytube/sprinklers[a]	$ 5000	$ 2500	
Total	**$ 7,950**	$ 7,719	$ 3,431

Footnotes:
[a] Supply cost in 2014 is for 2015 planting; Supply cost in 2015 is for 2016 planting Cost for fumigation from Custom
[b]Orchard Fumigation company
[c]Wages and benefits for labor for installing trellis, planting trees, installing irrigation and collecting data
[d]Standard annual plot fee, Sunrise Orchard

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.

Justification: Sweet cherry rootstocks that induce flowering by year three and significantly reduce tree size compared to trees on standard size rootstocks can enhance profitability. Early cropping will contribute to an early return on investment, and high flower numbers will increase the likelihood of setting a crop each year resulting in improved yield stability. Small tree size will result in reduced labor costs and the number of harvest laborers needed. Due to the high cropping potential of trees on dwarfing precocious cherry rootstocks compared to their vegetative vigor, new intensive training systems utilizing these rootstocks have been designed to achieve high yields of premium fruit.

Recently a new series of experimental cherry rootstocks were developed at Michigan State University (MSU). Five MSU cherry rootstocks were identified that produce small statured, precocious and high yielding trees based on a trial at WSU-Prosser planted in spring 2009 with 'Bing' scion. These rootstocks, named after Michigan counties, are CASS, CLARE, CLINTON, CRAWFORD, and LAKE. Like the currently available dwarfing rootstocks, these rootstocks can contribute to profitability by enhancing yield stability due to high flower numbers and reduced labor costs due to small tree size. One of the MSU selections (CASS) is significantly more dwarfing than Gisela 5 (Gi5), while the other four rootstocks induce similar dwarfing to Gi5. Two of the selections appear to encourage wider branch angles and flatter growth habit (CRAWFORD and CLINTON) more suited to a Vogel Central Leader (VCL)(Spindle) training system, and three of the rootstocks have a more upright growth habit that could be a plus in a Upright Fruiting Offshoot (UFO) training system (LAKE, CLARE, and CASS). Tree yields on the MSU rootstocks were either equivalent to that on Gi5 and Gi6 or significant greater than both control rootstocks. Mean 'Bing' fruit weights and row sizes from all five MSU rootstocks harvested in 2012 and 2013 were not significantly different from fruit produced on Gi5 and Gi6 trees. These results suggest that the MSU rootstocks have the potential to be productive and produce good fruit size when properly managed and when appropriate training systems are used.

Despite the potential of the MSU cherry rootstocks to contribute to profitability, numerous other performance-related questions have not yet been answered. These include performance with scions with different cropping potential, and suitability with different training systems, soils and growing conditions. All the data for the MSU rootstocks from the Pacific Northwest is from one plot at WSU-Prosser with 'Bing' scion trained to a multiple leader architecture. This proposed project is designed to fill these knowledge gaps and therefore inform the producer's decision of whether to plant trees on any of the new MSU cherry rootstocks. This proposed project will also support a successful rootstock introduction process through the use of virus-certified and genetically verified plant materials.

Commercial adoption of a new cherry rootstock is driven by the benefits of using the new rootstock compared to currently available rootstocks, tree cost, and availability. Therefore, the first proposed activity will compare the performance of the five MSU rootstocks to currently available rootstocks. We propose to evaluate the WSU-Prosser plot in 2014 (6^{th} leaf) and then remove the plot to focus on three experimental plots to be planted in 2015. The three proposed rootstock plots to be established in 2015 are the centerpiece of this proposal. The proposed plantings will be located in Oregon (The Dalles) and two in Washington (WSU-Prosser and WSU-Wenatchee). They will include four MSU rootstocks (CASS, CLARE, CLINTON and LAKE), and three rootstock controls (Gi5, Gi6, and a Krymsk rootstock). Three scions were chosen to represent a range in productivity and include 'Regina', 'Early Robin' and 'Sweetheart'.

Successful sweet cherry production with dwarfing precocious rootstocks requires the use of appropriate training systems to manage tree vigor and crop load. To compare the fruit yield and quality for the test rootstock scion combinations, different training systems will be used at each of the three plots. For the plot in Oregon, the chosen training system will be the VCL which is a spindle system familiar to Oregon growers. It provides for early high yields and is especially successful with pendant, non-spur type varieties like 'Regina'. Unlike a bush type tree, the single axe nature of the system will also provide good tree vigor on the most dwarfing rootstocks. For the plots in Prosser and Wenatchee, two intensive two-dimensional fruiting-wall training systems will be used. In Prosser the

UFO will be used, and in Wenatchee the Super Slender Axe (SSA) training systems will be used. The UFO system is a plant architecture comprised of unbranched vertical limbs. It is designed to incorporate automation/mechanization and has simplified pruning/training rules compared to other systems. The SSA is a new system developed by Stefano Musacchi whereby the fruit is produced from basal flower buds on the previous season's shoots, eliminating the problem of reduced fruit size associated with high spur numbers and fruit per spur relative to the leaf area available to support these fruit.

In 2016, we propose to plant three trials with the fifth MSU rootstock, CRAWFORD, which was not available in time for the 2015 plantings. The CRAWFORD plantings will include the two rootstocks with which it is most similar, CLINTON and Gi5, and two scions yet to be determined. The anticipated outcome for these trials will be knowledge of rootstock-scion performance that can be used by producers to determine whether any of the MSU cherry rootstocks would have value for their future plantings.

The second proposed activity will address tree cost and availability by determining the ease of liner production, liner vigor, and budding success. The third proposed activity will ensure that the MSU rootstocks are available for commercialization with certified virus-tested materials that are genetically verified. Significant progress has already been made in these last two objectives. Four of the MSU rootstocks (CASS, CLARE, CLINTON and LAKE) have been virus certified, genetically verified and distributed to seven liner nurseries (Cameron Nursery, Copenhaven Farms, Duarte Nurseries, North American Plants, Protree Nurseries, and Teak Nurseries). This distribution gives these liner nurseries the opportunity to experiment with the propagation of the MSU rootstocks and also provides a mechanism whereby liners for future experiments can be generated. To date, liner production has been most successful from tissue culture with limited success from softwood cuttings. Budwood of CRAWFORD was provisionally released in September 2013 and sent to three tissue culture nurseries (North American Plants, Protree Nurseries and Duarte Nursery).

Confirming genetic identify of the MSU rootstocks is critical to prevent any mix-ups that could result in a delay in rootstock availability for producers and financial losses to the nurseries. DNA markers are being used that distinguish the five MSU rootstocks. Genetic identity was verified for plants at the Clean Plant Center Northwest – Fruit Trees (CPCN – FT). Genetic identity was also verified for the CASS, CLARE, CLINTON and LAKE liners prior to transfer to the finished tree nurseries in spring 2013 for the production of the 'Regina', 'Early Robin' and 'Sweetheart' trees for the proposed 2015 plantings. This illustrates the strategy in place whereby DNA markers were and will be used to confirm identity during critical times in the rootstock project.

Ultimately the decision to put in an intensive sweet cherry orchard system with a MSU rootstock will depend on the ability to be profitable despite the high establishment cost associated with the high tree number. Therefore, it is important that the MSU rootstocks exhibit the following nursery attributes as any shortcoming has the potential to translate into higher tree costs: suitability for vegetative propagation, good liner establishment in the nursery, vigorous upright liner growth, rapid increase in girth in the nursery, and good bud take percentage. This proposal will allow a determination of the nursery potential of the MSU rootstocks by assessing these horticultural attributes. The anticipated outcomes of Objectives 2 and 3 will be a continual supply of genetically verified and virus certified liners and finished trees that can be used to estimate the future liner/tree price point and tree availability.

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 CPCN-FT and cooperating nurseries to insure MSU cherry rootstocks are available as certified virus tested and genetically verified.

Method by objective:

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

Trees will continue to be pruned and trained in 2014 according to a multiple leader architecture. This will be accomplished in collaboration with Matt Whiting, Tom Auvil and a crew from the WTFRC. Matt Whiting, Tom Auvil, Amy Iezzoni and WSU farm manager Clint Graf will meet at the plot to review pruning plans. In 2014, prior to bloom, two scaffolds per tree of the Gi5 and Gi6 controls and the five MSU dwarfing rootstocks (CASS, CLARE, CLINTON, CRAWFORD and LAKE) will be selected and the number of spurs and nodes will be counted. In addition, the flower buds on a maximum of 15 spurs will be recorded and the mean numbers of buds per spur will be calculated. Due to the high flower number that may occur on many of the trees, the fruit set will be thinned by hand when pea-size to achieve a fruit count that would be standard for the tree volume. This will involve thinning fruit from flowering zones that have insufficient leaf area. In June, the 'Bing' trees will be harvested and the individual tree yields will be recorded. For the two scaffolds per tree previously evaluated for flower traits, the total fruit number will be counted. The fruit will be transported to the WTFRC laboratory in Wenatchee for fruit quality evaluations. Evaluations will be done with a goal of 100 fruit per 5-tree replicate for the following traits: bulk fruit weight, cracking and brix. Next, a sample of 50 fruit will be evaluated for skin color, row size, individual fruit weight, stem pull force and firmness. After harvest, the tree trunk circumference will be measured 20 cm above the graft line.

The WSU-Prosser plot will be removed after the trunk circumference measurements have been taken. However, prior to tree removal, root distribution and size will be evaluated for 3 trees for each of the 5 MSU rootstock and the Gi5 and Gi6 controls. Trenches will be dug 1 m from the trunk on the east and west sides of each tree (in a north/south direction). The number of roots in different size classes will be observed in 0.25 m × 0.25 m grids within a 2 m wide and 1.5 m deep area will be counted. In addition the largest diameter of the largest 5 roots in each grid will be measured. A second trench will then be dug 1 m from the trunk on the south side of the tree and the roots will be evaluated in the same manner.

As in previous years, a plot tour will be conducted in 2014 (6th leaf) prior to 'Bing' harvest as part of the Cherry Field Day at Prosser. Plans for the proposed 2015 plantings will also be presented to the attendees at that time to allow a discussion of the future plots while having the opportunity to discuss what was learned from the existing plot. .

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. (WSU - Sunrise Farm) under the direction of 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 up on 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 × 15 ft = 363 t/ac)(2.44 m × 4.57 m = 897 t/ha); Gi5 and CLINTON (6 ft × 15 ft = 484 t/ac)(1.83 m × 4.57 m = 1196 t/ha), and CASS/LAKE/CLARE (4 ft × 15 ft = 726 t/ac)(1.22 m × 4.57 m = 1794 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 will include plant survival and trunk cross sectional area. Data to be collected in 2016 will include plant survival, trunk cross sectional area and suckering. Due to the precocious rootstocks 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 fifth MSU cherry rootstock, CRAWFORD, was selected for advancement a year later than the other four MSU rootstocks. CRAWFORD was not initially advanced due to what 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.). Options to make CRAWFORD trees for three plantings in 2016 will be explored. CRAWFORD will be compared with the two rootstocks with which it is most similar, CLINTON and Gi5. Two scions will be used and chosen at a later date. This trial will be replicated at three locations, two sites in Washington and one site in Oregon.

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 limited number of liners of CASS, CLARE, CLINTON and LAKE (Cameron Nursery, Copenhaven Farms, Duarte Nurseries, North American Plants, Protree Nurseries, and Teak Nurseries). Three of these liner nurseries (Duarte Nurseries, North American Plants, Protree Nurseries) received provisionally released budwood of CRAWFORD in Sept/Oct 2013 and will establish CRAWFORD in tissue culture. 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 be assessed through visits of A. Iezzoni to these nurseries.

The suitability of the MSU rootstocks CASS, CLARE, CLINTON and LAKE to make finished trees will be assessed using liners that were planted at three commercial nurseries in spring 2013. The background is as follows. To produce trees needed for the proposed 2015 plantings (Obj. 1.B.), in 2013, 1000 liners each of CASS, CLARE and CLINTON and 600 liners of LAKE were produced by North American Plants. The genetic identity of these liners was verified with DNA tests at MSU using three liners of each rootstock. In spring 2013, following these DNA tests, 333 liners each of CASS, CLARE and CLINTON and 200 liners of LAKE were shipped to each of three finished tree nurseries, Cameron Nursery, Gold Crown Nursery and Willow Drive Nursery. These nurseries will make the finished trees of the MSU rootstocks with 'Regina', 'Sweetheart' and 'Early Robin' scions, respectively.

Stand counts and visual observations of liner vigor and suitability for budding will be determined from the MSU rootstocks planted at these three nurseries. But bud take will be evaluated in spring 2014. Nursery performance for CRAWFORD will also be assessed once liners have been sent to finished tree nurseries. To achieve this objective, A. Iezzoni will visit each nursery that is producing trees of the MSU rootstocks either one or two times per year.

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

CRAWFORD, the fifth MSU rootstock, is in the final stage of virus-certification at the CPCN-FT. Full virus certification of CRAWFORD is projected for August 2014. 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 four markers [the self-incompatibility *S-RNase* locus and three SSR markers

(PceGA59, PMS40, and PMS67)]. 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.

LITERATURE REVIEW

Cherry rootstocks that produce dwarf trees that are precocious with abundant flowering enable cherry growers to adopt high efficiency orchard systems that enhance profitability. These advantages result from earlier and increased yields, higher quality fruit, and reduced labor costs (Lang and Ophardt 2000, Whiting et al. 2005). The most dwarfing rootstocks available are those from the Gisela series (Gi3 and Gi5) (Schmidt and Gruppe 1998), with Gi6 and those from the Krymsk series being semi-dwarfing (K5 and K6) (Long et al. 2007). These dwarfing sweet cherry rootstocks currently available in the U.S. were selected from a limited genetic background suggesting that breeding cherry rootstocks for specific production areas in the U.S. using a broader genetic base may provide improved options for cherry growers. By the mid-1990's, the MSU tart cherry scion germplasm collection included many wild species that were believed to be useful for sweet cherry rootstock development. Therefore, in 1997, prior to discarding plant materials from tart cherry scion development, cherry seedlings were selected for direct testing as sweet cherry rootstocks. The five MSU cherry rootstocks were identified through this effort (Iezzoni et al. 2013).

Very dwarfing precocious sweet cherry rootstocks, including those in the MSU series, can result in very heavy crop loads resulting in small fruit and loss of tree vigor. To avoid this situation, novel tree training and crop load management strategies to balance fruit number with canopy area have been developed (Whiting et al. 2005). One of these training systems is the VCL (spindle) system developed in Germany (Long 2003). Trees are trained to a central leader, branches are spread to achieve wide branch angles, and fruiting wood is continually renewed by removing and stubbing back branches. Dwarfing precocious rootstocks help reduce the height of the central leader and promote early cropping. Two other training systems, the UFO and the SSA, result in pedestrian orchards where the fruit is produced in a two dimensional "fruiting wall". In the UFO system, the trees are planted at an angle and the trunk is trained to a horizontal wire. Fruit is produced on spurs on each of the upright shoots. One fifth of the upright fruiting shoots are removed each year to achieve a 5 year cycle for renewal of the fruiting shoots. In the SSA system, the central leader is the only permanent wood; therefore the trees are planted very close, as close as 16 inches (0.4 m) apart (Lugli and Musacchi 2009, 2010 and Musacchi et al. 2012). The fruit is produced from basal buds on the previous season's shoots. Renewal cuts are made by pruning the current season's shoots to the basal flower buds plus one or two vegetative buds. These buds will regrow to produce the fruit and leaves that support these fruit. For both the UFO and SSA training systems, rootstocks that reduce vegetative vigor and promote flowering are a requirement.

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FINAL REPORT **YEAR**: 2013

Project Title: Prediction and mitigation of rain-induced cherry cracking

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Cooperators:

Product suppliers:	Garrett Bishop, Valent Biosciences (former Pace Intl.); Clive Kaiser, OSU; Adrian Roozen, Wilbur Ellis; Sean Musser, Cultiva IPM
Grower collaborators:	Jim Kelly, Ray Wolverton, John Verbrugge, Jaime Reyes, Denny Messimore, John Hefren, Rick Derry, Valerie Carlson Stanly
Other:	Michael Young, formerly Stemilt; Glade Brosi; Suzanne Niemann; internal program staff: Manoella Mendoza, Tory Schmidt, Sandy Stone, Felipe Castillo, Udel Mendoza, Alfonso Ruiz and WTFRC seasonal crew

Other funding sources

All supplies and chemicals were donated by industry suppliers (value: $ 2,500-3,000/year).

Budget history

Organization Name: WTFRC **Contract Administrator: Kathy Coffey**
Telephone: 509 665 8271 **Email address: Kathy@treefruitresearch.com**

Item	2011	2012	2013
Salaries	7,000	4,366	12,256
Benefits	3,000	1,872	1,294
Wages	22,000	11,745	11,745
Benefits	11,000	5,033	5,033
Equipment	0	0	0
Supplies	192	200	150
Travel	116	150	0
RCA room rental	360	360	360
Revenue	12,500	14,000	8,000
Total	**31,168**	**9,726**	**10,594**

Footnotes: Salaries are estimated based on actual time spent on project for internal program staff: Schmidt, Castillo and Hanrahan. Wages reflect actual timeslip costs. Revenue is based on reimbursement from Cultiva IPM (2011, 2012) and Pace International/Valent Biosciences (2011-13)

Note: Budget for informational purposes only. Research is funded through the WTFRC internal program.

RECAP ORIGINAL OBJECTIVES

Investigate rain cracking susceptibility and develop management strategies utilizing spray programs and prediction models.

1. Evaluate and optimize spray programs to reduce rain-induced cherry cracking.

2. Determine rain cracking susceptibility expression for common Northwest cherry cultivars during maturation and develop an easy test to determine cracking potential of individual blocks for grower use.

SIGNIFICANT FINDINGS

Objective 1 (spray programs):

- Significant field cracking pressure (10% of fruit) is needed for conclusive product performance evaluation.
- When applied according to manufacturer recommendations, hydrophobic coatings can significantly reduce field cracking incidence (25-67%). Maximum protection is achieved when applied close to a rain event (48 hours or less), and a single coating can maintain coverage of up to 10 days, depending on rate of fruit growth (Table 1, Fig. 1).
- Hydrophobic coatings do protect fruit from cracking when rain of 0.5 inches is received within 24 hours (Fig. 1).
- Fruit quality and storage performance was largely unaffected by preharvest coating application (Table 1&2).
- A reduction of the number of applications of RainGard® from three to two did not affect cracking incidence (Table1, Fig.1).
- Tank mixing of RainGard® and gibberellic acid (GA_3) is a practical way to apply the first coating.
- SureSeal (sold as Parka™ since 2013) can cause fruit burning when applied early and/or to sensitive varieties. Some discoloration may be masked by final fruit color.
- VAPORGARD did not reduce rain induced cherry cracking in Tieton. It did leave a sticky residue that decreased fruit shine and caused phytotoxicity.

Objective 2 (cracking prediction):

- Cracking sensitivity development of cherries can be plotted utilizing a modified cracking index based on Christensen (1972).
- High variability exists (both level of cracking susceptibility and onset of sensitivity) within blocks of the same cultivar and within the same block in different years.
- Cracking susceptibility for commercially important varieties was determined (Table 4).
- Cracking susceptibility levels determined with a bench top test correlated well to actual cracking incidence observed in the field after rain events (Fig.1).
- Management decisions regarding use of protective coatings in blocks threatened by rain may be informed by use of the grower bench top test.
- A simplified grower version of the bench top test was developed (www.treefruitresearch.com).

RESULTS & DISCUSSION

Sweet cherry has the highest per acre value of any specialty crop in the Pacific Northwest, but every year some orchards experience crop loss due to rain-induced cracking. Cherry cracking is a complex phenomenon, with a dynamic interplay of tree physiology, fruit surface morphology, and genetic predisposition of fruit (Christensen, 1996). A reduction of cherry cracking can be achieved by a variety of means such as use of protective orchard covers, high velocity air drying, application of osmolytes during rain events, or prophylactic use of hydrophobic coatings (Christensen, 1996; Pennell and Webster, 1996; Schrader et al., 2005). Standard industry practice in the Pacific Northwest has been to reduce the duration of fruit wetness by application of osmotic solutions such as calcium nitrate or by blow-drying the trees with air-blast sprayers or helicopters. Multiple applications of antitranspirants such as VAPOR GARD are used by growers as well, although efficacy against rain-induced fruit cracking is not well established (Richardson, 1998; Schrader et al., 2005; Hanrahan unpublished).

The first commercially available hydrophobic fruit coating is distributed by Valent (formerly sold thru Pace International LLC, Wapato, WA). RainGard® is a mix of natural fatty acids that reduce the direct water absorption through the cuticle (Schrader and Sun, 2006). It forms a waxy, invisible film of the fruit surface and delays the time until fruit cracks and/or reduces the overall amount of cracking (Schrader et al., 2005; Schrader and Sun, 2006). Another fruit coating product developed by Clive Kaiser at Oregon State University has been commercialized in spring 2013. Known previously as SureSeal it is sold as Parka™ by (Cultiva IPM). SureSeal is an elastic, organic biofilm made of edible components (stearic acid, cellulose, calcium) (Kaiser, 2013).

Objective 1: Evaluate and optimize spray programs to reduce rain-induced cherry cracking

Effects of hydrophobic coatings on rain-induced cherry cracking

Between 2009 and 2013, the WTFRC internal program executed 31 field trials, but only 12 sites received sufficient rain to cause 10% or more field cracking in untreated fruit. This threshold mark needs to be reached in order to conduct meaningful product comparisons, because cherry cracking is highly variable. Both hydrophobic coatings (RainGard® and SureSeal) performed well in reducing the incidence of cracking in cherries. In WTFRC trials from 2009-12, RainGard® consistently reduced field cracking by by 25- 57% in trials with significant cracking pressure (Fig. 1; Table 1) and SureSeal reduced cracking by 44-67%. Both coatings remained effective for 5-7 days (max. observed 10 days) and up to 0.56 inches of rain (Figure 1). Single rain events of more than 0.5 inches, like those observed in 2013, did overwhelm the capacity of both products to prevent cracking of fruit (data not shown). SureSeal reduced fruit cracking significantly and consistently in all experiments conducted by WTFRC (examples shown in Table 1), but has proven to be difficult to use under real orchard scenarios: a high volume of water (at least 200 gal/acre) is needed to achieve good performance regardless of planting system, and phytotoxicity may be an issue with some varieties when applied to light green fruit and/or higher than recommended product concentration.

Effects of variable application frequency of RainGard® and tank mixing with GA_3 on rain-induced cracking

One barrier for wider adoption of RainGard® has been the need for 3 separate weekly applications to maintain optimum product performance. Hence, in 2012 we tested two application scenarios: a) three weekly applications of RainGard® and b) two applications of RainGard®: first as a tank mix with GA_3, then followed by a second application of RainGard® alone once fruit had reached a cracking index reading of 20 and before a rain event (Figure 1). Both treatment scenarios resulted in significantly reduced fruit cracking in RainGard® treated areas of the trial orchard (Table 1).

Fruit quality and postharvest performance

Fruit quality was assessed in all five years of the study to ensure that none of the treatments negatively affected fruit quality. Commercially important quality parameters showed year to year variation (Table 1, Table 2). For example, mean fruit size in untreated control fruit ranged from 11.1g in 2012 to 14.5g in 2010. Fruit color had the least amount of yearly variability, while soluble solids content fluctuated considerably (Table 1).

- RainGard® : At harvest, fruit quality of RainGard® treated fruit remained unaffected (example for Tieton in Table 1). The lone exception was the increased mean titratable acidity level for RainGard® in 2011 (Table1). Maturity of fruit stored for two weeks in cold storage was not influenced by in-season RainGard® applications (data not shown). The amount of stem browning, fruit pitting and fruit weight loss in storage was equal between treated and untreated fruit (Table 2), except for 2011 and 2012 (handgun), when RainGard® treated fruit had greener stems after storage.
- SureSeal: At harvest, fruit quality of SureSeal treated fruit remained unaffected (example for Tieton Table 1). Maturity of fruit stored for two weeks in cold storage was not influenced by in-season SureSeal applications (data not shown). The amount of stem browning, fruit pitting and fruit weight loss in storage was equal between treated and untreated fruit (Table 2), except for 2012 (handgun), when SureSeal treated fruit had greener stems after storage.
- VAPORGARD: At harvest, fruit quality of VAPORGARD treated fruit remained unaffected (example for Tieton Table 1). Maturity of fruit stored for two weeks in cold storage was not influenced by in-season VAPORGARD applications (data not shown). The amount of stem browning, and fruit pitting in storage was equal between treated and untreated fruit (Table 2).

Both, Parka and VAPORGARD can cause burning of young fruitlets as reported in 2012 (see cont. report).

Table 1. Effects of preharvest RainGard™ , SureSeal , and VAPORGARD applications on harvest quality parameters of cherries. 'Tieton'/GiSelA6. Pasco, WA. WTFRC 2009-2012.

Treatment	Weight	Acids	Sugars	Firmness	Diameter	Row Size	Color	Cracking [Z]
	(g)	(% malic acid)	(% Brix)	(g/mm)	(mm)		(1-7)	(%)
2009 (grower applied)								
RainGard®	12.2 ns	0.691 ns	14.7 ns	277 ns	30.5 ns	9.1 ns	4.3 ns	15 a
UTC	12.8	0.757	15.7	278	30.9	8.9	4.4	31 b
2010 (grower applied								
RainGard®	14.5 ns	0.52 ns	18.9 ns	227 ns	31.7 ns	8.7 ns	5.3 ns	38 a
Control	14.5	0.49	18.8	238	32.1	8.6	5.4	51 b
2011(grower applied)								
RainGard®	13.7 ns	0.78 a	22.0 ns	348 ns	30.8 ns	8.9 ns	4.4 ns	10 b
SureSeal [Y]	12.4	0.77 ab	20.6	352	30.7	9.0	4.6	9 b
Control	13.4	0.68 b	20.9	322	30.5	9.0	5.4	16 a
2012 (handgun)								
RainGard®	11.1 ns	0.57 ab	14.7 ab	256 ns	26.8 ns	10.2 ns	5.1 ab	11 b
SureSeal	10.2	0.60 a	15.5 a	261	26.7	10.3	5.2 ab	9 b
VAPORGARD	11.9	0.55 b	14.5 b	238	27.0	10.2	4.9 b	31 a
Control	11.1	0.57 ab	14.7 ab	256	26.8	10.3	5.7 a	27 ab
2012 (grower applied)[X]								
RainGard®/GA_3	12.2 ns	0.59 ns	17.2 ns	262 ns	27.3 ns	10.1 ns	4.0 ns	15 b
RainGard®	11.9	0.62	17.3	259	26.8	10.2	4.0	12 b
Control	12.7	0.62	17.6	250	27.5	10.0	4.3	28 a

[Z]on tree reading based on 400 frt./rep; [Y] SureSeal is sold commercially as Parka since 2013; [X] RainGard™ ™ /GA_3 = 1st application as tank mix with GA_3' 2nd application when cracking index exceeded 20 and significant rain in the forecast; RainGard™ ™ = followed weekly application schedule starting at light green.

Table 2. Effects of preharvest RainGard™ , SureSeal , and VAPORGARD applications on stem browning, fruit pitting and weight loss after 14 days of cold storage at 1°C on cherries. 'Tieton'/GiSelA6. Pasco, WA. WTFRC 2009-2012.

	Stem browning				Pitting			Weight loss
	0-25 %	26-50 %	51-75 %	76-100 %	Clean %	Slight %	Severe %	%
				2009(grower applied)				
RainGard®	71 ns[Z]	17 ns	11 ns	1 ns	96 ns	4 ns	0 ns	2 ns
Control	63	21	12	5	92	7	1	3
				2010(grower applied)				
RainGard®	55 ns	27 ns	13 ns	5 ns	77 ns	23 ns	0 ns	2 ns
Control	51	26	13	10	51	32	18	2
				2011(grower applied)				
RainGard®	41 ns	14 b	22 ns	24 ns	90 ns	8 ns	2 ns	6 ns
SureSeal	37	17 ab	22	25	93	6	1	7
Control	25	23 a	18	25	92	6	2	3
				2012(grower applied)				
RainGard®	79 ns	14 ns	5 ns	2 ns	91 ns	7 ns	2 ns	-
RainGard™/GA_3	81	10	7	3	96	4	0	-
Control	83	9	5	2	93	6	2	-
				2012 (handgun)				
RainGard®	84 ns	12 ns	3 b	1 b	89 ns	9 ns	1 ns	-
SureSeal	82	14	3 b	1 b	95	5	1	-
VAPORGARD	77	15	8 ab	1 b	87	11	2	-
Control	72	14	9 a	4 a	92	8	0	-

Objective 2: Track and model rain cracking susceptibility development during maturation

We observed blocks of commercially important cultivars during the month before harvest from 2009-2013. Initial fruit weight averaged 3-4g and color was green to light green. Samples for the artificial cracking test were taken bi-weekly.

Bing cherries in both locations tested in bench-top assays in 2013 were already cracking sensitive at the start of the test series 24 and 25 days pre-harvest. Cracking index (CI) levels in excess of 20 were recorded for 21 or 7 days respectively (Table 3). Based on results from 5 consecutive years, Bing cherries vary considerably, both in the on-set of cracking sensitivity (33-14 days before harvest) and the duration of the phase of high sensitivity (0-21 days).

Of the two Tieton blocks observed in 2013, both were cracking susceptible over a long period of time (22 or 25 days). However, while the block in Zillah sustained CI levels above 20 for 9 days, the block in Sawyer recorded only 4 consecutive days, with one additional day 22 days before harvest. Although Tieton cherries are typically considered prone to rain induced cracking, our data from 5 years suggests variability between years and by block.

Sweetheart, Santina, and Skeena all had prolonged periods of cracking susceptibility, with 13-28 days of potential for cracking (Table 5). Benton was highly cracking sensitive only shortly before harvest in 2013 and 2012.This data corresponds well with industry's experience with these varieties. However, some varieties have not been consistent within years. For example, Sweetheart had a moderate cracking potential in 2012.

Overall, variability in cracking susceptibility as observed especially in Bing, Tieton, Santina, and Sweetheart, highlights the need to supplement general variety knowledge with year-to-year and block-by-block information regarding cracking potential. The bench top test has shown sensitive enough to pick up these swings and we recommend using it in blocks threatened by rain to determine the economic benefits/thresholds of applying protective coatings. For example, in 2012 a Tieton orchard in Pasco sustained 1.02 inches of total precipitation between May 23 and June 7, with three events at or above 0.1 inches, the general threshold for rain induced cracking (Figure 1). The fruit was susceptible during each of the main rain events, and sustained cumulative cracking. In both trials set-up in the Pasco Tieton block, applications of cracking protectants were made ahead (1 or 8 days) of the anticipated rainfall, and significant reductions in damage was observed (Table 1, Fig. 1).

To summarize our experience with locally grown cherry varieties, we have developed a table to show general varietal sensitivity (Table 4). We have added the colum 'variable' to highlight the seasonal swings of some varieties grown in the Pacific Northwest.

Table 3: Days of susceptibility (DOS; CI > 0), days of high susceptibility (DOHS; CI ≥ 20) and maximum cracking index (max CI) for cherry orchards in Washington. WTFRC 2013.

Variety	Location	DOS	DOHS	Max CI
Tieton	Zillah	22	9	22
	Sawyer	25	4*	65
Santina	Outlook 2	24	20	77
	Zillah	28	24	29
	Outlook 3	28	28	55
Benton	Zillah	15	5	70
	Outlook 2	21	7	59
Bing	Zillah	24	7	83
	Outlook 2	25	21	60
Skeena	Outlook 1	13	13	83
Sweetheart	Outlook 1	15	15	88

*1 DOHS @ 22 days before harvest

Table 4: Overall cracking sensitivity of cultivars grown in the Pacific Northwest

High	Variable*	Medium	Low
Early Robin	Sweetheart	Rainier	Regina
Van	Santina		Lapins
Skeena	Tieton		
Benton	Bing		

*Variable = can switch between medium to high sensitivity.

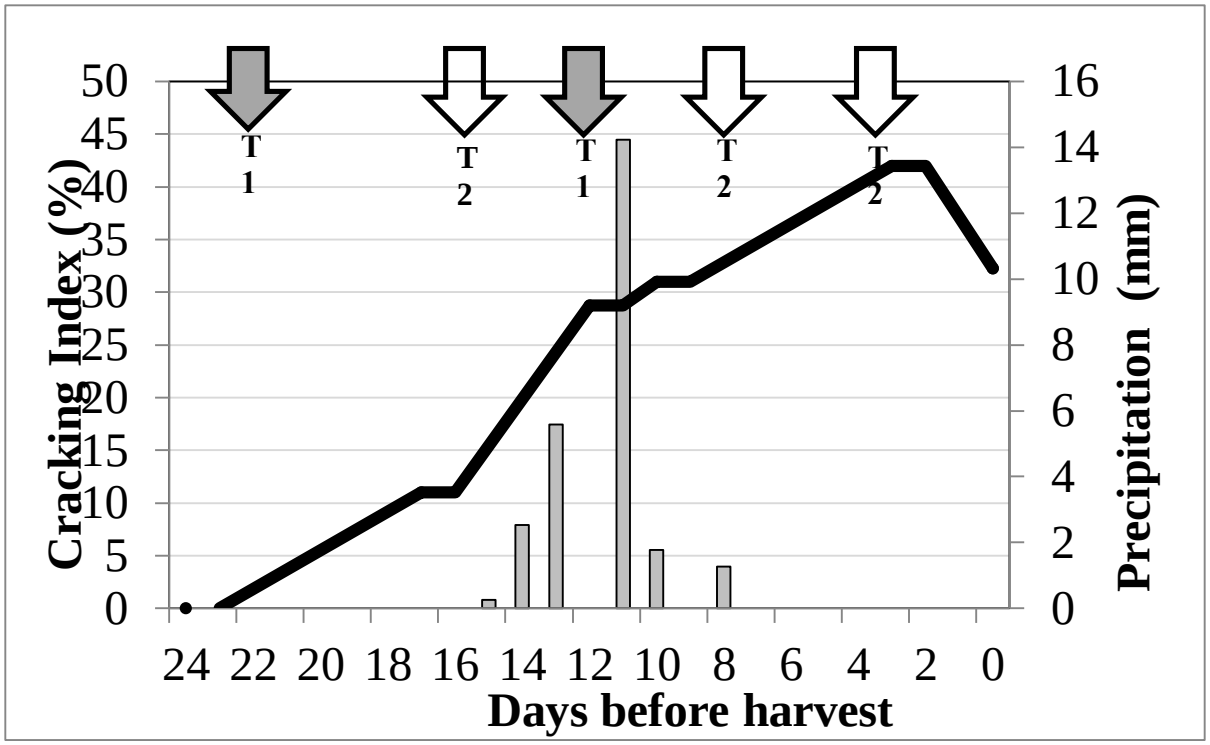

Fig.1. Development of cracking index (%), daily precipitation (mm) and sequence of treatments for 'Tieton'/GiSelA6. Pasco, WA. WTFRC 2012.

T1: First application = RainGard®+ GA_3; Second application: RainGard®
T2: Three times RainGard® application

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EXECUTIVE SUMMARY

Rain exclusion by means of prophylactic spray application of hydrophobic coating materials has been of interest to growers in the Pacific Northwest for the past decade (Schrader and Sun, 2006). Hence, applied horticultural field trials to test overall performance of RainGard®, the only commercially available product until 2013, were initiated by the WTFRC in 2007. In 2013 another hydrophobic coating, SureSeal (trade name: Parka™), was commercialized. Comparitive performance data between antitranspirants (VAPORGARD) and hydrophobic coatings (SureSeal, RainGard®) has been generated between 2012 and 2013.

Although we have conducted 31 trials in commercial orchards since 2007 (more than 50 overall), only a limited amount of information has been generated due to lack of adequate rain events. In the experiments described in this report, RainGard® and SureSeal reduced cracking incidence of fruit significantly and consistently, even under strong rain pressure (up to 0.56 inches).

However, RainGard® application schedules of three weekly applications, as suggested by the manufacturer, pose a significant cost to growers and reduce availability of equipment and personnel needed for other activities such as harvest. Hence, optimization of spray programs by 1) tank mixing product with GA_3, and/or 2) reduction of the number of total applications, can further increase the attractiveness of RainGard® and similar products. Our trials have demonstrated that a reduction of the number of applications from three to two did not reduce effectiveness of the product. If timed correctly, less applications may be sufficient to effectively coat the fruit. To achieve this, knowledge of actual cracking sensitivity of blocks prior to threatened rain events is needed and can be easily achieved with a simple grower version of the bench top test developed during the course of this study.

FINAL PROJECT REPORT (2013)

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

PI: Fredrick A. Bliss
Telephone: (530) 756-5154
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Address: 214 Inca Pl.
City: Davis
State/Zip: CA 95616

Cooperators: Jim McFerson, Cameron Peace, Nnadozie Oraguzie, Amy Iezzoni, Dorrie Main, Yanmin Zhu

Total Project Funding: $7,500

Budget History:

Item	2013	Year 2:	Year 3:
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel	$4,000		
Plot Fees			
Miscellaneous	3,500		
Total	$7,500		

ORIGINAL OBJECTIVES:

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

- 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.

- Facilitate adoption and use of technology and materials from the RosBREED project to support sweet cherry improvement.

- Interact with WSU, OSCC 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 with Jim McFerson

- Attended the NW Cherry Research Review Nov. 13, 2012 in Yakima WA
    - Presented assessment of and discussed proposals
    - Participated in Cherry GGB workshop prior to the Cherry Research Review

- Participated in RosBREED annual review as a member of the Scientific Advisory Panel January 10, 2013, San Diego, CA
    - Served on Scientific Advisory Panel
    - Evaluated results and outcomes of activities in the RosBreed project as a member of the Scientific Advisory Panel.
    - Evaluated results from the participating projects

- Facilitated interaction among breeders and scientists.
    - 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 and DISCUSSION

I provided scientific reviews to the commissions of proposals and reports for which I have expertise. In addition to those coming to this program, 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 show innovative ideas and approaches that I believe contribute to continued competitiveness and opportunities for funding.

The cherry GGB workshop organized by Dr. Oraguzie and held prior to the Research review provides 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 is in its final months, with many of the activities focused on achieving the multi-faceted milestones and goals. Several scientists associated with the PNW Cherry Improvement have played key roles in the success of RosBREED and the ongoing Genome Database for Rosaceae (GDR). Both of these projects have been very important for implementing the cherry breeding program and will have a continuing impact on the ultimate success, measured by the development and release of outstanding new cultivars for the cherry industry. The WTFRC and OSCC have been key supporters of these initiatives, providing 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.

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. This is the only public sweet cherry breeding program in the U.S. taking advantages of these key resources.

DNA-based information and technology are critical plant breeding capacity elements for success and efficiency. Diagnostic marker-locus-trait (M-L-T) associations are available for incompatibility/fertility alleles, fruit maturity date, fruit size, firmness, color, flavor components. Others nearing utility include flowering time, cracking, stem retention force. Using features 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 marker assisted selection of preferred genotypes, genetic verification of selected phenotypes, and genetic fingerprinting of elite selections for intellectual property protection.

Key collaborations 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). Interaction among these (and other) programs is critical to continued support and success. Especially with reduced budgets for research and development in the public sector, it is important to prioritize activities for 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 encouraging. 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. New elite selections will be identified each year in the breeding cycle. Thus, 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.

The 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 the RosBREED project to support sweet cherry improvement; and 4) interact with WSU, OSCC 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 Cherry GGB Workshop prior to the annual research meeting; 3) serving the RosBREED project as a member of the Scientific Advisory Panel; 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.

I provided consultant services to the WTFRC and OSCC about cherry improvement. My role is to provide information and feedback to Jim McFerson and Board members from Washington and Oregon 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 provide insight, guidance and ideas for identifying and applying appropriate technology to facilitate efficient cultivar development. I evaluate research proposals when requested.

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. This is the only public sweet cherry breeding program in the U.S. taking advantage of these valuable resources. DNA-based information and technology are critical plant breeding capacity elements for success and efficiency. Key collaborations 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). Interaction among these (and other) programs provides for continued support and success. The advancement of promising new selections into pre-commercial testing and evaluation is encouraging. 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.

The consulting project budget included $4,000 for travel to Wash. State for project review and related activities and $3,500 for miscellaneous expenses related to consulting. I will spend less than the amount budgeted.

NEW PROJECT PROPOSAL **PROPOSED DURATION**: 1 year

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

PI: Fredrick A. Bliss
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Address: 214 Inca Pl.,
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Cooperators: Jim McFerson, Cameron Peace, Nnadozie Oraguzie, Amy Iezzoni

Total Project Request: Year 1: 7,500

Other funding sources: None

Budget 1
Organization Name: Fred Bliss **Contract Administrator:**
Telephone: **Email address:**

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

Footnotes:

Justification

The focus of this proposal will be on consultation to the WTFRC and OSCC members and boards for assessing technical quality of proposals and reports and providing ideas and strategies to enhance adoption of new cultivars and new technology for greater productivity. I will provide information and feedback to Jim McFerson and Board members about progress toward breeding research objectives and give evaluation and critique of research proposals and reports as requested. Additionally, I will continue to work with scientists on issues about which they request my input.

The budget includes $4,000 for travel to the research project review (if needed) and $3,500 for miscellaneous expenses related to consulting. Much of my work is done from home in Davis, CA through electronic correspondence and use of conference calls for members of the project and industry. I am paid only for work and services actually provided; thus the annual cost depends on how much is requested of me.

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.

Methods

My role as a consultant to the WTFRC and OSCC will be to provide information and expert opinion about:

- Technical aspects of proposals and reports for competitive funding of research and development related to cherry improvement. I will review and analyze the proposals and reports for their appropriateness and likelihood of technical success and provide written and oral appraisals as requested.
- Optimal alignment of breeding program goals, target traits and operations with grower and consumer needs in sweet cherry commercial target market production areas in the PNW.
- Strategies and approaches to identify appropriate target market cultivars for testing and comparison to elite new selections and ways to facilitate adoption and use of new superior clones in key areas.
- Identification and integration of effective marker-locus-trait combinations for the breeding program.
- Optimal integration of genomics tools and information from research project for improving sweet cherry breeding operations and testing of elite selections to minimize time to commercialization and improve reliability of performance in commercial target markets.

- Suggestions for sustainable, long-term funding support for core breeding operations and evaluation of new elite cherry selections.

Liaison with the WTFRC and OSCC will be through Jim McFerson, with whom I maintain active contact. I will interact directly with other cooperators on an as-need basis. I will provide a liaison with various external groups on scientific matters. I expect to travel to the PNW for the research review and other related activities if requested. Prior to travel I will seek approval from Jim McFerson for any large amounts.

Proposed schedule of activities:

- Review research proposals, program plans, activities, documents and reports etc. for the Commission and breeding team members as needed throughout the year.
- Participate in the annual NW Cherry Research Review and GGB workshop and travel to other related events if requested.
- Interact and provide liaison with research projects about breeding, genetics and genomics of fruit crops, and with WSU faculty about education and training of plant breeding graduate students.
- Submit invoices for consulting on a quarterly basis.
- Provide final annual report of activities.

FINAL PROJECT REPORT

Project Title: Consulting for the Pacific Northwest sweet cherry breeding program

PI: Amy Iezzoni
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Address: 2075 Hamilton Road
City: Okemos
State/Zip: Michigan 48864

Cooperators: Nnadozie Oraguzie and other members of the cherry team (Matt Whiting, Cameron Peace, Amit Dhingra and Fred Bliss)

Other funding sources

Agency Name: USDA-CSREES NRI Plant Genome
Amount awarded: $400K, Aug 2009 – Aug 2013
Notes: "The development of COS markers for comparative mapping in the Rosaceae and their application for understanding variation in fruit size". PI: Iezzoni. Develop and validate fruit size genetic markers for sweet cherry and new state-of-the-art marker development for cherry. Leveraged with WTFRC/OSCC funding.

Agency Name: USDA-CSREES Specialty Crops Research Initiative
Amount awarded: $7.2 mil plus equal matching, Sep 2009 – Aug 2013
Notes: "RosBREED: Enabling marker-assisted breeding in Rosaceae". PI: Iezzoni. Co-PIs include Peace and Oraguzie. Broad umbrella project on genetic marker development and application. Leveraged with WTFRC/OSCC funding.

Total Project Funding: $ 13,000

Budget History:

Item	**2013**		
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel & expenses	3,000		
Consulting fee	10,000[a]		
Miscellaneous			
Total	$ 13,000		

[a]These activities, which began in 2004, have historically been funded as a consulting arrangement. This was done so that Michigan State University would not be a shared "inventor" of the forthcoming sweet cherry cultivars. I then waived my personal "inventor" rights to any cultivars in exchange for a consulting fee that I donate to MSU to help support the MSU tart cherry breeding program.

OBJECTIVES:

1. Assisted in generating breeding populations. This included developing the crossing plan, sourcing germplasm, and making crosses along with the breeding team.
2. Provided horticultural guidance. This was provided by site visits, phone consultations, and sharing results from my cherry research at MSU.
3. Provided genetic expertise. My cherry genetics team is currently developing the genetic infra-structure for the PNW sweet cherry breeding program in collaboration with C. Peace to include the development of DNA markers and genotyping of many of the parents used in the program. This work is funded by USDA grants.

SIGNIFICANT FINDINGS/ACCOMPLISHMENTS:

- Visited Prosser in March and discussed recent genetic findings and spring crossing plans. Specific parents that are likely to transfer early maturity, self-fertility and fruit firmness to their progeny were identified.
- The recommendation was made to re-evaluate the use of precocious abundantly flowering rootstocks for some of the seedlings predicted to have the most potential as parents. In certain cases, this benefit may be cost effective as it would reduce the number of years per generation.
- Continued to provide new specific information to C. Peace on new markers that can be used to refine performance predictions based on the region on cherry linkage group 2 that controls variation for fruit size.
- Three other regions on the cherry chromosomes were identified that contain loci associated with fruit size. Other traits associated with these same genomic regions were also summarized. Use of this new knowledge was shared with N. Oraguzie and C. Peace with the goal of them using this information to identify parents and seedlings that transfer large fruit size to their offspring along with other desired characteristics.

RESULTS and DISCUSSION:

Assist in generating breeding populations & provide horticultural guidance.

In March 2013 I traveled to Prosser to meet with N. Oraguzie, C. Peace and members of their teams to discuss progress in the genetic understanding of important traits and to plan for spring activities, specifically spring crossing. One of the topics of utmost importance was which parents to use for spring 2013 crosses to transfer early maturity along with self-fertility from the cultivar 'Cristobalina'. Results from the Peace lab had identified 16 offspring from the cross of 'Rainier' × 'Cristobalina' that were predicted to be self-fertile and have large fruit size alleles. Of these individuals, 5 also had very early maturity dates. Upon visiting these own-rooted seedlings in the field, it was determined that the flower numbers were very low, thus the pollen available for spring crosses was limited. This illustrates the benefit of using a precocious rootstock to increase flower number on potential parents. It was recommended that if DNA diagnostics can be used in the early stages of seedling growth, to identify a seedling as a potential parent, this seedling could be grafted on a precocious rootstock as soon as possible to ensure an early and abundant pollen supply.

A second topic of discussion was fruit firmness. The Spanish cultivar 'Ambrunes' has very firm fruit and many of its offspring have inherited this trait. However, none of these immediate 'Ambrunes' descendants are suitable as cultivar candidates due to small fruit size. Therefore, it is very important to continue the breeding with these 'Ambrunes' offspring used as parents for the next generation so that this firm-fruited characteristic can be transferred into future cultivar candidates.

As illustrated by these examples of early maturity and firmness, the most desirable parents to be used in crosses are now most likely seedlings that are determined to have the best breeding values (a quantitative genetics principle) and the most desirable trait locus alleles (a molecular genetics principle) for the traits of interest, rather than simply having the best performance (an antiquated pre-genetics principle). Therefore, a concerted effort is needed to evaluate the genetic potential of superior seedlings, not only for candidate cultivar status but as parents for crossing in spring 2014. Additionally, it would be important to re-evaluate the use of precocious, abundantly flowering rootstocks for some of the seedlings predicted to have the best potential as parents. In certain cases, this benefit will reduce the number of years per generation.

Provide genetic expertise

In 2012, a genome scan made up of thousands of anonymous markers was developed by the RosBREED genetics and genomics team. This genotyping array technology was made available through a commercial partner, Illumina Inc. These arrays were used to genome-scan 480 sweet cherry individuals. The arrays were run and scored by the team at Mich. State Univ., resulting in successful assessment of allelic states at ~1,900 positions along the eight cherry chromosomes of any individual scanned (Peace et al. 2012; Klagges et al. 2012). Collectively, these efforts provided the building blocks that allowed us to seek new practical knowledge of genetic diversity and trait inheritance in sweet cherry breeding germplasm. Examples of these outcomes are described below.

Using the available genetic marker data, 'Napoleon' was determined to be 'Bing's paternal parent (Rosyara et al. 201x). 'Napoleon' is also the grand parent of 'Stella', the self-fertile cultivar that is the ancestor of all the self-fertile cultivar released from the sweet cherry breeding program in British Columbia (BC) (Fig. 1). This indicates that the genepools used by both the BC program and the original WSU sweet cherry breeding program were very limited with extensive overlap. Our analysis also indicated that some of the parents that I used in 2004 to broaden the genetic base and introduce unique genetic diversity, such as 'Regina' and 'Ambrunes', are not related to 'Bing'. Understanding how to use genetic knowledge to transfer the superior attributes of these parents to elite cultivar candidates is a critical goal for which progress is being made.

Years ago, I initiated an effort to understand the genetic control of fruit size in cherry, because in my experience large-fruited progeny individuals were very rare. This suggested that marker-assisted selection could significantly increase the efficiency of achieving large fruit size. Fruit size data from the PNW sweet cherry breeding program supports this observation of the rarity of large-fruited seedlings. Therefore, using the available genetic data, we concentrated our efforts on the most important fruit size genomic region that is located on cherry linkage group 2. This genomic region is now used for marker-assisted selection in the PNW cherry breeding program as certain alleles for this trait locus are predictive of large versus small fruit size. However, this region also appears to be associated with other traits such as firmness, bloom time, and fruit cracking (Castede et al. 2012; Quero-Garcia 2012). Therefore we have increased marker density in this region with the goal of determining if the genetic control of firmness, for example, can be separated from that of fruit size (De Franceschi et al. 2013).

Although there is a major locus controlling fruit size on cherry linkage group 2, we identified other genomic regions also containing loci associated with fruit size. These are located on linkage groups 1, 3, and 6 (Fig. 2) in sweet cherry (Rosyara et al. 2013) plus linkage group 5 in tart cherry (Stegmeir 2013). The region on linkage group 3 that is associated with genetic variation for fruit size also contains the major locus for fruit skin and flesh color and the locus associated with 'Cristobalina' derived self-fertility (Sooriyapathirana et al. 2010; Fig. 2). The region on linkage group 6 containing the fruit size locus is linked to the self-incompatibility locus. Therefore, using DNA diagnostics to achieve desired phenotypes will require an understanding of segregation for multiple traits at a time and multiple genetic locations.

The importance of considering all the chromosomal regions containing loci associated with fruit size together is illustrated using fruit size data from progeny from the cross 'Regina' × 'Lapins' provided by E. Dirlewanger and J. Quero-Garcia (Rosyara et al. 2013) (Fig. 3). 'Regina' is predicted to be heterozygous for the loci associated with fruit size variation on linkage groups 1, 2, 3 and 6, where each trait locus can have alleles that either confer large fruit or small fruit. 'Lapins' is predicted to have two copies of the alleles associated with large fruit size on linkage groups 1 and 3, but only one allele for large fruit size for the loci on linkage groups 2 and 6. Of the ~200 offspring from the cross 'Regina' × 'Lapins', the seedling with the largest fruit was predicted to be homozygous for the large fruit allele at three of the four fruit size loci (Fig. 3). In contrast, the offspring with the smallest fruit had two copies of the small fruit allele for the trait loci of linkage groups 2 and 6 and only one copy of the large fruit allele for the other two loci. The fruit size difference between these two offspring was more than two-fold (5.4 g versus 11.1 g). This example illustrates how genetic knowledge of these four trait loci can predict a large difference in fruit size. Putting genetic knowledge of these other fruit size loci into application in the PNW cherry breeding program is a current goal.

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Klagges C, Campoy JA, Quero-Garcia J, Guzman A, Mansur L, Gratacos E, Silva H, Rosyara, UR, Iezzoni A, Meisel LA, Dirlewanger E. 2013. Construction and comparative analyses of highly dense linkage maps of two sweet cherry intra-specific progenies of commercial cultivars. PLoS ONE 8(1): e54743.

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Fig. 1. Pedigrees for sweet cherry cultivars bred in the PNW (modified from Rosyara et al. 201x). 'Napoleon' is the grandparent of 'Stella' and the newly identified paternal parent of 'Bing'. This shared ancestry illustrates the narrow gene pool used in breeding new sweet cherry cultivars for the PNW. The intensity of grey indicates the degree of relationship to 'Stella' according to pedigree records while a clear box indicates no known pedigree relationship to 'Stella'.

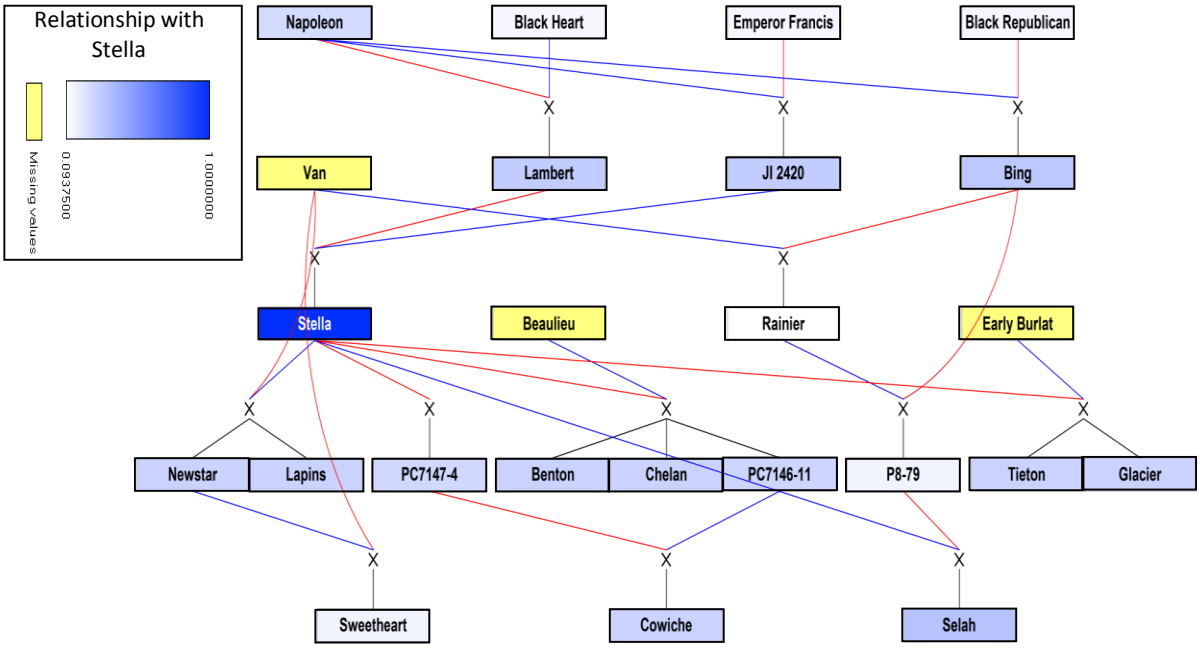


Fig. 2. Major loci associated with trait variation that have been identified on the eight cherry chromosomes (from 'Jewels in the genome: The Necklace, by A. Iezzoni in Weebadde et al. 2013). Fruit size trait loci have been identified on linkage groups 1, 2, 3, and 6 and on group 5 in tart cherry. The fruit size locus on group 3 co-locates with major loci associated with fruit skin and flesh color and 'Cristobalina'-derived self-fertility. The fruit size locus on group 6 co-locates with the locus controlling self-fertility and cross-compatibility. In tart cherry, a major locus associated with cherry leaf spot resistance was identified at the top of linkage group 4.

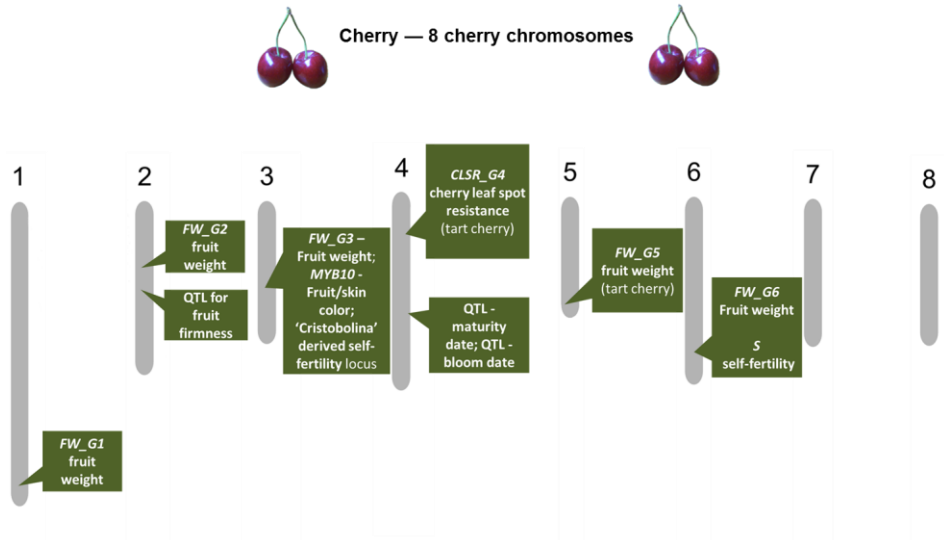
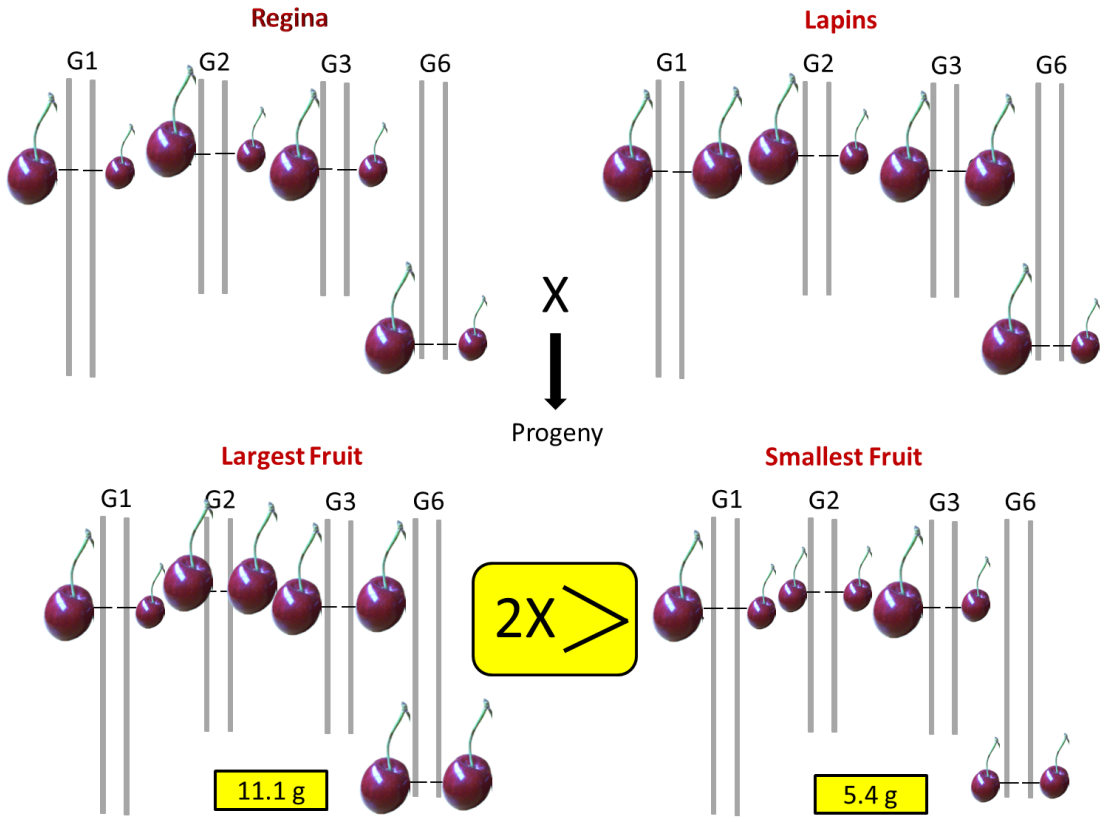


Fig. 3. Regions on cherry chromosomes 1, 2, 3, and 6 containing loci associated with fruit size in sweet cherry illustrated for 'Regina' and 'Lapins' and two of their offspring that contrast for fruit size (modified from Rosyara et al. 2013). The offspring that has the largest fruit size inherited the most large fruit alleles from its parents while the offspring that has the smallest fruit size inherited the most small fruit alleles from its parents.



Consulting for the Pacific Northwest Sweet Cherry Breeding Program

A. Iezzoni

EXECUTIVE SUMMARY:

The building blocks of a successful breeding program include the use of diverse germplasm, generation of large numbers of progeny populations for evaluation, appropriate horticultural management of the breeding materials, the ability to identify and commercialize superior cultivar candidates, and judicious use of genetics knowledge. The goal of my consultancy with the PNW sweet cherry breeding program was to assist in our ability to excel at all of these objectives so that we can deliver superior sweet cherry cultivars to the Oregon and Washington industries as quickly as possible. In 2013, parents were identified that confer early maturity, self-fertility, and firm fruit. These attributes were inherited from new germplasm introduced in the breeding program through crosses that began in 2004. This new germplasm and the novel attributes that this germplasm provides will increase the likelihood that the PNW program will identify elite cultivars that meet the maturity date and fruit quality targets. Major progress was made identifying genomic regions associated with fruit size. Knowledge for the linkage group 2 locus associated with fruit size is already being used routinely each year to select parents that are more likely to confer large fruit and eliminate seedlings predicted to have small fruit prior to field planting. These other fruit size trait loci are also targets for use in marker-assisted selection to increase the precision of fruit size predictions. Knowledge of what other traits are also associated with these loci has been summarized so that genetic improvement for multiple traits can occur simultaneously. Continued collaboration, whereby I contribute my time and knowledge of cherry germplasm, breeding, and genetics, will help us achieve our collective vision of a cost-effective aggressive and successful sweet cherry breeding program.

*RosBREED team members who have and continue to contribute substantially to this cherry genetics effort are Cameron Peace, Dorrie Main, Nahla Bassil, Umesh Rosyara and Audrey Sebolt.

CONTINUING PROJECT REPORT **PROPOSED DURATION**: Year 2 of 3
WTFRC Project #: CH-12-107

Project Title: PNW sweet cherry breeding and genetics program

PI:	Nnadozie Oraguzie	**Co-PI**:	Cameron Peace
Organization:	WSU-IAREC	**Organization**:	WSU-Pullman
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Address:	24106 N Bunn Road	**Address**:	39 Johnson Hall
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City/State/Zip:	Prosser, WA 99350	**City/State/Zip**:	Pullman, WA 99164

Cooperators: Todd Einhorn, Lynn Long**,** Ken Eastwell, James Susaimuthu, Amit Dhingra, Matt Whiting, Dorrie Main, Tom Auvil, Ines Hanrahan, Jim McFerson, Willow Drive Nursery, Amy Iezzoni, Fred Bliss

Budget: **Year 1**: $144,918 **Year 2**: $152,028 **Year 3**: **$204,100**

Other funding sources

Agency Name: USDA-CSREES Specialty Crop Research Initiative
Amt. requested/awarded: $3.4M plus equal matching Sep 2009-Aug 2013
Notes: "A total systems approach to developing stem-free sweet cherry production, processing, and marketing system". PI: Whiting. Co-PIs include Oraguzie and Dhingra

Agency Name: USDA-CSREES Specialty Crop Research Initiative
Amt. requested/awarded: $2.1M plus equal matching Sep 2009-Aug 2013
Notes: "Tree Fruit GDR: Translating genomics to fruit tree agriculture". PI: Dorrie Main. Co-PIs include Oraguzie and Peace.

Agency Name: USDA-CSREES Specialty Crops Research Initiative
Amount awarded: $7.2 mil plus equal matching, Sep 2009 – Aug 2013
Notes: "RosBREED: Enabling marker-assisted breeding in Rosaceae". PI: Iezzoni. Co-PIs include Peace, Oraguzie, and Main.

Agency name: WTFRC/OSCC
Amount awarded: $59K for 2012
Notes: "Targeting the ethylene response pathway to improve cherry quality". PI: Peace

Agency name: WTFRC/OSCC
Amount awarded: $79K for 2010-2012
Notes: Start-up funds and support for a full time technician with Oraguzie as PI

Budget Narrative

1. Core breeding activities ($101-110K)

Approximately ¾ of the budget is for core breeding activities including personnel costs, wages, land use fees, and plot establishment and maintenance. Personnel costs include salaries and benefits for 0.25 FTE technical support for breeding operations in Prosser, and 0.15 FTE technical support for the Genetic Screening Technician in Pullman to conduct DNA testing for marker-assisted seedling

selection (MASS) and marker-assisted parent selection (MAPS). Although in previous years the latter cost was included in research projects led by Dr. Peace, the now routine service provides DNA test results for thousands of germplasm individuals leading to significant resource savings over purely traditional breeding practices. As a case in point, WSU-IAREC, Prosser, introduced a land use fee of $475/acre/year in July 2010, which is the area taken up by every 900 seedlings. Further, plot establishment and maintenance cost has risen from $2.5K to $4.5K/acre/year to cover wages for orchard staff involved in plot establishment and maintenance, fees for hiring heavy orchard equipment, cost of consumables for plot maintenance, etc. For every one thousand seedlings culled using DNA test results, $5.5K is therefore saved per year, and because seedlings normally grow in the orchard for five years before the end of Phase 1, a projected $28K in orchard costs is saved for every 1000 seedlings culled. In 2013, 1400 seedlings were culled – providing a projected savings of about $40K, *four times* the total cost of supporting the DNA testing service (which also included genotyping of parents and advanced selections, of which the further economic value to the breeding program is not factored in here).

2. Evaluation of advanced selections ($40-45K)

This modern young breeding program has entered a new stage in its development with the commencement of multi-site replicated selection trials. Testing and evaluation of advanced selections in Phase 2 will require $40-45K. Propagation of breeding parents in 2013 was a one-time investment (~$9K) to provide grafted trees of promising F_1 progeny for inter-mating or back-crossing to parents with elite background. Use of propagated trees for crosses enhances flowering and improves fruit set unlike own-rooted seedlings. Planting of advanced selections at two trial sites, WSU Prosser and OSU MCAREC at Hood River, will cost ~$8K/year/site. Items of expenditure were mainly personnel costs, land use fees, and plot establishment and maintenance. The Prosser breeding technician was also partly involved in Phase 2 tree planting, maintenance, and performance evaluations (0.25 FTE).

Supporting funding

Funds from the stem-free sweet cherry SCRI project were used to provide critical upstream DNA information toward developing genetic markers and too develop a routine phenotyping protocol for pedicel fruit retention force.

Outcomes from the SCRI-funded RosBREED project for use in the breeding program included socio-economic values for trait targets and software-based tools for pipelining new genomics discoveries into breeding operations, and DNA information for high-value traits leading to new genetic tests. Funds from the project coming directly to the breeding program ($7K/year) supported fruit quality evaluations not covered in the breeding program, genomics discoveries, and the refinement of new genetic tests.

Washington State University-Irrigated Agriculture Research and Extension Center contributed $10,000 towards the installation of a system for bar-coded labels.

Wisdom of breeding consultants Drs. Bliss and Iezzoni was incorporated into core breeding activities, evaluation of advanced selections and allied research programs, and guided strategic planning for a transparent and streamlined breeding program that generates innovative genetic solutions for the coming decades of the PNW sweet cherry industry.

WTFRC Collaborative expenses:

Item	2012	2013	2014
Salaries +benefits	1,500	8,700	15,000
Wages	800	4,400	8,000
Benefits	320	1,700	3,200
RCA Room Rental			
Shipping			
Supplies		200	600
Travel	500	1,500	1,500
Miscellaneous			
Total	3,120	16,500	**28,300**

Footnotes: The funds are for phase 2 tree evaluation.

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

Item	2012	2013	2014
Salaries	24,646	25,632	46,035
Benefits	9,813	10,247	21,178
Wages	16,800	17,472	18,171
Benefits	12,953	13,471	14,010
Equipment			
Supplies	7,000	5,000	4,000
Elisa test	600	600	600
Land use fee	7,125	4,750	4,750
Plot establishment and maintenance	40,500	40,500	40,500
Travel	4,914	3,000	3,000
Miscellaneous			
Total	124, 351	120, 672	**152, 244**

Footnotes: Salaries include 1.0 FTE for Breeding Technician (combining CH-13-102 and this project) and 0.15 FTE for Terry Rowland (full-time genetic screening technician in Pullman's Pacific Northwest Tree Fruit Genotyping Lab). The other 0.5 FTE salary for Breeding Technician comes from WTFRC/OSCC funded project # CH-10-110. Wages include the equivalent of 5 temporary employees during spring and summer months. Supplies include propane, soil, stakes, chemicals and other lab consumables. Elisa test is conducted on approximately 20 cultivars at bloom time for $30/tree. Land use fee is $475/acre. Plot maintenance fee is ~$4,500/acre.

Budget 2: Willow Drive
Organization Name: Willow Drive Nursery Inc. **Contract Administrator:** Hal Leedy
Telephone: 509 787 1555 **Email address:** Hal@willowdrivenursery.com

Item	2012	2013	2014
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Tree propagation:			
Advanced selections	13,593	13,593	**13,593**
Parents	677	677	**677**
Breeding parents/Diversity set		9,024	
Miscellaneous			
Total	14,270	23,294	**14,270**

Footnotes: Tree propagation fee is $11.23 per tree. Sixty trees of 12 advanced selections will be propagated per year. Parents include market leading cultivars and checks planted alongside advanced selections. Breeding parents/diversity set includes F_1 progeny, modern cultivars and ancestors propagated for use as breeding parents, and for a workhorse pedigree set of multiple populations established in the RosBREED project for identifying and refining marker-locus-trait associations.

Budget 3: OSU (Todd Einhorn)
Organization Name: OSU-MCAREC **Contract Administrator:** L.J. Koong
Telephone: 541-296 5494 **Email address:** l.j.koong@oregonstate.edu

Item	2012	2013	2014
Salaries[1]	1,539	2,376	**2,447**
Benefits	1,154	1,782	**1,835**
Wages[2]	0	500	**1,500**
Benefits	0	50	**150**
Equipment			
Fees and Supplies[3]	3,604	3,354	**3,354**
Travel	0	0	**0**
Miscellaneous			
Total	6,297	8,062	**9,286**

Footnotes: [1]Salaries are for: 0.039 FTE (2 weeks) for technician in year 1, and 0.058 FTE (3 weeks) in years 2 and 3; to include planting, irrigation, tree training, data collection (bloom, harvest, fruit quality). OPE rate is 75%. A salary increase of 3% is factored into years 2 and 3. [2]Wages are for one part-time employee ($10/hr) to assist with tree planting, and data collection in years 2 and 3; OPE is 10%. [3]Supplies include bird netting, filters for juice analysis, lab tape, and labels. Fees include per acre research plot fee: $3,104.

Objectives

1. Develop and utilize best management practices for optimal seed germination and accelerated development of healthy seedlings in the greenhouses and for field maintenance and development of superior horticultural practices that accelerate seedling growth and development and reduce time to flowering and fruiting
2. Use elite selections from the breeding program and new external sources of genetic superiority as parents for hybridization and selfing to produce seedling populations that segregate for target traits critical to each target market group
3. Integrate genomics knowledge, marker-assisted breeding tools, and classical breeding methods into the breeding program to optimize use of resources and reduce time to release of elite selections with commercial potential
4. Develop and implement a cost-effective strategy for collaborative breeder-grower identification and evaluation of elite new selections from the breeding program
5. Identify in Phase 2 at least one elite selection from any target market class that exceeds the threshold values for the primary and secondary traits of that target market class for advancement to Phase 3

SIGNIFICANT FINDINGS AND ACHIEVEMENTS

- The breeding program has advanced to the next generation, using F_1 progeny as breeding parents –allowing us to focus on such traits as powdery mildew resistance and self-fertility while still maintaining effort in extending the early and late market windows.
- We have generated super-early progeny by integrating early-ripening trees produced in the 1970 and 1980s into the crossing strategy. Our experience with viability of the resulting early seed has been positive.
- A few of the available 300 'Rainier' x 'Cristobalina' offspring were identified via MAPS as being suitable as parents for introgression breeding for valuable new sources of extra-earliness and self-fertility.
- Several crosses involving 'Glacier', 'Kiona', and 'Tieton' were performed to increase the incidence of a relatively rare genotype which should confer large fruit size, and very firm fruit texture as well as the self-fertility of these cultivars.
- We continue our use and search for further optimal horticultural management practices that will ease controlled pollination efforts and encourage fruit set.
- Seed germination and seedling survival rates remain high.
- Genetic tests for self-fertility and fruit size were used for routine seedling selection. In addition, DNA evidence was used to confirm or deduce parentage. New marker-locus-trait associations are being discovered and new DNA tests are being developed to use in marker-assisted breeding strategies for additional desirable traits.
- Culling of genetically inferior seedlings using DNA tests now occurs before transition to the greenhouse, resulting in significant reductions in resource use. In 2013, more than 1400 seedlings were culled (80% of the 1800 tested), providing an estimated net projected savings of ~$30K in resource allocation.
- Tall (3- to 4-foot) saplings are routinely produced in the greenhouse, reducing generation time such that fruit is often now produced in the third year.
- Finding selections to fit the late-ripening market category has been a challenge. To address this issue in the next few years, many crosses have been made using 'Sweetheart' with other late varieties although the resulting progenies have not yet produced fruit. This year, using

DNA information, we chose a selection from the 1970s to cross with seedlings having high breeding value for lateness and firmness, and crosses were also made to capitalize on powdery mildew resistance along with lateness and good fruit quality.

- A total 32 advanced selections have been made from Phase 1, of which 16 are currently planted in Phase 2 trials at WSU-IAREC, Prosser, WA and OSU-MCAREC, Hood River, OR.
- Based on input from strategic BPAC members and continued observation of horticultural performance, fruit production, and quality, several advanced selections were identified as flawed and discontinued.
- For future Phase 3 plantings, collaboration between the breeding program and grower-cooperators is in progress at two sites: an early site represented by a farm in Pasco, WA and a late site represented by a farm in Wenatchee, WA.
- One Phase 2 selection was advanced to Phase 3 and will be planted into Phase 3 trials in spring of 2014 at two grower-cooperator sites in Washington. The selection, belonging to the ESM market class (early, self-fertile, and mahogany), is larger and firmer than 'Chelan' and has better flavor.
- Another early-ripening selection with fruit quality superior to 'Chelan' recently selected for advancement to Phase 2 may be fast tracked to Phase 3.

RESULTS AND DISCUSSION

1. Develop and utilize best management practices

We have continued to record more than 60% seed germination and more than 95% seedling survival in Phase 1 (Table 1) involving own-rooted trees. In addition, transition of seedlings from the growth room to the greenhouse is now limited to those that show DNA-based genetic potential, thus saving money on potting soil, labor, and greenhouse space. This savings is enabled by carrying out genetic tests and culling inferior seedlings when the seedlings are ~2 months old in the growth room. In the past, genetic tests were performed on 9-10 month-old seedlings prior to field planting, a slightly more costly stage. Another significant achievement is production of 3- to 4- foot tall trees in less than one year prior to field planting. This rapid growth helps to reduce generation time as these trees start flowering two years after field establishment and become fully productive in their third year, saving at least a year from previous practices.

For updates on outcomes of the efficient new bar-coding system, protective bird netting, and strategic tree pruning and training that also supported this modern, streamlined breeding program, please refer to the final report on the project titled "Support for a full time technician", CH-13-102.

Table 1: Summary of seedling material developed during 2010-2013 in the PNWSCBP

Characteristic	**Year of crossing**			
	2010	**2011**	**2012**	**2013**
No. of new parents used	29	6	61	85
No. of crosses made	107	74	50	165
No. seed	2610	1162[X]	4139	1325[X]
% germination	60	62	72	na
No. of seedlings	1580	724	1800[a]	na

No. of seedlings in field	776[y]	324[z]	399	na
No. of full sib families with >9 individuals	7	5	6	na

[a] Following germination, seedlings suspected to be open pollinated were removed; [X] Low fruit numbers recorded due to frost damage; [Y] Following marker-assisted seedling selection (MASS); z Early ripening crosses and mid-season and late powdery mildew resistant trees only; na = data not available.

2. Produce seedling populations that segregate for target traits critical to each target market group

Crossing decisions were guided by the breeding effort assigned to each of six target market classes (Table 2).

Table 2: Assignment of crossing effort in 2013

Market class	Breeding effort (%)	Target no. of seeds
ESM	30	1200
ESB	10	400
MSM	10	400
Mech-SM	10	400
LSB	10	400
LSM	30	1200
Total	**100**	**4000**

ESM = early, self-fertile, mahogany' ESB = early, self-fertile, blush; MSM = mid-season, self-fertile, mahogany;
Mech-SM = early, mid-, or late-ripening, self-fertile, mahogany, suitable for mechanical harvest;
LSB = late, self-fertile, blush; LSM = late, self-fertile, mahogany

The PNWSCBP has moved on from use as breeding parents of standard varieties and commercial standards in most cases to the common use in crossing of advanced selections and seedlings with unique traits or alleles of interest such as powdery mildew resistance and a new sources of earliness and/or lateness and self-fertility. This shift is further facilitated by propagating potential parents on Gisela 6® rootstock, as fruit set on own-rooted trees is poor due to erratic flowering and low fruit set.

In the last year, numbered selections from breeding efforts in the 1970s and 1980s, which are useful sources of earliness and lateness with fruit size above 10g and firmness >275 g/mm, were genotyped and intercrossed or mated with early advanced selections to generate progeny that are expected to include super earliness. Seed germination tests in our lab suggest that such progeny produce viable seed. Previously, due to embryo abortion and poor germination, breeding for early ripening was limited to crossing an early ripening variety to a late variety. For example, there is usually no pollination and fruit set when 'Chelan', the earliest commercial cultivar in the Pacific Northwest, is used as a seed parent so it is usually only used as a pollen source for earliness. However, either way, 'Chelan' is not suitable as a parent because it contains a double dose of allele "223" from marker BPPCT034 that is associated with small fruit size.

A new type of self-fertility differing from S4' (in the typical self-fertility source of 'Stella' and present in 'Lapins', 'Sweetheart', 'Benton' and 'Selah') in combination with earliness was efficiently introgressed into breeding germplasm by using directly as parents some 'Rainer' x 'Cristobalina' seedlings . This introgression enriches the early-ripening genepool for new cultivar development and diversifies the germplasm base. The 'Cristobalina' grandparent of the new breeding families is a Spanish landrace cultivar that itself is early and small-fruited with a unique source of self-fertility

that, unlike S4', is non-gametophytic and resides at a different locus to the *S* locus. Diversifying our germplasm base minimizes inbreeding and increases the chances of capturing heterotic (hybrid vigor) effects through development of progenies that out-perform their parents. Finally, we selfed self-fertile 'Glacier' and 'Kiona' (known for large fruit but poor firmness) and, in addition, mated these with 'Tieton,' to encourage development of self-fertile early, large, super-firm fruit due to recombinants possessing a double dose of allele "237" from BPPCT034. Allele "237" is rare in our germplasm. 'Tieton', 'Glacier', 'Kiona', and two other cultivar parents possess this allele in combination with other alleles but genotype 237: 237 (double dose) is lacking in the breeding germplasm.

New sources of late ripening are in short supply. We have only two advanced selections that are late ripening in Phase 2. This is because there is no cultivar as late as 'Sweetheart' and recently we discovered through DNA evidence that crosses made in the program in 2004 thought to be 'Sweetheart' x 'Regina' were actually 'Lapins' x 'Regina'. This could have been possible either through incorrect labeling of seedlings or by using a 'Lapins' tree as a seed parent mistaken to be "Sweetheart'. However, we have made many crosses in the past few years with 'Sweetheart' as a parent crossed with another late variety, although these are yet to fruit. Also, we have identified a certain numbered selection from the crosses made in the 1970s, that we used this year along with seedlings from an 'Ambrunes' lineage that have high breeding values (according to calculations using the Pedigree-Based Analysis software FlexQTL™ in the RosBREED project) for both lateness and firmness. 'Ambrunes' is another Spanish landrace cultivar that is late, firm, small fruited, and has low pedicel fruit retention force. Intermating the late selections and crossing with cultivars such as 'Sweetheart', where *S*-genotypes allow, should enrich the breeding populations with new sources of lateness superior to 'Sweetheart'. Further, crosses were made to combine powdery mildew resistance with good fruit quality using progenies of BB, CC, DD, AA, 'Moreau', and the MIM series accessions crossed with progenies of 'Ambrunes', 'Regina', 'Rainier', 'Sweetheart', and others known to be late and having high firmness and large fruit.

A major challenge for controlled crosses continues to be the moderate seed output due to adverse weather conditions during pollination. This has happened for two seasons in a row. In the last season, a combination of wind machine, irrigation water, and propane burners were not able to sufficiently mitigate the frost damage to thousands of hand-pollinated flowers. We are currently exploring other options to encourage pollination and fruit set. In the meantime, use of open-pollinated seed is a means of boosting seedling number, although such seedlings are DNA-tested just like controlled-cross seedlings to ensure that planted seedlings are enriched for desirable alleles.

3. Integrate genomics knowledge, marker assisted breeding tools, and classical breeding methods into the breeding program

Genetic tests for self-fertility and fruit size are routinely used for both parent selection and for culling inferior seedlings before field planting. We also use the DNA tests on advanced selections to establish genetic identity and for genetic potential characterization. As discussed in the previous activity, genomic information was used to re-assign parentage to seedlings for crosses made in 2004 that were thought to be 'Sweetheart' x 'Regina'.

Of 300 seedlings from the cross between 'Rainier' and 'Cristobalina', only 16 individuals were identified (using genetic tests for fruit size combined with markers for self-fertility from 'Cristobalina' to be suitable for use in introgression breeding. Introgression breeding is the incorporation of desirable alleles from a non-elite exotic source into an elite genetic background via at least two generations of crossing. The 16 seedlings had favorable genotypes for both fruit size and self-fertility, which was corroborated by their fruit size data. Numbered selections from breeding efforts in the 1970s and 1980s that are sources of earliness and lateness were also genotyped with

markers for fruit size and self-fertility and used in controlled crosses to target the genetics for early season fruiting.

We are developing new marker-locus-trait associations (addressed in a new WTFRC/OSCC proposal entitled "New genomic regions controlling production and fruit disorder traits") as well as new DNA tests (addressed in a new proposal entitled "After RosBREED: Developing and deploying new sweet cherry DNA tests") to extend marker-assisted selection to further valuable trait targets.

4. Develop and implement a cost-effective strategy for collaborative breeder-grower identification and evaluation of elite selections from the breeding program

To date, 32 advanced selections have been identified (Tables 3 & 4) while 16 have been planted in Phase 2 trials at WSU-IAREC experimental station in Prosser, WA and the Oregon State University (OSU) Mid-Columbia Agriculture Research and Extension Center (MCAREC) in Hood River, OR. Five trees of each advanced selection and one tree of a standard cultivar for comparison are planted in this trial. There are also two grower co-operator trials in north Wenatchee (providing a late site) and at Sagemoor Farms in Pasco (providing an early site). Late selections including FR001T070, FR001T074, and FR013T004 have been planted at the late site in north Wenatchee while the Pasco site has plantings of early advanced selections including FR001T007, FR009T033, and FR009T089. Input from BPAC members, in particular, Dena Ybarra, Dave Allan, and Jeff Cleveringa, have been very helpful in identifying flaws in some advanced selections and in pulling them out accordingly. For example, due to excessive heat last season, FR001T073, a late selection, had poor firmness and was discontinued. This was also the case with an early selection, FR001T005, which had more than 50% stylar splitting last year although the fruit size was greater than 10 g and firmness over 300 g/mm. This year, two advanced selections, FR049T083 and FR013T004, with a combination of too much blind wood and excessive splitting were pulled out. FR009T033 and FR009T089 were tentatively selected last year due to earliness, pending another year's data. Data recorded this summer showed that they are unworthy of advancement to Phase 2 based on the combination of small fruit size, excessive splitting, low firmness, and high surface pitting incidence.

Table 3: Current advanced selections of the PNWSCBP

Selection label	Year planted	Harvest date	Target market class	Location planted
FR001T002	2014	18-22 Jun	ESM	WSU, OSU, Pasco
FR001T004	2014	14-18 Jul	Mech-SM	WSU, OSU, Wen
FR001T007	2012	18-Jun	ESM	WSU, OSU, Pasco
FR001T036	2012	1-Jul	MSM	WSU, OSU
FR001T070	2013	18-Jul	LSB	WSU, OSU, Wen
FR001T074	2011/2012	6-Jul	LSM	WSU, OSU, Wen
FR002T030	2012	7-Jul	LSB	WSU, OSU
FR002T063	2012	3-Jul	LSM	WSU, OSU
FR002T074	2014	7-Jul	LSB	WSU, OSU,
FR004T029	2013	5-Jul	LSM	WSU, OSU,
FR006T059	2012	6-Jul	LSM	WSU, OSU
FR006T063	2012	7-Jul	LSM	WSU, OSU
FR009T033*	2013	10-Jun	ESM	WSU, OSU, Pasco
FR009T037	2013	12-Jul	LSB	WSU, OSU, Wen
FR009T049	2014	17-Jun	ESM	WSU, OSU, Pasco
FR009T089*	2013	13-Jun	ESM	WSU, OSU, Pasco

FR010T051	2012	23-Jun	ESM-MSM	WSU, OSU
FR011T059	2012	27-Jun	ESB	WSU, OSU
FR013T004*	2013	12-Jul	LSM	WSU, OSU, Wen
FR044T083	2014	27-Jun	MSM	WSU, OSU,
FR049T125	2014	3-Jul	MSM	WSU, OSU,
FR049T083*	2013	3-Jul	MSM	WSU, OSU

WSU = Washington State University-IAREC, Prosser, WA; OSU = Oregon State University, MCAREC, Hood River, OR' Wen = Wenatchee (north); * = Individuals to be discontinued. FR001T005 has already been pulled out.

Table 4: Phase 1 seedlings and their attributes, identified in 2013 for advancement to Phase 2. Standard cultivars are included for comparison

Selection label	Harvest date	Market class	Fruit weight (g)	Firmness (g/mm)	PFRF (kg)	SSC (°Brix)	TA (%)
Chelan	Jun 13	ESM	9.3	266	0.75	21.5	
FR014T012	Jun 5	ESM	9.2	393	1.05	22	0.46
FR035T087	Jun 20	ESB	13	308	0.64	16	0.77
FR036T035	Jun 19	ESB	18.2	315	0.80	23	0.51
Sweetheart	Jul 13	LSM	10.3	301	0.83	25	0.64
FR040T090	Jun 27	LSM	16	340	0.59	18	0.80
FR044T083	Jul 5	LSM	12	300	0.36	18	0.77
FR052T095	Jun 24	LSM	16.7	370	0.59	18	0.41
Rainier	Jul 2	LSB	13.5	331		15.4	
FR041T014	Jun 27	LSB	11	320	0.70	19	0.73
FR044T074	Jun 20	LSB	14.7	353	0.73	17	0.71
Bing	Jun 22	MSM	10.1	273	0.74	17.3	0.66
FR046T105	Jun 23	MSM	11	303	1.05	19	0.90
FR050T105	Jun 19	MSM	10.4	271	0.47	19	0.72
FR051T113	Jun 21	MSM	12	356	0.73	17.5	0.93

PFRF = pedicel fruit retention force; SSC = soluble solids content; TA= titratable acidity

5. Identify in Phase 2 at least one elite selection from any target market class that exceeds the threshold values for the primary and secondary traits of the target market class for advancement to Phase 3.

To date, we have selected one advanced selection, FR001T007, for advancement to Phase 3. This selection targets the ESM market class, being similar to 'Chelan' in harvest timing, but has fruit size and firmness greater than 'Chelan' as well as better flavor. This selection has been propagated on Gisela 6® by Willow Drive Nursery and will be planted into Phase 3 trials in the spring of 2014. One hundred trees of this selection and five trees of 'Chelan' will be planted at each of two grower-cooperator sites: one in Chelan (owned by Chelan fruits) and one in Pasco (owned by Sagemoor Farms). We have yet to identify an Oregon grower willing to take on this selection. This year we identified another early ripening selection, FR014T012, for Phase 2 advancement. This selection has similar fruit size to 'Chelan' (Table 4) but the firmness is close to 300 g/mm (without GA application) and ripens more than a week before 'Chelan'. This selection is already propagated on Gisela 6® rootstock in Phase 1 where it was first identified and, if the performance holds up next year, we may consider a fast track to Phase 3.

CONTINUING REPORT **YEAR: 1of 2**

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

PI: Todd Einhorn
Organization: OSU-MCAREC

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Total Project Request: **Year 1:** $59,910 **Year 2: $60,964**

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	28784	29648	
Benefits	18064	18604	
Wages	3520	3520	
Benefits	352	352	
Equipment			
Supplies	2310	1960	
Travel	1000	1000	
Miscellaneous			
Plot Fees			
Total	54030	**55084**	

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**
Telephone: 541 737-4866 **Email address: l.j.koong@oregonstate.edu**

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) 2000 to 3000 ovaries sampled randomly at 15 to 18 days after bloom were sufficient for crop estimates by dry weight per ovary

2) Bee exclusion bagging of limbs provided reference values for the growth of unfertilized ovaries

3) Ovary length to width ratios improved detection of potential fruit versus developmentally failed fruit

4) Crop estimates improved every five days, up to 30 days from bloom

5) Potential fruit size at harvest was determined 30 to 35 days from bloom

6) Fresh weight to dry weight ratios of ovaries differ between Fruit and Failures as early as 10 days from bloom and may lead to a new method using density of ovaries for crop estimates

7) Some bagged ovaries grew similar to fruit, especially in 'Sweetheart' indicating some self-fertilization in the absence of pollinators

8) '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

9) Early season application of Cytokinin increased fruit size at the pit hardening stage

Growth Analysis of Sweetheart. Calendar days versus Degree days

Growth analyses are necessary to objectively compare cherry growth behavior between different grow sites and seasons in order to develop predictive models that will inform growers and marketers of factors influencing cherry fruit quality. We performed such analyses for 'Sweetheart' at three grow sites with historical differences in bloom and harvest timing (fig.1).

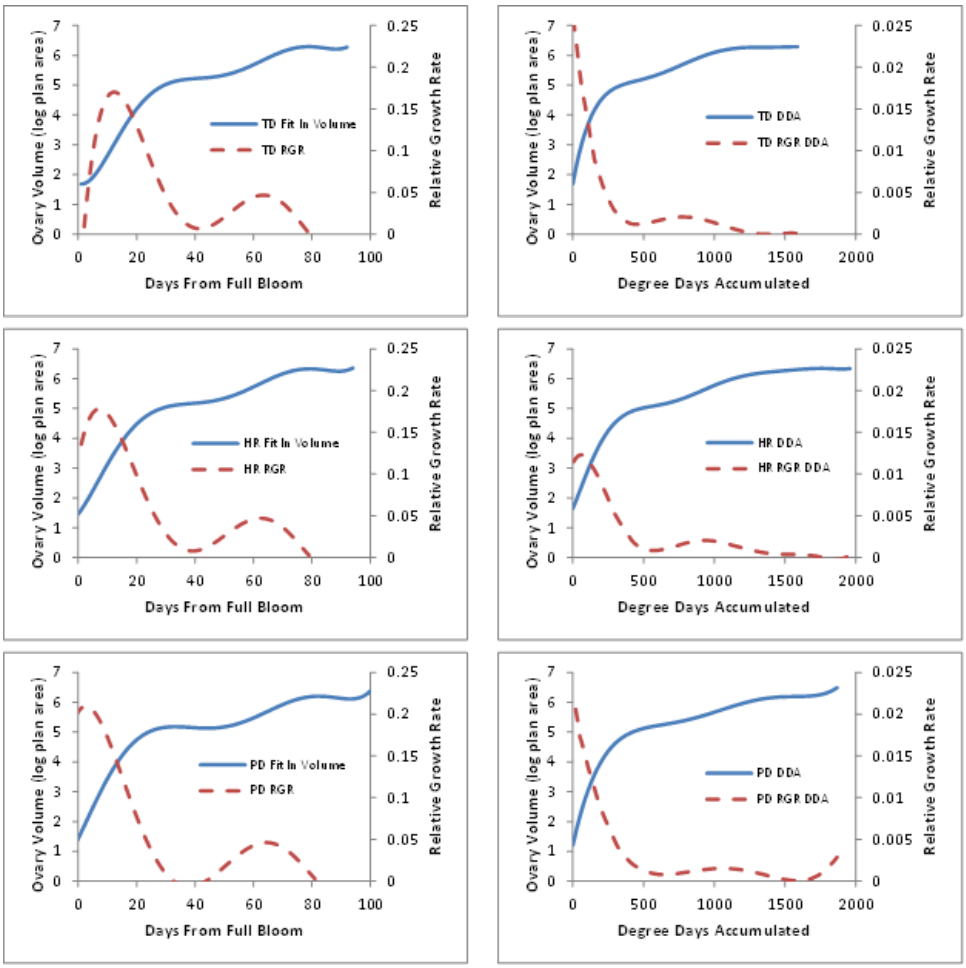

Figure 1. Growth analysis of 'Sweetheart' in three locations; TD (The Dalles) top panels, HR (Hood River) center, and PD (Parkdale) lower panels. Calendar date (left panels) and Degree Day (right panels). Ovary volume and Relative Growth Rates (RGR) are shown for comparison. Degree Days Accumulated (DDA) were calculated on a 43° F baseline.

Of great importance in producing growth models is the elimination of growth from unfertilized ovaries which lead to failed fruit development. Models which do not separate fertilized fruit from unfertilized fruit in the first 30 days from bloom will grossly underestimate fruit growth. This is the case since, on average, 25%-40% of the initial flowers set fruit (Table 1). Therefore, 60%-75% of the fruit in a random sample collected during the first 30 days from bloom will not be carried through to harvest, severely underestimating the potential growth rate and underscoring the importance of eliminating unfertilized growth from such analyses.

In this portion of the project we compared growth of open pollinated ovaries to ovaries enclosed in bee exclusion bags seeking to determine the size and shape differences that could be used to distinguish between fruitful ovaries and failed ovaries collected randomly. We measured size (plan area) and shape (length and width) photographically from approximately 300 ovaries at five day intervals for Chelan, Bing, Rainier and Sweetheart. The size and shape factors determined from the

bagged ovaries (data not shown) were then used to perform statistical cluster and discriminate analyses of the open pollinated ovaries, thereby eliminating unfertilized growth.

Fruit growth of sweet cherry is dependent upon temperature and thus can be modeled by growing degree units (fig 1). In 2013 there was approximately a two week difference in bloom timing, and a one week difference in the fruit development period between bloom and harvest when comparing The Dalles (TD) and Parkdale (PD). The timing for Hood River split the difference. TD site had the coolest temperatures at bloom and a delayed peak of relative growth rate (RGR), but the warmest summer resulting in the fewest days from bloom; whereas PD had the warmest temperatures at bloom with the most rapid increase in RGR but a cooler summer resulting in the longest time until harvest. Differences between the RGR curves, especially soon after bloom, and differences in the total degree days accumulated (DDA) at harvest time, indicate the baseline temperature used to calculate DDA should be adjusted downward.

Similar growth studies will be repeated next season. Each season and location that can be added to this study will add confidence to a DDA dependent model of cherry fruit growth.

Fruit Set Analysis. Size of fruit versus failures provides an early estimate of *Marketable Fruit*

Prediction of the potential crop and expected fruit size would aid growers in understanding and assessing the environmental factors and horticultural practices that limit fruit from reaching their predicted potential. Furthermore, prompt crop estimates would inform marketing strategies. It is important to note that most crop estimates neither account for aborted fruit that drop after 30 days nor for fruit that remains until harvest only to be culled at the packing house for lack of size. In the event that unfertilized ovaries or fertilized fruit that suffer some limitation in their development within the first 20 days from bloom, their growth rate will be reduced, and this measure can be used in crop estimates. We were able to generate these data and determine several groupings of fruit based on their weights (fig. 2). Dry weight was used (as opposed to fresh weight) because it provides the actual carbon gain of the fruit and, as a technique, it eliminates fruit weight loss (and measurement error) when significant time is required for analyzing fresh samples- as was the case for the processing and individual weighing of over 50,000 fruit in our 30 d sampling period.

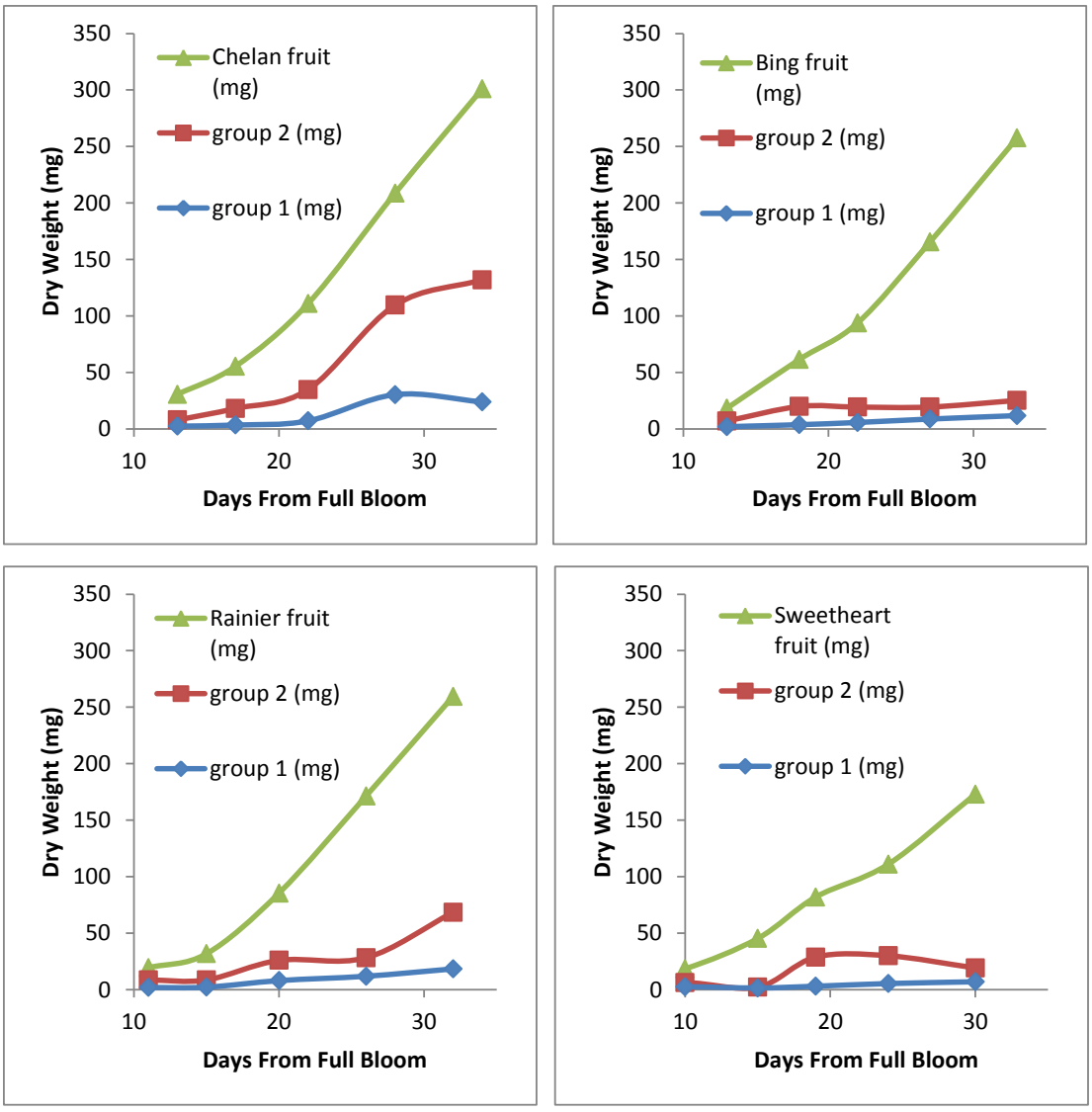

Figure 2. Growth analysis and fruit set of four varieties in The Dalles. Ovary dry weights were classified into three groups easily detected with statistical cluster analysis. Group 1 ovaries expand little beyond their size at bloom. Group 2 ovaries grow to the size of a 10 or 20 DFFB cherry then fail and drop. Chelan appears to have the greatest extent of growth of the Group 2 ovaries which explains the 'apparent' continual or 'late' drop often observed with this variety.

We also examined the relative water content of fruit during development (derived from fresh and dry weights) and have noted that a density difference distinguishes Fruit from Failures early in their development. A simple bucket procedure using liquids of varying density should allow Failures to float. Producers would then have a rapid test to estimate their crop in the orchard. We propose to develop this assay in 2014.

Table 1. Developing and undeveloped cherries from both unfertilized and fertilized ovaries were sampled at ~5 d intervals beginning ~15 d after full bloom. Ovaries were dried to constant weight in an oven and weighed individually (expressed in table as ovaries examined). These data were then subjected to statistical analyses to estimate the percentage of total fruit on the tree that will remain to harvest over time.

Variety	Days From Full Bloom	Ovaries Examined	Crop Estimate of Market Sized Fruit Remaining on the Tree	Fruit Set on Selected Limbs as a Percent of Bloom Count not Corrected for Market Size
	(days)	(no.)	(%)	(%)
Bing	18	2567	36%	
	22	2403	48%	
	27	1910	68%	
	33	1909	78%	
	39			38%
	50			30%
Chelan	17	2391	51%	
	22	2423	34%	
	28	1792	53%	
	34	1670	76%	
	41			30%
	52			24%
	62			24%
Rainier	15	2528	71%	
	20	2026	71%	
	26	1776	84%	
	32	1645	90%	
	39			41%
	50			36%
	78			39%
Sweetheart	15	3289	24%	
	19	2730	30%	
	24	1915	70%	
	30	1674	94%	
	36			36%
	47			32%

Ovary size was measured by dry weight allowing us to harvest several thousand ovaries on multiple dates. Ovaries collected from the field were brought to MCAREC where we removed styles and stems before drying the ovaries slowly in ovens. When dry, individual ovaries were weighed on an analytical balance connected to a computer for data acquisition.

PGR Experiments.

Eight single-tree reps in a 'Lapins' block were treated with various PGRs at 5 dafb with a pressurized hand gun. Treatment timing was based on our previous work, which identified early season maximum growth rates of sweet cherry fruit (irrespective of cultivar) to occur within the first week from bloom. Cytokinin (CPPU; KimBlue) and GA (ProGib) alone or in combination (Promalin) were applied to determine if fruit size could be increased at harvest. Fruit, randomly sampled at pit-hardening stage were significantly larger when treated with Promalin or CPPU, indicating a positive effect of cytokinin on early fruit growth (fig 3). This effect was not influenced by cropload and PGR treatments did not affect fruit set (Table 2). That GA (when applied on its own) did not have a positive effect on fruit growth suggests that this compound may not have a role in early fruit development at the rates applied. Zhang and Whiting (2011) observed a GA-induced increase in the

size of fruit at harvest when applied in a lanolin paste to the stems of cherry fruit at 9 dafb; however, in their study GA rate was 200 ppm. Though our applications were less direct (sprayed to entire canopies), GA clearly was taken up as shown by the significant enhanced stem growth (stems 13% longer) relative to other treatments (Table 2).

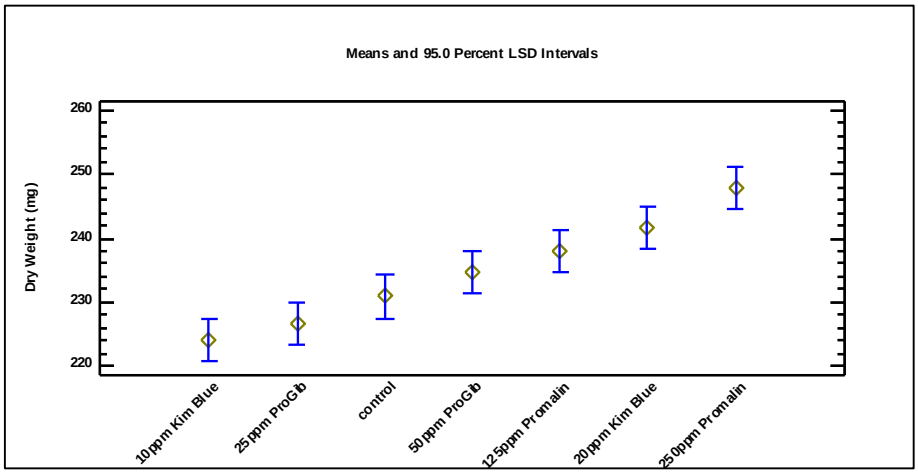

Figure 3. Fruit size (expressed as dry weight) was determined at pit hardening following PGR applications to whole trees 5 days after full bloom. Means are based on 240 fruit per treatment.

Table 2. Effect of PGRs applied to whole canopies of Lapins 5 days after bloom.

Treatments	Fruit set (%)	Yield (lbs/tree)	Fruit diameter (mm)	Fruit wt. (g)	FF (g/mm)	Stem length (mm)	SS (%)	TA (%)
Control	58	158.3	28.7	10.4 abc	213	45.8 c	16.6	0.58
GA (25 ppm)	62.7	196.4	26.8	9.1 c	217	49 ab	16.1	0.59
GA (50 ppm)	58.1	170.3	29	11.1 a	208	51.7 a	15.6	0.6
Promalin (125 ppm)	58.4	168.9	28.3	10.7 ab	194	50.8 a	16.5	0.59
Promalin (250 ppm)	67.9	140.9	28	10.1 bc	197	48.9 ab	17.3	0.6
CPPU (10 ppm)	72.2	171.3	28.2	10.2 bc	209	47.5 bc	16.3	0.6
CPPU (20 ppm)	59.2	150.7	28.2	10.3 abc	212	46.1 bc	16.2	0.58

data are means of 8 single tree reps; n=100 for fruit diameter, fruit weight, FF, and stem length; a segment of 2 and 3-year-old wood (1 per tree) was selected at bloom to determine fruit set. Flowers were counted at bloom and fruit (per segment) were counted at 40 dafb.

In fact, the influence of GA from Promalin treatments promoted increased stem length. At harvest no positive effects from PGRs were apparent on any of the fruit quality attributes analyzed; however, we feel that several key factors contributed to the apparent 'disappearance' of an early-season growth effect. Late-season climatic conditions were unfavorable, and likely adverse to cherry fruit growth. Between 28-June and 3-July daily maximum temperatures exceeded 95 °F, with maximum temperatures above 102°F on 30-June. These high temperatures followed a ~1/2 inch rain event the previous week. Marked splitting and sunburn injury was visually apparent at harvest (unaffected by PGRs) and fruit were exceptionally soft (~200 g/mm fruit firmness; Table 2), indicating severe heat stress. Fruit firmness over the past several years has averaged 270 g/mm in that block. Further evidence that development was impaired by these environmental factors was evident by low SS and TA, relative to past years. This was, in part, due to an earlier season; however, skin color at harvest was 5.5 on a ctifl color scale, which was similar to past years and suggests that similar fruit maturation was attained on the tree. We propose to expand our early-season PGR evaluations by selecting alternative sites (and cultivars) in 2014.

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

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

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Cooperators: Todd Einhorn, Lynn Long, David Felicetti (Pace International LLC), Ryan Durow (Orchard View Farm), Kumar Sellakanthan (Amcor), Ray Clarke (Apio Inc.), Xingbin Xie

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 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 is to minimize pitting, splitting, acid loss, dull color, and stem browning, therefore improve shipping quality of the PNW sweet cherry through (1) selecting the right modified atmosphere packaging (MAP) liner and zipper-lock bags/clamshells, (2) implementing calcium (Ca) in hydro-cooling and flume water, and (3) edible coatings and GRAS compounds.

The key objectives are to:

1. Understand the dynamics of cherry respiration physiology influenced by cultivars, ripeness, temperature, O_2 and CO_2 – an essential knowledge for improving shipping quality.

2. Determine efficacy of the major commercial MAP liners and the optimum MAP parameters (O_2, CO_2) for improving shipping quality of the major PNW and California cultivars at typical shipping conditions.

3. Optimize perforation ratios of zipper-lock bag and clamshell to maintain stem quality.

4. Study the mechanism and practical postharvest Ca treatments to minimize postharvest pitting, splitting, and stem browning.

5. Evaluate edible coatings and GRAS compounds applied post-harvest on shipping quality of PNW sweet cherries.

Goals, activities, and anticipated accomplishments for the next year:

- Determine the effect of simulated temperature fluctuations during commercial shipping on MAP efficacy, and optimize MAP parameters at typical shipping conditions.
- Optimize postharvest Ca application protocols on increasing Ca uptake, reducing pitting and splitting, and improving shipping quality of different PNW cultivars.
- Optimize application protocols of edible coatings and GRAS compounds to increase shipping quality of PNW cultivars.

SIGNIFICANT FINDINGS (year 2)

1. Respiration Dynamics

- At shipping temperatures (i.e., 32-40 °F), respiration rate of the major PNW and California cultivars was affected very little by reduced O_2 from 21 to 10%, but declined logarithmically from 10 to ~1%.
- Estimated fermentation induction points determined by a specific increased respiratory quotient (RQ) were <1% and 3-4% O_2 for most of the major cultivars at 32 and 68 °F, respectively.
- 'Skeena' has a higher Q_{10} from 32 to 50 °F and a higher RQ at elevated temperatures (i.e., 40 °F) than 'Lapins', 'Regina', and 'Sweetheart'. 'Skeena' fruit stressed by heat have a higher respiration rate and could show pitting on trees or after harvest without mechanical damage.

2. MAP Technologies

- The major commercial MAP liners (7) have extremely varied equilibrium O_2 and CO_2 concentrations for 'Bing', 'Regina', 'Skeena', 'Lapins', 'Sweetheart', and 'Coral' (California cultivar) at simulated commercial shipping conditions (i.e., 32-36 °F).
- **O_2 concentration affected flavor.** MAP liners with equilibrium 5-8% O_2 at 32 °F could reduce respiration rate and therefore maintain titratable acidity (TA) and flavor of the major

cultivars at commercial shipping temperatures (i.e., 32-40 °F). MAP liners with O_2 > 10% at 32 °F did not maintain flavor. MAP liners with O_2 < 5% at 32 °F may cause anaerobic fermentation due to temperature fluctuations during commercial storage/shipping.

- **CO_2 concentration affected fruit dull color.** MAP liners with equilibrium CO_2 > 10% at 32 °F could maintain the shiny fruit color at simulated shipping temperature (32-40 °F). MAP liners with CO_2 < 8% at 32 °F have little beneficial effect on maintaining fruit shiny color.
- 'Skeena' is more susceptible to anaerobic fermentation at elevated temperatures, therefore, needs MAP liners with relatively higher gas permeability (i.e., equilibrium 10-15% O_2 at 32 °F) to avoid anaerobic fermentation in commercial shipping.

3. Consumer packaging (see continuing report year-1)

4. Postharvest Ca application

- Adding Ca (0.2-0.5%) in hydro-cooling water efficiently increased fruit tissue Ca concentration and fruit firmness (FF), reduce pitting susceptibility, maintained stem quality and TA, and reduced decay of 'Lapins' and 'Sweetheart'. Ca application rate and temperature gradient between fruit pulp and solution are the key factors determining efficacy of the Ca treatments. Higher Ca rates (1.0-2.0%) damaged stems.
- Adding Ca in flume water at proper rates (i.e., 0.2-0.5%) reduced postharvest splitting and improved shipping quality (FF, total antioxidant capacity [TAC], stem quality, TA, and decay) of 'Skeena' and 'Sweetheart'. Higher Ca rates (i.e., 1.0-2.0%) damaged stems.

5. Edible coatings and GRAS compounds

- Semperfresh^{TM} at appropriate rates (i.e., 0.5% active ingredient [a.i.]) reduced moisture loss, maintained stem quality, and reduced pitting expression of 'Chelan', 'Lapins', and 'Sweetheart' packed in clamshells. However, Semperfresh^{TM} at its label rate of 1.0% a.i. increased pitting expression of 'Sweetheart'. Pitting expression seems to be associated with moisture loss and localized O_2 deficiency.
- Postharvest applications of salicylic acid (SA) and oxalic acid (OA) tended to reduce respiration rate and maintain higher TA, but did not affect total antioxidant capacity (TAC) of PNW cultivars following cold storage/shipping.

METHODS

1. Respiration Dynamics

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.

2. MAP Trials

Seven commercial MAP liners (ViewFresh, Xtend, LifeSpan, Breatheway, and Primpro, PEAKfresh, FreshLOK) with distinct technologies were obtained from 7 manufactures internationally (OVF, StePac, Amcor, Apio, Chantler, PEAKfresh USA, and Shields Bag and Printing CO.). fruit of different cultivars were either obtained from packinghouses shortly after packing or harvested from 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, anthocyanin, SSC, and TA immediately after cold storage and plus 2 days at 68°F. Fifty fruit were randomly selected for evaluations of pitting, splitting, stem quality, and decay. Ten fruit were randomly selected from each box for 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 Applications

1) Hydro-cooling water. Ca (Opti-CAL^{TM}) solutions at 0, 0.2, 0.5, 1.0, and 2.0% were cooled to 32 °F before treatments. 'Lapins' and 'Sweetheart' 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.

2) Flume water. Ca (Opti-CAL^{TM}) solutions at 0, 0.2, 0.5, 1.0, and 2.0% were cooled to 32 °F before treatments. 'Skeena' and 'Sweetheart' fruit harvested at commercial maturity from MCAREC were air-cooled with fruit pulp temperature at 35 °F and then dipped in the cold Ca solutions for 30 min to simulate the commercial on-line processing procedures.

4. Postharvest Applications of edible coatings and GRAS Compounds

Semperfresh^{TM}, 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 logarithmically from ~10% to ~1% significantly for 'Bing', 'Sweetheart', and 'Coral'. Estimated fermentation induction points determined by a specific increased RQ were less than 1% and 3-4% O_2 for both 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).

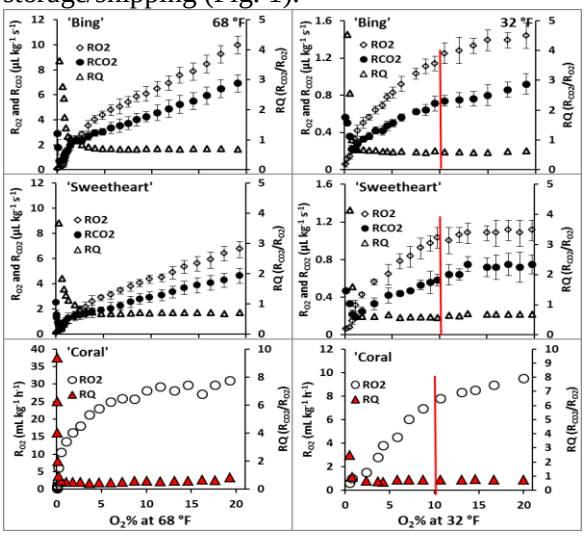

Fig. 1. Respiration dynamic of sweet cherry affected by variety, temperature, O_2 and CO_2.

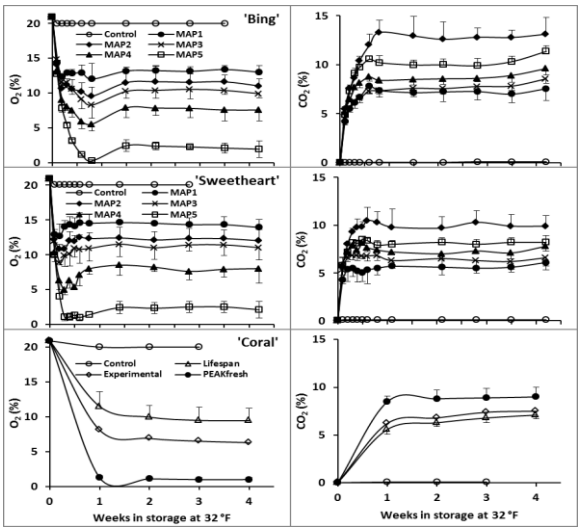

Fig. 2. O_2 and CO_2 concentrations in different MAP liners for 'Bing', 'Sweetheart', and 'Coral' at 32°F.

2. MAP Technologies

1). Gas permeability and efficacies of different MAP liners on maintaining fruit shipping quality.

The seven most popular 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. 2). O_2 ranged from 2-15% and CO_2 ranged from 5 to 13% for 'Bing', 'Lapins', 'Skeena', 'Regina', 'Sweetheart', and 'Coral'. While all the MAP liners maintained higher FF and reduced decay, only the MAP liners with lower O_2 permeability (i.e., equilibrated at 2-8% O_2 + 7-10% CO_2)

reduced fruit respiration rate and maintained TA and flavor of sweet cherries compared to the standard macro-perforated PE liners after 2-6 weeks of cold storage. In contrast, MAP liners that equilibrated with atmospheres of 10-15% O_2 + 5-13% CO_2 had little effect on inhibiting respiration rate and TA loss and maintaining flavor during cold storage.

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

Elevated transit temperatures from 32 to 41 °F reduced O_2 significantly (Fig. 3) but did not change CO_2 too much in MAP liners (data not shown). 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, respectively (Fig. 3). At 36 °F, the equilibrium O_2 was 4.5% and 1% in MAP4 and MAP5 during 2 weeks of cold storage and 'Sweetheart' fruit had no fermented flavor after 2 weeks of cold storage. At 41 °F, 'Sweetheart' fruit was tasted as fermented flavor in MAP5, but not in MAP4 after 2 weeks of storage. In conclusion, MAP with appropriate gas permeability (i.e., equilibrated at 5-8% O_2 at 32 °F) may be suitable for commercial application to maintain flavor without damaging the fruit through fermentation, even if temperature fluctuations, common in commercial storage/shipping, do occur.

'Skeena' has a higher RQ at elevated temperatures and is more sensitive to anaerobic fermentation due to temperature fluctuations during shipping (Fig. 4). MAP liners with equilibrium 10-15% 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' and 'Regina' fruit stressed by heat in the field had higher respiration rates and were more susceptible to anaerobic injury.

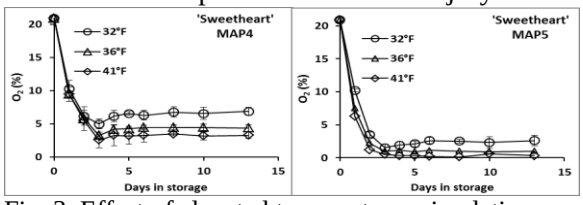

Fig. 3. Effect of elevated temperatures simulating commercial shipping on O_2 and CO_2 in MAP liners.

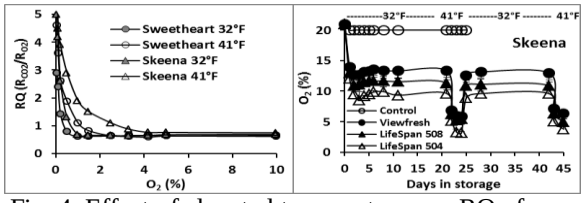

Fig. 4. Effect of elevated temperatures on RQ of 'Skeena' and O_2 and CO_2 in MAP liners.

3. Postharvest Ca Application in Hydro-Cooling Water and Flume Water

1) Hydro-cooling water

Adding Ca at 0.2-2.0% in hydro-cooling water efficiently increased Ca concentration in fruit tissue of 'Sweetheart' (Fig. 5) and 'Lapins' (Fig. 6). Fruit pulp temperature affected tissue Ca uptake, the greater the temperature gradient between fruit pulp and Ca solution, the higher the uptake rate of Ca into the tissue (data not shown). Fruit treated with Ca solutions maintained higher FF, reduced pitting susceptibility, reduced respiration rate, maintained higher TA, and maintained higher total antioxidant capacity (TAC) during 4 weeks of cold storage (Fig. 5&6). Stem quality of 'Lapins' and 'Sweetheart' were maintained by Ca at 0.2% and 0.5%, but damaged by Ca at 1.0% and 2.0% during 4 weeks of cold storage (Fig. 5&6&7).

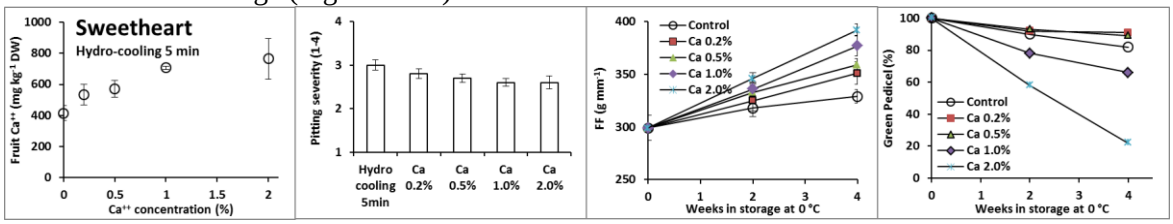

Fig. 5. Effect of Ca in hydro-cooling water on fruit tissue Ca content and shipping quality of 'Sweetheart'.

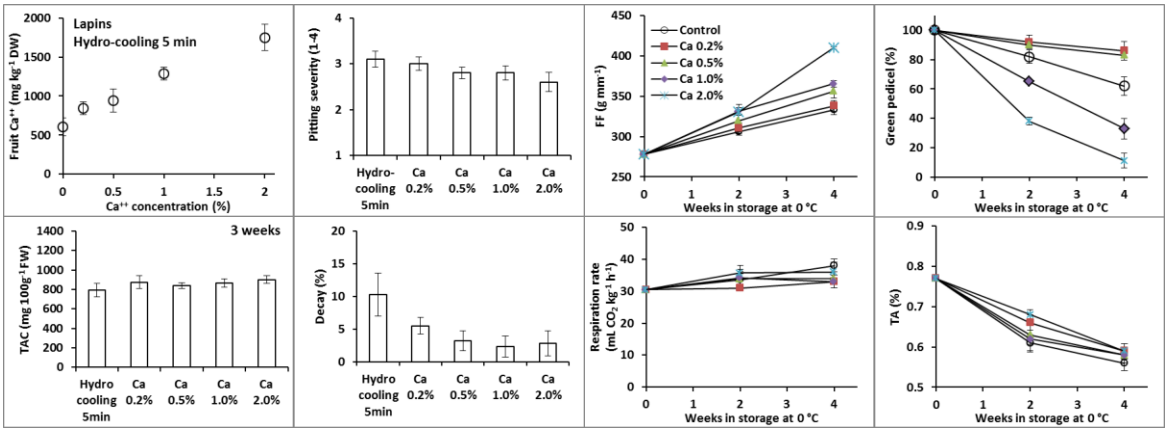

Fig. 6. Effect of Ca in hydro-cooling water on fruit tissue Ca content, shipping quality, and total antioxidant capacity (TAC) of 'Lapins'.

Moisture content after 4 weeks of cold storage:

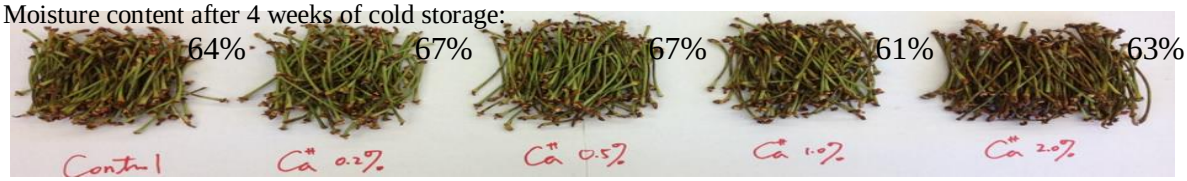

Fig. 7. Effect of Ca in hydro-cooling water on stem moisture content and color of 'Lapins' after 4 weeks in cold storage.

2) Flume water

Adding Ca at 0.2-2.0% in flume water increased Ca concentration in fruit of 'Skeena' (Fig. 8) and 'Sweetheart' (Fig. 10). Ca in flume water reduced postharvest splitting, increased FF, reduced respiration rate, maintained higher TA, and enhanced TAC of both cultivars during 4 weeks of cold storage. Stem quality was maintained by Ca at 0.2% and 0.5%, but damaged by 1.0% and 2.0% in flume water (Fig. 8&9&10). Ca in flume water did not affect water uptake but reduced soluble pectin compounds releasing from fruit of 'Skeena' and 'Sweetheart' into flume water (data not shown). In conclusion, Ca at 0.2-0.5% in flume water can reduce postharvest splitting, improve shipping quality, and enhance TAC of 'Skeena' and 'Sweetheart'.

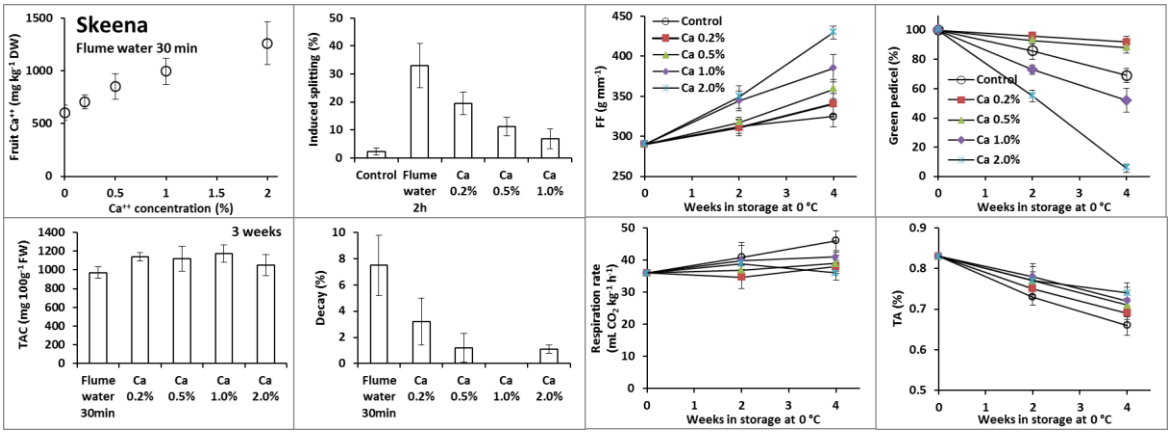

Fig. 8. Effect of Ca in flume water on fruit tissue Ca content, shipping quality, and total antioxidant capacity (TAC) of 'Skeena'.

Moisture content after 4 weeks of cold storage:

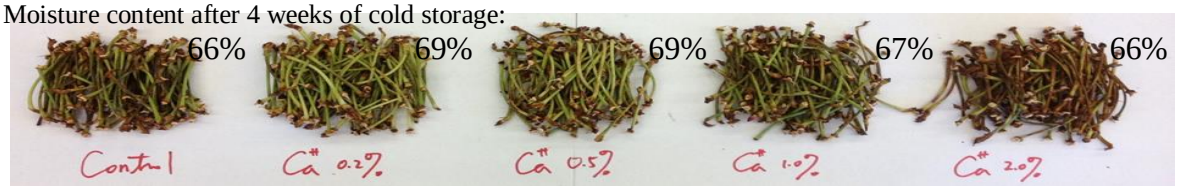

Fig. 9. Effect of Ca in flume water on stem moisture content and color of 'Skeena' after 4 weeks of cold storage.

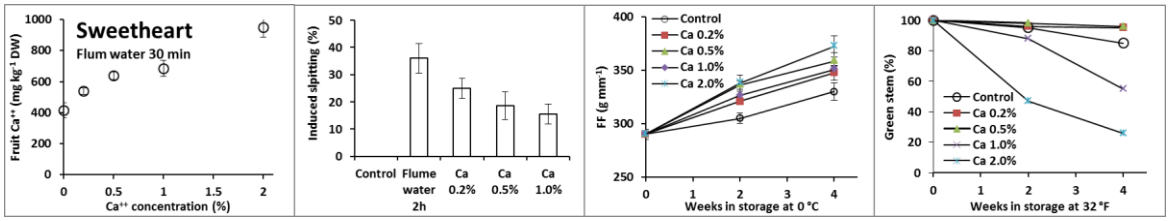

Fig. 10. Effect of Ca in flume water on fruit tissue Ca content and shipping quality of 'Sweetheart'.

4. Postharvest Treatments with GRAS Compounds and edible coatings

1) SA, OA, HBR,

Postharvest applications of SA and OA tended to reduce respiration rate and maintain TA of PNW cultivars packed in clamshells during storage (Fig. 11). It was reported that both SA and OA enhanced 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. 11). Postharvest treatment with HBR at 5 ppm had no effect on shipping quality of 'Lapins' and 'Skeena' (Fig. 11).

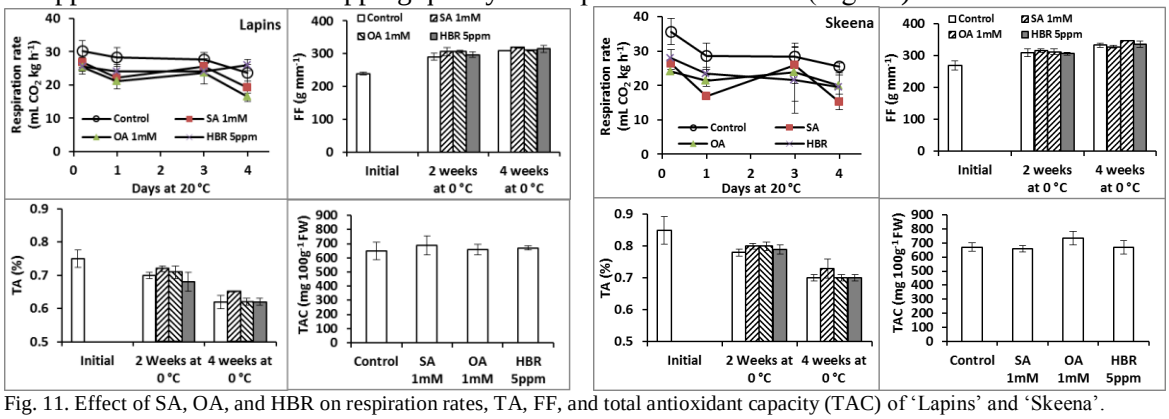

Fig. 11. Effect of SA, OA, and HBR on respiration rates, TA, FF, and total antioxidant capacity (TAC) of 'Lapins' and 'Skeena'.

2) Semperfresh™, GA_3

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. 12). 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. 13). Pitting formation seems to be associated with moisture loss and localized O_2 deficiency.

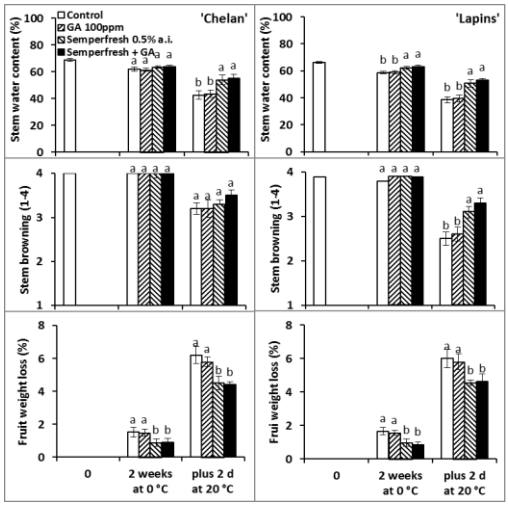

Fig. 12. Effect of Semperfresh™ and GA_3 on shipping quality of 'Chelan' and 'Lapins' at simulated marketing conditions.

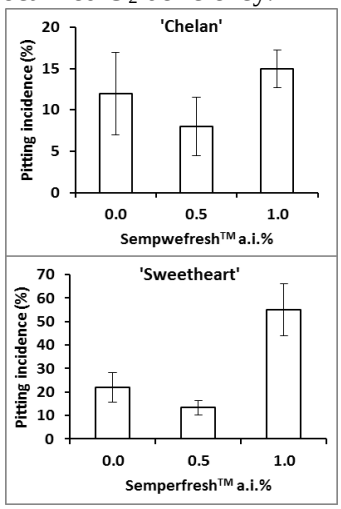

Fig. 13. Effect of Semperfresh™ on pitting incidences of 'Chelan' and 'Sweetheart' after 2 weeks of cold storage.

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

Project Title: Factors affecting the fruit phase of cherry mildew

PI: Gary Grove
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Co-PI (2): Yan Wang[1]
Organization: OSU-MCARES
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City/State/Zip: Hood River, OR 97031

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

Cooperators: Claudia Probst (IAREC technical assistance), Maurisio Garcia (field support and fungicide trial organization and application), Marcia Walters (TFREC technical assistance)

Graduate (PhD) Student: Binod Pandey, WSU-IAREC, Prosser, WA

Total Project Request: **Year 1**: $66,334 **Year 2**: **$67,822** **Year 3**: $70,000

Other funding sources

Agency Name: Washington State Commission on Pesticide Registration
Amt. requested: $19,958
Notes: **W**ill be submitted in November 2013 (quinoxyfen timing portions of study)

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

Year	**2013[1]**	**2014[1,*]**	**2015[1,*]**
Item	33,504[2]	34,836	36,229
Salaries	17,087	16,373	17,028
Benefits	7,075	7,076	7,075
Wages	667	686	686
Equipment	3,600	0	0
Supplies	1,000[3]	3,000[3]	3,000[3]
Travel[4]		1,000[4-5]	1,000[4-5]
Total	$62,933	**$62,971[6]**	$65,018[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, Mazzard, and Bing trees for inoculum production and increase, Nitex cloth replacement (8 um), maintenance parts for growth chambers)
[4]travel to WSU-IAREC Roza farm in 2014-2015 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	4209
Benefits	215[2]	940	500
Equipment			
Supplies	500[3]	500	500
Travel			
Miscellaneous			
Total:	$3,401	**$5,526**	$5,677

Footnotes:
[1]Wages: 300hr 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.

a) Detached fruit studies (growth chamber and laboratory)
b) Detached shoot studies (growth chamber and laboratory)
c) Orchard studies

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 'Bing' and 'Sweetheart' fruit to infection by *P. clandestina* in orchard studies.

4) Evaluate quinoxyfen as a key management component of the fruit phase of powdery mildew (PM), 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.

To establish a consistent and reliable methodology for the 1) infection of sweet cherry fruit with (PM), 2) optima for inoculum concentration, 3) periods of fruit susceptibility, 4) temperature, and relative humidity ranges must be determined. Once established, fruit from all growing regions, elevations, and levels of maturity can be utilized to extend the research season. During the first season new methods to infect sweet cherry fruit were developed and existing protocols were refined. Results, in particular those obtained from orchard studies (Objectives 1c, 3 and 4), provided additional insight into the biology and behavior of the fungus during cherry fruit development.

2013 challenges: Attaining *in vitro* infection of developing cherry fruit was difficult (Objectives 1a-b, and 2). The most reasonable explanation is an inherent resistance of immature cherries to fungal infection. This finding is supported by results from the orchard studies (Objectives 1c, and 3). Next season, timing of controlled-environment experiments (inoculum concentration and temperature/RH studies) will be modified based on 2013 results. Also, some additional experiments will be conducted on 'Bing' and 'Sweetheart' fruit picked in Prosser and Wenatchee to directly compare level of resistance and at different developmental stages.

The only deviation from the proposed objectives for the 2013 season was the lack of PM inoculum during cherry bloom due to difficulties maintaining temperatures conducive to production of conidia in growth chambers. Hence, floral clusters could not be inoculated and objective 5 was not executed in 2013. To avoid the same problem next year, two controlled-environment chambers have been repaired and PM inoculum will also be also continuously propagated on trees in the greenhouse. Additionally, a new (long-term) storage protocol is under evaluation. If proven successful, stored conidia could be used to start inoculum production in controlled environments well in advance of the growing season.

SIGNIFICANT FINDINGS (2013)

- Immature, developing cherry fruit appear to exhibit an innate resistance to PM infection in orchard studies.
- Susceptibility to PM infection increased with level of maturity (age).
- Severity of fruit infection may be partly dependent on disease pressure beginning 45 days after full bloom.
- The time lag between initial spore deposition and symptom development (incubation period) on fruit was longer than anticipated in controlled environment studies and overcame the capabilities of the experimental technique.
- Applications of quinoxyfen (Quintec®) decreased incidence and severity of PM in orchard studies on cv. 'Bing'.
- In naturally infected fruit (cv. 'Bing'), no significant differences of fruit firmness (FF), row size, soluble solid content (SSC), and titratable acidity (TA) were observed. On 'Sweetheart' cherries, fruit treated with one sequence of fungicides had lower soluble solids and higher fruit firmness.
- Pitting susceptibility was not affected by different fungicide treatments.
- In artificially inoculated fruit (cv. 'Bing'), incidence of PM corresponded to fruit SSC.

METHODS

Inoculum production: Immature foliage was used for inoculum production under controlled conditions and served as a source of fresh inoculum (of *P. clandestina*) of a known age. About ten trees cv. 'Mazzard' were planted every 7-10 days beginning in mid-March and inoculated by brushing conidia (conidia) of mass isolates of *P. clandestina* onto immature foliage. Trees were grown in a greenhouse at 64F – 72 F and shaken 24 hours prior to inoculum harvest to remove stale conidia. To harvest inoculum, about 10 infested, immature leaves were placed in sterile distilled water and comminuted using a blender. Inoculum concentrations were adjusted to specified levels using additional sterile distilled water and a hemacytometer.

Disease assessments: Disease on leaves in orchard studies was periodically assessed based on absence/ presence (incidence) and percentage leaf area infected (severity). Cherry fruit infected by *P. clandestina* 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 Also, a collodion epidermal peel technique was used along with dissection microscopy to evaluate fruit infection.

Inoculum concentration studies: Immature detached fruit and shoots (cv. Sweetheart) were harvested from trees by severing the fruit pedicel with a razor blade (Objectives 1a-b). Pedicels and shoot bases were immediately immersed in suitable containers containing water, placed in humidity chambers and inoculated with suspensions of conidia of 100, 500, 1000, 5000, and 10,000/ml. Detached fruit and shoots were incubated at 71.6 F at 80% RH in a controlled-environment chamber. Fruit were evaluated for evidence of colonies 7, 14, and 21 days after inoculation using a dissection microscope.

Similar studies were conducted in the orchard on attached fruit of 'Sweetheart' (Objective 1c). Fruit clusters were inoculated using various concentrations of conidia as described above. Two hours following inoculation fruit were covered with 8µm mesh Nitex cloth. About 10 fruit/replication were harvested 7 and 14 days following inoculation and observed microscopically for symptoms and signs

of PM. Inoculated fruit were incubated an additional 7 days as described above and again microscopically observed for symptoms and signs of the disease. The remaining 10 fruit/replication were observed at harvest for PM.

Humidity/Temperature Studies (Objective 2): Detached, immature fruit with petioles attached were collected as described above and inoculated with 5,000 conidia / ml. Fruit were placed in humidity chambers with varying relative humidities of 60%, 70%, 80%, 90%, and 95%. Fruit were incubated for 7 and 14 days at 7.5˚C (45.5F), 15˚C (59F), 22˚C (71.6F), 25˚C (77F) and 30˚C (86F) in controlled environment chambers and then observed microscopically for symptoms and signs of PM. Four replications of five fruits/replication were used at each temperature: relative humidity combination. If PM symptoms and signs were not evident after 14 days, fruit were incubated an additional 2-3 weeks and then reexamined.

Orchard inoculation timing studies (Objective 3): Initial experiments were conducted at inoculum concentrations of 5000 conidia/ml. Five single-tree replications (cv. 'Sweetheart') were used as fruit sources. Eight-µm mesh pieces of Nitex cloth were used to cover fruit clusters (about 50 fruit / tree) to contain deposited conidia and avoid natural infection. The remaining fruit on each replication/variety were left uncovered and exposed to natural airborne inoculum throughout fruit development (positive control). On each inoculation date Nitex covers were removed from one treatment and inoculated with a suspension of conidia (inoculated treatment) as described above. Covers were reapplied to the inoculated treatments within 30 min of inoculation; 25 additional fruit uncovered on that day were not inoculated but will remained exposed/uncovered for two subsequent weeks and then the covers reaffixed (natural exposure treatment). A set of clusters remained covered throughout fruit development (negative control). Fruit were harvested in July and disease was assessed as described above.

Orchard fungicide timing studies (Objective 4): Cultivar 'Bing' trees in a mature orchard at WSU-Prosser were utilized for studies on the application timing of Quintec (quinoxyfen; DowAgroSciences). Fungicide applications commenced at shuck fall bloom and continued biweekly until the beginning of June. Two sequential applications of Quintec were applied at different stages of fruit development beginning at shuck fall. Procure or Topguard was applied at all other stages in each treatment regimen. Disease was assessed shortly before harvest, and fruit were transported to Hood River, OR for fruit quality analyses. A replicated “sliding Quintec” trial was also conducted on cv. 'Sweetheart' in Wenatchee, WA using a commercial disease management approach.

RESULTS AND DISCUSSION

Much of the fruit quality data will be presented in poster format.

PM development was poor when fruit were harvested while immature, inoculated, and then subjected to various temperature and relative humidity regimens or inoculated under different inoculum concentrations. These fruit were harvested and inoculated about 5-6 weeks prior to harvest and deteriorated under the incubation conditions before disease could be observed on fruit surfaces. The results of 2013 orchard experiments and observations on the incubation period indicate the future controlled-environment studies should be conducted on more mature fruit when the incubation period appears to be shorter. In 2014 a large T/RH study will be conducted on fruit harvested and inoculated about 7-10 days prior to harvest.

In orchard studies immature, developing cherry fruit were relatively resistant to PM infection and there were no significant effects of inoculum concentration on infection and disease severity (data not

shown, see poster presentation). Susceptibility to PM infection increased with level of maturity (age). This finding is supported by results from orchard (Objectives 1c, and 3) and *in vitro* studies (Objectives 1a-b, and 2) and is in contrast to infections on leaves and fruit from other commodities (e.g. hop cones and leaves, grape leaves and berries). In those crops, susceptibility decreases with increasing age (termed ontogenic resistance). The increased susceptibility may be a function of physiological changes during cherry development, such as rapid changes in cuticular membrane (e.g. thickness, and chemical composition). While cuticular membranes generally increase in thickness in leaves and some fruit (e.g. apples), cuticular membranes rapidly decrease in thickness in cherries as they mature. The link between fruit infection and cuticular thickness requires further study.

Incidence and severity of fruit infection increased beginning about 45 days after full bloom. Fruit artificially inoculated with PM 45 or 60 days after full bloom had a higher incidence of infection than fruit inoculated in early May (Table 1). However, there is a time lag between initial spore deposition on the cherry surface and onset of visible disease. Results from the orchard studies showed that cherries infected with PM in May (30 days after full bloom) had the same level of PM infection at harvest than the negative control (fruit clusters never exposed to inoculum, always covered) and significantly less infection than the positive control (fruit clusters constantly exposed to natural inoculum). This is in contrast to fruit inoculated in early and late June (45 and 60 days after full bloom) where PM infection was significantly higher at harvest.

Applications of quinoxyfen (Quintec) limited incidence and severity of (Table 2). Trees treated with only Quintec had statistically less PM infection than trees treated with other fungicides and the untreated control. Fungicide rotations (Quintec, Topguard) showed potential but differences relative to the untreated control were only marginally significant. However, disease pressure in 2013 was much lower than normal and the variability in spatial disease distribution was higher than in previous years.

In naturally infected fruit (cv. 'Bing'), no significant differences of fruit firmness (FF), row size, soluble solid content (SSC), and titratable acidity (TA) were observed among the 11 treatments (Figure 1). Pitting susceptibility, indicated by percentage of severely induced-pits, was not affected by the different fungicide treatments. Fruit from all treatments had increased FF (~30%) and reduced TA (~18%) after 2 weeks of cold storage at 32°F (data not shown, see poster presentation). In artificially infected fruit (cv. Bing), higher infection index and incidences of treatments 4, 5, 6, and 8 (control) produced fruit with lower SSC. Trees which had lower disease severity and incidence tended to produce fruit with higher FF and lower pitting susceptibility, although the differences were consistently significant statistically. The leaves infected by powdery mildew may reduce carbon assimilation by photosynthesis, and therefore reduce fruit sugar content and FF at harvest. Previous research (Wang and Einhorn, 2013) indicated that FF is negatively related to pitting susceptibility of sweet cherry.

Both foliar and fruit PM incidence was extremely low or not observed (respectively) in the cv. 'Sweetheart' orchard trial (Table 3). The lack of PM precluded in depth disease incidence and severity evaluations although fruit harvested for quality evaluations were examined for symptoms and signs of PM. Fungicide treatment had no effect on pitting, but fruit treated with one sequence of fungicides (Luna > Tilt > Pristine > Procure > Quintec > Tilt > Pristine) had lower SSC and higher fruit firmness (Figure 1) compared to other treatments. The reason for the treatment effects are unclear but may be due to a PGR effect caused by DMI fungicides Procure (triflumizole) and/or Tilt (propiconazole).

*Inoculation date**	*Mean Incidence (%)*		*Severity*	
	Inoculated	Natural exposure	Inoculated	Natural exposure
5/28/2013	4.1 B**	25.9 AB	0.04 A	0.4 A
6/12/2013	29.4 AB	65.3 A	0.4 AB	1.0 A
6/27/2013	47.8 AB	43.5 AB	0.7 AB	0.7 A
Negative control, Always covered	6.5 B	6.9 B	0.1 AB	0.1 A
Positive control, Never covered	53.0 A	53.0 AB	0.7 B	0.7 A

Table I. incidence and severity of mature 'Sweetheart' cherry fruit at Prosser, WA, 2013.

* On the respective date, developing fruit clusters were either inoculated with a spore suspension (5000 spores/ml) (inoculated) or were naturally exposed to airborne inoculum (natural exposure). Both treatments were covered with Nitex bags after inoculation. Disease was assessed on 7/30/2013
**Results are averages of five replicates. Values within a column followed by a common letter are not significantly different based on a Tukey's T-test ($P < 0.05$).

Treatment #	*Treatment**	*Mean PM** Incidence (%)*	*Mean PM** Severity*
T11	Topguard [1]only	63 A	1.9 A
T3	Procure[2]-Procure-Quintec[3]	52 AB	1.8 BC
T2	Procure-Quintec-Quintec	44 ABC	2.0 ABC
T10	Topguard[4] only	43 ABCD	1.7 BC
T5	Procure only	40 ABCD	3.9 A
T4	Procure only	38 ABCD	3.9 A
T1	Quintec-Quintec-Procure	35 ABCD	1.7 BC
T6	Procure only	21 BCD	2.5 AB
T9	Topguard[4]-Quintec-Topguard[4]	10 CD	1.1 BC
T7	Quintec only	6 D	0.3 C
T8	Non-treated control	30 ABCD	2.0 ABC

Table 2. Effect of fungicide treatments (T1-T11) on incidence and severity
[1] Application rate: 7fl oz/A
[2] Application rate: 16fl oz/A
[3] Application rate: 7fl oz/A
[4] Application rate: 14fl oz/A
* Three applications total (5/7/13, 5/23/13. 6/6/13), in the specified order
** Results are averages of four replicates. Values within a column followed by a common letter are not significantly different based on a Tukey's HSD test ($P < 0.05$)

TMT	15-May	30-May	10-Jun	20-Jun	1-Jul	10-Jul	23-Jul	1-Aug
1	**Quintec**	Tilt	Pristine	Procure	Quintec	Gem	Tilt	Pristine
2	Luna	**Quintec**	Pristine	Procure	Quintec	Gem	Tilt	Pristine
3	Luna	Tilt	**Quintec**	Procure	Quintec	Gem	Tilt	Pristine
4	Luna	Tilt	Pristine	**Quintec**	Quintec	Gem	Tilt	Pristine
5	Luna	Tilt	Pristine	Procure	**Quintec**	Gem	Tilt	Pristine

Table 3. Fungicide program study on cv. 'Sweetheart' cherries conducted near Wenatchee, WA. Both fruit and foliage had no visible PM infection at harvest. Fruit were transported to Hood River, OR for quality analyses.

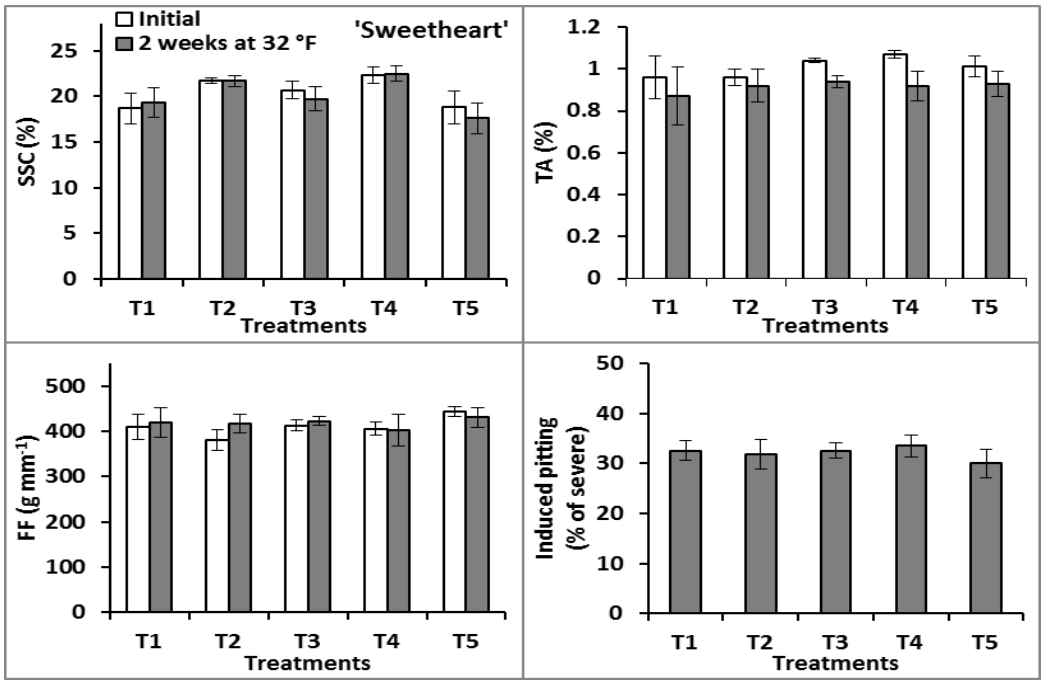


Figure 1. Effect of fungicide treatments (T1-T5, Table 3) on fruit quality at harvest and after 2 weeks of cold storage of 'Sweetheart' sweet cherry under natural inoculation conditions.

CONTINUING PROJECT REPORT **YEAR: 1 of 2**
WTFRC Project Number: CH-13-104

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

PI:	Spencer S. Walse	**Co-PI (2):**	David Obenland
Organization:	USDA-ARS-SJVASC	**Organization:**	USDA-ARS-SJVASC
Telephone:	559.596.2750	**Telephone:**	559.596.2801
Email:	spencer.walse@ars.usda.gov	**Email:**	david.obenland@ars.usda.gov
Address:	9611 S. Riverbend Ave	**Address:**	9611 S. Riverbend Ave
City/State/Zip:	Parlier, CA 93648	**City/State/Zip:**	Parlier, CA 93648

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

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

Other funding sources: None
WTFRC Collaborative expenses: None

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

Item	2013	2014	NA
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

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. Obanland) 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 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 objectives with another species of fruit fly pest, such as the Western cherry fruit fly (or the brown marmorated stinkbug), maintained at the Contained Research Facility at the University of California at Davis.

Significant findings:

- PH3 fumigation at cold-storage temp will control SWD in 36 to 48 h.
- Residues and worker exposure with PH3 are favorable (relative to MB)
- Fruit quality evaluations look promising; more varieties recommended

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. 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 (Figure 1). 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.

Fruit infestation. To simulate a naturally occurring infestation scenario, 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 and then infested cherries were transferred in pairs into a stainless-steel mesh ball cage (5.1-cm diameter). Mesh ball cages containing infested cherries were randomly selected, placed inside a pull-string cloth bag (~25 per bag), and used in laboratory-scale exploratory fumigations or buried throughout the load of commercial fruit bins in confirmatory-scale fumigations. Alternatively, mesh ball cages were not fumigated and held as untreated controls to estimate the number of individuals treated during a respective fumigation. For the exploratory fumigations, removal of cherries from rearing cages was synchronized to yield profiles of discrete development across all SWD life stages (less adults). For the confirmatory fumigations, cherries were removed from an enclosure after a 24-h ovipositional period so that only 0-24 h old eggs, the most PH3-tolerant age of SWD (*vide infra*), were present at the start of a pre-fumigation period of temperature equilibration (i.e., tempering).

Exploratory fumigations. To determine the treatment duration required to control the life stages of SWD with 1.6 mgL^{-1} (1000ppmv) and 3.7 mgL^{-1} (2500ppmv) phosphine (PH3) at 1.7 ± 0.5°C ($\bar{x} \pm s$) (~35°F), a series of exploratory fumigations were conducted in modified Labonco® 28.32-L vacuum chambers. Chambers were housed in a walk-in environmental incubator with tunable temperature, humidity, and pressure (USDA, 2010). Test specimens, non-fumigated control specimens, source-gas cylinders, and gas-tight syringes were acclimated, or tempered, to fumigation temperature of 1.7 ± 0.5 °C ($\bar{x} \pm s$)(~35°F) for 12 h prior to treatment. Sweet cherries infested with the various life stages of SWD were fumigated concomitantly within a chamber for a particular fumigation trial.

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 dose 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 the pressure needed for the respective trials 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.

Following the final sampling for fumigant concentration, chamber valves were opened to atmosphere and a 1-h aeration period was initiated. Chamber lids were then opened and the treated and non-treated infested sweet cherries were collected and transferred to an incubator at 27.0 ± 1.0 °C (~80°F) and 80 ± 2% RH ($\bar{x} \pm s$) prior to mortality evaluation.

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) set to treatment temperature of at 1.3 ± 0.5°C ($\bar{x} \pm s$)(~34.3°F). On the same day that they were packaged for export, either Bing or Coral variety sweet cherries were obtained from commercial wholesale sources. Cloth bags containing infested cherries were buried amongst noninfested cherries in wooden fruit bins (45.72l × 45.72w × 30.48h cm), which were constructed out of 1.3 cm –thick plywood as scaled-down replicates of those used in industry, to a level of ~75% capacity (Figure 2). The chamber was loaded with two fruit bins, bringing the chamber load to ~ 50 %($V_{commodity}/V_{chamber}$x 100), as calculated by the method of Monro (1969).

Chambers loaded with infested and uninfested cherries, cherries infested with control specimens, source-gas cylinders, and gas-tight syringes were acclimated to fumigation temperature, or tempered, for 12 h prior to treatment. 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 cherries distributed at different locations within bins of the infested cherries undergoing treatment. Temperature probes were then removed, circulation fans internal to the chamber were turned on, and chamber lids clamp-sealed in preparation for treatment. A slight vacuum of approximately 76-127 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 dose as predetermined in preliminary calibration studies. A syringe was fitted to a LuerLok ® sampling valve, which was subsequently opened so that PH3 was steadily drawn into the chamber. The syringe was then removed and normal atmospheric pressure (NAP) was reestablished in each chamber before the valve was closed; this 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, placed into respective pull-string cloth bags, and transferred into separate 0.03-m^3 nylon-mesh rearing cubicles maintained in an incubator at 27.0 ± 1.0 °C and 80 ± 2% RH ($\bar{x} \pm s$). 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 ($\bar{x}$ ± s) (~34.0°F) and temporally retrieved from storage and used for residue determination(s)(*not discussed*).

Mortality evaluation. Mortality of treated specimens was assessed at 1-d intervals post-fumigation for 21 d; cages were removed from the cloth bags, opened, and live adult specimens were tallied and discarded. The cages were then resealed, and placed back into the cloth bags for further incubation and evaluation. Quartered pieces of an uninfested cherry were added to the mesh ball cages approximately every other day to keep the test fruit and insects hydrated. The number of treated specimens was estimated by the cumulative number of adults that emerged from untreated controls.

Rearing and incubation conditions of 27.0 ± 1.0 °C (~80°F), 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 2-5 °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, we generally observed increases in the mortality of all SWD 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.

Chemical analysis. Fumigant levels in headspace of fumigation chambers were measured using gas chromatography; retention time were 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 (Walse et al 2012a & b). Detector response and retention indices were determined each day in calibration studies by diluting known volumes of gaseous 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 ($\bar{x}$ ± s) (~34.0°F) plus 16 hours at 22.2 ± 0.6 °C ($\bar{x}$ ± 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.

Results & Discussion:

Executive summary. Phosphine chamber fumigations were evaluated for postharvest control of spotted wing drosophila, *Drosophila suzukii*, in fresh sweet cherry exports from Western USA. A series of exploratory

fumigations were conducted to establish a toxicological response for pupae, larvae, and egg life stages. Fruit were infested with the various life stages and fumigated with 1.6 mgL^{-1} (1000ppmv) or 3.7 mgL^{-1} (2500ppmv) phosphine for 12, 24, 36, and 48 h at 1.7 ± 0.5 ºC ($\bar{x} \pm s$)(~35.0°F). The applied dose of cylinderized phosphine (1,000 or 2,500 ppm) did not affect the efficacy of fumigation, suggesting that the load factor and the load geometry are inconsequential, as long as the minimum headspace concentration at the end of fumigation is ca. 1000 ppm phosphine. In confirmatory fumigations, which simulated the commercial scenario, complete mortality of 35,265 ± 1,006 (*n* ± *SE*) eggs (ca. 12 to 36-h old at fumigation), the most tolerant SWD life stage, was achieved with an applied dose of 1000 ppm, a load factor of ~ 50%, and a treatment time of 48 h at 1.7 ± 0.5 ºC ($\bar{x} \pm s$)(~35.0°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.

Results from fruit quality evaluations following confirmatory SWD fumigations with 1000 ppm PH3 and treatment durations of either 24 or 48 h are detailed below.

Table 1. Subjecting rating scores for cherries.

Quality Attribute	Score	Description
Surface browning	0	No browning, full red color
	1	Slight browning, 1 - 25% of the fruit surface
	2	Moderate browning, 26 - 50% of the fruit surface
	3	Severe browning, >50% of the fruit surface
		Scores > 1 considered unacceptable
Stem browning	0	None
	1	1 - 25% brown
	2	26- 50% brown
	3	51 - 75% brown
	4	76 - 100% brown
		Scores > 2 considered unacceptable
Surface pitting	0	None
	1	Slight
	2	Moderate
	3	Severe
		Scores > 1 considered unacceptable
Surface cracking	0	None or insignificant cracking
	1	Slight, 1 - 2 mm long, shallow crack
	2	Moderate, 2 - 4 mm long, deep crack
	3	Severe (>5mm long, deep crack)
		Scores > 1 considered unacceptable
Surface shrivel	0	None
	1	Obvious shrivel
		Scores > 0 considered unacceptable
Decay	0	None
	1	Decay
		Scores > 0 considered unacceptable
Overall acceptability	0	Very good
	1	Some damage but still good and marketable
	2	Obvious damage but still marketable
	3	Unacceptable

'Bing' cherry quality following phosphine fumigation at fumigation times of 24 h and 48 h.
Subsequent storage was for 2 days at 34 °F plus 16 hours at 68 °F to simulate air shipment and marketing.

Treatment	Surface browning (% acceptable)[a]	Stem browning (% acceptable)[a]	Pitting (rating)[b]	Decay (%)	Overall acceptability (%)[a]	Color L (Lightness)	Color C (Chroma)	Color h (Hue)	Firmness (g)[c]	Soluble Solids (%)	Acidity (%)
Control, 24 h 35 °F	100a	33a	0.09a	0a	33a	33.35a	22.77a	18.16a	312.11b	18.50a	0.73a
PH3, 24 h 35 °F	100a	19a	0.09a	0a	19a	31.40b	17.74b	15.65b	365.53a	22.00a	0.87a
Fumigation effect[d]	NS	NS	NS	NS	NS	*	*	*	*	NS	NS
Control, 48 h 35 °F	100a	35a	0.09a	0a	35a	31.19a	16.10a	14.75a	338.68a	22.50a	0.88a
PH3, 48h 35 °F	99a	43a	0.09a	0a	45a	32.81a	20.46a	16.98a	297.79a	21.78a	0.81a
Fumigation effect[d]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

aPercent considered marketable.
bPitting was a subjective rating. Lower value = better quality. Pitting: 0, 1, 2 or 3 with 2 indicating that the fruit would likely not be acceptable to a consumer. Ratings are calculated indexes: (i.e. number of cherries with score 0*0) + (number of cherries with score 1*1)/total number of cherries.
cGrams required to cause a 1 mm deflection of the fruit surface.
dStatistical significance of fumigation treatments from controls. NS = not significant, * = significant. Red numbers show significance.

"Bing" conclusion: Some change in color was noted for the fumigated fruit but this was only the 24 h treatment and not the 48 h and is probably not an important factor. Firmness was enhanced in the fumigated fruit, but again only for the 24 h treatment. No change in any of the other quality attributes as a result of fumigation. Stems for both control and fumigated fruit were markedly browner in the fruit used for the phosphine tests as compared to simultaneous MB testing (for Korea export). The high amounts of stem browning are what caused the low levels of overall acceptability but there was no difference due to fumigation.

'Coral' cherry quality following phosphine fumigation at fumigation times of 24 h and 48 h.
Subsequent storage was for 2 days at 34 °F plus 16 hours at 68 °F to simulate air shipment and marketing.

Treatment	Surface browning (% acceptable)[a]	Stem browning (% acceptable)[a]	Pitting (rating)[b]	Decay (%)	Overall acceptability (%)[a]	Color L (Lightness)	Color C (Chroma)	Color h (Hue)	Firmness (g)[c]	Soluble Solids (%)	Acidity (%)
Control, 35 °F	100a	84b	0.10ab	0.00	83.75c	31.78a	20.64a	15.64a	307.66a	17.75a	1.04a
"7a", 24 h, 35 °F	100a	99a	0.13ab	0.00	98.75a	31.64a	20.36a	15.99a	301.62a	18.08a	1.01a
"7b", 24 h, 35 °F	100a	95ab	0.05b	0.00	98.75a	32.19a	21.85a	16.62a	309.84a	17.10a	1.04a
"8a", 48 h, 35 °F	100a	95ab	0.10ab	0.00	96.25ab	32.45a	22.69a	16.87a	299.34a	17.45a	1.09a
"8b", 48 h, 35 °F	100a	98a	0.21a	0.00	91.25bc	32.09a	21.91a	16.59	292.17a	17.73a	1.03a
Fumigation effect[d]	NS	*	NS	NS	*	NS	NS	NS	NS	NS	NS

aPercent considered marketable.
bPitting was a subjective rating. Lower value = better quality. Pitting: 0, 1, 2 or 3 with 2 indicating that the fruit would likely not be acceptable to a consumer. Ratings are calculated indexes: (i.e. number of cherries with score 0*0) + (number of cherries with score 1*1)/total number of cherries.
cGrams required to cause a 1 mm deflection of the fruit surface.
dStatistical significance of fumigation treatments from controls. NS = not significant, * = significant. Red numbers show significance.

"Coral" conclusion: Virtually no negative effect of fumigation with the exception of a very small increase in pitting in 8b.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: CH-12-105

Project Title: Spotted Wing Drosophila management on sweet cherry

PI: Elizabeth H. Beers
Organization: WSU-TFREC
Telephone: 509-663-8181 x 234
Email: ebeers@wsu.edu
Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee, WA 98801

Cooperators: Tim Smith, Chelan-Douglas Extension; Doug Walsh, WSU-IAREC

Total Project Request: **Year 1:** $50,000 **Year 2: $50,000** **Year 3:** $50,000

Other funding sources

Agency Name: WSDA Specialty Crop Block Grant
Amt. 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. 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 for subaward (funding is yearly, with a planned 3-year term).

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

Budget 1
Organization Name: WSU-TFREC **Contract Administrator:** Joni Cartwright; Carrie Johnston
Telephone: 509-663-8181 x221; 509-335-4564 **Email address:** joni.cartwright@wsu.edu; carriej@wsu.edu

Item	2012	2013	2014
Salaries[1]	12,000	12,480	12,979
Benefits[2]	4,829	5,023	5,224
Wages[3]	15,925	15,925	15,925
Benefits[4]	12,199	12,199	12,199
Equipment	0	0	0
Supplies[5]	2,395	1,722	1,022
Travel[6]	2,652	2,652	2,651
Plot Fees	0	0	0
Miscellaneous	0	0	0
Total	50,000	50,000	**50,000**

Footnotes: [1]Salaries: 0.25 FTE Research intern; [2]Benefits 40.2%; [3]Wages, 1 research assistant $12.25/h, 40 h/wk, 26 wk/year; 1 research assistant $12.25/hr, 20 hr/wk, 13 wk/year; [4]Benefits 76.6%; [5]Supplies - traps, apple cider vinegar, baits, lures, rearing supplies, plot charges; [6]Travel - to research plots, 200 miles/wk, 26 weeks/yr, $0.51/mile.

OBJECTIVES

1. *Provide a crop protection alert system to cherry/stone fruit producers and seasonal phenology information through a regional SWD trapping program.*
   This objective will continue through 2014, with the goal to transition the areawide monitoring/alert system over to the industry (if there is consensus on sharing information in this manner). Assistance may be in the form of website support, and training individuals responsible for identification of SWD in each participating organization.

2. *Determine timing of cherry fruit susceptibility in the field.*
   We have completed two years of studies on this objective using field-lab bioassays. This has provided us with 'worst-case' scenario data, where the female SWD are closely confined with fruit of various maturities. The 2014 studies will concentrate on using field cages to expose the fruit to laboratory flies in an orchard setting, where alternate food and oviposition resources will be available.

3. *Test standard trap types for capture efficiency of SWD (in collaboration with SCRI-SWD regional group).*
   This objective will continue through 2014 as technology in this area evolves. The 2013 tests employed several wet baits as in the past, and also two new dry baits. Trap design parameters have been explored for the wet baits, but future baits/lures may have a very different optimal design. In addition to maximizing trap capture, design considerations must include ease of use in the field or during transportation.

4. *Test pesticide efficacy for control of SWD in cherries in laboratory, field-laboratory, and field settings.*
   We will continue to test both full canopy and bait sprays in 2014, with emphasis on creating pressure through the use of field cages in unreplicated plots. For the bait sprays, meso-scale bioassays will be used to determine the relationship between droplet density and fruit protection.

SIGNIFICANT FINDINGS

- For the first time in 2013, SWD were caught in every month of the year (likely tied to a mild winter).
- Seasonal phenology followed a similar pattern to past years, with low populations through the winter, spring and summer, rising in August, and high during the fall.
- 'Bing', 'Lapins', and 'Rainier' cherries were susceptible to SWD by 4 June, 'Sweetheart' a week later.
- Higher trap catches were positively related to higher bait volumes.
- Two new Trécé dry lures provided similar trap catch to apple cider vinegar (ACV); the addition of the lures to an ACV trap enhanced capture.
- High levels of mortality in bioassays were found up to 21 days for many products, but high levels of fruit protection lasted no longer than 7 days.
- Canopy sprays and bait sprays provided similar levels of mortality in bioassays, as long as flies are exposed to fresh droplets.

METHODS

1. *Provide a crop protection alert system to cherry/stone fruit producers and seasonal phenology information through a regional SWD trapping program.* SWD populations were monitored in eastern Washington beginning in January with 359 traps placed strategically in fruit growing areas from the Canadian border to the Tri-Cities. The majority was in cherry orchards (172), but peach (20), nectarine (13), apple (2), apricot (17), blueberries (75), caneberries (20), grapes (38), and chokecherries (2) were also monitored. A group of core traps was monitored by WSU personnel year round (Beers, Walsh programs), with an additional group checked weekly by volunteer trappers during the growing season. The trap capture database was available on the

WSU-SWD website, along with an alert system. The first captures were listed in a table and chart on the splash page of the website, and an email was sent to the *SWDinterestgroup* list as each region caught the first fly. The custom graphics feature of the website, where growers or consultants can track their own trap catches, or look at aggregate catch, was available during the growing season.

2. *Determine timing of cherry fruit susceptibility.* Field-grown cherries (cvs 'Bing', 'Lapins', 'Rainier', 'Sweetheart') were collected at one-week intervals from 4 June through 23 July. The fruit were challenged with laboratory-reared flies in a bioassay arena consisting of a 16 oz plastic cup with three cherries suspended from the lid. Five female and two male SWD were introduced into the arena, and removed after 24 h. Oviposition punctures were counted, and fruit were incubated at 22 °C (72 °F) for 21 days, when the resulting adults were counted. A second test was conducted by releasing flies into field-grown caged single trees (Sweetheart only). Trees (1 tree/week, unreplicated) were caged at the beginning of the period (11 June), and 100 flies were released at the beginning of the exposure period of 1 week. After a week of exposure, the tree was stripped of fruit, which was incubated for 22 °C (72 °F) for 21 days, when the emerging flies were recorded.

3. *Test of baits, lures, and trap geometry.* Two new lures from Trécé were compared to wet baits (Monterey Ag Bait, yeast-sugar bait, wine-vinegar-molasses bait, and apple cider vinegar (ACV)), where the bait and the catching fluid are one in the same. Lures were placed in a 32 oz plastic jar with a red lid and a 1.5-in band of red duct tape around the middle of the trap (Fig. 1). Entry holes (1.5 in dia, 3/trap) covered with plastic mesh allowed diffusion of bait scent and entry of flies, while screening out larger arthropods. Traps were deployed in June, and checked weekly for various intervals depending on the test.

   Trap design parameters were tested by varying bait volume and surface area in a 3 x 3 factorial design, keeping the headspace above the bait constant. All traps used ACV as the bait/catching fluid. Three bait volumes (200, 350, and 500 ml [6.8, 11.8 and 16.9 fl oz, respectively]) and three surface areas (21.4, 47.8, and 82.5 cm^2 [3.3, 7.4 and 12.8 in^2, respectively]) were tested. Traps were deployed in August, with all replicates in a single sweet cherry orchard. Trap contents were retrieved and counted weekly. Parameters recorded included male and female SWD, other drosophila, Diptera, Coleoptera, and the bait volume remaining at the end of the week.

Fig. 1. The standard trap (aka the PBJ trap) used in the 2013 trapping program.

4. *Test pesticide efficacy for control of SWD in cherries in laboratory, field-laboratory, and field settings.* The lengths of residual control of candidate pesticides were tested in field-aged residue bioassays. Pesticides (Delegate, Entrust, Fyfanon, Sevin, and Diazinon) were applied to single tree plots with an airblast sprayer calibrated to deliver 100 gpa. Treated fruit and leaves were collected at intervals after the spray and challenged with laboratory-reared flies. The bioassay arena consisted of a 16-oz plastic cup lined with the treated leaves, and three treated fruit suspended from the lids. Ten female SWD were introduced into the arenas. Fruit were removed after 24 h of exposure, and oviposition punctures counted. Adult mortality was assessed after 48 h. Fruit were incubated for 21 d at 22 °C (72 °F), and emerging adults counted.

   A similar test examined the effect of application method on the length of residual control. The material Warrior II was applied airblast at 400 and 100 gpa, using the same rate/acre, and using a handgun (at the dilute, or 400 gpa concentration). Treated fruit and leaves were collected and challenged in bioassays as described above.

RESULTS & DISCUSSION

1. Provide a crop protection alert system to cherry/stone fruit producers and seasonal phenology information through a regional SWD trapping program. For the first time, SWD captures have been recorded in every month of 2013 to date (through October). Mild winter temperatures would appear to be responsible for this. The numbers of flies caught was low from January through July, with a sharp increase in August, and peak captures in October (to date) (Fig. 2). The general pattern (a single population peak in late summer through fall) is similar to that of previous years. However, the peak fall populations in 2013 were considerably higher than in 2012, with the early season and greater establishment perhaps contributing. The first capture occurred in most regions during the period of fruit maturity/susceptibility, and crop protection measures were warranted in most regions throughout this period.

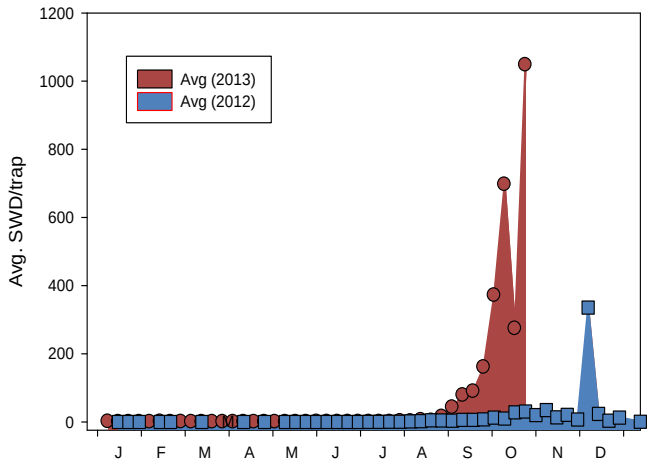
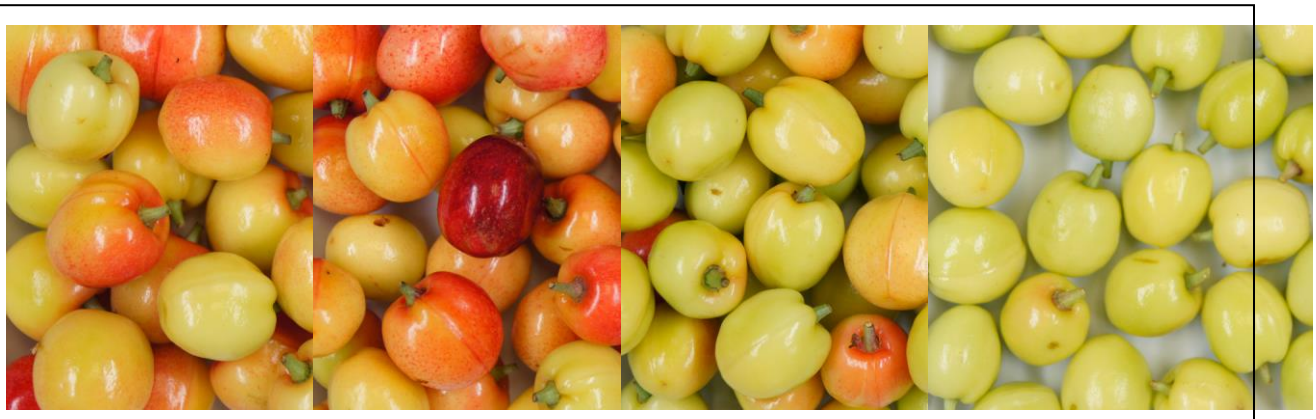


Fig. 2. Seasonal SWD captures in ACV traps in eastern Washington, 2012 and 2013.

As in previous years, first captures by region, and the seasonal phenology of SWD, was reported on the SWD website http://www.tfrec.wsu.edu/pages/swd.

2. Determine timing of cherry fruit susceptibility in the field. SWD were able to oviposit in 'Bing', 'Rainier', and 'Lapins' cherries (Columbia View) by 4 June, when fruit was green with a slight blush, and firmness ranged from 576-729 g/mm (Fig. 3). Only 'Sweetheart' was not attacked on that date; fruit were fully green, and firmness measured 1196 g/mm. However, 'Sweetheart' were susceptible to attack by the next sample date (11 June). Fruits in which oviposition punctures occurred also produced viable adults. The greatest numbers of eggs were laid in the first half of July, and attack rate decreased thereafter. Fruit were susceptible from ca. 21 to 42 days before commercial harvest, depending on the cultivar.



Bing Lapins Rainier Sweetheart

Fig. 3. Cherry color on 4 June, when 'Bing', 'Lapins', and 'Rainier' were susceptible to attack.

The field-caged 'Sweetheart' trees exposed to lab-reared flies were susceptible to attack beginning 17 June, with the peak numbers of adults produced during the first of July. The estimated commercial harvest date for this block was 23 July, thus the peak occurred ca. 3 weeks before harvest (Fig. 4).

Adult production was lower as the fruit senesced, apparently providing a poorer host for larval development.

3. Test standard trap types for capture efficiency of SWD (in collaboration with SCRI-SWD regional group). The SCRI research group did not have a trap test in 2013; instead, individual labs examined various baits and lures. Trécé is testing two new lures nationwide, 0890 and 0891. These were tested using water or ACV as the catching fluid. Trécé 0890 and 0891 over ACV had the highest captures, which were statistically superior to ACV alone (Fig. 5). The same lures over water, and superbait (a mixture of wine, water, molasses, and vinegar), were not statistically different than ACV alone. Yeast bait (a mixture of yeast, sugar, and water) caught the fewest SWD.

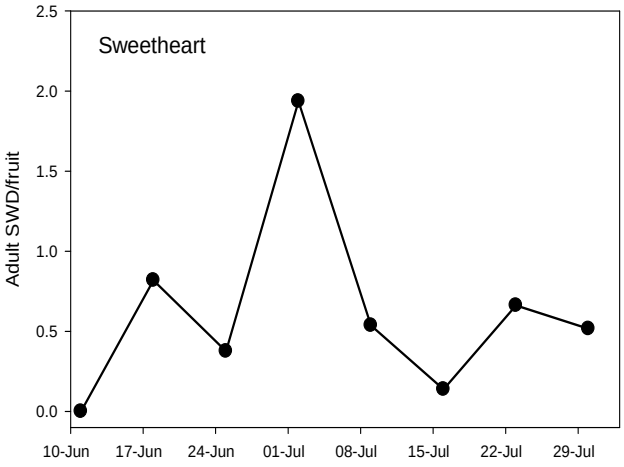


Fig. 4. Production of adult flies from field-caged 'Sweetheart' trees exposed to laboratory-reared SWD.

My lab also looked at the effect of bait volume and surface area on trap catch in ACV traps. Trap capture increased with increasing bait volume (200, 350, 500 ml [6.8, 11.8 and 16.9 fl oz, respectively] of ACV) (Fig. 6). There was also a significant effect of surface area, but the highest captures occurred in the intermediate surface area (47.8 cm^2 [7.4 in^2]) (data not shown). The 'headspace', or volume of air in the trap not occupied by ACV was held constant; this created some unusual trap geometries, which may account for the ordering of treatments.

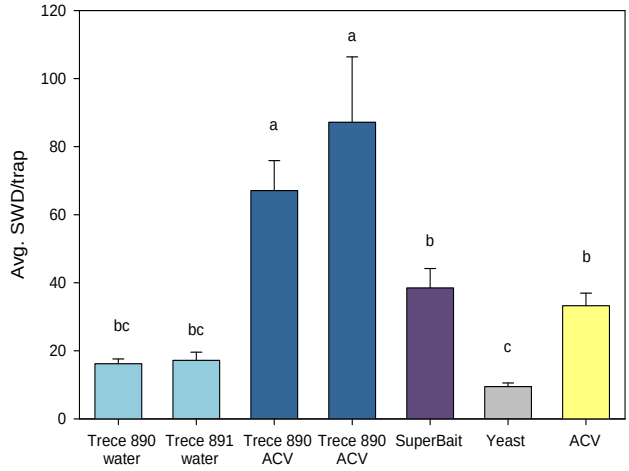


Fig. 5. Captures of SWD adults with various lures and baits, 2013.

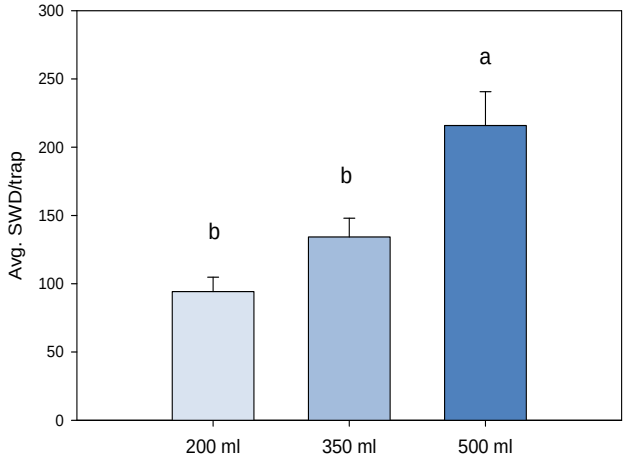


Fig. 6. SWD trap captures increase with increasing bait volume.

4. Test pesticide efficacy for control of SWD in cherries in laboratory, field-laboratory, and field settings. Warrior at 400 gpa, 100 gpa (applied airblast) and a handgun application provided similar levels of mortality throughout a 35-day post-treatment interval. Mortality was 100% through 10 DAT (days after treatment), but declined to 35-71% by 28 DAT. The 100 gpa and handgun applications provided statistically higher levels of mortality on several sample dates, but there was some variability in ranking of treatments depending on the date (Fig. 7).

Delegate provided high levels of mortality (>90%) through 14 DAT, as did Entrust. Sevin and Diazinon provided similar levels through 10 DAT, but activity dropped off precipitously by 14 DAT. Fyfanon gave good control through 4 DAT, but had dropped to 36% by 7 DAT. Rimon alone did not provide control of adults, but Rimon+Warrior gave high levels of control through 21 DAT (Fig. 8).

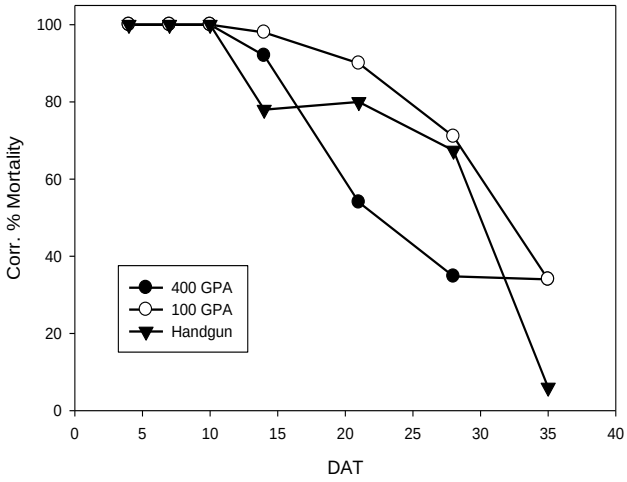


Fig. 7. Residual efficacy of Warrior applied at different gpa and sprayers.

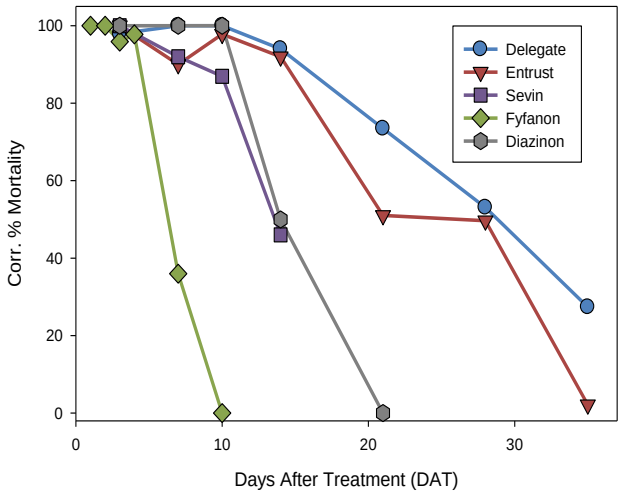


Fig. 8. Residual control of adult female SWD exposed to field-aged residues of various insecticides applied airblast to 'Sweetheart' cherry, 2013.

While the mortality levels remained high for a relatively long period of time, fruit protection (measured by the number of oviposition punctures in the fruit) decreased more rapidly (data not shown). Fruit attack in the Delegate and Entrust treatments rose after 7 DAT, while the attack rate in the Sevin and Fyfanon treatments was not significantly different than the check at 3 DAT. Diazinon had unacceptably high levels of fruit attack at 7 DAT, although it was significantly lower than the check.

The Entrust canopy spray treatment caused 100% mortality on all three bioassay dates (just prior to the 2nd spray, just prior to the third spray, and at harvest). The GF-120 treatment cause 100% mortality just prior to the third spray, but mortality was close to zero by harvest (the third spray was applied 3 DAT). The difference between the two assessments is most likely due to the difficulty of sampling in the field after multiple applications: the droplets on the leaves are still visible, but their age is unknown, and may not represent a fresh deposit. In addition, three precipitation events occurred during this period, although total rainfall was only 0.06 inches (Fig. 9).

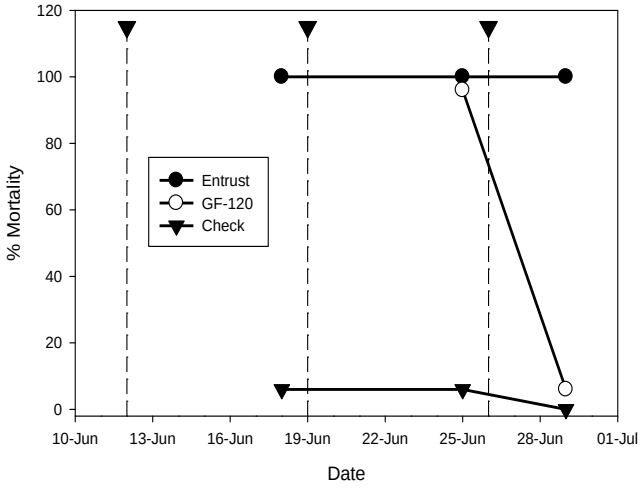


Fig. 9. Mortality of SWD in bioassays following airblast applications of Entrust, ATV applied GF-120, and an untreated check.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: CH-12-106A

Project Title: Identification of chemical lure for spotted wing drosophila

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Cooperators: Peter Shearer, OSU, Hood River; John Adamczyk of USDA, ARS Poplarville, MS;.

Total Project Request: **Year 1:** $35,000 **Year 2:** $34,000 **Year 3: $32,000**

Other funding sources: None

Budget 1
Organization Name: USDA, ARS **Contract Administrator: Chuck Myers**
Telephone: (510) 559-5769 **Email address: chuck.myers@ars.usda.gov**

Item	2013	2014	
Salaries			
Benefits			
Wages	10,100	10,100	
Benefits	900	900	
Equipment			
Supplies	4,500	2,500	
Travel	1,000	1,000	
Plot Fees			
Miscellaneous			
Total	16,500	**14,500**	

Budget 2
Organization Name: ODA **Contract Administrator: Kevin Slater**
Telephone: **Email address:** kslater@oda.stat.or.us

Item	2013	2014	
Salaries			
Benefits			
Wages	10,200	10,200	
Benefits	6,800	6,800	
Equipment			
Supplies	500	500	
Travel	500	500	
Plot Fees			
Miscellaneous			
Total	17,500	**17,500**	

OBJECTIVES

The objective of the project is to develop a reliable early detection system for cherry growers, which would allow them to respond to a spotted wing drosophila (SWD) infestation early and at low population densities. Technical objectives are to:

1. Isolate and identify volatile chemicals from wine that are attractive to SWD.
2. Isolate and identify volatile chemicals from vinegar that are attractive to SWD.
3. Determine an optimum combination of attractive chemicals for an effective lure.
4. Develop a controlled-release dispenser for use as a lure in a trap.

SIGNIFICANT FINDINGS

1. 13 wine and vinegar chemicals elicited fly antennal responses.
2. A four component chemical blend (acetic acid, ethanol, acetoin, and methionol) was as attractive to SWD in field tests as the starting material of a mix of wine and vinegar. This chemical blend was also a stronger lure than currently used baits at low SWD densities in early season cherry orchards.
3. Specifications were determined for inexpensive sachet dispensers of two of the four chemicals of the lure.
4. The 4-component chemical lure attracts fewer non-target insects (moths, yellowjackets, and muscid flies) than the combination of wine and vinegar.

METHODS

2012: Volatile or headspace samples from Merlot wine and rice vinegar were analyzed by GC-EAD (combination gas chromatography-electroantennography), using antennae of SWD flies as the detector. EAD-active compounds were identified by GC-MS, and all EAD-active compounds were purchased for confirmation of their identity and for testing in subsequent bioassays. A laboratory bioassay determined attraction or repulsion of flies to individual chemicals. Combinations of laboratory-attractive chemicals were then field-tested in traps to determine their importance as fly lures. The Agrisense dome trap was used, and traps were placed along roadsides, and outside of berry fields. This work involved the following five field experiments:

1. A comparison of combinations of EAD-active compounds found in wine and vinegar versus wine plus vinegar.
2. A comparison of combinations of the chemicals that were EAD-active minus those chemicals which were repellent in lab assays, versus wine plus vinegar.
3. A series of "add-on" tests which evaluated individual chemicals added to ethanol plus acetic acid.
4. A comparison of EAD-active chemicals which were active in the preceding experiment versus wine plus vinegar.
5. A drop out test, which evaluated the effects of removing each of the blend compounds, showing which compounds are necessary to maintain best attractiveness.

2013: Dispenser Development. To develop a less expensive lure and to optimize the attractiveness of the lure comprised of ethanol, acetic acid, acetoin and methionol, we evaluated the effect of varying the chemical release rates from plastic sachets on SWD flies trapped. This was accomplished by varying the release rate of one chemical while holding the release rates of the other three chemicals constant. Release rates of acetoin and methionol were varied by using different sizes and thicknesses of polyethylene plastic sachets. Similar work with acetic acid and ethanol continues.

Assessment of non-target insect responses. Four field tests evaluated the relative responses of certain non-target insects to the new chemical lure versus the wine/vinegar mix. It has been a

complaint that traps for SWD that utilize food type baits capture large numbers of other types of insects. These other insects in traps complicate the servicing of traps and make it difficult to sort and identify the SWD captured. The four experiments that we conducted in 2013 were set up at times of the season and in cropping systems that would provide good numbers of pest noctuid moths, muscoid flies, yellowjacket wasps, and other Drosophila species, which are routinely numerous in traps with fermentation type baits. The objective of this study was to determine if the chemical lure is more selective than the food type bait for these categories of non-target insects.

Early detection of SWD with a chemical lure. Our work to date has indicated that the dome type trap is superior to the cup type trap, the combination of wine and vinegar is superior to either wine or vinegar alone, and that the combination of acetoin, methionol, ethanol, and acetic acid accounts for SWD attraction to wine plus vinegar. However, this research was conducted when fly densities are high, although the most critical need for trapping SWD as part of a detection and monitoring effort in PNW cherries is in early spring. So we compared the new chemical lure to other baits and traps at this critical time of the growing season. This test was conducted in cooperation with personnel at OSU Hood River, in Hood River commercial cherry orchards. The four chemical lure (Cha lure) was compared to apple cider vinegar, and a yeast/sugar bait.

2014: Dispenser development. We will conduct a small number of additional field tests to further evaluate the use of the sachet dispenser method for acetic acid and ethanol. When that work is concluded, we will follow up with an additional field test to compare 1) a finalized sachet type lure with 2) our original method of dispensing the compounds (vials and drowning solution) and 3) the wine/vinegar mixture that we started with. We will not conduct additional evaluations of the chemical blend in water because of findings in 2013 of a very rapid rate of loss of ethanol from the water.

Early detection of SWD with a chemical lure. We will again work with cooperators, such as OSU Hood River, on comparative evaluations of trap and lure combinations for early detection of SWD in cherry orchards. The selection of these trap and lure combinations will take into consideration the continued experimental progress and development of commercial dispenser technologies.

Efficacy of the chemical lure. We will conduct field tests to determine the relative reliability of the chemical lure in relation to seasonal temperatures and fruit availability. Release rates of chemicals from lures are impacted by temperature, and fly response to the chemical lure may be impacted by the availability of food (reducing fly hunger) and the presence of competing attractive odors (such as decomposing fruits). This will be accomplished by a series of field tests that compare the chemical blend to the wine/vinegar mix, to a vinegar bait, and to a yellow sticky panel with no lure. We anticipate conducting this experiment every 4 to 6 weeks at 5 sites, from early spring into the following winter. This test will not provide a final answer to the question, but rather should indicate if there are serious shifts in fly response to the baits and lure in relation to ambient temperature and fruit presence.

RESULTS AND DISCUSSION

2012: Thirteen wine and vinegar chemicals elicited consistent fly antennal responses. A combination 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 however, because the GC-EAD assay determines chemicals detected (smelled) by the antennae, and does not indicate chemical attractiveness. There is potential for chemicals to be “smelled” by the fly and be repellent or behaviorally neutral.

A laboratory assay was developed and used to test all of the 13-EAD active chemicals individually to determine attractiveness and repellency of chemicals to SWD when added to the combination of acetic acid 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. 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 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 three chemicals that were both EAD-active and co-attractive in field tests. This third generation blend was equal in attractiveness to SWD compared to wine plus vinegar.

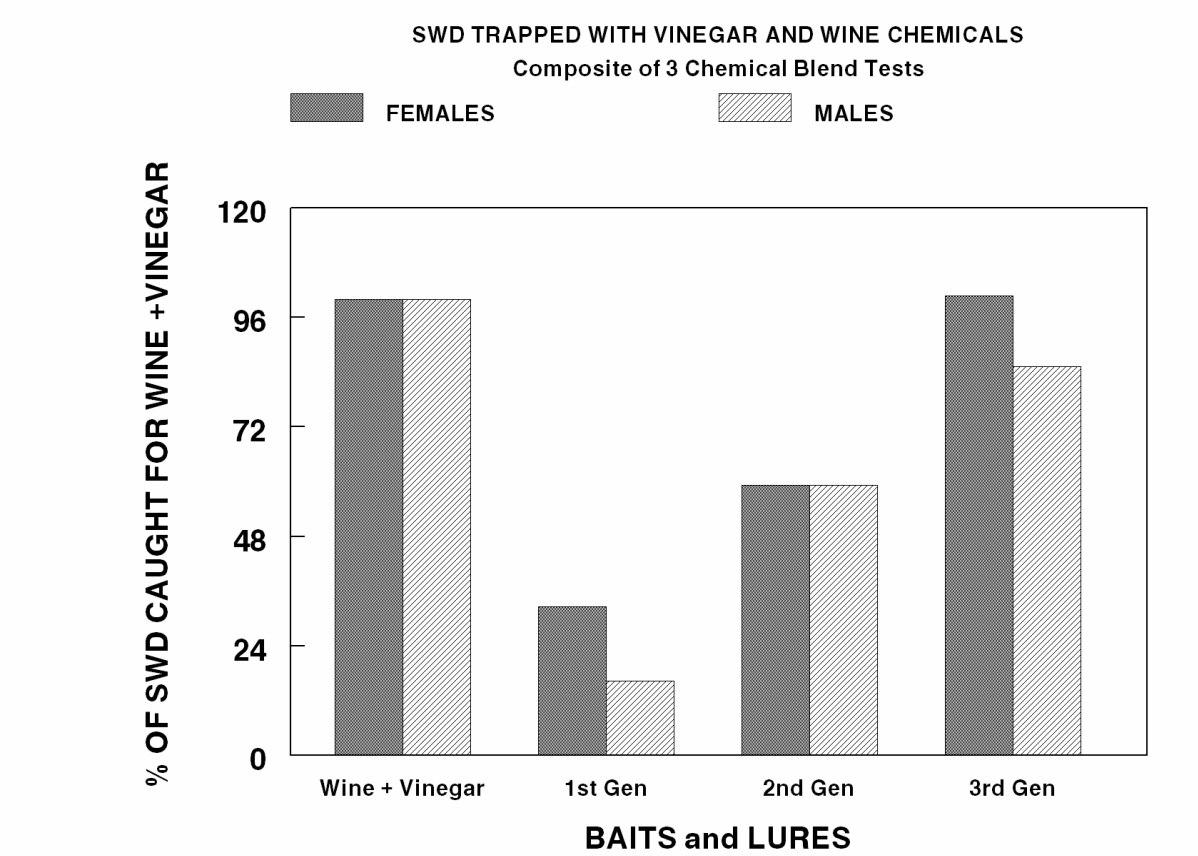

Figure 1. The attractiveness of the three blends of chemicals that were field-tested is shown in this graph, in relation to the attractiveness of wine plus vinegar. The first generation blend was the combination of EAD-active chemicals. The second generation blend was the EAD active chemicals minus the 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. In a series of trapping experiments, greater release rates of acetoin and greater doses of acetic acid and ethanol resulted in logarithmic 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.

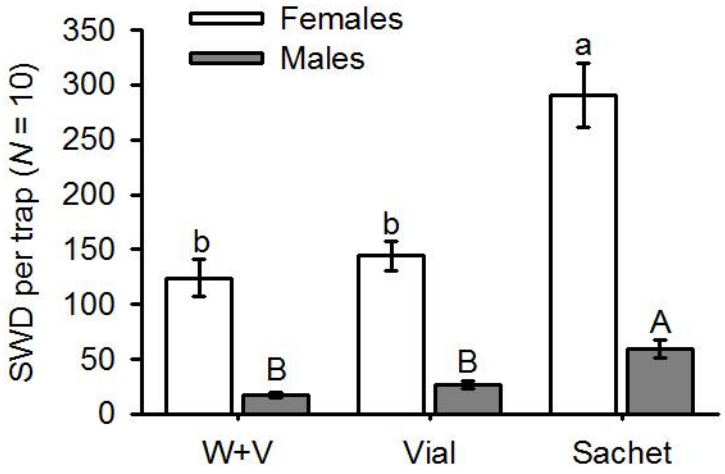

Figure 2. This graph shows SWD flies captured in traps baited with the combination of wine and vinegar (W + V), with acetoin and methionol in a vial and ethanol and acetic acid in the drowning solution (vial), and with acetoin and methionol in two individual sachets and ethanol and acetic acid in the 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 yellowjacket 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.

Table 1. 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 yellowjacket	19.0 ± 2.1a	5.0 ± 1.2b
Western yellowjacket	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 $P < 0.05$.

Early season detection of SWD with a chemical lure. 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.

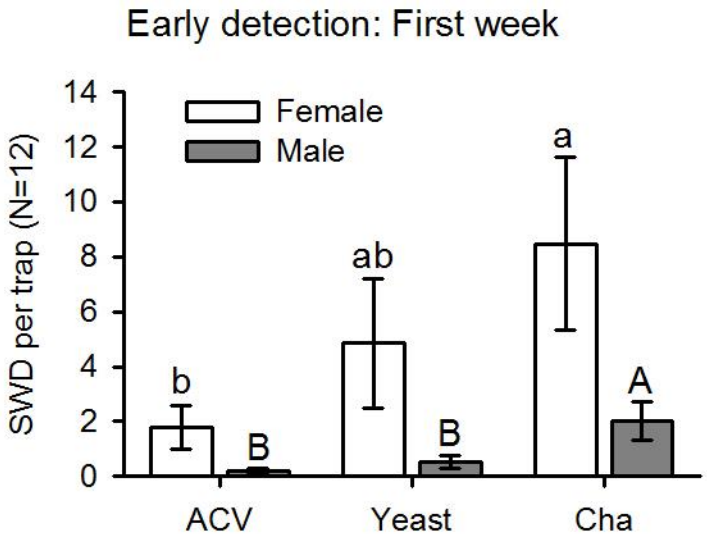

Figure 3. Mean numbers of SWD flies captured per trap per week, for traps baited with apple cider vinegar (ACV), a yeast/sugar formulation (yeast) and the SWD chemical lure (Cha).

These results provide a chemical lure for use in detecting and monitoring SWD. To date, 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. We have been contacted by four companies that have an interest in pursuing the development of commercial chemical lures for SWD and are working with two (Scentry and Trece) 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; 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.

PUBLICATIONS

Cha, D. H., T. Adams, H. Rogg, and P. J. Landolt. 2012. Identification and field evaluation of fermentation volatiles from wine and vinegar that mediate attraction of spotted wing drosophila, *Drosophila suzukii*. J. Chem. Ecol. 38: 1419-1431.

Cha, D. H., T. Adams, C. T. Werle, J. J. Adamczyk, Jr., H. Rogg, and P. J. Landolt. 2013. A four-component blend of fermented bait volatiles is attractive to spotted wing drosophila, *Drosophila suzukii* (Diptera: Drosophilidae). Pest Manag. Sci.

Cha, D. H., S. P. Hesler, R. S. Cowles, H. Vogt, G. M. Loeb, and P. J. Landolt. 2013. Comparison of a synthetic chemical lure and standard fermented baits for trapping *Drosophila suzukii* (Diptera: Drosophilidae). Environ. Entomol. 42: 1052-1060.

Landolt, P. J., T. Adams, and H. Rogg. 2012. Trapping spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) with combinations of vinegar and wine, and acetic acid and ethanol. J. Appl. Entomol. 136: 148-154.

Landolt, P. J., T. Adams, T. S. Davis, and H. Rogg. 2012. Spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Tephritidae), trapped with combinations of wines and vinegars. Florida Entomol. 95:326-332.