2009 NW Cherry Research Review
November 13-14, 2008
Hood River, OR

Thursday, November 13

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FINAL PROJECT REPORT

Project Title: Cherry Fruit Fly Control Options (CH-06-603)

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Cooperators: None

Other funding Sources

Name: Dow, DuPont, Bayer, Cerexagri, Chemtura, MGK, Gowan.

Amount awarded: $54,050 total over 3 years.

Total Project Funding:

Budget History:

Item	Year 1:	Year 2:	Year 3:
Salaries	$10,773	11,916	12,393
Benefits	4,094	4,051	4,214
Wages			
Benefits			
Equipment			
Supplies	300	300	300
Travel	1,940	1,869	1,869
Miscellaneous			
Total	$17,107	18,136	$18,776
3 Year Total			**$54,019**

Introduction and Justification

At the time this project was initiated, cherry fruit fly was identified as the top priority in the TFRC Cherry Research Committee yearly priority setting sessions. The objectives of this project were chosen because the carbamate and organophosphate class insecticides available at the inception of this work were (and continue to be) under regulatory pressure. This impending loss of key CFF control products could be offset by the discovery and demonstration of safe and effective new CFF control materials and methods. This project is the continuation of an 11 year effort, with financial support from the Washington Tree Fruit Research Commission for the past 5, to reach the objectives below as first stated in 2003.

Summary of Results:

Objective 1: Identify new conventional and organic cherry fruit fly control products and methods.

! Fifteen products within eight pesticide classes were identified as having potential for these trials, ten conventional and five organic, most had never been tested for efficacy on cherry fruit fly.

! A new method of cherry fruit fly control (GF-120 NF bait, ATV application) was tested for the first time, anywhere.

Objective 2*:* Assess these new insecticides and methods for cherry fruit fly control.

! All of the conventional products were effective, some equal or superior to standard products available in past decades.

Superior: imidacloprid (Provado), Delegate, Success and Entrust, Altacor, a numbered product "Z," and, based on one year's data, indoxacarb (Avaunt) and pyriproxyfen (Esteem).
Very good: GF-120 bait, Assail, Calypso, Actara.
Good, probably…: Rimon, Agri-Mek.

! Of the five organically acceptable products tested, two proved to be far superior to past choices.

Superior: GF-120 NF fruit fly bait, and Entrust, both of which have spinosad as their active ingredient. As fruit flies rarely develop pesticide resistance, this should not be a problem.
Good, but impractical: High rate per week of summer weight horticultural oil.
Fair: pyrethrum (Pyganic).
Poor: azadirachtin (neem, Aza-Direct)

! A product (imidacloprid, "Provado") was proven to be an effective alternative to the EPA-threatened dimethoate as an after-harvest "clean-up" spray. Two other products (Assail and Calypso) may also be adequately effective, though slightly less effective than imidacloprid.

Objective 3: To work toward the registration of effective new CFF control products.

! This project has had a positive effect on the registration and adoption of the following cherry fruit fly control materials:

Major effect: Success, GF-120, Entrust, Delegate, Assail.
Significant contribution: imidacloprid (Provado, various other brands), Avaunt.
Contribution: Actara.
Significant effort expended, but not registered yet: Altacor, Rimon, Calypso

Impact of this work:

! This project first recognized the potential and demonstrated the efficacy of GF-120 Bait, applied by ATV as a cherry fruit fly control. Adoption of this new technology by PNW Cherry growers has decreased yearly cost of controlling CFF by about $2.5 million each year. In the past four seasons since registration, total savings are about $6.7 million. These estimates are based on:
  - Reduced application costs, labor, fuel & tractor and sprayer, of about $20 / acre.
  - Lower spray material costs, at $20 / acre, or more.

! Other potential benefits of this technology include a 200,000+ gallon yearly fuel savings due to ATV application vs. tractor/sprayer. The rising cost of fuel increased the impact of this project by $500,000 / year in reduced grower fuel costs over the past two years.

! This bait may now be the most commonly used insecticide on Washington cherries, while it is used by virtually all organic cherry growers, by far the greatest acreage treated is in conventional orchards.

! Three products were identified as alternatives to dimethoate as after-harvest "clean-up" sprays. The EPA-proposed lower rate of dimethoate was found to be less effective. Growers now have "softer" choices (imidacloprid/Provado, Assail), making it more likely to be done. In the long term, this should reduce CFF numbers in a region the season after treatment, lowering risk of control failure to all.

! Some products were removed from organic grower options due to their failure to perform well in these trials. Azadirachtin (neem), previously recommended for organic control of CFF, was proven ineffective. Pyrethrum was found to be suppressive, but not sufficiently effective.

! In 2003, cherry fruit flies were commonly found in many organic cherry orchards. In 2007, industry leaders announced that this pest was "no longer a problem."

! Cherries can now be safely grown by any grower as "organophosphate-free."

! Black cherry aphid and leafrollers can be brought under control during the late spring as a side benefit of using some of the products newly registered as CFF controls (imidacloprid/Provado, Assail).

! Cherry fruit fly has recently dropped from #1 on the TFRC cherry committee priorities list to nearly last place. Growers are advised to avoid complacency when planning their cherry fruit fly control program. New tools will not work without proper use.

Results and Discussion:
Note: These data are reported as results of experimental trials. While many of these products can be legally used on cherries, some are not legal, and may never be legal. Always check to see if sweet cherry is listed on the product label prior to use of any pesticide.

Conventional Product in Trials and Rate/A	Years in Trial	Total Trees / Total Sites	Total Fruit Inspected	Total Larvae Found	Larvae Per 1000 Fruit
Untreated Checks	**2003-07**	**22 trees 22 sites**	**16,315**	**7,081 (43% Average)**	**434**
2008 Untreated Check	**2008**	**1 tree 1 site**	**1,000**	**573 (57% Average)**	**573**
Provado 3 - 6 oz. (imidacloprid)	**1999, 2003 04, 06, 07, 08**	**68 trees 34 sites**	**32,600**	**1***	**0.03**
Success (spinosad) **2, 4, 6 or 8 fl.oz 7 Day Intervals**	**1997, 98, 99, 2002**	**37 trees 5 sites**	**7,500**	**13** All in low rate trials**	**1.73**
Altacor (rynaxypyr)	**2002, 2005 2006**	**35 trees 31 sites**	**30,800**	**20** All in Low rate trials**	**0.65**
Delegate 4 oz. (10 day spray interval)	**2005, 2006**	**15 trees 15 sites**	**15,000**	**0**	**0**
Delegate 3 oz. (***10 day spray interval***)	**2008**	**4 trees 3 sites**	**4,000**	**0**	**0**
Delegate 4.5 oz. (***14 day spray interval***)	**2008**	**2 trees 2 sites**	**2,000**	**0**	**0**
Secret Product Z	**2005, 2006**	**11 trees 11 sites**	**11,000**	**0**	**0**
Rimon (novaluron)	**2005, 2006**	**7 trees 7 Sites**	**7,000**	**13**	**1.86**
Esteem 5 oz. (pyriproxyfen) 10 day intervals	**2008**	**2 trees 2 sites**	**2,000**	**0**	**0**
Agri-Mek 10 oz. (abamectin) 10 day intervals	**2008**	**1 tree 1 site**	**1,000**	**23****	**23**
Agri-Mek 20 oz. 10 day intervals	**2008**	**1 tree 1 site**	**1,000**	**1**	**1.0**
Avaunt 4.5 oz. (indoxacarb) 10 day intervals	**2008**	**4 trees 4 sites**	**4,000**	**0**	**0**

Table 1. Summary of cherry fruit fly control options trial data1999 through 2007, with most 2008 Year Data separated. * Single larva found when treatment tree was adjacent to a highly infested tree. **"Failures" were generally due to intentional research efforts while testing rates and spray intervals; the rate was too low, or interval too long, or both.

Organic Product in Trials	Years in Trial	Total Trees / Total Sites	Total Fruit Inspected	Total Larvae Found	Larvae Per 1000 Fruit
Untreated Checks	**2003-08**	**23 trees 22 sites**	**17,315**	**7,888 (46% Average)**	**456**
Aza-Direct / Neem (azadirachtin) Every 7 days	**2004**	**12 trees 6 sites**	**2000**	**102**	**51**
GF-120NF Bait (every 7 days) **Full Rate of 20 fl.oz/A**	**2002, 03, 04, 05, 06, 07, 08**	**128 trees 51 sites**	**46,400**	**2***	**0.04**
GF-120NF Bait – 7 days **Half Rate of 10 fl.oz/A**	**2007**	**3 trees 3 sites**	**3000**	**27***	**9.0**
GF-120NF Bait, 1st year on Extreme Infestations 20 fl.oz/A every 7 days	**2007**	**13 trees 2 sites**	**1000 1000**	**12 0**	**12 0**
GF-120NF Bait 20 fl.oz/A **every 10 days Not near CFF source**	**2008**	**5 trees 5 sites**	**5,000**	**0**	**0**
GF-120NF Bait 20 fl.oz/A **every 10 days Near CFF source**	**2007, 2008**	**3 trees 3 sites**	**3,000**	**36****	**12**
Entrust 1.9 oz @ 10 Days Interval (spinosad)	**2003, 05, 06, 2007**	**25 trees 16 sites**	**15,400**	**0**	**0**
Entrust 1.0 oz @ 10 Day Intervals	**2007**	**4 trees 4 sites**	**4,000**	**1***	**0.25**
Horticultural Spray Oil 1%, 300 gpa, @ 7 days	**1999**	**4 trees 1 site**	**800**	**6**	**7.5**
Pyganic 5 @ 7 days (pyrethrum)	**2006**	**6 trees 6 sites**	**6,000**	**10*****	**1.67**

Table 2. Organic CFF Control Product Summary: *Control failure due to research, while testing rates and intervals. **Untreated infested trees nearby. ***Five of six plots had low numbers of larvae in fruit, despite moderate CFF pressure, indicating that the product is suppressive, but not sufficiently effective.

After-Harvest Treatments

Three materials were demonstrated as effective for control of cherry fruit fly larvae inside the fruit, as possible alternatives for post-harvest dimethoate. One product, abamectin, was not adequately effective in the 2008 after harvest test.

After-harvest Product	Rate	Larvae Emerged / 1000 Fruit	As Percentage of the Untreated
Dimethoate 267	64 oz./200 gal./A	112	8.8
Provado 1.6F	6 oz./200 gal./A	64	5.0
Provado 1.6F	8 oz./200 gal./A	52	4.1
Agri-Mek 0.15 EC	10 oz./200 gal./A	608	47.8
Agri-Mek 0.15 EC	20 oz./200 gal./A	624	49.1
Untreated	0	1272	100

Table 3. A summary of 2008 after-harvest larva control trials, products applied to heavily infested cherries for control of larvae inside of fruit on the tree.

After-harvest Product	Rate/Acre	Number of Tests	Larvae Emerged per 1000 Fruit	As Percentage of the Untreated
Dimethoate 267	64 oz.	4	40	3.3
Dimethoate 267	48 oz.	2	48	4.0
Provado 1.6F	4 oz.	1	132	11.0
Provado 1.6F	6 oz.	2	84	7.0
Provado 1.6F	8 oz.	4	28	2.3
Calypso SC 480	8 oz.	2	46	3.8
Assail 70WP	2.3 oz	1	252	21.0
Assail 70WP	3.4 oz.	3	84	7.0
Agri-Mek 0.15 EC	10 oz.	1	608	50.6
Agri-Mek 0.15 EC	20 oz.	1	624	52.0
Untreated	0	4	**1201**	**100**

Table 4. A summary of 4 years after-harvest larva control trials, products applied to heavily infested cherries for control of larvae inside of fruit on the tree.

All after-harvest products tested appear to be very acceptable replacements for dimethoate, the only product currently recommended for controlling larvae in fruit remaining on harvested trees. The "post-infestation effect" of imidacloprid (Provado, etc.), acetamiprid (Assail) and thiacloprid (Calypso) may give products with this class of chemistry an advantage as a pre-harvest product, as application may control newly hatching eggs or larvae that may have slipped through earlier control programs. At this time, dimethoate is not a popular pre- or post-harvest choice, as it sometimes causes leaf yellowing, necrosis and drop. Many growers avoid using it.

Executive Summary:

Significant Progress and outcomes: Cherry growers now have a number of excellent control product choices, with a few more likely to come, and a new method of control due to this project. It is now possible to grow cherries free of any cherry fruit fly larvae without using any product listed in the 2003 WSU spray guide. While all pesticide use will continue to be a public concern, the products this cherry industry is now turning to for CFF control are judged to have low impact on the environment and the applicator. Especially important with a crop that must be protected from a key pest up to and through harvest, the alternative products registered with the help of this project are of very low toxicity, and, compared to traditional products, are used at very low rates per acre.

We now have effective control options for organic control of CFF, which had not been the case from the time rotenone was dropped from the organic use list until 2005, when GF-120 NF became available.

The options made available through this project should remain useful for decades, as fruit flies, as a group, do not tend to develop resistance to pesticides, even when exposed to the same product for many generations (example: the yearly near-universal use of ULV malathion for decades in The Dalles, Oregon.)

Future Directions: While there are always new ideas to work on, I believe the original three objectives had been achieved to a degree that relieves the impact of losing older class CFF control materials. While there are a few new products yet to be fully tested, most of those with an interested registrant company or organization have been evaluated.

I intend to continue working on control of cherry fruit fly, but at a reduced level, supported by grants from various sources, carrying forward some further investigations on current new products and waiting for new opportunities to develop. If any really significant technology or products develop, and I need the financial assistance of Washington and Oregon cherry growers to test these new possibilities, I will bring a new proposal to you and try to justify my plans and requirements.

Thank you for all of your support during the course of these trials. Your financial support led to the rapid development of the various cherry fruit fly control options. Without your support, much of the work would not be completed or may have never been initiated.

FINAL PROJECT REPORT

Project Title: Validation and implementation of the WA cherry powdery mildew model

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Other funding Sources:

Agency Name: Washington State Commission on Pesticide Registration
Amount requested/awarded: $16,030
Notes: 2008 only

BUDGET:

Organization: Washington State University	**Contract Administrator:** Stephanie Brock
Telephone:509 786 2226	**Email:** sabrock@wsu.edu

Item	Year 1: 2007	Year 2: 2008	Year 3: N/A
Salaries	28,309	29440	
Benefits	9,710	10,098	
Salaries (Hourly)		1,500	
Equipment			
Supplies	1700	1,700	
Travel	3,700	3,700	
Miscellaneous			
Total	43,419	46,438	

OBJECTIVES:

1) Continue model validation work on cv. 'Bing' and 'Sweetheart' and expand validation work to include other cultivars.

a. Determine the accuracy of the secondary infection risk index on Rainer cherries

b. Repeat studies designed to determine whether ascospore release and primary infection could result from the use of irrigation for frost protection.

2) Determine appropriate fungicides for use at critical points during mildew epidemics. In addition to repeating our 2007 work on overall program initiation, our 2008 work also focused on identification of the most efficacious fungicide class for use at the onset of secondary inoculum production (i.e. when the model indicates disease onset *after* primary infection).

3) Determine optimal spray intervals for various fungicide classes under ambient secondary mildew disease pressures as defined by the model's risk index. Identify potential interval variation across cultivars.

4) Establish online training resources for model usage and related powdery mildew management. Develop similar training material for distribution via DVD (and by extension, other digital formats).

Significant Findings (by objective):

1a. Under the weather conditions that characterized the 2008 season, the secondary infection risk index was found to be a reasonably accurate and *conservative* predictor of the both the initial occurrence and intensification of mildew on unsprayed trees in the orchard (**Figure 1**). The risk index was more conservative on the foliage of cv. 'Sweetheart' than cvs. 'Bing' and 'Rainier'. Results were similar in 2007 on 'Sweetheart' and 'Bing' (Rainier not tested in 2007).

1b. For the second year, evidence was collected that irrigation water used for frost protection contributes to ascospore release (**Table 1**). Propagules were detected using rotary impaction air samplers and PCR.

2. The powdery mildew model was used to initiate and schedule subsequent (objective 3) fungicide applications in 2008 (**Figures 2 and 3**). Because of their unique modes-of-action, quinoline (Quintec), QoI (strobilurin; Flint), DMI (Procure), and sulfur (Microthiol) fungicides were evaluated for their efficacy when used to *initiate* fungicide spray programs upon *primary infection* or *predicted disease onset*, both of which are critical [early] epidemiological events identified by the model. Specifically, the primary infection is predicted when 0.1" of precipitation is received at > 50 F. The second infection risk index is initiated at primary infection and looks for six consecutive hours between 59 and 81.3 F. These conditions need to be met for three consecutive days in order for the prediction of disease onset. In 2008, the secondary infection risk index was initiated at primary infection. Fungicide programs were initiated usually various fungicide chemistries at primary infection or disease onset. Subsequent applications were made at 14-day intervals through harvest. For example (**Figure 2**), a QoI program initiated at the first primary infection identified by the model resulted in disease incidence value of 25% and 0.4 disease severity index (DSI) while programs initiated according to crop phenology were 38.0% and 0.51 DSI respectively. As stated in above, the class of fungicide chosen to *initiate* spray programs was not significant when raw data was analyzed. *However, the application of a logit transformation (natural logarithm of incidencey/1-incidence) to the incidence data revealed that initiation of a fungicide program using Flint (QoI compound) or sulfur* **at disease onset** *resulted in control inferior to programs initiated using other modes-of-action at this critical epidemiological stage.*

3. Studies to ascertain the appropriate intervals between model-driven fungicide sprays continued in 2008 (**Figure 4**). In trials under extremely high disease pressure (i.e. high risk indices), QoI (Cabrio), QoI/pyridine (Pristine), DMI (Procure), oil (Stylet Oil), quinoline (Quintec), carbonate (Kaligreen), and sulfur (Microthiol Dispersee) fungicides appeared to require application intervals of 7 days (**Figure 4**) to provide adequate levels of control on both cvs. 'Bing' and 'Sweetheart'. Control was marginal at longer spray intervals. This finding stands in contrast to fungicide performance in 2007 when several compounds were effective at longer spray intervals. *Therefore, because of inconsistent performance 21-day spray intervals will not be recommended in conjunction with model usage*.

4. Personnel constraints precluded the completion of the model interface on the WSU Tree Fruit Decision Aid System (DAS; http://das.wsu.edu) in time for the 2008 growing season. However, the model on the AgWeatherNet (http://weather.wsu.edu) was improved in time for the 2008 growing season. Summary model outputs **(Figure 5)** were made available via text messaging **(Figure 6)** and a training video was completed using SnapZ Pro software. This software is utilized to make movies of the model configuration and output interpretation processes on the World Wide Web. Clients configure the text messaging function on the main AgWeatherNet web site. The distribution of summary outputs using text messaging and automated [PDF or text] email was tested several users during 2008 growing season. Upon completion of the DAS version of the model is complete, all model functionality will be transferred to the DAS site. A new (DAS-based) training module will be prepared prior to release by DAS. All training videos are (will be in the case of the DAS version) available on DVD upon request.

Results and Discussion

Model Rationale and Review

The cherry powdery mildew model is comprised of three components: 1) a growing degree day algorithm that tracks degradation of the overwintered inoculum supply following bud burst 2) a primary infection algorithm and 3) temperature-based Risk Assessment Index.

Component 1. The causal agent of grapevine powdery mildew, *Podosphaera clandestina* survives winter as cleistothecia in Eastern Washington. Cleistothecia persist until 250 growing degree days (base 50 F) have accumulated after bud burst.

Component 2. Studies in Eastern Washington have demonstrated that cleistothecia require 0.1" precipitation or greater at 50 F or greater in order to release ascospores. Component 2 of the cherry mildew model is a temperature/precipitation algorithm that looks for these conditions between bud burst and when 250 growing degree-days have accumulated. If the aforementioned temperature and moisture requirements are met within the specified time frame, primary infection occurs and the Risk Assessment Index (component 3) is initiated.

Components 1 and 2 are used to signal the beginning of the season's fungicide spray program. Although the post infective activity of the various fungicides needs to be determined for *P. clandestina* on cherries, work on other pathogenic fungi indicates that some synthetic fungicides (e.g. DMI) have higher post infective activity than contact fungicides and in some cases can be applied up to 96 hours after an infection event.

Component 3. Once powdery mildew is established, further fungicide treatments will be necessary because the fungus will continue to reproduce through the growing season. The rate of reproduction is temperature-dependent and best indicator of infection risk. The Risk Assessment Index ranges between 0-100 where indices of 0-30, 40-50, and 60-100 indicate low, moderate, or high disease pressure, respectively. The index measures how rapidly the fungus is reproducing and is used to provide general guidelines regarding the interval between fungicide applications. High risk indices result in shorter intervals between sprays whereas low indices allow "stretching" of application intervals. In general synthetic fungicides (Quinoline, DMI, QoI) compounds are more persistent that contact fungicides and protect fruit and foliage for longer periods of times.

After primary infection, an epidemic will begin when there are 3 consecutive days with 6 or more continuous hours of temperatures between 59 and 81.3° F. Starting with the index at 0 on the first day, 20 points are added for each day with 6 or more continuous hours of temperatures between 59° and 81.3°F. If fewer than 6 continuous hours of temperatures between 59° and 81.3°F occurs, 10 points are subtracted. If 6 or more continuous hours of temperatures between 59° and 81.3°F occurs, 20 points are added. If temperatures reached 90°F for more than 15 minutes, 10 points are subtracted.

If there are 6 or more continuous hours with temperatures between 59° and 81.3°F and the temperature rises to or above 90°F for at least 15 minutes, 10 points are added.

Relationship to 2008 results.
Primary infection was predicted and occurred on 3 May 2008, which initiated the secondary infection component (risk index) of the model. Disease onset was predicted on May 11 and was actually observed (without the aid of a hand lens) on all three cultivars on 16 May (**Figure 1**). High levels of risk (short spray intervals) persisted from that point through harvest and then declined with the onset of summer heat. The rate of foliar disease increase on 'Bing' and 'Rainier' was slightly more rapid than the rate of increase on 'Sweetheart'. Fruit infections were not observed on any cultivars cultivar. The model was *conservative* under the growing conditions of 2008.
Water-based frost protection was applied one of our IAREC research cherry orchards on 1,5, and 15 April (**Table 1**) while a second orchard was left protected. Rotorod air samplers were operated continuously through water-application periods and during drying periods the following morning. Using PCR, *Podosphaera clandestina* was detected in the air during consistently during watering. The causal organism was detected in the orchard left unprotected during frost periods. Powdery mildew was not detected in the orchard air prior to the application of irrigation water or (prior to primary infection) during cold evenings when water was not applied. Although additional work is needed in order to determine the effect of morning temperatures on primary infection (rather than just spore release), the results of this two-year study indicate the ascospore release can result from the application of irrigation water for frost protection.
Model studies were conducted in a Prosser orchard during 2008 (**Figure 1**). The model identified primary infection on 3 May. The powdery mildew risk index was initiated at this time. The model then predicted disease onset on 11 May following the first occurrence of 3 consecutive days of > 6 consecutive hours at the aforementioned critical temperatures (following primary infection). Weather- and detection-based fungicide programs were initiated on 4 May in response to the (3-May) rain event that promoted primary infection (0.31" of precipitation at 51.8 F) or at predicted disease onset on May 11. As in 2007, the effect of using various fungicide classes for program initiation in response to predicted *primary infection* was not significant. However, when control programs were initiated at disease onset (over 1 week after primary infection) with sulfur or QoI compounds disease levels were significantly more severe than in programs initiated with DMI or quinoline compounds (**Figures 2 and 3**). This finding could be particularly significant during years when abnormally warm or cool weather occurs immediately following primary infection. The incubation period between primary infection and disease onset is temperature dependent and is shortest when temperatures are in the 59-81.3 F range. Persistent temperatures outside of this range could extend the incubation period and perhaps eliminate a fungicide application provided that a DMI or quinoline fungicide was applied at disease onset.
Although all treatments were superior to the untreated controls, mildew levels were unacceptably high under 14- and 21-day application intervals (**Figure 4**, figure data expressed as % reduction from maximum disease severity value (4.2/5)).QoI, DMI, and sulfur fungicides provided the best levels of control on cv. 'Bing' and 'Sweetheart' when applied at 7-day intervals under the extremely high disease pressure. Disease severity in the untreated controls was 3.44 and 3.24 (maximum = 5.0) on cvs. 'Bing' and 'Sweetheart', respectively. At 7 day intervals severity ranged from 1.7 (Quintec) to 2.4 (Stylet Oil) on 'Bing' and 1.6 (Quintec) to 2.31 (Stylet Oil) on 'Sweetheart'. At 14 day intervals severity ranged from 2.18 (Pristine) to 2.74 (Kaligreen) on 'Bing' and 2.06 (Quintec) to 2.71 (Cabrio) on 'Sweetheart'. At 21 day intervals severity ranged from 2.46 (Quintec) to 3.14 (Stylet Oil) on 'Bing' and 2.37 (Quintec) to 2.97 (Stylet Oil) % (Kaligreen) on 'Sweetheart'. It was apparent that at the extreme disease pressure of 2008 that fungicides applied at 21-day spray intervals do not provide adequate control regardless of class and that (with the exception of Pristine @ 14-day intervals on 'Bing') Quintec consistently performed best of any compound. The demonstration of marginal fungicide performance at 21 day spray intervals is significant: some QoI compounds are effective at

these long intervals when used in conjunction with forecasting models developed for the powdery mildew of grapes and tomatoes.

Due to personnel constraints that precluded timely release of the full model on WSU-DAS in 2008, the model was improved on AgWeatherNet through the incorporation of new temperature algorithms, more detailed summary (**Figure 5**) and full model reports, and the addition of automatic email and text-message (**Figure 6**) functions. Coding used for all outputs were made available to the new WSU-DAS programmer for inclusion on the site for the 2009 season. All model outputs are hyperlinked to management information on disease biology, model rationale, and disease and fungicide management options. Training modules developed in 2007 Microsoft PowerPoint, Adobe Presenter, and SnapZ Pro are currently being updated using ScreenFlow software. Screen movies that actual depict real-time configuration of, and navigation through, the web-based modeling are made using ScreenFlow and SnapZPro. A ScreenFlow tutorial will be developed for DAS as the new interface is developed. The development of push technologies are particularly significant in the event of rapid change in the status of a pest or disease model: current information can be delivered directly to a client's cellular phone as conditions change. In certain instances (e.g. apple scab, where spray timing is *everything*) receipt of this timely information could significantly improve disease management.

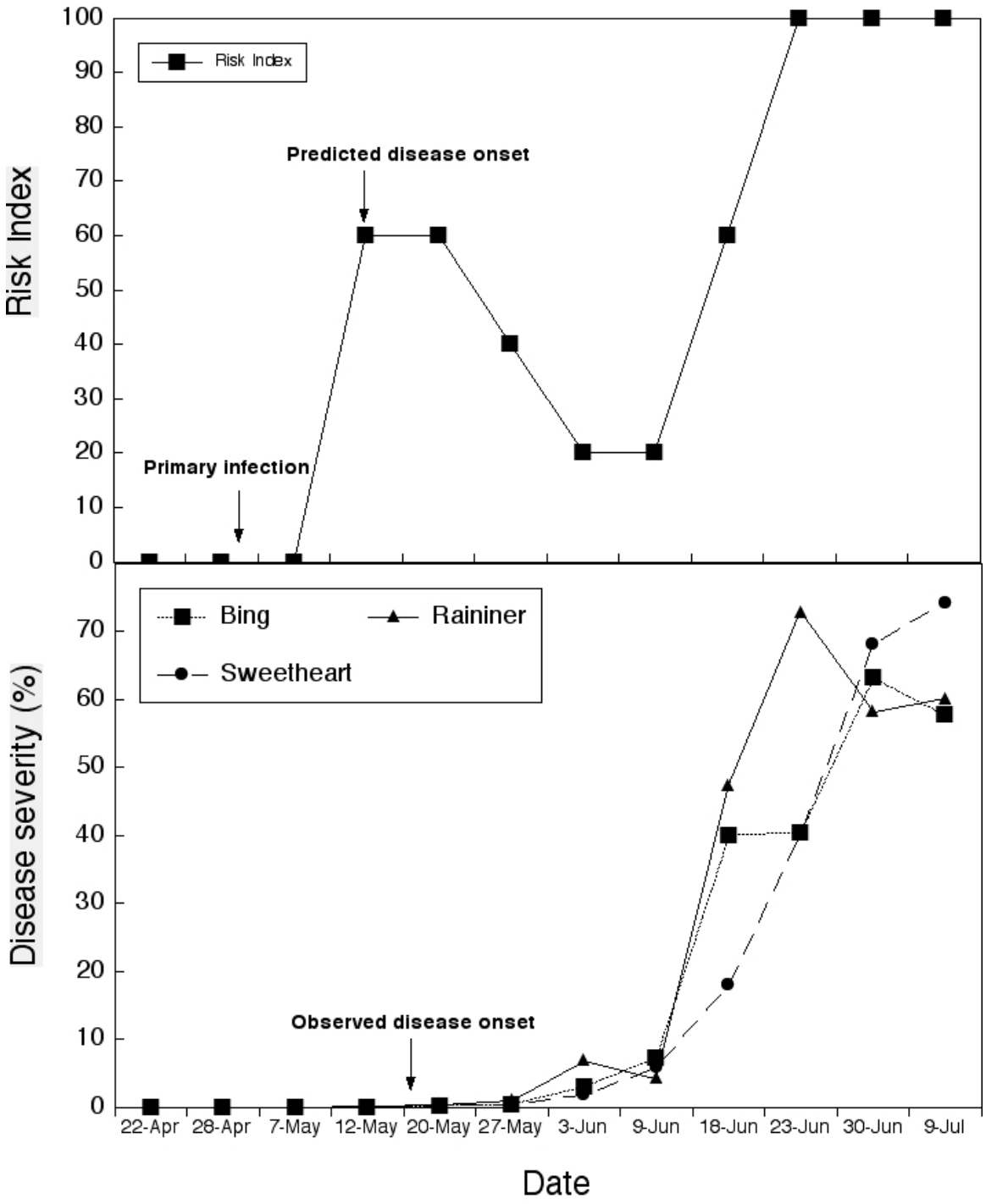

Figure 1. Progression of powdery mildew on cultivars 'Bing', 'Rainier', and 'Sweetheart' at Prosser. Presented in the upper graph are dates of predicted primary infection, disease onset, and the secondary risk index to harvest. Lower graph indicates disease severity on the various cultivars.

Table 1. Effect of early season irrigation (used for frost protection) on ascospore release by *P. clandestina*.

Date	Orchard	Water Applied	PCR Signal Strength	Minimum Temperature (F)
4/1	D39[1]	Yes	Bright	25
4/1	D51[2]	No	None	25
4/5	D39[1]	Yes	Bright	28.7
4/5	D51[2]	No	None	28.7
4/15	D39[1]	Yes	Bright	28.2
4/15	D51[2]	No	None	28.2

[1] water used for frost protection within orchard;[2] water not used

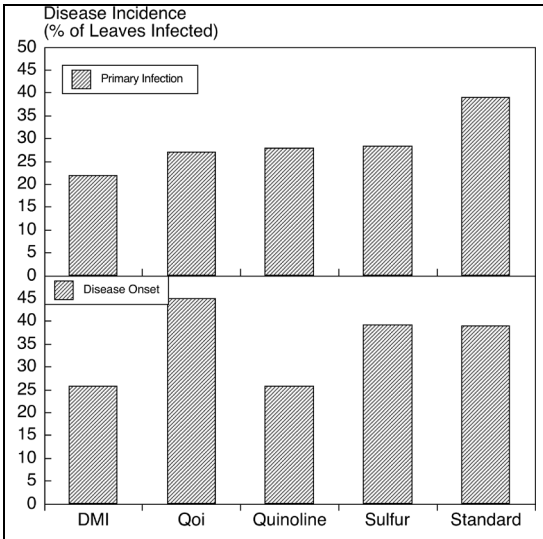

Figure 2. Incidence of powdery mildew on 'Bing' cherries when fungicide programs were initiated at predicted primary infection (upper graph) or disease onset (lower graph) using different fungicide chemistries.

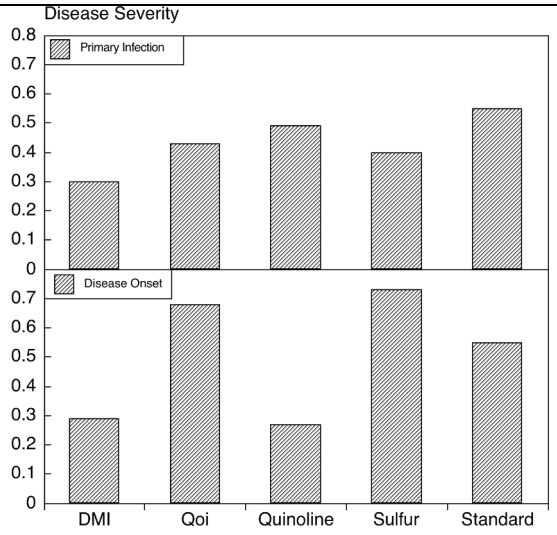

Figure 3. Severity of powdery mildew on 'Bing' cherries when fungicide programs were initiated at predicted primary infection (upper graph) or disease onset (lower graph) using different fungicide chemistries.

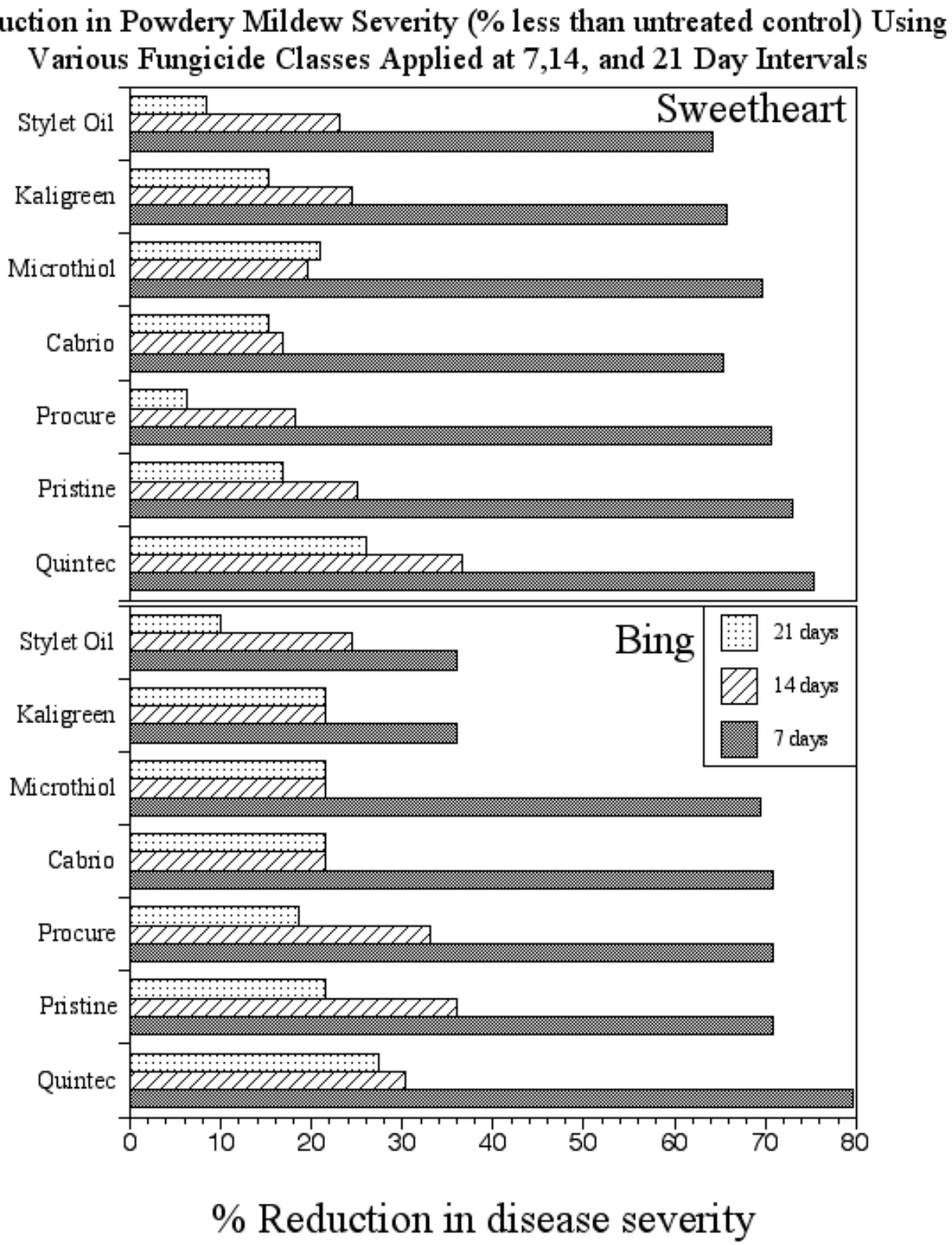

Figure 4. Effect of application interval on reduction of powdery mildew on 'Bing' and 'Sweetheart' cherries under extreme disease pressure. X axis expresses the % reduction in disease severity attained in untreated controls.

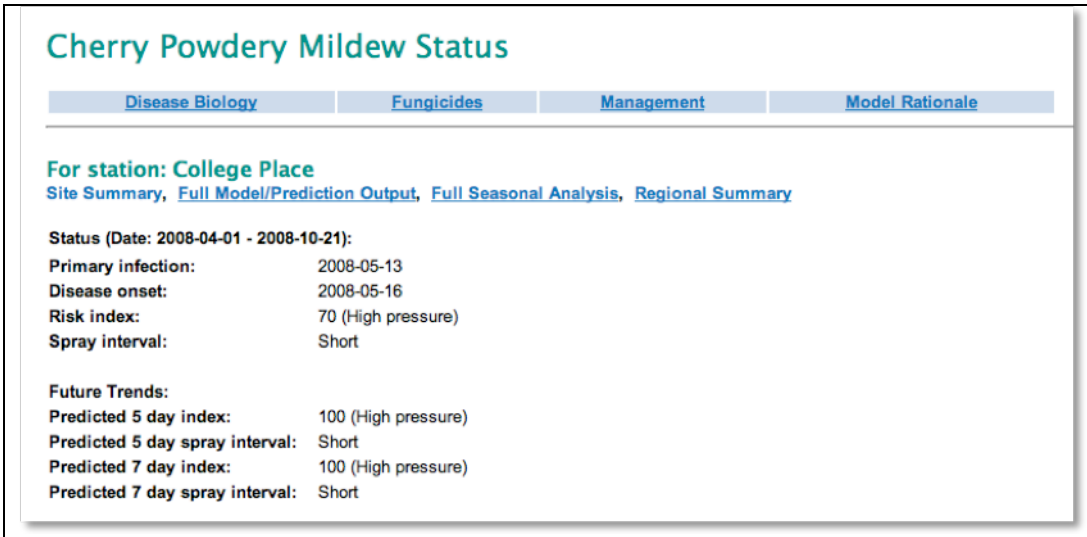

Figure 5. Summary model output from WSU-AWN College Place weather station. Note that dates of primary infection, disease onset, and current and predicted disease indices and spray intervals are presented. Summary outputs are also available using "push" technologies.

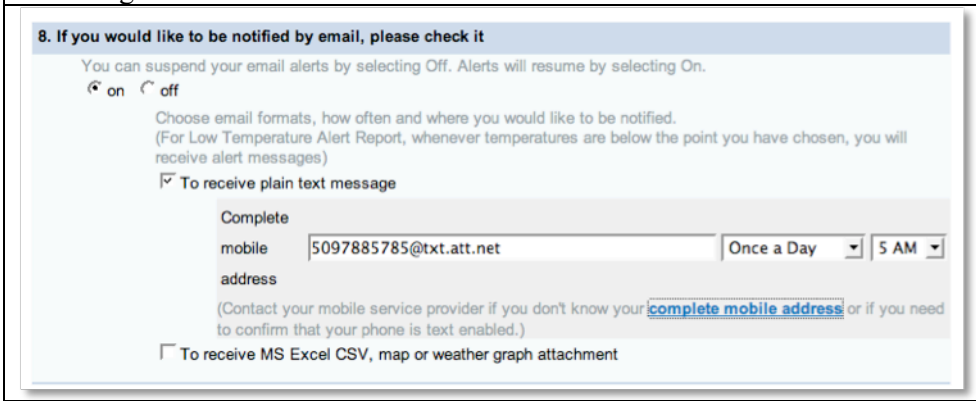

Figure 6. Configuration page for "push" model outputs available from WSU AgWeatherNet.

Executive Summary

Validation and Implementation of the WA cherry powdery mildew model

Gary G. Grove

The Northwest Powdery Mildew Model was developed over a period of years using controlled-environment and field studies. The current model is comprised of three components. Components 1 and 2 are used to predict primary infection while component 3 is used to predict the onset of disease in the orchard and to adjust the intervals between sprays. Components 1 and 2 were validated in the orchard using air sampling and PCR studies, while component 3 was validated were validated using disease progression studies across three cultivars. Efforts during this two- year study were focused in improving the model, developing management recommendations, and distributing model outputs to growers. Significant improvements were made in the temperature algorithms used to generate the second infection risk index and the identification of water-based frost protection as a mechanism to promote ascospore release. The model was also used to conduct disease control studies focused on the unique characteristic of each fungicide class. It was demonstrated that any of the synthetic fungicide classes and sulfur could be used to initiate fungicide programs if commenced at *primary infection*. Conversely, the initiation of programs *at disease onset* requires the use of a DMI or quinoline fungicide. Concurrent studies indicated that irrespective of fungicide mode-of-action application intervals should not exceed 14 days. Fungicide performance at 21-day intervals was inconsistent and is therefore not recommended. The model and associated recommendations were made available on the AgWeatherNet web site in 2007. The model was updated in 2008 with improved temperature algorithms, improved interface, and the inclusion of text message and email functions that "push" model outputs to client cell phones, PDA's, or computers. A new and inclusive interface is being developed for WSU-DAS. All model functionality will be transferred to DAS once the new interface is complete.

FINAL PROJECT REPORT

Project Title: Prevention of cherry fruit cracking using soluble potassium silicate

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Collaborators: **2007:** Pam Manning, Kevin Asai
2008: Pam Manning, Tim Dahle; John Morton, Randal Montgomery

Total project funding request:	**Year 1:**	**Year 2:**	**Year 3:**
	$6000	$6500	

Other funding Sources

Agency Name: **NONE**
Amount requested or awarded:
Notes:

Organization Name: OSU **Contract Administrator:** Dorothy Beaton
Telephone: 5417373228 **Email address:** Dorothy.Beaton@oregonstate.edu

Item	**(2007)**	**(2008)**	
Salaries	2000	2250	
Benefits			
Wages			
Benefits			
Equipment	1000		
Supplies	1500	1750	
Travel	1500	2000	
Miscellaneous		500	
Total	6000	6500	

Footnotes:

Objectives for 2008

1 To test the effects of soluble potassium silicate on cherry fruit quality paramters

- This research project had a main objective to control fruit splitting in 2007 (Priority 4).
- The research emphasis was changed by the WTFRC to that of fruit firmness.
- This research identified the effect of silicon on postharvest fruit quality (Priority 1).

Significant Findings and Accomplishments:

END OF YEAR 1 (2007)

Hood River 2007

- There were no significant differences in % fruit cracking between treatments at Hood River of 'Bing'/'Mazzard' (mean for cracked fruit = 23.6% $\pm$ 4.47%). Throughout the growing season in Hood River, there were no significant differences in 'Bing' fruit diameter (figure 1) however on the day of harvest, fruit from untreated control trees were 0.64 mm in diameter larger than those from trees treated with soluble potassium silicate (F. pr. <0.001).
- In Hood River, 'Bing' fruit from trees on Mazzard rootstock, treated with soluble potassium silicate were significantly firmer (409.4 $mm.g^{-1}$) than untreated control fruit (385.5 $mm.g^{-1}$) (F pr.<0.001).
- In Hood River, the TSS levels of 'Bing' fruit treated with soluble potassium silicate were significantly lower (19.15% Brix) compared to control fruit (19.99% Brix) (F. pr. <0.001).

Corvallis 2007

- There were no significant differences between treatments of 'Stardust'/'Gisela 6' (mean for cracked fruit = 14.7 $\pm$ 5.08) . However there was a major treatment difference for 'Stardust'/'MM14', where the application of potassium silicate (mean = 34.7%) actually resulted in more cracking than on control trees (mean = 14.7%) (Figure 5).
- 'Stardust' fruit from trees treated with soluble potassium silicate were significantly larger than control fruit (Figure 6) regardless of the rootstock cultivar.
- Furthermore, 'Stardust' fruit from trees on 'Gisela 6' rootstock were the firmest of the all the treatments (Figure 7). Those from trees on 'MM14' rootstocks were the least firm. Clearly rootstock effects are having a major impact on role of soluble potassium silicate.
- The incidence of disease in the fruit at harvest in Corvallis was such that postharvest disease assessments were not deemed necessary. It was concluded that the silicon soil applications had no effect on these diseases.

END OF YEAR 2 (2008)

The Dalles 2008

- There were no significant differences in percentage fruit cracking between treatments in The Dalles for either 'Royal Rainier' on 'Citation' interstocks nor for 'Sandra Rose' on 'Gisela 6' rootstocks as result of insufficient rain to induce cracking.
- In terms of fruit size, soil-drenching with soluble potassium silicate resulted in significantly more Row 9.5 'Royal Rainier' fruit than the untreated check. Only those trees soil-drenched with soluble potassium silicate had large fruit (Row 8). The converse was true for 'Sandra Rose'.
- Fruit TSS levels were not affected by soil drenching with soluble potassium silicate.
- 'Royal Rainier' fruit were softer than 'Sandra Rose' at harvest. Fruit firmness of fruit from trees soil-drenched with soluble potassium silicate, were however, significantly firmer than fruit from untreated check trees on the day of harvest and remained significantly firmer even when stored for two weeks in regular atmosphere storage at 2ºC.
- Stem pull force of 'Royal Rainier' and 'Sandra Rose' decreased most significantly for check fruit between the day of harvest and after two weeks in regular atmosphere storage at 2ºC. In

contrast, stem pull force of similar fruit from trees, soil-drenched with soluble potassium silicate, and stored in regular atmosphere for two weeks at 2ºC decreased slightly but these differences were not significant.

Milton Freewater 2008

- There were no significant differences in percentage fruit cracking between treatments in Milton–Freewater for 'Bing' on 'Mazzard' rootstocks as result of insufficient rain to induce cracking.
- In terms of fruit size, soil-drenching with soluble potassium silicate resulted in significantly larger 'Bing' fruit of size Row 8.5.
- Fruit TSS levels were not affected by soil drenching with soluble potassium silicate.
- 'Bing' fruit were firmer than 'Royal Rainier' and 'Sandra Rose' fruit.
- Fruit from 'Bing' trees, soil-drenched with soluble potassium silicate, were firmest after two weeks of regular atmosphere storage at 2ºC when compared to untreated controls either on the day of harvest or after two weeks of regular atmosphere storage 2ºC
- Stem pull force decreased significantly in untreated check fruit and those soil drenched with soluble potassium silicate, as a result of regular atmosphere storage at 2ºC however, decreases in stem pull force of fruit from trees soil-drenched with soluble potassium silicate were not as marked.

OVERALL CONCLUSION

- ***Soil Drenching on a regular basis with soluble potassium silicate during the growing season has a marked positive effect on cherry fruit quality at harvest and after two weeks regular atmosphere storage at 2ºC. Depending on the scion/ rootstock combination, increased fruit size, fruit firmness and stem pull force may be expected. Future research should aim at expanding the rootstock scion interactions.***

Materials and Methods:

The Dalles

In two completely randomized block design, ten eight-year-old 'Royal Rainier' on 'Citation' interstocks and 'Sandra Rose' on Gisela 6' trees in The Dalles were drenched three times on 05/9/08, 05/2308 and 06/06/08 with soluble potassium silicate during the growing season and compared against similar untreated control trees. Fruit were harvested according to industry standards and on the day of harvest, the total number of cracked fruit per tree were counted and expressed as a percentage of the total number of fruit on each tree. A sample of 50 fruit were harvested from each tree. On the day of harvest 25 fruit were analyzed for fruit size (as a function of Row size), fruit firmness, stem pull force and TSS. The remaining 25 fruit were stored in cold storage at 2ºC for two weeks, removed from the cold room and held at room temperature for 14 hr to equilibrate and then subjected to the same treatments as on the day of harvest. All results were analyzed by general analysis of variance using Genstat 11.1. In addition the covariance for each of these factors was tested for against the remaining factors.

Milton-Freewater

An identical trial was laid out in Milton-Freewater except that the trees used in this experiment were twelve-year-old 'Bing' on 'Mazzard' rootstock. Fruit were handled identically to those in The Dalles.

Results

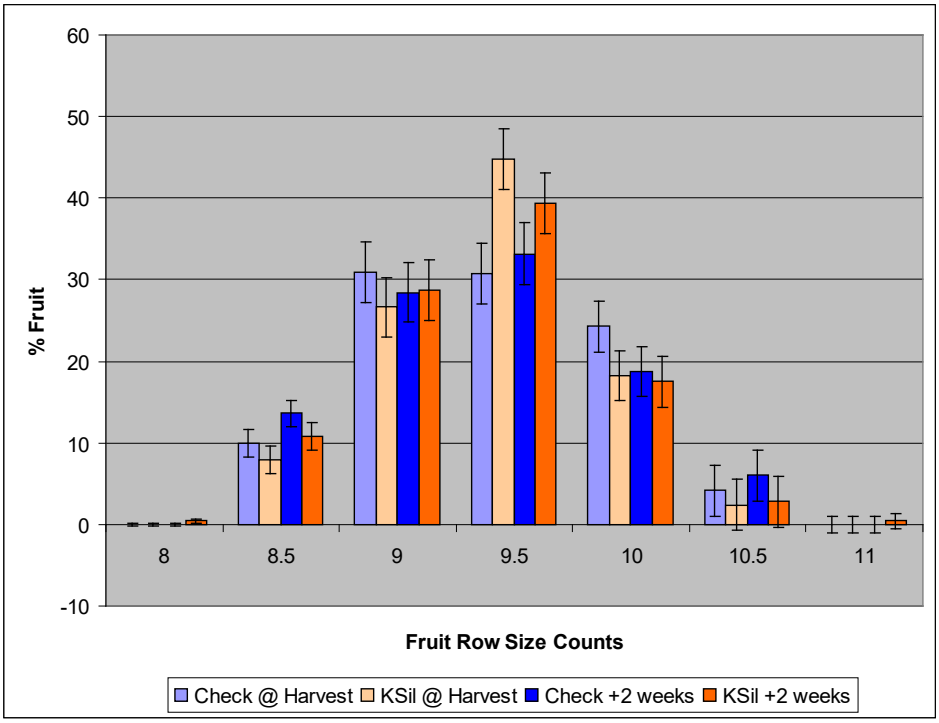

Figure 1. Average fruit size (Row counts) of 'Royal Rainier' fruit from trees with 'Citation interstem' in The Dalles, treated with or without soluble potassium silicate (KSil), on the day of harvest or stored for two weeks at 2ºC.

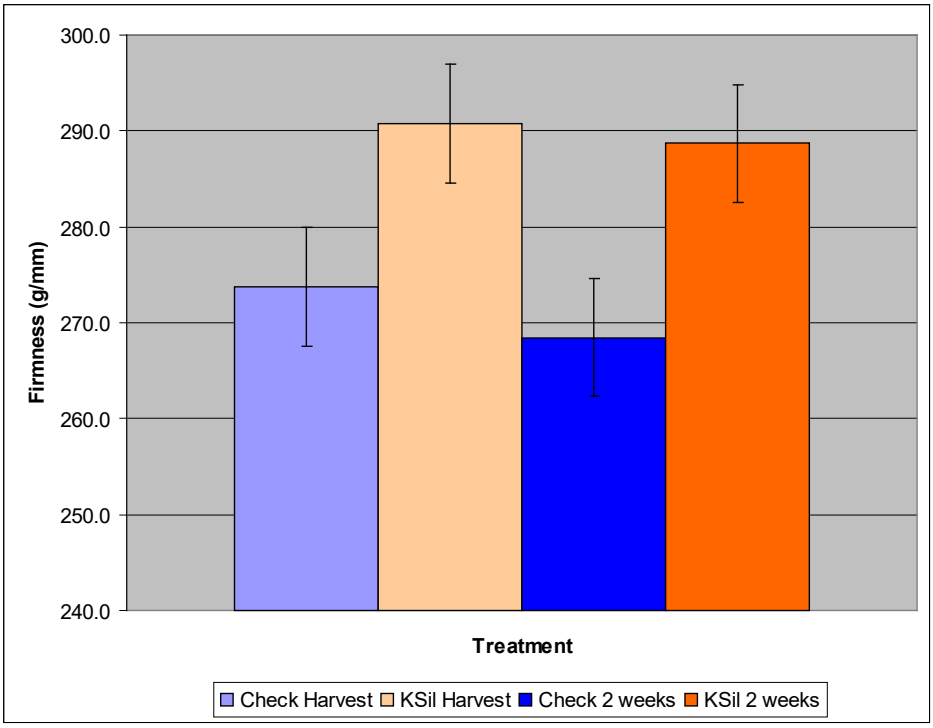

Figure 2. Average Firmness ($g.mm^{-1}$) of 'Royal Rainier' fruit from trees on 'Citation' interstem in The Dalles, treated with or without soluble potassium silicate (KSil), on the day of harvest or stored for two weeks at 2ºC.

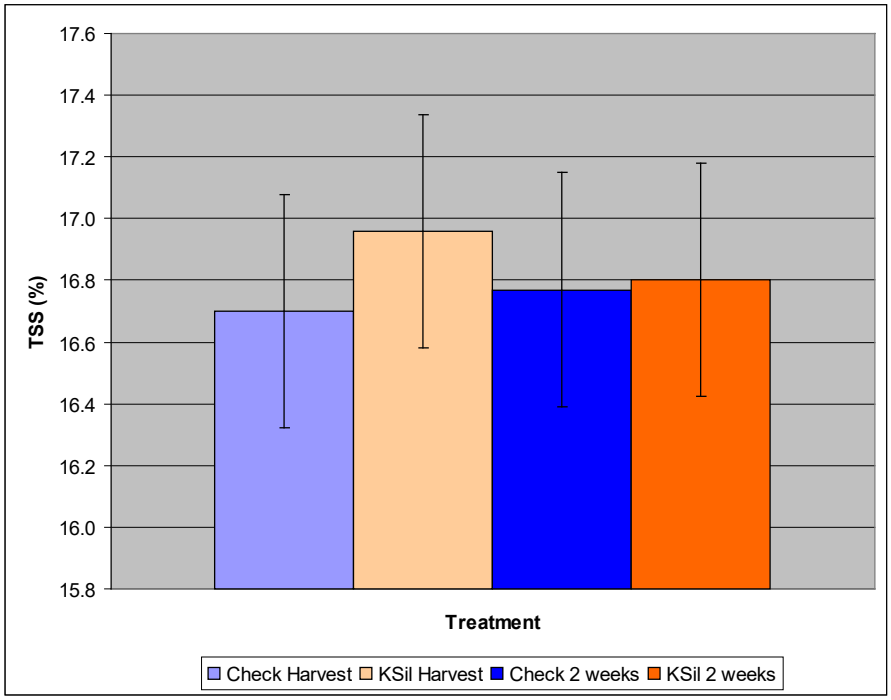

Figure 3. Average TSS (% Brix) of 'Royal Rainier' fruit from trees on 'Citation' interstem in The Dalles, treated with or without soluble potassium silicate (KSil), on the day of harvest or stored for two weeks at 2ºC.

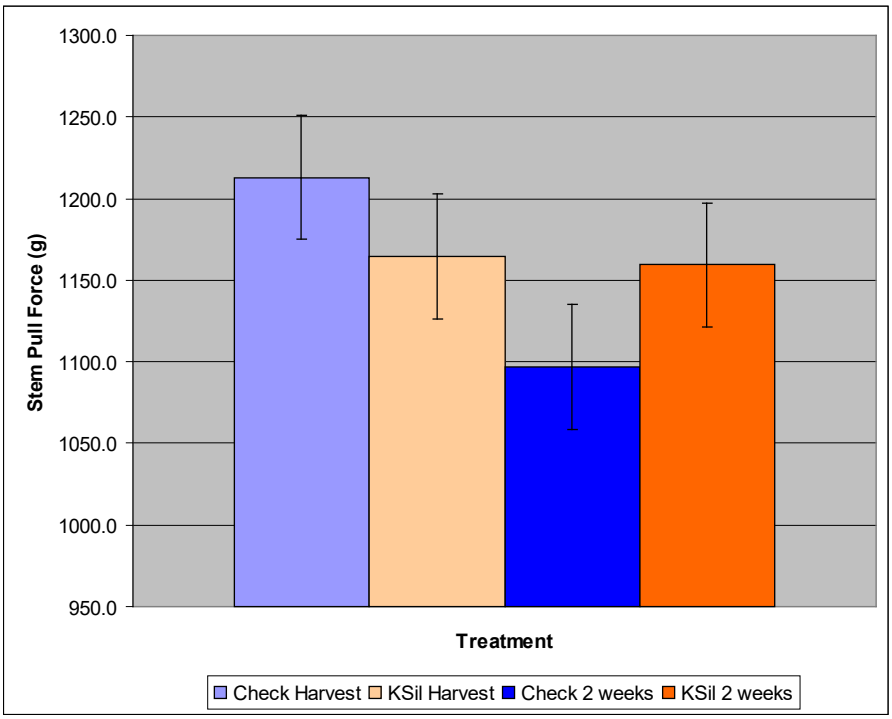

Figure 4. Average Stem Pull Force (g) of 'Royal Rainier' fruit from trees on 'Citation' interstem in The Dalles, treated with or without soluble potassium silicate (KSil), on the day of harvest or stored for two weeks at 2ºC.

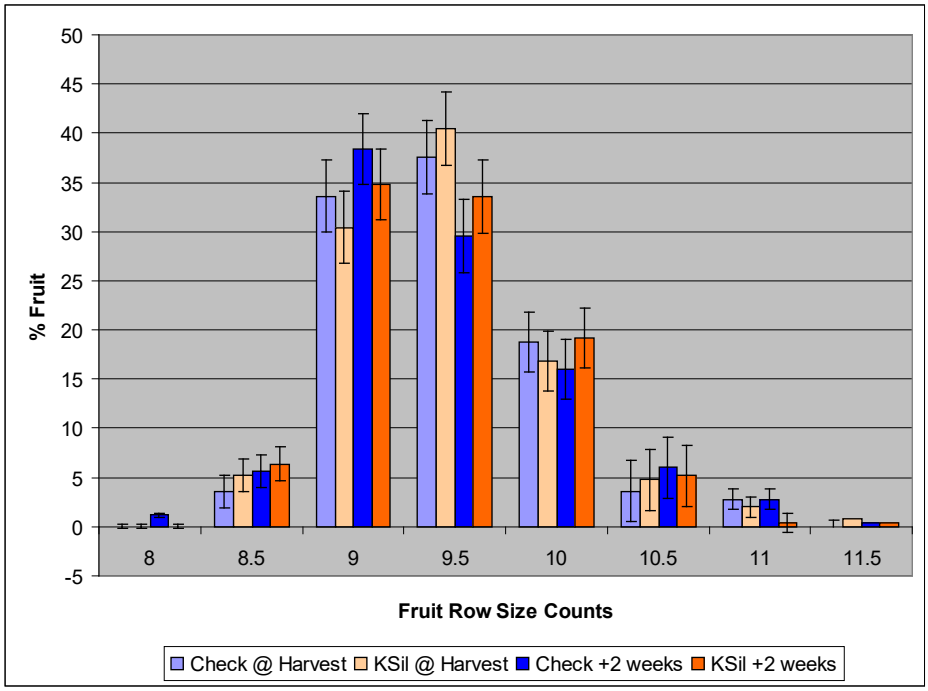

Figure 5. Average fruit size (Row counts) of 'Sandra Rose' fruit from trees on 'Gisela 6' in The Dalles, treated with or without soluble potassium silicate (KSil), on the day of harvest or stored for two weeks at 2ºC.

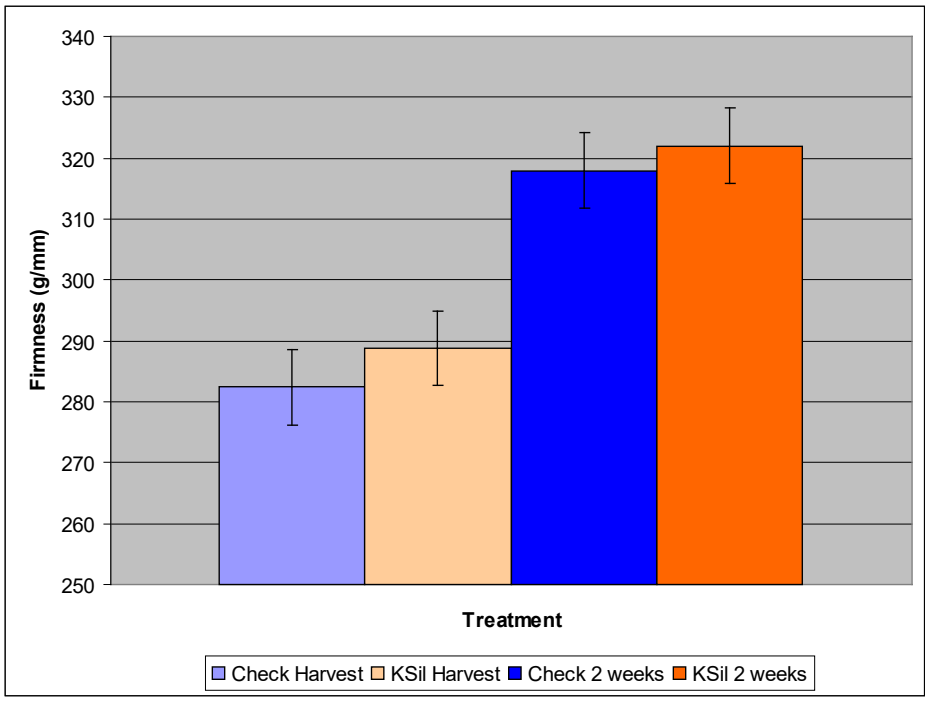

Figure 6. Average Firmness (g.mm^{-1}) of 'Sandra Rose' fruit from trees on 'Gisela 6' in The Dalles, treated with or without soluble potassium silicate (KSil), on the day of harvest or stored for two weeks at 2ºC.

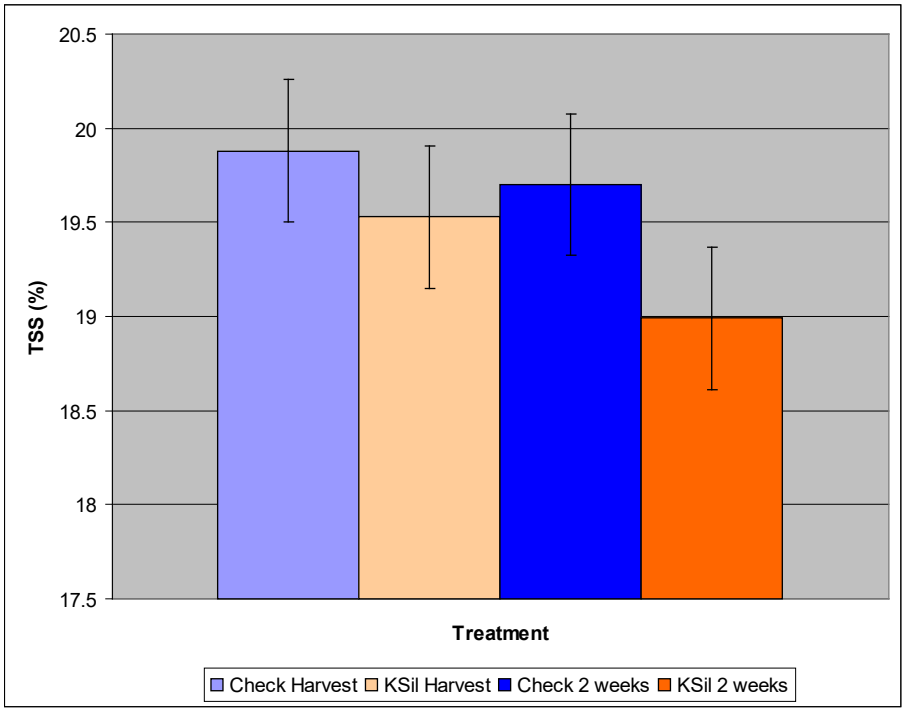

Figure 7. Average TSS (% Brix) of 'Sandra Rose' fruit from trees on 'Gisela 6' rootstocks in The Dalles, treated with or without soluble potassium silicate (KSil), on the day of harvest or stored for two weeks at 2ºC.

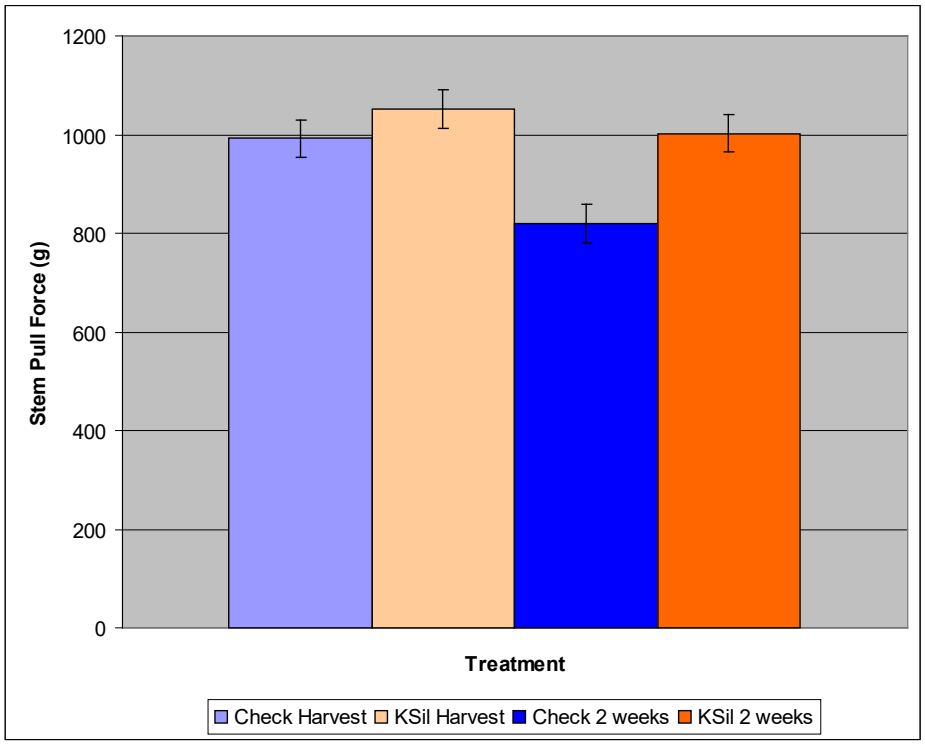

Figure 8. Average Stem Pull Force (g) of 'Sandra Rose' fruit from trees on 'Gisela 6' rootstocks in The Dalles, treated with or without soluble potassium silicate (KSil), on the day of harvest or stored for two weeks at 2ºC.

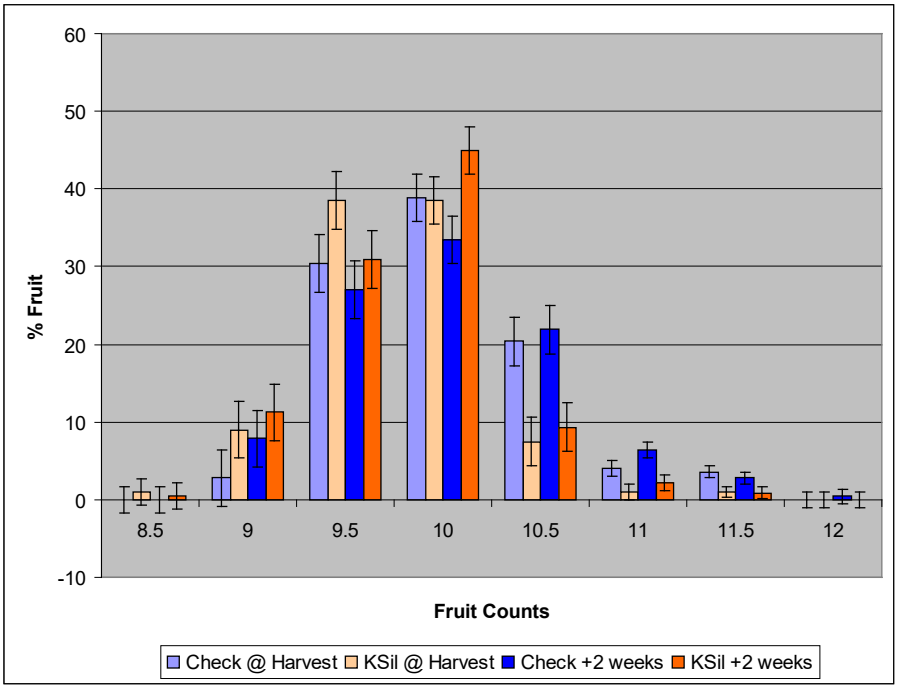

Figure 9. Average fruit size (Row counts) of 'Bing' fruit from trees on 'Mazzard' rootstocks in Milton-Freewater, treated with or without soluble potassium silicate (KSil), on the day of harvest or stored for two weeks at 2ºC.

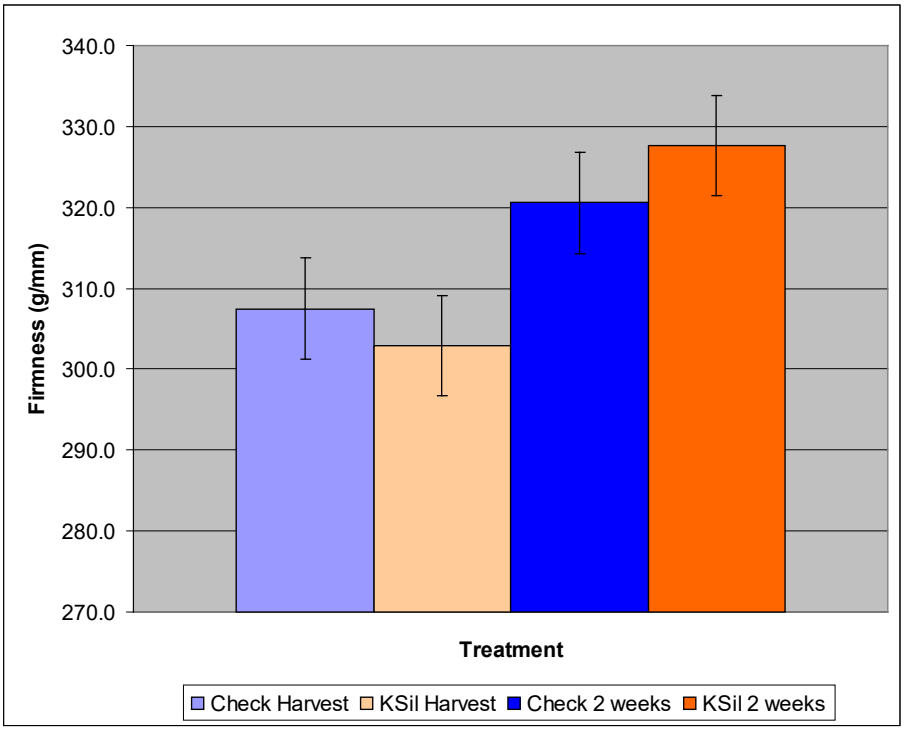

Figure 10. Average Firmness ($g.mm^{-1}$) of 'Bing' fruit from trees on 'Mazzard' rootstocks in Milton-Freewater, treated with or without soluble potassium silicate (KSil), on the day of harvest or stored for two weeks at 2ºC.

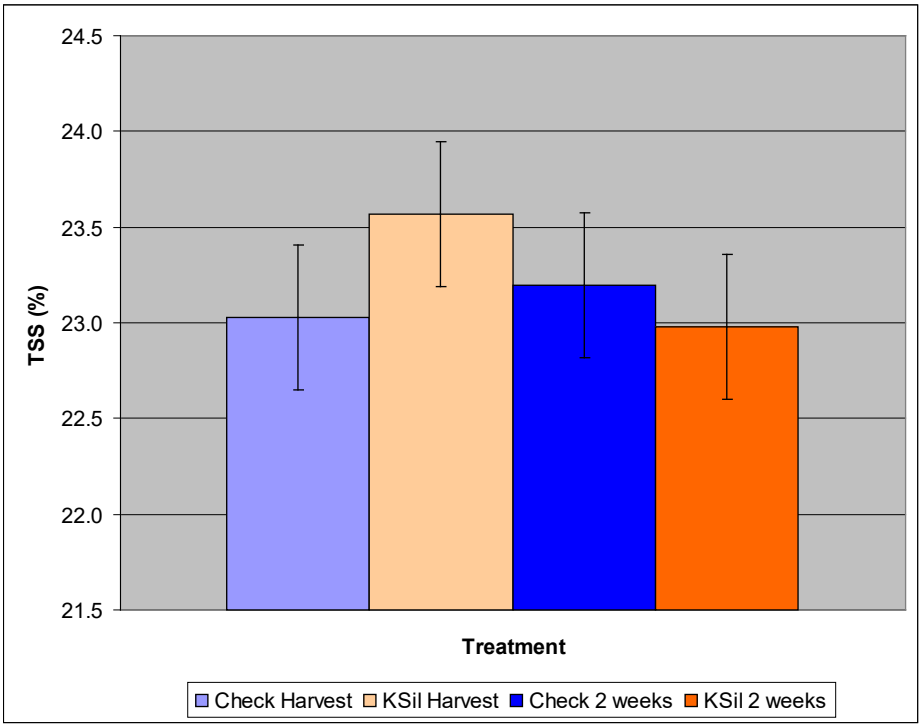

Figure 11. Average fruit TSS (%Brix) of 'Bing' fruit from trees on 'Mazzard' rootstock in Milton-Freewater, treated with or without soluble potassium silicate (KSil), on the day of harvest or stored for two weeks at 2ºC.

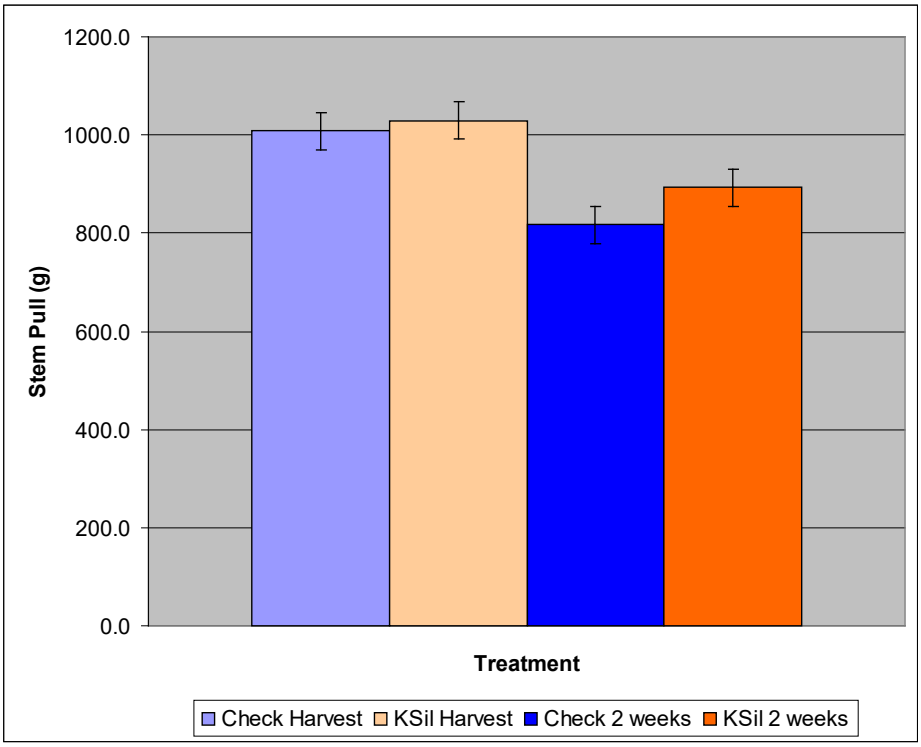

Figure 12. Average Stem Pull Force (g) of 'Bing' fruit from trees on 'Mazzard' rootstock in Milton-Freewater, treated with or without soluble potassium silicate (KSil), on the day of harvest or stored for two weeks at 2ºC.

Discussion & Conclusions

Unfortunately, there were no significant differences in rain-induced fruit cracking in the Pacific Northwest in 2008 in either Milton-Freewater or The Dalles. Indeed, the total percentage cracked fruit at harvest was less than 3% in both the untreated check trees, and those soil-drenched with soluble potassium silicate. Consequently, we are unable to make recommendations regarding the use of soluble potassium silicate for the prevention of cherry fruit cracking.

In terms of fruit size however, soil-drenching with potassium silicate in The Dalles in 2008, resulted in significantly (F.Pr.=0.015) more 'Royal Rainier' fruit of average diameter 28.17 mm (Row 9.5) than the untreated check. Furthermore, only those trees treated with potassium silicate had some very large fruit of average diameter 33.33 mm (Row 8) (Fig. 1). A similar finding was recorded for 'Bing' fruit in Milton Freewater in 2008, where soil-drenching with potassium silicate resulted in large fruit of average diameter 31.35 mm (Row 8.5) when compared to zero fruit of this size from untreated check trees (Fig. 9). This was a positive contrast to 2007, where potassium silicate actually resulted in slightly smaller fruit (0.644 mm on average) than untreated check trees in Hood River. In contrast, in 2008, untreated 'Sandra Rose' check fruit from The Dalles were larger than those treated with potassium silicate (Fig. 5). This may have been a response to cultivar or rootstock differences but at this point, the exact cause is speculative. The most important message to take home concerning the use of soluble potassium silicate on fruit size is that it did not result in smaller 'Bing' and 'Royal Rainier' fruit than the untreated checks.

Where total soluble solids (TSS) are concerned, there were significant differences (F.Pr.<0.001) between the different cultivars (Figs 3, 7 & 11) however, there were no significant differences between those fruit treated with potassium silicate or not, nor within any cultivar on the day of harvest v. those stored at 2ºC for two weeks. Testing for the effect of fruit firmness as a covariate found that there were no significant differences either. Consequently, we conclude that silicon applications had no effect on the TSS concentrations of any of the different cultivars whether stored in regular atmosphere for two weeks at 2ºC or not and that fruit firmness was not correlated with higher or lower TSS concentrations.

Where fruit firmness is concerned, there were highly significant differences between cultivars (F.Pr<0.001); effects of soil-drenching with soluble potassium silicate versus untreated checks (F.Pr=0.049); the day of harvest versus stored in regular atmosphere for two weeks at 2ºC (F.Pr<0.001), as well as the interaction between sampling on the day of harvest v. two weeks of storage in regular atmosphere at 2ºC and the different cultivars (F. Pr=0.002) (Figs 2, 6 & 10). 'Royal Rainier' fruit were the softest of all the fruit used in this trial. However, fruit from trees soil-drenched with soluble potassium silicate, but were significantly firmer than fruit from untreated check trees on the day of harvest (291 v. 274 $g.mm^{-1}$ respectively) and remained significantly firmer even when stored for two weeks in regular atmosphere storage at 2ºC (289 v. 269 $g.mm^{-1}$ respectively). Fruit from both 'Sandra Rose' and 'Bing' trees, soil-drenched with soluble potassium silicate, were firmest after two weeks of regular atmosphere storage at 2ºC (322 and 329 $g.mm^{-1}$ respectively) when compared to untreated controls either on the day of harvest (283 and. 308 $g.mm^{-1}$ respectively) or after two weeks of regular atmosphere storage 2ºC (318 and 321 $g.mm^{-1}$ respectively). These differences were however, not significant but still bode well for soil-drenching all these cultivars with potassium silicate. Furthermore, this contrasts well with the reduced fruit firmness, observed as a result of soil-drenching 'Stardust' on 'MM14' trees, with potassium silicate in 2007. This last result does however, suggest a measure of caution with this cultivar/rootstock combination. However, based on the majority of results obtained in 2007, where soil-drenching with soluble potassium silicate resulted in a significant improvement in fruit firmness of 'Bing' fruit on 'Mazzard' rootstocks

at Hood River and of ‘Stardust’ on ‘Gisela 6’ rootstock in Corvallis together with the significant increase in fruit firmness of ‘Royal Rainier’ on ‘Citation’ interstem in The Dalles 2008, we conclude that potassium silicate has a beneficial effect on most cultivar / rootstock combinations. Furthermore, this effect was further enhanced in all cultivars tested after two weeks of regular atmosphere storage at 2ºC in 2008.

Where stem pull force is concerned, there were highly significant differences between cultivars (F.Pr<0.001); effects of soil-drenching with soluble potassium silicate v. untreated checks (F.Pr=0.032); and between the day of harvest v. stored in regular atmosphere for two weeks at 2ºC (F.Pr<0.001). Furthermore, stem pull force was positively correlated with fruit firmness (covariate F.Pr=0.034) and was negatively correlated with TSS (F.Pr=0.046). This last correlation was most likely a function of fruit maturity. In all three cultivars tested in 2008, stem pull force decreased most significantly for check fruit between the day of harvest and after two weeks in regular atmosphere storage at 2ºC (Figs 4, 7 & 11). Indeed, stem pull force of ‘Bing’ check fruit decreased the most after two weeks regular atmosphere storage at 2ºC (from 1008 g to 816 g) compared to ‘Bing’ fruit from trees, soil-drenched with soluble potassium silicate (from 1026 g to 890 g). Stem pull force of ‘Sandra Rose’ check fruit decreased from 992 g to 815 g whereas stem pull force of similar fruit from trees, soil-drenched with soluble potassium silicate, decreased only slightly from 1052 g to 1002 g. Stem pull force of ‘Royal Rainier’ check fruit had the highest stem pull force at harvest 1213 g but this decreased significantly to 1097 g after two weeks of regular atmosphere storage at 2ºC compared to similar fruit from trees, soil-drenched with soluble potassium silicate, where stem pull force decreased only marginally from 1165 g to 1159 g. Clearly, stem pull force decreased significantly in untreated check fruit as a result of regular atmosphere storage at 2ºC. In contrast, although there were slight decreases in stem pull force of fruit from trees soil-drenched with soluble potassium silicate, these were only significant in fruit from ‘Bing’ trees on ‘Mazzard’ rootstock. Consequently, we conclude that soluble potassium silicate has a beneficial effect on stem pull force of ‘Royal Rainier’, ‘Sandra Rose’ and ‘Bing’ fruit and that this is especially marked after two weeks of regular atmosphere storage at 2ºC.

FINAL PROJECT REPORT

Project Title: Identifying sweet cherry fruit size genes and molecular markers

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City: Wooster
State/Zip: OH 44691

Cooperators: Marco Bink, Cameron Peace

Other funding Sources

Agency Name: USDA/CSREES/NRI Plant Genome
Amount requested/awarded: $400,000 requested on 2/14/08 and awarded 8/15/08
Notes: This WTFRC/OSSC project provided funds for us to get preliminary data that helped us successfully compete for this federal grant.

Total Project Funding: $ 24,830

Budget History:

Item	Year 1:	Year 2:	Year 3:
Salaries	7,158		
Benefits	3,342		
Wages	2,500		
Benefits			
Equipment			
Supplies	4,830		
Travel			
Consulting fee: M. Bink	2,000		
Other: DNA sequencing &	5,000		
primer synthesis			
Miscellaneous			
Total	**24,830**		

OBJECTIVES

1. Identify the genomic regions in sweet cherry that control fruit size.

- Regions of DNA that are associated with variation for a particular trait are termed quantitative trait loci (QTL).

2. Develop a conserved ortholog set (COS) of markers for cherry suitable for comparative mapping within the Rosaceae (partial funding is requested).

- COS markers are "state of the art" sequence based molecular markers this can be used for cherry mapping and for connecting maps between cherry and other rosaceous species.

SIGNIFICANT FINDINGS BY OBJECTIVE

1. Identify the genomic regions in sweet cherry that control fruit size.

- Four genomic regions (e.g. QTLs) were identified in sweet cherry that control fruit size. The QTL identified on cherry linkage group two appears to increase fruit size by increasing fruit mesocarp cell number. In contrast, the QTL identified on cherry linkage group six appears to increase fruit size by increasing pit size. Two more QTL were newly identified on linkage groups four and eight. The morphological basis of the fruit size increases associated with these newly identified QTLs has not yet been determined.

- The molecular markers flanking the QTLs on linkage groups two and six have been identified and will be used to examine the affect of these fruit size QTL in different genetic backgrounds.

- The newly identified fruit size QTLs on linkage groups four and eight will be validated in other germplasm used in the sweet cherry breeding program.

- A genetic database was generated to support the PNW sweet cherry breeding program. It includes marker genotyping data (e.g. genetic barcodes) for over 39 parents used in the breeding program plus progeny from the cross between PMR × Rainier. This database will serve as the foundation for future QTL discovery and marker assisted breeding through the use of pedigree based analysis.

2. Develop a conserved ortholog set (COS) of markers for cherry suitable for comparative mapping within the Rosaceae (partial funding is requested).

- From an existing set of 1041 Rosaceae COS, PCR primers were designed that successfully amplified 739 (86%) COS in *Prunus*. To date, 319 COS have been placed on the eight *Prunus* linkage groups with a density ranging from 1.19 to 2.10 markers per centimorgan (cM). The COS markers are also well distributed across the *Prunus* genome as COS markers were mapped in 53 of the 67 *Prunus* peach × almond (T×E) reference map bin positions (*Howad et al. 2005. Genetics 171:1305-1309*). A subset of these COS markers will also be mapped in sweet cherry, apple and strawberry, providing a lasting resource of high throughput markers.

- A survey of COS marker diversity was undertaken using seven sweet cherry selections (Bing, Van, Regina, Emperor Francis, NY54, Cristobalina, and Windsor). The majority of COS markers exhibited only two allelic variants per marker.

1 & 2. This WTFRC & OSSC grant provided seed money that helped us successfully compete for the following USDA-CSREES-NRI Award (Appendix I):

Title: The Development of COS Markers for Comparative Mapping in the Rosaceae and Their Application for Understanding Variation in Fruit Size.
PD: Amy Iezzoni
Co-PDs: Esther van der Knaap (Ohio State University) & Dechun Wang (Mich. State Univ.)
Award Amount: $400,000
Award Period: 08/15/08 through 08/14/11

RESULTS and DISCUSSION

Fruit Size: Findings

Cherry fruit size is not only a critical trait for market profitability, but it is the key trait altered during the domestication of sweet cherry from it wild forest tree relative. Our initial approach to elucidate the genetic control of fruit size in sweet cherry was to determine the genetic changes that accompanied domestication. With prior USDA-CSREES-NRI funding we conducted an analysis of the genetic control of fruit size in 2006 and 2007 using progeny from a cross between the domesticated founder cultivar 'Emperor Francis' (EF) and the forest sweet cherry 'NY 54' (NY). EF is in the ancestry of all the self-fertile sweet cherry cultivars as it was the material parent in the cross from which the self-fertile mutant was identified. In 2008, we repeated our fruit size analysis of the EF × NY population, and broadened our analyses to include progeny from the cross PMR × Rainier, commercial sweet cherry cultivars, and other parental cultivars used as parents in the PNW sweet cherry breeding program. Statistical analysis of the pedigree linked populations was possible with the implementation of pedigree based analysis software.

- *Segregation for fruit size*: Fruit weight was measured for the progeny from the two segregating populations, EF × NY (Fig. 1A) and PMR × Rainier (Fig. 1B). Progeny from both populations exhibited fruit weight values well below that of the large fruited parent. This tendency for the vast majority of the progeny to be small fruited has been observed repeatedly in studies in cherry and peach. This result suggests that large fruited progeny are rarely obtained in breeding populations. Therefore, it is critical that we put strategies in place to increase the percentage of large fruited seedlings that are planted in the evaluation orchards.

- *QTL discovery in EF × NY*: In 2006 and 2007, analysis of progeny from a population generated from the cross of EF × NY resulted in the identification of fruit weight QTLs on two of the eight *Prunus* linkage groups (Fig. 2). This analysis of the EF and NY progeny was repeated in 2008, and confirmed our previous findings. Fruit length and diameter QTLs were identified at similar genomic regions as the fruit weight QTLs, as would be expected due to the trait correlations (Fig. 2). A QTL for mesocarp cell number was also detected on EF 2 which suggests that the morphological basis of this QTL is to increase cell number by increasing cell division in the mesocarp. The fruit weight QTL identified on NY linkage group 6, co-located with pit size (linear measures) QTL. This suggests that the morphological basis underlying this QTL is an increase in pit diameter and length and not an increase in cell number (as measured on a radial section) or cell size.

- *QTL discovery in a PMR × Rainier progeny population*: In 2008, we extended this analysis to include the parental cultivars and progeny from the cross PMR × Rainier using pedigree based analysis. The 39 cultivars and 103 progeny individuals were genotyped for 61 and 49 marker loci, respectively (12 fewer markers were scored in the PMR × Rainier population as these markers did not segregate in this population.). Fruit weight was also measured. When this data set was combined with our data from the EF × NY population, fruit weight QTLs were also identified on cherry linkage groups four and eight (Table 1). Validating and fine mapping these newly discovered QTLs will be a major thrust undertaken with our newly acquired USDA-CSREES-NRI funding.

- *Determination of the fruit weight/cell number QTL allele*: Determining the favorable fruit size QTL allele(s), identified by its flanking markers, is critical for further QTL validation and eventually marker-assisted selection (MAS). For the fruit weight/cell number QTL on EF linkage group two, the unique PR96 allele present in EF is in coupling with the large fruit effect of the predicted fruit weight QTL. Future efforts will involve experiments to validate the presence and direction of this QTL in different genetic backgrounds using PR96 and other co-dominant markers linked in coupling with PR96.

- *Genome scans of 39 parental cultivars*: A description of the genetic make-up of 39 sweet cherry varieties and parental cultivars used in the breeding program was generated with past NRI funding and WTFRC/OSCC funding. This genetic data set (~ 5,000 genotypic data points) provides a critical foundation of genetic knowledge for the sweet cherry breeding program. This information will assist in our interpretation of genetic variation through the use of Flex QTL® and will inform future crosses and eventually strategies to pre-select superior seedlings prior to field planting. An example of how our genetic information is used to describe inheritance of genomic regions in cherry is illustrated in Fig. 3.

Fruit Size: Significance

Achieving large fruit size is an essential component for profitable fresh market sweet cherry production. Therefore, new cultivars to be released from the WSU sweet cherry breeding program must exhibit the large fruit size demanded by the growers and marketplace. Our work in cherry and other reports in both cherry and peach clearly indicate that large fruited progeny individuals occur only rarely in segregating populations. Two factors are likely contributing to this prevalence of small fruited progeny individuals: dominance of alleles conferring small fruit size, and the rarity of those unique allelic combinations that result in large fruit size. Our ability to understand the genetic control of fruit size in sweet cherry and pre-select for those desirable allelic combinations will dramatically increase the efficiency of sweet cherry breeding.

This project, in combination with prior NRI funding, resulted in the identification of four genomic regions that contain desirable genes controlling fruit size. Future NRI funding will allow us to expand this to additional breeding populations with the goal of identifying and defining those QTL that contribute to large fruit size so that they can be effectively manipulated in the breeding program. The genetic data obtained for the parental selections in the breeding program will be used to dissect the genetic control of other target traits, plan future crosses, and accelerate the implementation of marker assisted selection.

Fig. 1. Progeny distributions for fruit weight from the crosses EF × NY (A) and PMR × Rainier (B). Data is from 2008.

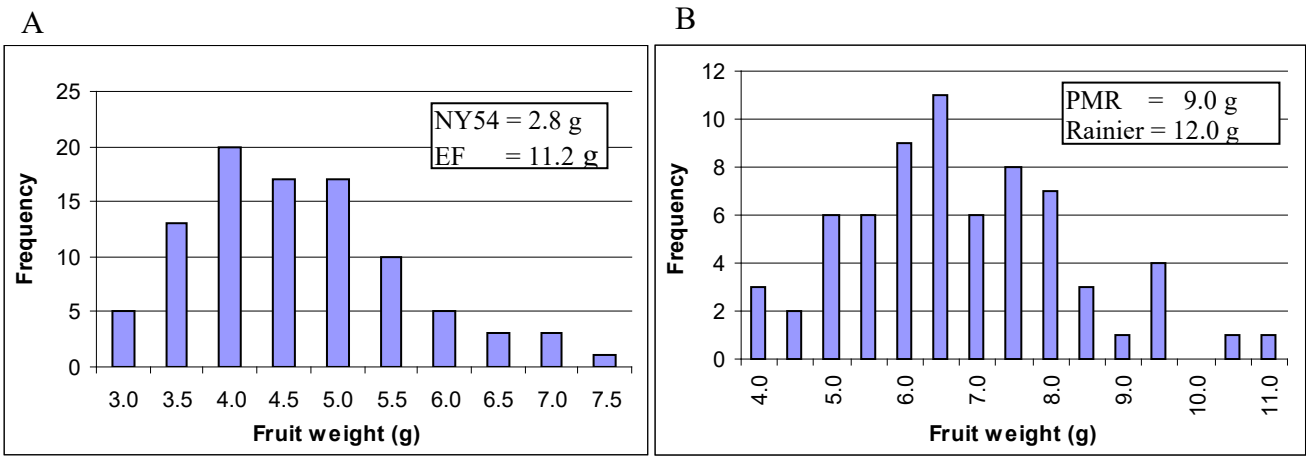


Table 1. QTLs identified using Flex QTL® and data from 41 parental cultivars and two mapping populations: EF × NY, PMR × Rainier.

Linkage group (parental map)	Bayes Factor[a]
2 (EF)[b]	6.4
2 (NY)	6.3
4 (EF)	3.1
8 (NY)	3.5

[a]QTL models interpreted based on the value of the 2ln Bayes Factor: 2.0-5.0 Positive, 5.0-10 Strong, > 10 Decisive.

[b]This QTL was also identified using fruit size and molecular marker data from progeny from the cross Regina × Lapins. The data from this population was generously provided by E. Dirlewanger and J. Queros, INRA, Bordeaux, France.

Fig. 2 Locations of QTLs for fruit weight (FW), fruit length (FL), fruit diameter (FD), mesocarp cell number (Cell), pit length (PL) and pit diameter (PD) in 2006, 2007, and 2008 using the multiple QTL mapping method. 1-LOD and 2-LOD support intervals of each QTL are marked by thick and thin bars, respectively. EF-2 and NY-2 represent EF and NY linkage groups 2, respectively, while NY-6 is NY linkage group 6.

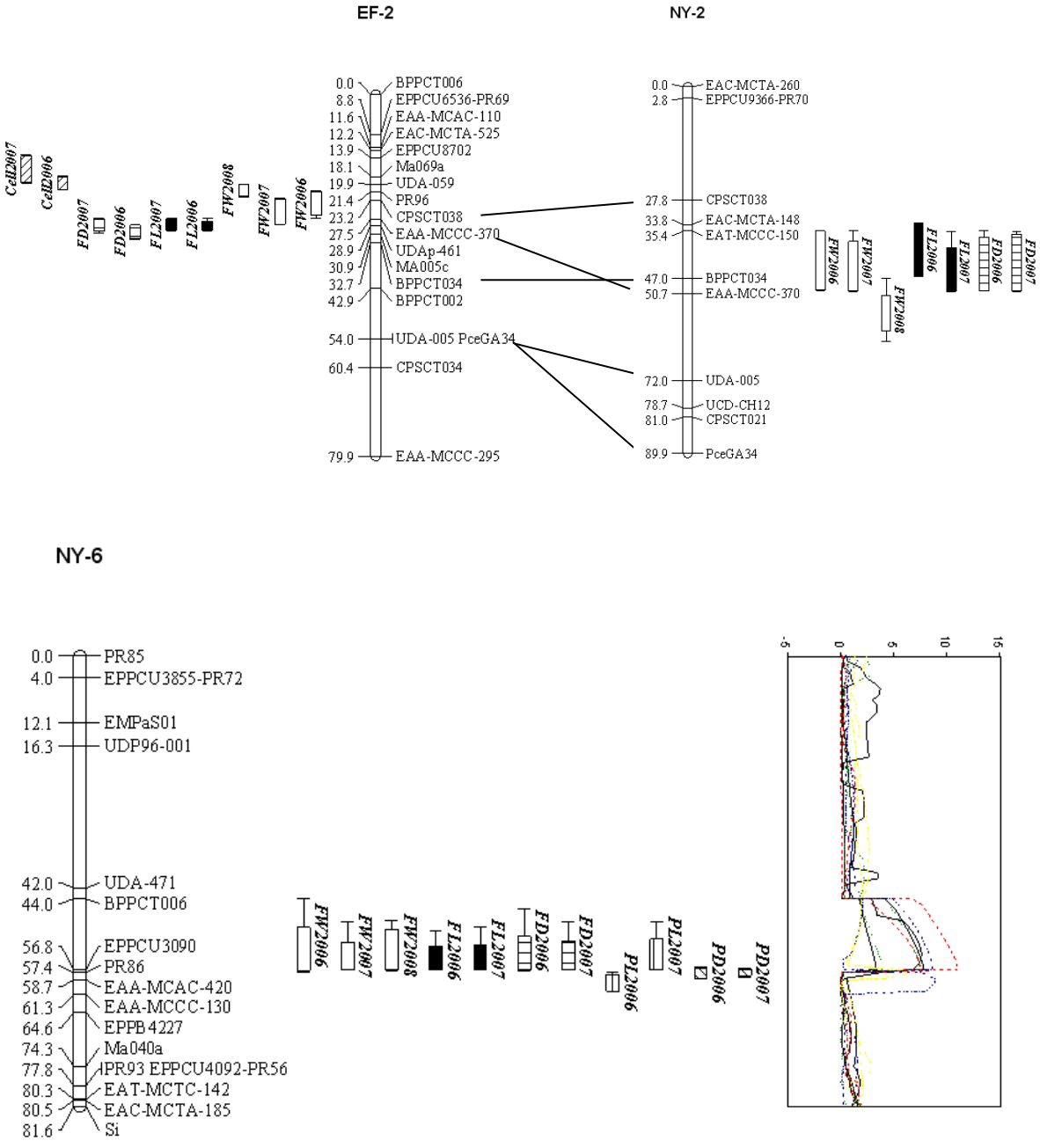
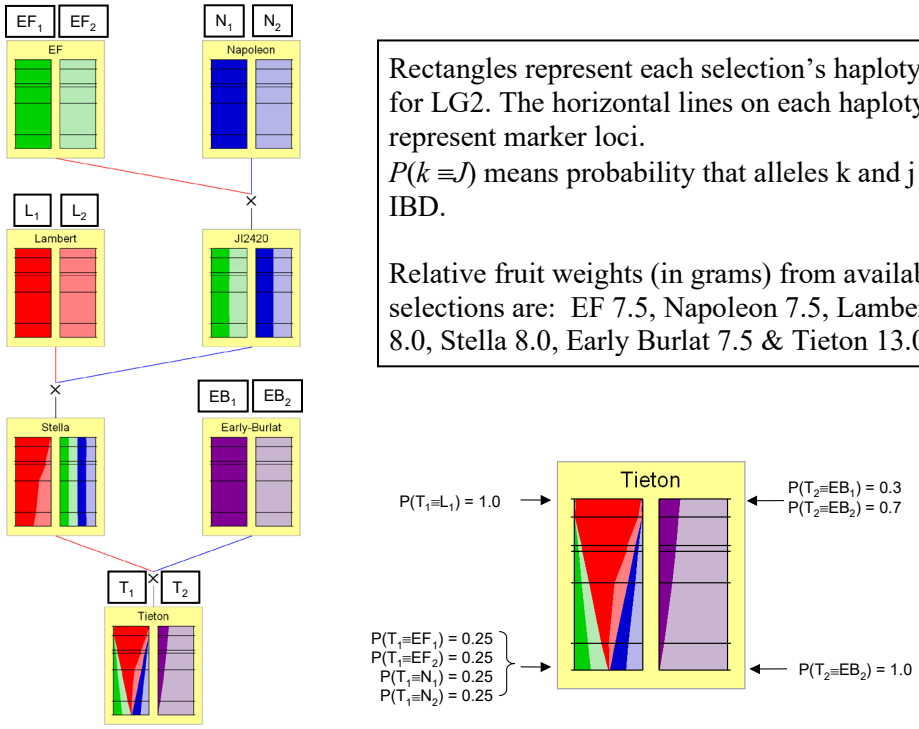


Fig. 3. Graphical representation of the Identity by Descent (IBD) probabilities for the marker alleles on LG 2 using the cultivars in the 'Tieton' lineage and our genotyping data.



COS Markers: Findings

PCR primer pairs were designed based on available sequence data and used to amplify 730 COS markers in the *Prunus* reference bin mapping population (T×E). Allelic polymorphisms, defined as single nucleotide polymorphisms (SNPs) or insertions/deletions (InDels), were identified using available software. A total of 221 polymorphic COS markers were mapped on the 8 *Prunus* linkage groups (Table 2) with an average marker/centimorgan (cM) density of 1.19 to 2.10 COS markers per cM. These markers also mapped to 53 out of 67 bins on the T×E map (Fig. 4). With newly acquired USDA-CSREES-NRI funding, a subset of the markers mapped to each of the T×E bins will be placed on our sweet cherry map and available strawberry and apple bin maps, providing a genome wide set of comparable high-throughput markers.

COS sequence data from seven sweet cherry cultivars identified, on average, two allelic variants within this sweet cherry germplasm. Therefore, our sequencing strategy should result in the identification of the vast majority of the allelic variation for these COS in the parents used in the sweet cherry breeding program.

COS markers: Significance

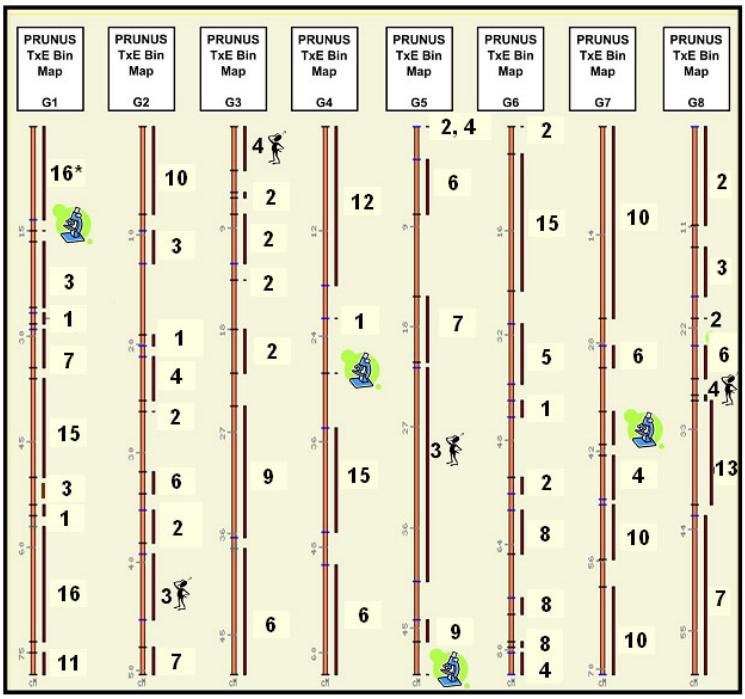
Dissection of the genetic basis of complex trait variation is accomplished by associating DNA variants with known linkage map location (e.g. markers) with phenotypic differences in the trait of interest. This strategy is used whether the target trait is in human genetics or cherry genetics. Unfortunately for sweet cherry, very few markers were available. To fill this void, the WTFRC/OSSC funded project provided us with seed money to obtain preliminary data that allowed us to successfully compete for NRI funds to generate and map additional markers in cherry and identify their comparative map locations in apple and strawberry. The COS markers that are being developed are state-of-the-art (identical marker type used in human genetics) and therefore amenable to high throughput phenotyping approaches (just ~ 10 cents per data point). With a detailed linkage map of

COS markers, we will finally have the markers density that we need in sweet cherry to do comprehensive genetic dissection for those traits so critical to future cultivars. In addition, as these markers will also be mapped in other rosaceous species, e.g. peach, almond, apple and strawberry, we will be able to immediately test any marker trait associations identified in these other crops for their relevance in cherry. This will speed up our ability to understand the genetic basis of important traits in the Rosaceae, and also provide a source of high-throughput markers for implementing MAS for desirable alleles.

Table 2. Number of COS markers per centimorgan (cM) placed on the 8 *Prunus* linkage groups utilizing the *Prunus* reference bin mapping population developed from a peach × almond cross (T×E).

Prunus Linkage Group	cM length from the T × E map	Number of COS markers mapped to this linkage group	Number. of COS markers mapped per cM
1	87.0	73	1.19
2	50.5	35	1.44
3	48.4	23	2.10
4	62.5	34	1.84
5	49.1	28	1.75
6	83.7	53	1.58
7	70.6	40	1.76
8	55.9	33	1.69

Fig 4. Locations of the COS markers on the *Prunus* reference (T×E) bin map. The eight vertical lines represent the 8 *Prunus* linkage groups while the lines to the right represent the *Prunus* bin locations. The numbers reflect the number of COS markers to date that have been mapped to each of the bins.



* Number next to the bin indicate amount of COS markers mapped

Bins without Rosaceae COS Markers in these bins could map elsewhere

EXECUTIVE SUMMARY

Key project goals were to identify favorable fruit size alleles segregating in sweet cherry breeding germplasm and determine the effects of these alleles in different genetic backgrounds. With the assistance of this project and prior USDA-CSREES-NRI funding we have identified and confirmed the presence of two fruit size QTLs on linkage groups 2 and 6. The QTL identified on cherry linkage group 2 appears to increase fruit size by increasing fruit mesocarp cell number. The molecular markers flanking this QTL have been identified and will be used to examine the affect of this fruit size QTL in different genetic backgrounds. The fruit size QTL identified on cherry linkage group 6 appears to increase fruit size by increasing pit size. Two more QTLs were newly identified on linkage groups 4 and 8. The morphological basis of the fruit size increases associated with these newly identified QTLs has not yet been determined.

A genetic database was developed to support the PNW sweet cherry breeding program. It includes marker genotyping data (e.g. genetic barcodes) for 39 parents used in the breeding program plus progeny from the cross between PMR × Rainier. This database of over 5,000 molecular marker data points, will serve as the foundation for future QTL discovery and marker assisted breeding through the use of pedigree based analysis

Whether one is a human geneticist or cherry geneticists, mapped markers (e.g. DNA variants with known genetic map locations) are a critical part of the investigator's "tool kit". It is this linked marker scaffold that is used to dissect the genetic basis of trait variation. With the genetic basis of the variation for a particular trait known, it is then possible to identify the desirable allelic variants and in cherry, select for those desirable allele variants prior to field planting. This has the potential to dramatically reduce the breeding cost and increase the chance of success, as those seedlings that are unlikely to have commercial potential can be discarded prior to field planting. With our prior NRI funding, bridging funds provided by the WTFRC/OSSC, and newly acquired NRI funding, we are building this scaffold for sweet cherry and linking it to peach, almond, apple and strawberry. With this toolkit in place, we can now move forward and use this resource for the genetic dissection of trait variation in sweet cherry, similar to our strategy for the genetic dissection of fruit size. To date, our accomplishments towards the construction of this marker scaffold for sweet cherry are as follows:

- Identification of a set of conserved othologous markers (COS) suitable for comparative mapping in the rosaceae.
- Generation of PCR primer pairs that successfully amplify 700 of these markers.
- Mapping of over 300 of these markers on the peach × almond bin map thereby setting the stage to map these in sweet cherry.
- Identification of allelic variants for these markers in sweet cherry thereby providing state-of-the art markers suitable for marker assisted selection in sweet cherry.

APPENDIX I

Title: The Development of COS Markers for Comparative Mapping in the Rosaceae and Their Application for Understanding Variation in Fruit Size.
PD: Amy Iezzoni
Co-PDs: Esther van der Knaap (Ohio State University) & Dechun Wang (Mich. State Univ.)
Award Amount: $400,000
Award Period: 08/15/08 through 08/14/11

The goals of this proposal are to accelerate Rosaceae comparative genomics through the development and mapping of a Rosaceae-Arabidopsis conserved ortholog set (COS) of markers. We will adopt these markers to enable our complementary long term goal to map and deploy beneficial alleles for increased fruit size in rosaceous crops. The COS markers developed will be a primary tool for integrating information across the family, and will provide additional markers for Rosaceae species for which current marker coverage is insufficient. For the first time, it will be possible to extensively align the linkage groups of the major fleshy fruited Rosaceae genera. The proposed research goals are to: (1) develop a Rosaceae-Arabidopsis COS marker set resource, (2) determine the comparative bin map locations of the COS markers in *Prunus*, *Malus* and *Fragaria*, (3) determine the sweet cherry linkage map locations for a set of COS markers with known bin map locations in *Prunus*, *Malus* and *Fragaria* to provide the basis for a genome-wide comparative gene-based linkage map, and (4) accelerate the discovery, quantification, validation, fine mapping and deployment of beneficial QTL alleles for fruit size in sweet cherry. This proposal addresses NRI priority 1: Use of genome-wide approaches for mapping and identification of important genes. The knowledge gained will enable comparative QTL mapping in Rosaceae species and significantly increase the efficiency of sweet cherry breeding in particular. Large fruit size, the focus of this proposal, is critical to the long term profitability of the U.S. Rosaceae fruit industries.

FINAL PROJECT REPORT

Project Title: Adapting available genomics tools to enhance PNW sweet cherry breeding

PI:	Cameron Peace	**Co-PI(2)**:	Jim Olmstead
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City:	East Lansing	**City**:	
State/Zip:	MI 48824	**State/Zip**:	

Cooperators: Matt Whiting (WSU-IAREC), Wayne Loescher (MSU), Fred Bliss (Davis, CA), Jim McFerson (WTFRC)

Other funding Sources

Agency Name: Prunus Crop Germplasm Committee
Amount requested/awarded: $8,833
Notes: Metabolite profiling of the National Clonal Germplasm Repository cherry collection

Total Project Funding: $67,900

Budget 1 History: WSU

Item	Year 1:	Year 2:	Year 3:
Salaries – postdoc* for 9 months	22,500		
Benefits	9,900		
Wages – for activity 2	3,587		
Benefits	413		
Equipment			
Supplies – activities 1,3,5	5,000		
Travel – in-state	4,000		
Travel - interstate**	7,500		
Miscellaneous – database software	5,000		
Total	57,900		

Budget 2 History: MSU

Item	Year 1:	Year 2:	Year 3:
Salaries	4,772		
Benefits	2,228		
Wages	2,000		
Benefits			
Equipment			
Supplies	1,000		
Travel			
Miscellaneous			
Total	10,000		

Footnotes:

*We hired an Agricultural Project Assistant (grad student level) from May 2008, which leveraged support from the Department of Horticulture and LA for a Teaching Assistantship starting in the Fall 2008 semester for this prospective PhD student. Summer salary for this person was instead paid by an existing federally funded project of the PI focusing on stone fruit texture genetics. A full-time technical assistant for this WTFRC/OSCC cherry project from August 2008.

**$1500 for one trip to CA (Davis repository), $6000 for PIs (and local collaborators) to meet once in Prosser and once in East Lansing

ORIGINAL OBJECTIVES

The main goal is to assess opportunities for genetic marker assistance in the Pacific Northwest sweet cherry breeding program (PNWSCBP) and develop the technical infrastructure for ready translation of genomics information and tools into practical breeding benefit.
Specific objectives are to:

1) Develop protocols for high-throughput genetic screening in the PNWSCBP.
2) Conduct flavor and texture phenotypic analysis of key breeding germplasm.
3) Collate, validate, and package available markers of value to the PNWSCBP through coordination with other sweet cherry projects on gene/marker identification.
4) Establish a database for the PNWSCBP that meets traditional breeding needs and is compatible with ongoing genomic analyses.
5) Assess internationally-renowned cherry germplasm collections for genetic diversity of potential value to the PNW.

SIGNIFICANT FINDINGS

Overall

- Numerous opportunities were identified for practical application of genomics tools and knowledge to enhancing sweet cherry breeding in the Pacific Northwest towards a tangible, and indeed revolutionary, effect on the sweet cherry industry. In 2008, we established some of the necessary technical infrastructure to translate genomics advances into industry impact. This project has helped leverage national efforts towards developing a nationwide" marker-assisted selection pipeline" approach, and helped maintain the strong collaboration between WSU and MSU for cherry genetic improvement.
- This project attracted and leveraged funding from the Department of Horticulture and Landscape Architecture for a PhD student Sanchita Haldar, under the direct supervision of PI Peace.
- Four large proposals for federal funding were submitted in 2008 that could help fulfill the goal of this current project. Only one of these proposals was funded, a 2-year USDA-NRI project beginning in 2009 focusing on the three major genes involved in Rosaceae tree fruit texture. In addition to a postdoc who will concentrate on apple, the PhD student mentioned above will focus on defining the role of these genes for practical application in the sweet cherry industry.

Objective 1: Technical infrastructure

- Objective in final stage of completion in October 2008.
- A method for high-throughput DNA extraction was tested for sweet cherry and is in the final stages of optimization. This method has a simple tissue sampling procedure, low start-up equipment costs, compatibility with our preferred high-throughput genotyping method, and is the same method to be used for the apple breeding program.
- A method for high-throughput genotyping was tested for sweet cherry and is in the final stages of optimization. We are currently seeking to purchase an ABI 3730 to service the PNW tree fruit breeding programs in collaboration with the USDA Small Grains Genotyping Laboratory on the WSU Pullman campus.

Objective 2: Phenotyping for flavor and texture

- Objective achieved for some germplasm, but spring freezes ruined the opportunity to obtain fruit quality data in Prosser, and reduced number of fruiting trees in Michigan.
- At Michigan State University (MSU), flavor phenotypic data (SSC, astringency, and GC measurement of major sugars and acids) were collected in the 2008 season for a key experimental population, NYxEF.
- Locating the genomic regions controlling these traits ("QTL analysis") will be performed in Nov-Dec 2008. Genetic markers are expected to be developed for flavor components, for utility in the PNWSCBP via marker-assisted selection (MAS) of seedlings and for describing the genetic predisposition of potential breeding parents and current cultivars of the PNW sweet cherry industry.
- Bird netting was used to cover the experimental orchard at MSU, ensuring sufficient fruit was available for multiple harvesting. Bird netting is recommended as a component of the Best Management Practices for the PNWSCBP.
- The 2008 spring freeze in Prosser destroyed our opportunity to collect texture and flavor phenotypic data on our pedigree-linked set of ~40 cultivars and selections growing at IAREC. We hope to gather this data in the 2009 season on an expanded set of cultivars, selections, and seedlings.
- An allied project conducted in 2008 by co-PI Olmstead and collaborator Dave Rudell, funded by USDA Prunus CGC funds, obtained interesting data on metabolic profiles (including sugars, acids, and aroma volatiles) on cherry germplasm from the Davis Repository and many parent cultivars used to date in the PNWSCBP. These results will feed into the PNWSCBP by identifying new sources of fruit flavor variation, developing protocols for flavor measurement, and enabling us to dissect the individual genetic components conditioning cherry fruit flavor.

Objective 3: Marker validation

- Objective achieved to the extent of known available markers. Collaborations and other projects identified provide an excellent source of new markers to be used in the PNWSCBP.
- Traits of highest priority for genetic testing in the PNWSCBP are: reduced tree juvenile period, self-fertility/cross-compatibility, fruit size, fruit firmness, sweetness, acidity, and rain cracking resistance. Recommendations for these traits are to develop and implement MAS as soon as possible. Of these, only one already has an available genetic test: self-fertility/cross-compatibility, previously developed for cherry in the programs of co-PI Iezzoni and researchers around the world. For the other traits, genetically variable plant material is available within the PNWSCBP for developing genetic markers, although improved phenotyping methods are required for each trait.
- The reliable "S-allele genotyping" for self-fertility/cross-compatibility was chosen as our baseline genetic test to ensure the effectiveness of our technical infrastructure establishment. We are currently verifying or determining S-allele genotypes of PNWSCBP parents.
- Chloroplast markers are being used to describe or verify maternal lineages for parents and selections of the PNWSCBP. Chloroplast markers will allow us to verify parentage, genetically group cultivars, and detect and monitor novel sources of germplasm used in breeding.
- Fruit size genetic markers are being developed within the federal- and WTFRC-funded program of co-PI Iezzoni, to be available for use on the PNWSCBP by the end of 2008.
- Other marker opportunities are arising from ongoing projects of the PIs and collaborators, with funding from various other sources. As marker-trait associations are discovered for high-priority traits, markers will be tested for validity and utility in the PNWSCBP.

- The FlexQTL software for validating marker-trait associations with the Pedigree Based Analysis approach was obtained from collaborator Marco Bink of Plant Research International in the Netherlands.

Objective 4: Breeding program database

- Objective not yet achieved – interrupted by start of a new breeder for the PNWSCBP, inability to secure access to an existing European-developed database package, and investment in developing a proposal to establish a US-wide common Breeders' Information Management System for Rosaceae ("RosBREED" proposal). We have pursued alternative options in the meantime for sweet cherry, with excellent progress and prospects.
- Working closely with the new breeder for the PNWSCBP, Nnadozie Oraguzie, we are creating a database that describes the pedigrees, performance, and genotypes of parents used in the breeding program. A Microsoft Excel-based database template was developed and is being filled with available data. This database template is compatible with Pedigree Based Analysis software.
- A genotyping database is being developed, designed to help efficiently process the genotyping of seedlings for the PNW tree fruit breeding programs.
- A breeders' Decision Support spreadsheet tool for MAS was developed in our program that determines the potential savings to be achieved by replacing phenotypic selection with marker selection, and determines the optimum stage for genotyping.

Objective 5: Allele mining in germplasm collections

- Objective half achieved thus far: US collections visited, but not yet genotyped with available high-priority markers and compared to PNWSCBP parents. To be completed later in 2008.
- Visits to the Davis Repository in California by PI Peace (funding from this project) and co-PI Olmstead (funding from the Prunus CGC project) allowed review of sweet cherry germplasm held in the Davis collection, California. A visit by Peace and Olmstead to Michigan identified further potential sweet cherry individuals that could be used as parents from 2009 onward.
- Metabolite and aroma profiling of sweet cherry germplasm held in the Davis collection was funded through the Prunus CGC. All available cherries were sampled in June 2008, shipped to WA and processed in Dave Rudell's laboratory at USDA-TFRL in Wenatchee. Relative concentrations of primary and secondary metabolites have been analyzed and aroma profiling is underway.

RESULTS AND DISCUSSION

Objective 1: Technical infrastructure

A method for high-throughput DNA extraction, which we call the silica bead method (SBM), was tested for sweet cherry and is in the final stages of optimization. SBM was our method of choice for the apple breeding program, compared to two other high-throughput methods and presented at the 4th International Rosaceae Genomics Conference. We prefer SBM for the PNW sweet cherry breeding program because it requires the simplest tissue sampling procedure, has low start-up equipment costs, is compatible with our preferred high-throughput genotyping method, and is the same method to be used for the apple breeding program. The most common high-throughput DNA extraction method used in other programs is the metallic bead method (MBM), but MBM requires sampled tissue to be kept cold and moist, and freeze-dried as soon as possible – a complication that is not compatible with routine breeding operations. The ultra-high-throughput extraction provided by the Theonyx automated system (TAS) developed at HortResearch in New Zealand was also tested (for apple). This method is perhaps too efficient for our needs, with robotics replacing the need for technician time, but with relatively high supplies costs, equipment costs, and it requires prior freeze-drying of tissue

samples. A poster presenting our high-throughput DNA extraction method trialing was presented at the 4th International Rosaceae Genomics Conference in Chile (March 2008), which generated much interest.

A method for high-throughput genotyping, using DNA Analyzing equipment from Applied Biosystems (ABI), was tested for sweet cherry and is in the final stages of optimization. The ABI genotyping system was compared to a dozen other possible approaches for per-sample costs, number of samples that can be tested per week, versatility to handle the types of genetic markers that are useful for the PNW sweet cherry breeding program, technical skill required, and availability and affordability of equipment. We are currently seeking to purchase an ABI 3730 to service the PNW tree fruit breeding programs (and associated research programs) in collaboration with the USDA Small Grains Genotyping Laboratory on the WSU Pullman campus.

Objective 2: Phenotyping for flavor and texture

At Michigan State University (MSU), flavor phenotypic data were collected in the 2008 season for a key experimental population, NYxEF. This cross was originally created for genetic analysis of fruit quality traits, as it represents a cross between the small-fruited astringent wild forest cherry 'NY54' and the large-fruited elite cultivar 'Emperor Francis'. Usually three, and up to five harvests (2-4 days apart) were conducted for each tree to get a handle on maturity changes on fruit quality attributes in this population. Once fully analyzed, these multiple harvest data are expected to provide valuable information regarding changes in fruit quality traits as maturity progresses, enabling development of optimized sampling protocols to more accurately detect genetic potential.

In the orchard, astringency was scored on a 0-2 scale. There was insufficient sensory variation detected for sweetness and acidity to allow scoring on a similar scale as originally planned. In the lab, SSC was measured for all fruit. Juice samples were collected for Gas Chromatography (GC) analysis to describe the concentrations of the predominant sugars and acids in cherry fruit, which will be analyzed in the final months of 2008. In an associated project of co-PI Iezzoni, data were also collected for other fruit quality traits: fruit size (weight and diameter), skin color (ground and blush), flesh color, and maturity date. Joint analysis will allow us to detect physiological and genetic relationships between these traits.

From sensory analysis (tasting the fruit), 38 trees had an astringency score of 0 (no astringency), 28 had a score of 1 (mild astringency), and 24 had a score of 2 (highly astringent). Results were consistent with high astringency being inherited in a recessive manner, indicating that astringency in wild germplasm should not significantly hamper its use in cultivar development. Astringency scores did not change for a tree from harvest to harvest. Therefore, astringency was very stable and not affected by fruit maturity – improving our chances to identify controlling gene regions.

SSC ranged from 13 to 22 ºBrix, averaging 19. Trees with highest astringency fruit tended to have a lower SSC (Figure 1), indicating a possible genetic correlation that a breeder could exploit. From other correlation analysis, we identified a significant positive correlation between SSC and fresh weight ($R=0.43$), a negative correlation with harvest date as described by growing degree days ($R=-0.37$), and no correlation with any measure of fruit color (skin blush, skin ground, or flesh). Trees in this population with smaller fruit tended to have highest astringency ($p<0.001$), while later maturing trees also tended to have more astringent fruit ($p=0.027$).

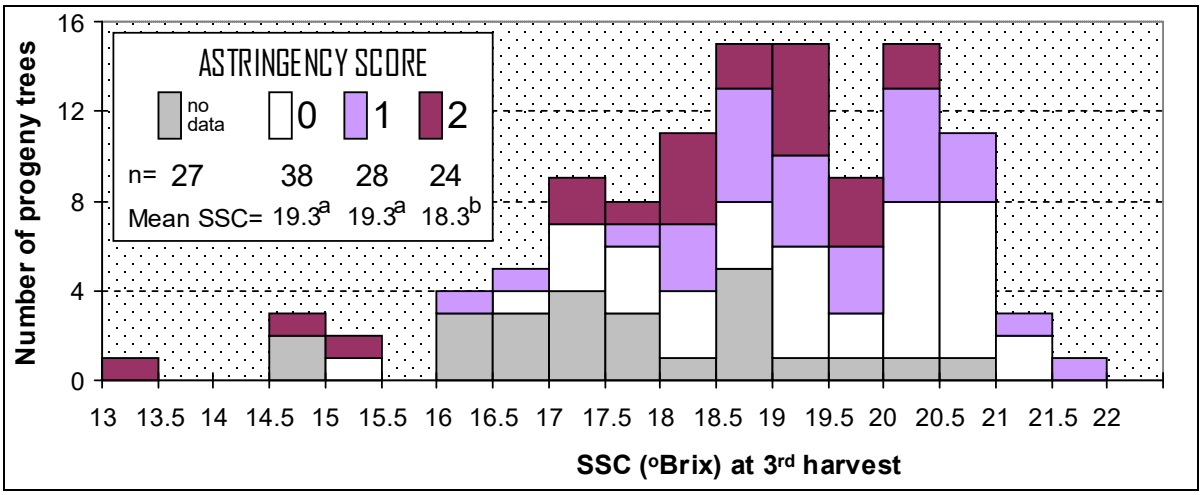

Figure 1: Genetic variation observed in flavor components of SSC and astringency for an experimental population, NYxEF. In the legend, numbers with different letters in superscript are significantly different ($p<0.05$).

After additional statistical analyses of the NYxEF phenotypic data later in 2008, we will develop recommendations for the PNWSCBP to genetically improve sweetness and TA while avoiding astringency. We will determine whether SSC is suitable enough as a quick laboratory screening of the sweetness trait rather than the detailed information that the GC acquires (although with much greater effort and expense). Already we have found that field tasting for sweetness is not sufficient, as this was deemed to not be scorable in 2008 on these trees. For breeding population in the PNWSCBP, field tasting may be worthwhile to assess genetic expression of sweetness only in crosses that vary widely for sweetness.

Locating the genomic regions controlling these traits ("QTL analysis") will be performed in Nov-Dec 2008, taking advantage of the powerful genetic resource previously developed by co-PIs Iezzoni and Olmstead and collaborator Audrey Sebolt: a genetic map of the genome of sweet cherry based on this NYxEF population. From this QTL analysis, we expect to develop genetic markers for flavor components that can enter the practical MAS pipeline and be tested for utility in the PNWSCBP seedling selection and for describing the genetic predisposition of potential breeding parents and current cultivars of the PNW sweet cherry industry.

A spring freeze in Michigan left about half the trees of the experimental population fruitless. Bird netting was used to cover the experimental orchard, ensuring a large crop set on those trees that had received effective pollination. Sufficient fruit was then available for multiple harvesting, and a solid data set was obtained that we can mine for useful information for many months to come. Bird netting is recommended as a component of the Best Management Practices for the PNWSCBP.

The 2008 spring freeze in Prosser destroyed our opportunity to collect texture and flavor phenotypic data on the "Pedigree Genotyping set" of approximately 40 cultivars and selections growing at IAREC. We hope to gather this data in the 2009 season on an expanded set of cultivars, selections, and seedlings, chosen for obtainment maximum genetic knowledge using the Pedigree Based Analysis approach.

An allied project conducted in 2008 by co-PI Olmstead and collaborator Dave Rudell, funded by USDA Prunus CGC funds, obtained interesting data on metabolic profiles (including sugars, acids,

and aroma volatiles) on cherry germplasm from the Davis Repository and many parent cultivars used to date in the PNWSCBP. These results will feed into the PNWSCBP by identifying new sources of fruit flavor variation, developing protocols for flavor measurement, and enabling us to dissect the individual genetic components conditioning cherry fruit flavor.

Objective 3: Marker validation
In collaboration with Dr. Fred Bliss and his WTFRC-funded projects in 2007 and 2008, we determined that the traits of highest priority for genetic testing in the PNWSCBP are reduced tree juvenile period, self-fertility/cross-compatibility, fruit size, fruit firmness, sweetness, acidity, and rain cracking resistance, for which the recommendations are to develop and implement MAS as soon as possible. Of these, only one already has an available genetic test: self-fertility/cross-compatibility, previously developed for cherry in the programs of co-PI Iezzoni and other researchers around the world. For the other traits, genetically variable plant material is available within the PNWSCBP for developing genetic markers, although improved phenotyping methods are required for each trait.

The reliable "S-allele genotyping" for self-fertility/cross-compatibility was chosen as our baseline genetic test to ensure the effectiveness of our technical infrastructure establishment. For most parents used thus far in the PNWSCBP, S-allele scores are available in published reports. We are currently verifying these genotypes for all PNWSCBP parents, and collecting new S-allele data for those parents and selections with unknown S-alleles. The S-allele genetic test was adopted as the primary test for verifying parentage of seedlings in the breeding program, which we have recently begun. We are using the S-allele genetic test to verify the ability of the ABI high-throughput genotyping system to efficiently provide genetic data for thousands of breeding program seedlings.

Sweet cherry chloroplast markers are also being used to describe or verify maternal lineages for parents and selections of the PNWSCBP. Published reports on such markers describe only three maternal lineages within cultivated sweet cherry, although another 13 were reported in wild populations of Europe. Chloroplast markers will allow us to verify parentage, genetically group cultivars, and detect and monitor novel sources of germplasm used in breeding. Thus far we have replicated the reported genetic tests, determined which of the three maternal lineages each member of the PNWSCBP parent cultivar belongs to, and verified that they will be an effective tool for genetic descriptions of cherry material.

Fruit size genetic markers are being developed within the federal- and WTFRC-funded program of co-PI Iezzoni. In that program, previously identified fruit size markers in the NYxEF population are currently being validated in sweet cherry germplasm that fully covers the breadth of the PNWSCBP. We expect that these markers will be available for use in the PNWSCBP by the end of 2008, at which time we will test them on parents and 2004 cross seedlings using the high-throughput ABI genotyping system.

Other marker opportunities are arising from ongoing projects of the PIs and collaborators, with funding from various other sources. We are conducting the basic discovery research toward identifying marker-trait associations for sweet cherry for the other high-priority traits. As such associations are discovered, markers will be tested for validity and utility in the PNWSCBP. Currently we are testing cherry for genes believed to control fruit texture, arising from a federally funded project by PI Peace. Phenotypic data with which to compare promising markers to identify and validate marker-trait associations is still lacking in most cases, and we are pursuing many angles to obtain this necessary performance data.

The FlexQTL software for validating marker-trait associations with the Pedigree Based Analysis approach was recently obtained from collaborator Dr. Marco Bink of Plant Research International in

the Netherlands. The free PediMap software was also obtained from another Dutch colleague, Dr. Eric van de Weg. PediMap was used to visualize the pedigree relationships among PNWSCBP parent cultivars, which was also overlain with information on maternal lineages (Figure 2).

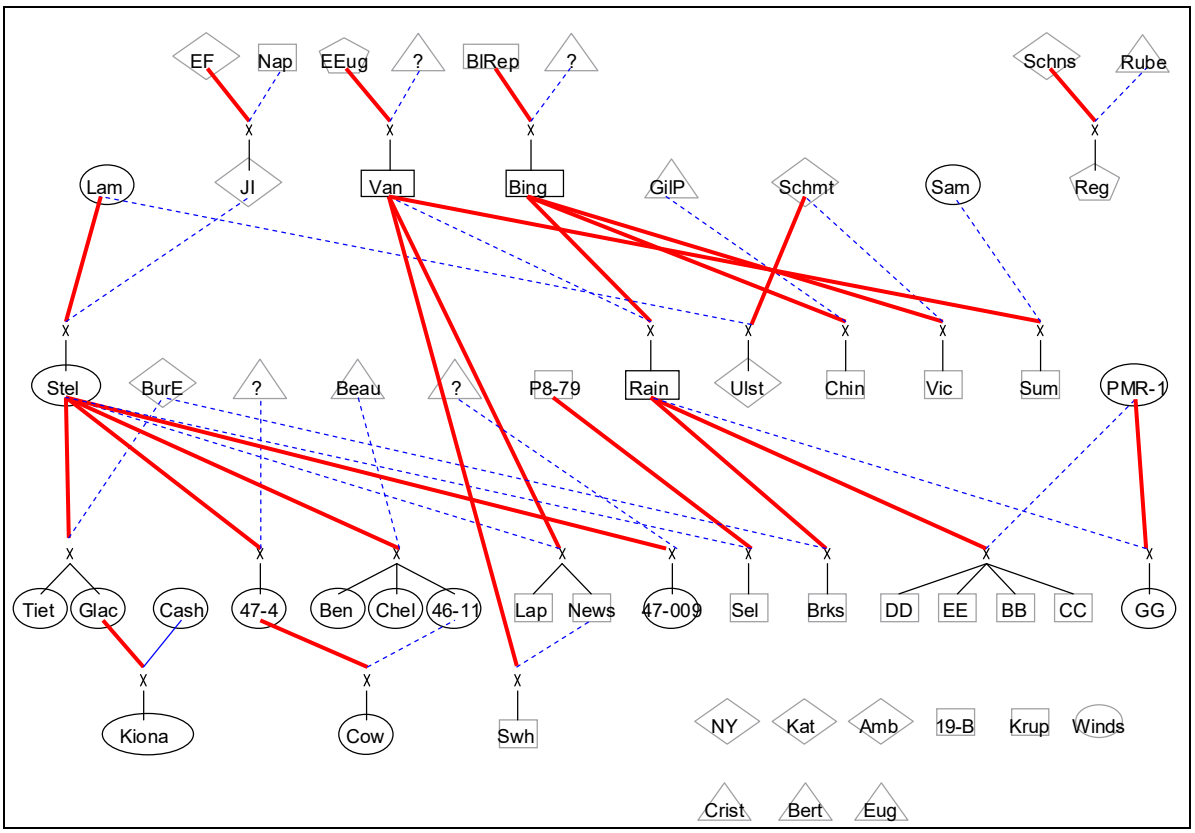

Figure 2: Pedigree structure of parent cultivars and selections of the PNWSCBP. Cultivar names are abbreviated. Solid lines represent maternal parentage, dotted lines pollen parentage. Cultivars in diamonds belong to the maternal lineage known as "H3", rectangles are H4, and ovals are H5. Triangles are unknown at present. Two cultivars (Regina and Empress Eugenie, in pentagrams) were detected with a maternal lineage different to reported information in the literature. Nine cultivars (lower right) have no known pedigree relationships to any other cultivars in the program.

Objective 4: Breeding program database

Dr. Nnadozie Oraguzie was hired as the new breeder for the PNWSCBP in May 2008. This new beginning, combined with a recently established breeding program for which the first limited fruit quality data was obtained in 2008, provides an opportunity to install a breeding database that is able to efficiently take advantage of genomic advances. We believe that this database should be based on the Pedigree Based Analysis (PBA) approach. The breeder requires knowledge about previously used and potential future parents to determine breeding value, including their pedigrees, their performance for traits of industry value, and their genotypes for traits with available predictive genetic markers. The supporting researcher requires the same knowledge to use PBA to validate that promising genetic markers are relevant for the breeding program. Therefore the breeder and supporting researchers can use the same database format for breeding program germplasm.

A fully functional breeding program database is not yet established for the PNWSCBP, but in the meantime we have a suitable solution for collating appropriate data and identifying knowledge gaps. We are working closely with Dr. Oraguzie, to create a database that describes the pedigrees, performance, and genotypes of parents used in the breeding program. A Microsoft Excel-based database template was developed and is being filled with available data with the help of our PhD student. This database template is compatible with Pedigree Based Analysis software.

We are still pursuing a database format known as AppleBreed developed for the European HiDRAS project. To date the developers are unable to commercially release the software, but we have made personal connections and hope to take advantage of the time and expertise put into the development of AppleBreed.

In collaboration with Pullman-based USDA-ARS scientist and high-throughput genotyper for regional wheat/barley breeding programs, Dr. Deven See, a computer science student has recently been hired to develop a genotyping database. This database will be designed to help efficiently process the genotyping of seedlings for the PNW tree fruit breeding programs. As the thousands of seedlings each year are sampled, DNA-extracted, and genotyped, the information will be logged and sorted, and resulting genotypic information will be provided in a breeder-friendly format to recommend which seedlings to cull and which to keep prior to field planting. We expect this genotyping database to be ready by mid-2009.

As part of the decision support package we are establishing for genomics-assisted breeding, an Excel spreadsheet has been developed for calculating the cost efficiency of MAS using available markers. Using input parameters that describe aspects of a breeding program – such as the stages involved, costs of each routine operation with traditional phenotypic selection (i.e. without markers), and proportions of seedlings expected to be maintained through each stage – we can determine the potential savings of replacing phenotypic selection with marker selection and the optimum stage for genotyping. So far we have used this for the Washington apple breeding program, and identified that if only a single genetic marker is available that detects 50% of the seedlings as undesirable, approximately 40% of the total cost after eight years (from crossing to deciding on which seedlings to advance to replicated trials) can be saved by using that marker. Also, we unexpectedly discovered that the optimum stage for genotyping is not always as early as possible. With the availability of more good markers comes greater savings and efficiency, which could be reinvested into larger seedling numbers for genotyping. We expect similar predicted outcomes for the PNWSCBP, for which S-allele genotyping is already available. Parameters for the PNWSCBP need to be determined and used in this MAS Decision Support spreadsheet tool.

We hope to leverage efforts made on the part of the larger Rosaceae community for database management tools. As part of the recent proposal, "RosBREED: Enabling Marker Assisted Breeding in Rosaceae" submitted to the USDA-CSREES Specialty Crop Resarch Initiative, we proposed a U.S.-wide common Breeders' Information Management System (BIMS). The BIMS concept builds upon our efforts to develop database and decision support packages for PNW apple and cherry breeding programs.

Objective 5: Allele mining in germplasm collections

Visits to the Davis Repository in California by PI Peace (funding from this project) and co-PI Olmstead (funding from the Prunus CGC project) allowed review of sweet cherry germplasm held in the USDA's National Plant Germplasm System. A visit by Peace and Olmstead to MSU's Clarksville Horticultural Experiment Station in Clarksville, MI, and Northwest Michigan Horticulture Research Station in Traverse City, MI, identified further potential sweet cherry individuals that could be used as parents from 2009 onward. DNA samples were obtained for some of this germplasm. Samples

were also obtained for related species in sweet cherry's readily crossable "primary genepool", including *P. fruticosa*, *P. canescens*, and tart cherry (*P. cerasus*). In 2009, we plan to test available genetic markers on this wider germplasm using existing/remaining funds to identify further sources of genetic diversity for high-priority traits, targeting the genes underlying the traits rather than relying solely on phenotypic assessment. We also plan a germplasm acquisition trip to Europe in 2009 or 2010 to gain access to further diversity, guided by genetic analyses.

Additional allele mining of flavor components was possible through a Prunus CGC project (PIs Olmstead and Rudell) to profile primary and secondary metabolites and aromas from the USDA clonal cherry germplasm collection. Samples at harvest maturity were collected and shipped to WA for analysis. Standard fruit quality measurements were made (size, color, firmness, total soluble solids, and titratable acidity) prior to gas chromatography-mass spectrometry analysis at the USDA-TFRL. Cherry accessions in the collection will be grouped according to sugar and acid levels, and novel and/or unfavorable flavor and aroma profiles will be identified. The information will be used to identify potential parents for use directly in crosses or to increase the level of diversity for flavor traits in the PNWSCBP.

EXECUTIVE SUMMARY

The main goal of this project was to assess opportunities for genetic marker assistance in the Pacific Northwest sweet cherry breeding program (PNWSCBP) and develop the technical infrastructure for ready translation of genomics information and tools into practical breeding benefit.

Specific objectives were to:

1. Develop protocols for high-throughput genetic screening in the PNWSCBP.
2. Conduct flavor and texture phenotypic analysis of key breeding germplasm.
3. Collate, validate, and package available markers of value to the PNWSCBP through coordination with other sweet cherry projects on gene/marker identification.
4. Establish a database for the PNWSCBP that meets traditional breeding needs and is compatible with ongoing genomic analyses.
5. Assess internationally-renowned cherry germplasm collections for genetic diversity of potential value to the PNW.

A summary of significant accomplishments from 2008 includes:

- High-throughput DNA extraction and genotyping methods were tested and are being optimized. These methods are essential for routine application of MAB on the thousands of seedlings in the PNWSCBP.
- Flavor phenotypic data were collected at Michigan State University (SSC, astringency, GC measurement of primary sugars and acids) for the NY×EF experimental population. Development of genetic markers for these traits is currently underway.
- The reliable "S-allele genotyping" for self-fertility/cross-compatibility was chosen as our baseline genetic test to ensure the effectiveness of our technical infrastructure establishment. Fruit size genetic markers are being developed within the federal- and WTFRC-funded program of co-PI Iezzoni, to be available for use on the PNWSCBP by the end of 2008. As marker-trait associations are discovered for other high-priority traits (for example, flavor phenotyping within this project) markers will be tested for validity and utility in the PNWSCBP.
- Chloroplast markers are being used to describe or verify maternal lineages for parents and selections of the PNWSCBP. Chloroplast markers will allow us to verify parentage, genetically group cultivars, and detect and monitor novel sources of germplasm used in breeding.
- The FlexQTL software for validating marker-trait associations with the Pedigree Based Analysis approach was obtained from collaborator Marco Bink of Plant Research International in the Netherlands.
- Working closely with the new breeder for the PNWSCBP, we are creating a database that describes the pedigrees, performance, and genotypes of parents used in the breeding program. This database template is compatible with Pedigree Based Analysis software. Also included is a breeders' Decision Support spreadsheet tool for MAS was developed in our program that determines the potential savings to be achieved with marker selection, and determines the optimum stage for genotyping.
- Visits to the Davis Repository in California by PI Peace (funding from this project) and co-PI Olmstead (funding from the Prunus CGC project) allowed review of sweet cherry germplasm held in the Davis collection, California. A visit by Peace and Olmstead to Michigan identified further potential sweet cherry individuals that could be used as parents from 2009 onward.
- This project attracted and leveraged funding from the Department of Horticulture and Landscape Architecture for a PhD student Sanchita Haldar, under the direct supervision of PI Peace.

Completion of this project has identified and put into practice several baseline steps for utilizing MAS in the PNWSCBP. Future efforts should focus on identifying useful germplasm variants for priority traits and validating the utility of existing and new markers in plant material from the PNWSCBP.

FINAL PROJECT REPORT

Project Title: Factors affecting meristem fate in Rosaceae

PI:	Amit Dhingra	**Co-PI(2):**	Matthew Whiting
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Cooperators:

Total project funding request: **Year 1:** 24,403 **Year 2:** 22,460 **Year 3**: $0

Other funding Sources

Agency Name: **WSU**
Amount awarded: **$ 50,000**
Notes: For genomics related equipment directly relevant to this project.
Budget data provided in "Other funding sources" is for informational purposes only, and for WTFRC to understand the scope of the project. These estimated costs are not presented as formal cost-sharing and therefore do not constitute a cost-share obligations on the part of WSU. Moreover, there is no requirement for WSU to document this other support of project as part of any cost-share or matching obligation.

Total Project Funding: 46,863
Budget History - Pullman

Item	2007	2008	
Salaries			
Benefits			
Wages	2,500	3,000	
Benefits	288	345	
Equipment			
Supplies	12,500	10,000	
Travel	2,000	2,000	
Miscellaneous[1]	5,000	5,000	
Total	22,288	20,345	

Footnotes: [1]Miscellaneous – funds requested for sequencing

Budget History - Prosser

Item	2007	2008	
Salaries			
Benefits			
Wages	1,000	1,000	
Benefits	115	115	
Travel	1,000	1,000	
Miscellaneous			
Total	2,115	2,115	

NOTE: This proposal was originally funded by the Technology Commission and later transferred to Cherry Commission. Therefore the timing for the Final Report on this proposal has arrived a few months in advance.

OBJECTIVES

Flower numbers in cherry determine overall fruit quality. Rootstocks have a major impact in determining the fate of the meristem as it transitions from a vegetative to a reproductive stage. This project was aimed at identifying the gene-level contribution of rootstocks towards the transition and consequently the flower number. For sweet cherry production, this is one of the important traits that controls flower set and understanding of this phenomenon is expected to inform us regarding thinning practices to obtain best quality fruit. Starting early May 2007 (period of floral bud initiation), entire 1-year-old spurs (fruiting wood in subsequent season) were collected from same-age trees for analyses. The samples were collected until December 2007 when the buds reach dormancy based based on the previous research carried out by Matt Whiting.

Briefly, the specific objectives and the progress made are listed below:

1. Generation of cDNA or EST (Expressed sequenced tag) libraries from floral tissues derived from specific scion and rootstock combinations.

2. The ESTs will be sequenced and made available as a community resource.

3. Cloning of the flower meristem identity genes from the EST libraries derived from different genotypes.

4. Quantification of gene expression levels for these genes at transcriptional level using real time-PCR and/or northern blot analysis.

(Please see glossary of technical terms used in this report)

SIGNIFICANT FINDINGS

1. Type of rootstock (Gisela or Mazzard) impacts the expression of flowering-related genes in the bud spurs of the same scion (e.g. Bing). What this means is that the DNA level variation in these genes can be potentially utilized for developing molecular markers to regulate crop load in new varieties.

2. Bud spurs are an important site where the rootstock effects manifest themselves. Our study has the potential of identifying additional genetic factors that may impact other flower or fruit related traits.

RESULTS AND DISCUSSION

1. Generation of cDNA (complementary DNA) libraries from floral tissues derived from the scion and rootstock combinations

RNA derived from DNA is used for developing cDNA libraries. We have established an economical and streamlined RNA extraction method from spur tissues, which is being prepared for a peer-reviewed publication. RNA yields are generally very low owing to the nature of the tissue. There were no previously published protocols for RNA extraction from this type of tissue. We have successfully prepared 8 cDNA pools from tissues collected in May and September 2007 representing

Bing/Mazzard, Bing/Gisela, Rainier/Mazzard and Rainier/Gisela. Capturing of cDNAs in a library is underway. This resource can be utilized in the breeding program activities for identifying important genes related to other desirable traits.

2. The ESTs will be sequenced and made available as a community resource

ESTs are unique short sequenced parts of cDNA. Generally the way EST libraries are made full length transcripts are not cloned. This generates a computational problem. No wonder there are 300,000 ESTs for apple in the database but only 30-40,000 genes are predicted to be present in the apple genome. Since the RNA isolation has been standardized, we have successfully generated cDNA pools and are now employing next generation sequencing technologies to sequence the cDNAs en masse. All the data analysis will be performed in the laboratory and will be available in a searchable database in four months. As in case of objective 1, the sequences derived from this objective will serve the needs of the breeding program to develop molecular markers for desirable traits.

3. Cloning of the flower meristem identity genes from the EST libraries derived from different genotypes.

Several genes out of the ones involved in transition of meristematic tissue from vegetative to reproductive stage namely, Apetala1 (AP1), Flowering Locus T (FT), Constans (CO), Leafy (LFY), Terminal Flower 1 (TFL1), Cauliflower (CAL) and Frigida (FRI) were amplified. The sequencing of these DNA fragments is currently underway. Comparisons of the sequences with those from Apple and Peach will provide insight into probable gene function. Once the nucleotide sequence is deciphered, computer based comparisons will enable identification of any nucleotide differences in these genes. This is an important piece of information required for the breeding activities.

4. Quantification of gene expression levels for these genes at transcriptional level using real time-PCR and/or northern blot analysis.

This work is currently underway. We have been able to use RNA to amplify the flowering-related genes in sweet cherry. Now we have to fine-tune the method for doing quantitative analysis. All the samples and RNA are available. We will be testing the relative expression of flowering-related genes in different scion/rootstock combinations. Based on these differences, nucleotide-level variations will be identified as in case of objective 3 and information provided to the sweet cherry breeder, Dr. Nnadozie Oraguzie for developing molecular markers for this useful trait.

ADDITIONAL DEVELOPMENTS

Leveraged Funding:
1. Graduate Student Support: This project is being carried out by Tyson Koepke who is a graduate student in the Dhingra Lab. Tyson is pursuing his graduate studies under the Molecular Plant Sciences Program that has been ranked 2nd in the nation recently. This proposal has been accepted for NIH Protein Biotechnology Graduate Training Program that provides Tyson 2 years of complete support for his Ph.D. work. That amounts to $ 70,000 for two years.

2. Equipment Grants: We have been able to leverage another $ 650,000 in equipment funds from the college and the department to enable genomics-related experiments that will directly benefit this project. Equipment includes Genome Sequencer, a high sensitivity spectrophotometer to accurately measure RNA and DNA, a freezer mill to grind hard tissue like the bud spurs and Bioanalyzer for RNA quality control.

PRESENTATIONS AND PUBLICATIONS

1. T Koepke, MD Whiting and A Dhingra. Discovery of Genomic Factors Regulating Flower Density in Sweet Cherry. Washington State Horticultural Association 103rd[d] Annual meeting, Wenatchee, WA. December 2007

2. T Koepke, MD Whiting and A Dhingra. Factors affecting meristem fate in Rosaceae. Oral presentation at the Annual Molecular Plant Science Retreat. Pullman, WA February 2008

3. T Koepke, MD Whiting and A Dhingra. Identification of Genomic Factors Regulating Flower Density in Sweet Cherry. 4th Rosaceae Genomics Conference, Pucon, Chile. March 2008

4. A Dhingra, MD Whiting and T Koepke. Using genomics tools to understand rootstock-induced floral bud initiation in Rosaceae. Oral presentation at the 9th International Symposium on Integrating Canopy, Rootstock and Environmental Physiology in Orchard Systems. Geneva, NY. August 2008. To be published in Acta Hort proceedings.

5. T Koepke, MD Whiting and A Dhingra: Streamlined protocol for isolation of RNA from woody perennial tissues for transcriptome analysis. Under preparation for Plant Methods (peer-reviewed)

EXECUTIVE SUMMARY AND FUTURE DIRECTIONS

This proposal is very unique as it attempts to understand the impact of rootstock on specific physical organs in the scion. The composite nature of the sweet cherry is biologically unique compared to other model systems where impact of roots has been studied on flowering. The findings from this project will have a major impact on our understanding of this phenomenon in apples and pears as well. While we are looking at a specific subset of genes involved in meristem transition, our approach is poised to identify all other genes that may play a role in other traits like fruit set, pollination or even fruit quality. Since the inception of this project, the technological infrastructure in our lab for genomics research has become cutting edge. What that means is that instead of just looking at a few genes at a time, we now have the capacity to study the entire complement of genes that are being impacted by the rootstocks in the same scion. The genome sequencer has the capacity to read the frequency of every gene's occurrence and thus provide a quantitative value on the expression of each gene.

Thus far, in our experiments we have confirmed that expression of certain meristem identity genes in one scion is impacted by the type of rootstock they are grafted on. This preliminary dataset has formed the foundational data for a federal grant proposal that we will submit to USDA-NRI in February 2009. The proposal is already prepared and is the research topic for the graduate student Tyson Koepke working on this project. Funding will be requested to extend this work to identification of rootstock induced phenomenon and characterize nucleotide-level polymorphisms for molecular marker development.

This research has been presented at national and international forums and has been very well received. A preliminary part of the research is being encapsulated into a peer-reviewed publication and a conference proceeding paper.

FINAL PROJECT REPORT

Project Title: Chemical Genomics

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Cooperators:

Other funding Sources

Agency Name: None
Amount requested/awarded:
Notes:

Total Project Funding: 60,000

Budget History:

Item	Year 1: (Aug., 2007)	Year 2: (Aug, 2008)	Year 3:
Salaries			
Benefits			
Wages	8708.78	4,124.37	
Benefits	648.58	320.08	
Equipment			
Supplies	20,543.60	4,586.61	
Travel	463.85	147.74	
Miscellaneous	46.00	29.00	
Total	30410.81	9,207.80	+20,381.39

Note: We did not pay PI salary of $5,000 plus $1,700 each year. The remaining money will be used for subtraction cloning studies.

ORIGINAL OBJECTIVES:

In this collaborative project, we proposed to apply a chemical genomics approach to rosaceous crops, and help solve some of the problems facing the Washington tree fruit industry. One of the major issues is how to improve fruit size and fruit quality. It has been well documented that fruit development and ripening are regulated by plant hormones such as auxin, gibberellins, and ethylene. For sweet cherry (*Prunus avitum*), we will focus on the effect of gibberellic acid (GA) on fruit size and quality, as well as tree size.

The plant hormone gibberellin has long been known to modulate development throughout the plant life cycle. Mutants that are impaired in GA biosynthesis or response tend to have small dark green leaves and reduced stem length. Thus understanding the regulatory mechanisms of GA could help to produce dwarf crops. GA mutants are also often defective in seed germination and floral development, and are delayed in flowering time (Fleet and Sun, 2005). In cherry, GA application is currently used by growers worldwide for improving fruit quality and delaying maturity (Lenahan et al., 2006; Maib et al., 1996). Vigorous shoot growth in sweet cherry trees can also be controlled with gibberellin-biosynthesis inhibitors such as such as prohexadione-Ca (Manriquez et al., 2004).

Our specific objectives were:

1. To screen the available chemical libraries and identify the chemical compounds which affect the GA pathway,
2. To study the effect of selected chemicals on gene expression and identify the marker genes involved in fruit development, ripening, and tree size using subtraction cloning and microarray technologies,
3. To study the effect of the chemical compounds on fruit shelf life, and quality, as well as tree size.
4. To train Washington State students in the cutting-edge discipline of chemical genomics.

SIGNIFICANT FINDINGS

1. Screened a 100,000 chemical library using strawberry and Arabidopsis.

2. 252 and 165 chemicals have been isolated from Arabidopsis and strawberry screenings, respectively. Among them, 125 chemicals exhibit the similar effects on both Arabidopsis and strawberry.

3. Of 125 chemicals, 77 have inhibitory effects, and 48 have stimulatory effects.

4. Twenty-five chemicals were selected for large scale field test in Bing in Prosser, WA, 2007. These chemicals were chosen because they showed best effects on seed germinations in both Arabidopsis and strawberry.

5. Several chemicals were effective in controlling skin color, flesh color immediately after application.

6. These chemicals affected the buds per spur and flower numbers per bud in following season.

7. Six chemicals were further selected for large scale field test in Pullman, WA, 2008. Selection of these chemicals was based on their performance in the field test of year 2007. The chemicals affected the fruit size, and fruit color, which were consistent to the results in Prosser, WA, 2007.

8. In conclusion, we have identified a few very effective chemicals which control fruit color and flower numbers.

RESULTS & DISCUSSION

In last report, we indicated that 25 selected chemicals were used to spray in Prosser orchard on May 30, 2007. The normal spray with GA3 was used as control. Each chemical was sprayed on the cherries in a branch of one tree. The experiment was repeated twice in two different trees. The cherries were harvested on June 22. We further analyzed the cherry weight, skin color, flesh color, firmness and Brix.

As shown in Figure 1-4, the 25 compounds had a variety of impacts on the traits we measured as compared to control. The most obvious effects were the skin color and flesh color which are desirable traits for consumers, while they did not show significant changes on the fruit firmness.

In 2008, we selected 6 chemicals for a large scale field test. These chemicals were selected based on their performance (positive and negative effects) in 2007 test. Since the Prosser orchard had no many fruits because of the bad weather this spring, we did the field test this year in Tukey Orchard, Pullman, WA in July 2008. We also changed the sweet cherry variety from Bing to Rainier in order to observe the color effects clearly. Two independent trees were used for all treatments.

Figure 5 shows that six chemicals can be separated into two groups, negative group (No. 2, 3) and positive group (No. 1, 4, 5, 6) based on their effects on the fruit weight. They all increased fruit color as compared with GA control. As for fruit firmness, No. 4, 5, 6 had no significant difference as compared with GA control. Among six chemicals, No. 4 showed the best in all measurements. Figure 6 are the photos exhibiting the effects of No. 4 chemicals on fruit ripening. In the same tree, the fruits sprayed with No. 4 chemical were ripen a week to 10 days later than no spray fruits in the same tree. The fruit weight in sprayed fruits was significantly improved (~40% increase). It also had better effects on fruit weight, skin color than GA control. However, the fruit firmness was comparable with GA control.

In conclusion, we have identified a few powerful chemicals which affect sweet cherry fruit quality and flower numbers. The tests on different locations and different varieties in different years indicate that these chemicals are more effective than GA. These chemicals may also have the potential for other tree fruits such as apple and pear. We still have not done the subtraction cloning yet to find which genes are affected by these chemicals. We have saved money to do this. Hopefully, we can find the important genes controlling fruit quality and flower number. These genes will be very useful for marker assisted breeding, and as management tools for industry.

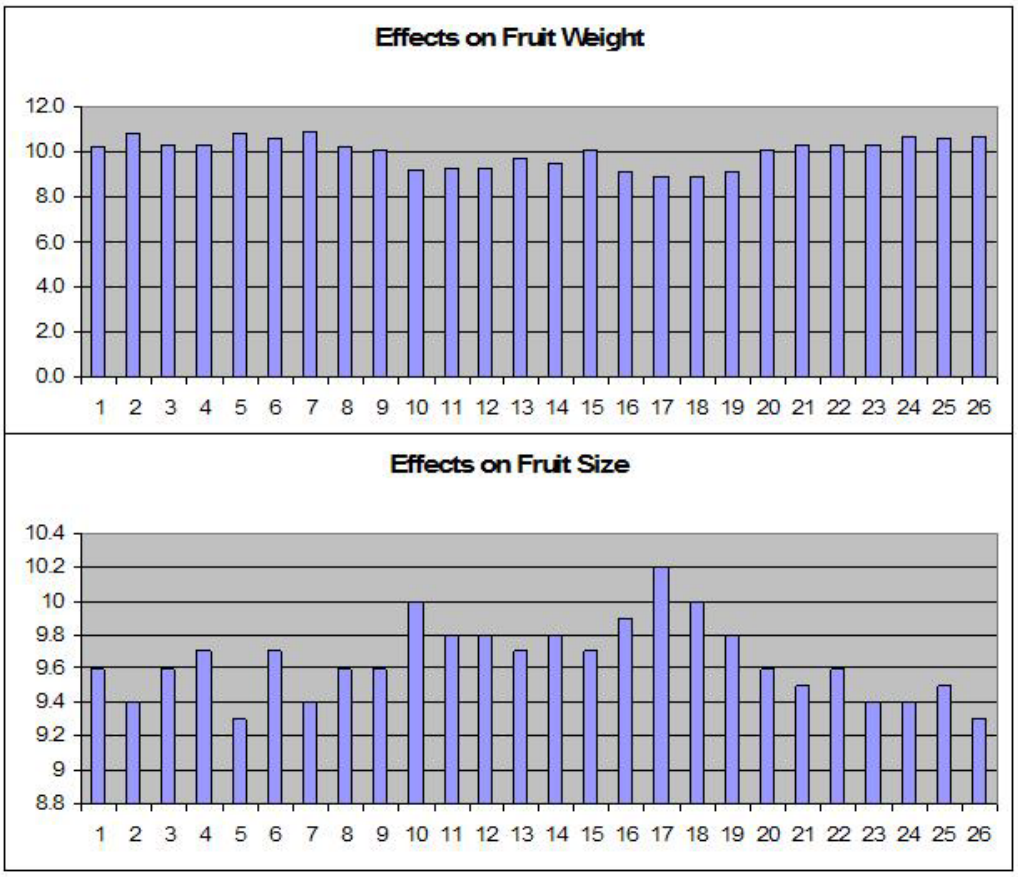

Figure 1. The effect of 25 selected compounds on the fruit weight and size. No. 26 represents the control which was treated with GA3. (Prosser, WA, 2007)

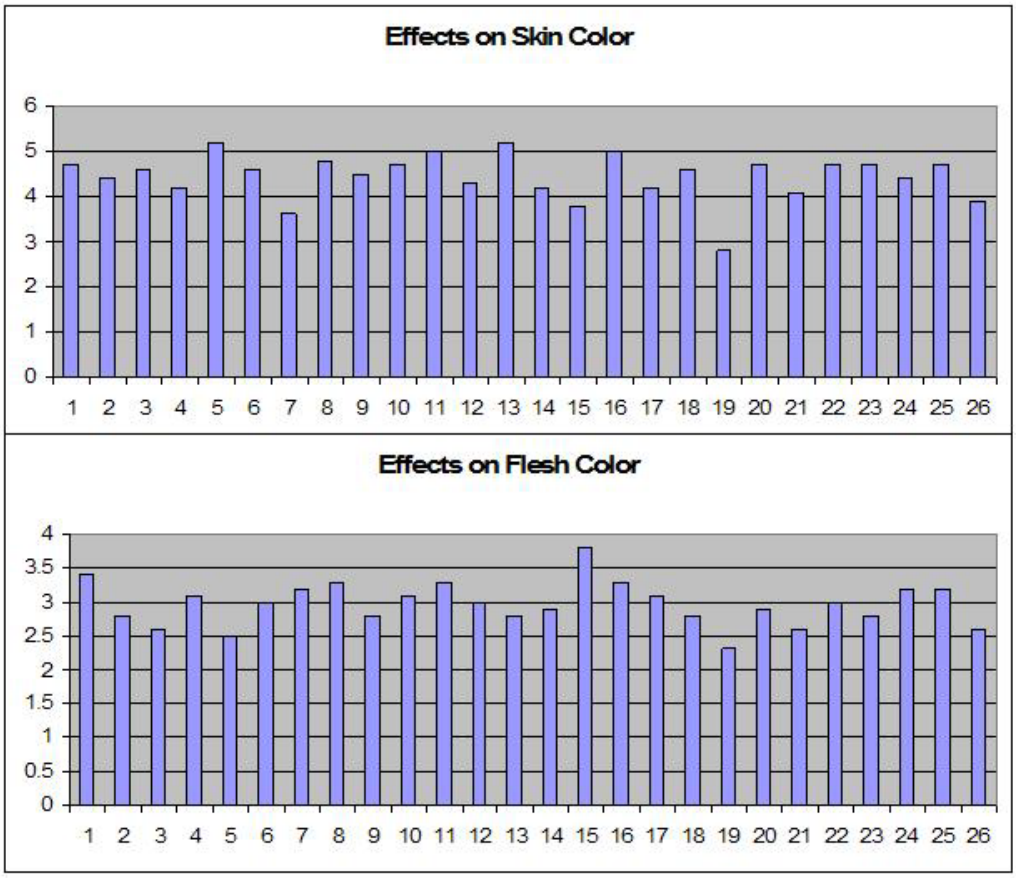

Figure 2. The effect of 25 selected compounds on skin color and flesh color. No. 26 represents the control which was treated with GA3. (Prosser, WA, 2007)

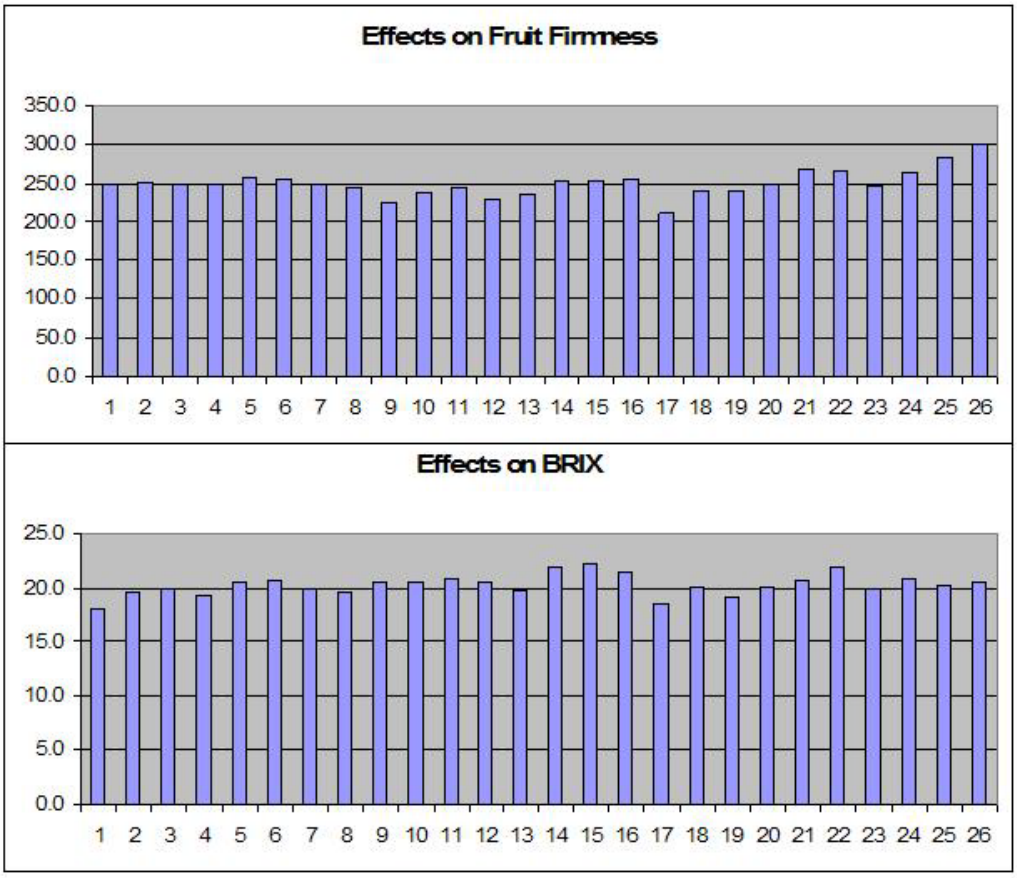

Figure 3. The effect of 25 selected compounds on fruit firmness and Brix. No. 26 represents the control which was treated with GA3. (Prosser, WA, 2007)

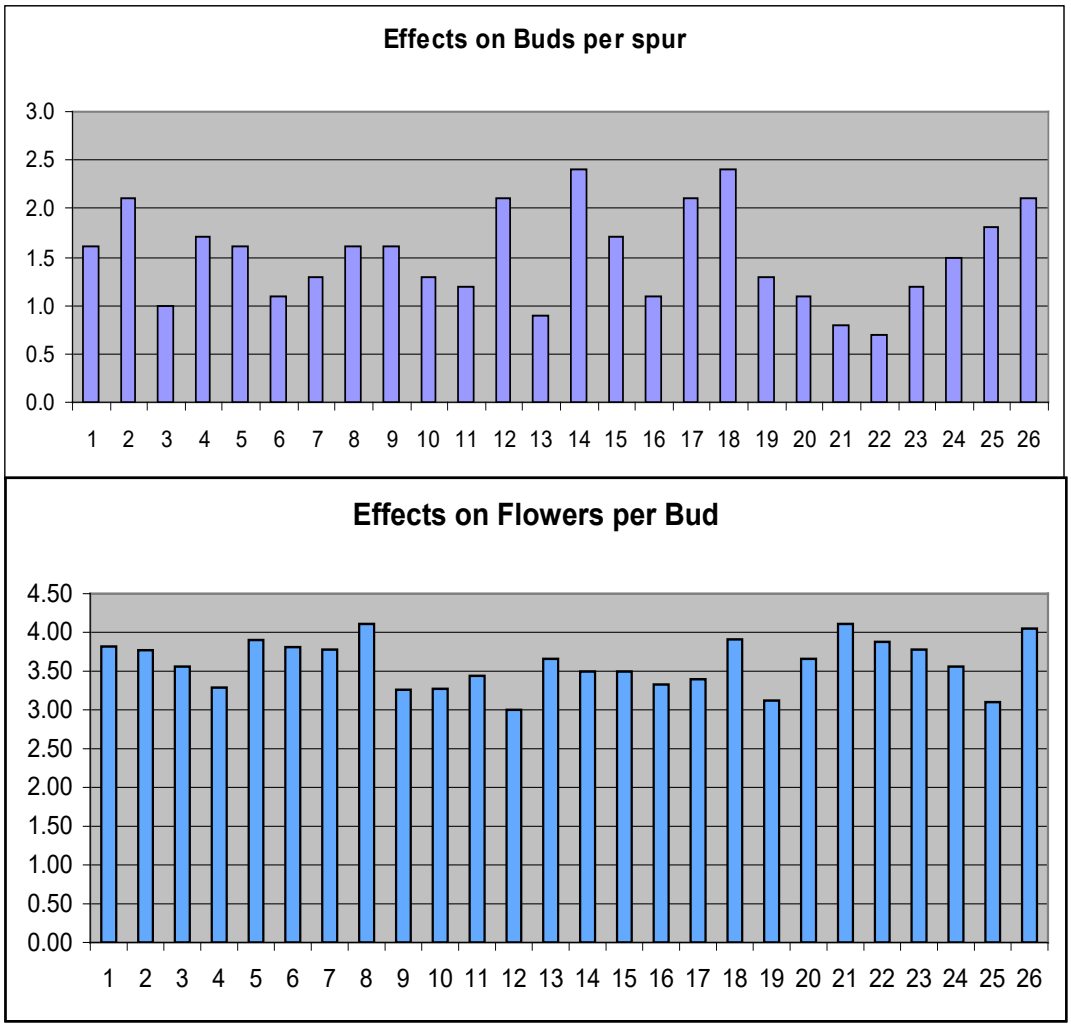

Figure 4. The effect of 25 selected compounds on bud numbers and flower numbers. No. 26 represents the control which was treated with GA3. (Prosser, WA, 2007)

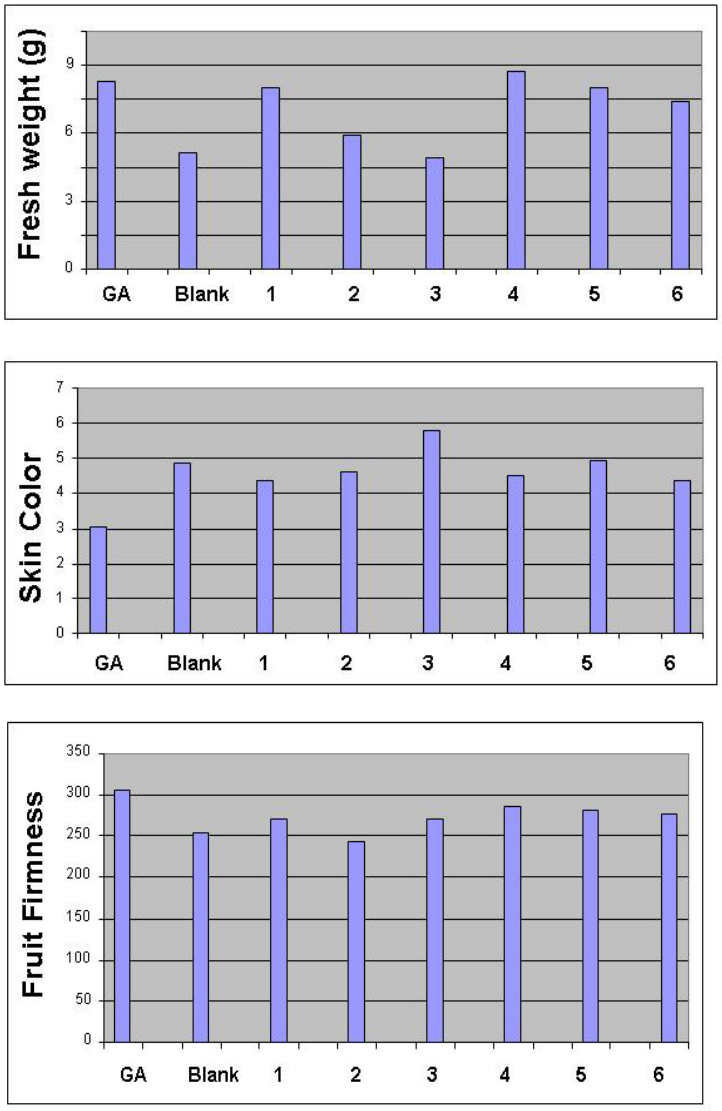

Figure 5. The effect of 6 selected compounds on the fruit weight, fruit size and fruit firmness. (Pullman, WA, 2008)

Figure 6. The effect of a small molecule (No. 4) on sweet cherry fruit ripening and fruit size. The photos show the fruits with treated and untreated from the same tree. This chemical can delay the fruit ripening and increase the fruit size. (Pullman, WA, 2008)

EXECUTIVE SUMMARY

Chemical genomics is a new high-throughput approach for determining gene function using small bioactive molecules to activate/inactivate gene products (i.e., proteins). Recently, chemical genomics has been used to better elucidate hormonal signaling in Arabidopsis. In this report we summarize our use of a chemical genomics approach for sweet cherry improvement.

From screening a 100,000 format chemical library, we identified more than 100 bioactive molecules that affect (elicitors and inhibitors) the gibberellin pathway. Twenty-five of these were applied to fruiting sweet cherry limbs in the field. We observed a variety of effects on fruit color, firmness, soluble solids, and weight. Furthermore, several compounds inhibited floral bud initiation and show potential as crop load management tools. A larger scale test using 6 selected chemicals in different location and different variety showed the similar results.

To sum up, we have identified a few very powerful chemicals which affect sweet cherry fruit quality and flower numbers. These chemicals are more effective than GA. After doing the subtraction cloning, we expect to find a set of genes affected by these chemicals. We have saved money to do this. It is anticipated that we can find the important genes affecting fruit quality and flower numbers which can be useful for marker assisted breeding, and as management tools for industry. Besides, these chemicals which are effective in sweet cherry may work in other tree fruits such as apple and pear, too. Our results indicate that using chemical genomics approach can save time and money for tree fruits gene disco very and crop improvement.

FINAL PROJECT REPORT

Project Title: Post-plant management of dagger nematodes

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Telephone: 509-786-9256
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Cooperators: Mr. Don Jagla; Cherry Grower, Wenatchee, WA; Dr. C. Ishida, Field R&D Scientist, Valent Biosciences Co., and Dr. Ken Eastwell, Virologist, Washington State University, IAREC, Prosser, WA.

Other funding Sources: VALENT
Agency Name:
Amount requested/awarded: Provided DiTera for free
Notes:

Total Project Funding: $ 7,495

Budget History:

Item	2006	2007	2008
Salaries			
Benefits			
Wages	4,185		
Benefits	460		
Equipment			
Supplies	450	1,000	0
Travel	400	1,000	0
Miscellaneous			
Total	**5,495**	**2,000**	**0**

Objectives: The objective is to use DiTera as means to control both *Xiphinema americanum* (dagger) and *Pratylenchus penetrans* (lesion) nematodes in post-plant cherry orchards. In addition, the effect of DiTera on non-target beneficial free living nematodes will be evaluated.

Significant Findings:

After 3 years of applying DiTera in cherry orchards, a significant reduction in dagger nematode population was achieved in comparison to the untreated controls. DiTera did not cause a significant reduction to the non-target beneficial free-living nematodes in comparison to the controls.

Results and Discussion:

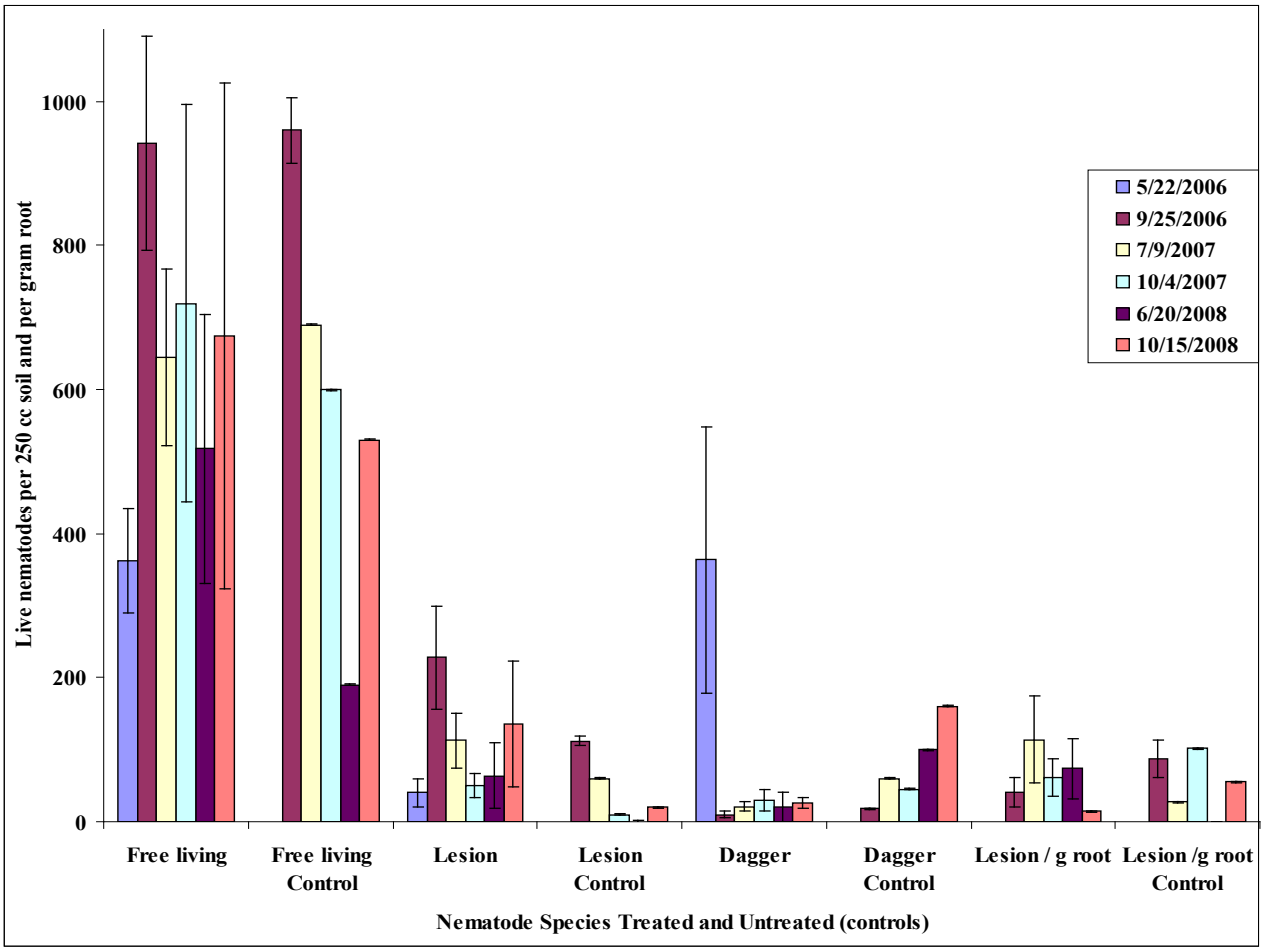
After 3 years of applying DiTera in a cherry orchard with high densities of dagger nematodes, a significant reduction in dagger nematode population was achieved in comparison to the untreated controls (Fig 1). On average, dagger nematodes were reduced from 400 individuals per 250 cc soil (initial samples were collected in May 2006) to 26 individuals per 250 cc (final samples were collected in October 2008). Similar reduction was recorded from all soil samples.

There was no significant reduction of lesion nematode populations in the soil or inside the cherry roots (this is a migratory endoparasite and it lives both inside the root and in the soil) (Fig. 1). We are hypothesizing that DiTera is reducing the dagger nematodes in the soil but does not affect lesion nematodes inside the roots; and once the nematicide effect of DiTera dissipates, then the lesion nematode leaves the roots and gets re-established in the rhizosphere soil. In addition, lesion nematodes have a shorter life cycle and a higher reproductive capacity than dagger nematodes so lesion nematodes can get re-established within one season in the soil.

DiTera did not cause a significant reduction to the non-target beneficial free-living nematodes in comparioan to the controls (Fig 1).

The reduction of dagger nematodes achieved in this study is of importance to established cherry orchards as controlling this nematode species will lead to reduction of virus transmission, yield increase and tree survival. Although DiTera did not control the lesion nematodes in this cherry orchard, the low lesion nematode densities found are not of significant concern to this established cherry orchard.

Fig 1. The effect of DiTera on *Xiphinema americanum* (dagger nematode), *Pratylenchus penetrans* (lesion nematode) and beneficial free-living nematodes – 2006, 2007 and 2008 Cherry Orchard field trial



Executive Summary

The dagger nematode, *Xiphinema americanum* is associated with virus transmission and yield reduction in cherries and other crops. Therefore, dagger reduction or elimination is necessary. After 3 years of applying DiTera in a cherry orchard with high initial dagger denstites, a significant reduction in dagger nematode population was achieved in comparison to the untreated controls.

Future Directions:

DiTera has been applied in a two additional cherry orchards; one orchard was treated in 2007 and 2008 and the second orchard was treated in 2008. The nematode densities in both orchards will be monitored over three years.

The high dagger nematodes found in the three cherry orchards we have sampled, indicate that pre-plant sampling for nematodes must become a routine measure to allow for pre-plant treatments against nematodes. Furthermore, pre-plant soil sampling must be followed by a synthetic nematicide or a bio-nematicide treatment to recude or eliminate nematodes prior to cherry planting.

A cherry orchard was fumigated in fall 2007 with Telone and Vapam to reduce dagger nematodes. Cherry seeedlings were planted in spring 2008 and DiTera was applied as an additional measure. This orchard will be monitored over three years.

We will seek more cherry orchards to sample for dagger nematodes and to apply synthetic or bio-nematicides in order to develop control strategies against nematodes.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: CH-07-706

Project Title: Efficient production of superlative fruit

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Cooperators: Amy Iezzoni, Clive Kaiser, Auvil Fruit Company, Allan Brothers, Rowe Farms, Roy Farms, Mark Hanrahan, WTFRC, John Verbrugge, Alejandro Antunez, Mauricio Frias Giaconi, Gemalier Lemus, Howard Hansen, Anna Steinheuser

Total project funding request: **Year 1:** 136,138 **Year 2**: 137,386 **Year 3:** **140,766**

Other funding Sources: NONE

Budget 1:
Organization Name: **Washington State University** **Contract Administrator: ML. Bricker**
Telephone: **509 335-7667** **Email address:** **mdesros@wsu.edu**

Item	2007	2008	**2009**
Salaries	20,309	21,121	**22,895**
Benefits	1,856	1,930	**1,925**
Wages	52,980	55,099	**57,303**
Benefits	6,093	6,336	**5,558**
Equipment	5,000	3,000	**3,000**
Supplies	14,500	14,500	**14,500**
Travel	22,500	22,500	**22,500**
Panel Testing	12,900	12,900	**12,900**
Total	136,138	137,386	**140,581**

Footnotes:

OBJECTIVES:

1. Improving efficiency (e.g., labor, pesticides, light use) through development of single-plane, compact orchard systems designed to incorporate mechanization and/or mechanical-assisted operations.
2. Develop pragmatic strategies for consistent and balanced cropping through understanding factors limiting fruit set and researching practical thinning strategies
3. Better understand critical fruit sensory attributes, consumers' perceptions of fruit quality, and their willingness to pay for those attributes

SIGNIFICANT FINDINGS:

Orchard systems

- High yields of quality fruit can be grown in compact fruiting wall architectures
- A novel tree architecture comprised of upright fruiting offshoots (UFO) continues to show promise in WSU and cooperator orchards – more than 12 cooperative orchards are established
- Recommended spacing is 10 feet between rows for upright fruiting walls and 14 feet between rows for angled fruiting walls; within row spacing should be ca. 6 feet for Gisela 5 and 7 – 8 feet for Gisela 6
- To encourage uniform growth, trees need to be planted at >45 degrees from vertical
- Fruiting uprights can be induced with 3-cm bands of 5,000 or 10,000 ppm Promalin + Pentra-bark (2% v/v) applied every 10 – 12 inches
- UFO-trained trees can bear fruit in the second year if there is no bud removal in the first year
- Summer pruning vigorous uprights helps balance vigor among uprights
- Timing of summer pruning affects the amount of regrowth

Fruit set/Effective pollination period

- Maternal factors (i.e., stigma receptivity, ovule viability) appear to limit fruit set under field conditions
- Among self-fertile cultivars, self-pollination resulted in a reduction in fruit set compared to pollination with different cultivars even if they had the same S-alleles.
- In field trials, elevating Bing and Sweetheart flower temperature at first white by 9ºF hastened flowering but cooling flower temperature by 9ºF did not significantly delay flowering compared to ambient
- 3 days after initiation of temperature treatments, heated Bing clusters had 76% open flowers vs. fewer than 20% open in ambient and cooled clusters
- In field trials, temperature had less effect than wind on fruit set in Sweetheart
- Fruit set of heated flower clusters was 20% less than fruit set of cooled flower clusters
- Fruit set of cooled flower clusters was 20% less than that of ambient, untreated flower clusters
- Constant, low velocity wind throughout anthesis reduced fruit set by 30% compared to untreated
- In growth chamber trials, temperature affected the rate of flowering and leaf development
- Pistil length was unaffected by temperature regime but pedicel length was ca. 2-fold longer in high temperature vs. low temperature environments
- Sweetheart and Regina flowers had a high percentage of protruding pistils at low temperature (13% and 22%, respectively)
- Spur leaf area development was positively related to temperature regime
- Benton spur leaves expanded the quickest and Regina expanded the slowest

- Temperature did not affect pollen viability for any cultivar tested
- We observed albino anthers in Benton that contained non-viable pollen
- Pollen tube growth in high and medium temperature treatments was rapid, reaching the ovule within 8 hours for all cultivars
- Pollen tube growth was only reduced slightly in cold treated flowers – most tubes had reached the base of the style 8 hours post pollination
- Stigma receptivity varied among cultivars
- Bing stigmas were receptive up to 96 hrs post anthesis and receptivity was reduced by cool temperatures
- Benton stigmas lost apparent receptivity within 8 hours after opening in cool and moderate temperature regimes and after 24 hours in high temperature environment
- Regina stigmas were receptive for 48 hr, 72 hr, and 96 hr in low, moderate, and high temperature regimes, respectively
- High temperatures hastened ovule senescence in all cultivars
- Bing and Benton exhibited significantly greater ovule longevity than Regina and Sweetheart
- Poor fruit set in Benton appears to be related to stigma receptivity
- Poor fruit set in Regina appears to be related to rapid ovule senescence

Balanced cropping

- ATS reduces stigma receptivity and pollen viability
- ATS is most effective at reducing fruit set potential of open, unpollinated flowers though it is also effective on open, pollinated flowers as well as unopened flowers
- 2 – 3% fish oil + 2 – 3% lime sulphur was inconsistent as a post-bloom thinner on Bing, Rainier, and Lapins – we recorded no reduction in fruit set but did see improvements in fruit quality
- Maxcel® was ineffective as a post-bloom thinner at 14 DAFB
- Applications of CPPU and CPA at full bloom increased Bing fruit set by ca. 25%
- Cytokinins Toplin and TDZ were effective bloom thinners, reducing fruit set by 20 – 30%
- Full bloom applications of GA_3 or GA_{4+7} increased Bing fruit weight by 15 – 20% without thinning
- Bing fruit treated with 10 ppm GA_{4+7} at full bloom had 50% 9 g or larger vs. only 10% of similar weight in untreated
- Applications of Prohexadione-Ca plus GA_3 or GA_{4+7} made at early stage III improved Bing fruit size and quality significantly

Sensory studies

- Harvest date had a significant effect on Sweetheart fruit quality and consumers' perceptions
- Early-harvest cherries (mid-harvest – 3 days) were perceived as lower color intensity, softer, and not as sweet as mid- and late-harvested fruit (mid-harvest + 5 days)
- Late-harvested fruit were rated higher in color intensity, sweetness and flavor intensity than early- and mid-harvested fruit
- Fruit sourness and juiciness was rated similarly for all harvest timings
- Cherries harvested at mid-season maturity had the highest overall acceptance, and highest acceptance for appearance and texture
- Early-harvest cherries were rated the poorest by consumer panels due to poorly rated flavor and fruit perceived as too sour
- Overall acceptance was strongly correlated to flavor acceptance ($r=0.94$)
- Preliminary analyses suggest that trained panelists could distinguish between ca. 40 - 60 g/mm firmness in cherry samples
- Panelists had difficulty distinguishing firmness differences between soft and moderately firm fruit (i.e., ca. 150 g/mm – 220 g/mm)

- Consumers did not accurately rate firmness of fruit
- About 60% of consumers correctly identified no difference in firmness between two samples with similarly low firmness – 40% however were incorrect in perceiving differences
- About 40% of consumers incorrectly rated a low firmness and moderate firmness sample as not being different
- About 38% of consumers incorrectly rated a moderate firmness and high firmness sample as not being different
- Consumers' perceptions of firmness were influenced by cultivar – more panelists correctly identified differences in firmness of Selah vs. firmness differences of Skeena
- Trained and consumer sensory panels can recognize attributes of cherry appearance, flavor and texture, and differentiate among different varieties of cherries
- Trained sensory panel responses showed clear relationships with corresponding empirical data of fruit attributes
- Flavor groupings based on perceived sweetness and sourness can be used to characterize cultivars
- Fruit sweetness, flavor, and juiciness, consumers showed strong positive relationships between intensity and acceptance of the attribute
- Maturity differences within a cultivar (as influenced by harvest timing) were perceived by trained and consumer sensory panels

METHODS: see below for brief descriptions and refer to proposal "Efficient production of superlative fruit"

RESULTS AND DISCUSSION:

Objective 1 Our evaluation of high efficiency fruiting wall architectures expanded in 2008. We continue to refine orchard establishment protocols to promote rapid canopy establishment and precocious fruiting. Critical to filling orchard space and early fruiting is planting unheaded whips. This requires communication with the nursery at the time of ordering trees. Whips are then planted at >45° from vertical and gradually trained horizontal along a low trellis wire (ca. 20″). Upright fruiting offshoots should be spaced ca. 6″ apart. These shoots can be induced with 3-cm bands of 5,000 or 10,000 ppm Promalin + Pentra-bark (2% v/v) applied every 10 – 12 inches. Summer pinching of vigorous uprights is recommended to balance vigor among uprights. In a Tieton/Gisela6 UFO orchard we pruned uprights to ca. 30″ at two-week intervals. The most regrowth (as number of breaks and length of breaks) from headed uprights occurred from pruning in mid-July (53% had new lateral growth initiated) and the least occurred following pruning in early August (30% of uprights had lateral growth). The effect of timing of heading uprights on flowering in the subsequent season will be documented. We continue to work with an illustrator to develop a grower's guide to UFO training.

Objective 2 In 2008 we continued our investigations of fruit set and pollination for sweet cherry, studying limiting genetic and environmental factors. Emphasis was given to understanding the role of temperature on the various components of fruit set: pollen germination, stigma receptivity, pollen tube growth rates, and ovule viability for inherently productive and unproductive cultivars. Trials were conducted in the field and in growth chambers on cut branches. We utilized the 'squid' to manipulate flower cluster temperature in the field and study its effects on fruit set in Sweetheart and Bing. Treatments included untreated control, heated (ambient + 9 F), cooled (ambient – 9 F), and ambient delivered via blower. Flowers were hand-pollinated daily. Fruit set of untreated Sweetheart

clusters was 73%. Fruit set was ca. 50%, 45%, and 30% for cooled, blower (ambient air temp.), and heated flowers, respectively. These results suggest that a light, constant wind (ca. 2 mph) can reduce fruit set significantly (ca. - 30%) and also that high temperatures reduce fruit set. The high temperature effect on Sweetheart fruit set is likely due to accelerated ovule senescence (see growth chamber results below). The role of windspeed on fruit set will be further investigated in 2009.

Growth chamber experiments were conducted using 4 cultivars representing high and low natural fruit set for both self-sterile and self-fertile types. Cut branches were placed in growth chambers programmed to high (24/12°C), medium (18/6°C), or low (10/2°C) temperature regimes with 12 hr day/night cycle. Populations of flowers were hand-pollinated and sampled post-pollination at 8 to 12 hr intervals to assess pollen tube growth, ovule viability, pollen viability, and stigma receptivity. Sample analyses are ongoing, however our preliminary analyses indicate that pollen tube growth is rapid at medium and high temperatures – in all cultivars we observed pollen tubes reaching the base of the pistil by 8 hrs post-pollination. This is considerably faster than previous reports. Pollen tube growth in low temperature environment was only slightly slower. We observed growth to the mid and base of the stylar tissue 8 hrs post-pollination. There did not appear to be any difference in pollen tube growth rates among maternal parents at any temperature regime. This suggests that for many cultivars (e.g., Bing, Benton, Sweetheart, Regina), fruit set is not likely limited by rate of pollen tube growth. Stigma receptivity was highest in Bing and lowest in Benton. Regina was intermediate. Interestingly, we were not able to assess Sweetheart stigma receptivity by the perex test. This suggests that stigmatic surface exudates are different among cultivars. In the other three cultivars, receptivity declined over time, post-anthesis and this was advanced with low temperatures. At high temperatures Bing and Regina remained receptive for 96 hr and 72 hr, respectively.

Ovule senescence was affected by temperature regime and the response was cultivar dependant. High temperature however, accelerated ovule senescence in every cultivar compared with moderate and low temperatures. Bing and Benton ovules remained viable up to 96 hr post-anthesis (ca. 55 – 70% viable) in high temperature environment. In contrast, we observed only ca. 5 – 15% viable ovules of Sweetheart and Regina by 96 hr post anthesis. Pollen germination was assessed at 24 and 72 hrs after initiation of temperature treatments and found to be unaffected by temperature. It appears that poor fruit set in Regina may be related to rapid ovule senescence and that poor fruit set in Benton may be related to poor stigma receptivity.

In 2008 we continued to investigate the role of maternal vs. paternal factors on fruit set potential. Pollen germination among the sources used for these field experiments ranged from 12% to nearly 79% (data not shown) and was unrelated to fruit set. As in 2007, our results show that fruit set in Regina is significantly different from the other female parents used in the crossing scheme. Again, variation in fruit set for these cultivars was largely a result of maternal effects; there were no significant differences in average fruit set among the different pollen donors. These findings were consistent with the results of a separate experiment to determine whether all sources of the same S-allele are equally effective with respect to fruit set. We utilized four different self-fertile cultivars in a mating scheme that resulted in only the S4' allele from each of the parents being compatible. Again, no significant differences were measured for pollen parent influence on fruit set. In all cases except 'Selah', fruit set was lowest when self-pollinations were made. This suggests some mechanism of selection against self-pollen other than that of the self-incompatibility locus, as in each of these cases, the pollen recognition function of the locus as been lost due to the self-fertility mutation.

In 2008 we also evaluated chemical thinner mode of action. Applications of either water or 2% ATS were made to Bing limbs with spurs which were comprised of closed flowers, open and pollinated flowers, or open and unpollinated flowers. We assessed fruit set just prior to harvest and found that ATS treated spurs had significantly lower fruit set than water treated spurs irrespective of flower type. Overall, the ATS treatment reduced fruit set from 66% to 24%. The greatest reductions in fruit set were on open and unpollinated flowers – fruit set of these flowers treated with ATS was 57% lower than water-treated (78% for water treated vs. 21% for ATS treated). In contrast, fruit set of flowers that were open but pollinated (manually) 24 hr previously and treated with ATS had 43%

lower fruit set and flowers that were closed at the time of treatment had only 24% lower fruit set. ATS reduced pollen viability (data not shown) though this is not likely to affect fruit set potential because our research has shown that maternal factors limit successful pollination (see above). ATS also reduced stigma receptivity significantly (data not shown). This is likely the most significant mode of action for this thinner though we did record significant thinning efficacy on flowers that had been pollinated 24 hrs prior to ATS application (and therefore presumably immune to reductions in stigma receptivity). This mode of thinning will be investigated further along with longer post-pollination intervals.

We also conducted several field trials evaluating the potential to manipulate fruit set and quality with many plant growth regulators. In a study of 22 PGR treatments (including auxins, cytokinins, and GAs) applied at full bloom to Bing we found significant improvements in fruit set (ca. + 25%) from CPPU at 15 ppm and CPA at both 10 and 30 ppm. Fruit quality was not reduced from these treatments despite their causing higher crop density. We also documented thinning potential with synthetic cytokinin toplin applied at 15 ppm – it reduced fruit set to ca. 10% from 35%. The most dramatic fruit quality improvements were from applications of GA_3 and GA_{4+7} at 10 ppm. Average fruit weight was 1 – 1.5 g greater from these treatments compared to the control (i.e., about a row-size improvement). Large scale field trials of these GA treatments were initiated in Tasmania in October on Lapins, Regina, and Sweetheart.

In a separate trial we screened similar PGRs (22 treatments in total) for their ability to increase Bing fruit size. Each treatment was applied in lanolin paste directly to fruit pedicels at 9 days after full bloom. This is a period of rapid cell division in sweet cherry and thus targeted as a key period to influence fruit size. Several cytokinins improved fruit weight significantly (ca. + 15%) with CPPU at 100 ppm being the best. Auxin treatments were ineffective at improving fruit quality but several GA treatments improved fruit size significantly – GA_3 at 200 ppm was most effective. Sixty percent of fruit treated with GA_3 at 200 ppm were 9 g or heavier while untreated control fruit had less than 5% fruit in the same category. These results are consistent with the previous study of applications at full bloom and show promise for early applications of GA_3 to improve fruit quality.

We investigated also the potential to improve Bing fruit quality with PGR applications during stage III of fruit development. Treatments included various GA isomers alone and in combination with Prohexadione-Ca (P-Ca). These late applications (ca. 28 days before harvest) of GA or P-Ca alone had no positive effect on fruit size. However, combinations of P-Ca + GA_3 and P-Ca + GA_{4+7} improved fruit weight and diameter by about 15%. Further, the percent of 9 g+ fruit from these treatments was ca. 45% vs. only 20% of the same size fruit in the untreated control. These combinations also improved fruit firmness from 245 g/mm in untreated control to 280 g/mm. The single application of P-Ca effectively reduced vegetative vigor – extension shoots were less than half the length of untreated extension shoots. These treatments will be tested in large-scale orchard trials in Tasmania in December and in Washington next season. We intend to further investigate the potential to control vigor in fruiting sweet cherry trees with applications of P-Ca.

Objective 3 In 2008 we conducted two trials that used trained and consumer panels to assess key components of fruit quality. The first evaluated the influence of harvest time on empirically-determined quality attributes and sensory attributes of sweet cherries. On three separate panel days, trained and consumer panelists evaluated 'Sweetheart' cherries that were harvested 3 days prior to estimated commercial maturity (early-harvest, 6 July), at mid-harvest (9 July), and 5 days post-mid harvest (late-harvest, 14 July). Fruit attributes from each harvest timing were characterized empirically by quantifying soluble solids concentration (SSC), acidity of juice, weight, diameter, exocarp color, and firmness. A sensory panel (n=12) was trained to recognize and evaluate the attributes of cherry appearance (color intensity), texture (flesh firmness and juiciness) and flavor/taste (sweetness, sourness, and cherry flavor intensity). Fruit were then evaluated by a consumer panel (n=272) for purchase intent, overall acceptance, appearance, flavor and texture. Empirical determinations of Sweetheart fruit quality show that fruit soluble solids were high but did not change

between the first and last harvests. Fruit exocarp color significantly changed from a light red in early and mid-harvests (CTIFL scale of 2.7) to dark red by the late harvest timing (CTIFL 3.5). Firmness declined significantly with each successive harvest timing, from 304 g/mm at the early-harvest timing to 265 g/mm at the late-harvest. The trained panel rated late-harvest cherries as highest in color intensity, sweetness and flavor intensity. Juiciness and sourness were rated similarly among the harvest timings. Interestingly, the trained panel rated mid-harvest fruit as the most firm – this contradicts the firmtech analysis of firmness (potential reasons for this are explained below). Further, sweetness was judged to be significantly high for mid- and late-harvest fruit compared to early-harvest, despite their being no difference based on refractometer data. Consumer panel assessments of the fruit indicated that mid-harvest fruit had the highest overall acceptance, and highest acceptance for appearance and texture. Consumers consistently rated the early-harvest fruit as lower quality than the other two harvests. Overall acceptance was strongly correlated to flavor acceptance (r=0.94). These results indicated that cherries harvested at commercial maturity were preferred among the three harvest times despite not having the highest intensities of color, sweetness or flavor (as judged by trained panel). This indicated the importance of color, sweetness and flavor of cherries on the overall acceptance and the possible interaction of these attributes in consumer acceptance. Further, the results suggest that standard harvest maturity indicator (i.e., red coloration of exocarp) was appropriate for optimum consumer acceptance of 'Sweetheart'.

Our second sensory study evaluated consumers' ability to identify differences in firmness among multiple cherry samples. Fruit firmness is consistently ranked as a critical fruit attribute and vague guidelines for interpreting Firmtech data have been described. However, to date no research has evaluated critically consumers' sensitivity to fruit firmness or their preference for firmness of sweet cherries using the current industry standard measure of g/mm. Following instrumental analysis of firmness by Firmtech, Selah and Skeena cherries were presented to trained and consumer panelists over a period of two days, with each session held at the same time each day. A directional paired comparison test was used to compare cherries from different firmness groupings; soft vs. soft (to evaluate the placebo effect), soft vs. intermediate, soft vs. firm and intermediate vs. firm. Thus for each varietal, a total of 8 paired comparisons were evaluated. As the ballot was not a forced choice, panelists were asked to indicate which cherry sample was firmer or if there was no difference between samples.

Using the analytical values of firmness (5, 50 and 95th percentile), the sensory perception of firmness, expressed as intensity along a 15-cm line scale was predicted. When the analytical firmness was value was 142.7 g/mm (5th percentile), the mean predicted sensory firmness was 5.4 cm. When the analytical firmness value increased to 198.39 g/mm (50th percentile), the mean predicted sensory firmness was 6.6. Finally, when the analytical firmness value increased to 464.221 g/mm, the mean predicted sensory firmness was 12.8. This suggests that panelists were not able to distinguish between cherries with low firmness as well as though that were firmer.

In consumer panel testing, the number of panelists selecting the "no difference" option decreased for the comparisons of soft vs. soft, soft vs. intermediate and soft vs. firm. This indicates that as fruit firmness differences between the cherries increased, panelists were more accurate at selecting one sample over the other as more firm. When comparing soft to soft cherries, the percentage of panelists selecting "no difference" was 63% and 53% for Selah and Skeena cherries, respectively. These percentages decreased to 40 and 42% for Selah and Skeena, respectively, when comparing soft to intermediate, and to 15% and 30% when comparing soft to firm. When comparing intermediate to firm, the percentage of panelists selecting "no difference" was to 33% and 45% for Selah and Skeena, respectively. Interestingly, results varied between cherry cultivars – significantly more consumers correctly identified firm Selah cherries as being firmer than intermediate firmness Selah cherries compared to Skeena. Overall, these preliminary analyses suggest that trained panelists could not detect differences in fruit firmness of 40 to 50 g/mm and that consumers have even more difficulty in perceiving firmness differences.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: CH-07-701

Project Title: Sweet cherry regeneration and transformation system

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Other funding sources: None

Total project funding request: **Year 1:** 10,000 **Year 2:** 10,000 **Year 3: 10,000**

Budget 1:
Organization Name: WSU **Contract Administrator:** Mary Lou Bricker
Telephone: 509 335 7667 **Email address:** mdesros@wsu.edu

Item	2007	2008	**2009**
Salaries			
Benefits			
Wages	2250	1,729	**1,729**
Benefits		271	**271**
Equipment			
Supplies	7000	7,000	**7000**
Travel	750	1,000	**1000**
Miscellaneous			
Total	10,000	10,000	**10,000**

OBJECTIVES

Our objectives for the proposal were:

A. We had proposed to employ a three-pronged approach in the first 18 - 20 months to establish an efficient regeneration system in sweet cherry.
   1. Identify the best media formulation for each variety being tested.
   2. Identify the best line for regeneration.
   3. Monitor the progression of regeneration using known markers of regeneration.

B. Establishment of an efficient transformation system.

In the last year of funding for the proposal our goal is to standardize conditions for efficient regeneration and transformation. We also plan to test impact of different light wavelengths on regeneration and micropropagation in general.

SIGNIFICANT FINDINGS

Last two years have been devoted to Objective A. Establishment of sweet cherry explants presented a challenge. Also, media formulations from published reports were used exhaustively with negative results. We have done extensive experimentation during the preceding growing season and have successfully overcome the challenges. Some of the significant findings are:

1. Decontamination of sweet cherry buds is best achieved using mercuric chloride treatment. It is less damaging than bleach and results in increased explant survival.
2. Time of collecting the cherry buds has a large impact on explant survival and its progression in tissue culture.
3. Successful establishment of Bing, Rainier and Lapin explants using our defined conditions has been achieved. Regeneration has been achieved but needs further refining.

These findings are explained in greater detail in the Results and Discussion section.

METHODS

Standard tissue culture methods have been used in this project. Exceptions are the use of temporary immersion system and specific light wavelengths to regulate plant growth. Methods include, explant retrieval from the trees in orchards, decontamination and transfer to the media. Some of the treatments that we have tried are:

1. Decontamination with Bleach or Mercuric Chloride
2. Photoperiod during growth
3. Light Intensity during growth
4. Buds with peeled bark in medium
5. Leaf versus Bud as explants
6. Temperature 24/16 deg C during Day and Night
7. Solid versus Liquid Media

We had initiated utilizing different chemicals that can enhance plant regeneration. This had resulted due to collaboration with another WTFRC funded project on chemical genomics currently being led by Dr. Tianbao Yang. Unfortunately we did not find any major effect of small molecules.

RESULTS AND DISCUSSION

We have successfully established sweet cherry tissue cultures for Bing, Rainier and Lapins. The next step is to enhance the efficiency of regeneration and establish transformation. Some of the results are discussed here.

A. Explant establishment and regeneration

Surface sterilization and explant establishment is established and currently regeneration part is being standardized. We have tested 36 media types for establishing explants and for plant regeneration as outlined in Table 1.

Table 1: Media Formulations Tested for Sweet Cherry Explant Establishment and Regeneration. All media contain 30 g/l of sucrose and 5.6 g/l of Tissue Culture grade agar.

Media	**Macro /Micro**	**Charcoal**	**Ascorbic Acid**	**BAP**	**TDZ**	**GA3**	**GA4+7**	**IBA**
MS+BAP	MS	n/a	n/a	0.675 mg/L	n/a	n/a	n/a	n/a
MS+BAP +charcoal	MS	10g/L	n/a	0.675 mg/L	n/a	n/a	n/a	n/a
MS+BAP +IBA	MS	n/a	n/a	1mg/L	n/a	n/a	n/a	0.1mg/ L
MS+IBA +GA3	**MS**	**n/a**	**n/a**	**1mg/L**	**n/a**	**0.5mg/ L**	**n/a**	**0.1mg/ L**
MSA	MS	1g/L	n/a	1mg/L	n/a	n/a	n/a	n/a
MSB	MS	1g/L	n/a	1mg/L	n/a	n/a	1mg/L	n/a
MSC	MS	1g/L	n/a	3mg/L	n/a	n/a	n/a	n/a
MSD	MS	1g/L	n/a	3mg/L	n/a	n/a	3mg/L	n/a
MSE	MS	n/a	200mg/L	2mg/L	n/a	n/a	n/a	n/a
MSF	MS	n/a	200mg/L	2mg/L	n/a	n/a	0.5mg/L	n/a
MSG	MS	n/a	100mg/L	1mg/L	n/a	n/a	n/a	n/a
MSH	MS	n/a	100mg/L	1mg/L	n/a	n/a	1mg/L	n/a
MSI	MS	n/a	100mg/L	1mg/L	n/a	n/a	n/a	n/a
MSJ	MS	n/a	100mg/L	1mg/L	n/a	n/a	1mg/L	n/a
MSJ-2	MS	n/a	100mg/L	1mg/L	n/a	n/a	2mg/L	n/a
MSK	**MS**	**n/a**	**100mg/L**	**2mg/L**	**n/a**	**n/a**	**1mg/L**	**n/a**
MSL	MS	n/a	100mg/L	1mg/L	n/a	n/a	0.5mg/L	n/a
MSM	MS	n/a	100mg/L	2mg/L	n/a	n/a	1mg/L	n/a
MSN	MS	n/a	100mg/L		n/a	n/a		
MSO	MS	n/a	100mg/L	1mg/L	n/a	n/a	1mg/L	0.1mg/ L

MSP	MS	n/a	100mg/L	1mg/L	n/a	n/a	n/a	0.1mg/L
MSR	MS	n/a	100mg/L	1mg/L	n/a	n/a	n/a	0.1mg/L
MSS	MS	n/a	100mg/L	2mg/L	n/a	n/a	n/a	0.1mg/L
MST	MS	n/a	100mg/L	2mg/L	n/a	n/a	n/a	n/a
MSU	MS	n/a	100mg/L	2mg/L	n/a	n/a	1mg/L	0.1mg/L
MSV	MS	n/a	100mg/L	1mg/L	n/a	n/a	0.5mg/L	0.1mg/L
MSW	MS	n/a	100mg/L	2mg/L	n/a	n/a	0.5mg/L	0.1mg/L
WPM-A	WPM	500mg/L	n/a	1mg/L	n/a	n/a	1mg/L	0.1mg/L
WPM-B	WPM	500mg/L	n/a	1mg/L	0.25 mg/L	n/a	1mg/L	0.1mg/L
WPM-C	WPM	500mg/L	n/a	n/a	0.25 mg/L	n/a	1mg/L	0.1mg/L
WPM-D	**WPM**	**500mg/L**	**n/a**	**n/a**	**1mg/L**	**n/a**	**1mg/L**	**0.1mg/L**
WPM-E	WPM	500mg/L	n/a	7mg/L	0.25 mg/L	n/a	1mg/L	0.1mg/L
WPM-F	WPM	500mg/L	n/a	n/a	1mg/L	1mg/L	1mg/L	0.1mg/L
WPM-G	WPM	500mg/L	n/a	n/a	1mg/L	1mg/L	n/a	0.1mg/L
N6MS	**N6/MS**	**n/a**	**n/a**	**7 mg/L**	**n/a**	**n/a**	**n/a**	**0.2 mg/L**
MS	MS	n/a	n/a	n/a	n/a	n/a	n/a	n/a

The best media for explant establishment are identified in the table above. Currently WPM-D is the media that support explant growth for Bing, Rainier and Lapin varieties. Other media may be useful as we expand the protocols to other important varieties, specifically the ones originating from the breeding program. N6MS is the best media for plant regeneration from leaf explants. This media is derived from our experiment on other Rosaceae plant regeneration and is the one that specifically suited sweet cherry. Further standardization will build upon this media type.

During these experiments it was observed that buds collected in September are at the appropriate developmental stage to move forward in tissue culture. Figure 1 (Opposite page) shows the buds in WPM-D media. A, B, C represent buds collected in August. Note the slow growth and health of buds. Figure 1 D and E are buds collected in mid September and the buds are growing well. Figure 2 shows the explant regeneration and shoot production. These buds were established in May.

FUTURE DIRECTIONS

We expect to initiate transformation experiments in a few weeks. Currently the Rainier, Bing and Lapin explants are being multiplied to have enough leaf material for the experiments. The regeneration of leaf explants needs to be standardized using different light regimes and fine-tuning of the N6MS regeneration medium.

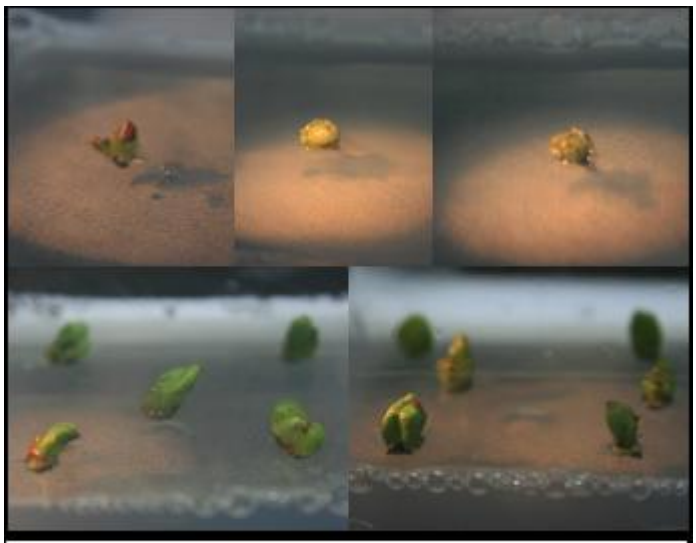

Figure 1. A, B, C: Buds collected in late August. D, E: Buds from mid September. Pictures from 10-13.

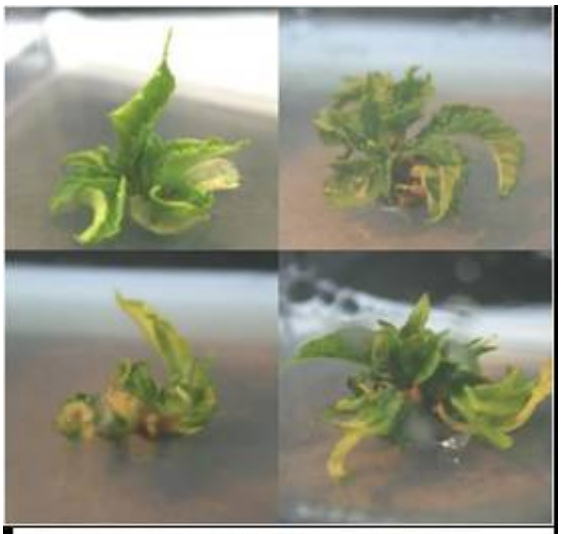

Figure 2. Buds collected in early May showing regeneration and shoot production. Pictures from 10-13.

PRESENTATIONS

The work being carried out under the support of this project has been presented at several national and internationals forums. Some of the approaches taken here will also be presented at the AEMP conference in December 2008.

1. J Poff, C Tong, C Wildenstein, T Koepke, J Milhollan, D Jiwan, S Schaeffer, N Tarlyn, T Yang & A Dhingra. Novel approaches for improving tissue culture, micropropagation and biotechnological applications in horticultural crops. 3rd AEMP 2007, Faro, Portugal. September 2007
2. DJ Druffel, J Poff, C Tong, C Wildenstein, S Moore, D Scarimbolo, K Nicholson, L Taylor, T Koepke, J Milhollan, D Jiwan, SM Schaeffer, N Tarlyn, T Yang & A Dhingra. Novel approaches for improving tissue culture, micropropagation and biotechnological applications in horticultural crops. Washington State Horticultural Association 103rdd Annual meeting, Wenatchee, WA. December 2007

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: CH-08-801

Project Title: Establishment of test plots for MSU sweet cherry rootstocks

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Cooperators: Todd Einhorn, Tom Auvil**,** Jim Olmstead

Total project funding request (Fall 2007): Year 1: $40,974 **Year 2**: $19,120 **Year 3**: $17,416
Revised project funding request (Fall 2008)[1]: Year 1: $14,574 **Year 2:** $24,589 **Year 3:** $33,011

Other funding Sources

Agency Name:
Amount requested/awarded:
Notes:

WTFRC Collaborative expenses:

Item	2008[1]	2009	2010
Stemilt RCA room rental			
Crew labor			
Shipping			
Supplies			
Travel			
Miscellaneous			
Plot costs[2]	0	10,725	5,775
Tree cost for PNW[3]	0	1,794	14,070
Total	0	12,519	19,845

Footnotes:
[1]The trees from Duarte Nursery were anticipated to be available for spring 2009 planting. However, due to a longer length of time for liner production, they will now be available for spring 2010 planting. This delay resulted in a shift to year 2 of this project for funds to cover plot establishment and tree cost.
[2]Plot cost for the sites in Oregon, Manson, and Mattawa. Plot costs are based on 550 trees per site; $6.50/tree for plot establishment in 2009 which covers site prep, fumigation and irrigation supplies; $3.50/tree in 2010 for planting, and first year general farming, water, taxes. The cost in this revised budget is slightly higher than predicted due to the inclusion of Gisela 12 control trees in each plot.
[3]Tree cost is based on 276 trees from Willow Drive Nursery @$6.50 tree in 2009 and 2,345 trees from Duarte Nursery @ $6/tree in 2010.

Budget 1

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

Item	2008	2009	2010
Salaries	5,163	5,317	5,477
Benefits	2,411	2,553	2,689
Wages	500	500	500
Benefits			
Equipment			
Supplies	500	500	500
Travel	1,000	1,000	1,000
Misc. (tree freight)	500[1]		
Plot cost	1,000	1,000[2]	1,000 / 1,500[2]
Gisela liners		1,200[3]	
Total	11,074	**12,070**	11,666

Footnotes:
[1] This freight fee has been encumbered to cover the cost of tree delivery in 2010.
[2] The 2009 request is reduced and the 2010 request is increased as tree planting has been delayed until 2010.
[3]Total cost of the 750 Gisela liners @ $1.60 per liner (no royalty fee).

Budget 2

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

Item	2008[1]	2009	2010
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel			
Plot charges	3500	0	1,500
Miscellaneous			
Total	3500	0	1,500

Footnotes:

[1] Due to the delay in planting, no funds were expended in 2008. These funds have been encumbered and year 2 and 3 requests have been reduced by $3,500.

OBJECTIVES

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

Specific Objectives for 2008:

1. Evaluate the existing trees of the 11 rootstock candidates to determine if they continue to show commercial promise.
2. Conduct DNA fingerprinting to assure that the genetic identity of the rootstock selections is correct.
3. Plot establishment to include site preparation and tree purchase, tree planting, and cultural management of the plots to assure the ability to assess rootstock performance.
    - The MSU rootstock liners at Duarte Nursery took longer than anticipated to reach the girth suitable for budding. This resulted in an unanticipated delay in the planting of the test plots from spring of 2009 to spring of 2010. Funds for this objective were not used in 2008, and appear on the 2009 budget request.
    - The MSU rootstock candidates at Willow Drive Nursery that were generated from liners produced at Michigan State University will be planted at the WSU-Prosser Roza Farm in spring of 2009.

Specific Objectives for 2009:

1. Evaluate the existing trees of the 10 remaining rootstock candidates to determine if they continue to show commercial promise.
    - All the rootstock candidates are currently under evaluation at MSU. Evaluation of all candidates in 2008 resulted in the elimination of one selection reducing the test number to 10.
2. Conduct DNA fingerprinting to assure that the genetic identity of the rootstock selections is correct.
    - Rootstock liners will be tested using DNA markers to assure correct identity prior to budding.
3. Plot establishment to include site preparation and tree purchase, tree planting, and cultural management of the plots to assure the ability to assess rootstock performance.
    - The test trees grown at Willow Drive Nursery will be planted at the WSU-Prosser Roza Farm in spring of 2009.
    - Preparations will be made to plant the test plots with the trees from Duarte Nursery in Spring of 2010.

SIGNIFICANT FINDINGS AND ACTIVITIES

- The MSU rootstock candidate Crawford was eliminated from future testing as it exhibited symptoms consistent with rootstock-scion incompatibility.
- Eight of the 10 MSU rootstock candidates produced trees with trunk cross sectional areas (TCSA) similar to or less than that of GI 6. Thus far, two candidate rootstocks, Kent and Cass, resulted in trees considerably more vigorous than GI6, with trees nearly 50% larger. Kent has consistently produced trees with wider branch angles than any of the other candidates, a trait that may be of interest for those scion cultivars with upright growth habits. Lake is the most dwarfing of the rootstock candidates, with an average tree size 69% of GI6.
- Three rootstock candidates, Lake, Clare and Lincoln, had been selected in 2007 for only limited testing. The results from 2008 support that decision.

- The remaining rootstock selections had acceptable flower bud numbers ranging from 8 – 18 (GI6=13) and fruit sizes ranging from 6.8 g – 8.8 g (GI6=7.2g) suggesting that they are of sufficient interest to enter into large scale testing.
- The rootstock test trees from Willow Drive Nursery will be dug this fall and planted at the WSU-Prosser Roza Farm in spring 2009. Due to unequal liner numbers, the rootstock candidates will be represented in this plot by a minimum and maximum of 5 and 46 trees, respectively. All rootstock candidates have 'Bing' scion and Kent also has 'Sweetheart' scion due to a large number of liners and excellent percentage bud take (90%).
- Based on discussions with growers in July 2008, Gisela® 12 (GI 12) was added as a fourth control for the rootstock plots to be planted in spring 2010. The other three controls are mazzard, Gisela® 5 (GI 5) and Gisela® 6 (GI 6).
- The rootstock liners at Duarte Nursery are produced by meristem culture and the final budded trees are grown in pots in the greenhouse. To assure comparable control trees, liners of GI 5, GI 6, and GI 12 were sold to me and shipped to Duarte Nursery in September 2008. Duarte Nursery will then produce the control trees using analogous horticultural practices.

METHODS BY OBJECTIVE

1. Evaluate the existing trees of the 10 rootstock candidates to determine if they continue to show commercial promise.

- All 10 of the MSU rootstock candidates are currently planted at MSU's Clarksville Horticultural Experiment Station (CHES). It is critical that these rootstocks continue to be evaluated as four of the selected rootstock candidates were only planted at CHES in 2004. Therefore continued monitoring of tree performance is necessary. Additionally, these trees are the oldest representatives of the MSU rootstock selections and therefore provide valuable data on tree size potential and tree longevity. These trees will be evaluated for the following parameters: tree health, structure, trunk cross-sectional area, visual estimates of bloom density and crop load, number of spurs on two branches of two and three year old wood, fruit weight, and annual growth of terminal and lateral shoots.

2. Conduct DNA fingerprinting to assure that the genetic identity of the rootstock selections is correct.

- All the MSU rootstock candidates at Duarte Nursery will continue to be tested for their DNA fingerprint to assure correct clonal identity.

3. Plot establishment to include: site preparation and tree purchase, tree planting, and cultural management of the plots to assure the ability to assess rootstock performance.

- In spring of 2009, the 276 trees grown at Willow Drive Nursery will be planted in a rootstock test plot at WSU-Prosser (Roza Farm). This test will include all 10 MSU rootstock candidates with 'Bing' scion, plus Kent with both 'Bing' and 'Sweetheart' scions. The control is GI 6. Each rootstock will be represented by a minimum of 5 trees to a maximum of 46 trees. The differing tree numbers are due to differences in the success of vegetative propagation and bud take.

- In 2009, land will be prepared for the experimental rootstock plantings with the trees from Duarte Nursery (Table 1). These trees will be planted in spring 2010 at a spacing of 8 ft × 15 ft. The trees will be managed (e.g. irrigation, pest control, etc.) following standard commercial practices. Trees will be trained to a multiple-leader, open-center canopy architecture.

Table 1. Plant material currently being propagated at Duarte Nursery for the MSU rootstock trials to be planted in spring of 2010. (See Table 5 for listing of the rootstock candidates).

Location	# of MSU rootstocks tested	Scions utilized	Replication	Total Tree Number[1]
Prosser WA	10	Bing on all 10 rootstocks, Sweetheart on 6 rootstocks	5 reps of 5 trees each	625
Manson WA	6	Bing & Sweetheart	5 reps of 5 trees each	550
Mattawa WA	6	Bing & Sweetheart	5 reps of 5 trees each	550
Oregon	6	Bing & Sweetheart	5 reps of 5 trees each	550
Clarksville MI	10	Bing & Sweetheart	5 reps of 5 trees each	70

[1] This number includes the addition of the following rootstock controls: mazzard, GI5, GI6, GI12 and mazzard.

Proposed accomplishments for Year 2 (2009):

- Evaluate the existing trees of the 10 MSU rootstock candidates.
- Conduct DNA fingerprinting to assure that the genetic identity of the rootstocks is correct.
- Plant and establish the trees from Willow Drive Nursery in the test plot at WSU-Prosser
- Prepare the test sites for planting the trees from Duarte Nursery in the five test plots.

RESULTS AND DISCUSSION

Rootstock performance: The MSU rootstock candidates were planted at MSU's Clarksville Horticultural Experiment Station (CHES) over a four year period (2001 – 2004), reflecting when they were selected from the MSU germplasm collection. In 2008, the selection Crawford that was planted in 2004, exhibited tree decline symptoms indicative of delayed graft incompatibility. Therefore, it was discontinued as an advanced selection. Data was collected in 2008 for the remaining 10 rootstock candidates and the GI6 control.

Eight of the 10 MSU rootstock candidates produced trees with trunk cross sectional areas (TCSA) similar to or less than that of GI6 (Table 2). Thus far, two candidate rootstocks, Kent and Cass, resulted in trees considerably more vigorous than GI6, with trees nearly 50% larger. Kent has consistently produced trees with wider branch angles than any of the other candidates, a trait that may be of interest for those scion cultivars with upright growth habits. Lake is the most dwarfing of the rootstock candidates, with an average tree size 69% of GI6. Additional vegetative measurements of lateral and terminal shoot extension averaged over three years were similar to the vigor relationships established from TCSA measurements except for Glenn and Clare. In both cases, the terminal and lateral shoot extension of scions propagated on Glenn and Clare were greater than that for GI6 despite smaller TCSAs.

Due to a spring freeze that killed over 90% of the flower buds, crop yields were not recorded in 2008. Instead, flower bud counts taken prior to the freezing temperatures are presented (2007 and 2008 flower bud counts were averaged over the two years) (Table 3). Lake and Clare, two rootstocks that had smaller TCSA than GI6, also had fewer flower buds on 2 year old wood (mean values of 8 versus 13). Clare also had smaller fruit than GI6 (Table 3). Lincoln, a selection with similar TCSA to GI6, had a mean value of 16 flower buds on two year old wood. However, this selection also had fruit smaller than GI6. These three selections, Lake, Clare and Lincoln, had been selected in 2007 for only limited testing. The results from 2008 support that decision. The remaining rootstock selections had flower bud numbers ranging from 8 – 18 (GI6=13) and fruit sizes ranging from 6.8 g – 8.8 g (GI6=7.2g) suggesting that they are of sufficient interest to enter into large scale testing.

Table 2. Three-year mean (2006-2008) for terminal and lateral shoot growth, 2008 trunk cross-sectional area (TCSA), and % size of the GI6 control for the 10 MSU rootstock selections planted in Clarksville, MI.

Rootstock	Terminal shoot length (cm)[z]	Lateral shoot length (cm)[z]	TCSA (cm^2)	Vigor (% of GI6)[y]
		2001[x]		
Lake[w]	35.7	28.3	84	69
Iron	32.3	24.3	113	93
Gi6	38.7	31.3	122	100
		2002		
King	31.7	18.0	79	73
Garfield	27.0	25.0	115	106
Lincoln[w]	39.7	25.0	102	94
Glenn	42.7	32.0	103	95
Gi6	36.0	26.3	108	100
Kent	43.7	36.0	156	144
		2004		
Clare[w]	43.7	42.0	30	88
Clinton	36.7	34.7	36	106
Cass	41.7	36.0	50	147
Gi6	39.3	32.7	34	100

[z] Mean from two replicate shoots per tree.
[y] Calculated from TCSA.
[x] Rootstocks were selected among groups planted at three different dates.
[w]Lake, Lincoln and Clare have been selected for limited testing at WSU-Prosser and MSU

Table 3. Mean flower bud number on 2nd year wood (averaged over years 2007 and 2008) and mean fruit size (2008) for 10 rootstock selections with 'Hedelfingen' scion compared to the GI6 control. Crop load is not reported as a severe freeze resulted in over 90% flower bud death.

Rootstock	Flower bud number	Mean fruit size (g)
GI6	13	7.2
Cass	18	7.0
Clinton	12	6.8
Garfield	12	8.8
Glenn	11	7.6
Iron	8	8.5
Kent	15	8.6
King	14	8.3
Lake[1]	8	7.5
Clare[1]	8	6.4
Lincoln[1]	16	6.5

[1]These three rootstocks will only be tested at WSU-Prosser and MSU.

Rootstock genetic check: To avoid any potential clonal mix-ups, DNA fingerprints of the rootstock selections were developed using molecular markers. With the combination of a primer set specific for the self-incompatibility *S-RNase* locus and three SSR markers (PceGA59, PMS40, and PMS67), all 10 rootstocks can conclusively be differentiated from each other and GI5 and GI6. Fingerprints done on plant material received from Duarte Nursery did not uncover any clonal mix-ups. One liner from each of the 10 MSU rootstock selections was received from Duarte Nursery in September 2008 and will be tested for its identity within the next few weeks.

Rootstock test plot establishment: Liners of the MSU rootstock candidates were propagated at MSU in 2006 and planted at Willow Drive Nursery in spring of 2007. The 276 trees (including GI 6 controls) from these test rootstocks were budded in fall of 2007 and are ready for planting at WSU-Prosser Roza Farm in spring of 2009. Due to unequal liner numbers, the rootstock candidates will be represented in this plot by a minimum and maximum of 5 and 46 trees, respectively (Table 4). All rootstock candidates have 'Bing' scion and Kent also has 'Sweetheart' scion due to a large number of liners and excellent percentage bud take (90%).

In 2008 it became clear that the MSU rootstock candidate liners at Duarte Nursery that were multiplied by meristem culture would not reach sufficient girth to bud in time for spring 2009 planting (Fig. 1). Therefore, the planting of the test plots was delayed until spring of 2010. This year's delay allowed me to make two changes in the rootstock trial. The first change was the inclusion of GI 12 as a fourth control rootstock along with mazzard, GI 5 and GI 6. GI 12 was included in the plots after discussions with growers during a cherry meeting held at Prosser in July 2008. Secondly, as the trees from Duarte Nursery are produced in pot culture it was desirable for the control trees to also be produced in pot culture under identical growing conditions. Control trees of mazzard were already being grown in such a manner. Fortunately, will the help of Wally Heuser (GISELA®, Inc) and Protree, I was able to purchase sufficient GI 5, GI 6 and GI 12 liners that were shipped to Duarte Nursery in September 2008. These liners are being grown and budded to provide the control trees for the test plots. A complete breakdown of the trees designated for each of the text plots is provided in Table 5.

Table 4. Percent bud take and final tree numbers for the 10 MSU rootstock candidates and GI6 control trees grown at Willow Drive Nursery. These trees will be planted at WSU-Prosser in spring of 2009.

Rootstock selection	Scion	# of rootstocks budded	% bud take	Number of trees for 2009 planting
Cass	Bing	6	56	5
Clare	Bing	63	43	27
Clinton	Bing	35	77	27
Garfield	Bing	15	87	13
Glenn	Bing	9	89	8
Iron	Bing	26	38	10
Kent	Bing	51	78	40
Kent	Sweetheart	51	90	46
King	Bing	17	76	13
Lake	Bing	9	67	6
Lincoln	Bing	53	60	32
Gisela 6	Bing	36	56	20
Gisela 6	Sweetheart	36	81	29

Fig. 1. Liners of the MSU rootstock selections Iron (A) and Clare (B) growing at Duarte Nursery in summer 2008.

A

B

Table 5. Plant material being produced at Duarte Nursery for the test plots to be planted in 2010

Rootstock	**Scion (tree numbers) Prosser, WA**	**Scion (tree numbers) Oregon, Manson, Mattawa**	**Scion (tree numbers) Michigan**
Clare	Bing (25)	-	Rainier (5)
Lake	Bing (25)	-	Rainier (5)
Lincoln	Bing (25)	-	Rainier (5)
Cass	Bing (25), Sweetheart (25)	Bing (25), Sweetheart (25)	Rainier (5)
Clinton	Bing (25), Sweetheart (25)	Bing (25), Sweetheart (25)	Rainier (5)
Garfield	Bing (25), Sweetheart (25)	Bing (25), Sweetheart (25)	Rainier (5)
Glenn	Bing (25), Sweetheart (25)	Bing (25), Sweetheart (25)	Rainier (5)
Iron	Bing (25), Sweetheart (25)	Bing (25), Sweetheart (25)	Rainier (5)
Kent	Bing (25), Sweetheart (25)	Bing (25), Sweetheart (25)	Rainier (5)
King	Bing (25), Sweetheart (25)	Bing (25), Sweetheart (25)	Rainier (5)
Mazzard	Bing (25), Sweetheart (25)	Bing (25), Sweetheart (25)	Rainier (5)
Gisela 5	Bing (25), Sweetheart (25)	Bing (25), Sweetheart (25)	Rainier (5)
Gisela 6	Bing (25), Sweetheart (25)	Bing (25), Sweetheart (25)	Rainier (5)
Gisela 12	Bing (25), Sweetheart (25)	Bing (25), Sweetheart (25)	Rainier (5)
Total tree # per plot	625	1,650 (550 per site)	70

CONTINUING PROJECT REPORT **YEAR**: Continuous
WTFRC Project Number:

Project Title: Horticultural management systems for high value fresh & brine cherries

PI:	Anita Nina Azarenko	**Co-PI(2):**	Annie Chozinski
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Cooperators: Todd Einhorn (OSU-MCAREC); John and Karen Carter; David, Karen and Stacey Cooper; Marcus Morgan; Mike, Mel and Linda Omeg; John McClaskey; Greg Johnson.

Total project funding request: **Year 1**: 56,000 **Year 2**: TBD **Year 3**: TBD

Other funding Sources

Agency Name: None
Amount requested/awarded:
Notes:

Budget 1
Organization Name: Agricultural Research Foundation **Contract Administrator:** Dorothy Beaton
Telephone: 541-737-3228 **Email address:** Dorothy.Beaton@oregonstate.edu

Item	2008	2009	2010
Salaries	26,700	29,500	
Benefits	17,890	18,585	
Wages	4,000	4,000	
Benefits			
Equipment			
Supplies			
Travel	450	500	
Misc. (plot charges)	7,200	4,200	
Total	56,240	**56,785**	

Footnotes:

Objectives:

1. Identify cherry cultivars and rootstocks suitable for the processing cherry industry (e.g. brine, freezer) and those that may have potential for fresh market production in the Willamette Valley and cooler cherry growing districts.
2. Evaluate the effects of training system, rootstock and variety on tree performance, fruit quality and yield.
3. Refine and test a growing degree hour model for fruit growth in 'Bing', 'Sweetheart' and 'Regina' sweet cherry for the PNW. Expand to include other commercial cultivars in 2009.
4. Evaluate alternative sweet cherry cropping systems for orchard performance and profitability.

Significant findings and results

1. *Rootstock and varieties*

a. *2002 'Sweetheart'/MxM trial*- MxM60 and MxM2 trees have the greatest trunk cross sectional area (TCSA) while MXM2 had the largest increase in girth (Table 1). Yields were lower in 2008 while pack-out was better ('Fancy' 91%, 5% stemless). Mean stem pull-force (1043 g) and firmness (348 g/mm) were excellent. Peak production was in 2006 followed by two years of poor production most probably a result of poor pollination weather and frost. MxM60 produced 7.5 tons/acre during peak production. The highest cumulative yields have been from trees grafted onto MxM 2 and 39. MxM39 tree size is substantively smaller and tree density could be increased to enhance orchard productivity. Bacterial canker is minimal in this plot which is noteworthy given 'Sweetheart' is an extremely susceptible cultivar. The combination of topworking a susceptible cultivar onto a tolerant rootstock appears to be a viable solution to increasing productivity and reducing the incidence of canker.

b. *2002 Top-worked trees* – All cultivars on MxM60 had the greatest TCSA, followed by MxM14, Mazzard and Giessen 196-4 (Table 2). 'Stardust', self-fertile, had the highest yield on MXM60 (94 lb/tree), the other varieties 22% or less. 'Royal Ann' and 'Sweetheart' had the highest cumulative yields on Gi196-4. 'Stardust' on MxM60 had the greatest cumulative yields. Firmness ranged from 207-223 g/mm for 'Royal Ann', averaged 278 g/mm for 'Stardust' and was over 300 g/mm for 'Sweetheart'. Pull-force was between 730-885 g for 'Royal Ann', averaged 765 g for 'Stardust' and was over 1000 g for 'Sweetheart'. 'Sweetheart' pull-force was up to 200 g lower than last years. 'Stardust' fruit from MXM14 trees had the lowest stem pull force (range: 728-805 kg/cm^2). Stem pull force for 'Stardust' was at least 250 g lower than 'Sweetheart'. In 2008, stem pull force in this trial remained over the 600 g threshold to insure optimum stem retention. There was no cracking.

c. *2003 'Skeena'/Krymsk rootstock trial* –Krymsk 6 trees have the largest TCSA (131 cm^2). Yields could be grouped into three categories: Krymsk 6 (83 lbs/tree), Gisela 6 and Krymsk 5 (57 lbs/tree and the smallest fruit with highest firmness) and Mazzard (26 lbs/tree). Krymsk 5 has 60% mortality and suckers more than Krymsk 6. Two weeks of storage decreased an already low stem pullforce of 'Skeena' fruit from an average 439 g to 334 g. The potentially low stem pull-force of Skeena fruit may need to be managed and might be of concern in some growing seasons.

d. *2005 'Regina' rootstock trial* –In 2008 Gisela 6 had both the greatest increase in TCSA and overall TCSA (data not shown). Gisela 5 had the smallest TCSA and change in TCSA. Gisela 6 trees bloomed significantly earlier by 10 days. This block failed to thrive and was removed in September 2008.

e. *2006 NY blush and variety trial on Gisela 6* – The 10 numbered NY selections, 'Rainier', 'Regina', and 'Skeena' trees that are planted in three Oregon locations (LBF, Omeg and Cooper) had their first bloom but produced negligible yields and are well established after completing their third growing season. Trees are substantively more vigorous at Cooper Orchards. Peak bloom at LBF separated into two groups: early- peaking on April 15; and those later, which included 'Regina', on April 27. We expect to collect bloom, harvest and fruit quality data at all locations next year.

f. *2006 Dark cherry cultivar and rootstock trial*- No measurable yields were obtained during the third leaf. Trees at the Cooper Orchard grew significantly more than other locations. Of the eleven selections tested, only five showed differences in vigor due to rootstock (Table 4). 'Tieton' and 'Sylvia' had highest TCSA on Mazzard while 'Regina' and 13N 07-39 grew most on Gi 196-4. 'Sunset Bing' grew equally strong on Gi 196-4 and Mazzard. Generally, Gi6 rootstock had the smallest TCSA for all selections when compared to Mazzard and Gi196-4.

g. *2008 Organic 'Regina' Rootstock Trial* – 'Regina' trees that were low-budded onto Gisela 6, Giessen 196-4, MxM14, MxM39 and MxM46 were planted in May 2008. Six replications of three tree plots each were planted at 8' X 16' spacing and are being trained to a modified central leader. The plot is being managed organically, using compost as mulch and drip irrigated. After one season of growth there are no statistical differences in TCSA but Gi196-4 is largest and MxM46 smallest. Tim Dahle also planted a large trial with the same scion and rootstock combinations.

h. *Ovule longevity and pollen tube growth rates for 'Regina'*–Fifty percent mortality of ovules occurred in '05, '06 and '08 bloom seasons between 775-900 GDHs that accumulated from peak bloom (Fig. 1). In '08, this was about 7.5 days after bloom. Complete (100%) mortality occurred between 1400-1575 GDHs after peak bloom. In 2008, this was at about 14.5 days. Pollen tubes from 'Stark's Gold' alone and in combination with 'Sam' and 'Schneider's Spaete Knorpel' required the least GDH (1066-1124 GDH) to reach the base of the style (Table 5) and occurred approximately 9-11 days after hand pollination when 30 to 40% of the ovules would have been viable (Fig. 1).

2. *Training systems*

a. *2003 Training systems and rootstock trial trees* – 'Stardust' was removed from the trial in September 2007 due to high mortality caused by bacterial canker. 'Royal Ann' and 'Sweetheart' multiple leader trees continue to have a greater TCSA than central leader trees (Table 3). MxM14 has the largest TCSA across both training systems. 'Sweetheart' produced more fruit than 'Royal Ann' but the effects of the previous year's frost during bloom and the subsequent *Pseudomonas syringae pv. syringae* infection remain evident (data not shown). Yield was higher on Gisela 6 for both systems and both varieties. For 'Sweetheart', fruit on Giessen 196-4 had the greatest stem pull-force. No significant differences in color or fruit weight were observed. 'Royal Ann' trees were removed from the trial in September 2008 due to lack of interest in evaluating this cultivar in an intensive fresh sweet cherry training system.

b. *2006 On-farm training systems trial* –Steep leader trees of 'Early Robin' and 'Rainier' on Gisela 6 were more vigorous than central leader trees, followed by multiple leader trees (data not shown). However, the change in TCSA for 2008 was greatest on multiple leader trees for 'Early Robin' compared to steep or central leader. Trees are well established at both on-farm trials.

3. *Growing degree hour model*- Washington cooperators did not collect bloom data due to freezing temperatures during bloom. Reporting producers from Oregon made GA applications later than the model's predicted "beginning of stage III" biofix and previous averages for 'Bing', 'Regina' and 'Sweetheart'(Fig. 2). 'Sweetheart' was harvested, on average, earlier than last year (Fig. 2). 'Regina' and 'Bing' were harvested on average, 400 GDHs after the end of Stage III (1-2 days). The range of growing degree hours (GDH) from peak bloom to GA application and harvest for 'Bing', 'Regina' and 'Sweetheart' are summarized in Table 6. We encourage producers to consider 17,000; 20,500; 22,500 GDH as benchmarks for harvest of 'Bing', 'Regina' and 'Sweetheart'. Although grower cooperation is present, work demands near bloom time often precluded recording crucial peak bloom and color change dates for our model verification. Five new varieties, 'Chelan', 'Early Robin', 'Rainier', 'Lapins', and 'Skeena' were added for future GA application and harvest predictions.

4. Alternative cropping systems

2005 Alternative orchard floor and fertility management –There was no difference in yield, yield efficiency, peak bloom or TCSA between the landscape cloth and the compost treatment. First bloom was five days earlier in the landscape cloth treatment. Yield was not sufficient for fruit analysis. All leaf nutrients except Mg and B were higher in response to the compost treatment. Soil organic matter (SOM) at the LBF has decreased from 4.0 to 3.6% under the landscape cloth whereas mulched areas had increased SOM (18%) (Fig. 3). N mineralization was increased by 67% and 28% in the compost amended soils in spring and in autumn, respectively. Application of organic mulch increased soil microbial activity as measured by β-Glm (N-acetyl-β-D-glucosaminidase) (Fig. 4). Fungi have a more prevalent ability to produce this enzyme than bacteria. β-Glm was highly correlated with N mineralization. Decreased relative amounts of Gram-positive and general bacterial markers were found in the mulch plots. The percentage of omnivorous nematodes in mulch plots exceeded that of the cloth treatment. The landscape cloth treatment showed a higher incidence of herbivorous nematodes and a higher plant predator index. On-farm replicated trials at Omegs and Coopers also had a 30% increase in N-mineralization rates with the addition of straw. The application of an organic mulch/compost increased soil microbial activity of a key N-cycling enzyme as well as N mineralization potential.

Materials and Methods:

- Train trees, maintain orchard and obtain data on yield, fruit size, tree vigor, bacterial canker tolerance and other relevant data from the existing cherry trials (1.48 ha) which include:

  Lewis-Brown Farm Trials
  2002 'Sweetheart'/MxM rootstock trial (0.09 ha)
  2003 Training systems and rootstock trial (0.24 ha)
  2006 Variety and rootstock trial (0.15 ha)
  2006 NY blush and dark cherry cultivar trial (0.20 ha)
  2005 Alternative fertility management trial (0.63 ha)
  2008 'Regina' rootstock trial (0.17 ha)
  On-Farm Trials
  2006 Variety and rootstock trial- Omeg and Cooper
  2006 NY blush and dark cherry cultivar trial- Omeg and Cooper

- Continue testing and refine *growing degree hour* model of cherry fruit growth. Test models over several sites where weather stations are located. Collaborating orchardists in OR and WA will provide peak bloom, GA application and harvest dates. Preliminary data was collected for 'Chelan', Early Robin', 'Rainier', 'Lapins', and 'Skeena' (Table 6). Determine ovule longevity of 'Chelan'.

- *Alternative cropping systems-*
  - Alternative orchard floor and fertility management Biological and economic effects of two different methods of orchard floor and fertility management during orchard establishment and early production are being compared. 'Regina' on Gisela 6 was planted in 2005 at LBF (Corvallis), with 'Sam', 'Skeena', 'Sandra Rose', 'Stark's Gold', 'Schneider' and 'Sylvia' pollinizers. Geotextile (landscape) cloth and bark mulch/compost are used in the tree row. Trees are being pruned and trained to a central leader system. In 2009,soil and leaf analyses will be obtained, as well as, tree performance, yield and fruit quality data.

Results: See detailed findings in the following figures and tables.

Table 1. Effect of rootstock on tree performance and fruit quality in 2008 of 'Sweetheart' trees topworked onto M x M rootstocks.

MxM Rootstock [z]	Yield (lb)[x]	TCSA (cm^2)	Fruit size (mm)	Cumulative yield (lbs)
14	34.6 a	160 b	22.4 ab	170 b
46	20.9 ab	184 b	21.8 b	209 ab
2	26.7 ab	260a	22.5 ab	254 a
39	27.1 ab	203 b	22.9 a	247 a
60	18.3 b	271 a	22.7 ab	225 ab
MSD[y]	14.6	58	1.0	73

[z]Rootstocks were planted in 2000 at a 18' x 18' spacing in a completely randomized design with 6 replications and topworked in 2001. Fruit were harvested July 9.
[y]Means separation is by the Waller Duncan k-ratio t-test, k-ratio=100.
[x]Packout as a composite: 91% fancy, 5% stemless.

Table 2. Effects of rootstock on the tree and fruit characteristics of topworked 'Sweetheart', 'Stardust', and 'Royal Ann' in 2008.

Rootstock [z]	Yield (lb)[x]	TCSA (cm^2)	Fruit size (mm)	Cumulative yield (lb)
'Royal Ann'				
MXM 60	14.8 ab	170 a	20.8 ab	17.9
MXM 14	19.4 ab	162 a	21.1 a	22.5
Mazzard	9.5 b	107 b	20.9 a	11.9
Giessen 196-4	23.4 a	77 c	20.3 b	34.0
MSD	10.4	19	0.6	
'Stardust'				
MXM 60	94.1 a	162 a	20.8 ab	106.5
MXM 14	53.8 b	117 b	20.2 b	61.7
Mazzard	54.0 b	96 bc	21.0 a	60.8
Giessen 196-4	62.8 b	82 c	19.3 c	96.1
MSD[y]	24.3	22	0.8	
'Sweetheart'				
MXM 60	13.9	197 a	22.3	39.7
MXM 14	17.0	170 b	22.3	43.2
Mazzard	9.3	106 c	22.5	20.1
Giessen 196-4	14.3	91 c	22.7	61.9
MSD[y]	ns	22	ns	

[z]Rootstocks were planted in 2002 at an 18' x 18' spacing. Trees were topworked in 2003. Trees were mechanically harvested.
[y]Means separation is by the Waller Duncan k-ratio t-test, k-ratio=100.
[x]Packout as a composite: 53% fancy, 29% stemless and 1% culls.

Table 3. Effect of training system and rootstock in 2008 on the performance of 'Sweetheart', and 'Royal Ann' trees planted in 2003.

Rootstock [z]	Yield (lb)[x]	TCSA (cm^2)	Fruit size (mm)	Firmness (g/mm)	Pullforce (g)
'Sweetheart'					
Central Leader					
MXM14	12.1 b	104	23.8	361 ab	489 ab
Mazzard	16.3 ab	99	24.5	315 c	500 ab
Gisela 6	20.1 a	86	23.8	327 bc	486 b
Giessen 196-4	8.4 b	88	24.0	381 a	579 a
MSD[y]	7.7	ns	ns	38	89
Multiple Leader					
MXM14	16.5 a	122 a	23.8 b	326 c	500
Mazzard	8.8 b	103 ab	25.2 a	352 ab	554
Gisela 6	21.6 a	93 b	24.0 b	340 bc	527
Giessen 196-4	5.3 b	98 b	24.2 b	368 a	591
MSD[y]	6.4	23	0.5	26	ns

[z]Rootstocks were planted fall 2003 at a 9' x 16' spacing. 'Royal Ann' was harvested July 9-14. 'Sweetheart'was harvest July 28-29, 2008.
[y]Means separation is by the Waller Duncan k-ratio t-test, k-ratio=100.

Figure 1. Ovule longevity from 2005-2008 for 'Regina' ovules evaluated in The Dalles and Corvallis, OR.

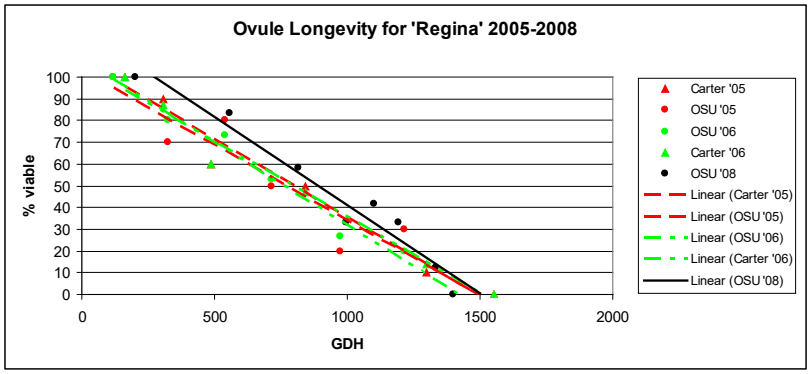


Table 4. Rootstock trial with 11 cultivars in three locations; LBF in Corvallis, and Omegs and Coopers in The Dalles, OR.

	TCSA (cm^2)			
Variety	Giessen 196-4	Gisela 6	Mazzard	MSD
'Tieton'	20.5b	20.8b	23.7a	2.2 [z]
'Early Robin'	22.0	22.2	20.5	ns
'Bing'	.	23.4	22.2	ns
'Benton'	21.4	22.0	20.6	ns
'Sylvia'	19.9b	18.7c	22.5a	1.1
'Rainier'	19.9	20.8	21.7	ns
13N 7-39	25.2a	20.4b	21.8b	2.4
'Sunset Bing'	23.6a	21.4b	23.2a	1.5
'Skeena'	20.7	19.9	21.1	ns
'Regina'	20.6a	18.3b	18.2b	1.0
'Sweetheart'	22.7	21.5	23.3	ns

[z]Means separation is by the Waller Duncan k-ratio t-test, k-ratio=100.

Table 5. Growing degree hours (GDH) and number of days (no.) required for pollen tubes to reach the base of the style after emasculation and hand pollination at the LBF in 2008. (28 April)

Pollen	GDH	No.
Sam	1238	11.5
Sam + Schneider	1238	11.5
Sam + Stark's Gold	1066	9.0
Schneider	1120	10.5
Schneider + Stark's Gold	1106	10.0
Stark's Gold	1124	10.5

Table 6. Ongoing GDH estimates for 'Bing', 'Regina',and 'Sweetheart' cultivars and preliminary estimates for GA and harvest GDH of 'Chelan', 'Early Robin', 'Lapins', 'Skeena' calculations for five more cultivars.

Variety	Grower I.D.	GDH to GA	GDH to Harvest
'Chelan'	TD1	10720	14388
'Early Robin'	TD5		15070
'Bing'	TD1	10025	17586
	TD2	11871	17484
	TD3	10521	16641
	TD3	.	18639
	TD3	11628	.
	TD4	10817	17970
	Average	10972	17644
'Rainier'	TD2	14369	18205
'Lapins'	TD1	10720	20659
	TD2	12949	20416
	TD3	13783	23309
	TD3	13505	18124
	TD3	13374	17956
	TD4	11361	20452
	Average	12615	20153
'Skeena'	TD1	11584	21112
	TD2	12403	20972
	TD4	10543	21194
	Average	11510	21093
'Regina'	TD1	11203	20730
	TD2	13696	21245
	TD3	14154	18576
	TD4	10243	21153
	Average	12324	20426
'Sweetheart'	TD1	12585	23748
	TD2	12871	23139
	TD3	14080	18474
	TD3	15814	21727
	TD3	.	19732
	TD3	12013	20562
	TD4	10817	22452
	Average	13181	21404

Figure 2. Fruit Growth Model to target timely GA sprays and predict harvest dates (GDH). Average grower dates for 2006 (long dashed line), 2007 (dash-dot line) and 2008 (black solid line) for these events. The short dotted line (OSU) indicates the beginning and end of Stage III as determined from 3 years of

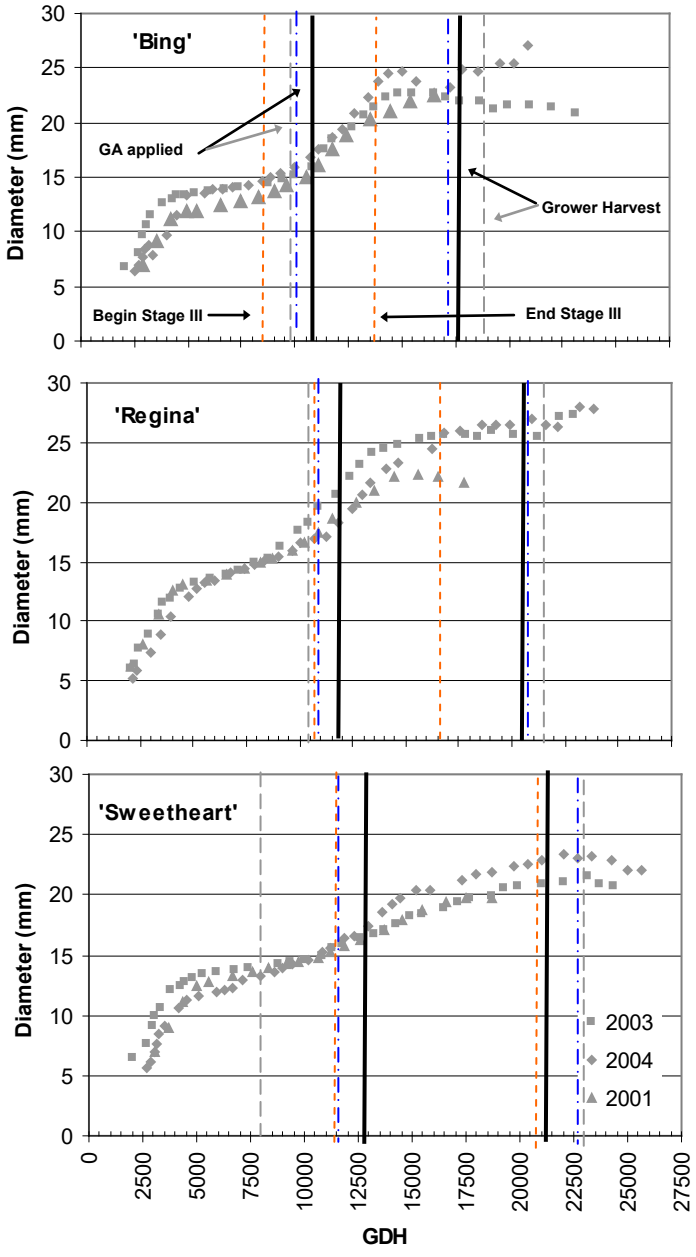


Figure 3. The influence of straw mulch/compost (OrAm) and landscape cloth (NoAm) on soil organic matter, potential mineralizable N, and soil NO_3^-.

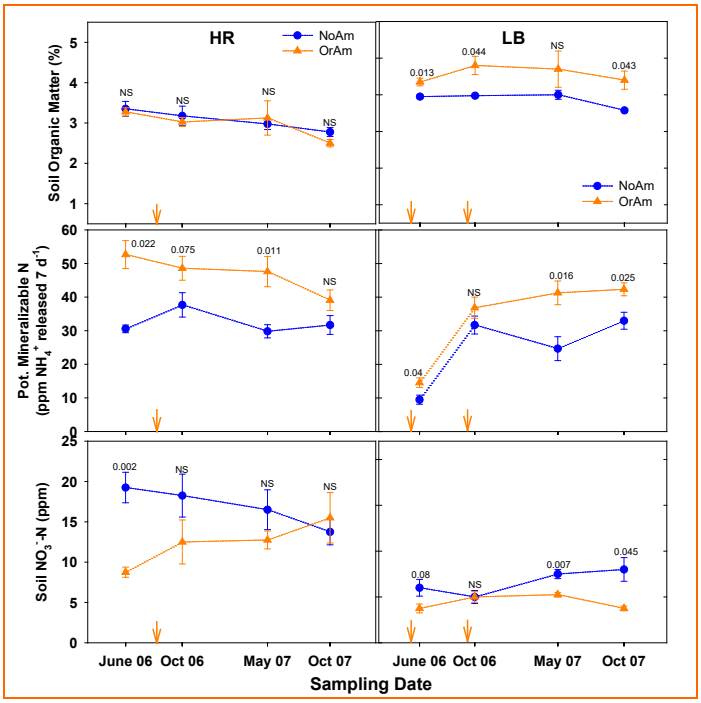
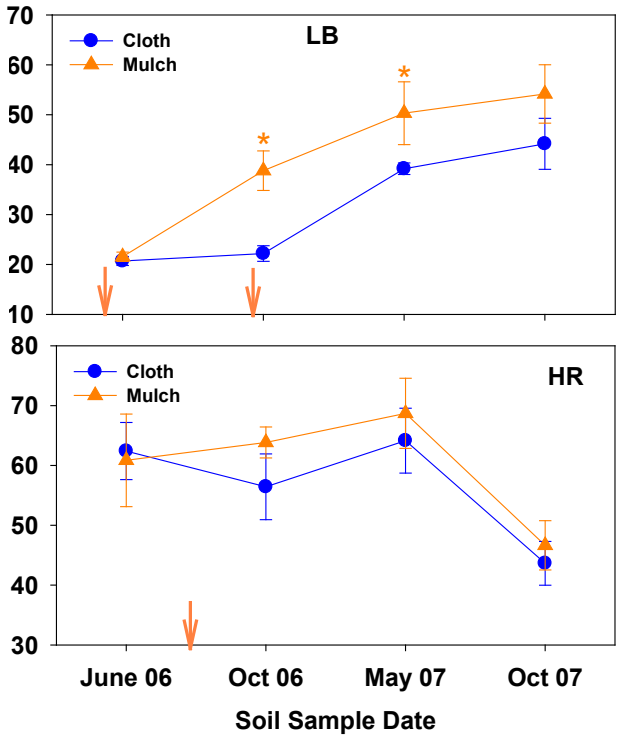


Figure 4. The influence of straw mulch/compost and landscape cloth on N-acetyl-b-D-glucosaminidase activity (µg/g soil) at HR and LB.



CONTINUING PROJECT REPORT **YEAR**: 2008

Project Title: Programs to increase yields of target fruit in cherries (WTFRC Internal Research)

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Budget 1:
Organization Name: WTFRC **Contract Administrator:** Kathy Schmidt
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Item	2008	2009	
Salaries	49,822	49,822	
Benefits	15,780	15,780	
Wages	8,182	8,182	
Benefits	3,389	3,389	
Equipment	424	786	
Supplies	4,255	1,245	
Travel	2,598	1,409	
Cooperator Payments	(16,500)	(25,600)	
Miscellaneous		600	
Total	67,950	**55,013**	

Footnotes: All chemicals and harvest supplies were provided by industry vendors. Clamshells were purchased. Costs were offset by $26,300 received for cherry cracking product testing from private companies.

NOTE: Budget for informational purposes only; research is funded through WTFRC internal program.

Acknowledgements: We would like to thank our grower collaborators Denny Hayden, Jim Kelly, Todd Badgely, John Verbrugge, Andy Arnold, Tim Perrault, Brent Sherer, Rick Derrey, Kyle Mathison, Jose Ramirez, Dave Poirier, and Dick Lutz.

OBJECTIVES

1. Investigate chemical blossom thinners to manage crop load in sweet cherry.
2. Evaluate spray programs to reduce rain-induced cherry cracking.

SIGNIFICANT FINDINGS

Chemical blossom thinning: No program tested in 2008 affected the crop load of Rainier or Sweetheart cherry trees.

Cherry cracking: Two products (P-08 and Biofilm) reduced natural cracking in Bing cherries. Bing, Rainier, Staccato, Tieton and Sweetheart show similar susceptibility to cracking in artificial cracking tests at harvest. Chelan is not susceptible to cracking when mature.

METHODS

Chemical blossom thinning: Trials were set up in two varieties (Rainier and Sweetheart on Mazzard, 7 years old, 9 x 18 spacing) as randomized complete blocks with six replications of 3 trees/plot. We evaluated four programs for their effectiveness in reducing cherry crop load: ATS (4%) or lime sulfur (2%, LS) plus Crockers Fish Oil (3%, CFO) were applied at 20 and 80% bloom; approximately 2 weeks after bloom, 2% LS and 3% CFO or 6 pt/acre BA (MaxCel) was applied. All applications were made by a PropTec sprayer at 100 gal/acre. Initial bloom counts and subsequent fruit counts were performed on two branches/plot. Standard harvest parameters including firmness, titratable acidity, sugar content, weight, diameter, defect incidence, and color were measured.

Cherry cracking: The trial series included 14 cherry cracking trial sites utilizing 6 cultivars and 5 products:

Material	Number of sites 2008					
	Bing	Rainier	Tieton	Sweetheart	Staccato	Chelan
BlueStim	2	3	2	1	1	
P-08	2		1		1	
Pace 1	3					1
Pace 2	3					1
Biofilm	1	1	1		1	

Trial designs were typically randomized complete block with 4 replications. All materials were applied by growers according to protocols developed collaboratively with product distributors:

Material	Concentration	# of appl.	Timing
BlueStim	4lbs/acre + 0.5pt. surfactant	2	light green; early pink
P-08	0.16 gal/acre	2-3	weekly
Pace 1	5% + water softener	4	weekly
Pace 2	0.80%	3	10 day intervals
Biofilm	1%	2	straw; 2 weeks later

Fruit was processed one day after harvest to determine standard maturity parameters and occurrence of natural cracks; some fruit was stored in regular atmosphere cold storage at 33F for 2 weeks for subsequent evaluation. Maturity parameters, weight loss, stem browning, and fruit pitting were

evaluated after storage. An artificial cracking test (modified after Christensen, 1972) was employed to assay cracking susceptibility under extreme osmotic gradients. Cherries were immersed in distilled water for up to five hours. After each hour, fruit that had split during that time period was removed and the numbers recorded. A weighted value (cracking index = CI) was generated from the results:

Hours submerged	1	2	3	4	5
Number of cracked fruit (Nc)	n_1	n_2	n_3	n_4	n_5
Factors for weighting (F)	5	4	3	2	1
Nc x F (weighted values)	n_1 x 5	n_2 x 4	n_3 x 3	n_4 x 2	n_5 x 1
Total weighted value					$\sum$ (Nc x F)
Maximum possible value					100*
Cracking index:				CI (%) =	$\frac{\sum(\text{Nc x F})}{100}$ x 100

* all 20 fruit/replication split after 1 hour: 20 * 5 = 100.

RESULTS AND DISCUSSION

Chemical blossom thinning: Aside from a reduction in firmness in MaxCel-treated Sweethearts, fruit set, weight, quality, and yield efficiency were not influenced by any treatment in either trial (Table 1).

Table 1: WTFRC cherry thinning results 2008.

TREATMENT	FRUIT SET (%)	FRUIT (g)	SUGARS (% Brix)	ACID (% Malic acid)	FIRMNESS (g / mm)	YIELD EFFICIENCY (kg/cm^2 LCSA)
Malaga Rainier / Mazzard - Malaga						
ATS	14 ns	9.3 ns	19.0 ns	1.109 ns	295 ns	0.05 ns
LS + CFO once	20	9.2	18.9	1.050	244	0.08
LS + CFO twice	19	9.3	18.6	1.034	278	0.06
MaxCel	21	9.2	19.7	1.122	288	0.07
Control	22	9.0	18.6	1.015	280	0.06
Malaga Sweetheart / Mazzard - Malaga						
ATS	15 ns	9.3 ns	21.2 a	1.541 ns	438 ab	0.04 ns
LS + CFO once	19	8.7	18.9 b	1.500	428 ab	0.04
LS + CFO twice	17	8.5	19.5 ab	1.505	413 ab	0.04
MaxCel	20	8.8	20.1 ab	1.445	406 b	0.05
Control	16	8.7	19.8 ab	1.507	445 a	0.03

Values with the same letter do not significantly differ.

As with the wider Northwest cherry industry, yields in our trial blocks were limited by frost and poor pollination conditions; low fruit set across both trials rendered marginal treatment differences difficult to observe (Table 1). The chemical thinning treatments used in these trials tend to be more effective in apple under warm conditions, especially those featuring lime sulfur and BA, but temperatures rarely exceeded 50F (Figure1). Given the historic inconsistency of cherry chemical thinning trials, we should not be surprised by lackluster results from programs applied in a spring like that of 2008.

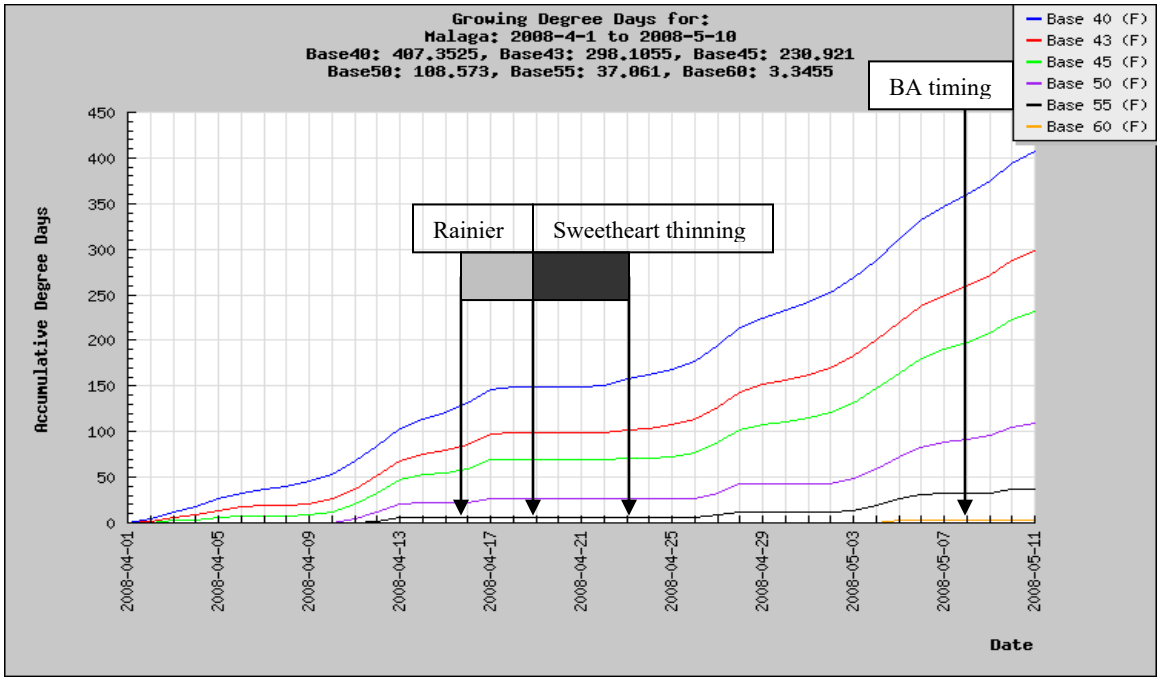

Figure 1: Growing degree days accumulated during the bloom and postbloom period and the relationship to the thinning spray applications (source: AgWeatherNet, www.weather.wsu.edu)

Cherry cracking: Only one trial site (Lutz) had background levels of natural cracking in excess of 10% (Table 4). The distribution of the natural cracks followed no specific pattern in Bing cherries. Chelan, Tieton, Rainier, Sweetheart, and Staccato split preferentially on the side or bottom. Lab-induced cracking revealed a general cracking susceptibility in all cultivars tested, except Chelan.

Bing (6 sites): Most blocks featured traditional open-center trees on Mazzard rootstocks and 20 x 20' spacing. P-08 reduced top cracking, which is likely indicative of rain-induced splitting. Biofilm reduced natural cracking and should be tested further in 2009. No other treatment effects were observed (Table 2). Natural cracking levels never exceeded 10% at any Bing site, making it difficult to assess product performance in field conditions. All products applied to Bing did increase the number of clean fruit, but we were unable to establish statistical significance. Further, prolonged bloom periods resulted in erratic fruit maturity, increasing variability and likely obscuring potential treatment effects.

The site with the most natural cracks for Bing (Hayden) had the most bottom cracks, which are typically more indicative of fluctuations in soil water potential rather than actual rain effects (Uriu et al., 1962). The cracking index did not consistently correlate well to field splitting. Inconsistent maturity likely affected postharvest cracking tests. We plan to repeat this evaluation next year and add a preharvest assay to a) establish periods of highest cracking susceptibility for different cultivars and b) track treatment effects over time.

Table 2: Harvest cracking evaluations and quality parameters for Bing cherries.

	SPLITTING					HARVEST FRUIT QUALITY		
	FIELD SPLITTING[z]				CRACKING INDEX			
TREATMENT	NONE (%)	TOP (%)	SIDE (%)	BOTTOM (%)	(%)	SUGARS (% Brix)	FIRMNESS (gm / mm)	COLOR (1 - 8)
Badgley Bing / Mazzard - Sawyer								
Bluestim	98 ab	1 ab	1 ns	1 ns	42 c	21.3 ns	277 a	6.15 ns
P-08	99 a	1 b	1	0	49 b	19.9	262 b	5.54
Control	95 b	4 a	1	1	60 a	20.6	266 b	5.54
Columbia River Bing / Colt - Pasco								
Pace 1	94 ns	2 ns	4 ns	0 ns	33 ns	22.9 ns	380 ns	5.83 ns
Pace 2	96	3	2	0	39	22.5	355	5.6
Control	91	4	5	0	32	21.5	364	5.92
Derrey Bing / Mazzard - Zillah								
Pace 1	95 ns	2 ns	2 ns	1 ns	27 ns	20.2 ns	289 ns	5.49 ns
Pace 2	96	2	2	1	33	21.1	291	5.7
Control	94	2	3	2	38	21.4	293	5.41
Hayden Bing / Mahaleb - Pasco								
Bluestim	92 ns	3 ab	1 ns	5 ns	36 ns	18.5 ns	301 ab	5.07 ns
Calcium	93	5 a	0	3	49	18.0	285 b	4.94
P-08	92	1 b	1	7	38	18.9	312 a	5
Control	90	4 a	1	5	41	17.5	300 ab	4.85
Manzana Bing / Mazzard - Royal City								
Pace 1	95 ns	2 ns	3 ns	0 ns	32 a	18.0 c	319 ns	4.7 ns
Pace 2	96	1	2	1	12 b	21.2 a	325	4.37
Control	96	3	2	0	29 ab	19.7 b	293	4.26
Stemilt Bing / Mazzard - Wenatchee								
Biofilm	100 a	0 ns	0 ns	0 ns	36 ns	18.2 ns	370 ns	4.89 ns
Pace1	100 a	0	0	0	30	18.8	355	3.97
Pace2	98 ab	2	1	0	40	18.0	357	3.89
Control	95 b	0	5	0	32	19.8	367	3.39

[z] Mean separation by Tukey's test and using arcsine data transformation for data presented as percentage ($p<0.1$). Values (n = 4) with the same letter do not significantly differ.

Rainier (3 sites): BlueStim and Biofilm did not reduce Rainier cracking beyond the low baseline levels of 94 and 98% undamaged fruit (Table 3).

Tieton (2 sites):. Neither BlueStim, P-08, nor Biofilm reduced field cracking; in one trial, BlueStim-treated fruit had more top cracks (Table 4). In one orchard, BlueStim increased postharvest stem browning, weight loss, and pitting. P-08 increased postharvest weight loss and Biofilm-treated cherries had browner stems (Table 5).

Sweetheart (1 site): This site had the highest natural level of cracking (27%), mainly expressed as side cracks. BlueStim did not influence the overall cracking incidence but did reduce side and top cracking. BlueStim-treated fruit had higher titratable acidity levels (Table 4). After storage, BlueStim-treated cherries exhibited less pitting (Table 5).

Staccato (1 site): BlueStim, P-08, and Biofilm did not affect cracking. Fruit firmness was increased by P-08 (Table 4). BlueStim and P-08 increased postharvest pitting (Table 5).

Chelan (1 site) : Both Pace materials improved sugar content at harvest (Table 4) and stem color postharvest (Table 5). While Chelan is generally not cracking sensitive, this cultivar might still benefit from preharvest applications for improved postharvest performance.

Table 3: Harvest cracking evaluations and quality parameters for Rainier cherries.

	SPLITTING					HARVEST FRUIT QUALITY	
					CRACKING		
		FIELD SPLITTING[z]			INDEX		
	NONE	TOP	SIDE	BOTTOM		SUGARS	FIRMNESS
TREATMENT	(%)	(%)	(%)	(%)	(%)	(% Brix)	(gm / mm)
Malaga Rainier / Mazzard - Malaga							
Bluestim	95 ns	2 ns	2 ns	1 ns	27 ns	19.5 ns	276 ns
Biofilm	95	2	2	2	24	19.4	274
Control	94	2	2	2	24	18.7	263
Perault Rainier / Gisela 6 - Harrah							
Bluestim	98 ns	1 ns	2 ns	0 ns	15 ns	18.2 a	277 ns
Control	98	0	3	0	20	17.2 b	264
Sherer Rainier / Gisela 5 - Zillah							
Bluestim	98 ns	0 ns	2 ns	0 ns	23 a	20.3 ns	286 a
Control	98	1	2	0	14 b	20.6	271 b

[z] Mean separation by Tukey's test and using arcsine data transformation for data presented as percentage ($p<0.1$). Values (n = 4) with the same letter do not significantly differ.

Table 4: Harvest cracking evaluations and quality parameters for Tieton, Sweetheart, Staccato, and Chelan cherries.

	SPLITTING					HARVEST FRUIT QUALITY		
					CRACKING			
		FIELD SPLITTING[z]			INDEX			
	NONE	TOP	SIDE	BOTTOM		SUGARS	FIRMNESS	COLOR
TREATMENT	(%)	(%)	(%)	(%)	(%)	(% Brix)	(gm / mm)	(1 - 8)
Carlson Tieton / G.6 - Pasco								
Bluestim	93 ns	0 ns	1 b	6 ns	34 ns	18.8 ns	265 ns	4.68 ns
P-08	89	0	5 a	7	39	18.7	247	5.01
Control	90	0	2 ab	9	30	19.4	260	4.68
Verbrugge Tieton / Gisela 6 - Buena								
Bluestim	91 ns	4 a	3 ns	2 ns	20 ns	19.0 ns	280 ns	5.96 ns
Biofilm	96	0 b	1	3	24	18.9	279	5.5
Control	92	1 b	3	5	39	18.8	282	5.16
Lutz Sweetheart / Mazzard - Azwell								
Bluestim	76 ns	5 b	16 b	3 ns	39 ns	24.0 ns	381 ns	5.51 ns
Control	73	7 a	18 a	3	39	22.7	354	5.67
Stemilt Staccato / Mazzard - Wenatchee								
Bluestim	98 ns	1 ns	1 ns	1 ns	9 ns	19.1 ns	355 b	5.34 ns
Biofilm	96	1	1	2	19	17.3	365 b	5.11
P-08	97	1	1	1	18	17.7	420 a	5.29
Control	95	1	3	2	18	16.6	361 b	5.26
Carlson Chelan / Mazzard - Pasco								
Pace 1	96 ns	2 ns	2 ns	1 ns	1 ns	21.3 a	286 ns	6.6 ns
Pace 2	96	1	3	1	0	20.7 ab	291	6.3
Control	97	1	2	0	2	19.0 b	308	6.23

[z] Mean separation by Tukey's test and using arcsine data transformation for data presented as percentage ($p<0.1$). Values (n = 4) with the same letter do not significantly differ.

Table 5: Evaluation of stem browning, fruit pitting, and weight loss after 14 days of cold storage at 33F for cherries treated preharvest.

	STEM BROWNING				PITTING			WT LOSS
TREATMENT[z]	0-25 (%)	26-50 (%)	51-75 (%)	76-100 (%)	CLEAN (%)	SLIGHT (%)	SEVERE (%)	(%)
Carlson Tieton / G.6 - Pasco								
Bluestim	98 ns	2 ns	0 ns	1 ns	18 ns	72 ns	10 ns	4 b
P-08	99	1	1	0	20	70	11	6 a
Control	98	0	1	2	23	58	19	3 b
Verbrugge Tieton / Gisela 6 - Buena								
Bluestim	38 b	42 ns	17 ab	5 ns	30 ns	48 ns	23 a	4 a
Biofilm	37 b	36	27 a	1	42	53	4 b	3 b
Control	61 a	29	10 b	1	44	51	6 b	3 b
Lutz Sweetheart / Mazzard - Azwell								
Bluestim	54 ns	25 ns	15 ns	7 ns	44 a	45 b	11 ns	5 ns
Control	56	24	15	4	24 b	62 a	14	3
Stemilt Staccato / Mazzard - Wenatchee								
Bluestim	81 ns	4 ns	9 ns	6 ns	9 b	84 a	7 a	3 ns
Biofilm	86	7	1	7	64 a	36 b	0 b	3
P-08	59	2	5	37	21 b	76 a	3 ab	4
Control	83	11	4	2	58 a	40 b	3 ab	3
Carlson Chelan / Mazzard - Pasco								
Pace 1	95 a	5 b	1 ns	0 ns	25 ns	68 ns	7 ns	4 ns
Pace 2	80 ab	11 ab	7	3	30	60	11	4
Control	62 b	21 a	10	7	38	51	11	4

[z] Mean separation by Tukey's test and using arcsine data transformation for data presented as percentage ($p<0.1$). Values (n = 4) with the same letter do not significantly differ.

It is exciting to have 5 new materials available to potentially reduce rain cracking. Further variety-specific testing should reveal best product/cultivar matches. For example, Biofilm performed well in Bing, but had no discernable effect in Rainier, Tieton, and Staccato. Our trials thus far have been hampered by a lack of significant summer rainfall (much to the relief of our grower-cooperators).

The artificial cracking test did not establish treatment effects on mature fruit at harvest. Based on past successes of this assay, we suspect a lack of treatment effect rather than a failure of the test. In-season testing might better capture changing cracking susceptibility during fruit development and demonstrate product efficacy.

Literature cited

Christensen, J.V. 1972. Cracking in cherries III. Determination of cracking susceptibility. Acta Agric. Scand. 22: 128-136.

Uriu, K., Hansen, C.J., Smith, J.J. 1962. The cracking of prunes in relation to irrigation. Proc. Amer. Soc. Hort. Sci. 80: 211-219.

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

Project Title: Branch induction in young sweet cherry trees without injury to bark

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Total project funding request: ***Year 1:*** 12,266 ***Year 2:*** **13,441**

Other funding Sources

Agency Name: None

Budget 1
Organization Name: WSU TFREC **Contract Administrator:** Mary Lou Bricker; Kevin J. Larson
Telephone: 509-335-7667; 509-663-8181x221 **Email address:** mdesros@wsu.edu; kevin_larson@wsu.edu

Item	2008	2009	
Salaries	6,000	**6,500**	
Benefits	2,100	**2,275**	
Wages	1,000	**1,000**	
Benefits	166	**166**	
Equipment	0	**0**	
Supplies	500	**500**	
Travel	2,500	**3,000**	
Miscellaneous	0	**0**	
Total	12,266	**13,441**	

Footnotes: n/a

Objectives:

1. Re-evaluate penetrants and/or high concentrations of BA/GA formulations to confirm the efficacy of either, or both, approaches for inducing lateral branching in sweet cherry trees without the requirement of damaging the bark.
2. Examine these modified treatment strategies for any undesirable effects on phytotoxicity in treated tissues of sweet cherry trees.
3. Confirm the branch-inducing properties of gibberellic acid alone; compare the branching responses from GA with the responses to standard BA/GA formulation treatments.
4. Test GA formulations to determine if any can be used for successful branch induction without the need for bark injury.
5. Assess whether applications to one side of one-year-old wood, without the use of bark cuts can produce one-sided branch induction, as is the case when small "nicking" cuts are used in conjunction with application of cytokinin/GA mixtures.

Significant findings:

1. Promalin (PR, 5,000 ppm) mixed with Pentra-bark surfactant (2% v/v) and painted on one-year-old vertical leader shoots of 'Skeena'/G.6 trees without nicking cuts was as effective as PR at the same concentration mixed with Regulaid (0.1% v/v) and applied to nicking cuts in the bark.
2. The best branching treatment in this trial was PR (5,000 ppm) + Syl-Tac surfactant (4% v/v) without nicking cuts. The higher concentration of Syl-Tac appeared to improve the response.
3. PR alone at 20,000 ppm (straight formulation) without nicking cuts was no better than control.
4. The cytokinins thidiazuron (TDZ) or forchlorfenuron (CPPU) at 5,000 ppm + Pentra-bark (2% v/v) applied without nicking cuts had no effect on branch development.
5. The GA formulations Novagib (Fine Americas) and ProVide (Valent Biosciences) were mixed with either Regulaid (0.1% v/v) or Pentra-Bark (2% v/v) and then applied to one-year-old shoots with or without nicking cuts. When combined with Regulaid, both formulations improved branching only when applied to nicking cuts. When combined with Pentra-bark, both formulations at concentrations of either 2,500 or 5,000 ppm improved branching to the same degree with or without nicking cuts. A control trial showed clearly that Pentra-Bark at 2% v/v alone or Syl-Tac at 4% v/v alone had no direct effect on branching.
6. No phytotoxic symptoms were observed in any of the treatments described above.
7. Second-leaf, UFO-trained trees of 'Early Robin' and 'Santina'/G.6 were treated on each of two dates (24 March or 9 April) with 3 cm bands of bioregulator solutions every 20-30 cm along the horizontal leader. PR was applied as follows: PR 5,000 ppm + Pentra-bark (2% v/v) banded on nicking cuts into the bark, the same solution banded without nicking cuts, PR 10,000 ppm + Pentra-bark (2% v/v) banded without nicking cuts or PR as the undiluted formulation (20,000 ppm) with no surfactant banded without nicking cuts.
8. Treatment on 24 March produced no branching effect at all from any treatment on either cultivar. During the ten day period following these treatments, the maximum daily temperature in the test orchard averaged about 45°F (7C) and the nightly minimum about 32°F (0C), with freezing temperatures every night but two during that period.
9. Treatment on 9 April resulted in all treatments more than doubling shoot formation on 'Early Robin' while only the nicking treatment increased branching on 'Santina'. During the ten day period following these treatments, the maximum daily temperature averaged about 62°F (17C) and the nightly minimum about 38°F (3C), with freezing temperatures on only one night during that period.

Methods:
Seven trials were initiated in 2008 to examine effects of cytokinins and gibberellins on branch induction on one-year-old sweet cherry leader shoots on conventionally trained trees or on the main horizontal leader shoot of UFO-trained (Uniform Fruiting Offshoots) sweet cherry trees. The trials focused on whether surfactants could substitute for bark injury in treating with cytokinin and/or gibberellic acid solutions for branch induction, whether GA alone could produce satisfactory branch development, and whether UFO-trained trees were at all amenable to the stimulation of offshoot development using bioregulators. We also were able to investigate the effects of temperature regime on branching response by the fortunate timing of bioregulator treatments on two cultivars in the same orchard.

Results and discussion:
Research in 2008 confirmed observations in 2007 that appropriate surfactants can substitute for cutting the bark in assuring that branch-inducing bioregulator products penetrate into living tissues in shoots. Several questions remain to be explored; perhaps the most important of those has to do with the relative importance of surfactant type vs. applied concentration. It may be that a variety of commonly-used surfactants will work if applied in high enough concentration. We have used up to 4% v/v (6 fl. oz./gallon solution) with good results and no phytotoxicity.

Gibberellic acid alone again proved effective for branch induction. In addition, GA was effective without the need for bark-cutting when either GA_4 (Novagib) or GA_{4+7} (ProVide) was combined with an effective surfactant, in this case Pentra-bark (2% v/v). In 2007, we showed that GA_3 (Pro-Gibb) was not a very effective inducer of lateral branching in sweet cherry; we have discontinued work with this formulation. We also observed that the cytokinins thidiazuron (TDZ) and forchlorfenuron (CPPU) were ineffective when applied at 5,000 ppm with a surfactant but without GA. In 2008, PR as the undiluted formulation (20,000 ppm) banded without the benefit of either surfactant or nicking was not impressive; this treatment induced branching in only one of five trials.

The work with UFO-trained trees in Buena allowed us to obtain at least a limited sense of differences in cultivar response as well as the effect of ambient temperatures on branch induction. 'Santina' proved to be generally less responsive in terms of branching than did 'Early Robin' to the same treatments applied on the same days. Temperature regimes following the two application dates were quite different; extended daytime cold temperatures and freezing overnight temperatures prevailed for the ten days following the first application date. For the comparable interval after the second set of applications, nighttime minima were not a great deal different from the same interval after the first application date. However, the daytime maxima averaged about 17°F (10C) higher than during the first interval, with the highest daytime maximum reaching 78°F (25C). These results indicate how critical it is that daytime warm temperatures follow immediately after a branch induction treatment. Growers planning to use this approach for branch induction should consult weather forecasting services and prepare to take advantage of any predicted warm periods while trees are in the green-tip stage. Waiting for optimum temperatures must be tempered with the knowledge that we have developed that if trees advance much beyond the green-tip growth stage, they become insensitive to branch-inducing bioregulator treatments.

Acknowledgments:
The assistance and support of the following people and organizations is gratefully acknowledged: Dean Christie, Kevin Forney, Marc Hanrahan, Dr. Ines Hanrahan, Dr. Chris Ishida, Bill Stringfellow, Dr. Matt Whiting, Bayer CropScience, Fine Americas, Valent Biosciences, Washington Tree Fruit Research Commission and the WSU Agricultural Research Center.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: CH-07-703

Project Title: Bioregulators, fruit loosening, mech harvest of sweet cherry

PI: Don C. Elfving
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Address: 1100 N. Western Ave.
City: Wenatchee
State/Zip: WA/98801

Cooperators: Dr. M.D. Whiting, WSU Prosser; Dr. E.M. Kupferman, WSU Wenatchee

Total project funding request: ***Year 1:*** 15,518 ***Year 2:*** 17,723 ***Year 3:*** **10,815**

Other funding Sources

Agency Name: NONE

WTFRC Collaborative expenses:

Item	2007	2008	2009
Stemilt RCA room rental			
Crew labor			**840**
Shipping			
Supplies			
Travel			**520**
Miscellaneous			
Total	**0**	**0**	**1,360**

Budget 1:
Organization: WSU TFREC **Contract Administrator:** ML. Bricker & Kevin Larson
Telephone: 509-335-7667 and 509-663-8181 **Email:** mdesros@wsu.edu and kevin_larson@wsu.edu,

Item	2007	(Granted for 2008 but not used)	2009
Salaries	7,000	7,500	**5,000**
Benefits	2,380	2,550	**1,700**
Wages	1,200	1,500	**1,000**
Benefits	138	173	**115**
Equipment	0	0	**0**
Supplies	800	1,000	**500**
Travel	4,000	5,000	**2,500**
Miscellaneous	0	0	
Total	15,518	17,723	**10,815**

Footnotes: Objectives scaled back in 2009, thus needs reduced.

Objectives:

1. GA may provide a tool for crop-load adjustment in sweet cherries by reducing return bloom, but it also affects the current season's crop quality. Explore the possibility of finding a suitable GA program that both contributes to reduced return bloom and favorably affects current season's fruit quality.
2. Alternative approaches to loosening sweet cherries for mechanical harvest will be explored using new bioregulator products that directly inhibit auxin transport from the fruit. When auxin transport is reduced, abscission layers are supposed to become active and loosening should occur. Such products might also be useful in conjunction with reduced rates of ethephon. Reducing the ethephon rate reduces its negative effects on fruit quality.
3. Alternative products will be examined for potential activity to offset or negate the negative effects of ethephon on fruit quality.

Significant findings:

1. 2008 was a difficult year for cherry growers. Extensive early cold and frost conditions compromised crop loads and crop quality in many orchards. No trials were carried out in 2008.
2. A GA trial on 'Sweetheart' cherry applied in 2007 to examine effects on crop load and fruit size in 2008 was damaged by cold temperatures and was not evaluated.

Methods:

Due to poor crop conditions in many orchards, no trials were initiated in 2008 to examine effects of various bioregulators on fruit loosening for mechanical harvest.

Results and discussion:

Objectives 1 and 2 have been completed. The work on GA for crop load control has not produced the desired results. Managing flowering with GA appears to be too variable to constitute a dependable technology. Plans are in place to continue work on Objective 3 of this project in 2009. In addition, I will be submitting a new proposal for an additional year, 2010, to allow a third year of trials in this project and to permit the follow-up of significant results from 2009 trials. In 2009, we are planning to focus on two factors.

1. An organic product called "Blue-Stim" (Monterey Ag. Resources) has been reported in California trials on sweet cherry to produce a small, but positive, effect on fruit flesh firmness in GA-treated fruit. It is possible that such an improvement could help offset the negative effect of preharvest ethephon treatment on flesh firmness. This research project and those that have preceded it have shown very clearly that preharvest ethephon under WA conditions typically produces significant reduction in fruit flesh firmness by the time of harvest. Other strategies for controlling firmness loss that have been tested in these projects have not proven effective for resolving that problem. We propose to evaluate the effect of preharvest applications of Blue-Stim in a commercial sweet cherry orchard on fruit flesh firmness at harvest and after air storage at 0°C. Standard ethephon treatments for fruit loosening and combinations of ethephon and Blue-Stim will also be made to determine if Blue-Stim can beneficially counteract the softening effect of ethephon.

2. Abscisic acid (ABA) is the last of the five naturally occurring plant hormones to become available commercially for exogenous use on plants. Several recent studies in Japan have shown an association between increased ABA levels in sweet cherry fruits and development of fruit maturity characteristics, including more red color and increased sugar content (see Kondo & Inoue, J. Hort. Sci. 72:221-227, 1997). A more recent European study (Blanusa et al., J. Hort. Sci. Biotech. 81:613-620, 2006) showed that application of ABA to sweet cherries near harvest stimulated rapid fruit loosening. No mention was made of effects on other fruit quality parameters. Formulated ABA material for exogenous application is now available so, in 2009,

we plan to compare the effects of ABA vs. ethephon for fruit loosening and effects on fruit quality.

3. We think both of these directions are of interest and may yield useful results that would warrant follow-up in 2010 to examine reproducibility of any beneficial effects observed in 2009. The proposed budget for 2009 and the planned budget for a new proposal in 2010 reflect the changes in plans documented herein.

4. Since the 2008 allocation of funds has already taken place, we request that no funds be allocated in 2009; funding intended for use in 2008 will be used in 2009 and, if approved, a new follow-up project in 2010 as described above.

Acknowledgments:
The assistance and support of the following organizations is gratefully acknowledged: Washington Tree Fruit Research Commission and the WSU Agricultural Research Center.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: CH-07-705

Project Title: Managing virus diseases detrimental to cherry production

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Cooperators: Mr. Bill Howell, Manager, NRSP-5, WSU-Prosser
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Dr. Lauri Guerra, WSDA, Prosser
Dr. Amy Iezzoni, MSU, MI
Dr. Nnadozie Oraguzie, WSU-Prosser
Dr. Tim Smith, WSU County Extension, Wenatchee

Total project funding request: $115,680 **Year 1**: $36,938 **Year 2:** $38,823 **Year 3:** 39,919

Other funding Sources
Agency Name: ANLA/HRI
Amount requested or awarded: $132,000 for each of first two years of this project (Project ended Sept 30, 2008)

Notes: WSU is including this information on other funding available for the support of similar research undertaken by the faculty member proposing this research. These resources are listed to identify other support granted for this research and are not included as a commitment of cost-share by the institution.

Budget 1:
Organization Name: Washington State University **Contract Administrator:** Mary Lou Bricker
Telephone: 509-335-7667 **Email address:** mdesros@wsu.edu

Item	Year 1 2007-2008	Year 2 2008-2009	Year 3 2009-2010
Salaries	15,523	16,292	$17,437
Benefits	6,324	6,983	$6,239
Wages	3,400	3,536	$3,600
Benefits	391	587	$648
Supplies	11,000	11,125	$11,695
Travel	300	300	$300
Total	36,938	38,823	$39,919

Footnotes: Itemized cost requirements for Year 3:
Salaries: 0.3 FTE Associate in Research and 0.2 FTE Nursery Specialist.
Wages: 360 hours of time slip labor.
Benefits: Rates are State-mandated.
Supplies: Greenhouse and field supplies ($3,000); molecular biology reagents and disposables ($8,695).
Travel: Travel to domestic plots and grower orchards.

Objectives:
Viruses induce losses over the life of an infected tree; recurring annual losses are cumulative and have a significant negative impact on the overall economic viability of farm operations. Despite past progress, some viruses that affect cherry production continue to challenge efforts to minimize their negative impact on profitability. Those that continue to be problematic from a management perspective include the viruses associated with little cherry disease, cherry leafroll, cherry raspleaf and the rusty mottle group of viruses (foveaviruses). Specific objectives of this project include:

1. To develop laboratory tests that increase grower accessibility to rapid virus diagnosis. The ability to correctly identify the underlying cause of poor fruit production is required for appropriate corrective measures to be implemented.
2. To develop alternative methods of managing virus diseases with particular reference to those where root-grafting and/or nematode transmission play significant roles in disease epidemiology. Rootstock selection may offer relief from the soil borne viruses.

Significant findings:

- The geographical distribution of cherry leafroll virus continues to expand in WA.
- Pollen transmission, considered a key element in cherry leafroll virus spread, is very inefficient.
- Root grafting is the primary mode of cherry leafroll virus transmission within an orchard.
- A broad spectrum assay was found to be effective in detecting viruses from three genera of viruses that infect sweet cherry. Thus, significant progress was made in establishing cost effective detection strategies for viruses.

Methods:
Objective 1. Working with growers and fieldmen, we examine new disease situations to identify the causal agent. Often, as is the case with little cherry disease, the only "symptom" is poor fruit yield and quality. If trees are not performing at acceptable standards of quality and quantity, and there is no apparent non-viral condition that would lead to this condition (e.g. cold injury, verticillium wilt), samples are collected for analysis. Several common viruses can be detected by cost-effective enzyme-linked immunosorbent assays (ELISAs). Additionally, extracts from field samples are prepared and analyzed at the molecular level for the presence of nucleic acids of known pathogens. The selection of a particular testing strategy is based on symptomatology, disease distribution, and orchard history. This information forms the basis from which assay selection and management strategies are developed.

In parallel with this collaborative work with growers, experimental extraction and assay protocols are being applied to a wide range of reference diseases maintained in a secure collection and to samples obtained from grower samples. Data is collected and collated to establish a database of reliable methods to evaluate future grower samples.

Objective 2. Cherry leafroll virus is increasing in its significance in the cherry industry of the Northwest. Two major routes of transmission are suggested for this virus: pollen transmission and transmission through root grafting. Experimental but controlled application of pollen from virus-infected trees into flowers of non-infected trees is being used to estimate the relative frequency of pollen transmission. For the management of soil-borne viruses, the abilities of various rootstocks and rootstock/interstock combinations to restrict virus transmission are being assessed. Our studies indicate that 'Colt' rootstock offers at least some field resistance to the transmission of cherry leafroll virus by root grafting. Therefore, we are systematically investigating resistance of 'Colt' rootstock to this virus. Other rootstocks will also be evaluated for possible field resistance. For consistency, 'Bing' is used as the scion throughout the trials. Separate trees will be inoculated above the graft union or below the graft union, and evaluated for sensitivity and monitored for the type of reaction to this virus. Combinations of rootstocks/interstocks with 'Bing' scions are also being established to evaluate horticultural performance.

Reports by other researchers in California and Colorado suggest that 'Colt' rootstock may offer some field resistance to cherry raspleaf virus, a virus that is also of increasing importance in WA cherry production and continues to be problematic in other western states. Therefore, in parallel with the experiments described above, individual 'Bing' trees with different rootstock/interstock combinations will be inoculated with buds infected with cherry raspleaf virus.

Results and discussion:

Objective 1. Development of laboratory tests to increase accessibility to rapid virus diagnosis.

There is ever increasing emphasis for large fruit in the sweet cherry marketplace. All viruses divert plant resources from growth and fruit production to virus replication, but the degree to which this affects returns on fruit production depends on the virus and the tree cultivar. There are several virus diseases that directly impact efforts to produce better quality fruit.

The principle obstacle in the routine use of molecular assays in evaluating grower samples is the relatively high cost per sample of extraction and isolation of the nucleic acids targeted by the assay. We evaluated the utility of a medium throughput automated system obtained through independent funding. This approach significantly reduced the cost of individual sample preparation and reduced the risk of sample cross-contamination. Results clearly demonstrated that this system is satisfactory as the basis for molecular analyses.

Over the last two years, our studies focused on little cherry disease caused by *Little cherry virus-1* (LChV-1). Concern is growing about the global distribution of LChV-1 for lack of adequate diagnostic methods. Last year we reported on the characterization of several strains of LChV-1 from Washington State and other cherry producing regions of the world. This resulted in the development of a reliable molecular assay for this virus. A molecular assay for Little cherry virus-2 (LChV-2) was developed by our project and published several years ago. Thus, reliable molecular assays are now available for both of the viruses known to cause little cherry disease.

During the first two years of this project, techniques developed in our laboratory were used to obtain sequence information from 18 isolates of foveaviruses associated with distinct disease symptoms in cherry, plus seven isolates of green ring mottle virus that are symptomless in sweet cherry. Analysis of these data confirmed the ability of the "universal" foveavirus primers to reliably screen for members of this virus genus. Recently, another molecular assay that will detect foveaviruses was published (Foissac et al., 2005) and has received increasing acceptance internationally. This is a polymerase chain reaction-based system that uses "TriFoCap" primers, and is capable of detecting members of multiple virus genera including trichoviruses, foveaviruses and capilloviruses. Thus, this single test would detect a wide range of viruses of concern to the fruit tree industry. We applied the "TriFoCap" assay to extracts of *Prunus* samples. Based on results from greenhouse woody indexing results, 18 virus isolates were selected for analysis. Extracts of the medium throughput automated system described above were analyzed with the "universal" foveavirus assay and the "TriFoCap" assay system. Any positive reactions were further investigated by sequence analysis to confirm test results. The "universal" foveavirus assays correctly identified three out of five trees identified by greenhouse indexing as likely being infected with foveaviruses. There were apparently two false negative results in the molecular assay. The "universal" foveavirus assay also gave five positive reactions for trees that were subsequently shown not to be infected with foveaviruses (false positives). These instances required sequencing to resolve the test results. In contrast, there was 100% agreement between results from the "TriFoCap" assay system and greenhouse woody indexing. Further analyses of "TriFoCap" positive samples revealed the presence of a unique virus in two samples. These same two samples were positive by woody indexing in the greenhouse, but negative by the "universal" foveavirus assay. Sequence analysis of the "TriFoCap" amplification products indicates that the two viruses are closely related to, but distinct from known foveaviruses. This critical observation highlights the danger of relying on molecular assays with narrow specificities for disease diagnosis. Although broad spectrum assays are very effective in revealing the presence or

absence of viruses of these virus genera in cherry samples, they do not inform which virus might be present. Assays are under development to amendments to this assay that would allow subsequent refinement of the diagnosis made with the broad spectrum "TriFoCap" assay.

Impact: Innovations in virus testing make the availability of rapid and accurate virus testing more accessible to growers. Once a virus diagnosis is confirmed, appropriate recommendations for a disease management strategy can be developed. The diseases associated with these viruses often resemble physiological conditions. Therefore, it is critical to understand the underlying cause of poor cherry production and tree growth. An incorrect diagnosis would result in ineffective and, frequently, costly investments in remedial treatments with little or no relief from poor production. Correct diagnosis of a viral agent allows the grower to make better economic decisions about managing his investment. The appropriate response to a disease situation depends on several factors including the virus and the scions and rootstocks that are involved, the proximity and type of wild vegetation and other crop plants, insect populations. Analyses of all these factors influence the most cost effective response.

Objective 2: Development of alternative methods of managing viruses.

Cherry leafroll virus (CLRV) continues to pose a serious risk to sweet cherry production in the Pacific Northwest. Assays performed by request in our program demonstrate that the geographic distribution of known CLRV infections is expanding. One of the major objectives of this project is to identify strategies to help minimize the effect of this virus on production. In an effort to obtain a measure of the risk of pollen transmission, in May 2006, 800 blossoms were pollinated with CLRV-infected pollen. As previously reported, at the time of shuck fall, 50% of the pedicels tested contained CLRV detectable by RT-PCR. In spring 2007, leaves adjacent to each of the labeled spurs were collected and tested for CLRV by RT-PCR. No samples yielded positive results. In 2008, leaves adjacent to the clusters were tested by ELISA; again, all were negative.

In 2007, the pollination experiment was repeated and flowers of 'Van' were hand pollinated with 'Bing' pollen collected from CLRV-infected trees. Each test cluster of approximately 20 blossoms was surrounded by an organdy cage to prevent the introduction of pollen from other sources and to limit movement of Western flower thrips. On each of four trees, five cages contained blossoms pollinated by hand with infected pollen; another five cages contained blossoms pollinated with infected pollen plus the addition of 100 Western flower thrips, and a final set of five cages contained blossoms, but no external pollen was introduced. Thus, there were a total of 400 blossoms (20 blossoms per cage; 5 cages per treatment on each of four trees) used in each of the three treatments. In the case where no pollen was introduced, there was no fruit set. This demonstrated that the organdy cages successfully excluded sources of compatible pollen from adjacent 'Bing' trees. In the cages into which infected pollen was introduced, there were 115 fruit in the absence of added thrips, and 117 fruit in the presence of added thrips. Each fruit cluster within a cage was tagged and labeled for later identification. In June of 2007, fruit was harvested from each cage and the pedicels extracted and tested by RT-PCR. Overall, 20% of the pedicels from cages into which CLRV-infected pollen was introduced yielded positive results by RT-PCR; there was no difference between the cages with or without added thrips. In contrast to the 2006 experiment, most fruit was carried to maturity. When leaves adjacent to spurs of fruit formed in 2007 were sampled in spring 2008, no samples yielded positive results. This was consistent with results from the pollination experiments begun in the previous season. Thus, in each of two years, although CLRV is present in the pedicels of fruit after blossoms are pollinated with virus-infected pollen, the virus is present in the stems of developing fruit during the year in which it is pollinated, but has not replicated to detectable levels in the adjacent vegetative tissue.

An additional experiment was initiated to monitor the rate of movement of CLRV through infected trees. This study was performed in the same orchard site as the pollen transmission experiments where trees are trained to four or five major scaffold limbs. Each of two major scaffold limbs was T-budded with a bark patch from an infected 'Bing' tree in August 2006. In spring 2007, CLRV could

be detected only in the shoots immediately adjacent to the buds. In spring 2008, leaves from the base of each scaffold limb were assayed by ELISA for CLRV (Table 1). After one full growing season, CLRV had not moved and replicated to a level detectable by ELISA in all parts of the tree. The amount of inoculum introduced by bark patch inoculations is much greater than that introduced through pollination. This suggests that at least another year of observation should be considered to determine if CLRV had been successfully transmitted from infected pollen during the experiments initiated in 2006 and 2007. In studies of CLRV pollen transmission in Europe, trees were monitored for four years after pollination in order to assess the rate of pollen transmission in other perennial species. Therefore, we propose to continue to monitor trees of the pollination experiment at the site annually for the development of detectable levels of infection.

Table 1. One-year old shoots of two major scaffold limbs of each tree were inoculated with cherry leafroll virus-infected bark patches in the autumn of 2006. In spring 2008, leaves at the base of each scaffold limb were assayed by ELISA for cherry leafroll virus.

Tree designation	# scaffold limbs positive / # scaffold limbs tested
R-6	1/4
S-6	4/5
T-6	2/5
U-6	5/5
Total scaffold limbs infected	12/19

Our previous research on the epidemiology of cherry leafroll virus demonstrated that transmission through root grafts is an important route of tree-to-tree spread within an orchard. Rootstocks that provide field resistance to cherry leafroll virus could minimize or even eliminate this significant route of infection. A small on-farm trail was established in 2000 to test the influence of rootstock on this mechanism of virus transmission. Twenty trees of 'Bing' on 'Colt' rootstock and 20 trees of 'Bing' on 'Mazzard' rootstock were planted in plots in three separate orchards. Each year they are monitored for cherry leafroll virus. Five of the 20 trees on 'Mazzard' rootstock became infected, some within the second growing season. None of the trees on 'Colt' rootstock became infected with cherry leafroll virus. During this same period, many trees planted by growers on 'Mazzard' rootstock at these locations have also become infected . All flowers that developed during the first four years of this study were removed so that the earlier bloom of 'Colt' did not influence results. These results suggest that 'Colt' offers some protection to root-graft transmission of cherry leafroll virus in the field setting. At this time, it is unknown if the 'Colt' rootstock is immune to cherry leafroll virus, if 'Colt' retards movement of virus from root grafts to the scion, or if 'Colt' is recalcitrant to root grafting.

Cherry raspleaf is a nematode transmitted virus that is problematic in discrete areas of production, although the incidence of raspleaf disease appears to be increasing. Anecdotal evidence from researchers in Colorado and California suggests that 'Colt' may also offer some field resistance to cherry raspleaf virus. This has not been addressed in a rigorous manner. The movement of cherry raspleaf virus in various rootstock, interstock and scion combinations will be examined in parallel with the studies on cherry leafroll virus.

In the research orchard at WSU-IAREC, finished trees have been established (Table 2). Additional trees were budded in 2008 (Table 3) to increase the complement of available trees so that a total of 117 trees will be available for studies commencing in 2009. All rootstock/interstock combinations will be evaluated for sensitivity and reaction to cherry leafroll virus or cherry raspleaf virus. Individual trees will be inoculated by chip budding to the rootstock to mimic the rootstock's response to root-grafting. Other trees will be infected by inoculation of the scion to determine tree response to

infection through pollen transmission. The Z-interstem was included to provide additional choices of rootstock and to increase the precocity of trees growing on 'Colt' rootstock.

Table 2. Rootstock/interstock combinations are budded with 'Bing'. The finished trees are established in the orchard and ready for inoculation.

Rootstock	Interstock	Number of trees
Citation	Z-Stem	16
Myrobalan 29C	Z-Stem	3
Gisela 5	n/a	8
Gisela 6	n/a	8
Gisela 12	n/a	5
Colt	n/a	10

Table 3. Rootstock/interstock combinations are budded with 'Bing'. The trees in the orchard or potted plants in the greenhouse were budded in summer of 2008. They will be ready for inoculation during 2009.

Rootstock	Interstock	Status	Number of trees
Citation	Z-Stem	Budded in field	1
Myrobalan 29C	Z-Stem	Budded in field	3
Gisela 5	n/a	Budded in field	33
Gisela 6	n/a	Budded in field	5
Colt	Z-Stem	Budded in greenhouse	12

In an effort to find a long-term solution to cherry leafroll virus in our industry, discussions with cherry breeders (Iezzoni and Oraguzie) led to the possibility of breeding for disease resistance. In 2008, pollen was collected from 'Colt' (*Prunus avium* × *P. pseudocerasus*) and Iezzoni used this to pollinate 'Montmorency' (*Prunus cerasus*). From this cross, Oraguzie produced 100 seedlings that will be tested for sensitivity and/or resistance to cherry leafroll virus. Plants with promising virus responses will be examined for their potential as a rootstock. Next season, crosses between 'Colt' and sweet cherry (*Prunus avium*) will be performed. In both cases, buds from seedlings will be chip budded to 'Bing' on 'Mazzard' rootstock. After the buds are allowed to heal, the 'Bing' will be chip budded with cherry leafroll virus-infected buds. The graft union between the seedling and the 'Bing' will be examined for it response to the virus.

Impact: Cherry leafroll virus continues to be a challenge in Washington's cherry production areas. Confirmed data relating to the aerial transmission of this serious virus will aid in management of the disease. Preliminary data suggest that the virus apparently is very inefficient in its ability to migrate past the abscission layer between the pedicel and the spur to establish infection in vegetative portions of the tree. Further observation is required. Strategies to minimize transmission via root grafting will slow the spread of the virus and its associated economic losses within infested orchards.

Cherry raspleaf virus is a nematode transmitted virus of sweet cherry trees with a wide host range. Its distribution is limited, but where it occurs, it is devastating. The host range includes apples and a number of weedy plants common in orchard flora. In combination with the nematode vector, this means that control options are very limited to the grower. The identification of rootstocks that offer field resistance to nematode transmission will provide the growers with the first real option for disease management. The use of interstocks to improve the horticultural production on the virus resistant rootstocks is an important component of this project.