2013 NW Cherry Research Review
Red Lion, Yakima

Tuesday, November 13, 2012

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	8:05	McFerson	Research update	
			Final Project Reports	
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11	8:30	Eastwell	Reducing the impact of virus diseases on cherry production	10-12
19	8:45	Eastwell	Developing a management strategy for little cherry disease	11-12
24	9:00	Oraguzie	Start-up funds and support for a full time technician	10-12
29	9:15	Iezzoni	Virus elimination/senstivity assessment of MSU rootstocks	12
32	9:30	Shearer	Insecticide management of SWD in cherry orchards	12
42	9:45	Einhorn	Influence of cropload level on fruit size and quality	10-12
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55	1	Iezzoni	Establishment and testing of MSU sweet cherry rootstocks	11-13
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Final Project Report
WTFRC Project Number: CH-10-105

Project Title: Improved management of powdery mildew of sweet cherry

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Other Funding sources: Washington State Commission on Pesticide Registration $37,500 (QoI resistance objective)

Total Project Funding: $161,019 **Year 1:** 57,280 **Year 2:** $46,834 **Year 3:** $56,905

Item	2010	2011	2012
Salaries1	35,281[1]	36,692	33,612[2]
Benefits	14,818	15,411	17,142
Supplies	5,181[3]	5,188[3]	4151[3]
Travel[4]	2,000[4]	2,000[4]	2,000[4]
Total	**$57,280**	**$59,291**	**$56,905**

Objectives

1) Determine the presence and regional extent of resistance to QoI fungicides in populations of *Podosphaera clandestina* in Eastern Washington.
2) Investigate early or mid-season cherries as potential sources of inoculum for infection of later cherry fruit. The initial step in the process will be determining whether there is a large inoculum increase in a cv. 'Bing' orchard once fungicide applications are terminated at harvest.
3) Investigate irrigation sets during late dormancy as a means to deplete overwintered inoculum supplies prior to the availability of susceptible host tissue.
4) Investigate various irrigation regimens and nitrogen fertilizer regimens, on the incidence and severity of powdery mildew on cv. 'Lapins' cherries.
5) Investigate full-season fungicide programs for effectiveness in reducing the production of ascocarps (cleistothecia) and therefore the amount of potential carryover inoculum.

Significant Findings

- A leaf disc bioassay developed in 2010 and 2011 was used to screen isolates of *P. clandestina* for QoI resistance. A total of 18 and 30 isolates were collected from the Yakima, Wenatchee, and Columbia Valleys in 2011 and 2012, respectively. Most isolates were sensitive to trifloxystrobin at up to 4x labeled rates but several appeared less sensitive at $\geq$ 160 ppm. This technique also resulted in the development new technique to study the effects of temperature and relative humidity on infection and pathogen sporulation.

- Initial attempts to store powdery mildew over time were successful: *P. clandestina* survived 12 months following freeze drying at -30 C and long-term storage at -80 C (-112 F).

- The new Burkard cyclonic air sampler was compared to rotary impaction traps for the molecular detection of *P. clandestina* in the orchard air. The new sampler was found to be vastly superior to because of improved dependability and the ability to take daily samples with far less labor input and fewer steps in the laboratory components of the assay. PCR Cp values obtained from these air samples were significantly correlated (r = -0.86; $P < 0.001$ in 2010; -0.65; $P < 0.01$ in 2011) with daily spore counts taken by volumetric traps. Use of the cyclonic trap confirmed a large increase in aerial spore populations following harvest.

- Experiments designed to force (using irrigation) ascospore (in order to deplete the overwintered inoculum source) were more promising in 2012 than in 2010 and 2011, probably due to improvements in the molecular air sampling technique. PCR signals increased immediately after a period when water was applied for frost control. The assay most likely detected an irrigation-induced ascospore release.

- Standard fungicide programs were applied from shuck fall to harvest in 50% of a cv. 'Bing' orchard at WSU-IAREC. The other 50% was left untreated for the entire season. Disease severity and airborne inoculum rapidly increased in the treated section after fungicide applications were discontinued at harvest. Levels eventually exceeded those in the orchard section where no fungicides were applied. These results indicate that (once fungicide applications cease) early- to mid-season cherries may serve as a potential source of inoculum for late-maturing cherries in the vicinity. The postharvest increase in disease severity may also serve to significantly increase the production of ascocarps, the overwinter survival propagule. These results indictate the full-season disease management, similar to approaches

used for grapevine powdery mildew, may serve to mitigate problems with cherry mildew over time and that an areawide approach to mildew management should be considered.

- Full-season fungicide programs (standard preharvest programs + postharvest oil applications) were found to have no significant effects on ascocarp (cleistothecia, chasmothecia) production in the orchard. Oil applications had a significant effect on chasmothecia production in the nursery, indicating that they timing of oil applications may be critical and the narrow window (10-14 days in the nursery) may have been missed in previous orchard studies.

- Irrigation / fertigation experiments were inconclusive in 2010 and 2011 due to low mildew incidence and severity but voluminous amounts of microclimatic data were collected. Mildew incidence and severity was again low in 2012, but additional microclimatic data were collected.

Results and Discussion

Objective 1. A leaf disk bioassay was developed to study practical resistance of orchard isolates. The technique involves inoculation of 'Sweetheart' leaf disks with a known quantity of conidia of *P. clandestina*. Leaf disks are incubated 10-14 days in petri plates containing 1.5% water agar. Fourteen of 18 isolates tested were sensitive to trifloxystrobin at all concentrations tested. However, isolates from 4 locations (2 from Mattawa and 2 from Benton City) grew on leaf disks treated with up to 320 ppm of fungicide. Two isolates grew at 1280 ppm trifloxystrobin. The obligate parasitic (needs to be cultured on an actively growing cherry plant) nature of *P. clandestina* makes studies of this nature inherently difficult. Methods to facilitate long-term storage of isolates were investigated during 2010-2011. Initial attempts to store powdery mildew over time were successful: *P. clandestina* survived 12 months following freeze drying (at -30 C) followed by storage at -80 C (-112 F). Further development of this technique should accelerate fungicide resistance studies and breeding efforts.

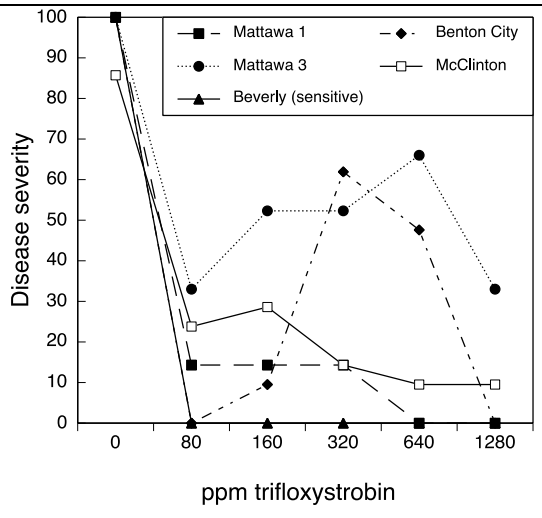

Figure 1. Growth of putative resistant isolates of *P. clandestina* on leaf disks of sweet cherry treated with 0-1280 ppm trifloxystrobin (Flint or Gem) in 2011. A sensitive isolate (Beverly) is included for comparison.

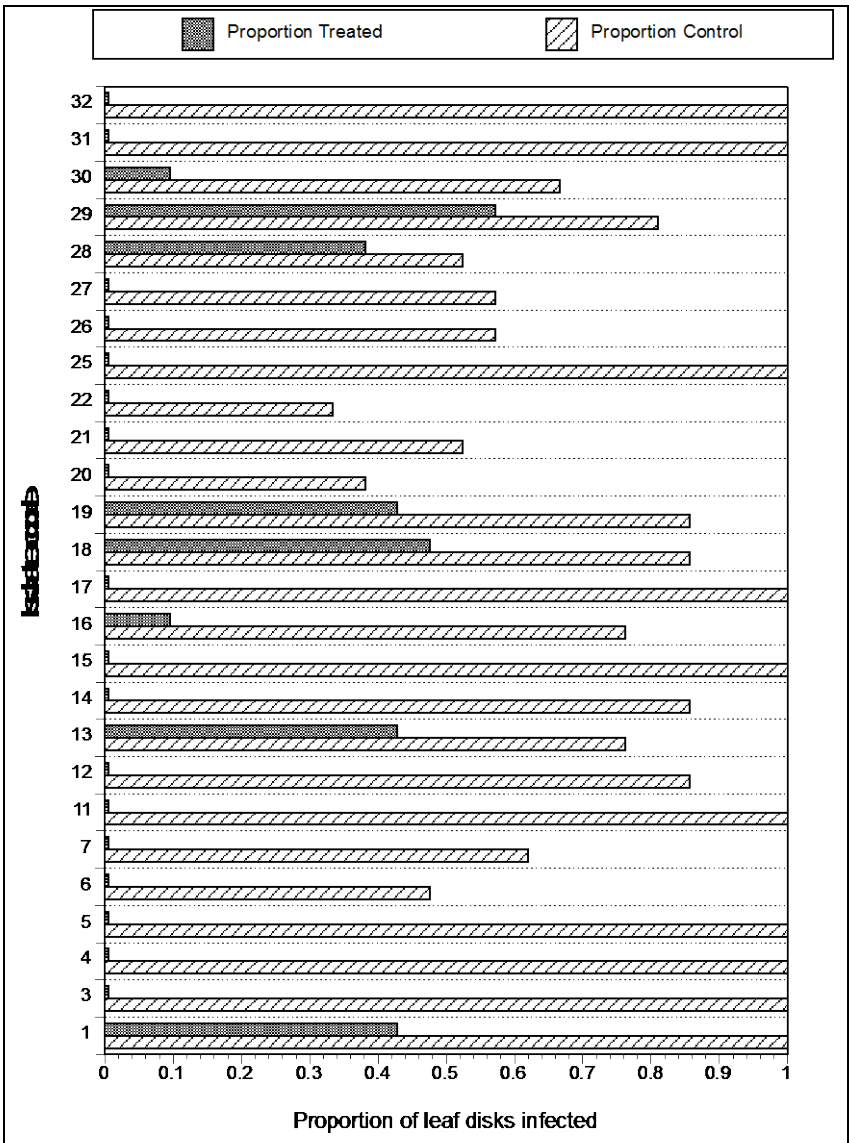

Figure 2. Proportion of leaf disks infected following inoculation with a suspension of spores of *P. clandestina* after 14 days incubation. Values "0" in the treated were given values of 0.05 (instead of 0) to provide contrast. Data shown are from 1280 ppm and untreated control treatments applied to foliage subsequently inoculated with *P. clandestina* from various orchards. Note growth of isolates 1,13,16,18,19, 28-30 on leaf disks treated with trifloxystrobin.

The majority of isolates were sensitive to trifloxystrobin at 1280 ppm, but 6 isolates appeared less sensitive (Figure 2). The isolates came from orchards in the Prosser (2), Mattawa (3), and Brewster-Omak area (2). We are confirming resistance with the qPCR assay developed in 2010-2011 but the assay requires a significant increase of inoculum of each isolate.

Objective 2. There were large increases in inoculum load in the orchard air during the 6-week period following harvest and the cessation of fungicide applications. In 2012, a more large-scale experiment was conducted. Orchard D-39 at WSU-Roza was separated into two equal segments. The north half received fungicide applications until harvest using standard grower practices. The south half remained untreated throughout the growing season. Disease severity in the *treated* section increased rapidly after harvest and eventually exceeded levels in the untreated section (Figure 3). PCR Cp values also showed a gradual downward trend in the treated section indicating the concentration of *P. clandestina* conidia in the orchard area increased following the cessation of fungicide applications (Figure 4). Results indicate that an areawide approach to mildew management should be considered and that season long disease management may serve to lower inoculum loads that could pose a risk to

later cherries in the immediate vicinity and also serve to lower the amount of survival inoculum going into the following growing season.

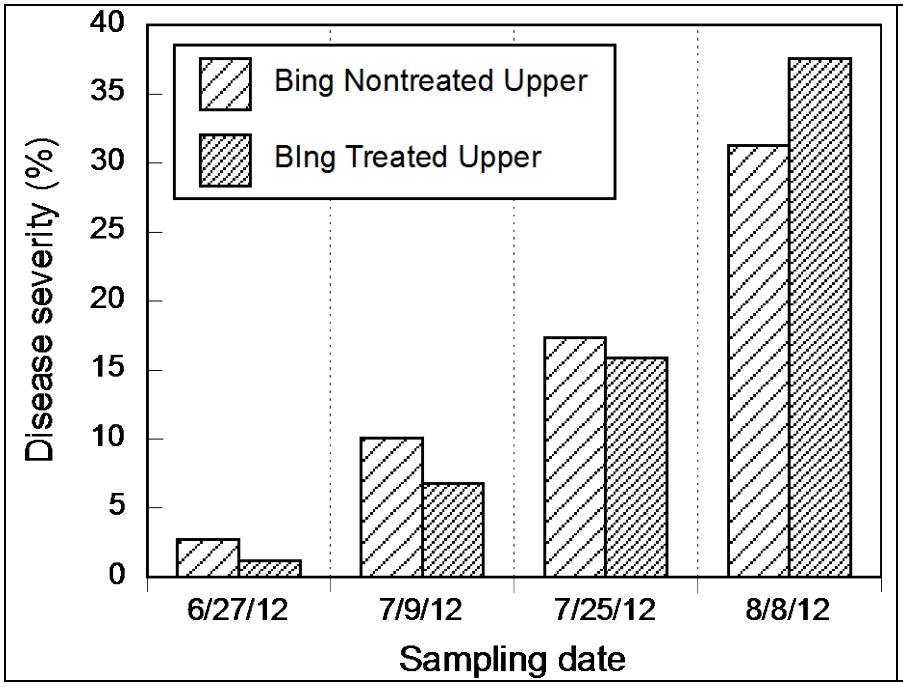

Figure 3. Powdery mildew disease severity progression following harvest. Bing nontreated upper received no preharvest fungicide applications while treated upper received standard grower regime. Note that disease severity in the preharvest treated actually surpasses that in the untreated sections of the orchard.

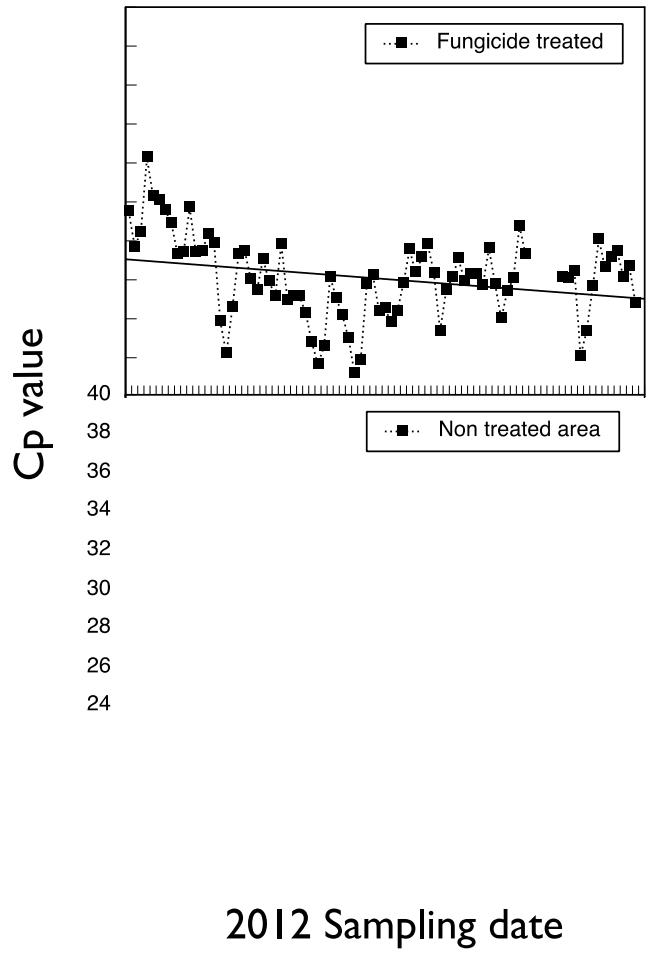

Figure 4. DNA signal strength in fungicide-treated (preharvest) and nontreated portions of a cv. 'Bing' orchard. The decreasing Cp value in the portion treated with preharvest fungicides is indicative of increases in airborne conidia concentrations following the cessation of fungicide applications.

Objective 3. Studies in 2012 were conducted using a modified (more efficient) Burkard cyclonic air sampler and improved qPCR techniques. Samplers were operated immediately prior to and through the only period of water-based frost protection in two (D-51 and D-39) mildew research orchards at WSU-Roza. Cp values decreased significantly during the 8 hours immediately following the irrigation water (Figure 5). Lower Cp values indicate stronger DNA signals during that period. We conclude that water used for frost protection increases the amount of *P. clandestina* DNA in the orchard air. Considering the overwintering inoculum load and the response to wetting, we conclude that the decreased Cp values reflect the presence of ascospores (primary inoculum) in the orchard air. Further studies should include successive applications of water in order to determine whether the practice can be used in conjunction with timely fungicide applications to deplete the amount of primary inoculum and therefore delay the onset of powdery mildew epidemics.

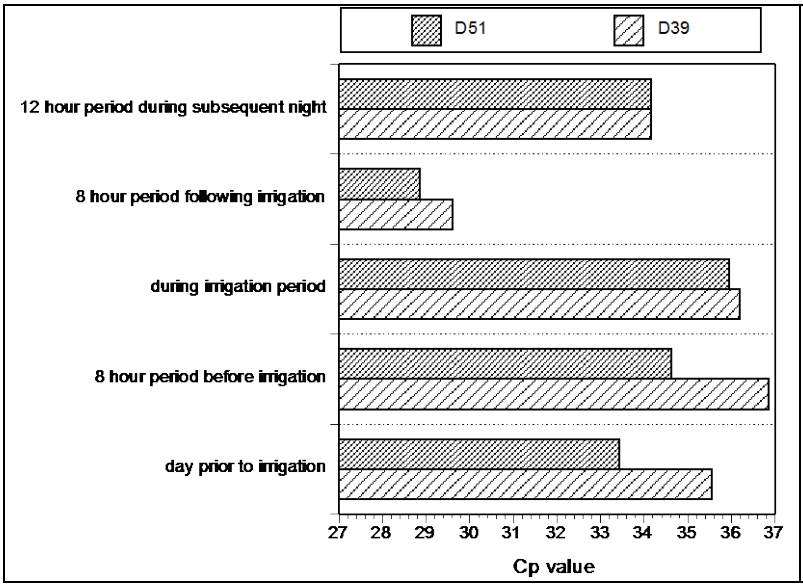

Figure 5. DNA signal strength (inversely proportional to Cp value) in WSU-Roza D51 and D39 when irrigation was used for frost protection. Note the decrease in Cp value (increase in signal strength) during the 8 hours following the application of water.

Objective 4. Experiments to designed to investigate the influence of irrigation and fertilizer regimens on powdery mildew incidence and severity were again unsuccessful due to low levels of disease. However, long-term experiments to determine the influence of various irrigation regimens on orchard microclimate established in 2010 and will continue over the course of the three-year study. Weather stations placed in various irrigation regimes collect temperature and relative humidity values at 15-minute intervals. Obvious differences were apparent during a period of summer heat in 2011 (Figure 6). Microclimatic data from 2012 will be downloaded during the first week of November.

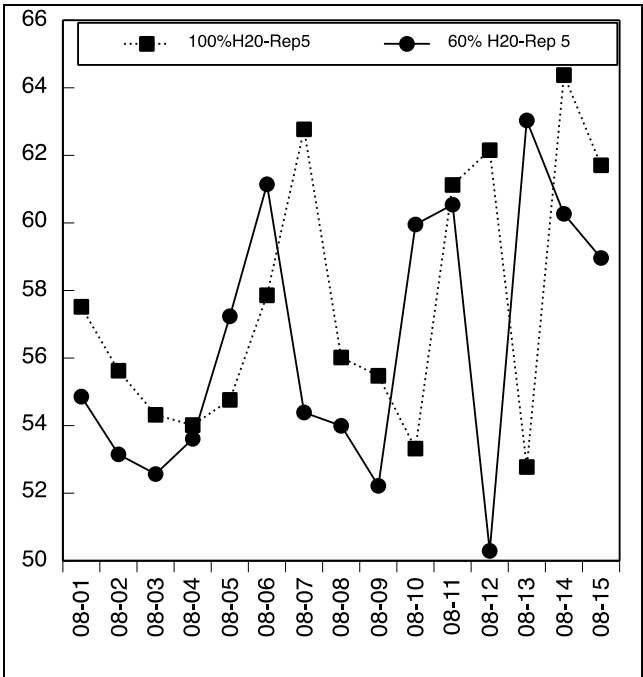

Figure 6. Relative humidity in 100% and 60% irrigation regimes during early August 2011.

Objective 5. Orchard studies 2010-2012 indicated that postharvest oil or Kaligreen applications made after standard fungicide applications were terminated resulted in no significant inhibition of chasmothecia formation. However, in 2012 we conducted parallel studies in Quincy, WA, nursery where we followed specific fungicide modes-of-action with late season oil applications. Although the chasmothecia counting process will not be complete until November 1, a significant oil effect was observed in the untreated controls (Figure 7). Furthermore, the number of chasmothecia increased exponentially between the September 15 and October 1 sampling dates, indicating the existence of a narrow window where late season fungicide applications may be most effective for lowering the amount of potential overwintering inoculum. We plan to present the entire set of data from various fungicide modes-of-action at the research review.

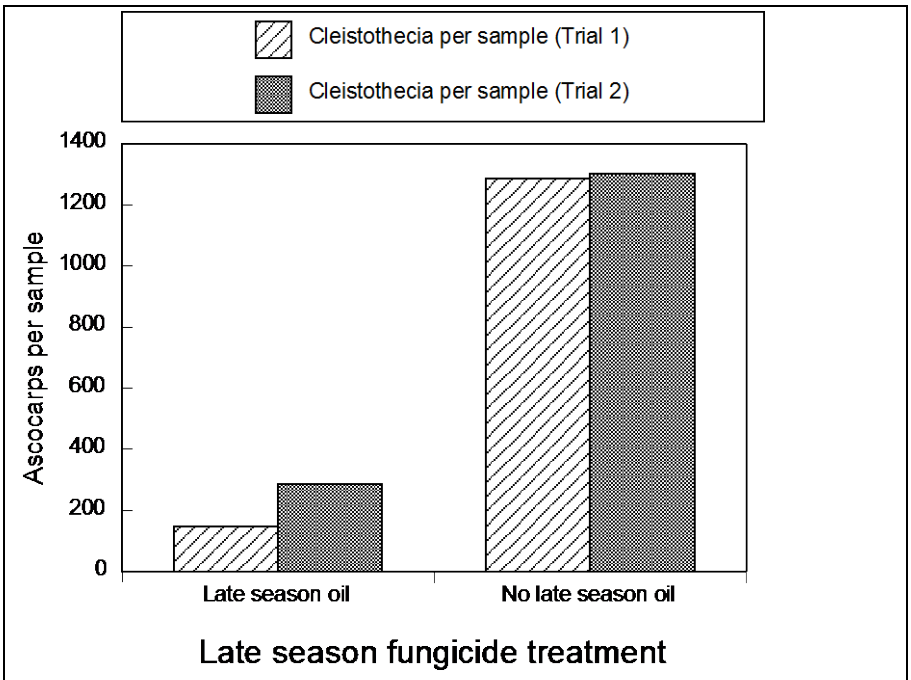

Figure 7. Effect of late season oil applications on ascocarp (chasmothecia or cleistothecia) production in untreated controls in a cherry *nursery*. Oil applications were inadvertently timed during a critical window for ascocarp formation.

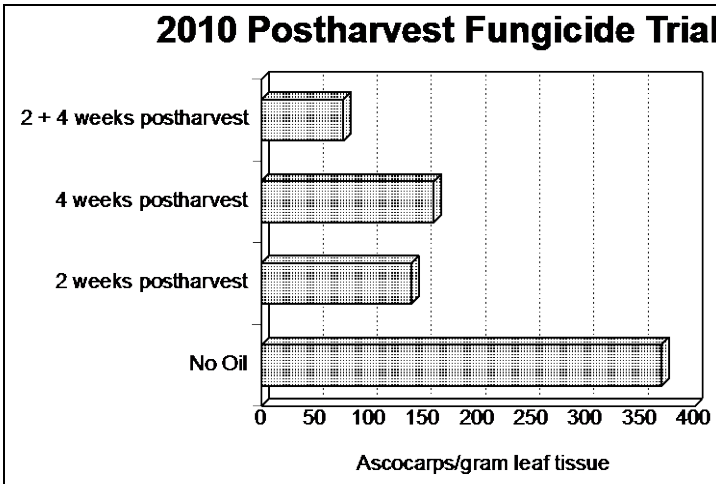

Figure 8. Chasmothecia (ascocarp) production on cv. 'Bing' trees receiving grower-standard preharvest fungicide program versus three full-season programs. There were no significant differences in chasmothecia production at $P < 0.05$

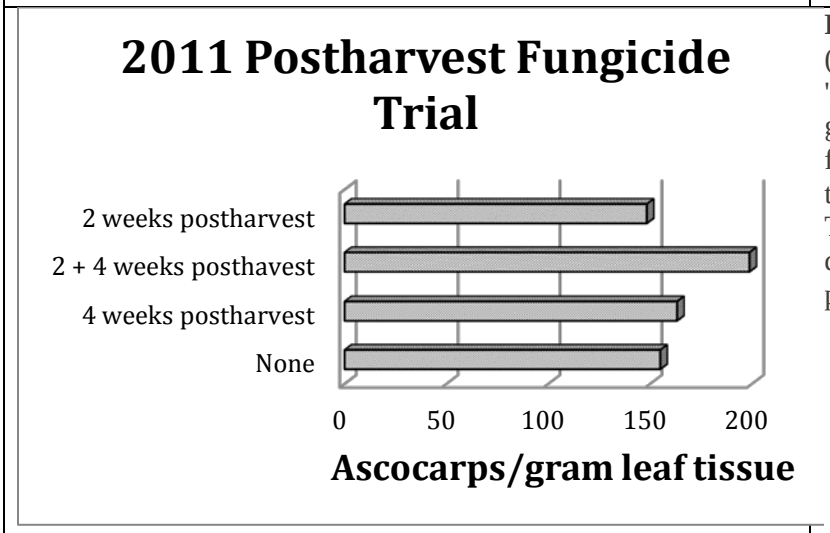

Figure 9. Chasmothecia (ascocarp) production on cv. 'Bing' trees receiving grower-standard preharvest fungicide program versus three full-season programs. There were no significant differences in chasmothecia production at $P < 0.05$

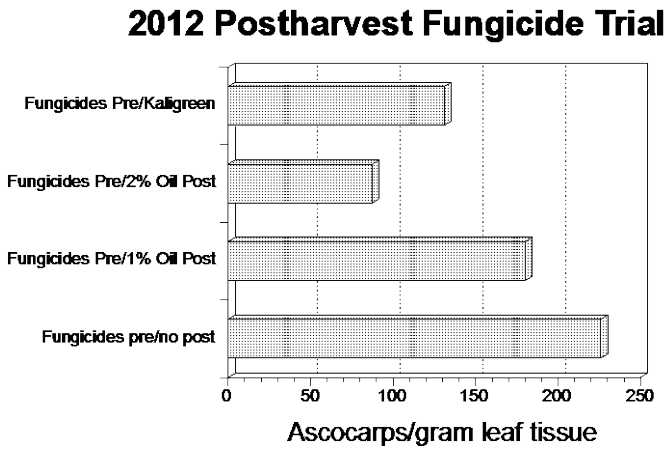

Figure 10. Chasmothecia (ascocarp) production on cv. 'Bing' trees receiving grower-standard preharvest fungicide program versus three full-season fungicide programs (1% and 2% oil applied postharvest.. There were no significant differences in chasmothecia production at $P < 0.05$

Executive Summary

Improved Management of Powdery Mildew of Sweet Cherry

Gary G. Grove, WSU-IAREC

Over 50 orchards in the Yakima and Wenatchee Valleys, Columbia Basin, and Omak/Brewster area were surveyed for resistance to QoI (strobilurin) fungicides. Evidence for resistance was found in a small minority of orchards and, following an inoculum increase, will be confirmed using a PCR assay. Water-based frost protection was shown to increase the amount of *P. clandestina* propagules in the orchard air, but further studies are needed to determine whether sequential irrigation sets can be used to deplete the primary inoculum source. Orchard experiments indicated that large increases in disease severity and airborne inoculum occur during the 6-week period following cessation of fungicide programs at harvest. This implicates early season cherries as an inoculum source for nearby later-developing varieties and intimates the need for full-season disease management in areas where multiple varieties are grown. Late season increases in disease levels were also shown to contribute significantly to the amount of survival inoculum going into the dormant season. Postharvest oil and potassium bicarbonate applications had no significant effect on the production of ascocarps (cleistothecia, chasmothecia) in the orchard but significantly late season oil applications significantly reduced numbers in cherry nurseries. A brief window of ascocarp formation occurred in the nursery indicating that orchard applications may have made outside of this brief window. Orchard experiments designed to identify irrigation and nitrogen factors affecting powdery mildew were unsuccessful due to lack of adequate disease. Our findings indicate possible future areas of research that include: 1) determining whether successive early-season water applications can be used to deplete the overwintered inoculum supply 2) studies to identify the narrow window of ascocarp formation in orchards and determine whether specific fungicide modes of action can be used to interrupt the process 3) investigate an area-wide approach to managing powdery mildew that utilizes full-season disease control without compromising (through resistance) our current fungicide arsenal. It is also apparent that a vigorous extension effort is needed in the area of fungicide resistance and the economics of full-season disease management.

FINAL PROJECT REPORT

Project Title: Reducing the impact of virus diseases on quality cherry production.

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Cooperators: Mr. Tim Smith, WSU-Extension, Wenatchee, WA
Dr. Tom Unruh, Dr. Wee Yee, USDA-ARS, Wapato
Numerous growers

Other funding sources

Agency Name: USDA-APHIS
Amount awarded: $42,300 as part of a much larger grant to support the efforts of the National Clean Plant Network at WSU-Prosser. This portion of the grant assisted in the maintenance of the orchard plantings.
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.

Total Project Funding: $133,560

Budget History:

Item	2010	2011	2012
Salaries	22,537	22,150	23,460
Benefits	8,198	9,711	9,853
Wages	0	0	0
Benefits	0	0	0
Equipment	0	0	0
Supplies	12,000	12,661	12,990
Travel	0	0	0
Plot Fees	0	0	0
Miscellaneous	0	0	0
Total	$42,735	$44,522	$46,303

OBJECTIVES:

The **overall project objective** is to identify viruses that cause low quality and quantity fruit production, and to develop an understanding of virus biology that will ultimately lead to the **development of effective management strategies for growers**.

Goal 1: Determine the ability of rootstock and inter-stock selections to limit the spread of cherry leaf roll virus and related viruses.

Goal 2: Determine the means of long distance transmission for cherry leaf roll virus.

Goal 3: Document the responses of new cherry cultivars to viruses.

SIGNIFICANT FINDINGS:

- Cherry leaf roll virus has appeared in several additional cherry production regions of the state.
- The selection of rootstock and scion significantly alters the expression of disease symptoms in response to cherry leaf roll virus.
    - Rootstocks could become the cornerstone of a long range management program to limit the transmission of cherry leaf roll virus under certain orchard settings and in the nursery industry
- A new native plant species has been identified as a host for cherry rasp leaf virus and may explain the intense virus pressure in certain areas of the state. This realization could impact site selection and preparation for new cherry or apple plantings.
- The viruses that cause two cherry diseases present in Washington State and the west coast have been identified. This permitted development of diagnostic tests for the accurate identification of pathogens associated with disease in cherry orchards.
    - Availability of diagnostic tests is fundamental to future research on disease epidemiology in the region including the identification of vectors and alternate hosts of the disease agents.

RESULTS AND DISCUSSION:

Cherry leaf roll virus:
Infection of cherry trees in Washington State by cherry leaf roll virus was first identified in 1998. Although cherry leaf roll virus was previously reported in golden elderberry in Washington, this was the first reported instance of cherry leaf roll virus in cherry. The incidence of the cherry leaf roll virus varies dramatically across different regions of the state. There are two areas of extremely high virus pressure: one in the lower Columbia Valley and one in the Yakima Valley. Outside of these distinct areas, the disease pressure is significantly lower but not absent. The virus is now found with increasing frequency throughout all cherry production areas. The most appropriate grower response to infection will vary depending upon in which location the infection is located.

Our analysis of the virus coat protein sequence, and eventually of the entire virus genome, revealed that the cherry isolate is genetically different from the golden elderberry isolate, suggesting a biological barrier has prevented the virus moving between these hosts. Further analysis revealed that native blue elderberry, ornamental black elderberry and rhubarb plants in Washington also harbor cherry leaf roll virus, but again these are genetically distinct isolates of the virus. Thus, the virus isolates that infect cherry appear to be isolated from the virus isolates infecting other host plants. The source of new infections in isolated cherry blocks remains unclear. It has been observed that a single, new infection can occur in an established orchard miles distance from the nearest known source of cherry leaf roll virus. This strongly suggests that an aerial transmission route exists. Based on similarity to the walnut isolate of cherry leaf roll virus where pollen transmission has been

demonstrated, controlled pollination of 10,000 cherry blossoms with virus-infected pollen was performed. This process resulted in the development of cherry leaf roll virus-infected seed, embryos, seedlings and fruit stems. However, this failed to result in transmission of virus to a single tree exposed to infected pollen. This suggests that if this is a route for transmission, it is very inefficient. Our data has demonstrated that the major means by which the virus spreads within an orchard is through root grafting. The efficiency of this process is determined in part by root zone architecture. Taken together, these observations suggest that if transmission by root-grafting can be eliminated, the impact and spread in a single orchard after a new infection could be substantially reduced. This is supported by experience with several growers. When the first infection in an orchard is detected, and the infected tree promptly removed using techniques recommended by this program, cherry leaf roll virus is easily contained.

Rootstock selection was examined as a parameter in determining the impact of cherry leaf roll virus on cherry production. The appropriate rootstock selection, independent of other horticultural properties of the rootstock/scion combination, is informed by the pressure of cherry leaf roll virus in the vicinity. In one of the concentrated areas of cherry leaf roll infection, choosing a tolerant rootstock/scion combination may permit economic success. This is predicated on the assumption that reduced yield and fruit quality caused by infection can be offset but superior performance of the trees. In an area of low virus pressure, a rootstock/scion combination that declines quickly after infection would be an effective means of quickly identifying new infections and preventing secondary spread. To evaluate these possibilities, nine blocks of trees were planted in 2009. Each block consisted of ten virus-free 'Bing' budded onto a rootstock. In August, 2010, the trees were inoculated by T-budding with cherry leaf roll virus infected buds. In four trees of each block, the bud was placed below the graft union to simulate infection through the root system. In another four trees of each block, the virus-infected bud was grafted above the graft union to mimic infection entering the tree through an aerial route. Two trees of each block were reserved as un-inoculated control trees. Exceptions were with 'Mazzard' rootstock where only three trees were inoculated instead of four, and with 'Gisela 12' where the set size was doubled.

Within one growing season, there was evidence of tree stress and reduced growth of trees on some rootstocks. The greatest reductions in growth were observed where the 'Bing' scion was inoculated and growing on 'Colt', 'Gisela 6' or Citation rootstock with Zee-stem interstock. In the beginning of the second season of observations, all of the trees (4 out of 4) growing on 'Colt' rootstock where the scion was inoculated had died but none of the trees in which the 'Colt' rootstock was inoculated were exhibiting any signs of stress or significant reductions in tree growth (Table 1). This confirms that 'Colt' responds to cherry leaf roll virus with a hypersensitive reaction, thus causing a layer of dead cells to form between the infected 'Bing' and the rootstock, resulting in rapid death and decline of the scion. In the case of the rootstock inoculated 'Colt', the same hypersensitive reaction slows or prevents the movement of the virus from the bud into the rootstock and thus protects the tree from infection. This is the desirable response that would enable a virus management program to be developed based on this rootstock since infections are quickly eliminated in the orchard and secondary spread through root grafting is prevented. In many sites in Washington State, trees growing on 'Colt' rootstock are not very precocious. We attempted to circumvent this by using the Zee-stem interstock on 'Colt' rootstock. This hybrid interstem has been used to increase precocity and decrease tree size on other rootstocks. However, the graft union between the 'Colt' and Zee-stem were very brittle and may not be workable in commercial production.

The only other rootstock to exhibit a rapid response to cherry leaf roll virus infection was 'Gisela 6' rootstock. All of the infected scions collapsed by the end of the second growing season after inoculation. However, unlike 'Colt', trees growing on 'Gisela 6' rootstock where the rootstock was inoculated also collapsed. Thus, 'Gisela 6' does not provide direct protection against root graft

transmission of cherry leaf roll virus, but would still assist in virus control by quickly eliminating sources of inoculum.

Trees growing on the Zee-stem interstock/Citation rootstock combination exhibit severe sign of stress (premature leaf reddening in late summer) and reduced growth as measured by trunk cross sectional area (Table1). Thus, growing trees on this combination may be used as sentinel plants to indicate the movement of cherry leaf roll virus into an orchard, but would not offer any direct control.

The results of this rootstock trial point the way for potential management strategies that could be implemented during orchard planning and redevelopment. The data also highlight the impact of cherry leaf roll virus on tree growth. For example, on 'Mazzard' rootstock, the trunk cross sectional area of inoculated trees after two years is approximately one-half relative to un-inoculate controls. All of these experiments were conducted under conditions where cherry leaf roll virus is the only virus in the research trees. Observation over the past 12 years has made it very clear that cherry leaf roll virus in combination with other common viruses of cherry such as *Prunus* necrotic ringspot virus and prune dwarf virus results in much more severe symptom development and tree decline than with cherry leaf roll virus alone. Therefore, the modest reductions observed in the tree growth in this study would be expected to be much greater in the practice where mixed infections are common place.

A small potted tree experiment indicates that scion also plays a role in disease severity. As previously reported, 'Lapins' was the most tolerant scion when tree growth is considered. The growth of 'Tieton' and 'Skeena' were most severely affected by cherry leaf roll virus. A longer term and larger research trial will be necessary to evaluate the effect of cherry leaf roll virus on the quality and quantity of fruit.

Table 1. The trunk cross sectional area of trees were measured and averaged two years after inoculation with cherry leaf roll virus. The scion in each case is 'Bing'.

Rootstock	**Average trunk cross sectional area –in.^{2} (number of surviving trees)**		
	Non-inoculated	Scion inoculated	Rootstock inoculated
Mazzard	5.68 (n=2)	2.17 (n=2)	3.04 (n=3)
Gisela 5	1.90 (n=2)	2.10 (n=4)	2.40 (n=4)
Gisela 6	3.89 (n=2)	0[a]	2.10 (n=3)
Gisela 12	5.55 (n=3)	3.37 (n=6)	5.91 (n=4)
Z-stem/Citation	6.92 (n=2)	3.50 (n=4)	5.32 (n=4)
Colt	7.36 (n=2)	0[a]	5.91 (n=4)
Krymsk 5	7.08 (n=2)	5.05 (n=4)	6.14 (n=4)
Krymsk 6	2.59 (n=2)	3.54 (n=4)	3.44 (n=4)
a. The four trees in each of these treatments died during the second growing season.			

Cherry rasp leaf virus:
Cherry rasp leaf virus and the diseases caused by it have been reported in several cherry production regions west of the continental divide. The first reports in Washington State arose during the virus disease surveys of 1942. At that time, cherry rasp leaf disease was reported to be extensive in

orchards of the Yakima Valley. Through years of orchard replacement, the virus has become very uncommon in the Yakima Valley and is now found primarily in Chelan County where it persists in localized areas. The explanation for this transition has been unclear. In 2012, investigation of areas surrounding a cherry rasp leaf virus-infected cherry orchard led to the observation of elderberry shrubs with deformed leaves. Virus profile testing revealed that these shrubs are infected with cherry rasp leaf virus. This virus is nematode transmitted so the presence of the virus in the wild native vegetation would serve as a reservoir of the virus from which it can enter the orchard. Most viruses related to cherry rasp leaf virus are also seed transmitted. This remains to be confirmed in the case of cherry rasp leaf virus-infected elderberry, but the wide dispersal of infected seed in the area by birds may account for the high incidence of the virus in this area. Wild blue elderberry is common in the canyon bottoms of the Wenatchee cherry growing area. This represents the first report of this shrub as a host of cherry rasp leaf virus. The potential ingress of cherry rasp leaf virus from shrub land into cherry orchards should be considered during the selection of the orchard site and the preparation of the orchard and adjacent land for planting.

Cherry rusty mottle disease group of viruses:
Cherry rusty mottle is a graft-transmissible disease of sweet cherries first described in the early 1940s in Washington State. Because of the graft transmissible nature of the disease, a viral nature of the disease has long been assumed but not demonstrated. The 3'-terminus regions of virus-like RNA were amplified from trees affected with cherry twisted leaf (CTL), apricot ring pox (ARP), cherry necrotic rusty mottle (CNRM), cherry rusty mottle (CRM) and cherry green ring mottle (CGRM) diseases. Phylogenetic analysis of virus-like sequences from a total of 24 trees representing these diseases along with published sequences of Cherry green ring mottle virus (CGRMV) and Cherry necrotic rusty mottle virus (CNRMV) from other geographic regions revealed segregation into four major populations, each corresponding to one of the diseases (Figure 1). Virus sequences within each of these clades were designated as clade I: Cherry twisted leaf associated virus (CTLaV), clade II: CNRMV, clade III: Cherry rusty mottle associated virus (CRMaV), and clade IV: CGRMV. Segregation into these clades correlated with symptom expression on *Prunus avium* cultivars 'Bing' and 'Sam', and *Prunus serrulata* 'Kwanzan'. Examination of frequency distribution data derived from pairwise sequence comparisons and symptomatology suggests that CTLaV, CNRMV, CRMaV and CGRMV are separate virus species. This is the first report of specific association of virus and virus sequences with cherry rusty mottle and cherry twisted leaf diseases.

Primers were designed to permit detection of each of the proposed virus species individually. This has allowed accurate assessment of the virus profile of viruses in orchard trees. Data from the study revealed that mixed infections, that is multiple viruses present in a single tree, are very common (Table 2), and that the interaction of the viruses within a single tree can modify disease symptoms, thereby making a diagnosis based on visual symptoms alone difficult.

Table 2. Symptomatic source trees used in the study of the family *Betaflexiviridae*.. The viruses listed are in addition to the virus-like sequences used in sequence analysis in Figure 1.

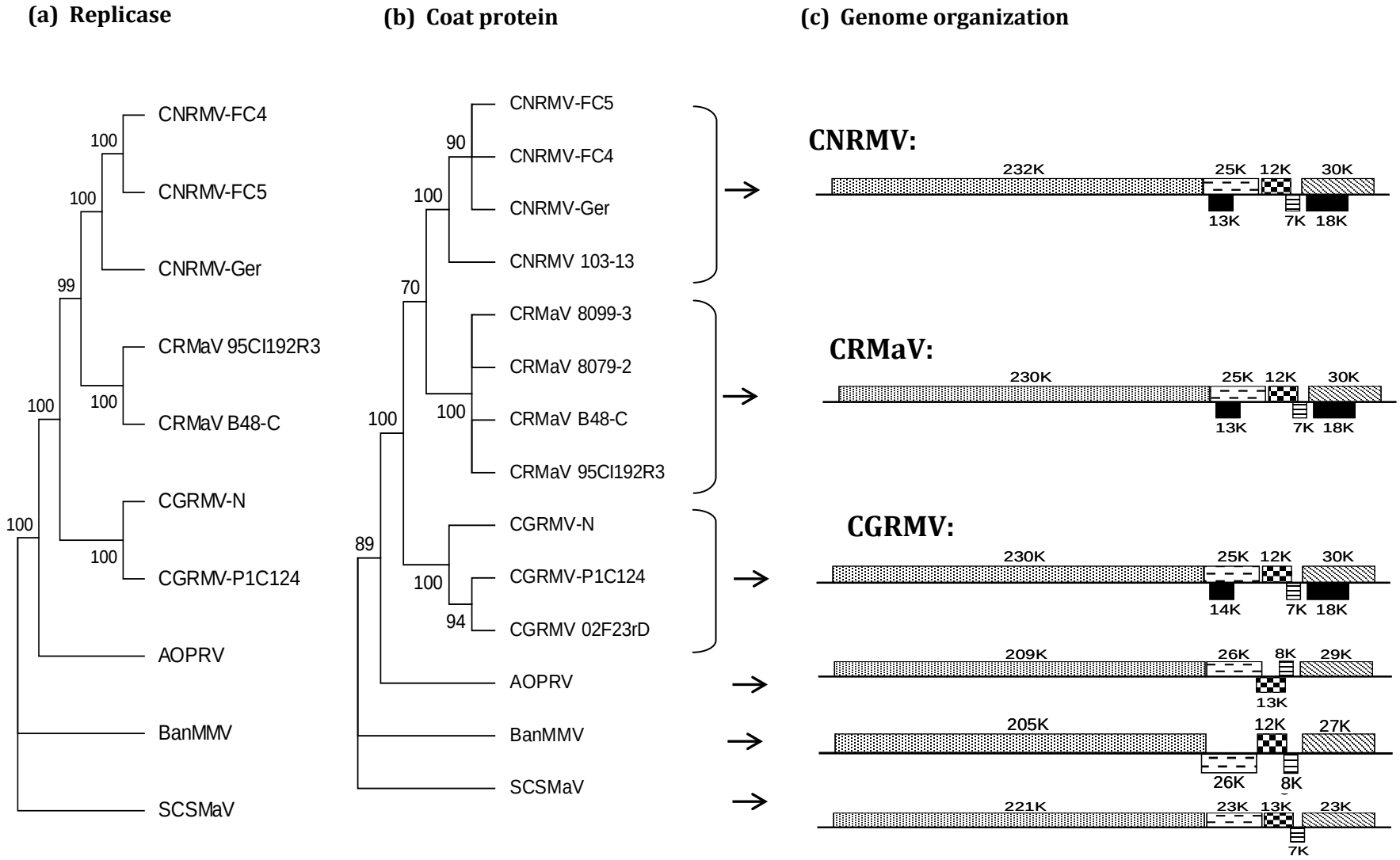
Source tree	Original collection site	Maintenance host	Other viruses detected[1,2]
Source trees maintained at the Clean Plant Center – Northwest, Washington State University			
B48-C	Benton Co., WA	*P. avium* 'Bing'	PDV
101-13	N/A[3]	*P. avium* 'Bing'	CMLV, CVA, PDV
103-13	N/A	*P. avium* 'Sam'	PDV
103-15	N/A	*P. avium* 'Bing'	ACLSV, CLMV
02F23rD	N/A	*P. avium* 'Bing'	PDV
04E17R1	The Dalles, OR	*P. avium* 'Bing'	ACLSV, PDV, PNRSV
04E36	Stockton, CA	*P. avium* 'Bing'	CVA, PDV, PNRSV
95CI189R2	Wenatchee, WA	*P. avium* 'Sam'	CVA, PDV, PNRSV
95CI190	Wenatchee, WA	*P. avium* 'Sam'	ACLSV, CVA, PDV
95CI191R3	Wenatchee, WA	*P. avium* 'Sam'	ACLSV, CVA, PDV, PNRSV
95CI192R3	Wenatchee, WA	*P. avium* 'Bing'	CVA, PDV, PNRSV
95CI205R1	Wenatchee, WA	*P. armeniaca*	None detected
98CI73R1	Moxee, WA	*P. mahaleb*	PDV
98CI194	Mattawa, WA	*P. avium* 'Bing'	CVA
99CI01	Granger, WA	*P. avium* 'Bing'	CVA, PDV, PNRSV
Symptomatic source trees from commercial orchards			
8079-1	Malaga, WA	*P. avium* 'Bing'	ACLSV, CLRV, CVA, PDV, PNRSV
8099-5	Prosser, WA	*P. avium* 'Bing'	CVA, PDV
8241-2	Wenatchee, WA	*P. avium* 'Bing'	CMLV, CVA PDV
8241-4	Wenatchee, WA	*P. avium* 'Bing'	ACLSV, CVA, PDV, PNRSV
8242-3	Wenatchee, WA	*P. avium* 'Bing'	CVA, LChV-2, PDV, PNRSV
8244-4	Malaga, WA	*P. avium* 'Bing'	CVA, PDV
8265	Granger, WA	*P. avium* 'Bing'	CMLV, PDV, PNRSV

1. ACLSV=Apple chlorotic leafspot trichovirus; CLRV=Cherry leaf roll nepovirus; CMLV=Cherry mottle leaf trichovirus; CVA=Cherry virus A capillovirus; LChV-2=Little cherry virus 2 ampelovirus; PDV= Prune dwarf ilarvirus; PNRSV=Prunus necrotic ringspot ilarvirus.

2. American plum line pattern ilarvirus; Cherry raspleaf cheravirus; Little cherry virus 1 unclassified Closteroviridae; Hop stunt pospiviroid; Peach latent mosaic avsunviroid, Xylella fastidiosa and phytoplasmas were not detected in these samples.

3. N/A indicates that information on the place of origin of samples is not available.

Figure 1. Sequence analysis of virus isolates from the rusty mottle group of diseases that affect cherry and their comparison to other members of the family *Betaflexiviridae.*



Project Title: Reducing the impact of virus diseases on quality cherry production.

EXECUTIVE SUMMARY:

Cherry leaf roll virus:
Disease caused by cherry leaf roll virus is appearing in additional cherry production regions of Washington State. The emphasis on premium cherry production increases the risk of cherry leaf roll virus-infected orchards being marginalized because the fruit does not meet current industry standards. Therefore, a management plan needs to be considered in planning new cherry plantings. The selection of scion and rootstock significantly alter disease expression. Moreover, the appropriate selection of rootstock could become the foundation of a cherry leaf roll virus disease management program. Some rootstocks such as 'Colt' and 'Gisela 6' respond very aggressively to infection by cherry leaf roll virus. This rapid response quickly eliminates the potential for secondary spread in the orchard. Alternatively, growth of trees on 'Gisela 5' rootstock does not seem to be impacted by infection. A longer term experiment will be needed to assess the impact on fruit production and in the presence of other viruses.

Cherry rasp leaf virus:
There has been a shift in the epidemiology of disease caused by cherry rasp leaf virus over the past 50 years. Our research revealed a new native plant species (blue elderberry) as a host for cherry rasp leaf virus. This may explain the intense virus pressure in certain areas of the state where elderberry grows in abundance. The potential for seed transmission increases this concern. Awareness of this newly reported host for the cherry rasp leaf virus could impact site selection and preparation for new cherry or apple plantings.

Cherry rusty mottle group of diseases:
The viruses that cause three diseases presence in Washington State and the west coast have been identified. Prior to this time the diseases were presumed to be caused by "virus-like agents" based on graft transmissibility. Our data led to development of virus-specific diagnostic tests for the accurate identification of pathogens associated with diseases in cherry orchards. Our study has shown that the presence of multiple viruses in a single tree can make disease diagnosis based on visual symptoms alone difficult and inaccurate. The virus-specific assays thus provide an accurate profile of the viruses present. This is important in knowing what cultural practices can and should be modified to minimize further spread of the disease. For future research, the availability of diagnostic tests is fundamental to studies of disease epidemiology in the region including the identification of vectors and alternate hosts of the disease agents.

FINAL PROJECT REPORT

Project Title: Developing a management strategy for little cherry disease

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David James, Entomologist, WSU-IAREC, Prosser, WA
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Grower cooperators

Other funding sources: None

Total funding: $56,083

Budget History:

Item	Year 1:	Year 2:	Year 3:
Salaries	143,464	13,056	
Benefits	5,655	5,484	
Wages			
Benefits			
Equipment			
Supplies	9,000	9,344	
Travel			
Plot Fees			
Miscellaneous			
Total	$28,199	$27,884	

OBJECTIVE:
The overall objective of this project is to **develop an industry-wide strategy to prevent the continued intrusion of little cherry disease into sweet cherry production regions**.

SIGNIFICANT FINDINGS:

- The resurgence of little cherry disease in Chelan and Grant Counties of Washington State is primarily associated with Little cherry virus 2.

- Smaller numbers of little cherry disease reports in Yakima County are associated with Little cherry virus 1 and the Western X phytoplasma.

- Grape mealybug was identified as a new and important vector that transmits Little cherry virus 2.

RESULTS AND DISCUSSION:

Little cherry disease is a serious economic disease of sweet cherry that has been present in Washington State since the 1940s but made a dramatic resurgence in 2010. Analysis in our laboratory demonstrated that this recent outbreak is associated primarily with Little cherry virus-2. Disease management is limited to destroying symptomatic trees, planting disease-free trees and controlling the insect vector.

The incidence of little cherry disease abruptly increased in many Washington commercial sweet cherry orchards during the 2011 and 2012 growing seasons. The result has been severe economic damage in affected orchards. Trees with little cherry disease produce small cherries with poor flavor development, and reduced sugar content making the fruit unmarketable, particularly with the current industry emphasis on premium fruit size and quality. However, some cultivars such as 'Bing' are fairly tolerant of little cherry disease. Once the trees become infected, they will exhibit the typical small fruit for one or two seasons; this is referred to as the shock phase. After this period, the fruit will return to near-normal size although the quality will still be sub-standard. The severity of little cherry disease expression varies depending on environmental conditions during the growing season, and colder than normal spring temperatures can induce the return to the shock phase of symptom expression in 'Bing'. It is believed that the cold spring of 2010 imitated the return of already infected trees to the "shock" phase of disease expression. As a result of this event, substantial amounts of fruit were sold at discounted prices or not packed at all. Additionally, many mature trees that are no longer commercially productive because of little cherry disease are being removed. The presence of infected orchards that serve as reservoirs of little cherry disease along with insect vectors creates a potential for extensive damage. Therefore, a sustainable integrated approach to control little cherry disease and its vector is needed, particularly for organic production.

Earlier studies in British Columbia identified apple mealybug (*Phenacoccus aceris* (Signoret)) as the primary vector associated with the previous little cherry disease outbreaks caused by little cherry virus-2. However, this insect species was not reported as a pest in Washington until the late 1990s and only a few apple mealybug populations have been recorded. The recent increase in the prevalence of little cherry virus-2 in the Pacific Northwest suggested that there may be changes in the vector population. Unlike populations of apple mealybug that have been declining, grape mealybug (*Pseudococcus maritimus* (Ehrhorn)) is becoming a common pest of both pome and stone fruits. The reduction in use of broad spectrum insecticides made possible by the use of spinosad bait (GF-120, Dow Agrosciences, Indianapolis, IN) for the key pest of cherries (western cherry fruit fly, *Rhagoletis indifferens* Curran) may have contributed to an increase in grape mealybug populations in sweet

cherry. The possible association between this insect and the spread of little cherry virus-2 was examined.

A natural infestation of mealybugs on myrobalan plum (*Prunus cerasifera*) trees was tentatively identified as grape mealybug based on general appearance. Slide mounts of several life stages confirmed the identity of grape mealybug and this colony was used for subsequent transmission experiments. Shoots from a sweet cherry tree inoculated with the North American LC5 strain of little cherry virus -2 was used as a virus source. In a growth chamber, crawlers were placed on shoots cut from the infected orchard tree and, after an acquisition period of seven days, approximately 50 insects were transferred to each potted 'Bing' cherry tree on Mazzard known to be free of little cherry disease. The young recipient potted trees (approximately 10 leaf stage) were growing in a second growth chamber at 23°C with a 16 hour light cycle. After one week, the trees were treated with a soil application of imidacloprid (Marathon 1% G; Olympic Horticultural Products, Mainland, PA) plus three times at one week intervals with horticultural oil to eliminate the mealybugs. This process was repeated on two separate groups of trees at different times during the growing season to yield a total of 20 young cherry trees that had been exposed to potentially viruliferous mealybugs. At 2 and 4 months after the transmission period; leaf tissue that developed after the transmission period was collected from each of the recipient trees for molecular testing. A similar test was done on a control plant that was not infested with mealybugs. After natural defoliation and a 3 month dormant period, emerging growth was again sampled and tested; this was 10 months after the initial transmission period. During each sampling, total RNAs were isolated from leaves and tested by reverse transcription polymerase chain reaction (RT-PCR) using two sets of primers in separate reactions. Identity of the RT-PCR amplicons was confirmed by sequencing.

Grape mealybug-mediated transmission of little cherry virus-2 to sweet cherry was confirmed by RT-PCR in 80% (16 of 20) of infected trees by molecular assays. Control samples, infested with no grape mealybugs, did not yield any bands. It is possible that the positive reaction in the RT-PCR was the result of virus carried by grape mealybug debris on the leaf surface but not transmitted. Hence, plants were allowed to continue to grow in the greenhouse and new growth was tested four months after inoculation to verify that the positive diagnostic reaction was the result of virus synthesis by the infected plant rather than surface contamination. To further negate this possibility, plants were allowed to go into dormancy at 6°C during winter and then transferred to a shade house to resume growth in the spring. When leaves collected from newly grown shoots were tested 10 months after the potential transmission period, identical results were obtained. The test results at different time points confirmed the establishment and spread of the virus in newly developed leaves of sweet cherry trees. The virus-specific amplicons from six independent trees were verified by sequencing. Both the partial replicase and coat protein gene sequences showed 99 to 100% sequence similarity among them and with corresponding regions of the LC5 strain of little cherry virus-2, confirming the identity of the virus. This work demonstrates that grape mealybug is an efficient vector of little cherry virus-2, one of the causal agents of little cherry disease. The confirmation of virus transmission by this common pest has great significance for sweet cherry producers in the Pacific Northwest.

This work demonstrates for the first time that grape mealybug is an efficient vector of little cherry virus-2. Grape mealybug has been recorded as a pest of tree fruits since the early 1970s, but pears were the primary host of concern. Recent versions of Washington's Crop Protection Guide do not include recommendations for grape mealybug control on cherry because its status as a pest is considered very minor. As a disease vector, its pest status is greatly elevated. This pest is most likely to be found in the tops of older trees, where spray penetration is poorest, and as such is considered difficult to control.

In 2012 alone, 72 cherry samples were submitted to our laboratory for virus testing in association with little cherry disease. More than two-thirds were infected with at least one virus-like agent. From these samples, 24 were selected and the coat protein determined. The nucleotide identity ranged from 83 to 100%. This information was used identify segments of the coat protein coding region that were conserved across all of the virus isolates and that could provide the reagents for recombinase polymerase assay, a cost effective diagnostic tool. The results are very promising and Washington State University is engaging in the next step of developing a diagnostic tool for little cherry virus-2.

EXECUTIVE SUMMARY:

Project title: Developing a management strategy for little cherry disease

A recent outbreak of little cherry disease in Washington State is associated with little cherry virus-2. The cherry cultivars that predominate in the region are less sensitive to this virus, but cool spring weather can induce development of severe symptoms. Such a cool spring occurred in 2010. As a result many trees in many orchards exhibited recognizable little cherry disease symptoms for the first time. The incidence of symptomatic trees has been over 30% in some orchards. The incidence of diseased trees has continued to increase since that first event. The possible involvement of a virus vector population was considered. The previously known vector, apple mealybug, is present in very low numbers. However, population of a different mealybug species, the grape mealybug, is increasing in fruit growing areas. Our study demonstrated that grape mealybug is an efficient vector of little cherry virus-2 and that the presence of this insect may be contributing significantly to the spread of little cherry disease.

In an effort to correctly identify whether poor fruit yield is the result of physiological conditions or infection by little cherry virus-2, a new molecular technology is being adopted that will offer growers and consultants access to a relatively inexpensive method to detect this virus.

FINAL PROJECT REPORT

Project Title: Support for a full time technician

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Cooperators: Jim McFerson, Amy Iezzoni, and Fred Bliss

Other funding sources

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

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

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

Agency name: WTFRC/OSCC
Amount awarded: $62K from 2011-2012
Notes: Understanding the genetic basis of powdery mildew resistance in sweet cherry: PI: Oraguzie

Total Project Funding: $79, 222

Budget History:

Item	2010	2011	2012
Salaries	16,340	17,372	18,067
Benefits	8,660	9,207	9,576
Wages			
Benefits			
Equipment			
Supplies			
Travel			
Plot Fees			
Miscellaneous			
Total	25,000	26,579	27,643

JUSTIFICATION

One of the objectives of the WSU sweet cherry scion breeding proposal funded in the last round (WTFRC # CH-09-902) was to assemble adequate personnel to ensure that healthy, vigorous plant materials of adequate size are produced. A lot of progress has been made in this respect over the past year and we now have 1.5FTE technicians assisting the breeder with the day-to-day management of breeding operations. One FTE technician (Blessing Athanson) is funded through WSU-ARC while the other 0.5FTE (Addie Dahl's position) is funded by WTFRC/OSCC. There is also an orchard manager (Clint Graff) funded by WSU-ARC who spends ~33% of his time in the breeding program helping with horticultural manipulations and general orchard management. Jan Burgess with 20 years experience working for the WSU National Clean Plant Network (NCPN) program is hired for an hour/day by the breeding program to advise on seedling development in the greenhouse and lathhouse.

In 2009, fruit were harvested from all crosses made in 2004 and from some 2005 crosses for the first time (i.e., since the breeding program started receiving funding from WTFRC/OSCC) and the best trees with commercial potential have been selected and propagated for more advanced tests. As the program moves to the stage where there is constant fruit production, fruit quality phenotyping, selection of best progenies, propagation, planting and testing of selections in more advanced trials, there is a need for another 0.5FTE technician. This person will be responsible for seed handling, horticultural manipulation of trees in the lathhouse and field, field plot management, and coordination of fruit sampling and tree propagation. The goal of this position is to ensure that healthy, vigorous, precocious and well managed plant materials of adequate size are produced and fast-tracked through to commercialization. The purpose of this proposal is to seek funds to support this position. Addie Dahl (currently funded part-time) is being trained to assume this role if funding request is approved, to provide continuity in the program.

OBJECTIVE:

The objective of this proposal is:

- To acquire support for a full time technician position to ensure that healthy, vigorous seedlings of adequate size and precocity are produced using best horticultural practices.

METHODS

1. **Support for a full-time technician**

The leadership of the WSU sweet cherry breeding program changed last year with the appointment of a permanent stone fruit breeder. This was followed by active recruitment of staff and infrastructure development to make the program more viable. We have made a lot progress in this direction over the past year (see WTFRC continuing report reference # CH-09-902 for details). For the first time (since the program started receiving funding from WTFRC/OSCC), fruit were evaluated in summer 2009 from breeding selections and their parents and 12 selections that fit into 4 of the target market cultivar groups were identified and propagated for more advanced testing. The installation of bird netting was very instrumental for getting sufficient numbers of fruit for assessment which was not possible the year before. We have also identified a new powdery mildew resistant selection from a "PMR-1" x :"Van" cross made in 1998. This is additional to the powdery mildew resistant advanced selections from 1998 crosses already in grower cooperator trials in Washington and at OSU experimental stations in Oregon.

We have initiated experiments in our lab to improve seed germination and seed handling in the green houses to facilitate development of larger numbers of healthy seedlings for field planting. One of the trials involves use of GA to promote the timing and uniformity of germination. Jan

Burgess, who works for the National Clean Plant Network (NCPN) program with 20 years experience working with sweet cherry has been hired for an hour per day, to assist with raising germinated seeds in the green houses. Horticultural manipulation of trees in the field has improved tree vigor and we anticipate that this will impact on precocity. At the moment, less than 5% of own-rooted sweet cherry trees come into bearing in their 4th year. Our goal is to get a larger proportion of these trees fruiting at that stage using horticultural manipulations both in the greenhouses and the field. Clint Graf, the vineyard and orchard manager spends 33% of his time assisting with horticultural manipulations and general orchard operations in the breeding program. Figure 1 shows a schematic diagram of activities in the breeding program from seedling development in a growth room supplied with 24 hours of lighting and maintained at 26 °C and 44% RH to raising the seedlings in the greenhouse and lathhouse before field transplanting.

Due to increased workload over the past year, we had to hire many more time slip employees to work in the breeding program nearly all year round over the past year. We would need a full time technician support to cope with the increased workload and having a permanent employee who is trained to do the job to assume this role would increase efficiency and timely delivery of milestones.

RESULTS AND DISCUSSION

Objective 1: **To acquire support for a full time technician position to ensure that healthy, vigorous seedlings of adequate size and precocity are produced using best horticultural practices.**

With the help of the breeding technicians, the program has developed a work in-progress best management practice document which provides guidelines on all aspects of sweet cherry breeding from seed collection to seed germination, seedling development in the green houses, tree management in the lathhouse, field planting and tree establishment, horticultural manipulations to encourage quick bloom and fruiting, fruit sampling and phenotyping protocols in P1. To date, we have recorded 60% seed germination which is up from 35% while seedling survival rate at baby stage is over 90%. Greenhouse management protocols result in accelerated development of large numbers of healthy seedlings less than a year for field planting. Previously, it took 2-3 years to generate seedlings of adequate size for field planting. Best practice guidelines have been prepared for slowing trees down in the fall months prior to winter. As the program enters Phase 3, we will be devel oping guidelines on field plot techniques, tree training, phenotyping protocols and selection criteria to ensure that only superior genotypes are identified and moved to Phase 3 and from there on to commercial release.

SIGNIFICANT FINDINGS AND ACCOMPLISHMENTS

- A new technician, Sue Watkins, joined the PNWSCBP in 2012. She replaced Andrea Young, who left the program to join WSU in Pullman in December 2011. Ms Watkins has been involved in R & D for ~17 years and knows how to get jobs done efficiently and in a timely manner
- Nine thousand trees in the breeding program have been tagged with bar-coded labels. Bar coding was also used in the lab for fruit quality evaluation to improve the efficiency of data collection.
- Two acres of a mother block for old cultivars at WSU-IAREC, Prosser have been pulled out this year
- A total of 324 F_1 trees including early maturing and powdery mildew resistant mid-season and late crosses were planted in the seedling block

- A total of 3000 seeds were collected from 2011 crosses of which 1162 were viable. Mean seed germination was 60% while seedling survival rate at the baby stage was over 90%.
- We have developed protocols in the greenhouses for raising seedlings over 3 ft tall in less than a year for field planting.
- We planted a total of 14 advanced selections into P2 trials at WSU-IAREC, Prosser and OSU MCAREC, Hood River. Three of these selections were planted at Norm Guitzweiler's orchard in N Wenatchee which represents a late site
- Elisa tests were conducted on 60 selections for use as breeding parents and those that tested positive for PDV, CLRV and PNRSV were eliminated.
- Pollen viability tests were conducted on 60 individuals used as breeding parents
- Hand pollinations carried out this year emphasized early and late ripening combined with high firmness. F_1 seedlings mainly from Sweetheart, Regina, Ambunes and Cowiche crosses with high breeding value for both firmness and late ripening were used for late ripening crosses. Six of those were advanced selections in P2. Individuals that have high breeding value for both earliness and firmness were also chosen for early ripening crosses. Breeding value was obtained from FlexQTL output in the RosBREED poject from genomic regions associated with ripening time and firmness. About 70,000 flowers were hand pollinated and fruit set was high although crosses for early ripening had a lower set than late crosses.
- Over 7000 seeds were collected from 2012 crosses while 4139 are viable.
- We thinned ~35 advanced selections identified for potential P2 planting to optimize fruit set
- We evaluated ~6000 trees in the field for fruit quality of which 2000 that had fruit size above 10 g were harvested for further evaluation in the lab. We also collected data from mother trees of advanced selections in the seedling blocks (P1) since P2 trees were not yet fruiting.
- We have sent budwood from the mother tree of FR001T007 for propagation for P3planting. Budwoods from FR001T002, FR001T004, FR002T074, FR009T049, FR044T083 and FR049T125 for propagation for P2.
- We budded 20 F_1 seedlings on Gisela 6 in a UFO system at IAREC Prosser to examine bud take and potential for cost savings compared to a seedling block.

EXECUTIVE SUMMARY

- The PNWSCBP has organized the human and physical resources necessary to have a world class program
- We have optimized seed germination procedures and a seed germination rate of 60% is now routine in the breeding program
- A seedling survival rate of >90% at the baby stage is common while our greenhouse techniques can generate seedlings of adequate size for field planting within a year
- We have developed and implemented bar coding for tree identification in the field and for fruit evaluation in the lab. This technology will improve tree identification and data recording and along with marker –assisted breeding will increase overall efficiency of the breeding program
- The breeding program has entered a new era with phase 3 operational in 2014. There are ~8000 seedlings in P1, 14 advanced selections in P2 and 5 more advanced selections will be planted in P2 in 2013. The P3 fast-track, FR001T007, is an early selection similar to Chelan in harvest timing but has larger fruit (>10 g) and higher firmness (~300 g/mm) without GA application.

FINAL PROJECT REPORT

Project Title: Virus elimination and sensitivity assessment of MSU cherry rootstocks

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Cooperators: Matt Whiting, Tom Auvil, Ken Eastwell

Total Project Funding Year 1: $2,600

Budget: James Susaimuthu
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Contract Administrator: James Susaimuthu
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Item	2012	2013	2014
Fee for heat therapy[1]	$1,800		
Fee for virus sensitivity tests[2]	$800		
Total	$2,600		

Footnotes:
[1] For the candidate rootstock CASS.
[2] For the four candidate rootstocks at $200/each.

ORIGINAL OBJECTIVES

(1) Conduct cold and chemotherapy for the MSU candidate cherry rootstock CASS to provide virus certified budwood for continued rootstock trials.

(2) Determine the virus sensitivity of the top four MSU candidate cherry rootstocks (CASS, CLINTON, CLARE and LAKE).

SIGNIFICANT FINDINGS

- Virus certified CASS rootstock material was identified and increased via tissue culture to provide both budwood and tissue culture material that was distributed to liner nurseries in September 2012.
- The second objective, determining the virus sensitivities of the other MSU candidate cherry rootstocks, will be done in 2013 as sufficient plant material for these activities was not available in 2012. This will be done using the previously awarded funding

RESULTS & DISCUSSION

Plants of CASS, one of the MSU cherry rootstock candidates maintained at the National Clean Plant Network – Fruit Trees (NCPN-FT), tested positive for *Hop stunt viroid* (HSVd) in 2011. Therefore, in 2012, the CASS plants at the NCPN-FT were repeatedly tested (a total of 21 separate instances) for different pathogens, including HSVd. Fortunately one of the four original CASS plants established at the NCPN-FT was eventually confirmed negative for HSVd and it was introduced into tissue culture. Virus certified budwood and tissue culture derived tubes from this one CASS plant were distributed to five commercial liner nurseries in September so that these nurseries could pursue propagation trials and provide liners for future test plots.

The second objective, determining the virus sensitivities of the other MSU candidate cherry rootstocks, requires a minimum of 20 liners per selection. As this number of liners was not yet available in 2012, the liners will be obtained in 2013 and these will be used for the virus screening. This experiment will be covered by the funding originally provided using the following protocol.

Inoculation and evaluation of test plants: Test plants of each of four MSU candidate cherry rootstocks and resistant and susceptible controls (see plant material list below) will be screened for their reaction to PDV and PNRSV separately and in combination. Each test will be replicated three times (Treatments = PDV, PNRSV, PDV & PNRSV and healthy control).

- CASS
- LAKE
- CLARE
- CLINTON
- Gisela 4 – Susceptible control
- Gisela 5 – Resistant control

Test plants will be evaluated for the virus sensitivity of the rootstocks through two growing seasons and a final report will be provided.

EXECUTIVE SUMMARY

CASS is one of the MSU candidate cherry rootstocks that shows commercial promise based on yield and fruit size observations from the trial at the Roza Station – WSU Prosser. Therefore, to provide a source of virus certified plant material for liner generation, these rootstocks were tested at the NCPN-FT for their virus status. Unfortunately, in 2011, CASS tested positive for *Hop stunt viroid* (HSVd) and therefore could not be distributed. However, after repeated testing in 2012, one CASS plant tested negative for HSVd and was therefore virus certified. As a result, CASS was successfully distributed to five liner nurseries in September 2012. These nurseries will experiment with CASS liner production and provide liners, as needed, for future trials.

All of the MSU sweet cherry candidate rootstocks were previously determined not to be sensitive to Prune Dwarf Virus (PDV) and *Prunus* Necrotic Ringspot Virus (PNRSV). A recent review of these tests that were conducted over 10 years ago, revealed that the characterizations of the rootstock reactions were minimal. Given that these MSU cherry rootstocks are being considered for commercialization, funds were awarded to obtain definitive characterization of their reactions to PDV and PNRSV infection. Knowledge of the reaction of these rootstocks to these common pollen borne viruses will be used to determine any potential risks to the commercial adoption of these rootstock candidates. These virus screens, originally scheduled for 2012, will be performed in 2013 as sufficient liners were not available for the 2012 growing season.

FINAL PROJECT REPORT

Project Title: Insecticide management of spotted wing drosophila in cherry orchards

PI:	Peter W. Shearer, Ph.D.	**Co-PI**:	Preston H. Brown
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Other funding sources

Agency Name: USDA SCRI SWD Grant: Biology and management of spotted wing drosophila on small and stone fruits: Duration 4.5 years

Amt. awarded: $598,144 / $564,959

Total Project Funding: $18,003

Budget History:

Item	Year 1: 2012
Salaries	
Benefits	
Wages	$11,520
Benefits	$1,005
Equipment	
Supplies	$600
Travel	$440
Plot Fees	$4,437
Miscellaneous	
Total	**$18,002**

RECAP ORIGINAL OBJECTIVES

1. *Determine effective insecticide rates for spotted wing drosophila management in cherry orchards*
    a. The original goal of this project was to conduct insecticide-rate studies in a large block of commercially managed Regina cherries maintained at MCAREC. The methodology included releasing large quantities of adult SWD to coincide with the insecticide applications and then assess the impact of these insecticides to protect fruit from infestation. Unfortunately, we were not able to obtain funding from the commission to reimburse MCAREC for using these trees. Instead, we conducted field-lab assays using four-single tree replicates to determine residual activity of insecticides against SWD. We evaluated efficacy of adult female SWD exposed to leaves and fruit out to 14 days after treatment (DAT).

2. *Evaluate if intensive sampling and monitoring for SWD will allow growers to incorporate GF-120 into their cherry IPM programs*
    a. This objective was modified for 2 reasons, 1) we detected SWD infested fruit in the earliest maturing blocks in Dallesport, and 2) we could not locate growers who would risk using GF-120 in light of the early documented SWD damaged fruit. Therefore, we took the opportunity to intensively monitor fruit from 12 commercial orchards; 3 early ripening blocks in both Dallesport and Hood River and 3 late harvest blocks in both The Dalles and Parkdale. We compared two types of apple cider baited traps at two densities and examined fruit for SWD eggs during the ripening period. In all 12 cases, we trapped and examined fruit at the orchards' periphery and interior.

SIGNIFICANT FINDINGS

Adult female spotted wing drosophila were used to evaluate the effectiveness and residual activity of various insecticides. Their response varied depending upon substrate (treated leaves versus fruit) and insecticide. The pyrethroids are the most efficacious and long lasting products tested. Spinosyns (Delegate and Entrust) are slower acting. Malathion provides quick knockdown but has extremely short residual activity.

Intensive trapping generally detected adult SWD before eggs were observed in cherry fruit. It is quite possible that with a better trap, increased trap numbers and possibly fruit sampling, growers and fieldmen may be able to use other products for cherry fruit fly when SWD is not present. Further research is needed to substantiate this.

RESULTS & DISCUSSION

1. Insecticides for spotted wing drosophila management

Various insecticides were applied to three ripening cherry trees per treatment using an airblast sprayer calibrated to deliver 100 GPA while traveling at 1.6 MPH. The application date was 24 July, 2012. Afterwards, we collected four leaves from each of the three replicates/treatment on 1, 3, 7, 10 and 14 days after treatment (DAT). Individually leaves were encased in small petri dishes containing five adult female SDW that were exposed to the bottom leaf surface plus a small piece of artificial diet for humidity control. Mortality was assessed after 24 and 48 h of exposure.

Adult female SWD mortality and egg production were also assessed by exposing adult female SWD to fruit collected from the same trees. At 1, 7 and 14 DAT, 5 fruit per replicate were collected

and placed into individual 1 oz portion cups. A single fly was added and then the cup was covered. Adult mortality was assessed at 24 h. The number of eggs laid in fruit was counted under a stereomicroscope. Average % mortality and average number of eggs laid was then calculated for each treatment.

The pyrethroids Warrior and Danitol and the OP malathion provided the best knockdown activity after 24h exposure 1DAT (Table 1). The pyrethroids provided the best residual activity with 90+ % mortality 10 DAT after 24 h exposure and over 85% mortality 14 DAT with a 48 h exposure to treated leaves (Tables 1 & 2). Malathion provided only marginal kill 3 DAT and mortality in this treatment was not different from levels of mortality observed in the untreated control leaves by 7 DAT. Leaves treated with Delegate or Entrust SC provided intermediate control at the 24 h assessment. However, the 48 h assessment revealed an increase in mortality of these products indicating that these spinosyns are not fast-acting when flies are exposed to treated leaves (Table 2). The nicotinoid insecticide, Belay, was not effective. Figures 1 (24 h) and 2 (48 h) presented this information as % corrected mortality, which factors in the levels of mortality observed in the control.

Interestingly, the effect of treated fruit as a substrate on mortality of adult SWD is somewhat different from what was observed on flies exposed to treated leaves. In this case, the spinosyns Delegate and Entrust and the carbamate carbaryl killed 100% of the flies within 24 h of exposure 1 DAT while during the same assessment period, only 67, 67 and 50% (respectively) of flies confined on leaves 1 DAT died, indicating these products were not as efficacious on foliage as compared with treated fruit (Tables 1 & 3, Fig. 3). Leaves treated with carbaryl were not as toxic as treated fruit to female SWD and fruit treated with carbaryl was more toxic than malathion 1 and 7 DAT (Table 3, Fig. 3). As in the leaf assay, Belay was not effective. This supports previous research indicating that nicotinoid are not effective against SWD.

Interpreting these results should be made with caution because of the differences in how adult female SWD flies responded to treatments on leaves versus fruit. As indicated, certain products such as spinosyns and carbaryl performed better when fruit were used as the assay substrate while the pyrethroids and malathion looked best when leaves were used. Another cautionary note is based upon how fast the residual activity of malathion declines. Results here indicate this product is active for 3 days or less. Growers that rely on malathion ULV with extended intervals may run into problems with infestations, especially late season.

Overall, these results parallel results from similar studies that indicate the pyrethroids, OPs and spinosyns have activity against SWD although residual control varies among the products. It may also be possible to use high rates of carbaryl as a rotational product for resistance management but not rely on it as a stand-alone product until further testing is conducted.

2. Evaluate if intensive sampling and monitoring for SWD will allow growers to incorporate GF-120 into their cherry IPM programs

This research was conducted in 12 commercially managed blocks of cherries. These sites were located in the two cherry districts, Dallesport/The Dalles and Hood River/Parkdale, OR. Within each district, three blocks were located in early-ripening areas (Dallesport and Hood River) and three blocks were located in late-ripening blocks (The Dalles and Parkdale).

At each orchard, we deployed 16 traps, all baited with apple cider vinegar; eight were the standard 32 oz deli trap with holes on the sides and the remaining were “Haviland” traps, a Rubbermaid container with a screen lid which has been shown to capture greater numbers of SWD. Half the traps at each block were deployed along a suspect border; the remainder was placed in the

interior of the cherry block. Traps were deployed at two densities; one trap per unit area versus three traps on three adjacent trees. The density component of this study allowed us to determine if we gain precision (first detection of SWD in traps, number of SWD per trap) by placing more traps in a localized area. The 192 traps were checked weekly.

Fruit samples were also collected and assessed for infestation. Each sample consisted of of 250 fruit collected haphazardly from periphery and interior trees. A sub-sample was observed for eggs under a stereo dissection scope, another subset was crushed crushed and inspected for infestation using the brown-sugar float method developed for cherry fruit fly (CFF) and a third subset was held in containers to rear out SWD. Phenology stages, color and soluble solids of the ripening cherries were recorded at sampling. Monitoring of traps and fruit sampling will continue through harvest

Female flies were trapped in all 12 blocks while male flies were trapped in 10 blocks. The first flies captured in a block were female in 6 blocks, males in two blocks and both sexes were first captured simultaneously in blocks 4 times (Fig. 4). There was no difference in when the first flies were captured between traps placed in border versus interior trees (Table 5). Flies were captured in border trees 7 times and in traps inside the orchard 9 times. In six instances, flies were detected on border and interior trees simultaneously. We caught significantly more flies in the Haviland traps compared with the deli cups. The average seasonal total for flies captured in the Haviland trap and deli cup was 42 versus 16, indicating that the Haviland trap is a superior trap. Additionally, the Haviland traps were more likely to capture the first fly in a block (Table 6). Regarding using traps in clusters to detect the first fly in a block, single traps captured the first fly 4 times while the clusters of three traps captured the first fly 13 times (Table 7). This indicates that the more traps placed in an orchard increases the likelihood one will capture the first fly.

In general, adult SWD were detected in traps (10 of 12 sites) in the study sites before eggs (1 of 12 sites) or larvae in fruit (1 of 12 sites). We observed SWD eggs in fruit collected from 5 blocks. In general, we captured adults in traps before we observed eggs in associated fruit (Fig. 5). Adults were detected in traps 7, 15, 28 and 34 days before we detected eggs in fruit on those 4 blocks. At one site in Dallesport, we detected eggs 7 days before we captured the first adult SWD in that block. In that sample, the average CTIFL fruit color was 1.9 indicating this block was attacked early in the ripening process. We suspect this damage was caused by the overwintering generation that survived a mild winter. The average CTIFL color of fruit in the other four blocks where we observed SWD eggs ranged from3.1-4.8. Six of 12 blocks had fruit in the green-pink stages when the first flies were captured.

Table 1. Mean % mortality (±SEM) of adult female *Drosophila suzukii* exposed to treated foliage for 24h 1, 3, 7, 10, or 14 after treatment (DAT).

		% Mortality (±SEM) of adult female *Drosophila suzukii*				
		DAT				
Insecticide	Rate/acre	1	3	7	10	14
Warrior II	2.56 oz	100.0±0.0a	100.0±0.0a	95.0±5.0a	90.0±5.8a	90.0±2.9a
Danitol 2.4 EC	16 oz	100.0±0.0a	96.7±3.3a	93.3±4.4a	90.0±7.6a	75.0±7.6a
Danitol 2.4 EC	21 oz	95.0±2.9a	100.0±0.0a	90.0±7.6a	100.0±0.0a	80.0±10.4a
Delegate WG	4.5 oz	66.7±19.2b	45.0±7.6b	18.3±3.3bc	11.7±1.7b	13.3±4.4b
Entrust SC	6.4 oz	66.7±11.7b	38.3±1.7bc	23.3±11.7b	23.3±6.7b	25.0±10.4b
Malathion 8 EC	80 oz	98.3±1.7a	53.3±15.9b	3.3±1.7c	16.7±12.0b	8.3±6.0b
Belay	6 oz	10.0±5.0d	10.0±2.9cd	5.0±2.9c	3.3±3.3b	11.7±9.3b
Carbaryl 4 L	2 qt	50.0±23.1c	33.3±4.4bc	13.3±4.4bc	20.0±13.2b	15.0±2.9b
Control	--	3.3±1.7d	5.0±2.9d	3.3±1.7c	3.3±3.3b	1.7±1.7b

Table 2. Mean % mortality (±SEM) of adult female *Drosophila suzukii* exposed to treated foliage for 48h 1, 3, 7, 10, or 14 after treatment (DAT).

		% Mortality (±SEM) of adult female *Drosophila suzukii*				
		DAT				
Insecticide	Rate/acre	1	3	7	10	14
Warrior II	2.56 oz	100.0±0.0a	100.0±0.0a	96.7±3.3a	96.7±3.3a	98.3±1.7a
Danitol 2.4 EC	16 oz	100.0±0.0a	98.3±1.7a	95.0±5.0a	93.3±6.7ab	85.0±7.6ab
Danitol 2.4 EC	21 oz	96.7±3.3ab	100.0±0.0a	91.7±8.3a	100.0±0.0a	86.7±8.8c
Delegate WG	4.5 oz	90.0±5.0ab	73.3±4.4ab	35.0±8.7bc	43.3±6.0c	31.7±11.7c
Entrust SC	6.4 oz	90.0±2.9ab	63.3±4.4b	66.7±8.8ab	46.7±6.7bc	36.7±13.6bc
Malathion 8 EC	80 oz	98.3±1.7a	63.3±19.6b	3.3±1.7d	18.3±11.7cd	16.7±7.3c
Belay	6 oz	23.3±8.8c	16.7±6.0cd	8.3±1.7dc	6.7±6.7d	16.7±14.2c
Carbaryl 4 L	2 qt	66.7±17.6b	50.0±5.8bc	51.7±17.4b	51.7±8.8	21.7±3.3c
Control	--	6.7±1.7c	8.3±1.7d	3.3±1.7d	3.3±3.3d	8.3±6.0c

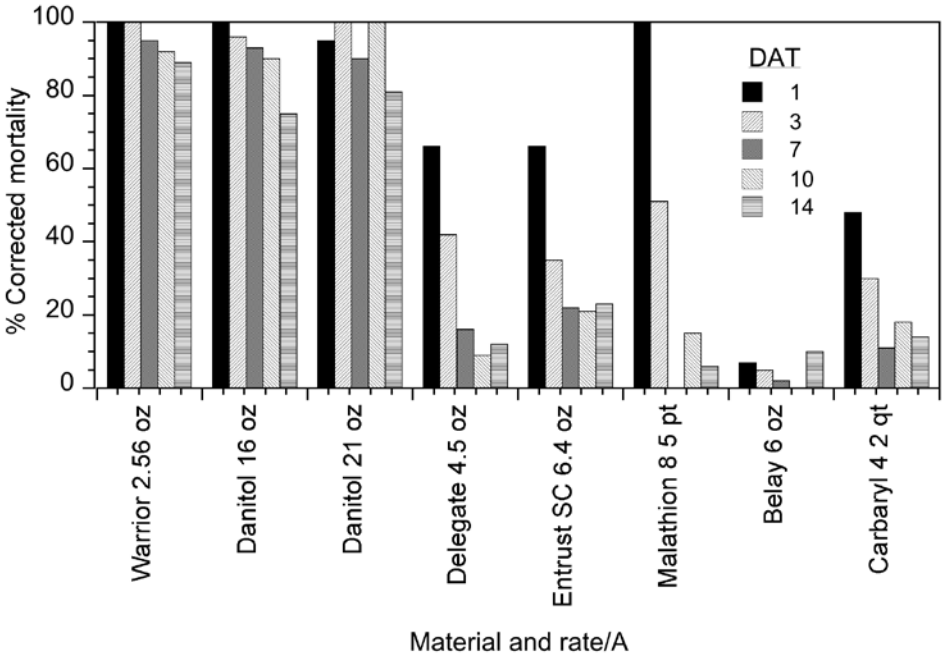

Fig 1. Mortality of adult spotted wing drosophila 24h after being placed on treated leaves 1, 3, 7, 10 and 14 days after treatment (DAT).

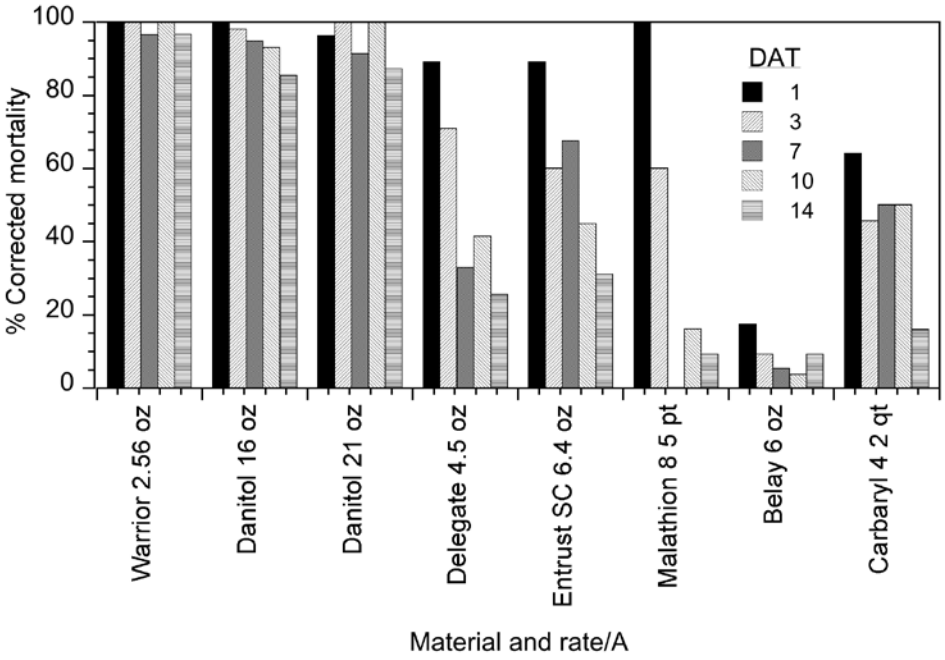

Fig 2. Mortality of adult spotted wing drosophila 48h after being placed on treated leaves 1, 3, 7, 10 and 14 days after treatment (DAT).

Table 3. Mean % mortality (±SEM) of adult female *Drosophila suzukii* exposed to treated cherry fruit for 24h 1, 7, or 14 after treatment (DAT).

		% Mortality (±SEM) of adult female *Drosophila suzukii*		
		DAT		
Insecticide	Rate/acre	1	7	14
Warrior II	2.56 oz	80.0±11.5ab	66.7±17.6ab	53.3±6.7abc
Danitol 2.4 EC	16 oz	80.0±0.0ab	33.3±17.6	20.0±11.5bc
Danitol 2.4 EC	21 oz	86.7±6.7a	53.3±6.7ab	46.7±26.7abc
Delegate WG	4.5 oz	100.0±0.0a	80.0±11.5ab	40.0±20.0abc
Entrust SC	6.4 oz	100.0±0.0a	73.3±13.3ab	86.7±6.7a
Malathion 8 EC	80 oz	73.3±17.6ab	20.0±11.5bcd	20.0±20.0c
Belay	6 oz	33.3±24.0bc	6.7±6.7cd	20.0±11.5bc
Carbaryl 4 L	2 qt	100.0±0.0a	86.7±6.7a	73.3±6.7ab
Control	--	0.0±0.0c	0.0±0.0d	13.3±6.7c

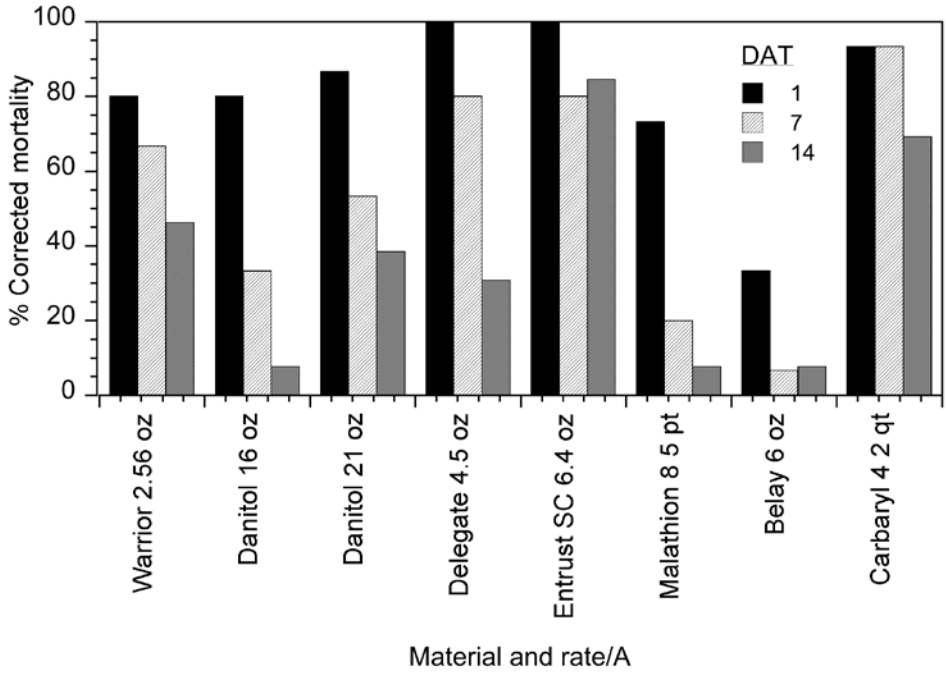

Fig 3. Mortality of adult spotted wing drosophila 24h after being placed on treated fruit 1, 7 and 14 days after treatment (DAT).

Table 4. Mean number (±SEM) of eggs laid 24h after exposure in cherry fruit collected 1, 7, and 14 days after treatment (DAT).

		Mean (±SEM) number of eggs		
		DAT		
Insecticide	Rate/acre	1	7	14
Warrior II	2.56 oz	0.3±0.2e	0.1±0.1c	2.6±0.9d
Danitol 2.4 EC	16 oz	0.4±0.4de	2.0±0.8bc	4.6±0.9bcd
Danitol 2.4 EC	21 oz	0.6±0.3cde	0.6±0.5c	2.7±0.5dc
Delegate WG	4.5 oz	2.0±0.9cde	2.7±1.2bc	7.1±2.8bcd
Entrust SC	6.4 oz	3.5±1.3bc	2.2±1.6c	12.3±4.8ab
Malathion 8 EC	80 oz	3.4±1.6bcd	12.9±4.0a	22.5±4.0a
Belay	6 oz	9.8±3.0ba	16.1±8.8a	25.3±4.8a
Carbaryl 4 L	2 qt	2.3±0.5cde	1.4±0.8c	9.4±0.4abc
Control	--	14.8±2.8a	9.0±2.0ab	23.9±4.5a

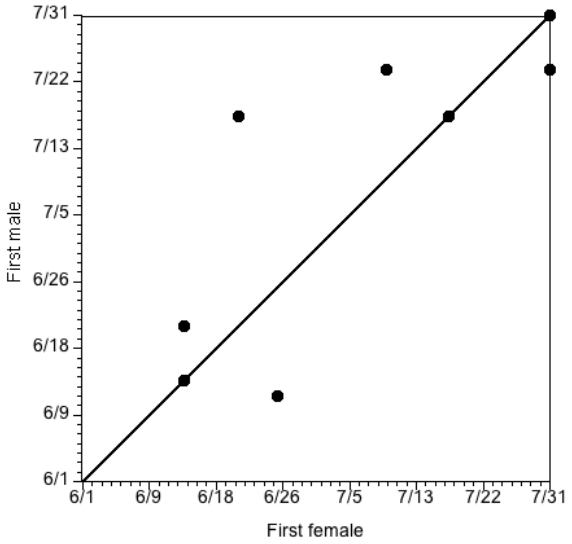

Figure 4. Relationship between first adult male and female SWD captured in ACV traps in the same block.

Table 5. Influence of orchard location on first capture of *Drosophila suzukii*

	Sex	
Field position	Female	Male
Border	5	2
Interior	6	3
Same time	1	5

Table 6. Influence of trap type on first capture of *Drosophila suzukii*

	Sex	
Trap type	Female	Male
Deli cup trap	3	0
Haviland trap	7	5
Same time	2	5

Table 7. Influence of trap density on first capture of *Drosophila suzukii*

	Sex	
Trap density	Female	Male
Single trap	3	1
Three traps	8	5
Same time	1	4

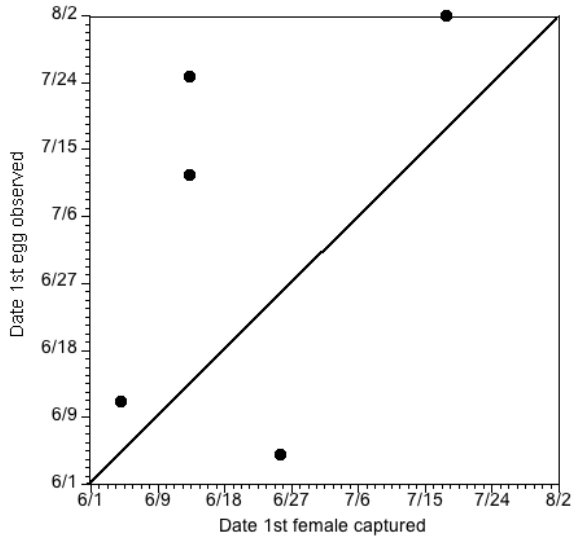

Figure 5. Relationship of first female captured in ACV baited traps with first observed egg.

EXECUTIVE SUMMARY

Project Title: Insecticide management of spotted wing drosophila in cherry orchards

PI: Peter W. Shearer, Ph.D. **Co-PI**: Preston H. Brown
Organization: OSU Mid-Columbia Ag. Res. & Ext. Ctr.

Early detection of commercial cherry fruit infested with spotted wing drosophila (SWD) and an agreed upon budget reduction forced changes to be made in how the two objectives were carried out. Instead of being able to buy the crop of commercial cherries at MCAREC for use in determining effective rates of a few insecticides, we looked at residual activity of several classes of insecticides to determine how long these were active against SWD. In this case, we determined that the pyrethroids provided the longest residual activity and that malathion was extremely short-lived. This later observation translates into a caution for growers that might want to rely on malathion ULV to provide extended control. It appears that when SWD is present, growers should not rely on this product exclusively. Similarly, while Delegate and Entrust are effective, they are not nearly as fast acting as pyrethroids and OP insecticides. This study has also indicates that high rates of carbaryl may provide some control of SWD, especially as a rotational material.

The original proposal also had a component in it to look at whether intensive trapping and monitoring for SWD would allow growers to use GF-120 for cherry fruit fly control if and when SWD was not detectable in the orchard. This project was modified after we detected SWD infested cherries in two early season blocks and we were not able to find growers that would risk their crops by using GF-120 for cherry fruit fly. So, instead of working in 4 orchards with intensive sampling in GF-120 and insecticide treated blocks, we expanded our study to 12 locations that represented early and late districts in Dallesport/The Dalles and Hood River County. Overall, we were able to detect flies in orchards with traps (16 traps per orchard) before we detected infested fruit. We also demonstrated that using more traps is better for detecting the first flies in cherry orchards. We also demonstrated that there is a better trap than the clear deli cup for detecting the first fly and for monitoring purposes.

Based upon these studies, we feel that with accurate monitoring, it is likely that growers may be able to treat for cherry fruit fly when SWD is not present. Before this happens, more research is needed.

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

Project Title: Influence of cropload level on fruit size and quality of sweet cherry

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Cooperators: Anita Azarenko

Total Project Request: $290,530 **Year 1: $**69,258 **Year 2:** $71,688 **Year 3:** $74,214

Other funding Sources: None

WTFRC Collaborative expenses: None

Budget 1 Todd Einhorn
Organization Name: OSU-MCAREC **Contract Administrator: Dorothy Beaton**
Telephone: 541-737-3228 **Email address: dorothy.beaton@oregonstate.edu**

Item	2010	2011	2012
Salaries[1]	37,350	38,844	40,397
Benefits	21,758	22,628	23,533
Wages[2]	1,500	1,560	1,622
Benefits	150	156	162
Equipment			
Supplies[3]	6,500	6,500	6,500
Travel[4]	2,000	2,000	2,000
Miscellaneous			
Total	69,258	71,688	74,214

Footnotes: [1]Salaries are for: 1) 0.75 FTE of a postdoc salary. OPE is 57 %, and 2) 0.15 FTE for faculty research assistant factoring an OPE rate of 65 %. [2]Wages are for part-time employee to assist with data collection, OPE is 10%. [3]Supplies include GA, chemicals for cell activity assays, and rates for microscopy lab use at OSU-Corvallis. [4]Travel includes visits to sites for sampling and trips to WA for laboratory analyses, and is 59 cents/mile.

Objectives

1) Understand timing of mesocarp cell division & expansion cycles, and their relative role in fruit quality, and determine the influence of cropload on these processes
2) Determine effective application timings and rates of GA for improved cherry fruit quality

Significant Findings

1. Fruit growth, cell number and size

- Growth characteristics of 'Chelan', 'Bing' and 'Sweetheart' were remarkably similar during early- and mid-stage growth. The difference between cherry varieties was in the duration of the stage III- final fruit swelling.
- 'Sweetheart' fruit size was negatively affected by high cropload level. Reduced fruit size on heavily cropped spurs was observed by 18 days after full bloom. These effects were sustained through the remainder of the season, and at harvest. Heavily cropped spurs reduced the average fruit size by about 15%.
- Heavy croploads limited pit size (endocarp). Pit growth ceased by 38 days after full bloom, and was positively correlated with final fruit size.
- The cell division period of 'Sweetheart' fruit was completed during mid-stage I growth, about 20 days after full bloom. Cell numbers do not appear to be affected by cropload.
- Roughly half of the cells comprising an individual fruit at harvest are already present at full bloom.
- Cells of the inner region of the mesocarp were elongated at harvest. Cells of the outer region also were elongated, but during the final fruit swelling became rounded as they expanded tangentially.
- Final fruit size was more strongly correlated with cell size than cell number. These results suggest that growers have a longer time frame to alter cropload than if cell number was the dominate factor in fruit size. However, early season deficits in growth will also be evident at harvest.
- The majority of the nuclei of 'Chelan', 'Bing' and 'Sweetheart' fruit become polyploid (i.e., cells possess multiple copies of chromosomes compared to vegetative tissues). After full bloom, polyploidy in fruit increased rapidly to > 60% by 7-10 days, in step with cell division activity. Fruitlets from flowers that were bagged to prevent pollination were far less polyploid which emphasizes the role of fertilization in promoting cell division and growth.
- Cropload level, and genotype, did not influence the magnitude or timing of polyploidy during fruit development when fruit size was considered.

2. GA Experiments

- The largest differences among all tested GA concentrations (10, 20, 30, 40, 60 ppm) and timings (single applications at straw color, or multiple applications split between straw color and mid-stage III) were observed between 0 and 20 ppm. The quality attribute consistently affected by GA was fruit firmness (higher when provided GA, but not consistently with rates beyond 10 ppm). This effect was observed on all cultivars evaluated (Lapins, Skeena, Sweetheart and Staccato).
- Multiple applications did not result in higher quality fruit.
- In most trials fruit size was not increased with GA. In trials where improvements in fruit size were detected, the response was not influenced by rate beyond 20 ppm.
- Skin color (darkening) was delayed with the application of GA; however, beyond the 20 ppm rate, the effects were highly variable, and difficult to qualify.
- Pitting was reduced in Lapins and Sweetheart at 25 ppm GA; however, concentrations exceeding 25 ppm (up to 100 ppm) did not improve the response.

  Return bloom (floral buds per spur and flowers per floral bud) was not reduced by rates between 10 and 60 ppm.

Results and Discussion

Fruit growth: Better uniformity in the timing of pollination and a greatly increased number of experimentally 'set' fruit were obtained by our modified procedures (refer to methods section). We estimate that full bloom of experimental-tagged flowers occurred within 1 day. A large number of un-tagged flowers and fruits were also sampled in an effort to capture the full range of fruit size on a given day. These samples included both early and late blooming fruits as well as un-fertilized fruitlets. Results confirm that better uniformity in fruit size of 'set' fruit was obtained throughout the growing season compared to the 'range' or crop average. Because the 'range' material displayed wide variability of sizes during the first few weeks after bloom, we were able to identify fruitlets that were likely un-fertilized. Upon re-examination of the 'set' fruit we realized that most of the 'set' material we had sampled in the first few weeks of growth were in fact un-fertilized and small in size. Once June-drop occurred the range of fruit sizes was greatly diminished because only successfully fertilized fruit were sampled. From this time onward the differences in variability between 'set' and 'range' fruit were evident, and we attributed the difference to individual bloom time. However, the question remained—what are the relative effects of individual bloom time and size of the ovary to final fruit size? To address these questions we needed to assess ovary size before bloom. Beginning before dormancy break this year, we began sampling buds and dissecting the ovaries for volume measurements. We also set up a large amount of material in bee-exclusion netting to measure the growth of what would only be un-fertilized fruitlets.

Growth analysis: Growth analysis, simply put, is the application of a mathematical function to growth data using curve fitting techniques. A necessary step in the analysis is an assessment of the variability of data from the beginning to end of the observations—and we achieved this from bud dormancy to harvest by combining last year's 'bloom to harvest' with this year's 'dormancy to bloom' data (Figure 1). Furthermore, because data of this kind spans several orders of magnitude, the variability of the raw data between sampling dates cannot be adequately described without mathematical transformation of the data. The transformation most commonly used is the $Log_{(e)}$, or 'natural log' because plant growth, especially in determinate growth organs such as fruit, follows a logarithmic pattern. This type of growth is also commonly described as the principle of compound interest. A pleasing result of this data transformation as applied to cherry growth is the observation that the magnitude of the variability in harvest fruit size is very similar, if not identical to the variability observed in dormant buds and blooms. This spurred us to attempt to segregate the data into what would be either successful 'fruit' or 'failures'. After performing these analyses separately for each cultivar of this study, as well as the cropload study, we realized that the patterns of growth were very similar. Therefore, we re-evaluated the data by combining all the data (a total of 12,099 individuals) and refitting it to a polynomial curve. Much can be said about the choice and validity of various curve fitting techniques but it suffices to point out the good correlation of the actual data points to the fitted curve for each variety.

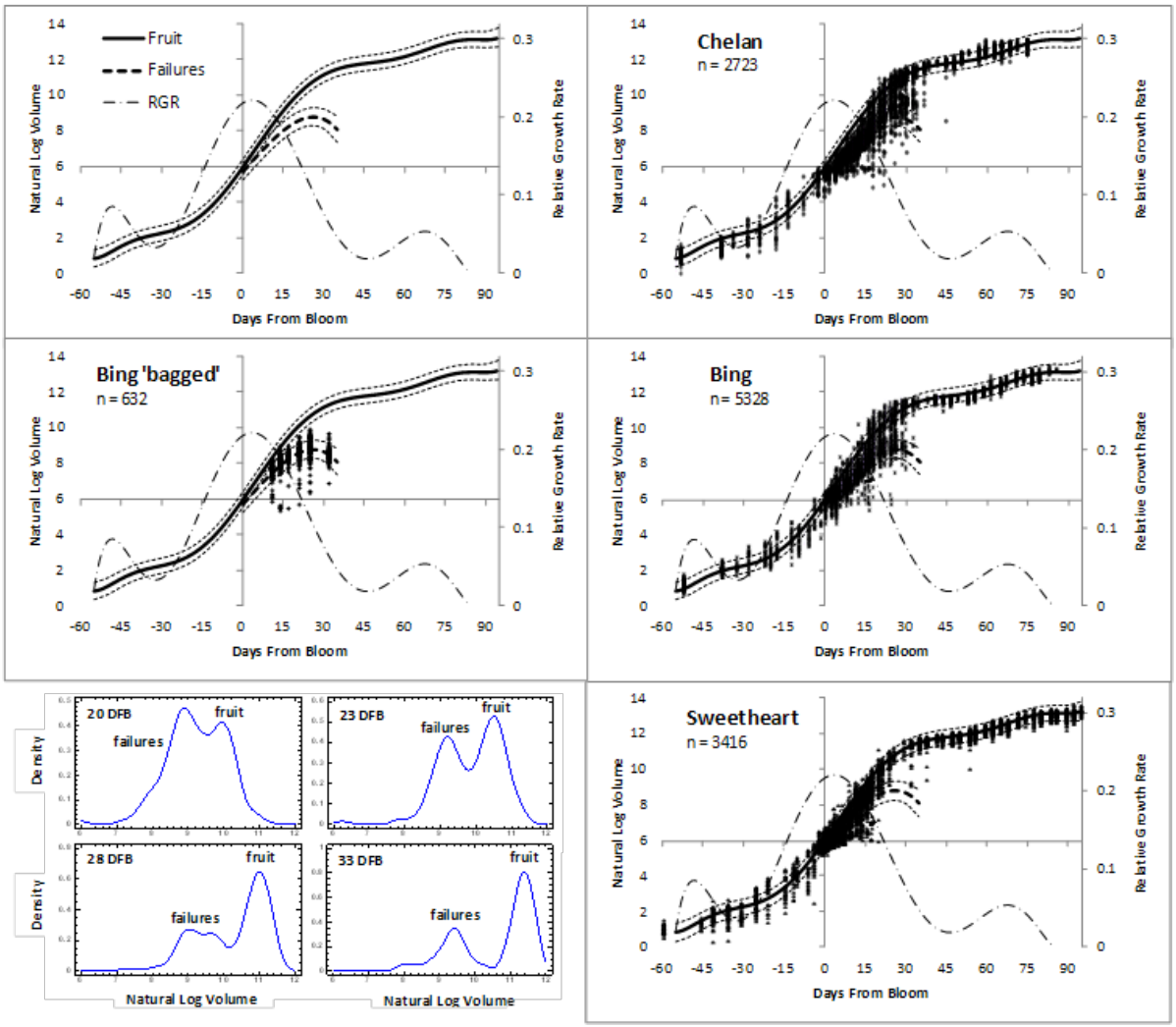


Figure 1. Growth analyses of 'Chelan', 'Bing' and 'Sweetheart' from bud-dormancy break to fruit harvest. Volume data were $Log_{(e)}$ transformed before polynomial regression of the means of 'fruit' (solid lines) and 'failures' (heavy dashed lines) with 95% prediction limits (thin dashed lines). Relative growth rates were calculated from the fitted curve (dot-dash lines). 'Fruit' and 'failure' data were distinguished by measurement of the ovaries in bee-exclusion 'bagged' limbs (i.e., unfertilized ovaries). Data from the time period when growth of 'fruit' and 'failures' overlap (4 to 17 DFB) were excluded from the polynomial regression. Data from 18 to 35 DFB were visually separated into 'fruit' and 'failures' using distribution density analysis (lower left panels), thereby defining the transition from rapid growth to pit hardening. Data for each cultivar are superimposed on the fitted curves.

Pit growth: Growth of the endocarp (pit) was completed by 38 DFB, and was negatively influenced by cropload level (Fig. 2). Differences in pit volume between heavy and light cropload treatments were observed by about 18 DFB (Fig. 2). Final fruit size was positively related to the size of the pit (Fig. 3). Approximately 94% to 95% of the final fruit volume was mesocarp, irrespective of cropload. These data support the contention that greater competition for carbohydrates by higher fruit load has an early, and marked, effect on fruit growth. They also imply that mechanisms controlling pit growth could be targeted to produce larger fruit.

We also evaluated the condition of the kernel throughout the growing season. Interestingly, kernel development ceased in about 50% of fruit during early-stage III growth and at harvest only about

50% of fruit had a full kernel. Therefore, once the pit has hardened a fertile kernel is not required for continued fruit growth.

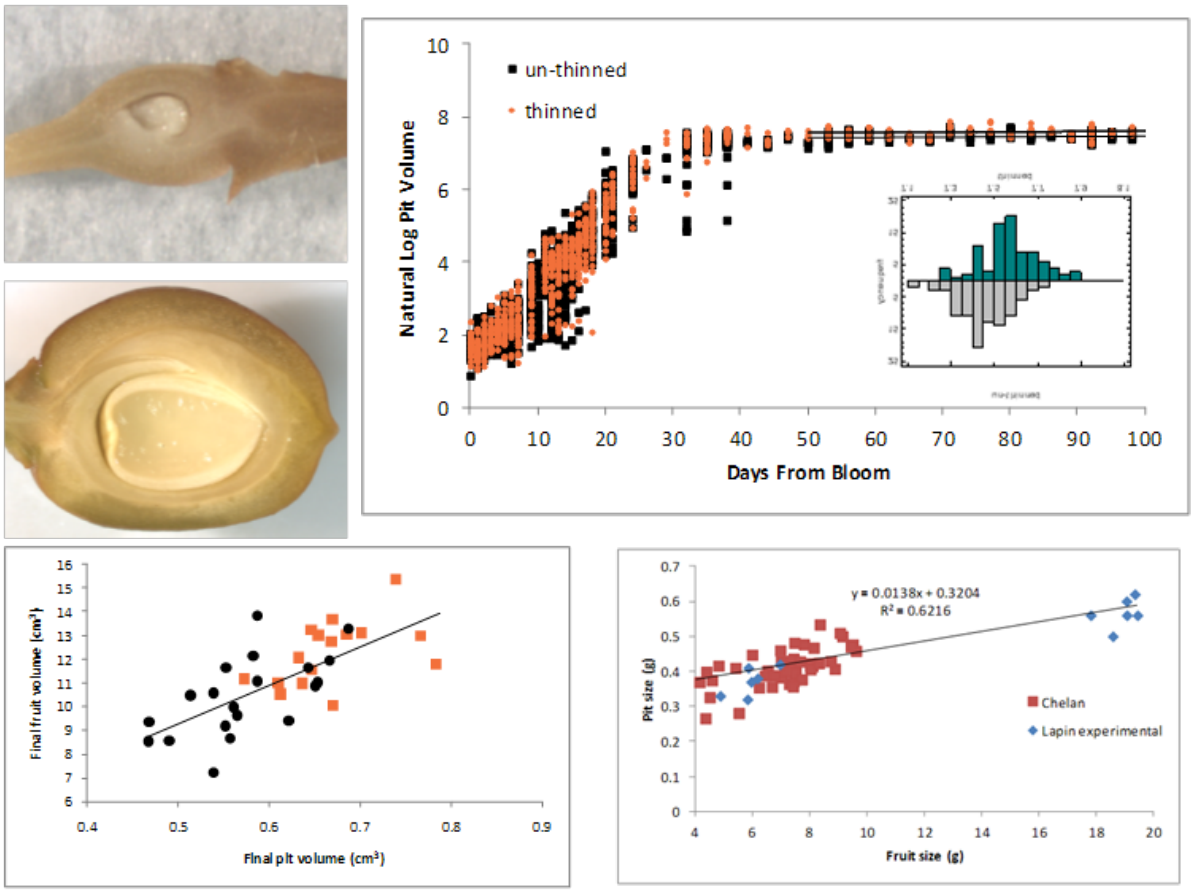


Figure 2. Pit size and fruit size are positively correlated. Ovaries were dissected (upper left panel) for measurement of pit volumes until pit hardening. After pit hardening, fruits were measured then the pits were cleaned for measurement of pit volume. Reduction in growth of the pit was observed as early as 18 DFB (upper right panel) in 'Sweetheart' when fruit per spur was greater than 12 as compared to less than 4. The average hardened pit size (inset histogram, 50-100 DFB) was significantly smaller with high cropload. Larger fruit had larger pits, irrespective of cropload in 'Sweetheart' (lower left panel). Comparison of average size Chelan, small Lapins and very large Lapins (lower right panel) demonstrate a positive pit to fruit size relationship.

SEM: High quality images were obtained using standard sample preparation techniques (Fig. 3). The region chosen for analysis was the widest breadth of the "cheek" perpendicular to the suture plane as has been done in several studies of stone fruit. Whereas most studies only observed a central region of the mesocarp for cell size measurements, we were able to obtain measurements across the entire mesocarp (Fig 3), and plot these measurements in relation to their position in the fruit (Fig. 4). From these relationships, it can be seen that cell divisions occur early and terminate early during fruit development. Further, cell expansion parallels the growth of the fruit throughout its entire development. With these measurements we determined that three regions (outer, inner and pit boundary) of the mesocarp showed differences in size, shape and growth. Cell shape was determined by the ratio of the radial to tangential diameter (data not shown). All cells at full bloom were slightly flattened tangentially then became isodiametric near the time of the cessation of cell division, about 20 days after full bloom. After cell divisions stopped, cells of the outer and inner mesocarp elongated

about 20 times their original size by the middle of stage III. During the final fruit swelling of late-stage III, mesocarp cells continued to elongate, including those of the pit boundary. However, only the outer mesocarp cells swelled tangentially forming once again, isodiametric cells. Furthermore, these outer mesocarp cells were the only cells that showed a treatment effect of flower thinning. Outer mesocarp cells of the thinned treatment were larger in both dimensions compared to cells of the unthinned treatment.

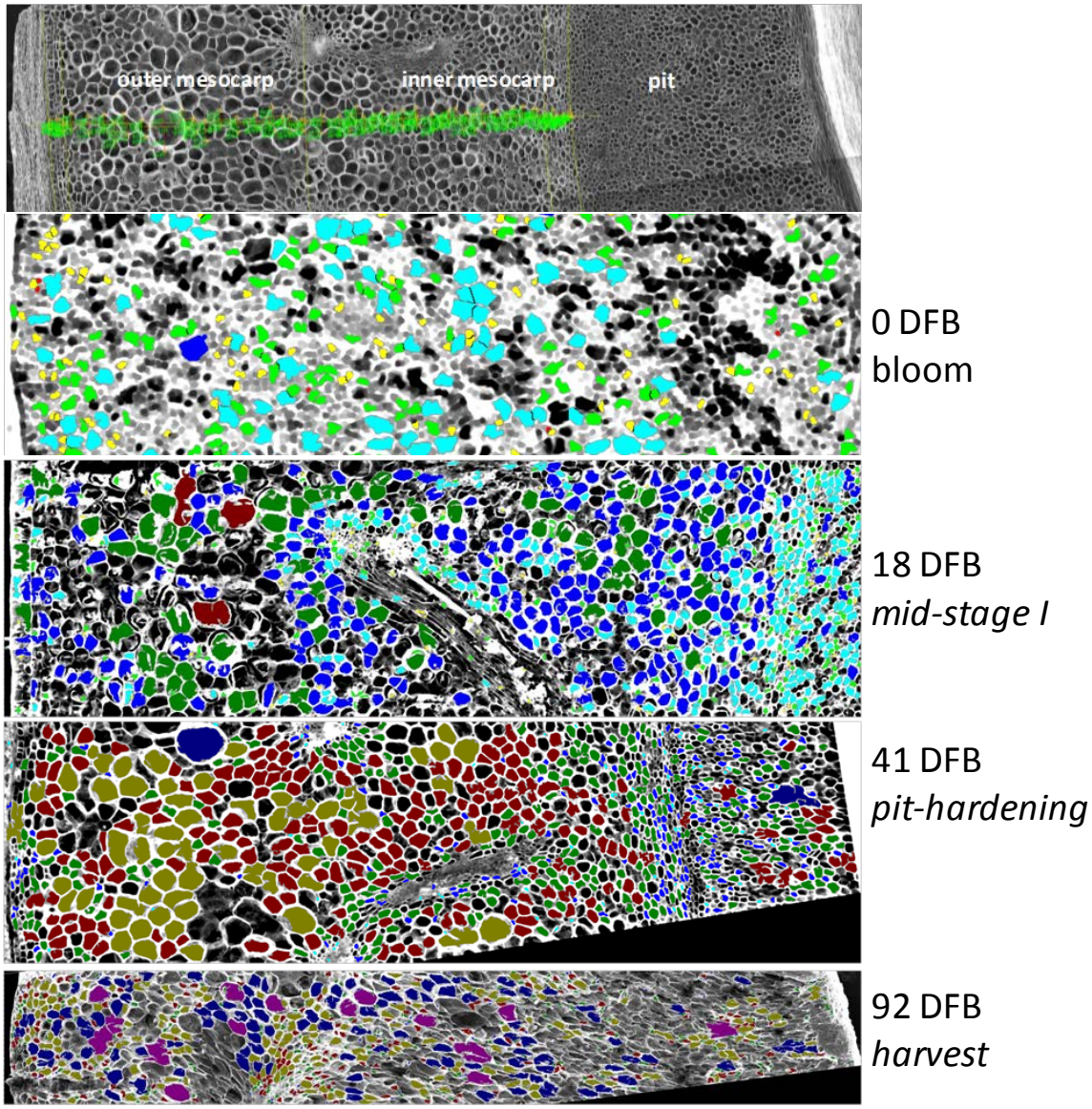


Figure 3. Image analysis for automatic detection of cell areas provided greater detail of cell size and position in cherry fruit compared to the 'line counting' method. The lower four panels show color coded size measurements of fruits 0, 18, 41 and 92 DFB.

Cell number was also determined in all regions revealing no differences between the cropload treatments of 'Sweetheart' (data not shown). However, insufficient SEM data in the earliest growth phase cannot rule out possible cropload effects on the timing of cell division; however, flow cytometry data do not indicate a cropload effect on the timing of cell division.

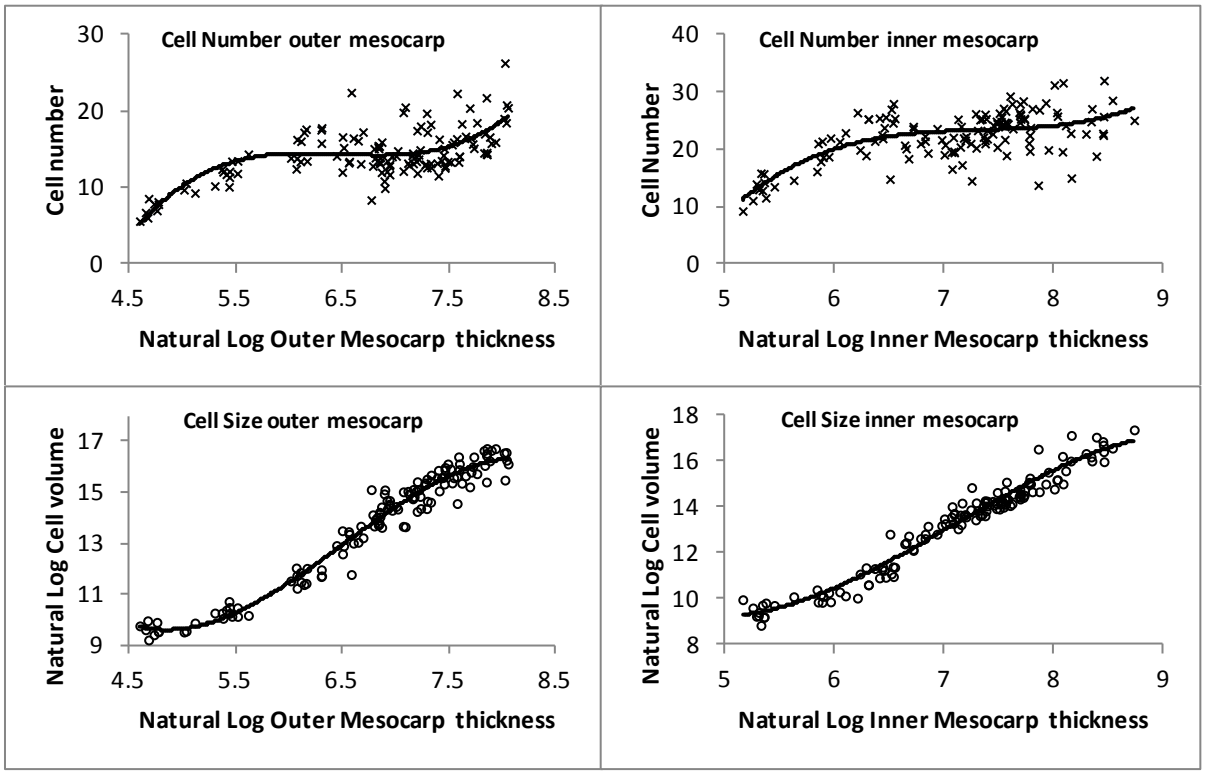
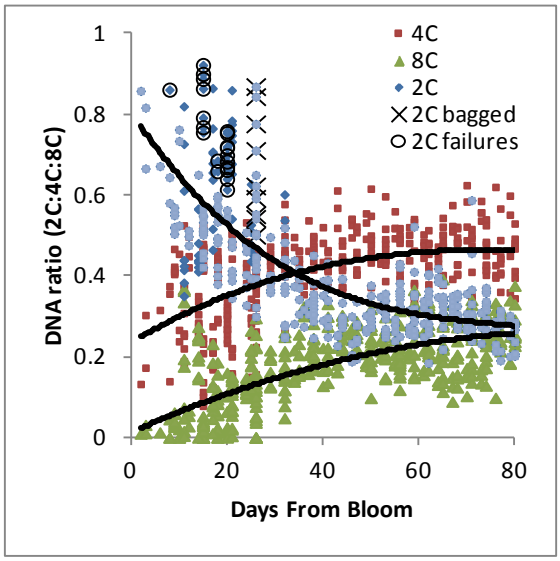


Figure 4. Estimates of cell number and cell size. Log transformation of the data allow comparison over several orders of magnitude.



	Ploidy distribution			
Green tissue	% 2C	% 4C	% 8C	% 16C
young leaf	80	20	0	0
ovary at bloom	80	20	0	0
	Ploidy distribution			
Ripe fruit	% 2C	% 4C	% 8C	% 16C
choke cherry	84	16	0	0
wild cherry	52	22	20	5
Chelan	32	39	27	2
Bing	26	49	24	1
Sweetheart	24	49	27	1
Lapin, small (6.0 g)	48	25	19	7
Lapin, very large (18.9 g)	39	20	26	13

Table 1. Sweet cherry endoreduplication

Figure 5. DNA content of nuclei

Flow cytometry: Heightened cell activity in 'Sweetheart' fruit can be observed immediately following bloom (decreasing 2C/increasing 4C and 8C), irrespective of cropload level (Fig. 5). These data

indicate increased cell division activity, and suggest that cell divisions are completed very soon after full bloom (within the first 3 weeks). Interestingly, >60% of the nuclei of all three cultivars evaluated were polyploid, beginning ~7-10 days after bloom, and remaining until harvest. When plant cells become polyploid they typically cease cell division. If this process, termed endoreduplication, could be delayed, then more cell divisions could potentially occur resulting in larger fruit. We are unclear as to whether cells with higher ploidy levels result in larger fruit size. Increased polyploidy in fruit was positively correlated with fruit size when comparing two strains of 'Gala' apple (Peter Hirst, personal communication); however, in this case the larger-fruited mutant was tetraploid. Though we did not observe significant differences in ploidy distribution among the cultivars that we investigated ('Chelan', 'Bing' and 'Sweetheart'), the finding that small-fruited *Prunus virginiana* (Chokecherry) were nearly entirely diploid [2C] (Table 1) prompted us to analyze ploidy levels for a few large-fruited and small-fruited genotypes in 2012 to determine whether differences in polyploidy occur at the extreme ends of the fruit size spectrum. Results from small-fruited wild cherry and very large Lapins did not support a positive correlation of endoreduplication and fruit size (Table 1).

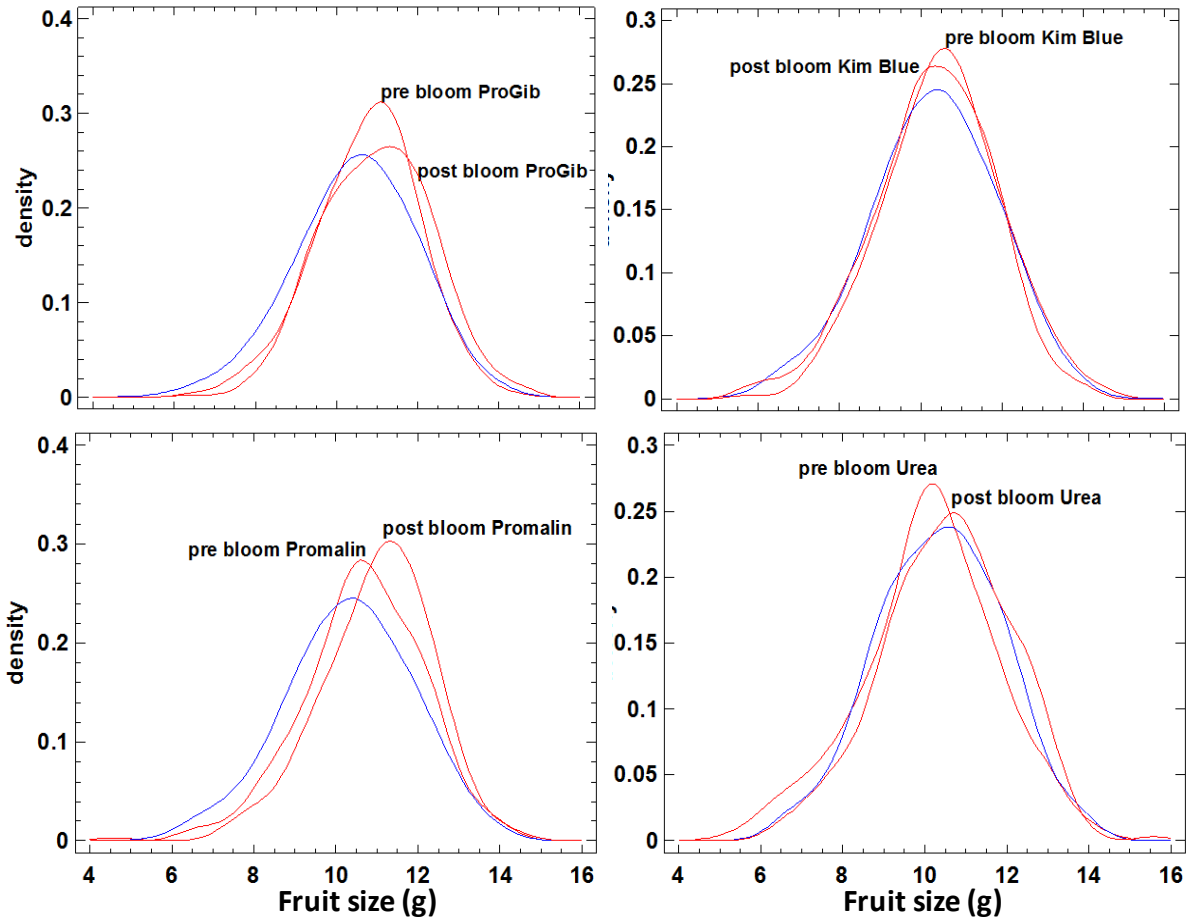


Figure 6. Distribution density (relative frequency) analysis of fruit size at harvest shows gibberellin containing PGR sprays (left panels) increased fruit size when applied soon after bloom. A synthetic cytokinin, Kim Blue and urea had little or no effect (right panels). Limbs were bud thinned two weeks prior to bloom to avoid high cropload, and PGR's were applied either -5 DFB or +12 DFB to six limbs per treatment. Fruit were collected at harvest time and weighed individually (n = 300 to 400 per treatment).

PGR trials: Because cell divisions occur early in cherry fruit growth we hypothesized growth regulators such as giberellin and cytokinin would be most effective around bloom time. In 2012, we conducted a preliminary investigation to test the effect of early applications of these different classes of PGRs on final fruit size. PGRs were applied to individual fruiting limbs. Both GA and Promalin (GA+cytokinin) improved final fruit size (Figure 6). Interestingly, cytokinin alone (CPPU) did not induce growth resulting in larger fruit. These results seemed counterintuitive, given that the maximum growth rate of sweet cherry 4-5 days after full bloom is due to cell divisions, and cytokinins augment cell division activity. Alternatively, bio-activity of GA, and the constituents of Promalin may have affected cell division, cell expansion, or a combination of the two to shift fruit into larger size classes. More testing is required to determine if significant, consistent increases in fruit size can be made through early applications of PGRs. Our urea (foliar application) was intended to provide additional nitrogen for growth at a time when maximum growth of fruit is occurring (see relative growth curves in Figure 1). This treatment did not appear to improve growth of fruits.

GA Experiments. We have tested applications of intermediate rates of GA (10-60 ppm) on a range of late-season cultivars: Skeena (2010 & 2011; two sites per year), Sweetheart (2010, 2011, 2012), Staccato (2010), and Lapins (2012). Our aim was to determine if increased fruit size from either indirect (delayed harvests via lighter skin color), or direct effects were attainable. Rates of 100 ppm and higher have been shown to markedly reduce return bloom. All applications were applied at ~straw color to scaffold limbs (with the exception of the 2012 Lapins trial where whole canopies were sprayed). In some trials, multiple applications were split between straw color and ~two or three weeks prior to harvest. Rates of 10 ppm and higher significantly improved fruit firmness, irrespective of cultivar, site, or year. It is the one fruit quality attribute that was consistently affected by GA. However, rates exceeding 20 ppm did not result in greater improvements in fruit firmness. Fruit size varied in response to GA: Sweetheart (2010) and Staccato (2010) fruit size was improved at 20 ppm relative to controls (no GA); larger fruit size of Skeena (both years), Sweetheart (1 year) and Lapins were not detected for any of the GA treatments relative to the control. In the case where an increase in fruit size was observed for 20 ppm GA, there were no additional significant gains at higher rates. Multiple timings had no additional effects on fruit size or firmness in any year of application. Other fruit quality attributes (sugars, acids) were not consistently affected by GA timings or rates.

Table 2. 2012 return bloom from 2011 GA applications to 7th leaf 'Skeena' / 'Gi6' scaffolds. Data are means of 6 replicate limbs (n= 25 for spurs & buds).

2012 Skeena	Return buds/spur		Return flowers/bud	
GA trt (ppm)	Site 1	Site 2	Site 1	Site 2
Control	4.9	5.4	3.5 c	3.6
Surfactant	4.3	5.1	3.7 bc	4.1
10	5	4.3	3.9 ab	3.7
20	5	4.5	3.7 bc	3.7
30	4.5	4.9	3.8 b	3.6
40	4.5	4.6	3.8 b	4
60	4.8	4.3	3.7 bc	3.7
20 + 20	5.3	-	3.8 ab	-
20 + 40	4.5	-	4.1 a	-
P >F	0.06	0.35	0.005	0.38

Timings for GA rates 20/20 and 20/40 were at straw/mid Stage III. Single rates were all applied at straw.

Skin color was typically lighter at commercial harvest timing for GA-treated limbs, regardless of the cultivar treated, but lighter skin colors were not observed with higher rates (i.e., those exceeding either 10 or 20 ppm, depending on the trial); the exception was for the Skeena 2010 trial (40 ppm fruit were significantly lighter than 20 ppm fruit). Depending on the season, fruit color was difficult to assess. In cases where protracted bloom periods occurred, variability in color was too great to detect significant differences for higher rates.

Intermediate rates (30 to 60 ppm) did not affect the number of return floral buds per spur, or the number of flowers per bud (shown for 2011 Skeena in Table 2); however, given the fruit size and quality results in the year of application, there would be no value in applying rates exceeding 20 ppm. In 2012, as part of a larger study, we explored the role of GA on pitting using Lapins and Sweetheart

(Table 3). GA did reduce pitting, but not at rates higher than 25 ppm. In fact, the fruit quality data from these trials agreed with our previous findings; low rates of GA are sufficient to saturate the response of measured variables.

Table 3. Fruit size and fruit quality attributes (FF, fruit firmness; SSC, soluble solids; TA, total acids; induced pitting on a 1-4 scale where 1 is no pitting, and 4 is severe pitting following pit induction; natural pitting of fruit from picking and handling [not run over a commercial line]; stem browning; and skin color, CTIFL [scale of 1-7 where 1 is light pink and 7 is black]*).

2012 Lapins	Fruit diam.	FF	SSC	TA	Induced pitting	Natural pitting	Stem Browning	Skin Color
GA (ppm)	mm	g mm^{-1}	%	%	1 to 4	%	%	CTIFL
0 Control	30.7	261 b	17.9	0.81 a	2.83 a	20.6 a	16.6 a	4.9
0 Surfactant	30.1	250 b	18	0.81 a	2.79 a	16.3 ab	15.5 a	5.0
25	30.7	297 a	17.9	0.74 b	2.61 b	10.1 b	9.1 b	5.2
50	30.0	281 a	17	0.74 b	2.6 b	12.2 b	9.6 b	4.9
100	29.6	262 b	16.7	0.74 b	2.68 ab	15.6 ab	8.5 b	4.9

2012 Sweetheart	Fruit diam.	FF	SSC	TA	Induced pitting	Natural pitting	Stem Browning	Skin Color
GA (ppm)	mm	g mm^{-1}	%	%	1 to 4	%	%	CTIFL
0 Control	27.1	298 b	19.3	0.89	2.61 a	12.8 a	20.5 a	4.0 a
0 Surfactant	28.0	305 b	21.1	0.91	2.65 a	8.3 ab	21.3 a	4.1 a
25	27.8	331 a	20.8	0.95	2.3 b	6.6 b	14.9 b	4.0 a
50	27.6	345 a	22.5	0.91	2.31 b	7.2 b	15.8 b	4.1 a
100	28.1	352 a	21.2	0.92	2.32 b	5.8 b	15 b	4.5 b

*In Lapins 25, 50 and 100 ppm fruit were lighter at commercial harvest timing, and were required an additonal 5 days to reach simialr CTIFL than control and surfactant fruit. In Sweetheart, skin color was lighter at harvest timing for 50 and 100 ppm fruit and were given 3 additional days to darken.

Methods

Fruit development studies: Methods were described in the 2010 report with the following changes. We did not hand pollinate flowers as done in 2010, due to the rather poor set that we observed. Alternatively, we selected flowers of similar age (within 1 day) borne on fruiting spurs of 2 and 3 year old sections of wood, located on adjacent trees within commercial blocks. Sections of wood were flagged for 'Bing' and 'Chelan', and all flowers in the balloon stage were left intact. Simultaneously, flowers that were either opened, or more tightly closed (less advanced) were removed. The following day we observed the flowers for uniformity, and removed all those that had not yet opened. At the completion of flower selection, all tagged spurs had roughly 2-4 flowers. For 'Sweetheart' two levels of cropload were established: 1) Heavy (unthinned), and 2) light (achieved by removing all but one of the reproductive buds per spur prior to bloom). Flower selection for the light cropload treatment was as described above. The heavy treatment flowers were selected at the balloon stage, and identified by lassoing the pedicel with a tag, being careful not to girdle the pedicel. All other flowers on the spur, either advanced or delayed in their development, were left intact so as to achieve a potential heavy fruit set.

Flower and fruit sampling occurred daily for the first 21 days after bloom, and then every 3 days until harvest. At each sampling date, 8 to 10 tagged fruit were selected for each cultivar. Half of the fruit were placed in fixative for imaging on the scanning electron microscope (SEM), while the other half were sectioned and stored in the freezer for flow cytometry analysis. At each sampling date, an additional 30 to 50 fruit from un-tagged spurs on similar 2 and 3 year old wood were stored in fixative. This second sampling revealed that two size populations, presumably fertilized or un-fertilized fruits, could be distinguished as early as 14 days after bloom. This finding prompted us to follow the growth characteristics of un-fertilized fruitlets during the 2012 season from limbs that were wrapped in bee-exclusion netting. During this season of 2012, we also collected floral buds that we dissected for measurement of ovaries.

Growth curves of whole fruit and its component tissues (mesocarp, endocarp and kernel) were obtained from measurements of photographs taken with a stereozoom microscope. Photos were taken of a whole fruit then the fruit (until pit hardening) was split lengthwise along the suture plane and photographed again. Next, a cross-section of one half of the fruit was obtained and photographed. After pit hardening, photos of the whole fruit were taken then the pit was cleaned and photographed. The pits were split open and the condition of the kernel (full or shriveled) was noted. This method gave measurements of the length (x-axis) and maximal diameters (y- and z-axis) of the whole fruit and its tissues. These values were used to calculate the equivalent volume of an ellipsoid. This method avoided the difficulty of fresh weight and caliper measurements, especially in very small fruit.

We continued to investigate ploidy levels of 'Chelan', 'Bing' and 'Sweetheart' fruit using flow cytometry. These experiments were carried out in Corvallis, Oregon in collaboration with Dr. Ryan Contreras. Methods were modified from 2010 with greatly improved results. Fresh fruit mesocarp tissue was dissected on the day of sampling then quickly frozen and stored until analysis. Frozen tissue was finely chopped in buffers that separate and stain cell nuclei. A solution containing these nuclei was then injected into a cytometer and passed through a laser. The stained nuclei fluoresce in the light source, and the amount of fluorescence is proportional to the DNA content of the nuclei. The number of nuclei is also obtained which allows a calculation of the ploidy distribution.

Scanning electron microscopy was performed at the EM facility at OSU. Fixed whole-fruit were dissected to reveal the widest extent of the "cheek" region, perpendicular to the suture plane. Following dehydration in a graded series of acetone, samples were critical point dried, mounted on stubs and sputter coated with gold and palladium. A series of digital images from the epidermis to pit were obtained at the appropriate magnification then assembled as a montage. Measurements of cell diameters and position within the fruit were accomplished with digital image analysis software.

EXECUTIVE SUMMARY

Project Title: Influence of cropload level on fruit size and quality of sweet cherry

Fruit growth, cell number and size

- Fruit size was negatively affected on spurs with more than 12 fruit.
- Heavy croploads limited pit size.
- Pit size was positively correlated with final fruit size.
- Half the number of cells comprising an individual fruit at harvest were already present at full bloom.
- Most cell division were completed during mid-stage I, but likely continue into early-stage II.
- Cell numbers do not appear to be affected by cropload.
- Cells of the inner region of mesocarp were elongated at harvest.
- Cells of the outer region were more rounded especially during final fruit swelling.
- Final fruit size more strongly correlated with cell size than cell number.
- Majority of the nuclei of 'Chelan', 'Bing' and 'Sweetheart' fruit become polyploid.
- Leaf tissue and flowers at full bloom showed no polyploidy.
- After full bloom, polyploidy in fruit increased rapidly to > 75% at harvest time.
- Non-pollinated flowers did not show polyploidy indicating fertilization was required.
  → *Polyploidy and activity of the genes involved could be used as early markers of fruit set.*
- Cropload level and genotype did not influence magnitude or timing of polyploidy during fruit development.
- Fruit set can be determined by segregating ovary size, well before shuck drop.
  → *This is markedly earlier than current assessment of fruit set (i.e., after June drop)*

Giberellic Acid (GA) Experiments

- Moderate rates of GA were applied to Lapins, Skeena, Sweetheart and Staccato over multiple years.
- The largest differences among all tested GA concentrations (10, 20, 30, 40, 60 ppm) and timings (single applications at straw color, or multiple applications split between straw color and mid-stage III) were observed between 0 and 20 ppm. The quality attribute consistently affected by GA was fruit firmness (higher when provided GA, but not consistently with rates beyond 10 ppm).
- Multiple applications did not result in higher quality fruit.
- In most trials fruit size was not increased with GA. In trials where improvements in fruit size were detected, the response was not influenced by rate beyond 20 ppm.
- Skin color (darkening) was delayed with the application of GA; however, beyond the 20 ppm rate, the effects were highly variable, and difficult to qualify.
- Pitting was reduced in Lapins and Sweetheart at 25 ppm GA; however, concentrations exceeding 25 ppm (up to 100 ppm) did not improve the response.
- Return bloom (floral buds per spur and flowers per floral bud) was not reduced by rates between 10 and 60 ppm.

PROJECT PROPOSAL **DURATION: 3 years**

Project Title: Establishment and testing of MSU sweet cherry rootstocks

PI:	Amy Iezzoni	**Co-PI(2)**:	Matt Whiting
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State/Zip:	OR 97031-9512	**State/Zip:**	WA 99350

Cooperators: Tom Auvil

Total <u>revised</u> project funding request: **Yr 1**: $30,919 **Yr 2**: $20,551 **Yr 3**: 21,000
Total <u>original</u> project funding request: **Yr 1**: $36,504 **Yr 2**: $26,208 **Yr 3**: 26,732

Other funding sources: None

WTFRC Collaborative Expenses

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

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

Budget 1: Amy Iezzoni

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

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

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

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

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

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

Budget 3: Matt Whiting

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

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

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

OBJECTIVES:

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

Specific objectives

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

SIGNIFICANT FINDINGS:

- (Obj. 1) Of the 11 MSU candidate rootstocks that were planted at Clarksville, Mich. in 2001 – 2004, only CRAWFORD showed signs of graft incompatibility with 'Hedelfingen' scion. However, because of the exceptional performance of 'Bing' on CRAWFORD at the Prosser location in both 2011 and 2012, with no signs of graft incompatibility, CRAWFORD was selected for further testing.
- (Obj. 2) Based on evaluations of TCSA, flower number, fruit yield, color, firmness, weight and row size, five of the MSU rootstocks candidates with 'Bing' scion show promise as new size controlling precocious rootstocks compared to Gi5 and Gi6. These five candidate rootstocks are CLINTON, CASS, CLARE, LAKE and CRAWFORD. In general, the number of flowers per node on these rootstocks was so high that a 50% thinning treatment was imposed for the entire plot so that fruit size potential could be assessed. All of these five MSU candidate rootstocks exhibited higher yield efficiencies than Gi6 and only CASS had a yield efficiency less than Gi5. The mean fruit size for three of these rootstocks (CASS, CLINTON and CLARE) was larger than that for Gi6, despite the higher yield efficiencies.
- (Obj. 3) All eight commercial liner nurseries that received budwood of CLARE, CLINTON and LAKE in September 2012 confirmed that the plant material was established and is being used in initial propagation trials. Budwood of CRAWFORD was obtained from the MSU rootstock mother block and set to the (NCPN-FT) for testing.
- (Obj. 4) DNA diagnostic tests for all five MSU candidate rootstocks were performed by two commercial companies to explore the option of having a private company perform the routine DNA diagnostics needed for monitoring genetic identity.

METHODS by OBJECTIVE

1. All nine 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.

2. *Experimental plot at WSU – Prosser*: Trees will continue to be pruned and trained yearly according to a multiple leader architecture. This will be accomplished in collaboration with Matt

Whiting, Tom Auvil and a crew from the WTFRC. Matt Whiting, Tom Auvil, Amy Iezzoni and WSU farm manager Clint Graf will meet at least once a year at the plot to review pruning and training plans. Flower and fruit evaluation in 2013 will be done using the protocol undertaken for 2012. Therefore, the protocol followed for 2012 is outlined below.

Prior to bloom, two scaffolds per tree were selected and the number of spurs and nodes were counted. In addition, the flower buds on a maximum of 15 spurs were recorded and the mean numbers of buds per spur were calculated. Due to the high flower number on many of the trees, the flowers were thinned by hand removing 50% of the flower buds. On July 9, tree trunk circumference was measured 20 cm above the graft line.

On June 28, 2012, the 'Bing' trees were harvested and the individual tree yields were recorded (fruit weight per tree). For the two scaffolds per tree previously evaluated for flower traits, the total fruit number was counted. The fruit were transported to the WTFRC laboratory Wenatchee for fruit quality evaluations. Evaluations were done with a goal of 100 fruit per 5 tree replicate for the following traits: bulk fruit weight, cracking and brix. Next, a sample of 50 fruit was evaluated for skin color, row size, stem pull force and firmness. Post-harvest storage data was recorded 13 days after harvest (July 11, 2012) from 100 fruit per 5 tree replicate for pitting, bulk weight, cracking and stem browning. A total of 50 fruit from the post-harvest treatment were evaluated for color and row size. As in 2012, a plot tour will be conducted in 2013 (5^{th} leaf) prior to 'Bing' harvest as part of the Cherry Field Day at Prosser.

4. *Virus testing*: CASS rootstock will be distributed by the NCPN-FT to the remaining liner nurseries. CRAWFORD will be tested at the NCPN-FT to meet all the requirements for certification. The testing procedures are outlined at: www.nrsp5.prosser.wsu.edu. If CRAWFORD passes virus certification, it will also be distributed to liner nurseries in September 2013.

5. *Conduct DNA fingerprinting to assure that the genetic identity of the rootstock selections is correct:* DNA fingerprinting was also done to assure correct clonal identity of the MSU candidate rootstocks that are distributed to the liner nurseries. All rootstock selections, including Gi 5 and Gi 6 can be differentiated with a combination of four markers [the self-incompatibility *S-RNase* locus and three SSR markers (PceGA59, PMS40, and PMS67)]. In 2013, the DNA fingerprinting will be done by the group at Michigan State University.

RESULTS and DISCUSSION:

Performance of the MSU candidate rootstocks: In 2009, a test planting of the MSU rootstocks with 'Bing', and in some cases 'Sweetheart' scion, was established at WSU- Prosser Roza Station. Eleven MSU rootstock candidates were included. IRON was subsequently discontinued due to poor propagation success. CRAWFORD had originally not be selected for further testing as it showed symptoms of graft incompatibility with 'Hedelfingen' scion in the Michigan plot. However, as CRAWFORD is performing well in the Prosser plot with 'Bing' scion, and shows no signs of graft incompatibility, it was selected for further testing (see below). All remaining nine MSU rootstock candidates continue to show no signs of graft incompatibility in the Michigan planting.

The trees in the Rosa Station planting were pruned in spring 2010 to obtain three main leaders. These leaders were headed about 50%, and shoots were thinned to allow best development of the leaders. In the spring of 2012 the leaders were headed back by 1/3 and side limbs 40 to 50%.

In 2012, 'Bing' grafted on five of the MSU candidate rootstocks had higher average numbers of flowers per node compared to Gi6 (Fig. 1) with CRAWFORD, CLINTON and LAKE having on average over four flowers per node compared to 2.4 flowers per node for 'Bing' on Gi6. This is the second year that these three rootstocks, plus CASS and CLARE, exhibited excellent flower density.

Therefore, these five rootstocks were chosen for continued evaluation as potential commercial rootstock candidates and data for only these five rootstocks, plus the Gi5 and Gi6 controls, are presented in the subsequent figures and tables. Because the number of flowers per node on these rootstocks was so high, a 50% thinning treatment was imposed for the entire plot so that fruit size potential could be assessed.

The trunk cross sectional areas (TCSA; cm^2) of all five MSU candidate rootstocks were less than that for 'Bing' on Gi6, and half of the MSU candidate rootstocks had TCSAs similar to that of 'Bing' on Gi5 (Fig. 2).

Mean yields for the two scaffolds per tree ranged from 2.95 kg to 5.67 kg for CASS and CRAWFORD, respectively (Table 1), with four of the MSU rootstock candidates having higher yields than Gi5 and Gi6. Despite the yield differences, the mean row sizes among the rootstocks did not very widely except for CASS that had a smaller mean row size (e.g. larger fruit). It was particularly noteworthy that the relatively high yields exhibited by CLINTON and CRAWFORD were not accompanied by a significant decrease in fruit size.

All five of the MSU candidate rootstocks had higher yield efficiencies (kg fruit/cm2) compared to Gi6 and only CASS had a yield efficiency less than Gi5 (Fig.3). The higher yield efficiencies of CRAWFORD and LAKE were accompanied by an average reduction in fruit weight; however, the average fruit weights for CLARE, CLINTON and CASS were higher than that for Gi6.

Gross returns for the Gi5, Gi6 and the five MSU candidate rootstocks were calculated based on total tree yield, percentage of fruit in each row size, and dollar price based on row size (Table 2). LAKE, CRAWFORD, and CLINTON had the highest gross returns which were significantly higher than those for Gi5 and Gi6. The gross returns for CLARE and CASS were less than that for Gi5. However, these two rootstocks significantly reduced tree size and increased precocity without a loss of fruit size; therefore, they warrant testing with additional training systems and cultivars.

Generation of virus-certified genetically-verified rootstock budwood for the MSU candidate rootstocks. One CASS plant was virus-certified and therefore distribution was initiated (see below). Because of the excellent performance of CRAWFORD, it was determined that it should be advanced for further testing. Therefore budwood of CRAWFORD was cut from the MSU rootstock mother block in Clarksville, Michigan and sent to NCPN-FT in September 2012 to be established subsequent to indexing

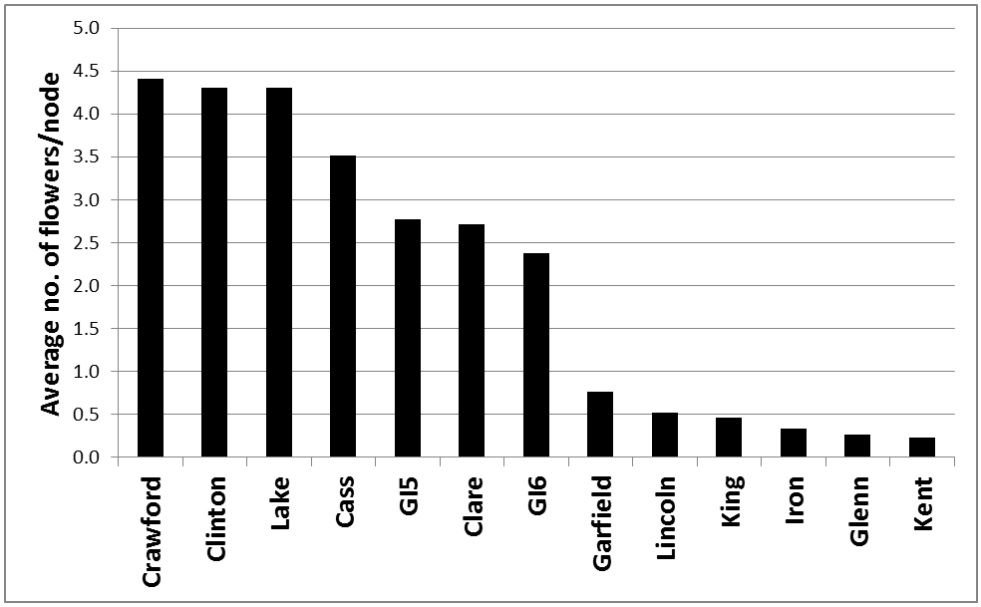
Distribution of rootstock budwood for pilot propagation trials and limited liner production. Distribution of the MSU candidate rootstocks to liner nurseries was accelerated to provide a mechanism for generating liners for future trials, give the nurseries an opportunity to gain experience propagating these rootstocks, and begin to establish stock plants in case of commercialization. The distribution of the most promising rootstocks that were already virus certified, e.g. CLINTON, CLARE, and LAKE, was done in September 2011 from certified material housed at the National Clean Plant Network.

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

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

Genetic-verified plant materials. Due to the recent establishment of the MSU rootstocks at the nurseries and limited tissue available for ID verification, we focused on exploring commercial options for genotyping services. Tissues from the candidate rootstocks were collected and selection names were coded. Ten coded tissue samples were sent to two commercial vendors, COMPANY A and COMPANY B[a], for SSR genotyping for the markers PceGA59 and PMS40. The cost for COMPANY A was $130 and COMPNAY B $574. After reviewing the results, it was determined that it would be better for my lab at Michigan State University to continue to do the genotyping. Verification of the plant materials at the nurseries will be done in 2013.

Fig 1. Average number of flowers per node* on 'Bing' trees grafted on 11 MSU rootstock candidates, Gi 5, and Gi 6 for trees planted in 2009 at WSU - Prosser Roza Experiment Station. Data was taken in April of 2012.



*Calculated from two scaffolds per tree using the following equation: (number of spurs × average number of flowers/spur) ÷ number of nodes.

Fig 2. Trunk cross sectional area (TCSA; cm^2) of 'Bing' trees grafted on 5 MSU rootstock candidates, Gi 5, and Gi 6 for trees planted in 2009 at the WSU - Prosser Roza Experiment Station. The top black bars indicate mean TCSA recorded on 9 July 2012. The bottom bar is the mean TCSA measurement on 28 September 2011. Therefore, the black bar indicates the TCSA increase during the first part of the 2012 growing season.

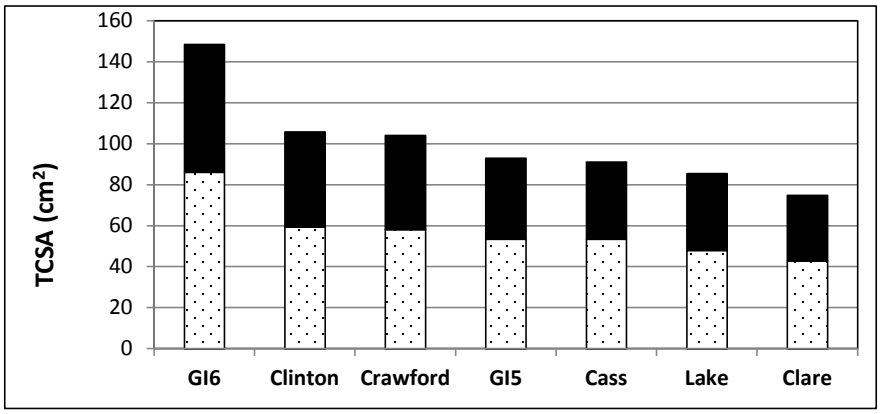


Table 1. Mean tree yield, row size, percentages of fruit, and fruit number for each row size category for five MSU Rootstocks, Gi5 and Gi6. Flowers were thinned to 50% and the fruit was harvested on June 28, 2012.

Rootstock selection	Mean tree yield (kg)	Mean row size	Row size							
			9	9.5	10	10.5	11	11.5	12	13
Gi6	3.20	9.9	3% (3[1])	31% (32)	52% (53)	9% (9)	4% (4)	2% (2)	1% (1)	0%
Gi5	3.47	9.8	6% (8)	45% (62)	37% (51)	8% (11)	3% (4)	0%	1% (1)	0%
Cass	2.95	9.7	4% (5)	60% (80)	28% (37)	8% (11)	0%	0%	0%	0%
Clare	3.58	9.9	4% (6)	36% (54)	46% (68)	11% (16)	2% (3)	0%	0%	0%
Clinton	5.03	9.8	13% (27)	31% (63)	43% (88)	10% (19)	2% (4)	1% (2)	0%	1% (1)
Crawford	5.67	10.0	2% (4)	30% (65)	44% (95)	12% (26)	8% (17)	4% (9)	0%	0%
Lake	4.92	10.1	0%	18% (48)	51% (137)	22% (58)	6% (16)	4% (11)	0%	0%

[1]Based on the fruit number on the two scaffolds

Fig 3. Relationship between average yield efficiency and fruit weight of 'Bing' trees grafted on MSU rootstock candidates and Gi 5, and Gi 6 for trees planted in 2009 at WSU - Prosser Roza Experiment Station. Flowers were thinned to 50% and fruit was harvested on June 28, 2012.

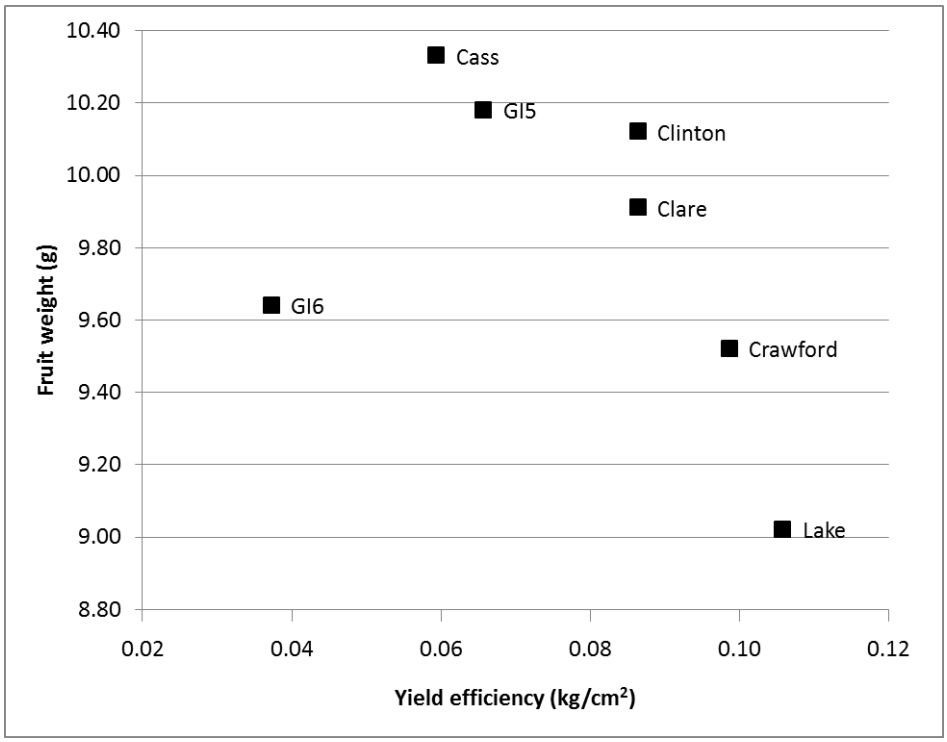


Table 2. Gross returns for 'Bing' trees grafted on 5 MSU rootstock candidates, Gi 5, and Gi 6 for trees planted in 2009 at WSU - Prosser Roza Experiment Station. The flowers on the trees were hand thinned with half of the flowers removed, and fruits were harvested on June 28, 2012.

Rootstock selection	Mean tree yield (kg)	Gross returns*
Clare	3.58	$13.32
Gi6	3.20	$14.22
Cass	2.95	$14.59
Gi5	3.47	$16.40
Lake	4.92	$20.37
Clinton	5.03	$23.17
Crawford	5.67	$24.66

*The gross returns were calculated by summing the products from the following equation for all row sizes: the average tree yield (lb) × % fruit for that row size category × $ price for the row size. Row size values used were as follows: Row Size 9= $2.50/lb, Row Size 9.5 = $2.50/lb, Row Size 10 = $1.80/lb, Row Size 10.5 = $1.80/lb, Row Size 11 = $1.50/lb, and Row Sizes 11.5-13 = $1.20/lb.

CONTINUING PROJECT REPORT **PROPOSED DURATION:** 1 of 3
WTFRC Project #: CH-12-107

Project Title: PNW sweet cherry breeding and genetics program

PI:	Nnadozie Oraguzie	**Co-PI**:	Cameron Peace
Organization:	WSU	**Organization**:	WSU
Telephone:	509 786 9271	**Telephone**:	509 335 6899
Email:	noraguzie@wsu.edu	**Email**:	cpeace@wsu.edu
Address:	24106 N Bunn Road	**Address**:	39 Johnson Hall
Address 2:		**Address 2**:	Hort/LA WSU
City/State/Zip:	Prosser, WA 99350	**City/State/Zip**:	Pullman, WA 99164

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

Budget: **Year 1**: $144,918 **Year 2**: **$152, 028** **Year 3**: $145,901

Other funding sources

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

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

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

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

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

Budget Narrative

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

About ¾ of the budget is for core breeding activities including personnel costs, wages, land use fees, and plot establishment and maintenance. Personnel costs include salaries and benefits for 0.25 FTE technical support for breeding operations in Prosser, and 0.15 FTE technical support for the Genetic

Screening Technician in Pullman to facilitate marker-assisted seedling selection (MASS) and marker-assisted parent selection (MAPS). The latter cost in previous years was included in research projects led by Dr. Peace, but in any case, genetic marker assistance represents resource savings over purely conventional breeding practices. A land use fee ($475/acre/year) was introduced in July 2010 by WSU-IAREC, Prosser. Further, plot establishment and maintenance cost has risen from $2.5K to $4.5K/acre/year to cover wages for orchard staff involved in plot establishment and maintenance, fees for hiring heavy orchard equipment, cost of consumables for plot maintenance, etc.

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

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

<u>Supporting funding</u>

Funds from the stem-free sweet cherry SCRI project will be used to develop genetic markers and phenotyping protocols for pedicel fruit retention force

Funds from the SCRI-funded Tree fruit GDR project will be used to develop a multi-tooled database for efficient management of sweet cherry breeding program data.

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

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

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

WTFRC Collaborative expenses:

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

Footnotes: The funds are for phase 2 tree evaluation.

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

Item	2012	**2013**	2014
Salaries	24,646	25,632	26,657
Benefits	9,813	10,247	10,657
Wages	16,800	17,472	18,171
Benefits	12,953	13,471	14,010
Equipment			
Supplies	7,,000	5,000	4,000
Elisa test	600	600	600
Land use fee	7,125	4,750	4,750
Plot establishment and maintenance	40,500	40,500	40,500
Travel	4,914	3,000	3,000
Miscellaneous			
Total	124, 351	**120, 672**	122, 345

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

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

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

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

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

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

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

Justification:
The Pacific Northwest sweet cherry breeding program (PNWSCBP) has made considerable progress during the last three years in infrastructure development and personnel recruitment. Further, a best management practice document has been developed resulting in high seed germination rate and accelerated development of large numbers of healthy seedlings in the greenhouses for field planting. In the field, superior horticultural manipulations facilitate accelerated seedling growth and development and reduce the time to flowering and fruiting. Marker-assisted parent selection (MAPS) is used to encourage the generation of a high proportion of genetically superior progeny seedlings while marker-assisted seedling selection (MASS) is used to cull genetically inferior seedlings before field planting (with current genetic tests predicting small fruited and self-incompatible seedlings for culling). The first selections are ready for replicated multi-location field trials. The breeding program is now positioned to take advantage of these advances to develop new high quality sweet cherry cultivars to keep the PNW sweet cherry industry profitable and globally competitive.

This proposal requests funds to propagate and evaluate new advanced selections, continue evaluation of existing seedlings, and further develop the germplasm base through continued hybridization of commercial cultivars, newly developed advanced selections, and exotic germplasm with novel sources of desirable traits. The proposed project addresses the evaluation of existing cultivars and the breeding and testing of new scion cultivars, both priority issues identified in the 2011 cherry priority-setting session.

Objectives:

The goal of the PNWSCBP is to develop and release new high quality sweet cherry cultivars to replace and/or complement current market leading cultivars in key Pacific Northwest regions. The specific objectives of this project are to:

1. Develop and utilize best management practices for optimal seed germination and accelerated development of healthy seedlings in the greenhouses and for field maintenance and development of superior horticultural practices that accelerate seedling growth and development and reduce time to flowering and fruiting

2. Use elite selections from the breeding program and new external sources of genetic superiority as parents for hybridization and selfing to produce seedling populations that segregate for target traits critical to each target market group

3. Integrate genomics knowledge, marker-assisted breeding tools, and classical breeding methods into the breeding program to optimize use of resources and reduce time to release of elite selections with commercial potential

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

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

Results and Discussion

Develop and utilize best management practices

The best management practice document which was developed over the last few years includes all aspects of sweet cherry breeding from seed handling to seedling development in the greenhouses, lathhouse seedling management, field planting and management, hand pollinations, fruit harvest to fruit evaluations in the lab. These guidelines ensure that plant material of adequate size and high quality are produced and managed using best practice techniques. in 2012, bar coding was implemented both for tree identification in the field and to aid fruit evaluation in the lab. Due to unusually high fruit set in the seedling block fruit thinning down to 3-4 fruit per cluster was carried out to optimize fruit size. This work-in progress document initially focused on phase 1 of the PNWSCBP, but as the program evolves and trees are planted in P2, we need to also develop guidelines for tree management that encompasses all phases of the program. Several discussions with some members of the breeding program advisory committee (BPAC) resulted in the adoption of a three leader training system for P2 trees which will be implemented in the winter of 2012. We have organized a transportable easy-to-fit bird netting prototype developed by Allan brothers for bird netting in P2. This system appears cheaper and less labor intensive than the type currently used in the seedling blocks at the Roza orchards, WSU-IAREC Prosser. Another advantage of this netting system is portability and can be dismantled at the end of the season to prevent frost and wind damage. We will continue to update this document d in consultation with the BPAC as new ideas and technology emerge.

Table 1. Summary of seedling material developed during 2009-2011 in the PNW sweet cherry breeding program.

	Year			
	2009	2010	2011	2012
No. of new parents used	5	29	6	61
No. of crosses made	55	107	74	50
No. seed	5000	2610	1162[x]	4139
% Germination	61	60	62	na
No. of seedlings	3000	1580	724	na
No. of seedlings in field	1994[y]	776[y]	324[z]	na
No. of full sib families > 9 individuals	26	7	5	na

[x] Low fruit numbers recorded due to frost damage

[y] following marker-assisted seedling selection (MASS).

z Early ripening crosses and mid-season and late powdery mildew resistant trees only

na = not available.

2. Use elite selections from the breeding program and new external sources of genetic superiority as parents for hybridization and selfing.

Since 2010, F_1 seedlings have been consistently used as both seed and pollen parents in crosses to provide a new generation of seedlings genetically superior to their parents in fruit quality. The emphasis of the crossing plan in 2012 was to develop more early and late ripening seedlings using F_1 seedlings with high breeding value for both firmness and early/late ripening traits as parents to ensure a high proportion of seedlings that are firm and at the same time early or late ripening. Breeding values of individuals with high firmness and early/late ripening were obtained from FlexQTL ouput in the RosBREED project from genomic regions associated with these traits.

Eight advanced selections (half of these still in P2) including FR001T007, FR001T036, FR001T050, FR001T073, FR002T063, FR006T059, FR006T063, FR006T093 and FR007T037 were used for

pollen. Fifty three (53) F_1 individuals mainly from Ambrunes, Regina, Sweetheart or Cowiche crosses as well as Ambrunes and Regina were intercrossed for late ripening crosses. Only F_1 individuals were used as seed parents. For early ripening, 23 families were created using mainly Benton, Bing, Index, FR14T28 and FR14T37 as seed parents. A total of ~4000 seedlings were generated (Table 1).

We harvested seed in summer from some early maturing F_1 seedlings but mostly from Rainier x Cristobalina cross for germination tests. Cristobalina is a land race cultivar from Spain that is early maturing and believed to carry a different source of self-fertility presumably non-gametophytic. . If these seeds are successfully germinated and the seedlings have a favorable genotype for fruit size and self-fertility, these individuals will be used in 2013 in crosses for early ripening.

3. Integrate genomics knowledge, marker-assisted breeding, and classical breeding tools

Based on preliminary powdery mildew (PM) screening conducted in the program in 2009 and 2010 (with ~7000 seedlings from crosses made in 2004, 2005 and 2006) using a 4-point scale, where R=resistance, S-L= low susceptibility (less than 25% of foliage infected), S-M=medium susceptibility (26-70% of foliage infected) and, S-H=high susceptibility (more than 70% of foliage infected), we found that varying proportions of progenies of Regina crossed with different cultivars were resistant to PM (See Table 2). When we screened Regina we found no symptoms of PM in both years. Chi-square analysis of segregation ratios (resistant vs suscpetible) within progenies of selected cultivars such as Chelan, PMR-1 and Regina, crossed with common quality susceptible cultivars including Sweetheart, Bing, Lapins, Rainier, Selah, Cowiche and Benton showed significant differences among cultivars suggesting that Chelan, PMR-1 and Regina may have different genes/alleles that control resistance to PM. Also, in some cases, Regina and Chelan seem to produce populations with a higher proportion of resistance depending on the other parent- a case of specific combining ability for PM resistance (data not shown). However, this result is preliminary and may need to be confirmed with DNA markers. Further, there were no populations large enough from crosses including Regina x PMR-1, Chelan x Regina and Chelan x PMR-1 to look for non-segregants. Note that each of these sources of PM being used as parents also contribute other essential traits such as fruit quality (in the case of Regina) and earliness (for Chelan). In fact, a few of the elite selections from the breeding program that are in P2 tests have putative resistance to PM. The plan is to identify more resistant individuals that are outstanding for other critical fruit quality traits in the breeding program for use as breeding parents.

Table 2: PM segregation data in PNW breeding program including data showing Chi Square tests for independence among segregation ratios resulting from crosses among different sweet cherry parents.

	No. of Seedlings				
	R	**S=L**	**S=M**	**S=H**	**Total**
Rainier x Regina	39	74	32	1	146
Rainier x Chelan	28	25	2	0	55
χ^2 = 20.1 at 3 df. , P=0.001					
Lapins x Regina	1	56	2	0	59
Lapins x Chelan	14	21	6	0	41
χ^2 = 26.8 at 3 df.; P=0.001					
Selah x Regina	24	36	17	5	82
Selah x PMR-1	25	31	5	0	61
χ^2 = 9.009 at 3 df., P=0.05.					

In another project on PM, 'Understanding the genetics of powdery mildew in cherry (CH-11-103)', funded by WTFRC and OSCC in 2011, we have screened ~ 500 sweet cherry accessions including

sweet cherry founders, old cultivars, breeding parents and progenies for PM resistance/susceptibility using a 6-point scale. Where, 0 = no symptoms, 1 = leaves very slightly infected, 2 = leaves slightly infected, 3 = leaves moderately infected, 4 = leaves severely infected, and 5 = leaves completely infected. This pedigree-linked germplasm respresents the US Sweet Cherry Crop Reference Set and the PNWSCBP Pedigree Set. This phenotypic data and the genotypic data set generated in RosBREED (based on a 6K SNP array) will be integrated in FlexQTL and pedigree based analysis carried out in the winter of 2012 to identify genomic regions associated with powdery mildew resistance. The results will be used to validate the classical genetics results above and the information will facilitate PM resistance gene pyramiding in the breeding program.

In the ROSBREED project, using the germplasm described above, we have collected extensive phenotypic data on bloom date, firmness, ripening date and fruit size, and are currently analyzing these to identify the genomic regions associated with these traits for use in breeding. Where QTLs for PM resistance and fruit quality co-locate on the same chromosomes, germplasm that have both attributes will be identified and used as breeding parents to speed up breeding for combined PM resistance and superior fruit quality.

In the SCRI-funded stem-less cherry project, we have reported a significant genotypic variation for pedicel fruit retention force (PFRF) as well as a strong genotype x year interaction component (Zhao *et al*. 2012). We also found that PFRF was independent of fruit quality suggesting that low or high PFRF will not impact fruit quality negatively or positively. Preliminary QTL analysis using PFRF data collected in the RosBRREED project and one year's data from a Selah (low PFRF) x Cowiche (high PFRF) mapping population obtained in the Stem-less cherry project, identified common QTLs on LG 2 and LG 8 associated with PFRF. These results will be validated in the future for use in developing a MAB strategy for PFRF in the PNWSCBP.

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

We have developed standardized phenotyping protocols for all traits of interest in the PNWSCBP except pitting and are continuing to search for protocols that will accurately simulate both pre-harvest and post-harvest pitting. A protocol for rain induced cracking has been developed in collaboration with Dr. Ines Hanrahan (WTFRC), however, this was not implemented this year due to the unusually high rainfall in summer which made cracking easier to assess on the tree in the field.

Five trees of each of 14 advanced selections chosen from a total of 29 identified in 2009 and 2010 were planted in P2in the spring of 2012 at the WSU IAREC Prosser and the OSU MCAREC Hood River experiment stations along with 2 trees each of the market leading cultivars including 'Bing,', 'Sweetheart,' 'Rainier,' Early Robin and Chelan. Three of these advanced selections including FR001T068, FR001T073, and FR001T074 were also planted at Norm Gutzwiler's orchard in N. Wenatchee.
We obtained fruit quality data from the mother trees of advanced selections in the seedling block since these are not yet fruiting in P2. Based on this data, a decision was made to discontinue some selections in P2 leaving only 9 genotypes that persistently performed well (Table 3a). The five genotypes we discontinued had a combination of high incidence of splitting (>20%) and low firmness (<260 g/mm) which is likely due to a high number of rain events and heat stress. We also chose 7 genotypes out of the 20 identified in 2011 for planting in P2 in 2013 (Table 3b). Finally, we identified 6 genotypes (Table 3b) for advancement to P2. These have outstanding fruit quality in combination with early or late harvest date.

In line with increased priority on early or late ripening new cultivars to extend the marketing window of PNW cherries, we have identified additional 18 early genotypes (data not shown) with fruit quality attributes better than Chelan. The decision to propagate these will be based on consistent performance for another year or two to keep costs down.

Table 1: Sweet cherry advanced selections planted in P2 trials at WSU Prosser and OSU MCAREC, Hood River. Means in most cases are based on 4 years data on fruit traits.

Tree id	Cross	Target market	Harvest date	Fruit wt (g)	Firmness (g/mm)	SSC (%)	TA (%)	PFRF (Kg/F)
FR001T007	Swt x Chelan	ESM	18-21 Jun	10.6	304	17.6	0.85	0.60
FR001T036	Swt x Regina	LSM	9-11 Jul	12.2	334	19.9	0.79	0.75
FR001T074*	Swt x Regina	LSM	5-7 Jul	12.4	297	20.3	0.99	0.91
FR002T030	Rainier x Sunburst	LSB	5-6 Jul	11.2	276	18.5	0.76	0.98
FR002T063	Lapins x Regina	LSB	30 Jun-	12.5	299	18.7	0.68	0.78
FR006T059	Swt x EE	MSN	30 Jun-2 Jul	11.2	289	20.2	0.99	1.08
FR006T063	Swt x EE	MSN	30 Jun-2 Jul	13.4	326.3	20.2	1.14	1.06
FR010T051	EE x Lapins	MSN	27-29 Jun	11.3	320	19.8	0.82	0.58
FR011T059	Rainier x Regina	MSN	28 Jun-5 Jul	13.5	331	19.4	0.81	0.65

*Planted in a grower cooperator trial in N. Wenatchee
ESM=Early, self-fertile, mahogany. Chelan is the market leading cultivar
ESB=Early, self-fertile, blush. Early Robin is the market leading cultivar
LSB=Late, self-fertile, blush. Rainier is the market leading cultivar
LSM=Late, self-fertile, mahogany. Sweetheart is the market leading cultivar
Mech-SM= A range of early, mid and late season varieties suitable for mechanical harvesting. Selah is the market leading cultivar
MSM = Mid-season, self-fertile, mahogany. Bing is the market leading cultivar

Table 2: Sweet cherry advanced selections identified for planting in P2 trials at WSU-Prosser and OSU, MCAREC, Hood River in 2013. Means are in most cases based on 3 years data on fruit traits.

Tree id	Cross	Target market	Harvest date	Fruit wt (g)	Firmness (g/mm)	SSC (%)	TA (%)	PFRF (Kg/F)
FR001T070	Swt x Regina	LSB	18-24 July	12.7	291	22.2	0.73	0.93
FR004T029	Selah x Krup	LSB	9-11 July	12.8	302	22.0	0.64	0.39
FR009T033	Swt x Moreau	ESM	12-15 June	10.1	296	16	0.75	
FR009T037	DD x Lapins	LSB	2-4 July	11.0	318	19.8	0.74	1.03
FR009T089	Kiona x Chelan	ESM	12-15 June	10.1	301	16.0	0.64	
FR013T004	Swt x BB	LSM	14 July	14.5	303	21.0	0.75	0.71
FR049T083	Swt x Tieton	ESB?	30 Jun	11.8	318	19.1		0.94

See key for target markets in Table 1. Note that early selections FR009T033 and FR009T089 may be delayed for another year to collect more data.

Table 3: Sweet cherry genotypes identified in 2012 for propagation and planting in P2 trials in 2014. Means are based on one or two years data on fruit traits.

Tree id	Cross	Target market	Harvest date	Fruit wt (g)	Firmness (g/mm)	SSC (%)	TA (%)	PFRF (Kg/F)
FR001T002	Swt x Tieton	ESM	25 Jun	12.8	341	16.5	0.64	0.67
FR001T004	Swt x Tieton	Mech-SM	13 Jul	11.5	300	19.5	0.69	0.28
FR002T074	Selah x Van	ESB	30 Jun	10.7	337	20	0.79	0.79
FR009T049	Rainier x 1921B	ESM	12 Jun	10.0	308	18	0.55	1.06
FR044T083	Selah x Regina	MSM	30 Jun	10.8	298	18	0.48	0.85
FR049T125	Cowiche x Regina	MSM	30 Jun	11.0	313	16	0.76	0.71

See key for target markets in Table 1.

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

In consultation with BPAC, FR001T007, an early genotype that matures 1-2 days after Chelan that is already in P2 was recommended for fast-tracking to P3. Apart from having fruit quality attributes superior to Chelan without GA application, this selection can hang on the tree up to 3 weeks after commercial harvest without significant loss of firmness (see table 1). Initial tests indicate that this genotype is free of PNRSV, PDV and CLRV, however, we have contacted NCPN to initiate a full virus therapy while bud wood from the mother tree has been sent to WDN for propagation for P3 tests in 2014.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number: CH 12-103

Project Title: Investigating post-bloom thinning

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

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

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

Other funding sources: none

Budget 1
Organization Name: WSU **Contract Administrator: Carrie Johnston**
Telephone: 5093354564 **Email address: carriej@wsu.edu**

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

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

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

SIGNIFICANT FINDINGS:

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

METHODS

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

I – TIMING OF APPLICATION

Treatments:

- unthinned control (water sprayed)
- hand thinning to 30 fruit per foot
- 'rake' thinner targeting 30 fruit per foot
- Ethephon at 150 ppm

Timing of application:

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

Methods:

Applications will be made using a pressurized spray gun or commercial airblast sprayer to 'Sweetheart', 'Rainier', and 'Skeena' trees that exhibit heavy fruit set (we have several orchards identified but won't be able to determine which to use for these trials until after bloom). Two experiments will be conducted for each cultivar – one in a commercial orchard and one at the WSU-Roza experimental orchards. In addition, we will work opportunistically with additional growers interested in evaluating post-bloom thinning strategies by providing suggestions for protocols and helping with data collection on efficacy. On each application date, treatments will be made to entire trees, with 6 whole-tree replications. Hand thinning will be accomplished by manually removing fruit from throughout entire trees with a goal of leaving ca. 30 fruit per foot (preliminary work shows this is a reasonable target to balance fruit number with quality). The 'rake' tool (http://www.goodfruit.com/Good-Fruit-Grower/Web-2011/New-cherry-thinning-tool/) will target a similar final crop density. Depending on the orchard, we will use either a completely randomized design or a randomized complete block design, with at least 2 border trees between adjacent treatments. We will require 96 trees in each orchard (4 treatments x 4 timings x 6 reps). Key

environmental conditions (e.g., wind speed, temperature, humidity) during and following application will be monitored using AgWeatherNet stations in the vicinity.

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

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

II – RATE OF ETHEPHON

Treatments:

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

Methods:

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

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

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

RESULTS

Fruit set

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

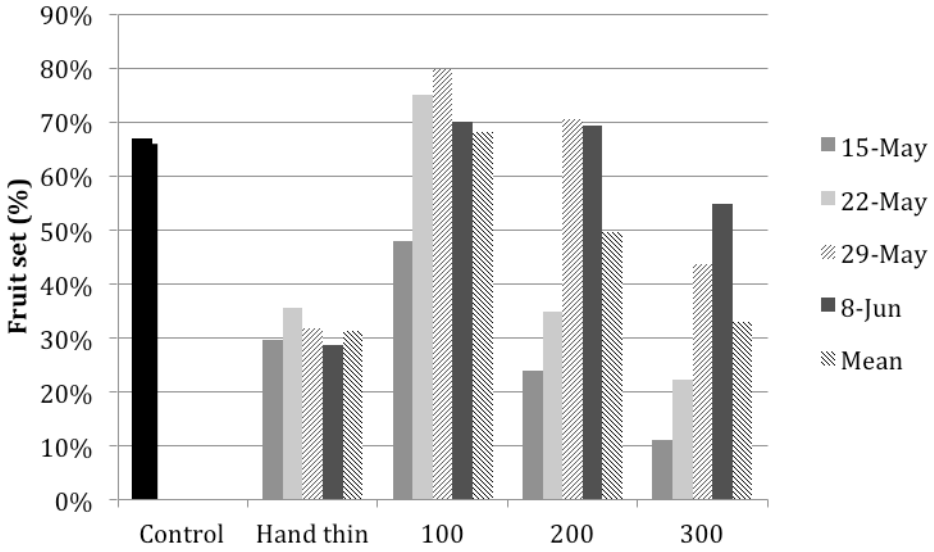


Figure 1. Fruit set of Skeena limbs that were hand-thinned or treated with various rates of Ethephon. Fruit were harvested at commercial maturity on 27 July, 2012.

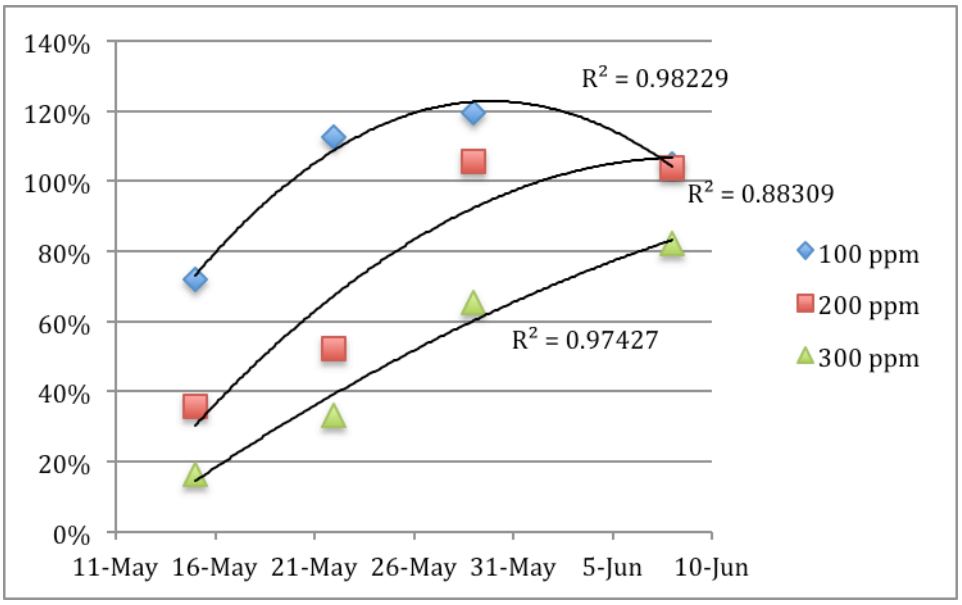


Figure 2. Skeena fruit set over time from treatment with various rates of Ethephon. Fruit were harvested at commercial maturity on 27 July, 2012.

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

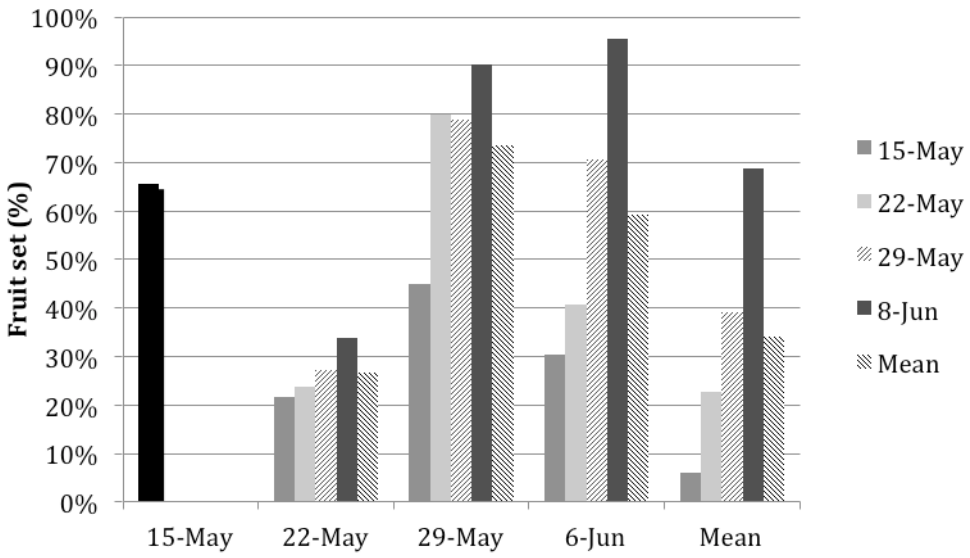


Figure 3. Fruit set of Sweetheart limbs that were hand-thinned or treated with various rates of Ethephon. Fruit were harvested at commercial maturity on 2 August, 2012.

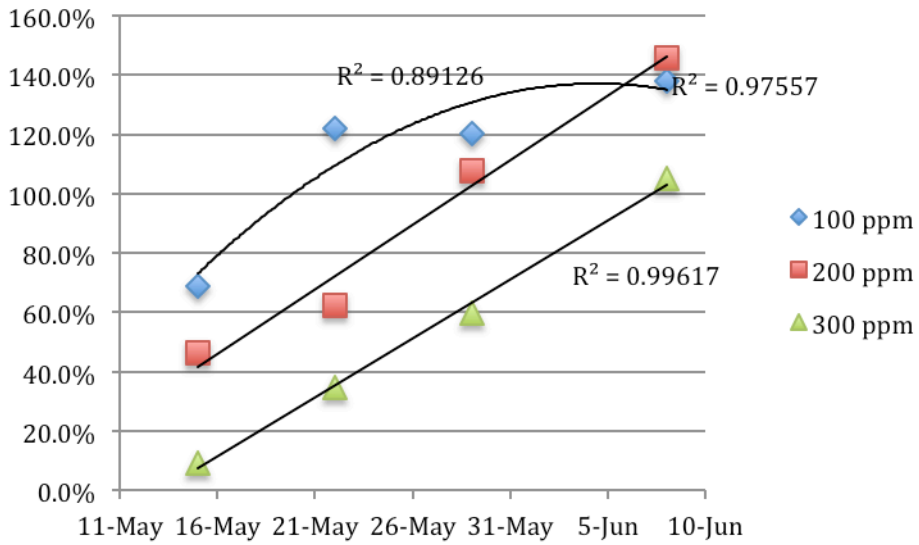


Figure 4. Sweetheart fruit set over time from treatment with various rates of Ethephon. Fruit were harvested at commercial maturity on 2 August, 2012.

Fruit quality

Ethephon applications had a varied effect on fruit quality. In Skeena, fruit weight in untreated control was ca. 8.4 g. Hand thinning treatments improved final fruit weight by about 14% (fruit weight = 9.6 g overall), across all treatment timings (Figure 5). In comparison, mean fruit weight across all timings was 9.3 g, 9.2 g, and 9.4 g in response to treatment with 100 ppm, 200 ppm, and 300 ppm Ethephon, respectively. Each application rate of Ethephon therefore induced some subtle improvement in fruit weight. Timing of Ethephon application was important – fruit weight increase was greatest on the later application dates in general (data not shown) with improvements of ca. 24% at 100 ppm applied on 8 June (4th timing), ca. 26% at 200 ppm applied on 29 May (3rd

timing), and 22% from 300 ppm applied on 22 May (2nd timing). These results reveal a disconnect between reduction in fruit set and improvement in fruit weight/size (i.e., significant reductions in fruit set did not lead to significant improvements in fruit size). Indeed, the relationship between fruit weight and fruit set is not significant (Figure 5). A similar relationship was found for other fruit quality attributes including fruit firmness, color, and soluble solids.

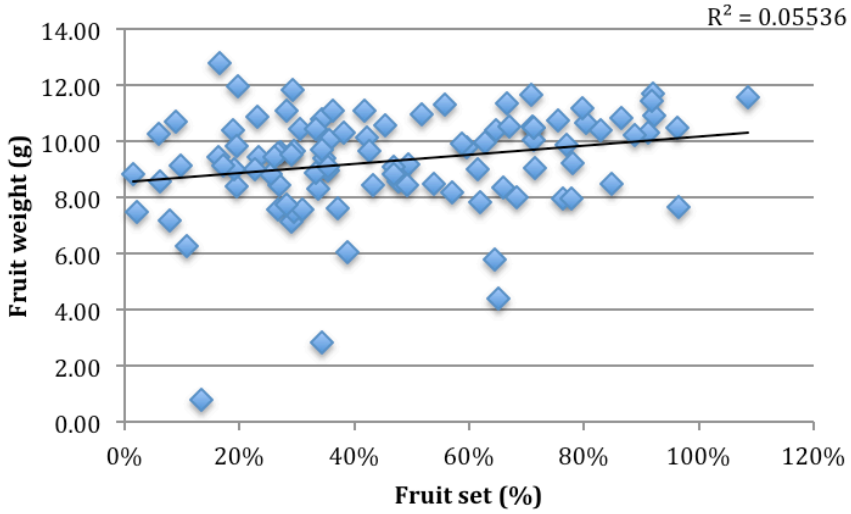


Figure 5. Relationship between Skeena final fruit weight and fruit set (% available flowers) across all rates of Ethephon and application dates.

In Sweetheart, fruit weight of untreated control was ca. 5.0 g – a clear indication of over-cropped trees. Overall, the unthinned trees had 77 fruit/foot. Hand thinning treatments improved final fruit weight by about 22% (fruit weight = 6.2 g overall), across all treatment timings. In comparison, mean fruit weight across all timings was 5.6 g, 6.5 g, and 5.8 g in response to treatment with 100 ppm, 200 ppm, and 300 ppm Ethephon, respectively. The effect of application timing was variable – fruit weight increase from treatment with 100 ppm was greatest (+43%) on the latest timing, whereas with 200 ppm and 300 ppm the greatest improvements in fruit weight/size were on the 2nd application date (22 May). Therefore, despite significant reductions in fruit set, treatment with 300 ppm Ethephon did not improve fruit quality proportionally. This may be due to a phytotoxic effect, and will be investigate further in 2013.

CONTINUING PROJECT REPORT **YEAR**: 2012

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

PI: Ines Hanrahan
Organization: Washington Tree Fruit Research Commission
Telephone: 509-669-0267
Email: hanrahan@treefruitresearch.com
Address: 2403 S. 18th Street, Suite 100
City/State/Zip: Union Gap, WA 98903-1637

Cooperators:

Product suppliers:	Garrett Bishop, Pace Intl.; Clive Kaiser, OSU; Adrian Roozen, Wilbur Ellis
Grower collaborators:	Jim Kelly, Ray Wolverton, John Verbrugge, Jaime Reyes, Denny Messimore
Other:	Michael Young, formerly Stemilt; internal program staff: Manoella Mendoza, Tory Schmidt, Sandy Stone, Felipe Castillo, Udel Mendoza, Alfonso Ruiz

Other funding sources

All supplies and chemicals were donated by industry suppliers (value: $ 2,827).

Budget 1
Organization Name: WTFRC **Contract Administrator: Kathy Schmidt**
Telephone: **Email address:**

Item	2012	2013
Salaries	4,366	4,366
Benefits	1,872	1,872
Wages	11,745	11,745
Benefits	5,033	5,033
Equipment		
Supplies	200	200
Travel	150	150
RCA room rental	360	360
Revenue	14,000	10,000
Total	**9,726**	**13,726**

Footnotes: Salaries are estimated based on actual time spent on project for internal program staff: Schmidt, Castillo and Hanrahan. Wages reflect timeslip costs. 2013 costs are estimated and will be adjusted (±25%) based on industry feedback and priorities, and accounting for 2013 seasonal challenges.

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

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

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

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

SIGNIFICANT FINDINGS
Objective 1: Two out of five trials demonstrated significant field cracking (>10%) of fruit. RainGard and SureSeal, reduced cracking incidence by 47-66%. Fruit quality and storage performance was largely unaffected. A reduction of the number of applications of RainGard from four to two did not reduce effectiveness of product.

Objective 2: General variety knowledge should be combined with year-to-year and block-by-block information regarding cracking potential. Cracking susceptibility levels determined with a bench top test correlated well to the actual cracking observed in the field. We recommend using it in blocks threatened by rain to determine the economic benefits/thresholds of applying protective coatings.

METHODS
This project featured four cherry cracking trials utilizing two cultivars (Tieton and Skeena) and three products or product combinations (Table 1). Trial designs were typically randomized complete blocks with four replications. In another trial, GA and RainGard were applied alone or in combination to Tieton trees (Table 2). All materials were applied by a) grower cooperators or b) WTFRC staff with an AccuTec or handgun sprayer according to protocols developed collaboratively with product distributors. If more than 10% of fruit was affected by cracking at harvest, on-tree readings of cracking incidence were performed.

Table 1: Spray materials used to prevent rain-induced cherry cracking. WTFRC 2012.

	Spray schedule Wks before harvest				Concentration	Active ingredient(s)
Material	4[z]	3	2[y]	1		
RainGard	x	x	x	x	0.8 gal/acre	Natural fatty acids
VaporGard	x		x		1 gal/acre	Di-1-p-menthene
SureSeal	x		x		1% solution	Copolymer: stearic acid, cellulose and calcium

[z] equals light green; [y] equals early pink

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

Table 2: Material, concentration, and timing of gibberellic acid (GA) and RainGard (RG)

Material	Concentration	Timing
GA/RG mix + RG standard Program	GA: 20 ppm; RG: 0.8 gal/acre	GA timing* + 4 weekly apps
(GA/RG mix) + RG before rain	GA: 20 ppm; RG: 0.8 gal/acre	GA timing + RG before rain
GA/RG mix	GA: 20 ppm; RG: 0.8 gal/acre	GA timing
(GA only) + RG standard Program	GA: 20 ppm; RG: 0.8 gal/acre	GA timing + 4 weekly apps
GA + RG before rain	GA: 20 ppm; RG: 0.8 gal/acre	GA timing + RG before rain
GA (Control)	GA: 20 ppm	GA timing

*refers to approximately 3 weeks before harvest

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 cracking index (CI) was calculated from the results as follows:

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)	$n_1 \times 5$	$n_2 \times 4$	$n_3 \times 3$	$n_4 \times 2$	$n_5 \times 1$
Total weighted value					$\sum$ (Nc x F)
Maximum possible value					100*
Cracking index:				CI (%) = $\frac{\sum(Nc \times F)}{100} \times 100$	

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

RESULTS & DISCUSSION

Cherries are a valuable crop and every year some orchards experience crop loss due to rain-induced cracking. Standard industry practice has been to reduce the duration of fruit wetness by blow drying the trees or to apply osmoticum solutions such as calcium nitrate. Rain exclusion in form of hydrophobic spray materials has been of interest for the past decade. Several products are available (RainGard, VaporGard, SureSeal) and there is interest in comparative performance data. Thus far, we have only had limited success with these direct product comparisons, mainly due to lack of adequate rain events. Another factor influencing the choice of method to prevent rain damage is the high cost of the products available. Knowledge of actual cracking susceptibility of individual blocks prior to threatened rain events (such as CI data), could minimize the number of applications of hydrophobic materials while optimize the rain-cracking prevention.

Objective 1: Evaluate and optimize spray programs to reduce rain-induced cherry cracking
Two out of five trials demonstrated significant field cracking (>10%) of fruit (Tables 3 + 4). RainGard and SureSeal (a new product developed by Clive Kaiser, OSU) reduced cracking incidence significantly twice and once respectively by approximately 47-66%, even under strong rain pressure (3-4 rain events with more than 0.1inches of precipitation each). Mixing RainGard and GA to gain application efficacy did not influence GA performance (Table 4). In fact, tank mixing resulted in firmer fruit overall, though not to a level of statistical significance. Fruit treated with VaporGard and SureSeal exhibited chemical burns on the stylar end of the fruit in all Tieton blocks (example shown in Figure 1), but not in the Skeena orchard. SureSeal was applied at twice the recommended concentration at the first application timing, and all fruit damage with that material was incurred because of the application error. Second, VaporGard treated fruit had a significantly reduced shine of the fruit, especially in Tieton (Figure 2). Skeena showed diminished luster with all three products tested, indicating that this variety may be especially sensitive. We will test most commercially important varieties in 2013 to determine if other varieties are sensitive also.

All other commercially important quality parameters remained unaffected at harvest (Tables 3 and 4) and after two weeks in cold storage (not shown).

Corroborating results from 2011, both hydrophobic coatings (Raingard and SureSeal) performed well in reducing the incidence of cracking in cherries in 2012. In WTFRC trials from 2009-12, RainGard consistently reduced field cracking by up to 59% in trials with significant cracking pressure (Figure 3). Both coatings remained effective for 5-7 days (max. observed 10 days) and up to 0.56 inches of rain (Figure 4). Product efficiency is maintained, even if the product is applied only twice: first as a tank mix with GA, and second before a rain event. (Table 3). SureSeal performed very well in 2011 and 2012 (Figure 3), but has proven to be difficult to use under real orchard scenarios: the tank has to be extremely clean (triple rinsing recommended) to avoid adverse reactions of the spray solution with other chemical residues, a high volume of water (at least 200 gal/acre) is needed to achieve good performance, and phytotoxicity may be an issue with some varieties and/or higher than recommended product concentration.

Table 3: Cracking severity and at harvest fruit quality of cherry. WTFRC 2012.

Treatment	Weight	Acids	Sugars	Firmness	Diameter	Row Size	Color	Cracking*
	(g)	(% malic acid)	(% Brix)	(g/mm)	(mm)		(1-7)	(%)
Pasco 'Tieton'/G6 (handgun)								
RainGard	11.1 ns	0.57 ab	14.7 ab	256 ns	26.8 ns	10.2 ns	5.1 ab	11 b
SureSeal	10.2	0.60 a	15.5 a	261	26.7	10.3	5.2 ab	9 b
Vaporgard	11.9	0.55 b	14.5 b	238	27.0	10.2	4.9 b	31 a
UTC	11.1 ns	0.57 ab	14.7 ab	256 ns	26.8 ns	10.3 ns	5.7 a	27 ab
Stemilt Hill 'Skeena'/Mazzard (handgun)								
RainGard	8.4 ns	0.81 ns	14.8 ns	385 a	23.8 b	11.4 a	4.5 ns	5 ns
SureSeal	9.0	0.80	16.1	360 ab	25.6 a	10.7 b	4.4	4
Vaporgard	8.7	0.79	14.8	351 b	24.3 b	11.2 ab	4.6	4
UTC	9.1	0.80	15.4	380 ab	24.7 ab	11.1 ab	4.4	4
Sawyer 'Tieton'/G6 (handgun)								
SureSeal	8 b	0.47 ns	14.7 ns	259 ns	24.3 ns	11.2 ns	4.7 ns	4 a
UTC	9 a	0.49	16.2	253	24.9	11.0	4.8	2 b
Pasco 'Tieton'/G6 (grower applied)								
RainGard 2x	12.2 ns	0.59 ns	17.2 ns	262 ns	27.3 ns	10.1 ns	4.0 ns	15 b
RainGard 4x	11.9	0.62	17.3	259	26.8	10.2	4.0	12 b
UTC	12.7	0.62	17.6	250	27.5	10.0	4.3	28 a

*on tree reading based on 400 frt./rep

Table 4: Cracking severity and at harvest fruit quality of cherry. WTFRC 2012.

Treatment	Weight	Acids	Sugars	Firmness	Diameter	Row Size	Color
	(g)	(% malic acid)	(% Brix)	(g/mm)	(mm)		(1-7)
Sawyer 'Tieton'/G6 (Accutec)							
GA + RG before rain	8.8 ns	0.47 ns	14.7 ns	257 b	24.7 ns	11.0 ns	3.4 c
GA + RG standard	8.6	0.47	15.0	262 b	24.2	11.2	3.7 ab
GA/RG mix	7.9	0.48	14.7	266 ab	24.0	11.3	3.5 bc
GA/RG mix + RG before rain	8.6	0.48	15.0	282 a	24.2	11.2	4.0 a
GA/RG mix + RG standard	9.1	0.49	15.0	267 ab	24.7	11.0	3.5 bc
Control GA	9.4	0.48	14.9	260 b	24.8	11.0	3.6 bc

When deciding whether or not to use hydrophobic spray materials, one should take into account the actual susceptibility of the block in question as described under Objective 2. Considering a maximum 50% reduction in cracking and estimating the total production cost for a 6-year-old sweet cherry block at $10,120 per acre (http://www.tfrec.wsu.edu/pdfs/P569.pdf), hydrophobic spray materials become cost effective at production volumes of 10.5; 5.5; or 3.5 tons/acre when considering 50 cents, $1, or $1.50 returns respectively. In other words, if you have a high yielding block (> 10.5 tons/acre) or expect high net-returns (>$1.50) it is always worth using such materials.

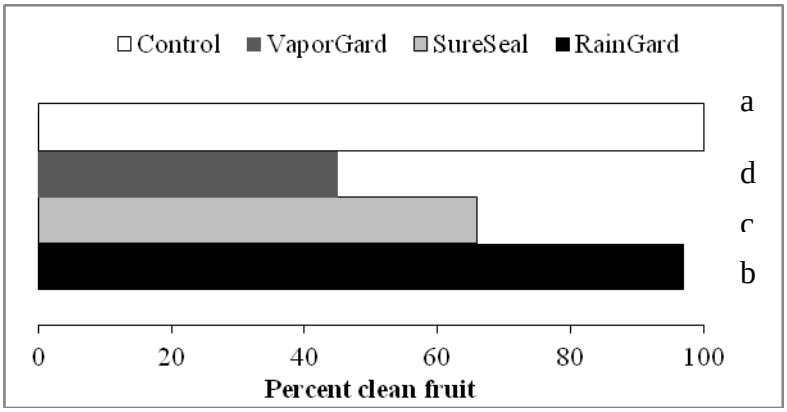


Figure 1: Amount of fruit not affected with chemical burn (in percent of clean fruit) after application of rain cracking suppressants to Tieton cherries. WTFRC 2012.

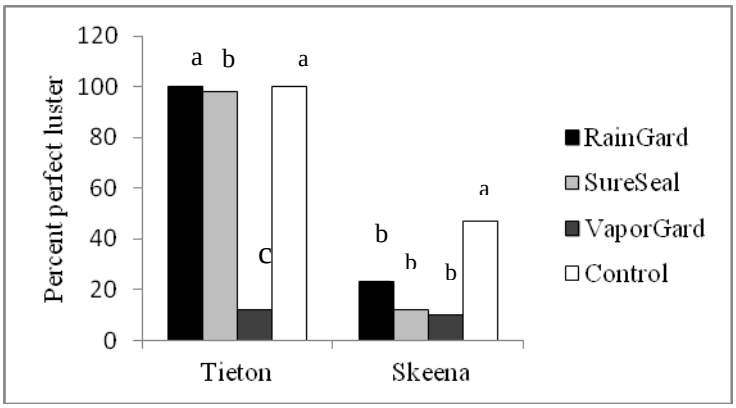


Figure 2: Amount of fruit with perfect shine at harvest. WTFRC 2012.

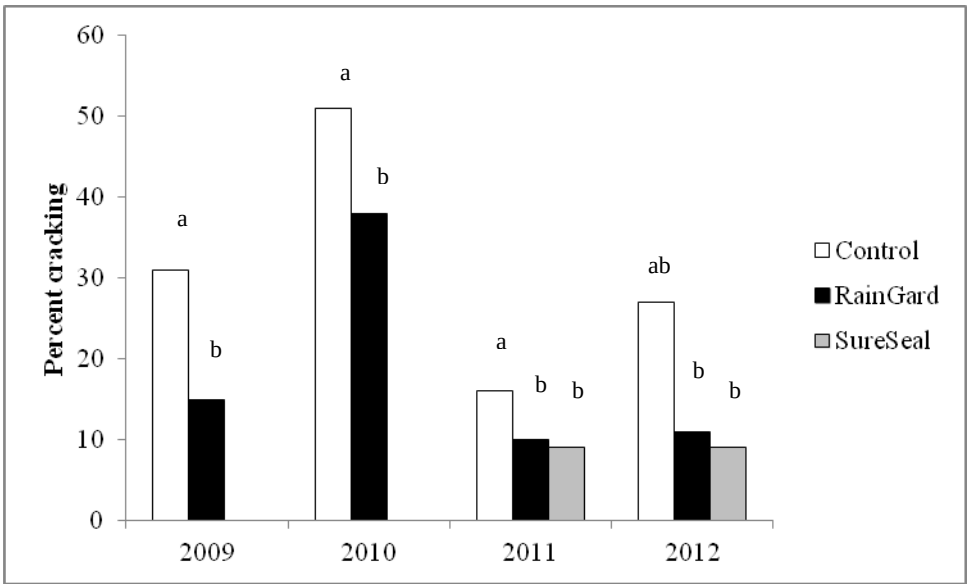


Figure 3: Percent of fruit affected by rain induced cherry cracking from 2009-12. WTFRC 2012.

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

We observed 10 blocks during the month before harvest in 2011 (two Bing, two Skeena, two Tieton, one each of Santina, Benton, Early Robin, Sweetheart). Initial fruit weight averaged 3-4g and color was green to light green. Samples for the artificial cracking test were taken bi-weekly.

Bing cherries in both locations started to crack in bench-top assays 22 - 31days before harvest with rapidly increasing susceptibility, including sustained CI levels in excess of 20 for 15-17 days before harvest (Table 5). Compared to last year (DOS: 14-23, DOHS: 7 days), Bing were at much higher risk for cracking development in 2012.

Of the two Tieton blocks observed, both were cracking susceptible over a long period of time (16-17 days). However, while the block in Pasco sustained CI level above 20 for 12 days, the block in Sawyer recorded only one day (Table 5). Although Tieton cherries are typically considered prone to rain induced fruit cracking, the data from 2011 and 2012 indicates that there are block-by-block variations to be accounted for as well. Further, CI levels observed in these blocks correlated well with the actual cracking observed in the field: the block in Sawyer had natural fruit cracking levels below 5% at harvest (data not shown), while the block in Pasco had up to 28% cracked fruit without protection (Table 3). Pasco sustained 1.02 inches of total precipitation between May 23 and June 7, with three events at or above 0.1 inches, the general threshold for rain induced cracking (Figure 4). The fruit was susceptible during each of the main rain events, and sustained cumulative cracking. In both trials set-up in the Pasco Tieton block, applications of cracking protectants were applied ahead (1 or 8 days) of the anticipated rainfall, and significant reductions in damage was observed (Table3).

Early Robin, Benton, Santina, and Skeena all had prolonged periods of cracking susceptibility, with 16-23 days of potential for cracking (Table 5). This data corresponds well with industry's experience with these varieties. Conversely, Sweetheart would be grouped as having moderate cracking potential based on 2012 data. Although fruit was susceptible throughout our observation period of 36 days, it only reach high levels of susceptibility for a few days before harvest (Table 5). Overall, variability in cracking susceptibility as observed especially in Bing and Tieton, highlights the need to supplement general variety knowledge with year-to-year and block-by-block information regarding cracking

potential. The bench top test described above has shown sensitive enough to pick up these swings and we recommend using it in blocks threatened by rain to determine the economic benefits/thresholds of applying protective coatings.

Table 5: Days of susceptibility (DOS; CI > 0), days of high susceptibility (DOHS; CI ≥ 20) and maximum cracking index (max CI) for cherry orchards in Washington. WTFRC 2012.

Variety	Location	DOS	DOHS	Max CI
Tieton	Pasco	17	12	42
	Sawyer	16	1	21
Early Robin	Buena	18	11	50
Santina	Zillah	21	18	43
Benton	Zillah	16	5	65
Bing	Zillah	31	17	59
	Stemilt Hill	22	15	47
Skeena	Outlook	21	11	58
	Stemilt Hill	23	23	36
Sweetheart	Stemilt Hill	36	8	56

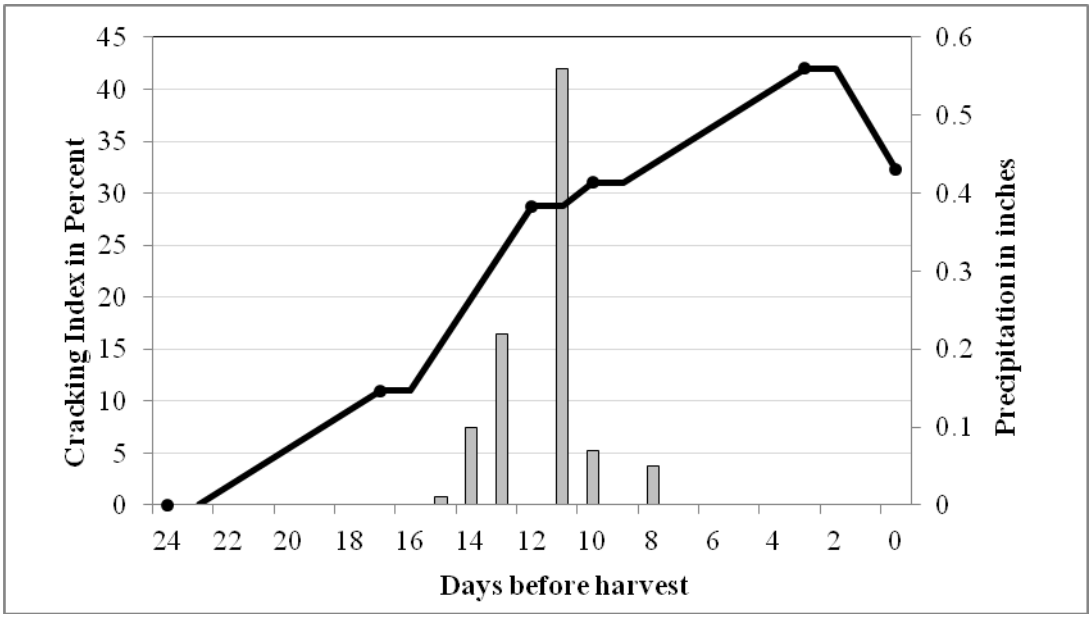


Figure 4: Development of cracking potential (cracking index) and timing of rainfall for Tieton/Pasco. WTFRC 2012.

Literature cited

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

CONTINUING PROJECT REPORT - *Extension* **YEAR: 3 of 3**
WTFRC Project Number: CH 10-112

Project Title: Hand-held mechanical thinning device for cherry production

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Cooperators: **WA Producers**

Total project funding: **Year 1:**45,378 **Year 2:** 46,443 **Year 3:** 7,000

Budget
Organization Name: WA State University **Contract Administrator:** Carrie Johnston
Telephone: 509.335.4564 **Email address:** carriej@wsu.edu

Item	2010	2011	2012
Salaries[1]	24,460	25,438	0
Benefits	1,918	1,995	0
Wages	0	0	0
Benefits	0	0	0
Equipment	2,000	1,000	0
Supplies	10,000	11,000	3,000
Travel (Zhang)	3,000	3,000	1,000
Travel (Lewis)	2,000	2,000	3,000
Publications	2,000	2,000	0
Miscellaneous	0	0	0
Total[1]	45,378	46,443	7,000

Footnotes:[1] **Graduate Assistantship**

OBJECTIVES

The primary objective is to develop the fundamental technology and fabricate prototype devices for hand-held cherry blossom thinning. The devices should have ability to remove bloom with controlled thinning power, reach into inner layers of trees trained to different systems, and expand its applicability to other fruit trees with limited modifications. The selective thinning capability will be furnished through human intelligence of the operator using the device. Tool would also be a good fit in all current sweet cherry production systems, could be used by workers on the ground or on platforms including mobile multi-worker and single worker bucket type.

SIGNIFICANT FINDINGS

In the past two years, **three designs of hand-held cherry blossom mechanical thinning prototype device have been developed and tested in commercial production** in Washington, Pennsylvania and Chile. Trials and demonstrations have been conducted in blocks trained to the UFO, Steep Leader, Central Leader and Spanish Bush systems at different bloom stages. Major findings include:

1. Pre- and post-thinning blossom counts indicate that the all four thinning spindle designs could effectively remove bloom, and that spindle rotating speed could noticeably impact the amount of bloom removed. Test data revealed that in a low (500 – 800 rpm), medium (1500 – 1800 rpm) and high speed (2500 – 3000 rpm) ranges, the devices could easily remove about 18%, 31% and 62% blossom, respectively.
2. Configuration of thinning spindle head noticeably affects thinning efficiency, but had less impact than spindle speed. Thinner spindles with three rows of plastic strings have better flower removal capability. String length longer than 3 inches and shorter than 8 inches has no noticeable difference on flower removal capability.
3. Prototype one (gasoline engine powered prototype) could achieve the basic functionality requirement, but the load-sensitive speed variation and resulting lack of operator control was a weakness. The relatively heavy weight (~13 lbs)) of the device and the inevitable vibration also lowered the worker's operation efficiency.
4. Prototype two (battery powered prototype with built-in DC motor controller) could achieve reasonable spindle speed control in the entire speed range (0-8,000 rpm). However, the relative short battery life, the short continuing-work time limitation of the DC motor, and the relatively heavy weight of the battery were major obstacles.
5. In the design of the third prototype, the battery was detached from the thinner and a battery with greater capacity was connected by wire and put into a backpack. This simple solution effectively removed two major obstacles of Prototype-2: the weight of hand-held device dropped to ~5 lbs and the battery could support an 8-hr day operation. The problem of short continuing-work life of the DC-motor remains unsolved.
6. Hand held prototypes were effective at removing bloom in cherry, apricot and apple across bloom stages and impacted final fruit set and final fruit size.
7. In all species, hand held is a versatile tool that could effectively be used in many strategies: one time hand held plus green fruit hand thinning, Darwin with hand held follow up, hand held plus BA, handheld plus post bloom etc.
8. Hand bloom thinning and green fruit thinning in cherries is slightly more effective than hand held in terms of final fruit size, but the time to complete the tasks is reduced significantly with the hand held.

9. Grower and employee response indicates a continued need for hand held device.

METHODS

Thinning Actuators Development

A blossom thinning actuator, or *spindle head*, is an end-effector mounted on top of a hand-held device to actually remove blossoms from the tree, and is the core element developed in this research. The specific design objective was to find optimal parameters of a spindle capable of removing bloom effectively. A few aluminum spindles with a few narrow metal pieces fix strings were designed and fabricated. Regardless of the specific design of a spindle, the length of those spindles was set 10 inches. Each metal piece fixed strings on the spindle to form one trimmer line. With different numbers of trimmer line being fixed, the spindling force of strings removed the blossom.

Several string configurations were designed and a set of development tests were conducted both in laboratory setup and in actual field conditions to evaluate corresponding performance of those designs. This year, according to the performance results, the configurations was narrowed down to

- Two different trimmer line configurations were selected: two lines of 180° installation, three lines of 120° installation;
- Two types of strip materials, 0.1" diameter plastic strip and 0.14" diameter plastic covered wires, were selected and tested on their suitability for this particular application; and
- Different string lengths from 3 to 7 inches were tested in field operations.

Figure 1. Examples of thinning spindle design: A 3D sketch (left) with 4 trimmer lines, eight 6 inches long strings in each line; and a thinning head (right) with 2 trimmer lines of 6 inches long plastic covered wire

Prototype Two Development

In an effort to find solutions to solve control, weight and vibration problems, prototype-2 was designed to use a battery powered DC motor. This new prototype was also developed based on a commercially available DC motor powered grass trimmer (Figure 2). An electronic speed controller was developed (also based on a commercially available model) and integrated into the prototype to control the DC-motor speed. By supplying driving power from 0 to 24V, this prototype could control thinning spindle speed from zero to the highest speed limited by the DC motor. Weight was dropped to about 6 lbs and vibration was noticeably reduced.

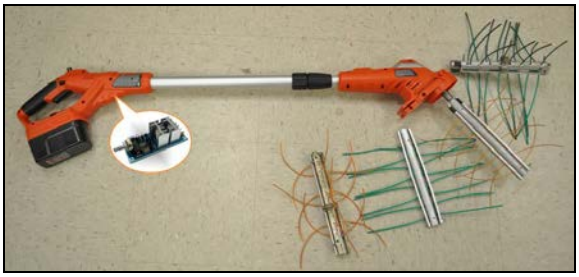

Figure 2. Prototype-2: DC motor-powered hand-held mechanical blossom thinner with four spindles

Prototype Three Development

Though the thinning spindle speed of prototype-2 could be steadily adjusted and the vibration was reduced, a few other issues for the battery powered prototype were identified. Firstly, battery life was too short to perform thinning task for extended period of 4-8 hours. Secondly, the weight was still relatively heavy. In the design of prototype-3, the DC motor powered grass trimmer that was used for building prototype-2 was used, with a major modification. The original battery was removed from the device and a larger capacity battery was placed in a backpack. This prototype could be used in two different ways: operated by single person by putting battery in backpack or operated by multiple persons using a vehicle to carry the batteries as shown in Figure 3. Both configurations could support thinning operations for an 8 hour + work schedule.

Figure 3. Single-person prototype with backpack (left) and two-person prototype with platform carried batteries

Product Commercialization

As a concept-proof research project, this project not only created several conceptual designs of hand-held mechanical blossom thinners which can be used in orchards effectively it also provided guidelines to a manufacturer in China who has taken the concept to commercialization. The first version of this commercial product was extremely light compared to the WSU prototypes. The manufacturer is working with industry to improve the robustness of the device. Figure 4 shows the commercial product.

Figure 4. Commercial product of the hand-held mechanical thinner.
Dr. Qin Zhang introducing the commercial device to Governor Chris Gregoire.

Experimental design

Field tests were designed to evaluate the functionality and performance of the developed prototype-2 and prototype-3, and were conducted in ‘Sweetheart’, ‘Chelan’ and ‘Lapin’ at both research and commercial orchards in Washington, Pennsylvania and Chile in 2011. The validation tests for

developed prototypes were conducted on different tree structures, including UFO (Upright Fruiting Offshoots), V-trellis, Steep Leader and Spanish Bush systems, and at 20-40% and 60-80% bloom stages. The hand held device was compared to hand bloom thinning, hand green fruit thinning and control depending on the trial. Two-spine and three-spine heads were compared in one trial.

Data collection included: pre and post bloom counts for removal rates, final fruit set, yield and fruit quality to include size, firmness and brix. Time required to apply thinning treatments was recorded in several trials.

In one trial, hand held thinning was evaluated with 4 treatments 1) control – no thinning, 2) light thinning – lower side of spur branches, 3) Heavy thinning – light thinning plus crotches and pendent wood and 4) Fast thinning – areas with a lot of white targeted. This trial only collected data on final row size.

RESULTS AND DISCUSSION

For prototype-1, the low, medium and high spindle speed (as defined in previous section) removed 18%, 31% and 62% flowers from a branch with one swing by the same operator (see Figure 5). The configuration of test device was three row plastic strings. The results indicate that the designed thinning spindle could effective remove cherry blossom to different percentage by controlling spindle speed.

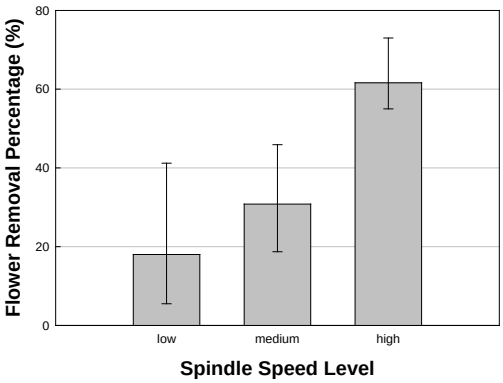

Figure 5. Removal percentage of flowers with different spindle speeds (prototype-1).

In one study, the average time needed for thinning a whole tree using the hand-held mechanical thinner was 85 seconds. In comparison, when the same operator used a brush tool, the operator had to use a ladder to reach the branch to sweep the flowers. The recorded time for the brush thinning operation was over 300 seconds per tree.

In the spindle head removal capability tests, two sets of evaluation were conducted: the first set was measured by the fruit flower ratio (FFR) of cherry after the thinning using spindle heads of four different design configurations (A, B, C and D4) with the same length of strings (6 inches), and the second set was measured by FFR of cherry after the thinning using configuration D spindle head but with different lengths of thinning strings (3, 4, 5, 6 and 7 inches). In the first set of tests, the results (FFRs corresponding to configurations A, B, C and D4 were 16.2, 20.1, 14.2 and 10.6% as shown in Figure 6) revealed that the spindle head configuration A (two rows of 6 inches plastic thinning strings) had better removal capability than configuration B (three rows of 6 inches plastic covered metal wires). Configuration D4 (three rows of 6 inches plastic thinning strings) had better removal capability than configuration B (two rows of 6 inches plastic covered metal wires). These results imply that the string materials could affect the flower removal capability. In comparing the two

tested materials, the plastic string showed a better flower removal capability than the plastic cover metal wires which indicated that the metal wires could be too rigid for blossom thinning uses.

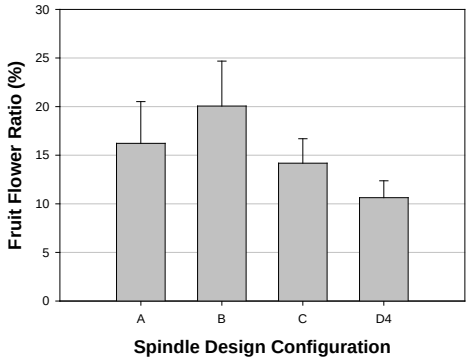

Figure 6. Average fruit flower ratio of cherry post thinning with spindle head configurations A, B, C and D4 with roughly the same swiping speed around 0.5 m·s-1. In this test, nine replications were conducted at each configuration.

From the results presented in Figure 6, one could also find that the average FFRs on branches thinned using spindle configurations A and B (two rows of strings) had less removal capability than configurations C and D4 (three string rows) for both thinning string materials. It indicates that the thinner spindles with three rows of strings have better flower removal capability than two-row string configurations.

The results obtained from the second set of comparison tests (spindle configurations of D1 to D5) indicated that there is no noticeable difference in FFR among different string lengths except the 3 inch strings (as shown in Figure 7). Except for a 16% FFR for branches thinned using the spindle of 3 inch long strings, the FFRs on rest of branches were ranged from 7.3 to 10.3%, with the 5 inch strings resulting in the lowest FFR (implies the highest flower removal rate) and the 6 and 7 inch strings resulting in the similar level of higher FFR (the lowest flower removal rate). One possible explanation for the results was that the accessibility of the spindle of 3 inch strings was limited and could not reach all of the flowers on the branch during the thinning. String lengths of more than 6 inches (configurations D4 and D5), provided sufficient string to bloom contact to achieve effective thinning, with a thinning efficiency very close to the most effective design (configurations D2). The longer strings create some possibility that the long strings can easily become twisted into neighboring branches. Considering efficiency and accessibility, the spindle of three rows of 6 inch plastic strings (configuration D4) was selected as the optimal design.

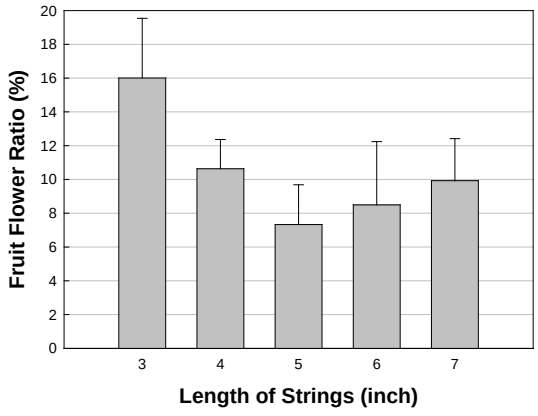

Figure 7. Average fruit flower ratio of cherry after post thinning with spindle head configurations D1 to D5 with roughly the same swiping speed around 0.5 m·s-1. Three replications were conducted at each setting in this test.

It was also visually observed that (1) shorter strings needed the spindle to be placed closer to the branch and it would slow rotational speed down dramatically when engaging the strings to the flowers; (2) longer strings could easily get intertwist branches next to the target branch. Therefore, a string length of 5 to 6 inches was within an appropriate range for a hand-held mechanical thinner of similar design as we have tested.

WA Cherry trial and demonstrations will be presented at the Cheery Institute in January 2013. Additional replicated trial results will be presented through the Innovative Strategies for Thinning Fruit SCRI final report in Jan 2013.

EXECUTIVE SUMMARY

The primary objective of this work was to develop prototype devices for hand-held mechanical bloom thinning in sweet cherry. Three versions of prototype devices were developed and one product is being commercialized.

Trials were conducted in WSU research blocks and commercial orchards. Results indicate that the designed thinning spindle could effectively remove cherry bloom, and the spindle rotating speed could noticeably impact the amount of bloom being removed. Operators could control the percentage of bloom removal within a cluster by controlling spindle speed and could control total bloom removal by the strategic placement of the device in a tree. The configuration of the thinning head could also affect thinning efficiency.

Development tests conducted in research orchards indicated that a thinning spindle configuration with three rows of 6 inch plastic strings could offer sufficient accessibility and a satisfactory flower removal capability when compared to all other tested configurations. Under commercial orchard operation conditions, obtained test results indicated that the thinning spindle speed had a dominating impact on the effectiveness of blossom removal. Higher spindle speed did increase flower removal capability in thinning operations within the tested speed range: an average flower removal rate of 61.1%, 30.8% and 18.0% was observed at high (2500-3000 rpm), medium (1500-1800 rpm) and low (500-800) speed ranges. Results also showed that different percentage removal requirements could be satisfied through spindle speed control as the results from commercial orchard tests revealed that different spindle speeds could remove different amount of flowers. The efficiency test results revealed that the use of a hand-held blossom thinner could increase thinning efficiency by 3 times compared to manual thinning using a hand brush and a ladder, which proved that the use of hand-held blossom thinners could reduce the labor costs associated with thinning operations. In summary, this study proved the concept of hand-held blossom thinning device, and verified the usefulness and effectiveness of a hand-held mechanical blossom thinner in tree fruit production.

There are two extra objectives need to be completed. One is investigation of the relationships between string stiffness and product performance, which will be conducted on a laboratory platform. The other is investigation of operation parameters which directly impact flower removal rate in orchard. A few parameters have been defined. Both objectives will be achieved in the coming year.

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

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

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Cooperators: Todd Einhorn

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

Other funding sources: None

WTFRC Collaborative expenses: None

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

Item	2012	2013	2014
Salaries			
Benefits			
Wages	9,600[1]	9,888	10,185
Benefits	8,275[2]	8,525	8,781
Equipment			
Supplies	8,000[3]	8,000	5,000
Travel	500[4]	500	500
Miscellaneous			
Total	26,375	**26,913**	24,466

Footnotes:
[1]Wages: 320hr each for 2 part-time employees at $10/hr and $20/h, respectively. 3% increase is factored into Year 2 and 3.
[2]OPE: calculated OPE for both employees.
[3]Supplies: maintaining cold rooms, GC column, buying fruit, gases (helium, nitrogen, hydrogen, air, and standard gases), and chemicals.
[4]Travel: field trips to orchards and packinghouses.

OBJECTIVES

The goal of this project is to improve shipping quality and minimize postharvest splitting, pitting, and stem browning of the major sweet cherry varieties grown in PNW. The key objectives are to:

1. Understand the dynamics of cherry respiration physiology influenced by cultivars, ripeness, temperature, O_2 and CO_2 – an essential knowledge for improving shipping quality.
2. Determine effectiveness of the major commercial modified atmosphere packaging (MAP) technologies on maintaining fruit quality of the major PNW varieties at shipping conditions.
3. Optimize consumer packaging to maintain cherry stem quality during shipping/marketing.
4. Study the mechanism and practical solutions to postharvest splitting and pitting.
5. Evaluate PGRs and GRAS compounds on postharvest shipping quality.

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

1. Study respiration dynamics of stressed products (heat/rain) and determine the requirements of stressed product for MAP. Determine and select the right MAP for long-distance shipping of stressed products.
2. Continue to study how to prevent/reduce Regina and Lapins from developing bitter taste after long-distance shipping. Determine and find out the low O_2 threshold of developing bitter taste at shipping condition.
3. Continue to study the mechanism and practical solutions to postharvest stem browning, splitting and pitting. Determine the effect of temperature management, moisture status, and GRAS compounds on stem quality, splitting and pitting of the susceptible varieties.

SIGNIFICANT FINDINGS (year 1)

1. Respiration Dynamics

- Respiration rates of Bing, Regina, and Sweetheart were inhibited linearly by reduced O_2 from 21% to 3% at 68°F, **however, only from ~10% to 1% at 32°F**. Reduced O_2 from 21% to ~10% did not reduce respiration rate of sweet cherry fruit at 32°F.
- Elevated CO_2 (0-15%) did not inhibit respiration rates of Bing and Sweetheart. Interestingly, respiration rate of Regina was reduced by CO_2 at 5-15%.
- Respiration quotient (RQ) of Skeena started to increase at O_2 of ~3% at 41°F, which was higher than other late season varieties at the same temperature.

2. MAP Technologies

- The major commercially available MAP technologies had extremely varied equilibrium O_2 and CO_2 concentrations within bags for Bing, Regina, Skeena, Lapins and Sweetheart at shipping condition (32°F).
- **O_2 concentration affected flavor.** Fruit flavor and titratable acidity (TA) were influenced significantly by different equilibrium O_2 concentrations generated by different MAP bags after 4 and 6 weeks at 32°F. Fruit flavor maintained better in MAP with O_2 at 2-3%, followed by O_2 at 5-6%, and O_2 at 10-15%, and O_2 at 21% (control), after 4 and 6 weeks at 32°F.
- **CO_2 concentration affected anthocyanin synthesis.** Fruit in MAP with CO_2 at 12-13% had lower anthocyanin content, therefore, a brighter color compared to fruit in MAPs with CO_2 at 6-10% after 4 and 6 weeks at 32°F.
- **Regina and Lapins** produced a bitter taste after 4-6 weeks shipping at 32°F. MAP bags with O_2 at 3-6% prevented or reduced bitter taste development.
- **Skeena** could suffer anaerobic injury in most of the commercial MAP bags at 32°F with temperature fluctuated to 41°F. Skeena needs MAP with higher gas permeability to avoid anaerobic respiration during shipping.

3. Consumer Packaging

- Cherry stem moisture loss and quality were linearly related to WVPD (water vapor pressure deficit).

- Zipper-lock bags and clamshells with perforation ratio of 0.5% (3mm diameter) maintained stem healthier than the commercial ones (perforation at 2-5%, 8mm diameter), without generating extra condensation after a simulated storage/shipping/marketing period.

4. Postharvest Splitting & Pitting

- Varieties varied in susceptibility to postharvest splitting. GRAS compounds in processing water could reduce postharvest splitting of Bing and Skeena by reducing the release of soluble pectin compounds from fruit into processing water.
- Moderate moisture loss (0.5%) by air-cooling overnight before online processing reduced pitting susceptibility of Lapins.
- Box filling after the second hydro-cooling is one of the major processing points at which sweet cherry fruit suffer pitting damage. Reducing the drop height or proving cushion to fruit during box filling reduced pitting significantly.

METHODS

1. Respiration Dynamics

Bing, Skeena, Regina, Lapins and Sweetheart were harvested from orchards in The Dalles, Hood River, and Parkdale, OR. Cherries were picked in the morning and immediately transported to lab at MCAREC, Hood River, OR. Cherry samples of ~500g were placed in hermetically sealed glass containers (960mL) equipped with 2 rubber sampling ports at 32, 50, and 68°F. Headspace O_2 and CO_2 concentrations were periodically monitored by an O_2/CO_2 analyzer.

2. MAP Trials

Five commercial MAP bags (ViewFresh, Xtend, LifeSpan, Breatheway, and Primpro) with distinct technologies were obtained from 5 manufactures internationally (OVF, StePac, Amcor, Apio, and Chantler). Bing, Skeena, Sweetheart were obtained from OVF (The Dalles, OR) shortly after hydro-cooling and commercial on-line processing and transported to MCAREC (Hood River, OR). Fruit were immediately packed into different MAP bags (18lb/box) at 32°F. Regina and Lapins were harvested directly from orchards in MCAREC and packed in different MAP bags (18lb/box) after fruit were cooled down to 32°F. Bing and Sweetheart in 5 different MAP bags were stored at 32°F constantly until assessments. Skeena, Lapins and Regina in selected different MAP bags were transferred to 41°F for 3 days after 3 weeks at 32°F and transferred back to 32°F until assessments. The concentrations of O_2 and CO_2 in MAP bags were determined every day in the first week then every 3-5 days until at the end of the tests. At 4 and 6 weeks, 50 fruit were randomly selected from each box for determinations of respiration, FF, anthocyanin, TSS, and TA immediately after cold storage and plus 2 days at 68°F. Fifty fruit were randomly selected for evaluations of pitting, splitting, stem quality, and decay. Ten fruit were randomly selected from each box for sensory evaluation. Experimental units were boxes and there were three replications per treatment at each evaluation period. The experimental design was completely randomized.

3. Consumer Packaging

Chelan fruit were obtained from OVF (The Dalles, OR) shortly after packing. Two pounds of fruit were placed in commercial zipper-lock polyethylene (PE) bags with perforation ratio 2% (hole size at 8 mm), and PE bags with perforation ratio at 0.5% and 0.05% (hole size at 3 mm). Packaged fruit were stored at 32°F for 1 week, and then transferred to 50°F for 2 days, and then transferred to 68°F for 2 days. RH (TinyTag: Gemini Data Loggers, London, UK) within packages, fruit moisture loss, stem water content, pitting percentage, stem visual quality (1= totally browning; 5=healthy green), fruit firmness, and decay percentage were evaluated after 1 week at 32°F, plus 2 days at 50°F, and plus 2 days at 68°F. Experimental units were consumer zip-lock bags (2lbs) and there were three replications per treatment at each evaluation period.

4. Postharvest Splitting and Pitting

Chelan, Bing, Skeena, Lapins, Regina, and Sweetheart were harvested at commercial maturity from orchards in The Dalles, Hood River, and Parkdale, OR. Water uptake rate of each variety was

determined by immersing fruit in distilled water for 3 h. Fruit were weighed periodically and splitting was evaluated after 2 and 3 h. Bing and Skeena fruit were immersed in solutions with GRAS compounds and pH adjustment for 2 hrs. Fruit splitting, moisture gained, and pectin compounds released into water were evaluated. Experimental units were 50 fruit and there were three replications per treatment at each evaluation period. Pitting was induced using the device designed by the Pacific Agri-Food Research Center, Summerland B.C. with modified procedures to simulate pitting occurring at commercial harvesting and on-line packing.

RESULTS AND DISCUSSION

Respiration Dynamic

Respiration rates [CO_2 production (R_{CO2}) and O_2 consumption (R_{O2}) rates] of sweet cherries were inhibited linearly by reduced O_2 from 21% to 3% at 68°F, however, only from ~10% to 1% at 32°F. O_2 from 21% to ~10% did not affect R_{CO2} and R_{O2} of sweet cherries significantly at 32 °F (Fig. 1). **Under shipping conditions, as a consequence, the gas permeability of MAP has to be modified to reducing O_2 under ~10% within the package to inhibit cherry fruit respiration activity to maintain fruit quality (flavor).** Elevated CO_2 (0-15%) did not inhibit respiration rates of Bing and Sweetheart significantly at 32°F and 68°F. Interestingly, CO_2 concentrations between 5-15% reduced respiration rate of Regina at both 32°F and 68°F (Fig. 1).

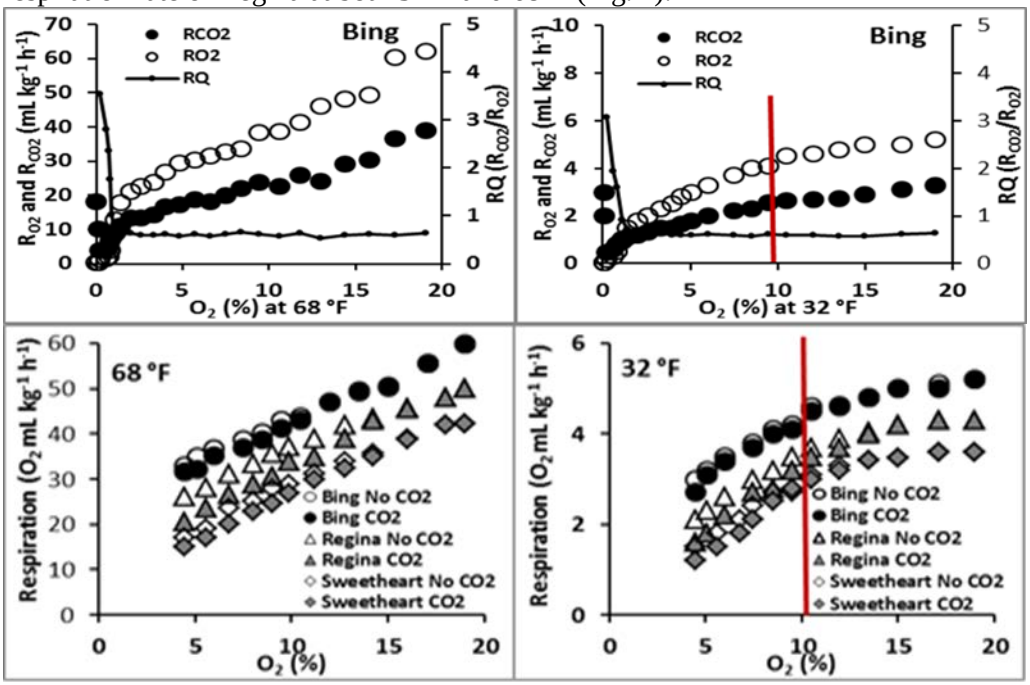

Fig. 1. Respiration dynamic of sweet cherry affected by variety, temperature, O_2 and CO_2.

MAP Technologies

1. Gas analysis in MAP bags containing Bing and Sweetheart at 32°F for 6 weeks.

The concentrations of O_2 and CO_2 in each of the 5 MAPs reached equilibrium by the second week at 32°F and were maintained throughout the remaining 5 weeks of simulated shipping period for Bing and Sweetheart (Fig. 2). **The 5 MAPs generated varied equilibrium O_2 and CO_2 concentrations for each variety. O_2 ranged from 2-13% for Bing and 2 to 15% for Sweetheart. CO_2 ranged from 7 to 13% for Bing and 5 to 10% for Sweetheart.** There was no accumulation of CO_2 or reduction of O_2 in the macro-perforated plastic bags (control) compared to environment.

The equilibrium O_2 and CO_2 for each of the MAPs for Bing were: MAP1 (12.5%, 7%), MAP2 (11%, 13%), MAP3 (10%, 7.5%), MAP4 (7%, 8%), MAP5 (2%, 10%); Sweetheart: MAP1 (14.5%, 5%), MAP2 (12%, 9%), MAP3 (11%, 6.5%), MAP4 (8%, 7%), MAP5 (2%, 8%).

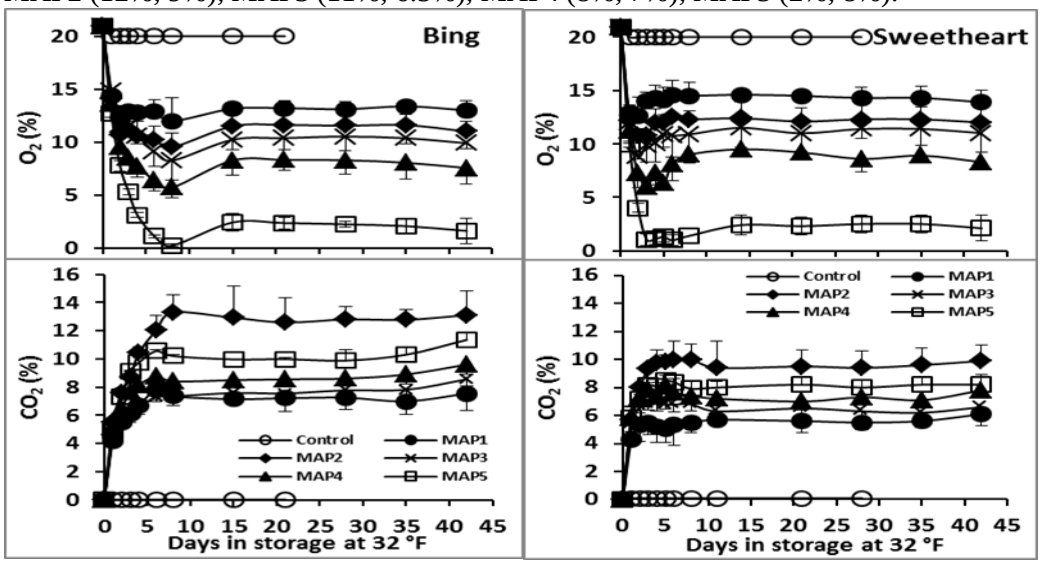

Fig. 2. O_2 and CO_2 concentrations in different MAP bags containing Bing and Sweetheart fruit at 32°F.

2. Effect of MAP on fruit respiration activity of Bing and Sweetheart fruit after 6 weeks at 32°F.
Sweetheart fruit had lower respiration rate than Bing for each particular MAP treatment and control fruit at 32°F. For each variety, fruit packed in MAP5 with O_2 at 2% had the lowest respiration rate followed by that in MAP4 with O_2 at 7-8% and MAP1-3 with O_2 at 10-15%. MAP1-3 did not reduce respiration rates of Bing and Sweetheart fruit at statistically significant level ($p \leq 0.05$) compared to the macro-perforated polyethylene bags (control).

3. Effect of MAP on fruit quality of Bing and Sweetheart at 32°F for 4 and 6 weeks.
MAPs had similar effects on quality of Bing with that of Sweetheart, therefore, only data of Bing are presented in this report. Compared to control, all MAPs did not influence the incidences of pitting and splitting after 4 and 6 weeks at 32°F. All MAPs reduced decay incidence, but there was no statistical difference ($p \leq .0.05$) among the MAPs. Compared to the control, all MAPs reduced the incidence of stem browning numerically, but **only MAP4 and MAP5 reduced the incidence of stem browning at statistically significant level** ($p \leq 0.05$) (Fig. 3).

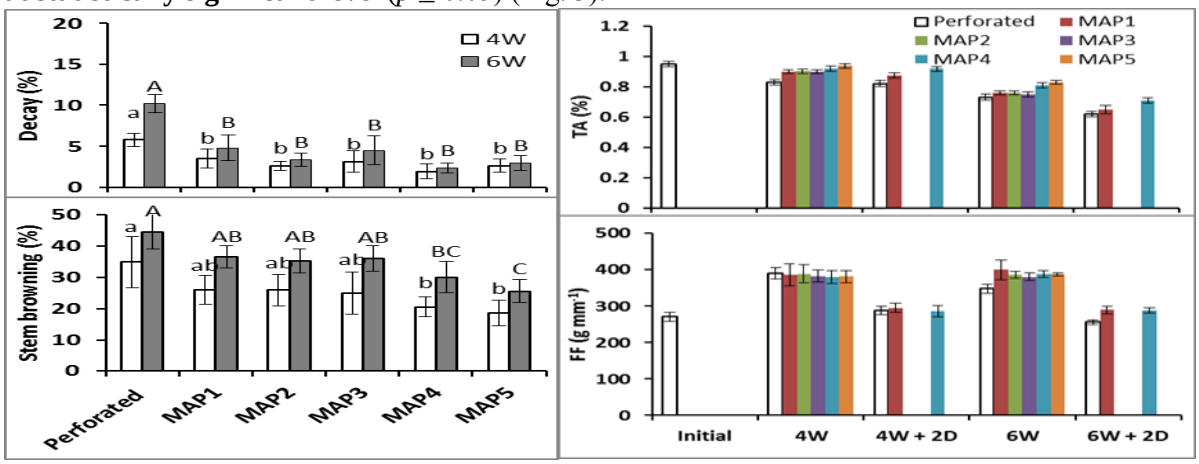

Fig. 3. Effect of different MAPs on fruit quality of Bing at 32°F for 4 and 6 weeks.

Compared to the control, all the MAPs slowed down the rate of TA loss after 4 weeks, but **only MAP4 and MAP5 maintained higher TA after 6 weeks at 32°F. MAP5 maintained higher TA than MAP1-3 after 4 weeks and both MAP4 and MAP5 maintained higher TA than MAP1-3 after 6 weeks at 32°F. Panelists gave higher sensory scores to fruit packed in MAP4 and MAP5 than MAP1-3 after 4 and 6 weeks**.

Cold storage increased fruit firmness (FF) dramatically in control and MAPs, i.e., ~30% after 4 and 6 weeks at 32°F. FF reduced back to the initial values at harvest after 4 and 6 weeks plus 2 days at 68°F. There was no difference of FF between control and MAPs after 4 weeks at 32°F, but MAPs maintained higher FF than control after 6 weeks at 32°F. There was no difference of FF among the 5 MAPs after 4 and 6 weeks at 32°F (Fig. 3).

4. Effect of MAP on anthocyanin synthesis of Bing at 32°F for 4 and 6 weeks.
Anthocyanin in fruit peel continued to synthesize, therefore cherry fruit color was becoming darker during storage at 32°F. All MAPs slowed down the increasing rate of anthocyanin concentration in fruit. **Fruit packaged in MAP2 with CO_2 at 13% contained the least concentration of anthocyanin compared to other MAPs with CO_2 less than 10% after 6 weeks at 32°F** (Fig.4).

5. Effect of MAP on bitter taste development of Regina and Lapins.
A bitter taste was developed in Regina and Lapins of control after 4 and 6 weeks at 32°F. **MAPs with O_2 at 2% and 8% prevented or reduced Regina and Lapins from developing the bitter taste.**

6. Effect of temperature fluctuation on gas composition in MAP bags with Skeena.
Skeena has a higher low-O_2-threshold for anaerobic respiration compared to other late season varieties. The respiration quotient (RQ) of Skeena at 41°F started to increase at ~3% O_2. O_2 in MAP2 and MAP3-A reduced to lower than 3% when temperature increased to 41°F from 32°F for 1 day. MAP1 and MAP3-B with higher gas permeability could maintain O_2 high than 5% at 41°F (Fig. 5). **Skeena may need MAPs with higher gas permeability to prevent anaerobic respiration for long-distance shipping.**

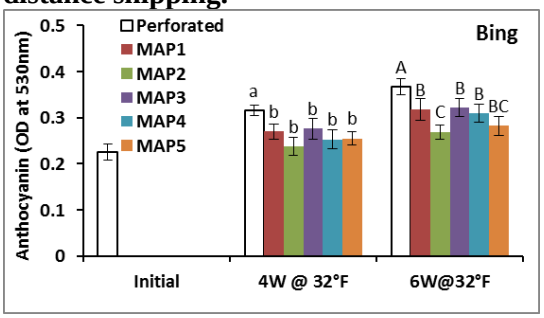

Fig. 4. Effect of different MAPs on anthocyanin.

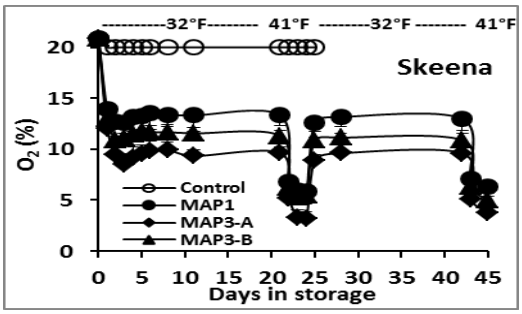

Fig.5. O_2 concentrations in MAPs at 32 °F and 41°F.

7. Effect of the ways to closing MAP liner on gas composition.
The equilibrium O_2 and CO_2 in MAP5 (cv. Lapins) closed by twist-and-tie were 3% and 8-9%, respectively. Closure the liners by folding gave average O_2 and CO_2 concentrations at ~12% and ~4%, respectively, with extremely high standard deviations. **The fruit quality varied in MAP5 closed by folding due to the variation of gas compositions.** Therefore, closing MAP by folding is not recommended.

Consumer Packaging
1. Effect of perforation ratio on RH within Zipper-Lock consumer bags with Chelan fruit.
The perforation ratio of commercial zipper-lock bags were ranged from 2-5% (hole size 8mm). The RH within zipper-lock bags with perforation of 2% were 96%, 93%, and 91% at environment

temperatures being 32°F (RH 88%), 50°F (RH 75%), and 68°F (RH 65%), respectively. The RH within zipper-lock bags with perforation of 0.5% (hole size of ~3mm) were 99%, 98%, and 96% at 32°F, 50°F, and 68°F, respectively. RH within the bags with perforation of 0.05% was close to 100% at each of the temperatures tested.

2. Effect of zipper-lock bags with different perforation ratios on stem and fruit quality of Chelan. Chelan fruit packed in zipper-lock bag with perforation at 2% lost moisture significantly higher than in bags with perforation at 0.5% and 0.05% ($p \leq 0.05$) after the simulated marketing period. Fruit pitting incidence was higher and FF was lower for fruit packed in the bags with perforation at 2% than that packed in bags with peroration at 0.5% and 0.05% after the marketing period. Stem showed a much higher moisture loss rate compared to fruit at the same conditions. Stem moisture loss was higher in bags with 2% perforation than 0.5% and 0.05% at each of the simulated marketing stages. **Stem visual quality was higher in bags with perforation at 0.5% than at 2% after 1 week at 32°F + 2 days at 50°F + 2 days at 68°F.** Bags with perforation at 0.05% had higher condensation and higher decay incidence than bags with perforations at 0.5% and 2% after the simulated marketing periods.

Postharvest Splitting (PS) and pitting

1. Varieties varied significantly on susceptibility to PS.

Skeena, Bing, Chelan and Sweetheart showed higher water uptake rates and more susceptible to PS than Regina and Lapins.

2. GRAS compounds and high pH solutions reduced PS.

Calcium chloride, bicarbonate, and a buffer solution at pH=8 reduced, but carbonate, potassium meta-bisulfite, EDTA and a buffer solution with pH=4 increased PS incidence of Bing and Skeena. $MgCl_2$ at 0.2% did not affect PS incidence. The PS incidence of Skeena was lower in calcium chloride solution at 2% than at 0.2% (Fig. 6).

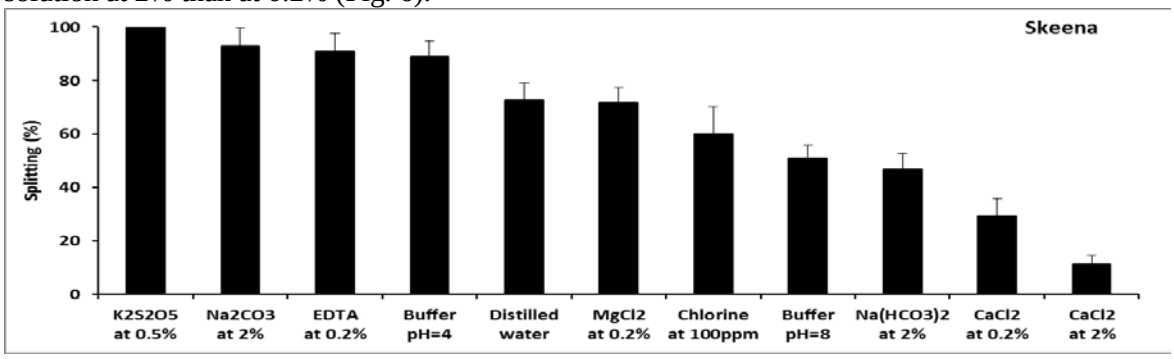

Fig. 6. Effect of different compounds in solutions on PS of Skeena at room temperature.

3. Calcium chloride reduced pectin compounds releasing from fruit to solution.

Calcium chloride at 0.2% and 2% reduced the content of pectin compounds in solutions. Calcium in solution did not affect water uptake of Bing and Skeena. It is possible that calcium reduced PS through inhibiting pectin compounds releasing from fruit into solutions.

4. Fruit pulp temperature and moisture status affected fruit susceptibility to pitting.

Fruit pulp temperature at the time of impact damage influenced pitting susceptibility of Lapins and sweetheart. Fruit at box filling after the second hydro-cooling on commercial packing line were extremely sensitive to pitting damage. Reducing drop height or providing cushion to the fruit during box filling reduced pitting significantly. An overnight air-cooling (24h) with fruit moisture loss of 0.5% reduced Lapins fruit pitting susceptibility significantly than fruit hydro-cooled immediately after harvest. The aircooling for 24h reduced stem quality after a simulated marketing period at a minimum level. Hydro-cooling increased pitting susceptibility was not related to water uptake.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: CH 12-105

Project Title: Spotted wing drosophila management on sweet cherry

PI: Elizabeth H. Beers
Organization: WSU-TFREC
Telephone: 509-663-8181 x234
Email: ebeers@wsu.edu
Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

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

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

Other funding sources

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

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

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

WTFRC Collaborative Expenses: None

Budget 1
Organization Name: **WSU-TFREC** **Contract Administrator:** Kevin Larson; Joan Root
Telephone: 509-663-8181 x221; 509-335-2885 **Email address:** kevin_larson@wsu.edu; rootj@wsu.edu

Item	2012	2013	2014
Salaries[1]	12,000	12,480	12,979
Benefits[2]	4,829	5,022	5,223
Wages[3]	15,925	15,925	15,925
Benefits[4]	12,199	12,199	12,199
Equipment	0	0	0
Supplies[5]	2,395	1,722	1,022
Travel[6]	2,652	2,652	2,652
Total	50,000	**50,000**	50,000

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

Objectives

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

Significant Findings

- Regional emergence of SWD in 2012 was earlier than in 2011, more similar to the pattern seen in 2010.
- Numbers found in traps to date are also more similar to the 2010 levels,
- Two cherry cultivars ('Bing' and 'Sweetheart') are fully susceptible to SWD 21 days before harvest, with no discernible increase/decrease through postharvest,
- Trap color, surface area of the bait, and location of entry point (top v sides) did not affect capture. A new bait, Monterey Ag Bait, appears to be more attractive and selective than apple cider vinegar.
- Warrior (single application) provides excellent mortality and fruit protection immediately after application; both decline in 14 days (the PHI); Entrust at weekly intervals also provides high levels of mortality, but fruit protection is not as good.
- Low rates of Entrust combined with a bait applied with an ATV provided similar levels of mortality and fruit protection as the full rate of Entrust applied airblast,
- Diazinon and Warrior had the longest residual control of SWD on foliage,

Methods

1. *Provide a crop protection alert system to cherry/stone fruit producers and seasonal phenology information through a regional SWD trapping program.* SWD populations were monitored in eastern Washington from May through frost with traps placed strategically in fruit growing areas from the Canadian border to the Tri-Cities. Cherry orchards were the sites of most of the traps, however, other stone and pome fruit crops will be monitored, as well as other cane/vine crops. Traps will be deployed in April, and checked weekly from May 1 through frost. The trap capture database will be made available on the WSU-SWD website, along with an alert system. The alert system will notify viewers of the first catch in each region, which triggers crop protection measures for vulnerable crops in that region. Growers and consultants can track their own traps via a customized list, and create custom graphs of either their own traps, or look at trapping data on a regional/crop/regime basis.

2. *Determine timing of cherry fruit susceptibility in the field.* Field-grown cherries were sleeve caged 21 days before harvest to ensure that ambient infestation did not interfere with the bioassays. The cultivars tested were 'Bing' (CV-14) and 'Sweetheart' (Sunrise-4). Cherries were collected from the field on 21, 14, 7, days before harvest, day of harvest, and harvest+7 day for bioassay with lab-reared flies. Three cherries were suspended from the lid of a 16-oz deli cup, and placed inside a cherry leaf-lined cup. Five adult females were added to the cup and allowed

to oviposit for 16 h. Cups were provisioned with 2.5 ml drosophila medium and a vial of 50% honey-water. Cherries were removed from the arena after 16 h, and the number of oviposition stings was counted. After counting, the cherries were held at 22 C for 21 d to allow development and emergence of adult flies. Fruit maturity indices (firmness, size, weight, color, brix, titratable acidity, pH) were taken on each bioassay date on a sample of 150 fruit.

3. *Test trap types for capture efficiency of SWD (in collaboration with SCRI-SWD regional group).* Ten trap types in three comparison groups were evaluated in 2012. The first group was comprised of 500 ml Solo cups in 5 colors (black, white, red, yellow, and clear). The second group tested a standard Haviland trap, and a Haviland trap modified so that the surface area of the bait was reduced. The third group tested the orientation of the entry point (top vs. side, with lid), with an additional side-entry trap (without lid).

   In addition to the three groups tested by SCRI collaborators, I tested two other groups. The first was two dry lures, one with a floral scent, the other with ripening fruit odor, compared with ACV. These were tested in 1-qt jars with 10 3/16th in. holes in the sides; the dry lures use water with unscented dishwashing soap as a surfactant. The second group compared a commercial bait adjuvant (Monterey Ag bait) with ACV in a standard Haviland trap.

4. *Test pesticide efficacy for control of SWD in cherries in laboratory, field-laboratory, and field settings.*

   Laboratory tests: I tested registered and unregistered pesticides for efficacy via contact and ingestion in laboratory bioassays. The first series tested numbered formulations of two organic pesticides. They were tested by contact, contact+ingestion, ingestion, and contact with residues in a 50 ml plastic portion cup. All sprays were applied with a Potter Spray Tower operating at 6.5 psi (2 ml/arena). Mortality was evaluated after 48 h.

   I also tested a soap formulation (Des-X) that is registered for use in organic cherries as an adjuvant for Entrust. All sprays were applied contact as described above, and mortality evaluated after 48 h. In addition, I compared two formulations of Entrust (SC and WP) in anticipation to the switch to the SC formulation.

   Field test #1(replicated, conventional pesticides): A medium-plot field study was conducted in the 'Sweetheart' block of the Sunrise orchard. Plots were three rows x four trees, with four treatments and four replications. The treatments were three applications of Entrust (the SC and the WP formulation at 17, 10, and 3 days before harvest or a single application of Warrior 14 days before harvest. All treatments were applied airblast at 100 gpa. Laboratory-reared SWD were released weekly in the block to increase pest pressure. Fruit samples were taken the day prior to each pesticide application to determine infestation. Fruit were held for 21 day at 22 C, at which time emerged adult SWD were counted.

   Field test #2 (unreplicated, bait spray): A 1.5 acre 'Bing' cherry block (CV-14) was divided into three subequal sections. Each plot received one of three treatments: Entrust SC applied airblast at 8 fl oz/acre; Entrust SC 1 fl oz/acre+1 gallon of Monterey Ag Bait applied with a 6-nozzle ATV sprayer; and an untreated check. Each treated plot received a weekly application at 17, 10, and 3 days before harvest. SWD releases and fruit samples were taken as described above.

   Field-aged residue bioassays, medium plot study: Leaves and fruit treated and aged in the field were taken from Field-Test #1 to determine if residues could kill flies and protect fruit from damage as a supplement to the information on attack and damage in the field. Leaves and fruit were collected from the treated plots the day before a pesticide application. Leaves were used to line the deli cup arena, and fruit were suspended from the lid as described under the fruit susceptibility timing test. Fruit were removed after 16 h, ovipositions counted, and held for 21 days for flies to emerge. Mortality was evaluated after 48 h.

Field-aged residue bioassay, unreplicated bait spray study: Bioassays of fruit and leaves were taken in the same manner as for the medium-plot study.

Field-aged residue bioassay, single tree plots. A field-aged residue bioassay was conducted in the 'Sweetheart' orchard at Sunrise in the eastern rows of the block not in use in the replicated field study. Treatments were applied airblast (100 gpa) to single tree reps on 9 July, and sampled at 1, 7, and 14 DAT. Treatments included Sevin 4F (3 and 1 qt/100 gal), Fyfanon (1.75 pt), and PermaGuard, a diatomaceous earth product. An additional series of bioassays was started during the postharvest period in the check plot of the 'Bing' orchard at Columbia View to explore the possibility of requesting an extension on the use of azinphosmethyl on cherries, prompted by the control failures experienced in 2012. The test included most of the pesticides likely to be used by eastern Washington cherry growers (Guthion, Diazinon, Warrior, Entrust, Delegate, Sevin, and Malathion). Applications were made to single-tree plots by handgun (to drip). Fruit was no longer available, so bioassays used leaves and mortality evaluations only.

Results and Discussion

1. *SWD trapping program*. A total of 334 traps (Beers+Walsh) were deployed and checked weekly from Jan. 1 through present. The majority of the traps (57%) were in sweet cherry orchards, followed by blueberry (14%), grape (11%), peach (6%), caneberries (5%), nectarine (4%), apricot (2%), apple (1%), and feral hosts (1%).

First capture in 17 cherry-growing regions was earlier in 2012 than in 2011, with 71% of regions positive before 1 August (vs. 25% in 2011). Technically, the first capture of 2012 was in January, but for the growing season, the first capture was 30 April in Prosser. The spread over time of first captures was much greater than in previous years, with the final first capture in Entiat on 27 September. The greater spread may reflect lower pressure, or lower trapping density overall.

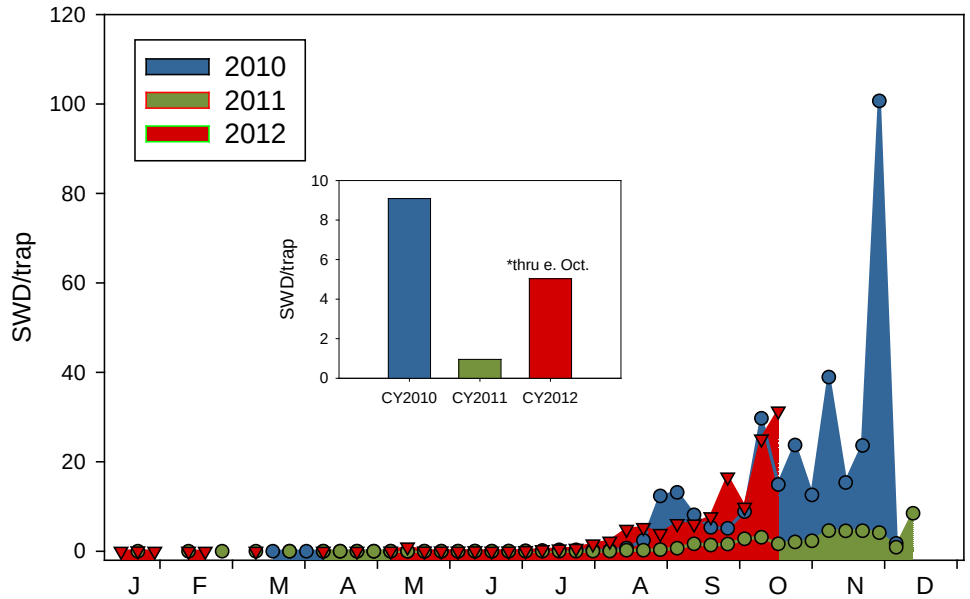

Fig. 1. SWD trap captures in eastern WA, 2010-2012

The pattern of trap captures was similar to the two previous years of trapping, with low numbers through mid-July, and increasing steadily thereafter (Fig. 1). At the date of this writing, the trap captures are intermediate between 2010 and 2011, however, the period of highest trap catches in

previous years (late October-early November) is not included. It should be noted in addition that the trap type in the Beers program has changed from one year to the next, and cannot be compared directly; however, the Walsh program data reflect capture in Nalgene bottles in all three years. While it will take several more years of trapping to establish a pattern definitively, 2012 is likely a representative year.

The SWD website was available to track first captures over the region; information was given in tabular and map formats. Alerts were posted within 24 h of the recorded capture, and new records were highlighted in a blog and sent out through the SWD mailing list.

2. *Timing of cherry fruit susceptibility*. Both 'Bing' and 'Sweetheart' cherries were fully susceptible to attack by SWD at 21 days before harvest (firmness 364 g/mm, 12.7% brix; firmness 516 g/mm, 14.2% brix for 'Bing' and 'Sweetheart', respectively). The rate of attack fluctuated throughout the period from Harvest-21 d to Harvest+7 day, but not in a consistent manner (Fig. 2). Although not significantly different, the emergence success was lower earlier in the season, and with the exception of the day of harvest sample, was higher as the fruit matured.

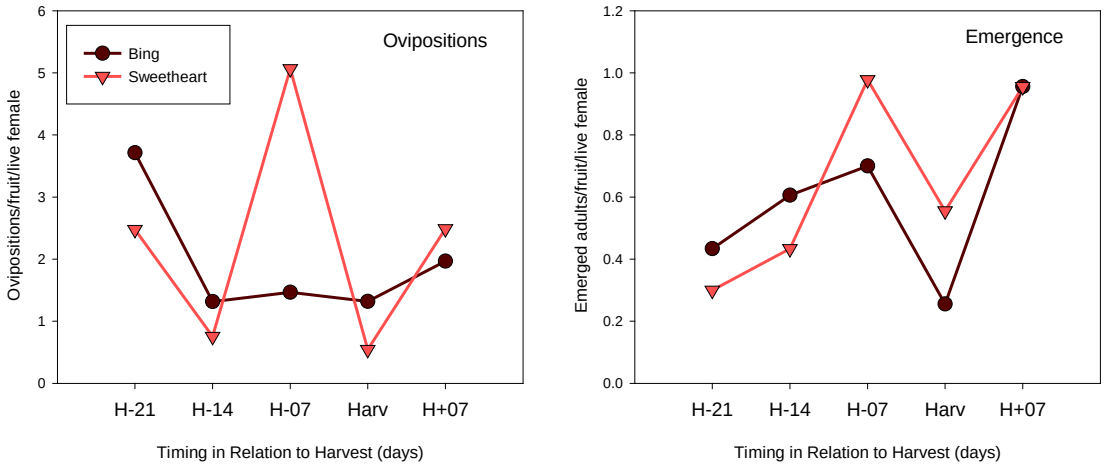

Fig. 2. Fruit susceptibility to SWD in relation to harvest maturity, 2012

3. Trap types. Trap color (red, yellow, black, white, clear) did not influence the total or proportion of SWD females captured. There was a slight, but significant trend for the black cup to be more selective for SWD than the clear cup. The location of the entrance (top vs. side, with or without rain cover) did not influence the total capture of SWD. These traps were slightly less selective than the other types. A 1-qt screw top jar caught ca. 2x as many total SWD as the BioBest or Captiva traps. The Scentry floral lure and benzaldehyde lure caught relatively few SWD in comparison to the standard ACV. The sex ratios in the SWD captures by the floral lure were skewed toward females, but this may have been an artifact of the low numbers. A 1:5 dilution of Monterey Ag Bait in water caught 3x as many SWD than ACV, however, this difference was not significant due to the low numbers of replications (3) (Fig. 3). Monterey Ag Bait was also significantly more selective for SWD than ACV, with 2.8x higher proportion of SWD compared to other *Drosophila* species. However, selectivity continues to be an issue with these traps; generally about 3-7% of the *Drosophila* captured are SWD (18% for Monterey Ag Bait). This bait represents a potential replacement for ACV; it is commercially available, does not contain alcohol, and does not have the same odor problems as the yeast baits. Although more turbid than ACV, specimens can be readily seen with the addition of water.

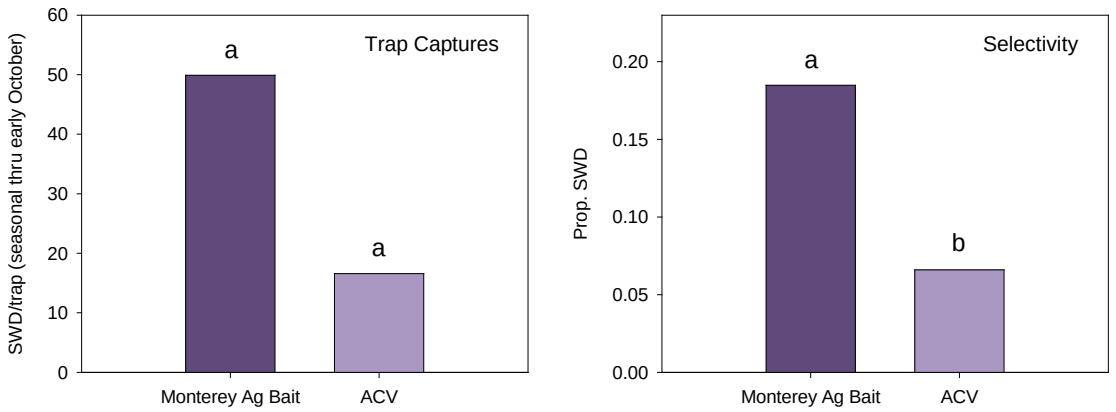

Fig. 3. Trap captures and selectivity of Monterey Ag Bait vs ACV in a standard Haviland trap

4. *Chemical control*.

Bioassays. There was a low level of mortality in the MBI-203 and MBI-206 (two organic pesticides derived from soil bacteria) in the contact, residual, and contact plus residual bioassays, never exceeding 23%. The materials tended to be more toxic by ingestion, with 10-27% mortality at 48 h. However, the toxicity was consistently less than the standard, spinosad, regardless of exposure route. Both the 80W and 2SC formulations of Entrust provided 100% control at both rates tested (2.5 and 1 oz of the 80W; 8 and 3.2 fl oz of the 2SC). The high rate of Sevin 4F (3 qt/100 gal) caused more mortality than the low rate (1 qt/100); the latter was only 70%, significantly lower than the low rates of Entrust. Entrust SC combined with DES-X soap caused 100% mortality at 48 h regardless of the rate of either the AI or the adjuvant; typically the low rate of Entrust (2.66 fl oz of the SC) would allow some survival.

Field-aged residue bioassays (Sunrise, airblast): By one day after treatment, Sevin 4F (3 qt and 1 qt) caused high levels of mortality, as did Fyfanon (1.75 pt); only PermaGuard did not provide significant mortality at this or any other post-treatment interval. By 7 DAT, mortality in the Sevin treatments had dropped to 52 and 36% for the high and low rates, respectively; mortality in the Fyfanon treatment was zero at this time and thereafter. By 14 DAT, none of the treatments was different than the untreated check. *(Columbia View, handgun*): Rates were calculated on a 100 gpa basis, but were applied by handgun (gpa unknown); thus the amounts/acre applied were potentially much higher than would be normal on mature trees, although within the range of what might be applied to young trees. All treatment caused ca. 100% mortality at 1 DAT. By 7 DAT, mortality was the same in all treatments with the exception of Delegate, which caused significantly lower mortality (93%) than the other treatments. By 14 DAT, both the Fyfanon (65%) and Delegate (82%) treatments were lower than the others. By 21 and in subsequent bioassays, only Diazinon and Warrior continued to cause levels of mortality near 100%; these two treatments and the check continued to be sampled as of this writing (mid-October).

Field trial #1, replicated, conventional insecticides. Despite the release of laboratory-reared flies in the block, field infestation in the checks was negligible. Fruit in the checks were highly susceptible from the first bioassay date (15 DBH), with no consistent trend in susceptibility. The decline in activity of single application (14 DBH) of Warrior during the test was the most consistent trend; after 10 days, mortality was not different than the check. In general, mortality remained higher in the two Entrust treatments applied three times at 7-day intervals (Fig. 4).

Field trial #2, unreplicated bait spray test: As with the conventional insecticide trial, releases of flies did not produce a useful level of insect pressure in the field; all meaningful data are from the corresponding bioassays. Mortality was close to 100% immediately prior to the 2nd and 3rd applications; by 7 days after harvest, however, mortality in the bait treatment was significantly lower (62%) than in the Entrust/airblast treatment (Fig. 5). Both treatments suppressed fruit damage (ovipositions/fruit) to about the same extent on all bioassay dates; however, there was slightly higher emergence of flies from the fruit in the bait plot. However, differences among treatments were not statistically significant in the latter two measurements on any bioassay date. These data are a preliminary indication that ATV-applied bait sprays with low rates of Entrust may be equivalent in efficacy to higher rates applied airblast.

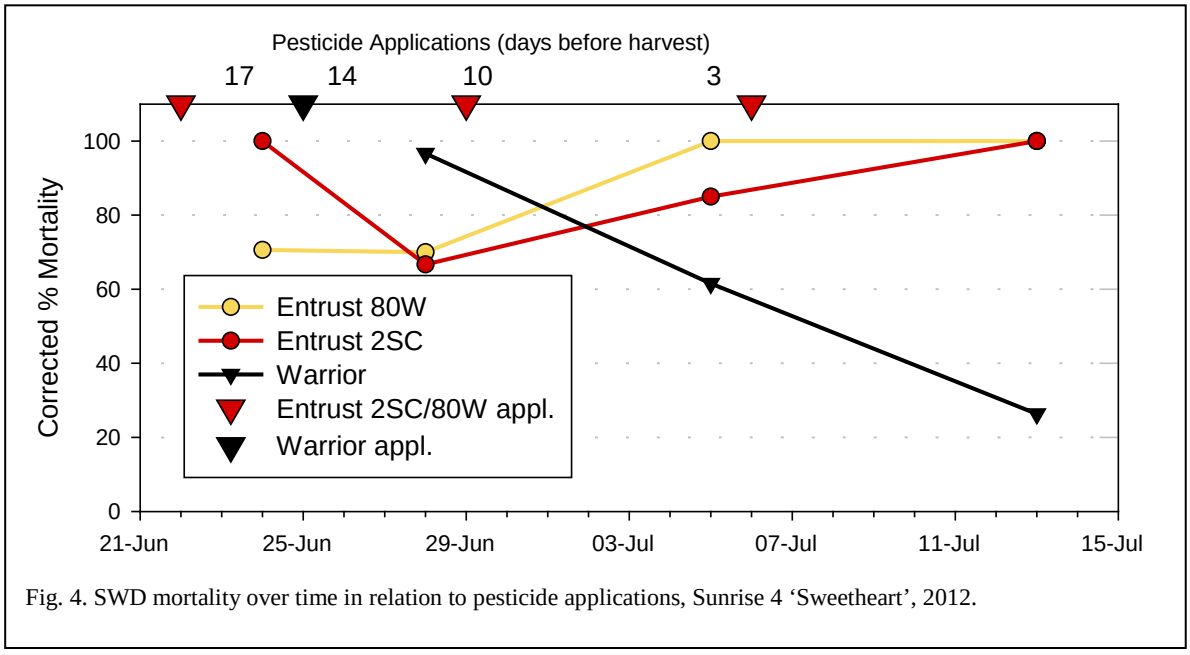

Fig. 4. SWD mortality over time in relation to pesticide applications, Sunrise 4 'Sweetheart', 2012.

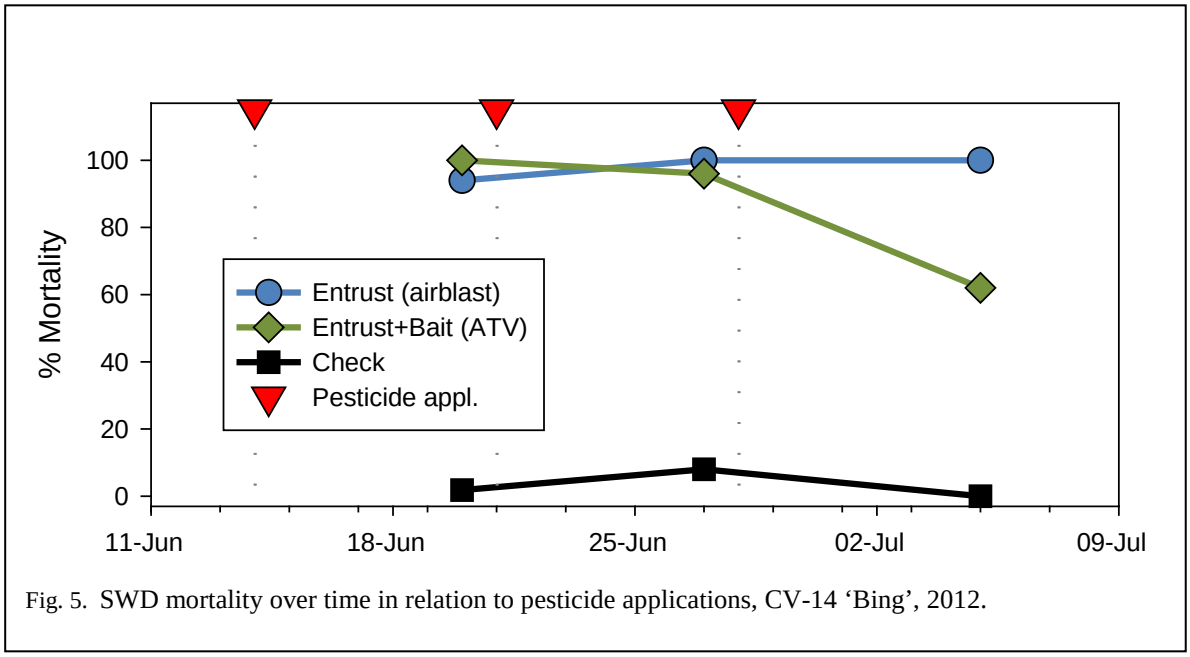

Fig. 5. SWD mortality over time in relation to pesticide applications, CV-14 'Bing', 2012.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: CH-12-106A

Project Title: Identification of chemical lure for spotted wing drosophila

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Cooperators: None
Total Project Request: Year 1: $35,000 **Year 2: $34,000** **Year 3:** $32,000
Other funding sources: None

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

Item	2012	2013	2014
Salaries			
Benefits			
Wages	10,100	10,100	10,100
Benefits	900	900	900
Equipment			
Supplies	5,500	4,500	2,500
Travel	1,000	1,000	1,000
Plot Fees			
Miscellaneous			
Total	17,500	**16,500**	14,500

Footnotes:

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

Item	2012	2013	2014
Salaries			
Benefits			
Wages	10,200	10,200	10,200
Benefits	6,800	6,800	6,800
Equipment			
Supplies	500	500	500
Travel	500	500	500
Plot Fees			
Miscellaneous			
Total	17,500	**17,500**	17,500

Footnotes:

OBJECTIVES

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

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

SIGNIFICANT FINDINGS

1. 13 wine and vinegar chemicals elicited fly antennal responses.
2. A five component chemical blend was as attractive to SWD as the starting material of a mix of wine and vinegar, in field tests.
3. The chemical lure attracts fewer non-target insects than the combination of wine and vinegar.

METHODS

2012:

Volatile or headspace samples from Merlot wine and rice vinegar were analyzed by GC-EAD, using antennae of spotted wing drosophila as the detector. EAD active compounds were identified by GC-MS, and all EAD active compounds were purchased for confirmation of their identity and use in subsequent bioassays.

A laboratory bioassay was used to determine attraction or repulsion of individual EAD-active chemicals.

Combinations of EAD-active chemicals were tested in traps in the field to determine their importance to fly attraction to the wine and vinegar. The Agrisense dome trap was used, and traps were placed along roadsides, and outside of berry fields. This included the following five field experiments:

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

2013:

Laboratory work will include developing controlled release information for optimization of a lure. We will determine release rates of the attractive chemicals from formulations and dispensers, and set up formulations and dispensers with known release characteristics for field assays. Measurements of release rates will be made by a combination of three methods. Loaded dispensers will be weighted initially and at intervals of time to determine weight loss as an indicator of attractant emission. For measurements of emission of mixed chemicals or blends, volatile collections will be made and analyzed to determine quantities of chemicals emitted. This technique is time consuming and relatively expensive, so it will be used at intervals in combination with the weight loss measurements. Formulation/dispenser methods will include polyethylene sachets, vials, and incorporation of chemicals into the drowning solution of wet traps. There is a potential to involve an industry partner during this time frame, to begin development of a commercially viable lure. In that

case, such an industry partner might have a preferred approach or method of formulating or dispensing the attractive chemicals.

Much of the field work will involve the testing of release rates, ratios, and formulation methods to optimize the fly attraction to the final blend of chemicals. This will be comparisons of blend release rates to determine a best release rate, comparisons of chemical ratios, and field validation of formulation methods developed in the lab. As described in the original proposal, this will include formulation of the chemicals in the drowning solution of a wet trap and the development of a dispenser for use in a dry trap. Throughout these tests, we will use the combination of wine and vinegar as a positive control or standard for comparison.

Preliminary questions regarding the use of the identified attractive chemicals will be dealt with experimentally depending on time and fly populations. We will evaluate the captures of non-target insects in traps baited with the chemical lure, compared to wine plus vinegar to determine if the numbers of non-target insects are reduced with the chemical lure, as expected. Such a comparison might need to be done at multiple locations and times of the year because the diversity of non-target insects varies with the season and on a landscape level. In 2013 we will begin work with other researchers to determine how this lure performs in comparison to the trap and bait in general use in 2012. This preliminary work will involve a) comparisons of trap power in luring SWD, b) comparisons of first detections for the season, and c) comparisons of non-target insect numbers.

RESULTS AND DISCUSSION

Thirteen wine and vinegar chemicals, in addition to ethanol and acetic acid, elicited consistent fly antennal responses. A combination of these chemicals tested in the field was not as attractive as the starting material of wine and vinegar, and indeed was quite weak. This problem was anticipated however, because the GC-EAD assay determines chemicals detected (smelled) by the antennae, and does not indicate chemical attractiveness. There is potential for chemicals to be "smelled" by the fly and be repellent or behaviorally neutral.

A laboratory assay was developed and used to test all of the 13-EAD active chemicals individually to determine attractiveness and repellency of chemicals to SWD when added to the combination of acetic acid and ethanol. Results of this series of assays indicated that seven chemicals reduced the fly response to acetic acid and ethanol, while the six other chemicals either improved the attraction response or were neutral. A second generation chemical blend, consisting of 6 chemicals plus acetic acid and ethanol, was field- tested as a bait for a trap. This blend was improved over the previous blend but was still significantly less attractive than wine plus vinegar.

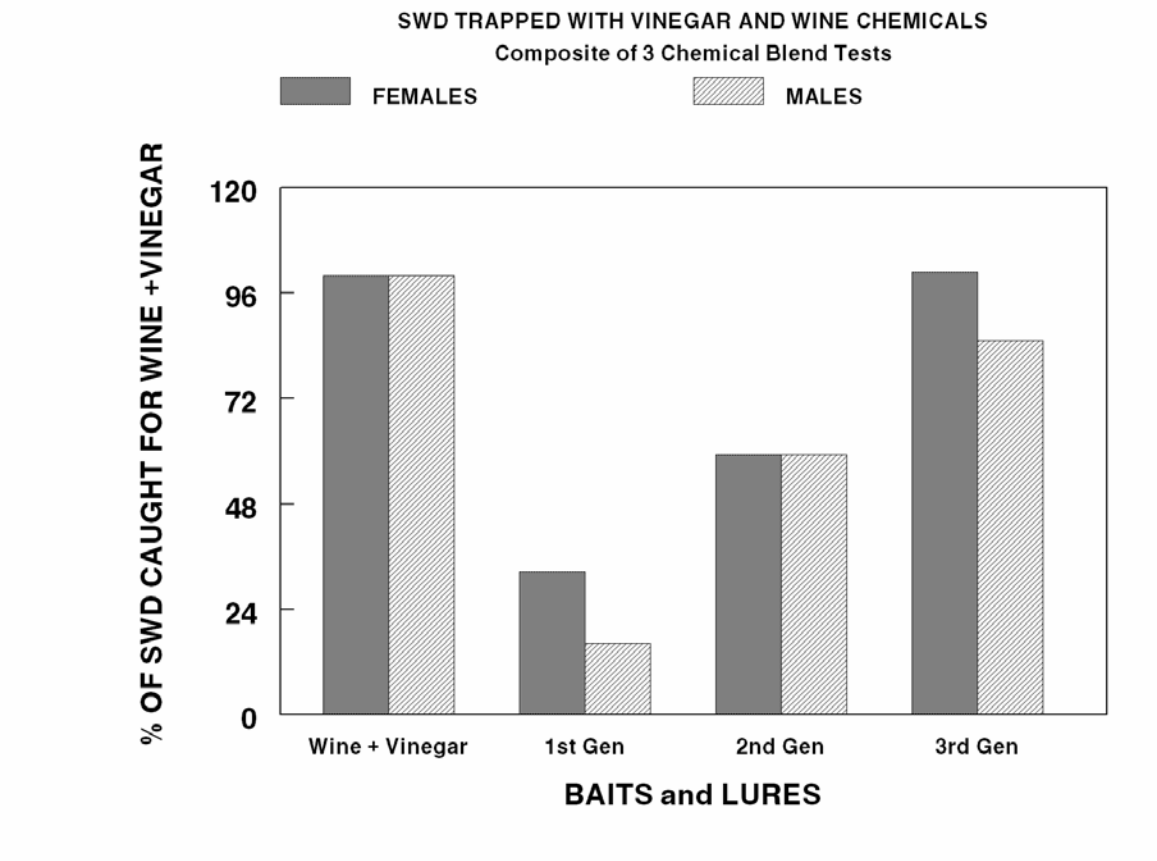
A series of field tests showed that only three of thirteen EAD-active wine and vinegar chemicals significantly improved fly captures in traps when added to acetic acid plus ethanol. A third generation chemical blend then was comprised of acetic acid, ethanol, and those three chemicals that were EAD-active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

These results provide a basis for refining and optimizing a chemical lure for use in detecting SWD. Our previous work showed that the combination of wine and vinegar is much more attractive to the fly than either wine or vinegar. To date, that combination of materials is by far the best bait for SWD. We are quite pleased that the combination of chemicals that we identified is as attractive as that best bait. This result provides a clear opportunity to develop and use a lure that is powerful in luring both sexes, can be formulated to provide even attractiveness for long periods of time, and can be used in a dry trap or a wet trap. All of the active chemicals are commercially available and are relatively inexpensive.

We expect that planned evaluations of the importance of chemical release rates and ratios to lure attractiveness, as well as work with combinations of lures and trap designs will further improve the sensitivity or power of this attractant.

This work is conducted with the purpose of providing a powerful chemical lure for reliable early season detection of SWD for cherry orchards, information that is needed for making sound pest

management decisions. We anticipate that the approach and strategy taken here also will provide a trap/lure combination that is easier to use and more consistent in its attractiveness compared to current monitoring methods in use. The direct practical impact will be to reduce crop damage and losses due to undetected SWD populations, and also to reduce costs of pest control incurred when a fly population is not present.



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

PUBLICATIONS

Cha, D. H., T. Adams, H. Rogg, and P. J. Landolt. Identification and field evaluation of fermentation volatiles from wine and vinegar that mediate attraction of spotted wing drosophila, *Drosophila suzukii*. J. Chem. Ecol. (in press).

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

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