2012 NW Cherry Research Review
Hood River, OR Best Western
Tuesday, November 8, 2011

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FINAL PROJECT REPORT

Project Title: Improving sweet cherry yield security and fruit quality

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Other funding sources

Agency Name: Horticulture Australia Limited (HAL)
Amt. awarded: $231,000
Notes: this funding supported a highly-rated counter-seasonal research project with similar objectives in Australia

Budget 1
Organization Name: WSU **Contract Administrator: Mary Lou Bricker/Lisa Bruce**
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Item	(2010)	**(2011)**	
Salaries	28,914	**35,071**	
Benefits	4,365	**4,539**	
Wages	24,264	**25,235**	
Benefits	3,301	**3,433**	
Equipment	6,500		
Supplies	2,000	**2,000**	
Travel	5,000	**7,500**	
Miscellaneous			
Total	74,344	**77,778**	

Footnotes: Salaries is for Ph.D. student salary and benefits (include health insurance and 1.5% med aid), Research Assistant Allyson Leonhard (0.15 FTE benefits at 36%); Wages are for equivalent of 3 students for the summer months (15.0% benefits) and Ph.D. student summer wages (9.6% benefits); supplies includes EM center microscopy fee and lab consumables. Travel is for domestic, to plots ($5000) and international ($2500 for 1 trip annually to Tasmania).

OBJECTIVES

This research project is a logical evolution from previous research by PI Whiting and other TFRC-funded work that have highlighted the need to achieve yield security, develop precision thinning strategies, and better understand components of fruit size. Towards achieving these goals, this project has the following key objectives:

1. Understand role of environment on fruit set and effective pollination period.
2. Identify the best time to thin.
3. Investigate potential post-bloom thinners.
4. Understand timing of mesocarp cell division & expansion cycles and their relative role in fruit quality.
5. Develop counter-seasonal collaboration with University of Tasmania and leverage WTFRC/OSCC funding via Horticulture Australia Limited.

SIGNIFICANT FINDINGS

- Daily, fruit set varies significantly
- Natural fruit set is low when flowers open during windy, hot conditions
- Pollen germination rate and growth rate don't appear to limit fruit set in cultivars with low productivity
- Short period of ovule longevity appears to limit productivity (fruit set) in Regina, Benton
- Fruit set in Regina, Tieton, Benton can be improved with PGRs applied during bloom
- Fruit quality potential is related to timing of flowering at high crop load
- Fruit quality potential is unrelated to timing of flowering at low crop load
- Fruit quality potential is similar for all buds in a spur
- Fruit quality is highest for single-fruit 'clusters' (i.e., 1 fruit set per floral bud) compared with multiple-fruit 'clusters' (i.e., several fruit set per floral bud)

- The earlier the thinning, the better the fruit quality response
- The benefit of thinning on fruit quality depends on the fruit density – there are benefits from thinning after pit hardening if crop density if high
- Trials with BA, ABA, methyl jasmonate, and NAA showed no efficacy as post-bloom thinners
- Ethephon and PCa + ABA show potential as post-bloom thinners applied 2 – 3 weeks after full bloom

- Counter-seasonal collaboration with University of Tasmania is established – we have project funded by Horticulture Australia Limited for complementary work

RESULTS & DISCUSSION

1. Role of environment on fruit set and effective pollination period

Field studies All previous studies of fruit set have been accomplished by counting flowers on a single day early in bloom, and counting final fruit numbers near harvest – a technique that reveals nothing about the variability in fruit set nor the underlying causes. From tagging individual flowers on the day of opening we were able to study fruit set on a daily basis, throughout the flowering

period. In addition, we have a preliminary dataset for modeling flowering progression with key environmental parameters. Across both years, we documented tremendous variability in fruit set under field conditions in Lapins, Kordia, Van, and Sweetheart throughout the bloom period. For example, fruit set varied from a low of 10% to 100% in Lapins, across the 18-day bloom period (Fig. 1). At this stage we are analyzing variability in fruit set with daily weather conditions to identify patterns and key environmental factors. Preliminary analyses show no relationship between temperature on the day a flower opens and fruit set. Interestingly, fruit set from hand pollinations was similar to that of open pollinated flowers on most days. This suggests there weren't many days when pollinator activity was limiting to fruit set.

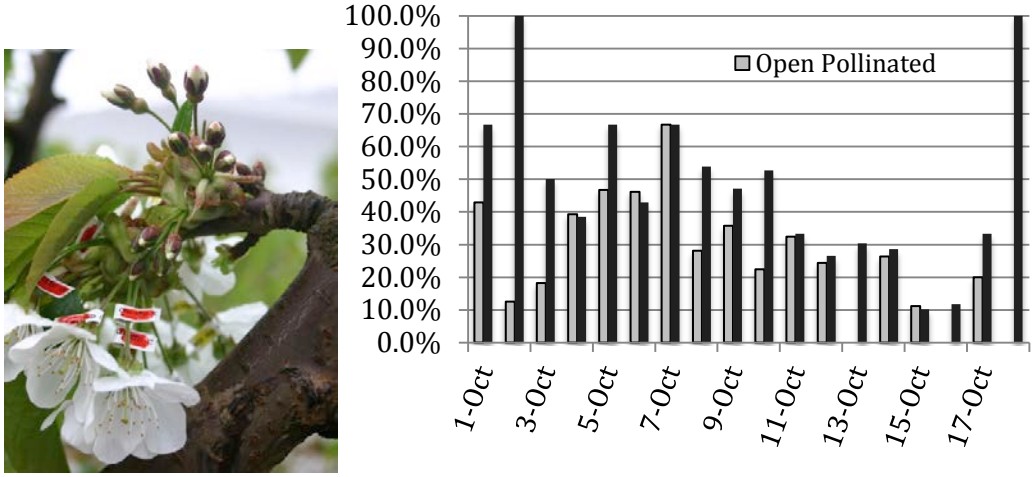

Figure 1. Variability in fruit set (% available flowers) throughout the bloom period in 'Sweetheart' sweet cherry in a commercial orchard in the Huon valley, Tasmania. Individual pedicels were labeled on the day of opening (photo).

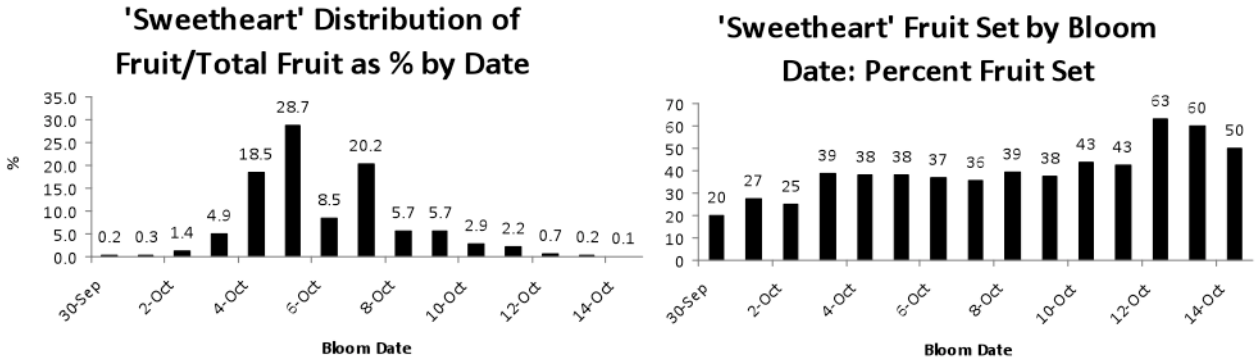

Figure 2. The percent of final fruit count by date of flowering (Left) and the daily variation in fruit set (% available flowers) (Right) for 'Sweetheart' in 2011.

In 2010/2011 flower tagging studies revealed that 'Sweetheart' fruit set was about 37% overall. Daily range in fruit set ranged from about 20 to 63%. Flowering began slowly, peaking on day 6 and declining slowly thereafter (Fig. 2). Nearly 30% of all fruit at harvest were set from flowers that opened on a single day (Fig. 2). If only the flowers that opened on that day were to have set fruit, overall fruit set would have been about 12%, only slightly less than we estimate being a desirable balance for 'Sweetheart'. This flowering pattern was common for most cultivars studied and suggests that a commercial crop may be set within a day or two, if conditions are favorable. We anticipate

developing targeted crop load management strategies with this knowledge combined with data from our pollen tube growth studies (see below). This may include removal of pollinators past peak bloom or application of caustic thinners. Rigorous models of bloom progression and a clear understanding

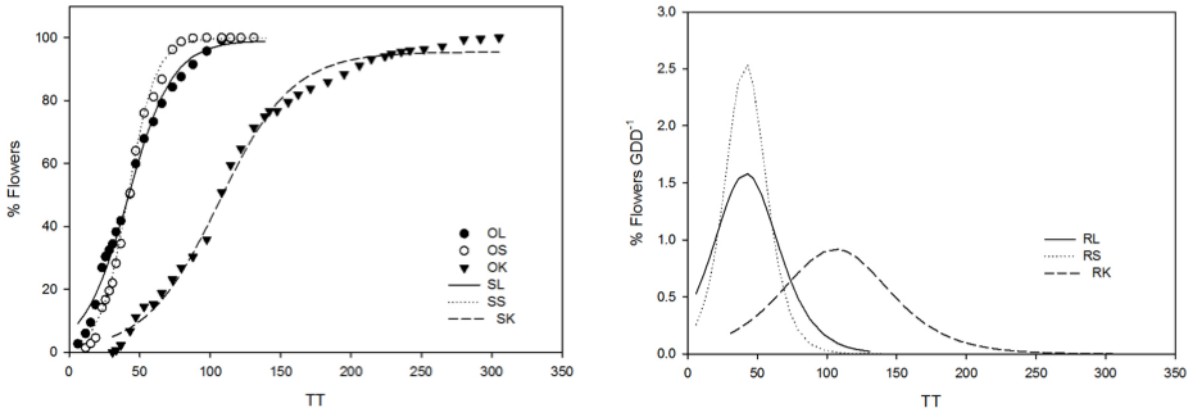

Figure 3. Logistic model for flowering of 'Lapins' (OL), 'Sweetheart' (OS), and 'Kordia' (OK) as a function of thermal time (TT) (left). Rate of flowering (flowers/growing degree day) as a function of thermal time (TT) (right).

of effective pollination period will facilitate the development of effective crop load management strategies. Our preliminary attempts to model flowering of sweet cherry with thermal time reveal a sigmoidal curve with Lapins and Sweetheart blooming earlier than Attika (Fig. 3). We are currently including growth chamber studies with field observations to strengthen these flowering models. In addition, we are pursuing funding from USDA to continue the effort.

At commercial harvest there was no apparent relationship between fruit quality (any attribute evaluated) and the date that the flower opened during bloom (Fig. 5). This was true for each cultivar evaluated and contradicts one dataset collected in Prosser that showed that quality potential was highest in the earliest-opening flowers. We believe this is due to differences in crop load, which was heavy in Prosser and light in Tasmania. Trials established in Tasmania evaluated the role of crop load on the effect of timing of flowering on fruit quality potential, however, fruit load was light overall and we observed no effect. Regardless, the variability among fruit at 'commercial' harvest maturity is tremendous, even among fruit from flowers that opened on the same day (Fig. 5). We recorded nearly a 3-fold variability (e.g., 5 to 15 g per fruit) in fruit weight that is obviously not related to timing of flowering. Variability in other key attributes was significant as well. Combined, these data suggest fruit quality potential is determined, in part, at the time of flowering (a possibility we are investigating) and not by the timing of anthesis. A preliminary investigation into the potential for bud hierarchy within cherry spurs revealed no consistent difference in fruit quality between fruit from the apical-most fruit bud (i.e., that nearest the vegetative bud) vs. the basal-most bud (Table 1).

In Prosser in 2011 we harvested fruit from several cultivars at commercial maturity, selecting fruit borne from the same floral bud but with different numbers of fruit. These were categorized as single-, double-, triple-, or quadruple-fruit 'clusters'. This observational study's results are currently being analyzed but a notable trend is apparent – there is a negative relationship between the number of fruit set in a bud and the size/weight of those fruit (Table 2). When only one flower in a bud set fruit, the quality of those fruit was always better than quality of fruit from multiple-fruit clusters, regardless of cultivar. Previous work funded by the WTFRC in PI Whiting's lab showed that applications of GA during floral bud initiation could reduce the density of flowers per bud without impacting the number of buds per spur. This may be a practical strategy to achieve improvements in quality by favoring fewer flowers per bud. We will investigate this further along with other analyses into fruit quality potential that are needed to elucidate factors accounting for the tremendous

variability.

Table 1. Quality attributes of 'Sweetheart' sweet cherry fruit borne on apical or basal floral buds. Data are means from individual fruit analyses, N = 22 (apical), N= 24 (basal), N=48 (apical-thinned), N=42 (basal-thinned).

	Weight (g)	Diameter (mm)	Skin colour	Soluble solids
Apical	12.4	30.0	5.5	17.1
Basal	13.3	31.3	5.6	18.5
Apical – thinned	12.2	30.1	5.3	15.9
Basal – thinned	11.9	29.7	5.3	16.3

Table 2. Fruit weight of sweet cherry cultivars harvested at commercial maturity from either a single-, double-, triple-, or quadruple-fruit cluster.

	Fruit weight (g)				
Cluster type	'Benton'	'Chelan'	'Cowiche'	'Rainier'	'Tieton'
Single	9.88	7.11	11.15	8.46	11.59
Double	8.90	6.55	10.15	7.64	10.70
Triple	7.00	6.53	9.56	7.25	9.59
Quadruple		5.68		4.67	7.78

In another field experiment we covered limbs of emasculated flowers with bee exclusion netting and populations of flowers were hand pollinated at 1-day intervals to study stigma receptivity/ovule longevity. Results indicate an extended period of stigma receptivity/ovule viability in all cultivars – 'Tieton', 'Benton, 'Rainier', and 'Sweetheart', with fruit being set from pollinations made 5 days after emasculation (roughly equivalent to 4 days after the flower opened).

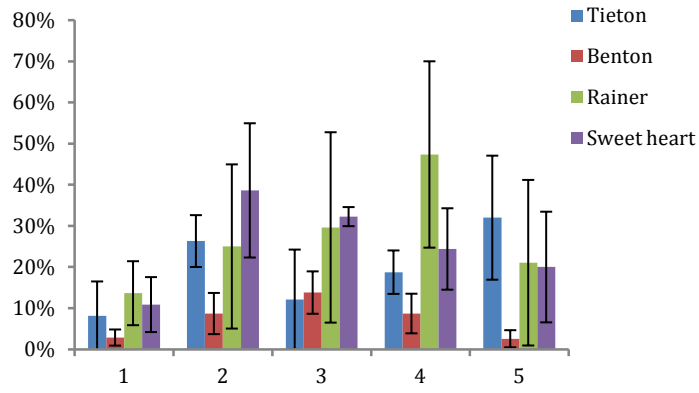

Figure 4. Fruit set (% available flowers) from hand pollinations made at daily intervals. Day 1 – flowers emasculated at full white. Day 2 – date of opening. Flowers were isolated from bees so that pollination was initiated manually. Data are means +/- SE.

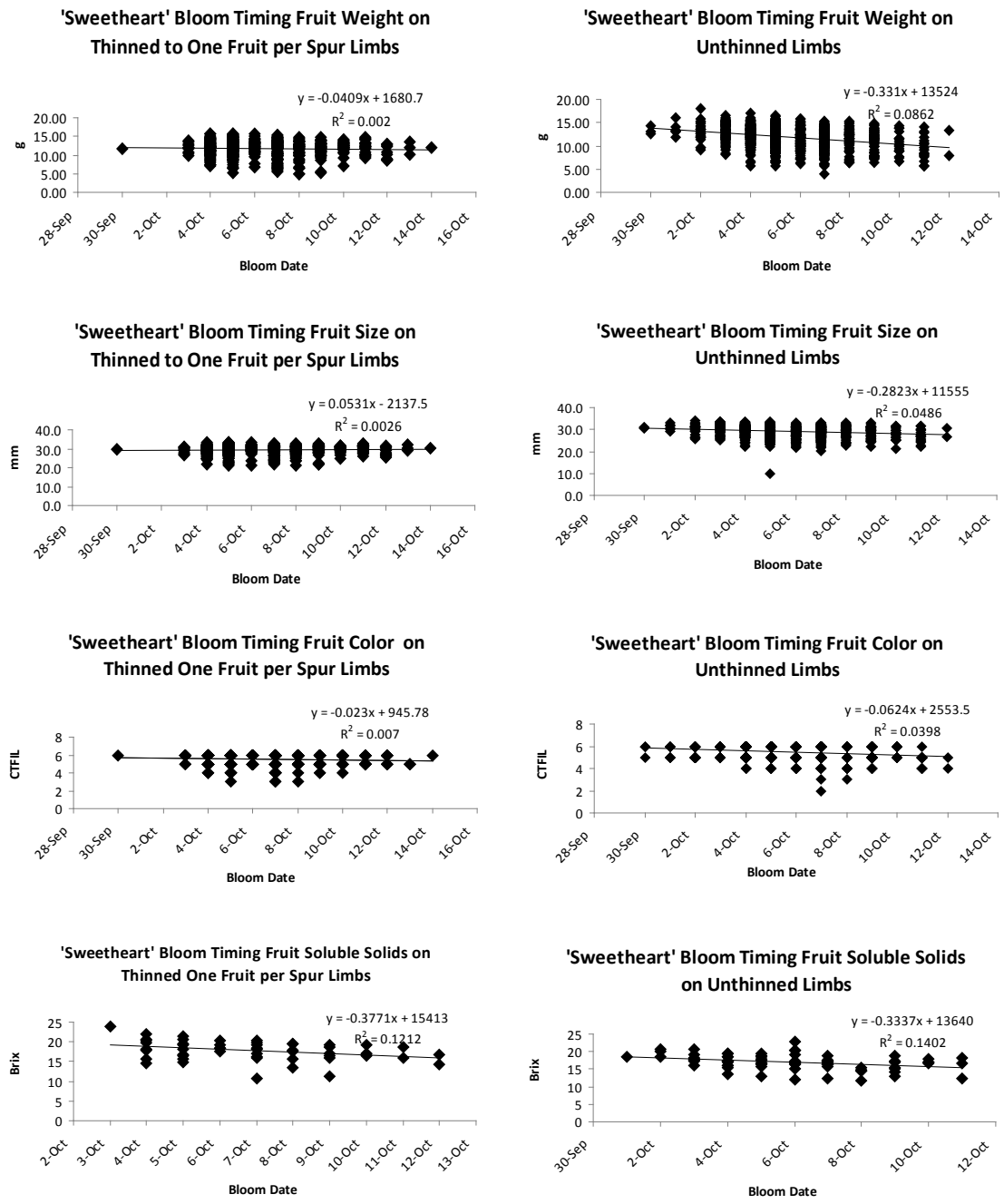

Figure 5. Relationship between fruit quality attributes and date of flowering for 'Sweetheart' trees that were thinning to 1 bud/spur (left) or unthinned (right).

Growth chamber studies In 2011 we conducted several studies in plant growth chambers to evaluate the effective pollination period for sweet cherry and to understand the role of temperature on fundamental elements of fertilization. Our assessments of pollen germination, pollen tube growth, stigma receptivity, and ovule viability of 'Benton', 'Bing', 'Regina', and 'Sweetheart' reveal differences between 'productive' cultivars (e.g., 'Bing' and 'Sweetheart') and unproductive cultivars (e.g., 'Benton' and 'Regina'), though our analyses are ongoing. It appears that pistil factors are important in cultivars with low fruit set – we observed lower receptivity of the stigma and faster degeneration of the ovule in 'Benton' and 'Regina' compared with 'Bing' and 'Sweetheart'. Low

temperature reduces the rate of pollen germination and growth through the style and extends the viability of the ovule whereas high temperature accelerates these components. Under low temperatures, we observed no pollen germination by 8 hours after hand pollination, irrespective of cultivar. In contrast, more than 60% of the pollen grains had germinated on 'Sweetheart' stigmas after 8 hours of high temperature treatment. Under our average temperature regime, designed to mimic 'normal' spring conditions, we recorded pollen tube growth to the base of the style by 96 hours in 'Bing' and by 72 hours in 'Sweetheart'. In contrast, in 'Benton' and 'Regina', we did not record similar pollen growth until 120 hours post pollination. Our observations from lab and field studies, as well as anecdotal evidence from growers, indicate that low temperature conditions are favorable for achieving high fruit set. This is likely due to prolonged viability of the ovules.

Complete results of our growth chamber trials will be posted on our program's website once complete – please visit http://fruit.prosser.wsu.edu for more information.

Our investigations into practical strategies for improving fruit set have been based upon our discovery that ovule longevity appears important to cultivars exhibiting low fruit set. In 2010 we treated 'Tieton' at about 75% full bloom with 4-CPA (a synthetic auxin), GA_3+GA_{4+7}, and AVG (Retain®). Each treatment improved final fruit set significantly (Table 3). Both GA treatment and CPA have yielded inconsistent results. Two years of field trials in Tasmania have also confirmed the efficacy of AVG for improving fruit set of 'Regina'. We have anecdotal evidence from two orchards that two applications of AVG, made at about 20% and 50% of full bloom are effective for improving fruit set. At this stage the most promising program for improving fruit set is two applications of AVG made during early stages of flowering (ca. 10-20% and 40-60%).

Treatment	Fruit set (%)
Control	25 a
4-CPA	36 b
AVG	40 b
GA_3+GA_{4+7}	44 b

Table 3. Effect of PGRs applied to whole trees at about 75% full bloom on fruit set of 'Tieton' sweet cherry. Data with different letters are significantly different at P < 0.01)

2. Timing of thinning - we investigated the effects of the timing of thinning at key phenological stages of fruit development on fruit yield and quality relationships for Bing and Sweetheart in 2010, and Van and Sweetheart in 2011. In addition, we investigated target crop loads by thinning entire trees to leave 1, 2, or 4 floral buds per spur. This work is intended to answer a few simple questions – when is the best time to thin, and, to what targets should we thin?

In every case, earlier thinning was beneficial compared with thinning later in the season. For example, when crop load was adjusted by thinning dormant buds or flowers at full bloom, 'Sweetheart' fruit weight was about 17% heavier compared to later thinning timings, which were similar (Table 4). The results with 'Sweetheart' contradict slightly our previous results that showed benefits from thinning up to early stage II of fruit development (see previous reports). This may be due to the relatively light crop load in the 'Sweetheart' trial – when crop load is heavier, later thinning may be beneficial, as late as early stage III in heavily cropped trees. However, our results do underscore the importance for thinning programs to be imposed as early as possible in the fruiting timeline. The significant challenge of course is not knowing what fruit set is until well past full bloom. Our future work will continue to investigate post-bloom thinning strategies.

Interestingly, we observed a clear relationship between crop load and susceptibility to cracking – incidence of split fruit was dramatically higher in trees with low fruit density and large fruit size.

Table 4. Effects of fruit bud density and time of thinning on yield and fruit quality attributes of 'Sweetheart' sweet cherry.

Treatment	Estimated fruit per tree	Estimated fruit per cm^2 TCSA	Mean fruit weight (g)	Yield efficiency (kg/cm^2 TCSA)	% cracked fruit
Crop load (CL)					
1 bud/spur	1441 a ***	9.53 a ***	11.75 b **	0.109 a ***	58.6 c ***
2 buds/spur	2157 b	13.54 b	11.56 b	0.149 b	39.5 b
4 buds/spur	3810 c	22.58 c	10.29 a	0.222 c	18.0 a
Thinning time (TT)					
Dormant	2192 ab *	13.62 ns	12.28 b ***	0.155 ns	34.0 a **
Full bloom (FB)	2135 a	13.01	12.11 b	0.152	40.8 ab
2 wAFB	2679 c	16.36	10.52 a	0.161	33.0 a
4 wAFB	2792 c	17.32	10.26 a	0.163	35.8 a
6 wAFB	2613 bc	15.78	10.83 a	0.170	50.1 b

ns, *, **, ***, non significant or significant at P≤0.05, P≤0.01, P≤0.001. Within a single column and main effect only, means sharing the same letter are not significantly different at P=0.05 using the LSD test. Main effects of CL, or TT represent data averaged TT, or CL, respectively.

3. Investigate potential post-bloom thinners In 2011 we repeated trials of various PGRs as post-bloom thinners for sweet cherry. Trials were conducted on 'Sweetheart', 'Bing', and 'Rainier', all on 'Gisela' rootstocks. Single applications were made at about 3 weeks after full bloom, approximately the stage I – stage II transition (i.e., pit formation was beginning in some fruit). In contrast to 2010 results, we documented effective thinning with Ethephon in all cultivars (Fig. 5). None of the other PGRs (BA, ABA, methyl jasmonate, NAA) were effective though BA did improve fruit size slightly without inducing any thinning. There did not appear to be any collateral damage to the Ethephon-treated trees – leaves did not abscise and shoot growth continued. Thinning was clearly excessive with Ethephon – we propose to investigate rate and timing response for multiple cultivars in the new proposal. The development of an effective post-bloom thinner for sweet cherry would give growers a convenient tool for managing crop load.

Figure 5. Comparison of limbs shortly after treatment with Ethephon (left) and about one month following treatment with Ethephon (right).

4. Understand timing of mesocarp cell division & expansion cycles and their relative role in fruit quality – see report from Einhorn and Gibeault for progress on this collaboration.

5. Develop counter-seasonal collaboration with University of Tasmania and leverage WTFRC/OSCC funding via Horticulture Australia Limited – considerable effort was made this year to work through contract negotiations for the collaboration. A project that complements the current project was submitted to HAL with PIs Whiting and Close. The full amount of funding from the WTFRC awarded to the current project was sent to HAL as a 'voluntary contribution' to the HAL project. These funds will be matched with HAL funds at about 41% (i.e., the $74,344 funded in year 1 will be leveraged to about $104,000). These matched funds will then be returned to the WTFRC to be issued to WSU. The HAL proposal has been funded fully for 2 years and puts in place technical support in Tasmania to work on issues of concern common to Tasmania, Washington, and Oregon.

EXECUTIVE SUMMARY

This research has improved our understanding of factors that limit fruit set in sweet cherry. Cultivars characterized as poor producers appear to be so from short viability of the ovules. With this knowledge we have begun research into practical strategies to increase productivity in key cultivars. Field trials have shown promise for AVG applications made early in flowering to improve fruit set.

Every element of fruit set/pollination is affected by temperature – pollen tube growth rates increase with temperature, but so does the rate of ovule senescence. Our results suggest that fruit set will be greater in cool springs due to delayed ovule senescence despite slower pollen tube growth. Warm, and windy weather during bloom will decrease fruit set due to accelerated ovule senescence, despite increased pollen tube growth rates.

If crop load is balanced, timing of flowering does not affect fruit quality (i.e., fruit from the earliest and latest opening flowers will be similar quality). However, if crop load is high, fruit set from the earlier opening flowers will be better quality than those from late-opening flowers.

Thinning early in fruit development (e.g., dormant buds, flowers) is more beneficial than later thinning (e.g., post pit hardening) for improving fruit quality.

For most cultivars, a well-balanced crop load is 2 – 4 fruit per spur. Fruit quality from single-fruit clusters is better than from multiple-fruit clusters (i.e., several fruit set from same bud). There appears to be no effect of flower bud position on fruit quality potential in sweet cherry.

Ethephon applied 2 to 3 weeks after full bloom shows promise as a post-bloom thinning agent.

FINAL PROJECT REPORT

WTFRC Project Number: CH10106

Project Title: Branch induction in two-year-old wood of sweet cherry

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Cooperators: Dr. M.D. Whiting, WSU Prosser

Other funding sources: NONE

Total Project Funding: $5,875

Budget History:

Item	2010	2011
Salaries[1]	0	0
Benefits[1]	0	0
Wages[2]	1,000	1,500
Benefits[2]	150	225
Equipment	0	0
Supplies[3]	200	300
Travel[4]	1,000	1,500
Miscellaneous	0	0
Total	2,350	3,525

[1] No technical help indicated since Technician position no longer exists. Time-slip help is absolutely essential to collect the volume of data needed to set up trials and evaluate growth responses to the various bioregulator applications involved.

[2] Time-slip help substitutes for unfilled Technician position. Time-slip benefit rate is calculated at 15%.

[3] This category includes miscellaneous supplies, non-capital equipment, consumables, repairs, etc. that are needed to carry out the research project.

[4] Treatment application and data collection at distant sites, all off-station. Includes vehicle lease-to-purchase, operating, repair costs.

Objectives:

1. Test cytokinins without GA to determine efficacy for stimulation of lateral branch development on two-year-old wood using both cuts and high surfactant concentration additives to evaluate efficacy of cytokinins for bud activation and penetrability of older bark.
2. Assess whether supplementation or substitution of cytokinin-based treatment solutions with GA produces any beneficial effect on branching of older wood.
3. Evaluate the characteristics of induced branches on older wood and determine follow-up strategies for modification of branch growth habit if needed.
4. Evaluate effects of treatments to older wood on pedicel development of flowers borne on treated wood sections.

Significant findings 2010:

1. All of the three orchards used for these studies experienced significant cold damage to buds and/or woody tissues from the Oct. 11, 2009 freeze event. The three orchards were located from Stayman Flats near Chelan, WA to the Sunrise orchard near Moses Coulee. In all three locations, the minimum temperature that night reached between 21 and 15°F during the freeze, and in all three locations the rate of temperature decrease overnight equaled or exceeded -1.8°F (-1°C) per hour, a rate sufficient to produce significant damage to unacclimated tissues.
2. In a comparison of Promalin, Maxcel and ProVide (Valent BioSciences) applied to scoring cuts on two-year-old wood of 'Sweetheart' trees, only Maxcel (5,000 ppm) showed some increase in branching over control, but extensive wood damage from cold (low of 21°F on Oct. 11, 2009) significantly compromised the branching potential in this trial.
3. Promalin (5,000 ppm) applied to scoring cuts only modestly increased lateral branching on two-year-old wood of 'Sweetheart' cherry trees compared to untreated control trees. Combining Promalin with Pentra-bark surfactant (Quest Products Corp.) at up to 15% v/v and applying these bioregulator/surfactant mixtures as bands to two-year-old wood of 'Sweetheart' cherry trees was completely ineffective for branch induction. Again, significant wood and bud damage, severe enough to result in the removal of some trees, compromised the results.
4. In a block of 'Early Robin'/Mazzard trees near the Columbia River (Stayman Flats), Promalin (5,000 ppm) applied to scoring cuts only increased branching from two-year-old wood by about two-fold. Bud damage due to cold appeared to limit branching potential. Mixing Promalin with Pentra-bark at up to 15% v/v and applying these mixtures as bands at intervals on two-year-old wood had no effect on branching.
5. Applying either scoring or bioregulator banding to two-year-old wood of 'Early Robin' trees either every 15 or every 30 cm along the two-year-old wood made no difference in branching response.
6. Two trials examined the effects of the surfactants Syl-Tac (Wilbur-Ellis) or Yucca-Aide (Monterey Ag Resources) as supplements for Promalin (2,000 ppm) when applied to scoring cuts or as bands on one-year-old wood of 'Sweetheart' cherry. All the experimental trees were subjected to a low of 15°F on Oct. 11, 2009, resulting in some dieback on terminals of one-year-old wood and an unknown amount of internal tissue damage. The death of the terminal portion of the one-year-old leader acted much as a heading-back cut, producing some stimulation of branching among the remaining live buds. Promalin plus scoring produced about twice the branching of untreated controls, suggesting that cold injury combined with the heading-back effect may have compromised the potential for additional branch induction with bioregulators.
7. In both of these trials, Syl-Tac at 2, 5 or 10% v/v and Yucca-Aide at 0.25, 2 or 15% v/v improved branching as much as did scoring plus Promalin. The other surfactant-concentration

treatments were ineffective. Terminal dieback on one-year-old wood was present in almost every tree in each trial. The uneven branching response to surfactant supplementation may have been due in part to non-visible vascular damage in the treated branch sections.

Significant findings 2011:

1. Four trials, two on one-year-old wood and two on two-year-old wood, were established in a young orchard of 'Bing'/G.6 trees near Wenatchee, WA. Two trials on two-year-old wood of 4th-leaf 'Chelan'/Mazzard trees were established in Pasco. One trial was established on two-year-old wood on 5th-leaf 'Selah'/Mazzard trees in East Wenatchee. The trees turned out to have suffered variable amounts of tissue and bud damage from the late Nov. 2010 freeze event, with the trees near Wenatchee more severely affected. Although the leader shoots on the Wenatchee 'Bing' trees were unpruned, every tree suffered some killing of the upper portion of the new leader shoot that grew in 2010. Thus the trees in spring, 2011 behaved as if they had been headed back in the winter, creating a stimulus for lateral-branch development due to interrupted apical dominance.
2. In a comparison of several different cytokinin/gibberellic acid products applied to scoring cuts at green-tip on two-year wood of 'Chelan' trees that suffered only minor cold damage, scoring alone was no better than no treatment for induction of branching.
3. On both two- and three-year-old 'Chelan' wood, any bioregulator product (Maxcel, Promalin, Pro-Gibb, ProVide, Novagib or GA_7 alone) combined with Syl-Tac surfactant (0.5% v/v) and applied to scoring cuts 15 cm apart resulted in improved branch induction.
4. Surprisingly, on older 'Chelan' wood, any gibberellic acid formulation applied to scoring cuts produced better lateral-branch induction than 6-benzyladenine (Maxcel) alone.
5. In contrast, on two-year-old wood of winter-injured 'Bing'/G.6 trees, any GA + scoring did not induce branching as well as Maxcel (6-BA only) + scoring. Is this a varietal difference or somehow related to the winter damage situation?
6. Increasing the concentration of Promalin combined with Regulaid surfactant applied to scoring cuts on two- and three-year-old wood of 'Selah' trees resulted in a comparable improvement in branching despite some cold injury to buds. Quality of branching at the highest Promalin concentration (20,000 ppm, undiluted product straight from the bottle, no Regulaid) was similar to that from lower concentrations (wide crotch angles, no upright suckers). Branch induction on older wood may be enhanced by higher bioregulator concentrations.
7. In a test of a variety of surfactants combined with Promalin (5,000 mg a.i./liter) and applied as sloppy bands every 15 cm without scoring cuts on 'Bing' trees near Wenatchee, no treatment produced any improvement in lateral branching.
8. Crotch angles of induced branches on two-year-old wood on young 'Bing' trees were unaffected by any treatment. In addition, no induced branches developed into upright suckers. The average crotch angle of induced branches was around 70° - 80°, resulting in desirably flat induced shoots with no evidence for promotion of undesirable sucker growth.
9. Despite post-treatment temperatures in the acceptable range, branching response of two-year-old wood of 5th-leaf 'Chelan'/Mazzard trees was quite limited, due in part to killing of some lateral buds by cold the previous November. Nevertheless, Promalin + scoring produced about a 6 to 10-fold increase in branching compared to untreated controls, scoring + surfactants only, or Promalin + surfactants painted onto unscored bark.
10. In April, 2011, two trials were conducted on one-year-old wood of young 'Bing'/G.6 trees on which a variable amount of that one-year-old wood had been damaged by cold the previous November.
11. Combining various surfactants with Promalin (5,000 mg a.i./liter) and applying those solutions as sloppy bands every 30 cm on the living portion of the one-year-old wood, lateral branching was improved by supplementation of Promalin with either Syl-Tac (5% v/v),

Pentra-bark (5% v/v) or Rocket DL (4% v/v). Lateral branching was similarly stimulated by scoring every 30 cm and painting the scoring cuts with Promalin plus Regulaid (1% v/v). Mixing the surfactants Prolec (0.5% v/v) or Canhance (10% v/v) with a similar concentration of Promalin and applying as sloppy bands did not result in improved branching.

12. Combining the surfactant Canhance (10% v/v) with various bioregulators, each at 5,000 mg a.i./liter and applying each solution to scoring cuts on one-year-old wood of young 'Bing'/G.6 trees, Promalin and Pro-Gibb produced an improvement of over 50% in lateral-branch development from treated wood. The gibberellins ProVide and GA_7 alone were nearly as effective. Canhance alone and Maxcel plus Canhance were completely ineffective for stimulation of branching.
13. Limited observations indicated that the presence of GA in a branch-induction treatment could increase pedicel length on fruit set on spurs on treated wood.

Methods:

Three trials were initiated in 2010 and five in 2011 to examine effects of cytokinins vs. gibberellins along with scoring vs. surfactant treatments on branch induction on two-year-old wood. Two additional trials were initiated in 2010 and two more in 2011 to examine in greater detail the potential for surfactants to substitute for scoring or nicking cuts in one-year-old wood in stimulating lateral branch development. The trials focused on whether surfactants could substitute for cutting the bark on two-year-old wood for encouraging penetration of bioregulators into active tissues, whether GA alone could induce branching on two-year-old wood as has been demonstrated for such treatments on one-year-old wood, whether the distance between scores or banded bioregulator treatments on two-year-old wood had any beneficial effect on branch induction, and whether concentration of Promalin influenced branching success on older wood.

Results and discussion:

One goal of the program was to determine whether gibberellic acid (GA) alone can induce lateral branching in two-year-old wood of sweet cherry. Previous research has clearly shown that GA alone is about as effective as cytokinin for branch induction in one-year-old wood. One advantage this finding confers is that GA products are OMRI-approved, and thus can be used in organic orchards. They are also a bit cheaper than Promalin. Winter injury precluded clear conclusions in 2010. In 2011 the branching results, although diminished to some degree by winter injury sustained in late Nov. 2010, showed that GA products alone were effective for branch induction on two-year-old wood in 'Chelan' cherry, but less strongly in 'Bing'.

In several of the trials, comparisons of surfactant concentrations vs. using scoring cuts to improve bioregulator penetration were undertaken. Despite some cold damage effects in these trials, it was clear that when we applied Promalin to scoring cuts, branching was improved to some extent in every case. These results showed that if there were live buds present on two-year-old wood and that wood had not been killed outright by either the 2009 or 2010 cold events, those living buds could be activated if the Promalin could penetrate into active tissues. Results of the two trials with one-year-old wood confirmed this observation.

In the case of the one-year-old wood, killing the terminal portion of those shoots altered the apical dominance situation by producing the equivalent of a heading-back cut. This physiological change resulted in a certain amount of increased branching, thus limiting the degree to which additional branching could be induced by the bioregulator applications themselves. On one-year-old wood, three surfactant treatments, Promalin plus either Pentra-bark (Quest), Rocket DL (Monterey) or Syl-Tac (Wilbur-Ellis) resulted in sufficient

bioregulator penetration into one-year-old wood to stimulate branching over and above the stimulus produced by cold damage to the upper portion of that wood.

None of the surfactant-supplemented treatments showed significant branching activity on two-year-old wood in the absence of scoring. It appears clear that surfactants alone, even at high concentrations (up to 15% v/v), do not provide a reliable method for assuring bioregulator penetration through the bark and into active tissues on two-year-old or older wood. Our trials indicate that successful branch induction on branch sections older than one year require some form of bark injury to open a path for successful penetration of bioregulators.

Acknowledgements:

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Reports Published:

Elfving, D.C., D.B. Visser and J.L. Henry. 2011. Gibberellins stimulate lateral branch development in young sweet cherry trees in the orchard. **International Jour. of Fruit Sci. 11:41-54.**

Elfving, D.C. 2010. Plant bioregulators in the deciduous fruit tree nursery. **Acta Horticulturae 884:159-166.**

Elfving, D.C. and T.R. Schmidt. 2010. Bioregulator sprays. p. 133-146. In: M. Bush (coord.), **2010 Crop Protection Guide for Tree Fruits in Washington. EB 0419.**

Executive Summary

1. No surfactant tested, even at high concentration (up to 15 % v/v), was capable of producing sufficient penetration of cytokinin- or gibberellin-based bioregulators through the bark to successfully induce lateral branching on two- or three-year-old wood in young sweet cherry trees. Only when such bioregulators were combined with scoring cuts to permit penetration into living tissues did lateral branching occur on older wood.
2. Gibberellic acid (Pro-Gibb, Novagib, ProVide or GA_7) alone proved effective for induction of lateral branching on two- or three-year-old wood of sweet cherry trees when applied to scoring cuts. This observation suggests that these products may have a role for branch induction in organic sweet cherry orchards.

FINAL PROJECT REPORT

Project Title: Irrigation and fertilization for optimal cherry fruit quality

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Cooperators: Jac le Roux, Don Fesler

Total project funding request: $109,061 **Year 1**: $35,874 **Year 2**: $36,127 **Year 3**:$37,060

Other funding sources

Agency Name: NRCS
Amount requested/awarded: $5,000
Notes: Funds were used to offset irrigation supplies and labor costs for reconfiguration of the irrigation system to accommodate the experimental design.

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

Item	2009	2010	2011
Salaries[1]	**15,000**	**13,800**	**14,352**
Benefits[3]	**9,330**	**9,522**	**9,903**
Wages[2]	**4,000**	**5,000**	**5,000**
Benefits[3]	**1,044**	**1,305**	**1,305**
Equipment	**0**	**0**	**0**
Supplies	**4,500**	**4,500**	**4,500**
Travel	**2,000**	**2,000**	**2,000**
Miscellaneous	**0**	**0**	**0**
Total	**35,874**	**36,127**	**37,060**

Footnotes:
[1] .50 FTE Technician (D. Laraway), yr 3 includes 4% pay raise
[2] Hourly labor, .20 FTE (temporary technician)
[3] Technician OPE rate is 69% based on actual, hourly OPE rate is 26%

Objectives

1) Optimize irrigation scheduling and fertilization of sweet cherry through measuring and monitoring soil moisture and plant growth and development, and develop a predictive model for cherry fruit and shoot growth based on soil moisture and plant measurements.
2) Determine the effect of drip irrigation on fruit and shoot processes.
3) Determine the appropriate allowable depletion of soil moisture for optimizing cherry fruit quality and yields, and managing vigor.

Significant Findings

- For two cherry orchards planted on Mazzard rootstock, on fairly deep soils (3-4 feet of soil), significant water savings were achievable by application of deficit irrigation.
- Deficit treatments replaced between 45% to 65% of Lapins/Mazzard cumulative reference evapotranspiration (ET0).
- Early spring monitoring of soil moisture resulted in water savings by delaying the start of irrigations.
- Yields of Lapins were not significantly affected by deficit irrigation of 55% of ET0 in each of the three years of the study, though when water was withheld to 45% to 50% of ET0, yield reductions approaching 10% were observed in year 3.
- Fruit quality of Tieton and Lapins was neither improved, nor limited, by deficit irrigation at harvest, or after three weeks of postharvest storage.
- In 2011 the T1 treatment level of irrigation was increased to replace 96 % ET0.
- Fruit growth rates were not affected at any point throughout the season for deficit irrigation treatments.
- Shoot growth was not significantly affected by deficit irrigation.
- For 'Tieton' and 'Lapins' trials, stem water potential (measure of water stress) declined as the season progressed, irrespective of treatment. In 2011, the Lapins T1 treatment (receiving 96 % ET0) had significantly higher stem water potential than the deficit treatments. No differences were observed among the other levels of deficit irrigation (reaching values as low as -1.3 MPa). Trunk growth was limited in 2011 in all deficit treatments relative to T1.
- In all years of the study, deficit treatments utilized significantly more water to meet their evaporative demand from greater soil depths than the T1 treatment. Subsequently, the heightened activity of deep roots mitigated the onset of water stress. These findings support the general lack of differences observed for yield and fruit quality of deficit irrigated treatments. 'Tieton' had the additional benefit of an early harvest date before significant soil moisture depletion occurred.
- Tieton trees receiving irrigation at higher frequencies (4x per week), but with less water per event experienced greater water stress than those receiving low frequency applications (once per week). Yields of these treatments were slightly reduced. Quality was not affected.
- Overall three-year yields were not significantly influenced by nitrogen rate (100 lbs or 60 lbs actual N per acre) or by delivery technique (Split broadcast application vs. fertigation).
- Shoot growth of 60 lb N per acre was slightly reduced relative to the 100 lb rates.

Results and Discussion

Site 1. Lapins/Mazzard-Irrigation. In 2011, irrigation volume of the T1 treatment was increased to provide non-limiting irrigation supply, and when summed with precipitation events after bloom, received 96% of total seasonal reference evapotranspiration (ET0) (Fig 1). In each of the previous two seasons, ET0 replacement for T1 was ~65%. 2011 seasonal irrigation supply and postbloom precipitation (summed) provided 55%, 45%, and 50% of ET0 to T1, T2 and RDI treatments, respectively (Fig 1); the latter three treatments received similar volumes of irrigation in each year of

the three-year study. The decision to increase the volume of T1 in 2011 was based on the nonsignificant differences observed among treatments for yield and fruit quality in 2009 and 2010. During the previous years we had also observed a lack of difference in stem water potential (measure of plant water status and stress) among treatments, and attributed this to the large reserve of soil water built up from winter and spring rain events. In other words, our measurements of soil moisture extraction showed that Mazzard roots were actively utilizing water at depths of 3 feet (plausibly at depths exceeding 3 feet), thus supplementing our deficit treatments with adequate water to produce good yields of high-quality fruit. However, our hypothesis that provision of irrigation between 40% and 65% ET0 would not adversely impact yield or fruit quality could not be proven without comparing these treatments against a non-limiting irrigation 'control', since it is possible that all of the treatments were limited by the relatively low percentages of ET replacement.

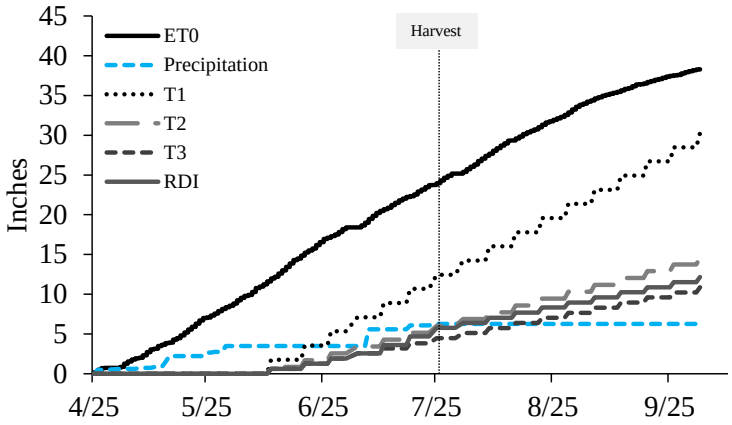

Fig 1. 2011 cumulative water lost through evaporation/transpiration [reference ET (ET0)], or gained via precipitation, and irrigation events received by four different irrigation treatments (T1, T2, T3, RDI) for Lapins/Mazzard. An acre inch of water=27,154 gallons.

At full bloom (April 25) ~ 3 inches of water per foot of soil (25% by volume) was present throughout the soil profile (Fig 2). For this soil, field capacity is ~3.4 inches, or 28% by volume. Irrigations were delayed (47 DAFB) to allow for some utilization of water in the top 2 ft. of soil (Fig 2). The relatively cool temperatures and precipitation between bloom and 47 DAFB, in combination with soil moisture monitoring, facilitated early-season water savings. In situations where nitrogen is broadcasted in one to two applications in spring, early irrigations to 'full' profiles can exacerbate leaching (particularly when N is applied in the form of nitrate). An increase in soil moisture for the RDI treatment can be observed after 68 DAFB, when it was increased from 45 % ET replacement to 65% (Fig 2). This corresponded with the end of pit-hardening, and the period of rapid fruit growth (Fig 3). In general, control (T1) soil moisture was maintained above 85% FC, and was significantly higher than other treatments from 50 DAFB through the remainder of the season. Increased soil water depletion, at all depths monitored, was observed for the deficit treatments as the season progressed (Fig 2). Between 68 DAFB and harvest (92 DAFB), soil moisture differences could be seen relative to the degree of deficit. The post-harvest period was associated with greater evaporative demand, and all deficit treatments were observed to equally deplete soil water reserves. These data were in agreement with results from 2009 and 2010; the only differences were those of the T1 treatment, which received ~65% ET in 2009 and 2010. In those two seasons T1 had statistically higher soil moisture levels than deficit treatments, but intermediate between deficit treatments and the 2011 T1. These data, together, show the value of soil moisture measurement as an effective irrigation scheduling tool. Monitoring soil water levels to depths of three feet are advisable for vigorous rootstocks such as Mazzard. With more dwarfing rootstocks greater root activity will likely take

place at shallower depths. However, knowledge of 'storage' water between 2 and 3 feet is still important since it can move through capillary forces to the shallower profile.

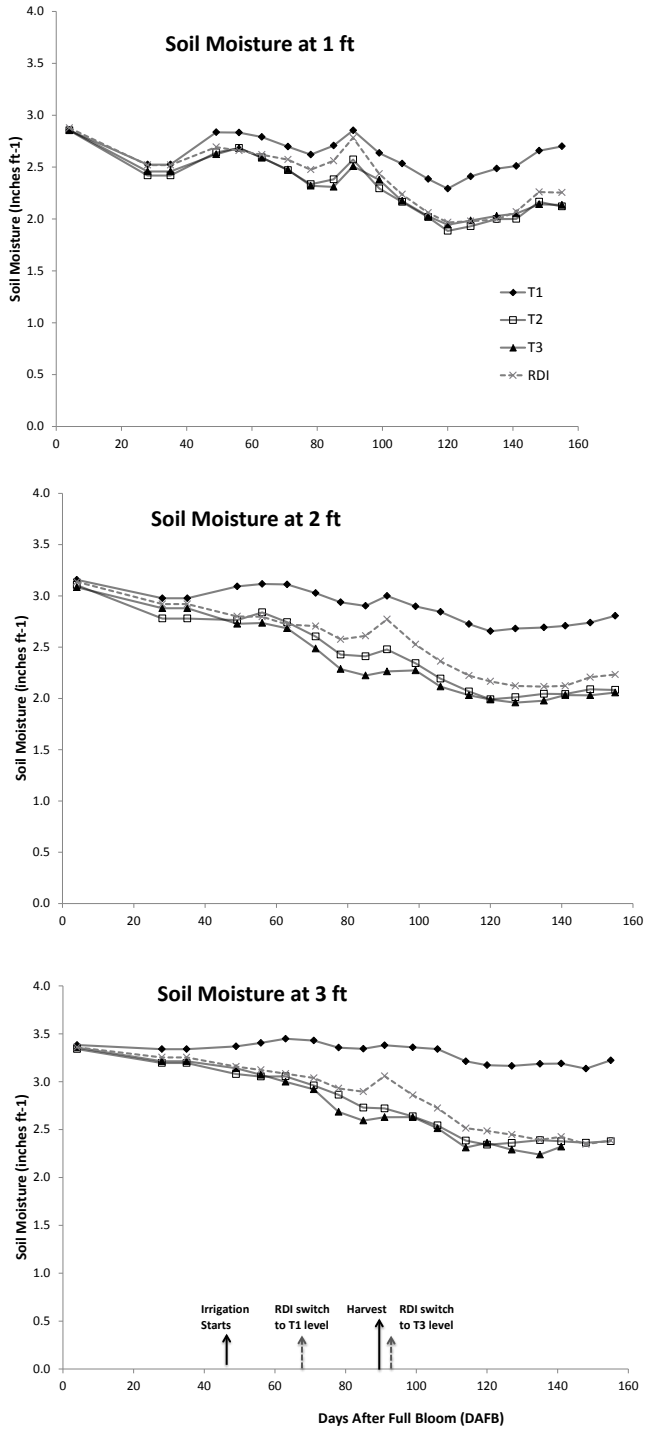

Fig 2. Effect of control (T1) and deficit irrigation treatments (T2, T3, and RDI) on volumetric soil moisture content (inches per foot) of the soil profile at 1 ft (upper), 2 ft (center), and 3 ft (lower) depths for 'Lapins'/ 'Mazzard' trees. Each data point is the mean of 5 replicates (n=3).

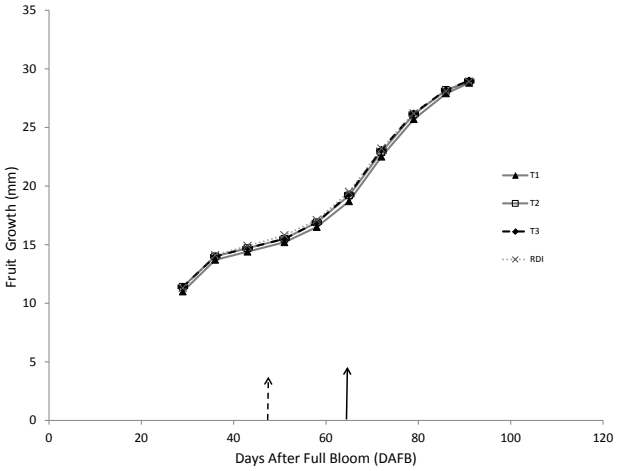

Fig 3. 2011 fruit growth of Lapins/Mazzard as affected by irrigation treatment. T1, 96% ET; T2, 55% ET; T3, 45 % ET; RDI, 45% ET between 47 DAFB and 68 DAFB, 65% ET between 68 DAFB-Harvest, and 45% ET after harvest. Data are the means of 5 replications (n=15). Dashed arrow at bottom signifies start of irrigation treatments; solid arrow signifies RDI switch from 30% to 60 % ET. Harvest was one day following last data point.

Monitoring fruit growth rates provided a plant-based indicator of water stress. As in 2010, we observed no treatment differences in cumulative fruit growth rate (Fig 3). Shoot length was also not significantly affected by irrigation level (data not shown). Trunk cross-sectional area increase, however, was highest for T1 trees in the postharvest interval in 2011 only (5.5% increase compared to 3.7% for T2 and T3, and 3% for RDI). Trunks have previously been shown to compete poorly with shoots and fruit for carbohydrates (Whiting et al.). Subsequently, a significant proportion of their annual growth occurs after harvest; a time which coincides with greater water stress in the deficit treatments.

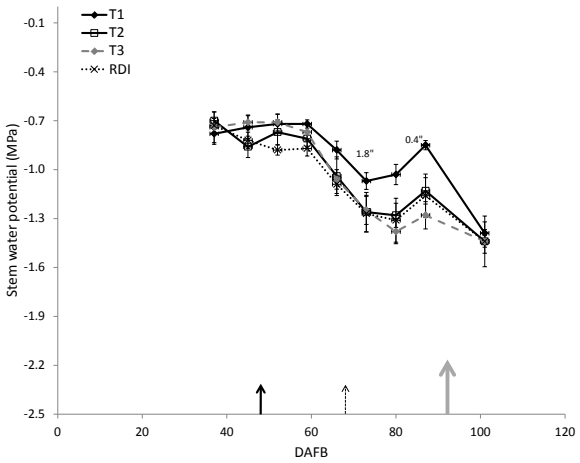

Fig 4. 2011 stem water potential (-MPa) of Lapins/Mazzard in response to irrigation treatment. T1, 100% ET; T2, 50% ET; T3, 30 % ET; RDI, 45% 47 DAFB-68 DAFB, 65 % 68 DAFB-Harvest, 45% Postharvest. Data are the means of 5 replications (n=4). Black solid arrow at bottom (47 DAFB) signifies start of irrigation treatments; dashed arrow (68 DAFB) signifies RDI switch from 45% to 65% ET; tall grey arrow (92 DAFB) signifies harvest.

In early spring (until 59 DAFB) all treatments had high (less negative) stem water potential values, signifying non-limiting hydraulic conditions for growth (Fig 4). No differences among treatments were detected until 73 DAFB, when the control (T1) had significantly higher values than the deficit treatments [i.e., less stress] (Fig 4). Stem water potential can be seen to become progressively more negative as the season advanced, irrespective of irrigation treatment (Fig 4). The declining stem water potential of Control trees was a response to an increasing atmospheric demand (i.e., hotter and dryer). In woody plants, the flow of water from the soil to the leaf encounters a higher resistance than that between the leaf surface and the atmosphere. Therefore, under higher evaporative conditions, water is being lost from the leaf faster than it can be taken up from the roots and translocated back to the leaf. The consequence is a lower water potential, despite the presence of adequate available soil moisture, as can be seen in control (T1) trees (Figs 2 and 4). The lower water potential of the deficit treatments improved the ability of these trees to extract soil water in the upper profile which was becoming increasingly dryer (Fig 2). Previous work with sweet cherries suggests that photosynthesis is not limited until stem water potential drops below -1.5 MPa. During the pre-harvest interval, trees in 2011 never reached values this low (Fig 4), as was similarly observed in previous years of the study (data not shown). In 2009 and 2010 photosynthesis measurements were taken on a few pre-harvest dates. No differences in photosynthesis were observed (data not shown). Following harvest, stem water potential reached minimum values of -1.9 MPa in 2009, and -1.6 MPa in 2010 and 2011 (data not shown) for the Lapins site. It is plausible that low stem water potential coinciding with the postharvest period could have reduced floral bud development for the following year, as previously documented for other deciduous fruit trees. Return bloom dynamics were not investigated in the present project. The increase in water potential for all treatments just prior to harvest was the result of untimely rain events.

Table 1. Effect of irrigation treatments on average tree yield of 'Lapins'/'Mazzard' for each year of a 3-year study. Data are means of 5 replications (n=5).

Treatment	Yield (lbs per tree)			
Irrigation	2009	2010	2011	2009-2011
T1	181	141 a	100 a	422
T2	179	115 b	105 a	399
T3	185	123 ab	91 b	399
RDI	175	118 ab	89 b	382
Stat.signif.	ns	*	*	ns

ns=not significant; * significant at $P < 0.05$

Treatment yields for the entirety of the project are provided in Table 1. Overall, tree yields appear to be declining throughout the experimental period. It should be noted, however, that uncharacteristically high croploads occurred in 2009. Average 2009 yields were not affected by irrigation treatment, and equated to 14.5 tons per acre at the planting density of the orchard. The fact that 2010 yields were reduced is likely a direct response to the high croploads of 2009. However, 2010 yields were reduced for all treatments relative to T1. In 2011, yields were ~10% reduced for T3 and RDI (Table 1). The yield deficits observed may be attributed to differences in fruit set, since there were no negative effects of irrigation treatment on fruit size in 2011 (Table 2), or in either of the two previous years (data from 2009 and 2010 were provided in earlier reports, and omitted here for space). Lapins fruit quality in 2011 was excellent (Table 2). Soluble solids content was reduced in T1, likely due to active accumulation of soluble solids in deficit fruit (Table 2). All other quality attributes were unaffected by irrigation treatment (Table 2). The occurrence of several separate rain events totaling 2.5 inches (Fig 1) during rapid fruit growth in early to mid-July, and a 0.25 inch event the day preceding harvest, provided an opportunity to observe the influence of irrigation treatment on cracking. The total percentage of cracking (sum of side cracks and stem-bowl cracks) was ~ 22 %, and was not related to irrigation volume (Table 2), even though statistically significant differences in soil water moisture (Fig 2) and stem water potential (Fig 4) were found during these events.

Table 2. Effect of 2011 irrigation treatment on fruit quality attributes (fruit wt. and diameter; FF= firmness; SS=soluble solids; TA= total acids; cracks [ttl side and stem bowl cracks]) at harvest for 'Lapins'/'Mazzard'. Data are means of 5 replications (n=4 for wt; n=200 for FF and mm fruit size; n=2 for SS and TA; n=100 for cracking analysis).

Treatment Irrigation	Avg. fruit wt. (g)	Avg. fruit size (mm)	FF g/mm	SS %	TA %	Cracks %
T1	12.8	30.4	332	17.2 b	0.47	24
T2	12.5	30.1	325	18.1 ab	0.52	19
T3	12.9	30.4	332	18.5 ab	0.48	24
RDI	12.8	30.4	335	19 a	0.46	23
Statist. signif.	ns	ns	ns	*	ns	ns

ns=not significant; * significant at $P < 0.05$

Post-harvest fruit quality was not affected by irrigation treatment in 2009 or 2010 (data not shown). Postharvest quality was not analyzed in 2011; however, differences in postharvest quality would not have been expected given the lack of differences in quality attributes at harvest, notwithstanding SS content.

Site 1. Lapins/Mazzard-Nitrogen. The influence of nitrogen rate and delivery method on shoot growth, yield and fruit quality was also investigated at the Lapins site. Trees were either provided 100 lbs of actual nitrogen per acre through microsprinklers (fertigation), or broadcast in a split application. An additional treatment of 60 lb nitrogen per acre (fertigation) was also evaluated. All nitrogen treatments were superimposed on the irrigation treatments outlined above. Shoot growth was slightly reduced for the 60 lb N treatment (Fig 5), albeit nonsignificantly.

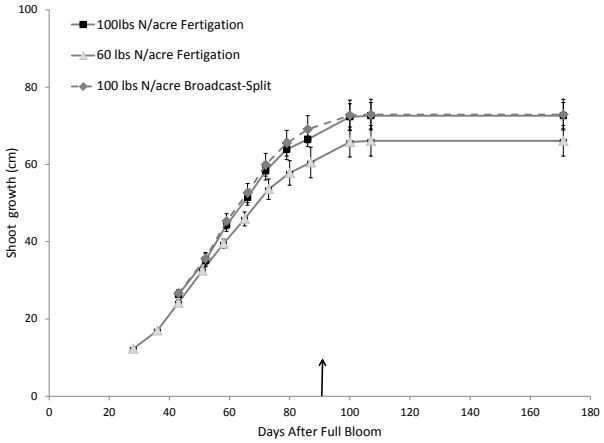

Fig 5. 2011 shoot length of Lapins/Mazzard as affected by nitrogen treatment. Nitrogen was delivered through irrigation lines [i.e., fertigation] at100 lbs or 60 lbs actual N per acre or broadcast at 100 lbs actual N per acre in a split application; first application 10 days after bloom, second application 24 days after full bloom. Data are the means of 5 replications (n=12 [3 shoots per cardinal direction]). Arrow at bottom signifies harvest (92 days after bloom).

In 2010, yields were greatest for the high N treatments, irrespective of delivery mode; however, these results were not repeated in 2011 (Table 3). That the three-year yield was not further reduced by the low nitrogen treatment is surprising. These data would suggest that either improved nitrogen use efficiency was achieved by fertigating the lower rates of N, or that sufficient N pools existed to support growth of shoots and fruit under the current croploads. As a standard practice, foliar urea (20 lbs actual N) was applied postharvest in late summer, and might have raised the total seasonal N pool of the low N treatment to adequate levels. The significant yield effects observed in 2011 could have been attributed to nitrogen losses from harvested fruit alone, given that yields were >14 tons per acre. The results also suggest that fertigation of 100 lbs N per acre had no distinct advantages over broadcast applications for yield, or fruit quality (data not shown). It is unlikely that significant leaching would have occurred for any of our irrigation treatments, though this parameter was not

evaluated. Practices which improve nitrogen use efficiency, and result in reduced application rates deserve further attention.

Table 3. Effect of fertilization treatments on total tree yields (lbs) of 'Lapins'/'Mazzard'. Data are means of 5 replications (n=3).

Treatment	Yield (lbs per tree)			
Fertilization	2009	2010	2011	2009-2011
Broadcast (100 lb N)	179	123 ab	93	396
Fertigation (100lb N)	183	132 a	101	416
Fertigation (60 lb N)	177	117 b	96	389
Stat.signif.	ns	*	ns	ns

ns=not significant; * significant at $P<0.05$.

Site 2.Tieton/Mazzard. A three-year experiment comparing different rates of drip irrigation, at two different frequencies, was initiated in a Tieton/Mazzard block in 2009. Marked variability in yield limited our ability to resolve significant differences among treatments throughout the experiment, despite treatment means being comprised of 25 trees. Three-year yields were only significantly reduced for the HF T3 treatment (Table 4). In all years of the study, significant water depletion of the soil profile occurred as the season progressed (data not shown). Concomitantly, increased water stress was observed relative to the degree of deficit irrigation [stem water potential values of ~2.0 MPa for T3 treatments occurred by mid-summer] (data not shown). The soil for this site is lighter (higher percentage of sand) in comparison with Site 1, and has less water holding capacity. Water depletion below 50% field capacity was observed for deficit treatments in the 3 ft profile in each year (data shown previously, but omitted for space considerations). The lack of considerable adverse effects on yield is likely attributed to a combination of early harvest timing, and low productivity associated with Tieton.

Table 4. Effect of 2011 irrigation treatments on average tree yield of 'Tieton'/'Mazzard'. LF = low frequency (one irrigation event per week); HF= high frequency (equivalent amount of total weekly irrigation supplied to LF, but provided in small doses every other day). Data are means of 5 replications (n=5).

Treatment	Tree yield (lbs per tree)			
Irrigation	2009	2010	2011	2009-2011
T1 LF	93	69	94	256 a
T1 HF	92	61	82	235 a
T2 LF	92	76	83	251 a
T2 HF	93	74	84	251 a
RDI LF	86	79	97	262 a
RDI HF	94	76	89	259 a
Statist. signif.	ns	ns	ns	*

ns=not significant; * = significance at $P<0.05$.

Fruit quality was also not affected in 2011 by irrigation treatment (Table 5), as was the case in the two preceding years of the study (data not shown). Fruit were softer in 2011, regardless of treatment, which is interesting given the relatively cool year. No effects on postharvest fruit quality were observed in 2011 (data not shown).

In all years, results have been similar to those observed with Lapins, and can be attributed to a vigorous Mazzard root system, active at the 3 foot depth. By the time water stress develops, shoot growth is complete, and fruit has been harvested. Irrigation frequency did not consistently affect yield or fruit quality (Tables 4 and 5).

In 2010 we reported slightly lower water potential (greater stress) values for HF treatments compared to LF, and attributed increased evaporative losses from the wetted surface of the HF soil (wetted 4 times per week more frequently than LF). However, in 2011 these results were not observed.

We previously documented that water stress coinciding with high temperatures during early August did not exacerbate doubling/twinning of fruit the following year, as has been linked to peach and nectarine varieties.

Table 5. Effect of 2011 irrigation treatment on fruit quality attributes (fruit wt. and diameter; FF= firmness; SS=soluble solids; TA= total acids) at harvest for 'Tieton'/'Mazzard'. Data are means of 5 replications (n=4 for wt; n=250 for FF and mm fruit size; n=2 for SS and TA).

Treatment Irrigation	Avg. fruit wt. (g)	Avg. fruit size (mm)	FF g/mm	SS %	TA %
T1 LF	11.7	29.3	244	16.4	0.65
T1 HF	11.7	29.3	250	16.3	0.65
T2 LF	11.7	29.2	241	16.8	0.65
T2 HF	11.4	29.2	242	17.0	0.65
T3 LF	11.5	29.2	251	16.5	0.64
T3HF	11.6	29.1	233	16.8	0.65
RDI LF	11.5	29.2	246	16.4	0.65
RDI HF	11.7	29.4	246	16.7	0.65
Statist. signif.	ns	ns	ns	ns	ns

ns=not significant; * = significance at $P < 0.05$.

Methods

Objectives 1 and 3: A ten-year-old 'Lapins'/'Mazzard' orchard, located in The Dalles, OR, and trained to a multi-leader system, was used for a fertilization x irrigation experiment. The experimental design was a 2 x 2 factorial, split plot with four levels of irrigation volume and three levels of fertilization. Main plot treatments (irrigation volume) were arranged in an RCBD, with five replicates. Subplot treatments were fertilization. Each replicate comprised of four trees, with the two center trees used for data collection. Four levels of irrigation amount, based on replacement of a percentage of tree water use, were delivered once weekly via microsprinklers, and were: 1) T1 (65% ET applied in 2009 and 2010, and 96% ET applied in 2011), 2) T2, 55% of ET, 3) T3, 45% of ET and, 4) regulated deficit irrigation (RDI), in which trees received 45% ET between the first irrigation and pit-hardening, 65% ET from pit-hardening through harvest, and 45% ET postharvest. Irrigation sets were controlled by automated valves.

Nitrogen was either broadcast to experimental plots in a split application roughly two weeks apart, beginning within one to two weeks from full bloom, or provided through the irrigation system (fertigation). Fertigation events occurred once per week for an eight-week period. For each event, nitrogen was injected over a four hour period during the middle of the irrigation set. The fertigation pump was controlled by an automated programmer. Rates were 100, 100, and 60 lbs/a, for the broadcast, fertigation-high, and fertigation-moderate treatments, respectively.

Objectives 2 and 3: A nine-year-old drip irrigated 'Tieton'/'Mazzard' orchard, located in Mosier, OR, and trained to a multi-leader system, was used for an irrigation volume x frequency experiment. The experimental design was a 2 x 2 factorial, split plot with four levels of irrigation volume and two levels of frequency. Main plot treatments (irrigation volume) were arranged in an RCBD, in five replicates. Subplot treatment was frequency. Each treatment/replicate was applied to an individual row (13 trees), and 5 trees per row were chosen for measurements based on similar trunk size and canopies. Four levels of irrigation volume were applied to replace tree water use via drip irrigation either once weekly (Low frequency- 12 hour set), or every other day (High frequency- 3 hour set; totaling an equivalent amount of weekly irrigation as Low frequency).

Soil moisture was measured at three sites per replicate (Lapins), or one site per replicate for Tietons, to a depth of 3 feet, in 6 inch intervals using a neutron probe. Stem water potential was measured using a pressure chamber every 7-10 days, to study plant water status. Briefly, shoot leaves were selected in the mid portion of one-year-old shoot sections, bagged, and allowed to equilibrate for a minimum of 30 minutes prior to measurement. Four leaves per replicate tree were measured. Leaves were bagged roughly 1 hour prior to solar noon so measurements could bracket solar noon (+/- 1 hr).

Fruit and shoot growth was measured weekly during 2010 and 2011 at the Lapins site. Trunk circumference was recorded in spring, at harvest and in the fall, each year, and converted to cross-sectional area. At harvest, individual tree yields were recorded (5 per replicate for Tieton, and 6 per replicate for Lapins) and 100 fruit subsamples per replicate were collected for evaluation of fruit quality attributes (size, soluble solids, total acids, and firmness). Fruit quality attributes were evaluated similarly following four weeks of storage at 1° C.

Executive Summary

A three-year study was initiated in 2009 to investigate the effect of deficit irrigation treatments on sweet cherry yield, fruit quality and vegetative growth. Experiments were conducted at two sites. The first site consisted of microsprinkler irrigated, 9-year-old Lapins/Mazzard, planted at a density of 161 trees per acre. Treatment applications replaced different percentages of season-long reference evapotranspiration (ET0) and were, T1) 65% ET0 in 2009 and 2010, and 96% ET0 in 2011, T2) 55% ET0 (2009-2011), T3) 45% ET0 (2009-2011), and regulated deficit irrigation (RDI), which replaced 45% ET0 from the first irrigation in spring through pit-hardening, 65% ET0 from the end of pit-hardening until harvest, and 45% ET0 throughout the entire postharvest period (2009-2011). In addition, a nitrogen rate by delivery experiment was superimposed on the irrigation treatments. Nitrogen was applied at 100 lbs actual N per acre via microsprinklers (fertigation), or by ground application. A low rate of 60 lbs N per acre was also fertigated.

In 2011, yields of T3 and RDI were reduced ~10%, relative to T1 and T2. Over the three-year experiment, cumulative yields were reduced by 5% for all treatments relative to T1, albeit non-significantly. Yield reductions were attributed to differences in fruit number. No consistent differences were observed in fruit quality throughout the experiment. Fruit growth rate during 2010 and 2011, and final fruit size at harvest, were not affected by irrigation treatments, in any year. Shoot growth was not negatively affected by deficit irrigation. Trunk growth was limited in 2011 during the postharvest period by all deficit treatments, relative to T1. Stem water potential (indication of plant water status) declined (more stressed) as the season advanced, irrespective of treatment. In 2009-2010 treatment differences were slight and nonsignificant. However, when T1 was increased to 96% ET0 replacement in 2011, significant differences were observed by 3 weeks prior to harvest, where T1 trees had the highest water potential (less stressed). Water potential differences among the three deficit treatments were not significant. Measurement of soil moisture showed that roots of deficit treatments extracted significantly more water at the 2 and 3 foot depths. The additional water from deep soil reserves compensated for the reduced irrigation supply to deficit treatments.

Nitrogen (N) treatments (rate and delivery) did not consistently alter yield or fruit quality. In 2010, 60 lbs N via fertigation reduced yields. Differences between the two 100 lb per acre treatments were not significant. The reduction in 2010 yield followed high croploads in 2009 (14.5 tons per acre). There were no significant interactions between N and irrigation on yield or fruit quality.

The second site consisted of drip irrigated, 9-year-old Tieton/Mazzard. Trees were provided similar levels of replacement irrigation as described above, but delivered either once per week, or in four applications per week (same total volume per week). Frequency of irrigation did not consistently affect yield or fruit quality. Similar results were observed as described for Lapins above.

Overall, significant water savings were achieved at both sites. Fruit growth and quality was unaffected by deficit irrigation. Slight reductions in cumulative yield were observed in the most severe treatments. Drip irrigation resulted in greater water savings than micro-sprinkler irrigation, though this was enabled by the very short fruit development period, and low productivity, of Tieton. At both sites, deep soil moisture reserves served to limit the development of tree water stress during the pre-harvest interval. Caution is required when interpreting our results. Application of low percentages of ET0, such as those reported herein, to plantings in either shallow or light soils, or to dwarfing rootstocks and/or productive, late-season varieties (i.e., Sweetheart) would not be expected to produce similar results, and could in fact result in severe stress. Moreover, much our results can be attributed to good soil recharge occurring from adequate precipitation during dormancy and early spring. Future research should focus on deficit irrigation in high-density orchards planted on dwarfing rootstocks.

FINAL PROJECT REPORT
WTFRC/OSCC Project Number: CH-09-902

Project Title: Breeding and genetics program for PNW sweet cherry

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Other funding sources

Agency Name: USDA-CSREES Specialty Crops Research Initiative
Amt. 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-PIs include Dhingra and Oraguzie

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

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

Agency name: WTFRC/OSCC
Amount awarded: $62K from 2011-2012
Notes: Understanding the genetic basis of powdery mildew resistance in sweet cherry: PI: Oraguzie with Peace, Dhingra and Grove as Co-PIs

Total project funding: 286, 738

Budget

Organization Name: WSU-Prosser **Contract Administrator:** Mary Lou Bricker

Telephone: 509 335 7667 **Email address:**mdeseros@wsu.edu

Item	2009	2010	2011
Salaries	15,960	16,598	**17,262**
Benefits	9,895	10,291	**10,702**
Wages	13,000	13,000	**13,000**
Benefits	2,340	2,340	**2,340**
Equipment	5,000	5,000	**5,000**
Supplies	6,100	6,300	**6,500**
Travel	5,750	7,750	**5,750**
Virus-indexing services	3,000	3,000	**3,000**
Plant material	2,500	2,500	
Plot establishment and maintenance	25,500	29,500	**37,500**
Total	89,405	96,279	**101,054**

Footnotes: Salaries include a 1/2 time associate-in-research (2010) responsible for seed collection, raising seedlings in the lathhouse and greenhouse, and tree maintenance in the orchards. Wages are for the equivalent of 4 temporary assistants during bloom and 4 during the summer months Equipment includes propane tanks and frost pots. Supplies include propane, fertilizers, soil, pots, stakes, tree guards, tree labels, nets, chemicals and other lab consumables. $5, 750 is for domestic travel to see various production areas and micro climates, and visiting with operators and handlers while the additional $2000 for travel in 2010 is to attend the Rosaceae genomics conference (RGC5) in South Africa. Virus indexing services include annual ELISA testing of parents used in the breeding program and establishment of virus-free clones in NRSP5 for WSDA virus-free certification status.

OBJECTIVES

The goal of this project is to develop high-quality sweet cherry cultivars ideally suited for PNW growing regions. The specific emphasis of this project will be to:

- Establish and implement best management practices that insure optimal plant materials and protocols for sweet cherry breeding along with active renovation of seedling selection blocks to assure efficient use of field space.
- Assemble support personnel, establish linkages with other researchers and identify resources required for the breeding program.
- Produce genetically-variable sweet cherry selection populations that segregate for important target traits, then select best individuals within outstanding families for those traits.
- Propagate selections that out-perform target market-leading cultivars for performance and adaptation trials in a range of environments.

PROPOSED SCHEDULE OF ACCOMPLISHMENTS

End of year 1 (2009)

1. Establish and implement a written protocol for best nursery and field management that will ensure optimal tree growth for trait selection.
2. Germinate and maintain in the greenhouse ~1200 seed from crosses made in 2008
3. Develop a crossing plan emphasizing inter-mating of cultivars and other germplasm with novel fruit traits and pest and disease resistances.
4. Plant the remaining ~1000 seedlings from 2006 crosses and ~1000 seedlings from 2007 crosses in the field.
5. Evaluate fruit from fruiting seedlings after 5 days storage at 0-4°C for flavor, firmness, fruit size, bitterness, astringency, and skin and flesh colors.
6. Identify superior selections from 2004 & 2005 crosses
7. Propagate elite selections from 2004 & 2005 crosses.
8. Propagate trees to establish a new crossing block at WSU-Prosser.

End of year 2 (2010)

1. Update best management protocol
2. Germinate and maintain in the greenhouse ~2000 seed from crosses made in 2009.
3. Plant ~1000 seedling trees generated from 2008 crosses in the field
4. Develop a crossing plan emphasizing inter-mating of superior selections identified in the previous year.
5. Validate superior selections from 2004 & 2005 crosses and identify superior selections from 2006 crosses that will begin fruiting.
6. Implement selection tests for postharvest pitting and rain cracking for superior selections identified from 2004 & 2005 crosses.
7. Propagate more elite selections from 2005 crosses and superior selections from 2006 crosses.

End of year 3 (2011)

1. Update best management protocol
2. Germinate and maintain in the greenhouse ~2000 seed from 2010 crosses
3. Plant ~2000 seedlings from 2009 crosses in the field
4. Cross superior selections identified in the previous years
5. Implement selection tests for postharvest pitting and rain cracking for superior selections identified from 2004, 2005 & 2006 crosses.

6. Validate superior selections from crosses made in 2004, 2005 and 2006 and identify superior selections from 2007 crosses fruiting for the first time
7. Plant elite clones from 2004 & 2005 crosses at multiple testing locations.
8. Remove seedlings from 2004 & 2005 crosses and prepare land for planting.
9. Propagate superior selections from 2006 & 2007 crosses

The section below on results and discussion will focus on the milestones for 2011.

1. Update best management protocol

A draft of the best management protocol (BMP) for sweet cherry breeding has been developed to provide practical guidelines on breeding program operations such as seed collection and handling, seedling maintenance in the greenhouse and the lath house, tree planting and maintenance in the field, horticultural manipulations to encourage bloom and fruiting, fruit sampling and evaluation to pollen collection and artificial pollination. Additions to this document this year include new protocols for seed planting and seedling maintenance that guarantee ~95% seedling survival at the baby stage under controlled conditions including a temperature of 76 F, RH of 40-45% and 24 hours lighting supplied by grow lights. In the greenhouses, we can generate trees that are over 3 ft tall within 2 months. This accelerated seedling development necessitated field planting of less than a year old trees that are over 3 ft tall. In the previous years it took 2-3 years to generate seedlings of adequate size for field planting. In collaboration with Drs Iezzoni and Peace, standardized phenotyping protocols have been developed for both sweet and tart cherry breeding. These methods were used for the first time in 2010 for fruit quality assessment. Included in the BMP also are horticultural manipulations to slow trees down in the fall months prior to winter.

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

	Year		
	2009	2010	2011
No. of new parents used	5	29	6
No. of crosses made	55	107	74
No. seed	5000	2610	1162
% Germination	61	60	na
No. of seedlings	3000	1580	na
No. of seedlings in field	1994*	776*	na
No. of full sib families > 9 individuals	26	7	na

* following marker-assisted seedling selection (MASS). na = not available.

2. Germinate and maintain in the greenhouse ~2000 seed from 2010 crosses

Approximately 4000 seeds were generated from crosses made in 2010 of which 2610 were viable (Table 1). Mean seed germination across crosses was 60% which was very close to the number recorded in 2009. Over 1500 seedlings were raised in the greenhouses. The current main challenge for accelerated seedling development is prolonged seed germination. More than half of the seeds can germinate within six months following cold stratification while about 20-30% depending on cross may not germinate until a year or so later. Experiments are on-going in the

breeding labs to achieve more uniform germination to facilitate more streamlined seedling establishment and field planting.

3. Plant ~1000 seedling trees generated from 2010 crosses in the field

We have continued to stream line tree planting with just two field plantings performed this year (in spring and fall) unlike last year that we had 3 field plantings. Observation of vigor on trees planted in the October of 2009 suggested that the effect of winter freeze was minimal. The staggered nature of the planting was due to a combination of prolonged germination period as mentioned above and accelerated seedling establishment whereby trees grow over 3 ft tall in the greenhouses within 2 months of transplanting. Marker-assisted seedling selection (MASS) was used to cull inferior seedlings and of 1580 seedlings submitted for genetic tests based on DNA markers for self fertility and large fruit size, 776 that had favorable alleles for these traits were identified and planted in the field. To our knowledge, this is the only sweet cherry breeding program in the world that practices MAB. Apart from reducing orchard costs, MAB guarantees that only genotypes that have the potential to yield desired phenotypes are field planted.

4. Develop a crossing plan emphasizing inter-mating of superior selections identified in the previous year.

The crosses performed this year emphasized powdery mildew resistance, large fruit, pistil doubling and both early and late ripening. For large fruit size, advanced selections including FR1T7 (Sweetheart x Chelan), FR2T68 (Sweetheart x Ambrunes) and FR2T72 (Selah x Van) were pollinated with FR3T31 (Lapins x Chelan (13 g), 'Jubile' (14 g fruit) and 'Salihi' (18-20 g fruit) both from Turkey. Crosses for early ripening utilized FR2T10 (Gold x Dzherlo), Chelan, Brooks, Big Burlat, Index and 7146-11 crossed onto 'Kiona', an early variety. Late ripening was combined with powdery mildew resistance using advanced selections and/or cultivars such as DD, BB, Regina and Sweetheart, to ensure late progeny that are powdery mildew resistant since late cultivars are more vulnerable to powdery mildew attack than early ones. We did not use a lot of F_1 seedlings as seed parents due to poor fruit set. Also, many of the seedlings had low flower numbers and were better kept for fruit production. For successful inter-mating of F_1 seedlings it would be worthwhile to bud them on a rootstock.
Hand pollinations started in early April and continued up until the 25th of April. Approximately 70,000 flowers were hand pollinated in Prosser in the rainy and cold weather with a crew of 16. Up to 10 frost events were recorded during this period.

5. Validate superior selections from 2004 & 2005 crosses and identify superior selections from 2006 crosses that will begin fruiting.

Three advanced selections identified in 2009 from 2004 crosses were planted in Phase 2 trials in 2011 while the remaining 4 will be planted in the spring of 2012 at WSU Prosser, OSU MCAREC, Hood River, OSU The Dalles and in grower trials. A majority of these advanced selections had the '255' allele of 'BPPCT034' associated with large fruit. The 5 selections that were discontinued performed below the threshold value for fruit size (10 g), firmness (250 g/mm), soluble solids content (20%) and acidity (0.5%) (Table 2). Based on fruit evaluation of selections identified in 2010, we were able to select 8 individuals for planting in Phase 2 trials in 2012 (Table 3). The selection criteria focused mainly on the threshold values for fruit size, firmness, soluble solids content and titratable acidity. Other criteria for elimination of genotypes include pitting and cracking incidence as well as bitterness and astringency. Apart from fruit quality issues, FR2T38, FR3T75 and FR10T23 were eliminated

because they tested positive for *Prunus* necrotic ring spot virus (PNRSV) and *Prunus* dwarf virus (PDV). FR3T41 and R33T75 were eliminated as well because they had the '223' bp allele of BPPCT034 associated with small fruit. As you will notice, some parents and/or standard cultivars have larger fruit size than these selections. This is because the selection criteria focused not only on large fruit size but also on threshold values of other desirable traits as well. Two of the individuals chosen for powdery mildew resistance (from 'PMR-1') have 'Sweetheart' as a common parent. Although these are not as late ripening as 'Sweetheart', they do constitute an important part of our breeding strategy to develop a suite of new mid- and late-season disease resistant 'spray-free' selections. Finally, 'FR11T59', was selected for higher brix (28%) than those of the two parents including 'Rainier' and 'Regina'. Some fruit from this individual had brix up to 35%.

Another set of 20 individuals were identified for advancement to the Phase 2 following fruit evaluation in 2011 (Table 4). Several of these selections have already been evaluated for 3 years while the majority will be evaluated again in 2013 before identifying selections for planting into Phase 2 trials using a combination of DNA marker information and phenotypic data. It is important to note that the individuals identified fit into 5 target market categories and 5 of these including FR6T97 (Sweetheart x CC), FR7T26 (Sweetheart x CC), FR8T74 (GG op), FR9T89 (Kiona x Chelan) and FR13T54 (Sweetheart x BB) have powdery mildew resistance.

6. Implement selection tests for postharvest pitting and rain cracking for superior selections identified from 2004, 2005 & 2006 crosses.

We are still working on our methodology for recording pitting and rain cracking. In the mean time, we use 'incidence' which is calculated as the number of fruit that had pitting or cracking divided by the total number of fruit evaluated, to record these attributes on seedlings and advanced selections.

7. Propagate more elite selections from 2005 crosses and superior selections from 2006 crosses.

Twenty advanced selections identified in 2011 from 2004, 2005 and 2006 crosses (Table 4) have been propagated on Gisela 6 at Willow Drive Nurseries. These will be evaluated one more time in 2012 to choose individuals for planting into Phase 2 trials.

Table 2: Sweet cherry advanced selections first identified in 2009, their target market group and market leading cultivars for comparison.

Selection Tree Id. No.	Target market category	SI/SF	Harvest date			Fruit wt (g) Threshold 10g			Fruit firmness Threshold 250 g/mm			Fruit SSC Threshold 20%			Fruit TA (%) Threshold 0.5%		
			2009	2010	2011	2009	2010	2011	2009	2010	2011	2009	2010	2011	2009	2010	2011
	Chelan	*SI*	*25 Jun*	*17 Jun*	*27-Jun*	*8.7*	*10.0*	*8.5*	*251*	*>250*	*282*	*17.9*	*18.0*	*21.6*	*0.98*	*0.73*	*0.78*
FR3T62	ESM	SF	18-Jun		27-Jul	8.0		10.6	239		360	22.3		20.3	0.88		0.62
FR3T13	Valentine[X]		23-Jun		21-Jul	12.0		14.8	196		260	15.7		19.3	0.59		0.58
	Early Robin																
FR1T7	ESB	SF	12-Jun	2-Jul		11.7	10.5		314	317		16.9	20.6		0.93		
FR1T36	Freestone	SI	6-Jul	8-Jul	25-Jul	10.2	11.4	11.9	329	285	331	20.6	18.8	18.0	0.52	0.64	0.69
FR3T10	Freestone	SI	22-Jun		18-Jul	12.1		12.4	209		206	21.0		19.0	0.62		0.63
	Rainier	*SI*	*20-Jul*	*12-Jul*	*14-Jul*	*6.8*	*10.6*	*11.2*	*295*	*300*	*312*	*18.2*	*23.0*	*22.8*	*0.8*	*0.67*	*0.87*
FR2T30	LSB		6-Jul	15-Jul	27-Jul	10.0	9.2	12.8	233	329	287	19.6	20.7	16.0	0.85	0.63	0.87
FR1T14	LSB	SI	1-Jul	8-Jul		11.9	12.6		177	201		21.4	18.6			0.71	
FR2T63	LSB	SF	1-Jul	8-Jul	21-Jul	10.6	11.6	14.0	253	292	331	22.1	21.7	17.0	0.59	0.66	0.61
	Sweetheart	*SF*	*17-Jul*	*!4-Jul*	*29-Jul*	*7.7*	*9.71*	*10.6*	*272*	*297*	*348*	*16.1*	*18.0*	*17.1*	*0.9*	*0.78*	*0.61*
FR1T73	LSM	SI	9-Jul	21-Jul	27-Jul	11.2	10.3	15.0	209	251	258	24.5	27.2	22.0	1.12	0.89	0.89
FR1T74	LSM	SF	2-Jul	21-Jul	21-Jul	13.1	11.6	14.0	250	294	336		23.0	19.0		0.78	1.09
FR1T68	LSM	SI	14-Jul	21-Jul		11.7	11.0		226	254		20.5	21.4		1.1	0.82	
FR1T65	LSM	SI	1-Jul	8-Jul		10.0	12.5		194	204		25.9	18.5		0.7	0.62	

Individuals in red have been discontinued. TA=titratable acidity; SSC-soluble solids content.

Table 3: Sweet cherry advance selections first identified in 2010, their target market groups and market leading cultivars for comparison.

				Harvest date			Fruit wt. Threshold 10g			Fruit firmness Threshold 250 g/mm			Fruit SSC Threshold 20%			Fruit TA Threshold 0.5%		
Tree Id. No.	**Target market category**	**Cross**	**SI/SF**	2009	2010	2011	2009	2010	2011	2009	2010	2011	2009	2010	2011	2009	2010	2011
	Chelan		***SI***	*25-Jun*	*17-Jun*	*27-Jun*	*8.7*	*10*	*8.5*	*251*	*>250*	*282*	*17.9*	*18.0*	*21.6*	*0.98*	*0.73*	*0.78*
FR3T41	ESM **Early Robin**	Lapins x Chelan	SI	1-Jul	14-Jun		6.3	10.5		257	~280		20.7	20.0		1.09	0.56	
FR2T72	ESB	Selah x Van	SI	30-Jun	29-Jun	25-Jul	11.5	13.0	15.3	166	~270	271	21.4	25.0	21.0	1.05	0.88	0.61
	Bing		***SI***	*24-Jun*	*1-Jul*	*27-Jul*	*10.8*	*9.0*	*12.8*	*266*	*271*	*283*	*22.3*	*20.0*		*0.67*	*0.73*	*0.87*
FR1T5	MSM	Swrt x Kiona	SI	12-Jun	27-Jun	6-Jul	8.0	10.5	9.9	333	~280	297	16.7	19.0	16.0		0.74	0.83
FR10T51	MSM	EE x Lapins			1-Jul	21-Jul		11.0	10.2		>300	299		20.0	20.0		0.90	0.89
FR6T93	MSM	Swt x CC			1-Jul	21-Jul		12.0	11.0		300	267		19.0	22.5		0.68	0.71
FR7T37	MSM	Swt x CC			1-Jul	21-Jul		11.5	11.9		300	288		20.0	23.0		0.68	0.98
	Rainier		*SI*	*20-Jul*	*12-Jul*	*14-Jul*	*6.8*	*10.6*	*11.2*	*295*	*~300*	*312*	*18.2*	*23.0*	*22.8*	*0.80*	*0.67*	*0.87*
FR11T59	LSB	Rainier x Regina			8-Jul	21-Jul		11.0	14.8		>250	263		28.0	24.0		0.83	0.81
	Selah		*SF*	*7-Jul*	*8-Jul*	*21-Jul*	*11.7*	*13.5*	*16.8*	*184*	*219*	*275*	*18.8*	*20.0*	*23.4*	*0.75*		*0.97*
FR3T75	E-MSM	Selah op	SF	26-Jun	29-Jun	18-Jul	11.2	11.0	13.6	199	300	210	20.5	19.0	21.0	0.88	0.52	0.61
FR2T68	E-MSM	Selah x Van	SF	30-Jun	21-Jul	25-Jul	10.2	12.0	12.9	200	>270	292	17.6	19.0	18.8		0.73	0.76
	Sweetheart		*SF*	*17-Jul*	*!4-Jul*	*29-Jul*	*7.7*	*9.7*	*10.6*	*272*	*297*	*348*	*16.1*	*18.0*	*17.1*	*0.90*	*0.78*	*0.61*
FR6T59	LSM	Swt x EE			5-Jul	18-Jul		11.5	10.0		>300	288		19.0	22.0		0.74	1.01
FR6T63	LSM	Swt x EE			5-Jul	18-Jul		11.5	12.2		>300	292		22.5	20.4		0.85	1.1
FR10T23	LSM	Lapins x BB			19-Jul			11.0			>270			20.0				
FR2T38	LSM	Lapins x Ambrunes	SI	21-Jul	22-Jul	3-Aug	7.1	10.5	9.2	256	~220	307	19.8	20.0	14..9	0.67	0.61	0.49

FR3T075 and FR10T023 tested positive for PDV while FR2T038 have PNRSV, and have been discontinued along with FR2T68 and FR3T75. TA=titratable acidity; SSC=soluble solids content.

Table 4: Sweet cherry advance selections first identified in 2011, their target market groups and market leading cultivars used for comparison.

Tree Id. No.	Target market category	Cross	SI/SF	Harvest date			Fruit wt. (g) Threshold 10 g			Fruit firmness (g/mm) Threshold 250 g/mm			Fruit SSC (%) Threshold 20%			Fruit TA (%) Threshold 0.5%		
				2009	2010	2011	2009	2010	2011	2009	2010	2011	2009	2010	2011	2009	2010	2011
	Chelan		***SI***	***25-Jun***	***17-Jun***	***27-Jun***	***8.7***	***10.0***	***8.5***	***251***	***>250***	***282***	***17.9***	***18.0***	***21.6***	***0.98***	***0.73***	***0.78***
FR9T89	ESM	Kiona x Chelan				23-Jun			10.1			301			16.0			0.64
FR9T33	ESM	Swt x Moreau				23-Jun			10.1			296			16.0			0.75
	Rainier		***SI***	***20-Jul***	***12-Jul***	***14-Jul***	***6.8***	***10.6***	***11.2***	***295***	***~300***	***312***	***18.2***	***23.0***	***22.8***	***0.80***	***0.67***	***0.87***
FR1T70	LSB	Swrt x Regina	SI	26-Jun	19-Jul	29-Jul	8.5	10.1	13.1	283	232	300	21.9	23.6	21.0	0.59		0.86
FR2T24	LSB	Selah x Sunburst	SF	23-Jun		29-Jul	12.5		13.6	178		277	17.8		23.0	0.76		0.51
FR9T37	LSB	DD x Lapins				18-Jul			11.4			302			21.0			0.69
FR4T29	LSB	Selah x Krup				25-Jul			13.1			312			22.0			0.76
FR49T83	LSB	Swtt x Tieton				3-Aug			12.9			339			20.0			0.4
	Bing		***SI***	***24-Jun***	***1-Jul***	***27-Jul***	***10.8***	***9.0***	***12.8***	***266***	***271***	***283***	***22.3***	***20.0***		***0.67***	***0.73***	***0.87***
FR8T74	MSM	GG op				18-Jul			12.5			286			20.0			0.85
	Selah		***SF***	***7-Jul***	***8-Jul***	***21-Jul***	***11.7***	***13.5***	***16.8***	***184***	***219***	***275***	***18.8***	***20.0***	***23.4***	***0.75***		***0.97***
FR5T40	E-MSM	Selah x Selah				25-Jul			11.3			330			20.0			0.49
FR13T71	E-MSM	Selah x Moreau				25-Jul			10.2			272			27.0			0.81
	Sweetheart		***SF***	***17-Jul***	***!4-Jul***	***29-Jul***	***7.7***	***9.7***	***10.6***	***272***	***297***	***348***	***16.1***	***18.0***	***17.1***	***0.90***	***0.78***	***0.61***
FR6T97	LSM	Swt x CC				18-Jul			12.4			307			20.0			1.00
FR7T26	LSM	Swt x CC				27-Jul			13.3			285			22.0			1.04
FR7T45	LSM	Regina op				3-Aug			10.4			338			20.0			0.62
FR7T47	LSM	Regina op				18-Jul			11.3			329			21.0			0.87
FR8T28	LSM	Rainier x Summit				21-Jul			11.1			312			23.0			0.49
FRIT50	LSM	Swrt x Regina	SI	26-Jun	6-Jul	27-Jul	9.9	13.3	13.2	209	278	346	18.0	19.9	20.0	0.83	0.81	0.8
FR2T62	LSM	Lapins x Ambrunes			15-Jul	27-Jul		8.2	10.6		282	360		19.7	20.0		0.64	0.62
FR13T4	LSM	Swrt x BB				29-Jul			15.3			329			21.0			0.77
FR13T10	LSM	Swt x BB				25-Jul			10.7			319			27.0			0.97
FR13T54	LSM	Swrt x BB				25-Jul			10.2			303			24.0			0.77

TA=titratable acidity; SSC=soluble solids content.

EXECUTIVE SUMMARY

- 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 to selection criteria.
- There are 2 full time technicians in the program; one funded by WTFRC/OSCC and the other by the University. There is active collaboration with scientists within and outside of WSU as well as with overseas scientists. Infrastructure such as post-harvest fruit quality and molecular labs, greenhouses, shade house and orchard facilities constitute the foundation of a state-of-the art sweet cherry breeding and genetics program.
- Crosses have been made to date that emphasize novel sources of self fertility, large fruit size, powdery mildew resistance, novel flavors, bacterial canker resistance, cracking resistance, extended ripening date, high firmness, high soluble solids content, mechanical harvesting, reduced pistil doubling, cold tolerance, less bitterness and astringency. The germplasm base includes commercial cultivars, advanced/elite selections, wild and/or exotic germplasm, and ancestor cultivars. The crosses made in the last 2 years resulted in a total of 6580 seedlings of which 2772 were field planted following marker-assisted seedling selection. The crosses made in 2011 resulted in 1162 seeds which are currently under stratification in the cold room.
- Fruit evaluation was conducted for the first time in the program in 2009 and in subsequent years resulting in the identification of 15 advanced selections. Three of these have already been planted into Phase 2 trials at WSU Prosser, OSU MCAREC Hood River and The Dalles, as well as, in a grower trial in N Wenatchee. The remaining 12 genotypes will be planted in the spring of 2012. The 20 potential advanced selections identified in 2011 will be evaluated one more time in 2012 to eliminate individuals that do not have consistent performance or those that perform below threshold values for priority traits. Chosen individuals will be planted in Phase 2 trials in 2013.
- A parental crossing block has been established at the Roza orchards with 66 cultivars/F_1 seedlings and advanced selections. The first parental set planted in 2009 fruited for the first time in 2011 and these will be used for crosses in the spring of 2012.
- Approximately 1.0 acre of the seedling block at Roza orchards including 2004 and 2005 crosses have been pulled out following completion of fruit evaluation and identification of advanced selections in those blocks.
- Marker assisted breeding (MAB) is now routine in the breeding program. S-locus markers for self (in) compatibility have been in use since 2009 and these markers help to determine cross-compatibility and /or self fertility. Marker-assisted parent selection (MAPS) based on G2 markers is used to select parents to cross to encourage a high proportion of genetically superior progeny while marker-assisted seedling selection (MASS) is used to cull genotypes that have undesirable alleles for fruit size and firmness before field planting.
- The first set of virus clean advanced selections (18 in number) were planted at the pear acre orchards to provide a source of virus clean wood for phase 3 tree propagation.
- Bird netting has been installed at the seedling block at the Roza orchards covering an area of 8.0 acres.
- The powdery mildew advanced selections including AA, DD, GG and JJ from Sagemoore farms in Pasco as well as from WSU Prosser orchards were evaluated again in 2011. We will be compiling data from these genotypes collected since 2006 for presentation to the Cherry advisory committee who will then make a final decision to either advance or discontinue these genotypes.

FINAL PROJECT REPORT

Project Title: Marker-assisted breeding strategies for large fruit and self-fertility

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Cooperators: Fred Bliss (Davis, California), Dorrie Main (WSU-Pullman), Jim McFerson (WTFRC), Jim Olmstead (Univ. Florida)

Other funding sources

Agency Name: USDA-CSREES NRI Plant Genome
Amount awarded: $400K, Aug 2009 – Aug2011
Notes: "The development of COS markers for comparative mapping in the Rosaceae and their application for understanding variation in fruit size". PI: Iezzoni. Leveraged with WTFRC/OSCC funding. Developed and validates fruit size genetic markers for sweet cherry, providing the marker tools for this project.

Agency Name: WTFRC Apple Review
Amount awarded: $77,616, Feb 2009 – Dec 2010
Notes: "Developing an online toolbox for tree fruit breeding". PI: Main. Co-PIs include Peace and Oraguzie. Databasing support for WSU cherry and apple breeding programs.

Agency Name: USDA-CSREES Specialty Crop Research Initiative
Amount awarded: $2.0 M plus equal matching, Sep 2009 – Aug 2013
Notes: "Tree Fruit GDR: Translating genomics to fruit tree agriculture". PI: Main. Co-PIs include Peace and Oraguzie. Leveraged with WTFRC funding. For practical application of bioinformatics to tree fruit crops.

Agency Name: USDA-CSREES Specialty Crop Research Initiative
Amount awarded: $3.8 M plus equal matching, Sep 2009 – Aug 2013
Notes: "A total systems approach to developing a sustainable, stem-free sweet cherry production, processing and marketing system". PI: Whiting. Co-PIs include Oraguzie. Leveraged with WTFRC/OSCC funding. In addition to developing genomics knowledge on cherry abscission for

amenability to mechanical harvesting, includes cell number and size measurements of local cultivars that integrated with this project.

Agency Name: USDA-CSREES Specialty Crop Research Initiative
Amount awarded: $7.2 M plus equal matching, Sep 2009 – Aug 2013
Notes: "RosBREED: Enabling marker-assisted breeding in Rosaceae". PI: Iezzoni. Co-PIs include Peace and Oraguzie. Broad umbrella project on genetic marker development and application. Leveraged with WTFRC/OSCC funding. Our MAB here for fruit size in Dr. Oraguzie's breeding program is enhanced with higher-resolution genetic markers, and used in RosBREED as a MAB success story.

Agency Name: WTFRC NW Cherry Review
Amount awarded: $95K in 2010, $100K in 2011
Notes: "Breeding and genetics program for PNW sweet cherries". PI: Oraguzie. Beneficiary of MAB for large fruit and self-fertility; phenotypic performance data of seedlings mostly provided by this program.

Agency Name: WTFRC NW Cherry Review
Amount awarded: $13K in 2010
Notes: "Consulting for the Pacific Northwest Sweet Cherry Breeding Program". PI: Iezzoni. Consultancy to enhance success of the PNWSCBP.

Total Project Funding: $88,600

Budget History:

Item	2010	2011	
Salaries			
Benefits			
Wages[1]	26,484	27,032	
Benefits	2,516	2,568	
Equipment			
Supplies[2]	11,000	11,000	
Travel[3]	4,000	4,000	
Miscellaneous			
Total	44,000	44,600	

Footnotes:
[1] Technical assistance - molecular work (Pullman): $15,000 (wages and 9.5% benefits) in year 1 and 4% increase in year 2; Technical assistance - phenotyping work (Prosser): $14,000 (wages and 9.5% benefits) each in year 1 and year 2.
[2] Reagents and consumables - molecular work (Pullman): $1500 for DNA extraction, $2000 for S-genotyping, $14,500 for fruit size marker genotyping, spread evenly over the two years; Consumables for phenotyping (Prosser): $2000 per year
[3] Within-state: Pullman-Prosser for coordination of experimental work: $2000 per year; Interstate: WSU-MSU for coordination among PIs: $2000 per year

RECAP ORIGINAL OBJECTIVES

The primary goal of this project was to finally apply DNA markers for improved efficiency of Pacific Northwest sweet cherry breeding (PNWSCBP), putting to use many years and dollars invested in developing the tools and infrastructure.

Specific objectives were to:

1. Deliver marker-assisted breeding (MAB) strategies for both large fruit and self-fertility to the PNWSCBP.
2. Exploit additional opportunities for key trait improvement in the PNWSCBP using MAB.
3. Coordinate MAB strategy development, implementation, and outreach in the PNWSCBP with Dr. Iezzoni's NRI project and RosBREED, and Dr. Main's Tree Fruit GDR project.
4. Deliver knowledge on cultivar genetic potential for fruit size to PNW cherry growers.

SIGNIFICANT FINDINGS

Objective 1: **Deliver MAB strategies for both large fruit and self-fertility to the PNWSCBP**.

- Strategies for DNA-informed breeding were delivered to the young, modern PNW sweet cherry breeding program, and enacted in the program since spring 2010 to provide more efficient and precise breeding.
- A net projected savings of more than $80K was estimated from the culling of more than 1500 seedlings predicted to be self-infertile or genetically inferior for fruit size ('09 and '10 crosses).
- Segregating populations for the next wave of seedlings ('10 and '11 crosses) were efficiently enriched for self-fertility and large fruit genetic potential by incorporating DNA information into crossing decisions of the last two years.
- The list of advanced selections destined for Phase 2 replicated trials was reduced by consideration of DNA information on fruit size and self-fertility. The potential savings here have not yet been calculated.
- Other aspects of breeding are now conducted more easily, precisely, and cost-effectively through MAB applications such as parentage verification and deduction.
- A full-time Genetic Screening Technician was hired at WSU for a critical role in sustained operations of the PNW Tree Fruit Genotyping Laboratory.
- Successful protocols for high-throughput DNA extraction and high-throughput genotyping were established for sweet cherry, meeting the logistical needs of routine MAB.
- The PNWSCBP is the first stone fruit breeding program in world to routinely conduct high-throughput marker-assisted seedling selection.
- Refinement of the fruit size genetic tests continues. Large datasets were obtained over the last two years for several thousand seedlings ('04, '05, and '06 crosses). Numerous fruit quality traits were evaluated. Genotypes were obtained at three genomic associated with fruit size, and *S*-genotypes. Statistical analyses in late 2011 are expected to provide a comprehensive understanding of the potential for fruit size MAB particularly in the context of other important traits.

Objective 2: **Exploit additional opportunities for key trait improvement**.

- Several further potential genetic tests have arisen and are being translated for use in the breeding program, including for the traits of firmness, sweetness, acidity, fruit color, and a non-'Stella' source of self-fertility.

Objective 3: **Coordinate MAB strategies with large federal projects**.

- Coordination with several large federal projects continued, with information on genetic markers shared among our projects.
- Advances were reported to industry and scientific audiences.
- The PNWSCBP is strategically positioned to utilize large federal investments in Rosaceae genomics, genetics, and breeding.

Objective 4: **Deliver knowledge on cultivar fruit size genetic potential to PNW cherry growers**.

- Components of fruit size are being dissected in detail for representative current industry cultivars, and combined with fruit size genotypes at three genomic regions. Statistical analyses will continue in late 2011, followed by delivery of knowledge to PNW cherry growers.

RESULTS & DISCUSSION

1. Deliver MAB strategies for both large fruit and self-fertility to the PNWSCBP.
Strategies were delivered, positively impacting the efficiency of breeding operations. Further refinement of the strategies will arise from large-scale data analyses in late 2011.

MAB strategies in action:

Crossing decisions in spring 2010 and spring 2010 were supported by DNA information. Examples include:

- Using knowledge on fruit size (and firmness) genetic potential of parents to produce families enriched with genetics for large (and firm) fruit.
- As above to discontinue use of parents with poor genetic potential.
- Using *S*-genotype knowledge of parents to avoid incompatible crosses.
- Using *S*-genotype knowledge of parents to produce families with 25%, 50%, 75%, or 100% of seedlings predicted to be self-fertile, as desired.
- Where selfing is not desired (e.g. to make a specific cross between two different parents), avoiding the use of self-fertile mothers in crosses because they tend to produce a large proportion of selfed seedlings
- Selfing certain self-fertile parents, as resulting seedlings will always be self-fertile and other useful genetics from that parent can be concentrated.
- Using certain recent selections (from ’04 crosses) as new parents – those with predicted superior breeding value based on all available phenotypic, pedigree, and DNA information.

Interpretation of seedling performance was supported by DNA information. *S*-genotypes and fruit size genotypes of ’04-‘06 seedlings are known. Parentage of seedlings can now be verified by genetic markers. Examples of using this information include:

- Better estimates of parental breeding values for various traits.
- Verifying germplasm sources of certain valuable and rare traits: very firm fruit, freestone.
- Understanding cases of seedlings that don’t perform as predicted from their supposed parents – because their deduced true parentage is different to that originally recorded.

Advancement to Phase 2 selection decisions were supported by DNA information. Examples include:

- Elevation to selection status of several seedlings with large and firm fruit that also possessed the genotypes predicting such genetic potential.

- When all else is equal, selecting seedlings that are self-fertile (carrying the S_4' allele) rather than self-incompatible.
- Excluding from further consideration an early-season selection that was predicted by genetic markers to have small fruit size genetic potential.

Planting decisions in fall 2010 and twice in spring 2011 were supported by DNA information:

- In fall 2010, 800 seedlings growing in the lath house and destined for fall planting were genotyped with two available predictive markers of genetic potential, and most of those with predicted small to medium fruit size and self-incompatibility were culled. Only 340 seedlings were subsequently planted. This effort was calculated to provide a net projected savings (in efficiency, via reallocation of resources) of around $25,000 by avoiding costs involved in planting and future maintenance and evaluation of inferior seedlings. The cost of DNA analysis of these seedlings was less than 10% of those projected savings!
- In May 2011, 1500 seedlings growing in the lath house and destined for spring planting were genotyped with the above-mentioned genetic tests. Less than half the seedlings were subsequently planted, providing an estimated net savings in future breeding expenditure of about $45,000!
- In June 2011, another 400 seedlings were genetically screened. Less than half of those seedlings were subsequently planted, providing an estimated net projected savings of more than $10,000!

Progress was made toward further refinement of our knowledge of genetic control of fruit size, the effects of selfing, and utility of this knowledge for the PNWSCBP. Our current understanding of the utility of the fruit size genetic tests comes from analyses of 2009 season data for more than 200 fruiting seedlings of ’04 crosses. Many hundreds more seedlings have since fruited, and thousands of seedlings are available. We are using the thousands of fruiting ’04, ’05, and ’06 seedlings of the PNWSCBP as the much-expanded “training population” on which to calculate the explanatory power of DNA tests for fruit size.

Performance evaluation of the training population:

- In 2010, fruit size performance data was obtained for 355 fruiting seedlings (189 of seedlings created from ’04 crosses, and 166 seedlings of ’05 crosses), using phenotyping protocols standardized with MSU. Traits evaluated were ripening date, fruit firmness, fruit weight, fruit length and width, pit weight, pit length and width, stem length, stem pull force, fruit shape, fruit skin color and fruit flesh color (based on both spectrophotometer reading and visual rating), freestone/clingstone, titratable acidity (TA), pH, and total soluble solids (TSS).
- For the ’04 seedlings which were also phenotyped in both the 2009 and 2010 seasons, fruit size was fairly consistent between the two seasons (R=0.64 based on each seedling’s maximum observed fruit weight).
- In 2011, many more seedling trees were fruiting for the first time, the most to date in the history of this young breeding program. Seedling numbers are summarized in Table 1. Evaluated fruit quality traits were the same as in 2010, although given the overwhelming number of fruiting trees this year, only seedlings with field-assessed fruit averaging 10 g or larger were harvested and evaluated.
- Fruit quality data from the 2011 season is still being error-checked at the time of writing. Observed distributions of traits and correlations among them cannot be reported at this time. Such summarization of performance data will be conducted in late 2011.

Table 1. Numbers of seedlings in the breeding program undergoing performance evaluation and genetic screening in recent seasons.

Cross year	No. of seedlings				
	Available	Genotyped	Phenotyped in 2009	Phenotyped in 2010	Phenotyped in 2011[a]
'04	250	all	219	189	96
'05	1500	all	0	166	220
'06	3600	2120	0	0	330

[a] Only a subset of fruiting seedlings, those with superior field performance, were evaluated for fruit quality in 2011

Genetic screening of the training population:

- A full-time Genetic Screening Technician was hired at WSU: Terry Rowland, who reliably performs routine genetic screening for both the PNWSCBP and the Washington apple breeding program (approx. half-time) as well as working on separately funded research projects (approx. half-time). The establishment of this critical permanent position helps ensure that the skilled labor for genetic screening is now available throughout the year when needed by WSU's tree fruit breeding programs.
- Successful protocols for high-throughput DNA extraction (using the silica bead method) and high-throughput genotyping (using an ABI 3730xl DNA Analyzer purchased through a WTFRC grant in 2009) were developed for sweet cherry in our PNW Tree Fruit Genotyping Laboratory in Pullman (managed by C. Peace's Associate in Research, Daniel Edge-Garza). Such processing ability had eluded us in previous years. The protocols can now be routinely conducted for *S*-genotyping and fruit size genotyping, using leaf material collected in spring through to September. In the meantime, we continue to tweak the protocols for maximum efficiency.
- DNA was extracted from approximately 4000 seedlings of '05 and '06 crosses, with a success rate of >95%.
- Genotyping of these seedlings (success rate >95%) was conducted for the *S* locus controlling self-fertility/cross-compatibility (*S*-universal and S_4' genetic tests) and three fruit size genetic loci (five markers, mostly obtained from Dr. Iezzoni's federal NRI project). Scoring of this data is ongoing.
- Seedlings with unintended parentage remains a significant proportion in '06 populations as in '04 (Figure 1), but at least the genetic screening can identify them.
- Much genetic variation, in the form of multiple alleles, has been observed in the '04, 05, and '06 breeding populations for markers at the fruit size genetic loci and at the *S* locus. An example is shown in Figure 2. This genetic variation in our training population represents opportunities to discover or verify "jewels in the genome". This genetic variation evident in breeding populations also represents opportunities for MAB to fine-tune crosses and planted trees to focus later performance evaluation efforts on the very best genetics.

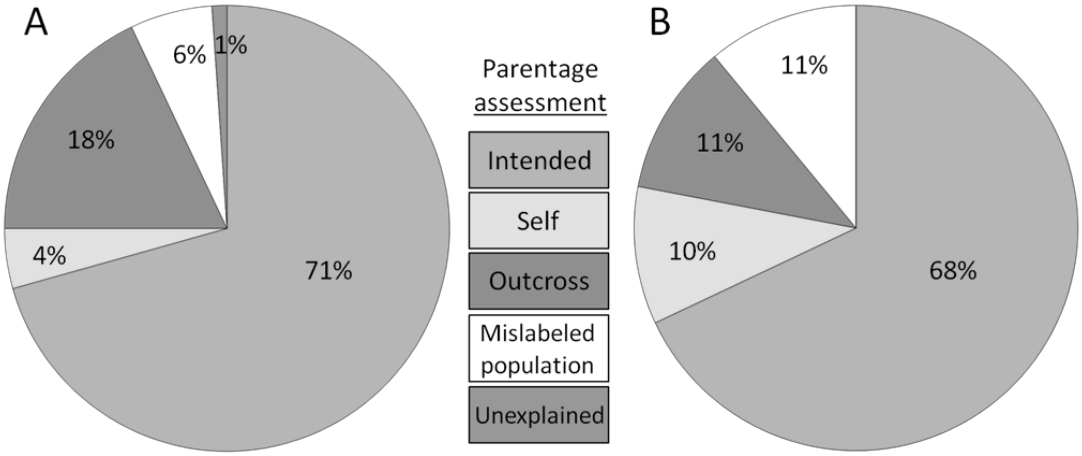

Figure 1. Parentage proportions of some sweet cherry seedlings of the PNW sweet cherry breeding program as determined by genotypes at seven markers. **A**. For a 375 seedling subset of ’06 progenies, resulting from seven crosses among nine parent cultivars. **B**. For all 243 of the ’04 seedlings, resulting from 22 crosses among 17 parents (data from 2009 final report, “Establishing the Marker-Assisted Breeding Pipeline for sweet cherry”). All parentage types other than “intended” are unintended crossing outcomes.

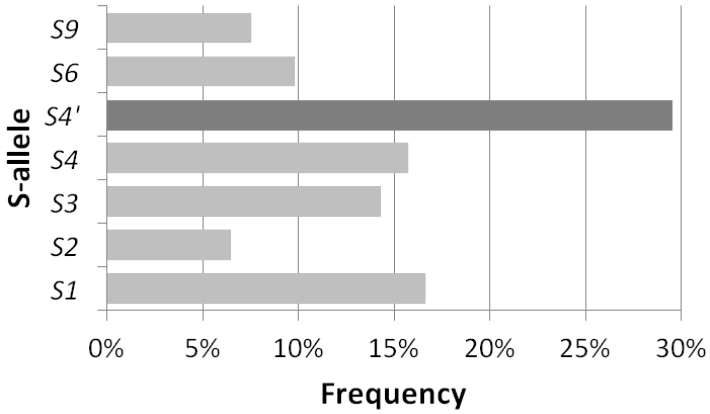

Figure 2. Allelic frequency at the *S* locus for 375 seedlings from seven ’06 crosses among nine parent cultivars, a snapshot of the 4000 seedlings under scrutiny for seven genetic markers. The S_4' allele here provides self-fertility to seedlings possessing it, and was the most common allele observed.

Explanatory power of DNA tests for fruit size

- Analyses to determine the explanatory power of DNA tests for fruit size will be conducted in late 2011. The results will be used to further refine MAB strategies will arise from large-scale data analyses in late 2011.
- In the meantime, the categories of largest and smallest fruit described by the genotype at a single DNA marker from the 2009 season data were again predictive of fruit size in 2010.

Further refinements and delivery of MAB strategies for fruit size, self-fertility, and other traits

- Statistical analyses in late 2011 are expected to provide a comprehensive understanding of the potential for fruit size MAB particularly in the context of other important traits. Specific aims will be to:
    - Determine the impact of marker-assisted seedling selection (MASS) for fruit size on achieving sufficient genetic gain in other essential breeding target traits (i.e., firmness and flavor components).
    - Determine the genetic basis for low occurrence of large-fruited seedlings in sweet cherry crosses.
    - Determine the effect of MASS for self-fertility on obtaining enough seedlings with large fruit size from which to make selections for other traits.

2. Exploit additional opportunities for key trait improvement in the PNWSCBP using MAB.
Several further potential genetic tests have arisen and are being translated for use in the breeding program. A genetic marker for acidity and fruit color (the genomic region happens to be associated with these two otherwise independent traits) was taken further though the MAB Pipeline toward routine use in the PNWSCBP. This marker has been given extra attention in the RosBREED project (together with our main fruit size markers), which is helping its Pipelining. Firmness and sweetness are associated with our main genetic test for fruit size on chromosome 2, and because those traits are routinely evaluated on seedlings in the breeding program, genetic tools for their improvement are also proceeding. A marker for a novel source of self-fertility from the Spanish cultivar Cristobalina was reported by a European lab, and will be used in late 2011 to identify Cristobalina seedlings that can be immediately used as parents in spring 2012 to confer their self-fertility. Efforts in the RosBREED project are furthering the development of markers for all of the PNWSCBP's Primary traits, Secondary traits, and Market-defining traits, and we expect major/many discoveries by early 2012 using RosBREED, resources, approaches, and momentum.

3. Coordinate MAB strategy development, implementation, and outreach in the PNWSCBP with Dr. Iezzoni's NRI project and RosBREED, and Dr. Main's Tree Fruit Genome Database Resources project.
Coordination with other projects continued throughout the last two years, with information on genetic markers shared among our projects. Latest advances were described in the 2010 and 2011 Cherry Field Days at IAREC in Prosser, WA. Our main fruit size genomic region is a target for detailed genomic dissection in both if Dr. Iezzoni's federally funded projects, to find the underlying genes as well as better understand its predictive power. This genetic test was chosen as the major example of impactful MAB in presentations and reports to scientific audiences by Dr. Peace: a RosBREED talk by at the American Society for Horticultural Science annual conference in Aug 2010 (Palm Desert, CA), a talk at the International Rosaceae Genomics Conference in Nov 2010 (Stellenbosch, South Africa), a poster at the American Society for Horticultural Science annual conference in Sep 2011 (Waikoloa, HI), and the Community Breeders' Page article for the Nov 2011 quarterly RosBREED Newsletter. Other genomic regions influencing cherry fruit quality also currently targeted in RosBREED. In Dr. Main's federal project, genotypic data collected on the parent pool of the breeding program is being uploaded to the secure Breeders' Toolbox database for this breeding program, for ready access to DNA information by Dr. Oraguzie. Further functionalities in the Breeders' Toolbox to facilitate ready incorporation of DNA information into crossing decisions are being developed in RosBREED. The PNWSCBP is strategically positioned to utilize large federal investments in Rosaceae genomics, genetics, and breeding.

4. Deliver knowledge on cultivar genetic potential for fruit size to PNW cherry growers.
To determine what physiological components of fruit size are associated with genotypic categories for fruit size potential, 15 cultivars were evaluated over two seasons. Considerable phenotypic variation was observed, with some consistency between years. However, to be able to place any cultivar into a

genetic category that can predict fruit size and levels of its physiological components, full results from Objective 1 activities are required.

Cultivars and selections were chosen to cover the available range of our current idea of fruit size genotype groups, with one to three representative cultivars per group. To maximize expression of fruit size genetic potential, whole trees or two large branches were bloom-thinned to one bud per spur for 15 cultivars and selections: 14 in 2010 (Benton, Bing, Cashmere, Chelan, Cowiche, Rainier, Selah, Skeena, Summit, Tieton, Van, BB, CC, and DD) and 11 in 2011 (Benton, Bing, Cashmere, Chelan, Cowiche, Kiona, Selah, Skeena, Summit, CC, and DD); underlined cultivars were those not evaluated in the other year. Recorded for these individuals were various components of fruit size: fruit weight, fruit shape, fruit width at cheeks, fruit width at suture, fruit length, fruit volume, pit weight, pit width in two different planes, and pit length (fruit samples were frozen for later determination of cell number and cell size). Cowiche consistently had the largest fruit, but only a modest fruit weight to pit weight ratio due to relatively large pits, whereas Selah had large fruit and an exceptionally high proportion of that being flesh and not pit (Figures 3 and 4).

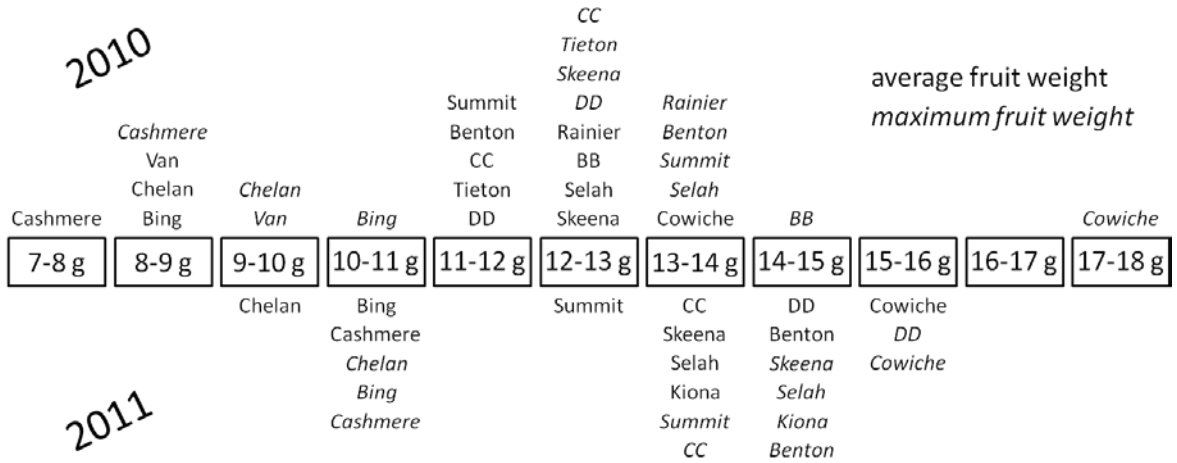

Figure 3. Fruit weight observations for 15 sweet cherry cultivars in two fruiting seasons. Shown are weights for an average of the largest five fruit on a tree ("average fruit weight") and the largest single fruit on a tree ("maximum fruit weight"), following thinning manipulations to maximize fruit size.

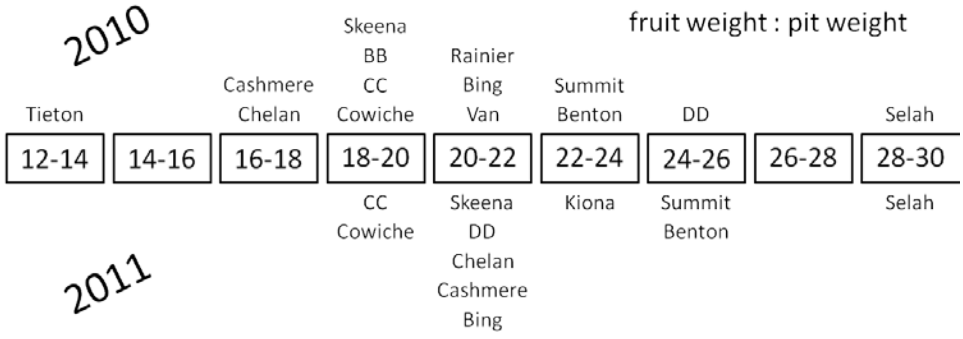

Figure 4. An example of cultivar variation for physiological components of fruit size: ratios of fruit weight to pit weight for 15 sweet cherry cultivars in two fruiting seasons. Measurements are from the average of the largest five fruit on a tree, following thinning manipulations to maximize fruit size.

EXECUTIVE SUMMARY

Our 2010-2011 project on developing and delivering marker-assisted breeding (MAB) strategies for the Pacific Northwest sweet cherry breeding program (PNWSCBP) achieved several important milestones for this young, modern cultivar development program. The primary goal of this project was to finally apply DNA markers for improved sweet cherry breeding efficiency, putting to use many years and dollars invested in developing the tools and infrastructure. This goal was achieved.

The PNWSCBP now applies DNA-based genetic markers to enhance the efficiency and precision of breeding operations. DNA information is incorporated in crossing decisions, seedling field evaluation decisions, and selection advancement decisions. Marker-assisted seedling selection on several thousand seedlings at a time helps eliminate genetically inferior material. *S*-genotyping and fruit size genotyping of new cultivar releases is also available. The PNWSCBP is the first stone fruit breeding program in world to routinely conduct high-throughput marker-assisted seedling selection. Leadership of the PIs and Cooperators in allied projects has strategically positioned the PNWSCBP to utilize large federal investments in Rosaceae genomics, genetics, and breeding.

MAB delivery accomplishments include:

- A net projected savings estimated at more than $80K, by culling more than 1500 seedlings predicted to be self-infertile or genetically inferior for fruit size.
- Efficient enrichment for self-fertility and large fruit genetic potential of the next wave of seedlings, by incorporating DNA information into crossing decisions of the last two years.
- Reduction of the number of advanced selections destined for Phase 2 replicated trials, by consideration of DNA information on fruit size and self-fertility.

Other accomplishments include:

- Filling the full-time Genetic Screening Technician position for a critical role in sustained operations of the PNW Tree Fruit Genotyping Laboratory.
- Establishment of successful protocols for high-throughput DNA extraction and high-throughput genotyping for sweet cherry, meeting the logistical needs of routine MAB.

Projected cost savings are already reflected in the requested budget for the next three years of the breeding program because having fewer planted trees is cheaper (lower land use fees and lower maintenance costs). Savings should also become evident in future years as the trees that survived the genotyping cull begin to fruit, with fruit quality evaluation efforts not wasted on many genetically inferior seedlings. Savings can also manifest as resource reallocation, if a certain limited amount of land, labor, and other resources are used as efficiently as possible for generating superior new cultivars. Such decisions are still in flux as the repercussions of routine MAB sink in for all involved.

Some major aspects of the project are continuing through to the end of 2011. Refinement continues for three fruit size genetic tests. Large phenotypic and genotypic datasets obtained over the last two years, for cultivars, selections, and several thousand seedlings of the earliest crosses and, will be statistically analyzed in late 2011. The major expected outcome is a comprehensive understanding of the genetic potential for fruit size, particularly in the context of other important traits. Such knowledge will be immediately developed into refined MAB strategies in breeding, and delivered to the PNW sweet cherry industry as genetic categories that define cultivar fruit size genetic potential.

Future Directions:
Determine the role for MAB in a cost-efficient PNWSCBP; Optimize replicated trials and support cultivar release and adoption decisions by revealing and communicating genetic potential for commercial performance; Take promising new trait markers through the MAB Pipeline; Enhance and utilize bioinformatics support for maximized access to performance and DNA-based data; Coordinate with the RosBREED and tfGDR projects to maximize benefit for the PNW sweet cherry industry.

FINAL PROJECT REPORT

Project Title: Targeting the ethylene biosynthetic pathway to improve cherry quality

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Other funding sources

Agency Name: USDA-CSREES Specialty Crop Research Initiative
Amount awarded: $3.8 mil plus equal matching, Sep 2009 – Aug 2013
Notes: "A total systems approach to developing a sustainable, stem-free sweet cherry production, processing and marketing system". PI: Whiting. Co-PIs include Oraguzie and Dhingra. Develops genomics knowledge on cherry abscission for amenability to mechanical harvesting, with ethylene physiological analyses of local cultivars.

Total Project Funding: $56,000

Budget History:

Item	**Year 1: 2010**	**Year 2: 2011**	
Salary	5,312	5,524	
Benefits	2,019	2,099	
Wages	4,959	5,157	
Benefits	471	490	
Equipment			
Supplies	10,739	10,230	
Travel	4,500	4,500	
Miscellaneous			
Total	28,000	28,000	

RECAP ORIGINAL OBJECTIVES

Our overall goal is to improve the storage and shelf life of sweet cherry cultivars through an understanding of the ethylene genetic control in this apparently non-climacteric crop. Specific objectives were to:

1. Characterize differences in the ethylene biosynthetic pathway between sweet cherry and peach—*why do they ripen differently?*
2. Identify ethylene physiology categories of cherry cultivars that simplify development of strategies to extend market life—*does increased synthesis and/or sensitivity to ethylene determine ultimate postharvest response?*
3. Provide specific selection criteria in breeding for improved market life based on ethylene biosynthesis and response—*can a simple high-throughput physiological or genetic assay help predict market life?*

SIGNIFICANT FINDINGS

- Sweet cherry is confirmed as a non-climacteric fruit.
- Mature ripe sweet cherry fruit did not respond to ethylene treatment by any changes in respiration or ethylene production.
- An early peak in ethylene production in tiny fruitlets subsided well before, just before, or soon after color break (depending on the cultivar) and became undetectable upon further maturation.
- Differences in several *ACS* genes were observed among three cherry cultivars over six sampling times throughout fruit development
- Activity of ACO (the "ethylene forming enzyme") in sweet cherry fruit declined to zero with advancing fruit development. In stark contrast, ACO activity in peach was high in early season fruit, leveled off, then had a medium activity in mature ripe fruit.
- ACO activity patterns for nine sweet cherry cultivars throughout fruit development showed several patterns. However, no significant associations of these patterns with fruit quality traits were detected, including after a 1-week storage regime.
- The lack of endogenous ethylene production or ethylene response indicates that there are no off-tree quality improvements to be realized for sweet cherry fruit beyond color break.
- These results lead us to believe that the difference between cherry and its climacteric relatives is due to synthesis over-regulation and/or is in the ethylene signal response pathway such as the absence of critical response elements.

RESULTS & DISCUSSION

Sweet cherry and peach fruit were compared for their ethylene physiology and responses, which determined that sweet cherry is truly non-climacteric. Next, components of ethylene biosynthetic machinery were examined to narrow down the missing component in sweet cherry compared to its classically climacteric relative, peach. In cherry, *ACS* gene expression was detected in developing

fruit, but ACO activity beyond color break was low to zero. Therefore, ethylene production machinery appears functional until color break. Different patterns of ACO activity were observed among diverse cultivars, but no fruit quality differences were found to be associated.

Fruit sampling, respiration, and endogenous ethylene production: Cherry fruit were collected from WSU, IAREC, Prosser twice per week beginning at full bloom (April 2010) until the commercial harvest (July 2010). Three cultivars 'Bing', 'Chelan' and 'Skeena' with differential abscission characteristics, as abscission is related to ethylene levels, were included in the present study. 'Early Elberta' peach was harvested from a nearby commercial orchard for a comparative fruit during the 2010 season. During the 2011 growing season, cherry fruit of 9 varieties, including 'Bing', 'Chelan', and 'Skeena' were harvested weekly at WSU, IAREC, Prosser. 'Early Elberta' peach was harvested from the same orchard as in 2010.

In contrast with climacteric fruits in general, immature (green) stages of sweet cherry fruit were associated with higher rates of respiration and low ethylene production. Respiration and ethylene production declined with fruit development. Ethylene levels were undetectable following color break. In the climacteric peach fruit studied, fruit in early developmental stages exhibited a high respiration rate with no ethylene production. As peach fruit developed to maturity and beyond, both respiration and ethylene increased concomitantly.

Response to added ethylene: Exogenous ethylene was treated on early season 'Bing' cherries at 75 DAFB, pre-climacteric 'Early Elberta' peach at 131 DAFB, and post-climacteric 'Early Elberta' peach at 146 DAFB. Etherel (Sigma Aldrich, St. Louis, MO) at 21% AI was diluted to a concentration of 250 ppm. The solution was mixed with ddH_2O and then titrated with 10 M KOH to a pH of 6.0. At this pH the solution releases ethylene gas. Individual dilutions were made to achieve 125 ppm and 12.5 ppm. Each of the ethylene concentrations were placed into 50 ml beakers with a 20 ml etherel solution and set into 6.0L respiration chambers holding the fruit. The final concentrations provided 1.7 ppm, 0.7 ppm, and 0.11 ppm in a constant flow system of 100 ml/min air. The ethylene and CO_2 levels were monitored by gas chromatography as specified in year 1 of our report as (n=3). Each of the chambers receiving ethylene was quantitatively reported by subtracting the etherel supplied ethylene, from each data point and reported a ul/Kg/Hr. Ethylene was reported for pre-climacteric fruit treatments.

To study the non-climacteric nature of cherry fruit in relationship to the climacteric nature of peach, applications of continuous ethylene were established (Fig. 1). At the onset of color break, 3 concentrations of ethylene were treated to freshly harvested cherry fruit at 58 DAFB. Respiration rate and ethylene production are shown if figures 5A and 5B. All 3 concentrations of ethylene induced a higher respiration rate on fresh cherry after 3 days at 66^{o}F (Fig. 1A). Similarly, pre-climacteric peach (131 DAFB) responded to ethylene treatments as the respiration rates all increased due to ethylene concentration within 1 day at 66^{o}F (Fig. 1C). The ethylene production from early season cherry fruit was zero over 5.5 days and no ethylene was produced due to ethylene treatment (Fig. 1B). Similarly, the pre-climacteric peach showed nearly zero levels of ethylene for the control and all ethylene treatments (Fig. 1D). It has been documented that in non-climacteric fruit and the pre-climacteric state of a climacteric fruit, a dose of continuous ethylene treatment triggers increased respiration (Kays, 2005). Upon removal of the exogenous ethylene and the respiration rate will resume to normal control levels.

Upon treatment of 'Early Elberta' peach in the climacteric state (146 DAFB) the dose of ethylene had very low effects on stimulating respiration up to 14 days, however, the lowest doses stimulated respiration greater than the higher doses (Fig. 1E). Similar effects on ethylene production were shown up to day 11 at which time the lowest ethylene does (0.11 ppm) slightly stimulated ethylene in climacteric peach (Fig. 1F).

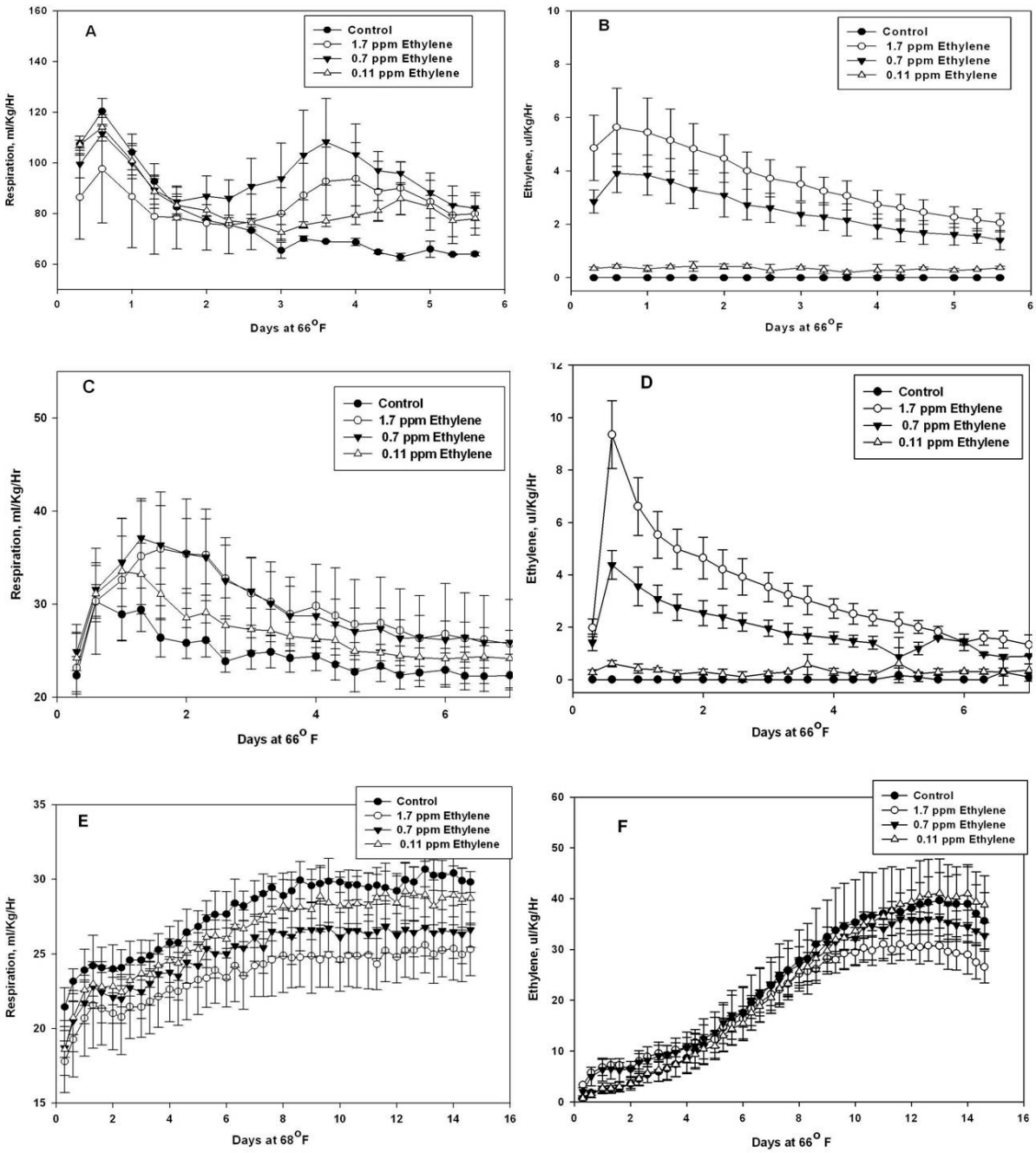


Figure 1. Respiration rates and ethylene production monitored on 'Bing' sweet cherries (A-B), pre-climacteric peach (C-D), and post-climacteric peach (E-F) with continuous ethylene treatment using ethephon solutions at 1.7 ppm, 0.7 ppm, and 0.11 ppm (n=3).

There are several reports as to the effects of dosing mature sweet cherries with ethylene, and monitoring respiration and ethylene production. (Li et al., 1994) applied ethephon to various stages of 'Bing' cherry maturities, but at the ripe state, the ethephon treated fruit did not change in respiration rate or ethylene production over the control after 8 days at 66°F. In fact ethylene levels were zero for the ethephon treated fruit. Gong, et al. (2002) showed similar results using mature commercial harvest 'Bing' sweet cherries. The ethylene exposure was applied at 80 ppm ethylene gas for 6 hours. The

ethylene production increase lasted 1 day, and the increase was extremely low at 0.02 umol/Kg/Hr (Gong et al., 2002). The control fruit produced zero ethylene in the experiment (Gong et al., 2002).

A research study where continuous ethylene gas was exposed to a climacteric fruit such as 'Anjou' pear over 4 harvest timings suggested similar results as shown by this study with 'Early Elberta' peach. Up to 5 concentrations of exogenous ethylene treatment onto 'Anjou' fruit in the pre-climacteric state showed increases in respiration over 18 days, and no increases in ethylene production until 6 days (Wang et al., 1972). Similar applications of exogenous ethylene onto mature stage IV fruit showed increases in respiration immediately up to 18 days and increases in ethylene at 4 days (Wang et al., 1972).

Testing if ethylene pathway genes are functional in cherry: The genes from several *Prunus* equivalent *Md-ACS1-5* sequences were analyzed for 'Chelan', 'Bing', and 'Skeena' in fruit from the 2010 season (Fig. 2). The *ACS 1* and *ACS 2* gene expression assays were not successful, perhaps indicating the absence of these genes. However, optimization did show success for gene expression products describing *ACS 3-5*, as well as the ribosomal *r18S* control (Fig. 2). *ACS 3-5* expression in 'Bing' samples was evident until at 56 DAFB, then again showed up at 76 DAFB (Fig. 2). 'Chelan' and 'Skeena' showed similar *ACS 3-5* gene expression patterns; however, signal disappearance occurred at a different stages than 'Bing' (Fig. 2). In a study detailing the level of *ACS* transcripts during a climacteric fruit development cycle, Yokotani et al. (2009) showed that *ACS 1, 2, 4,* and *6* have different levels of expression between early immature tomato stages, to mature green, and finally into the red ripe stages, similar to the variable levels we observed for cherry.

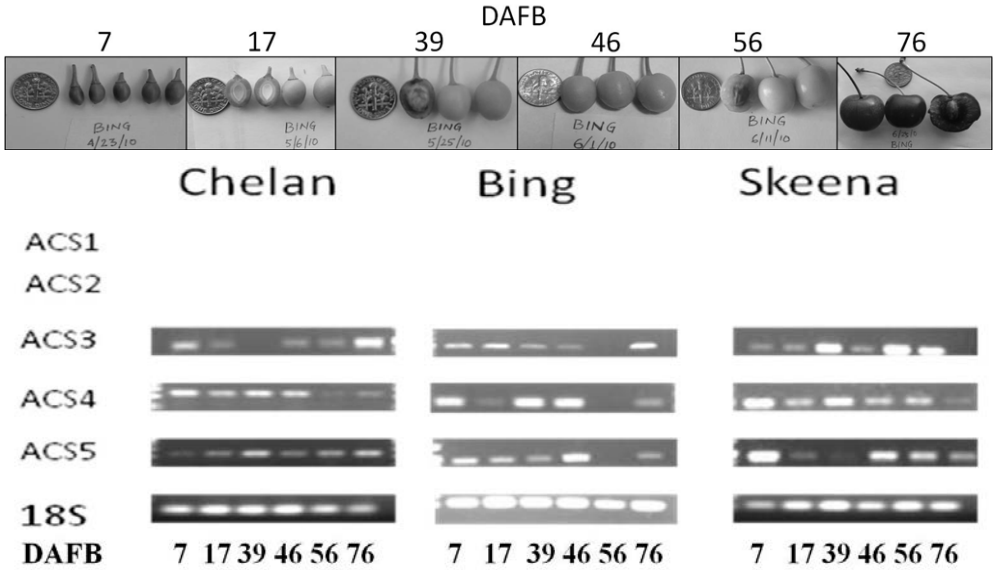


Figure 2. (A) Fruit development stages used for gene expression studies. (B) Semi-quantitative *ACS* gene expression for three sweet cherry cultivars.

Ethylene enzymes and products: The enzymatic regulation of ACC (1-aminocyclopropane-1-carboxylate) synthase (ACS) and ACC oxidase (ACO) during fruit growth and development of cherry were compared to that of peach. During the most recent growing season, the ACO disk assay was optimized to assure the ethylene produced is due to *in vivo* feeding with (+) or without (-) the addition of the enzyme's substrate, ACC. Other pathways exist whereby ethylene may be produced to due oxidation of ACC to ethylene by superoxide radical generation upon tissue wounding (Kumar and Knowles, 2003)**.** The use of *n*-propyl gallate under assay conditions was fed to 'Regina' cherry disks,

with and without ACC. Ethylene is produced under assay conditions, at nearly zero levels without ACC, and up to 12 nl/g/hr. with ACC, as well as with ACC and propyl gallate. The assay therefore is not limited by superoxide radical generation due to wounding as a mechanism to produce ethylene after 5-24 hours under *in vivo* conditions.

As described in year one of the study, by following ACO activity levels with and without ACC, varying peak levels of ACO activity were observed for ACO. Endogenous ACO activity, obtained without adding ACC, peaked for 'Chelan' at 35 days DAFB, at 45 DAFB for 'Bing', and 40 DAFB for 'Skeena' (Fig. 3). These peaks occurred near color break. ACO activity levels, with or without ACC, leveled off to zero near physiological and commercial maturity (Fig. 3). This result concurs well with whole fruit ethylene and respiration in cherry, where both level off to undetectable levels and very low levels, respectively, just before cherry harvest (data shown year 1).

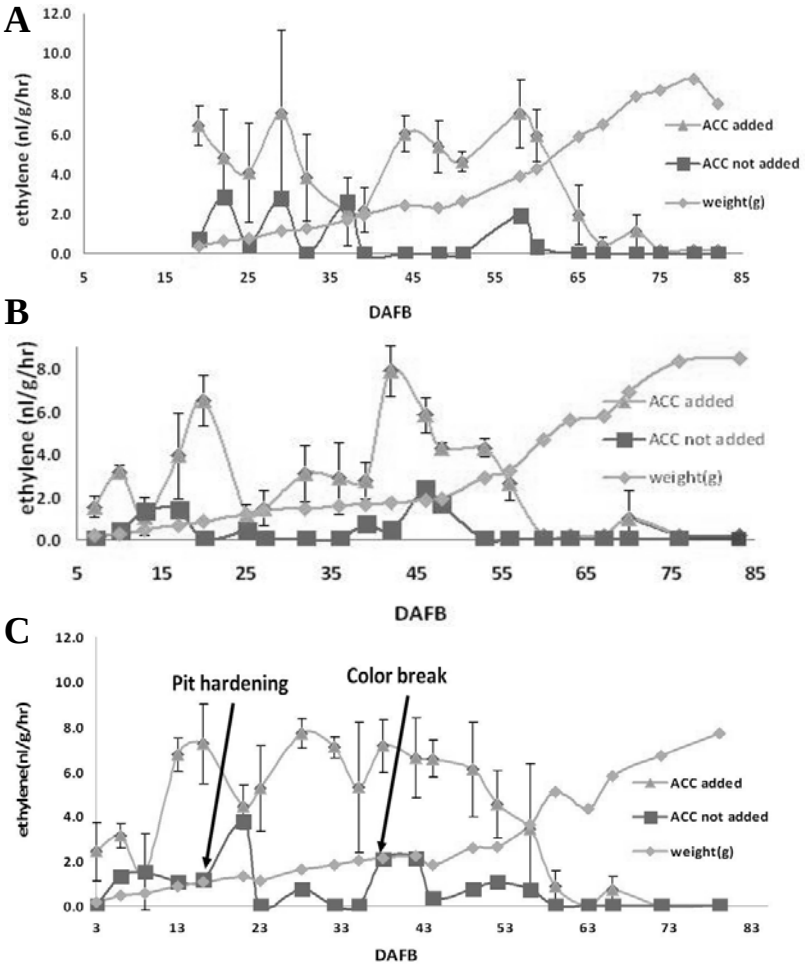


Figure 3. ACO enzyme activity and fruit weight changes during fruit development of three sweet cherry cultivars in 2010. (A) 'Chelan'. (B) 'Bing'. (C) 'Skeena'.

Peach was assayed at six points during fruit development in 2010 (Fig. 4A) and four points, plus an additional 146 DAFB ripening at 66°F for 23 days, in 2011 (Fig. 4B). The ACO level as +ACC changed from nearly zero at 113 DAFB to 10 nl/g/hr at 125 DAFB then leveled off to nearly 9 nl/g/hr at 146 DAFB (Fig. 4A). The ACO levels a –ACC data for 2010 showed an increase at 125 DAFB, and again a 146 DAFB, closely paralleling the +ACC data (Fig. 4A). The peach ACO activity as

+ACC had very high levels in 2011 at 110 DAFB, then leveled off to nearly 14 nl/g/hr at 146 DAFB. The ACO activity as –ACC did not parallel the +ACC enzyme activity. By maturity, the 146 DAFB fruit for 23 days at 66°F, the ACO as –ACC increased to 6 nl/g/hr (Fig. 4B). At this same time point the ACO as +ACC decreased to nearly 6 nl/g/hr (Fig. 4B). Although the ACO data as +ACC and as –ACC over two harvest seasons showed similarities, data at 146 DAFB, the climacteric state of peach was similar (Fig. 4A-B). The ACO as +ACC was the highest for both years at this time point (Fig. 4). This result was similarly shown by Zhang and Hui-Juan (2005) in peach as the ACC content, the *in vitro* ACO, and whole fruit ethylene increased at the mature ripe state. Additional work by Tonutti et al., (1996) showed *in vivo* levels of ACO increase along with ethylene generated from –ACC-spiked fruit disks, at the time of early ripening in three peach varieties.

The ACO enzyme activity as +ACC in three sweet cherry cultivars was zero at mature ripe (Fig. 3), therefore, a major difference to the peach ACO at mature ripe (Fig. 4). Several authors have described ACS activity, ACO activity, and ethylene levels in peach, but at this time no information exists for sweet cherry ACS or ACO levels through harvest. The ACO with and without ACC, whole fruit ethylene levels, and respiration comparisons between sweet cherry and peach over the growth and development period are the first such studies.

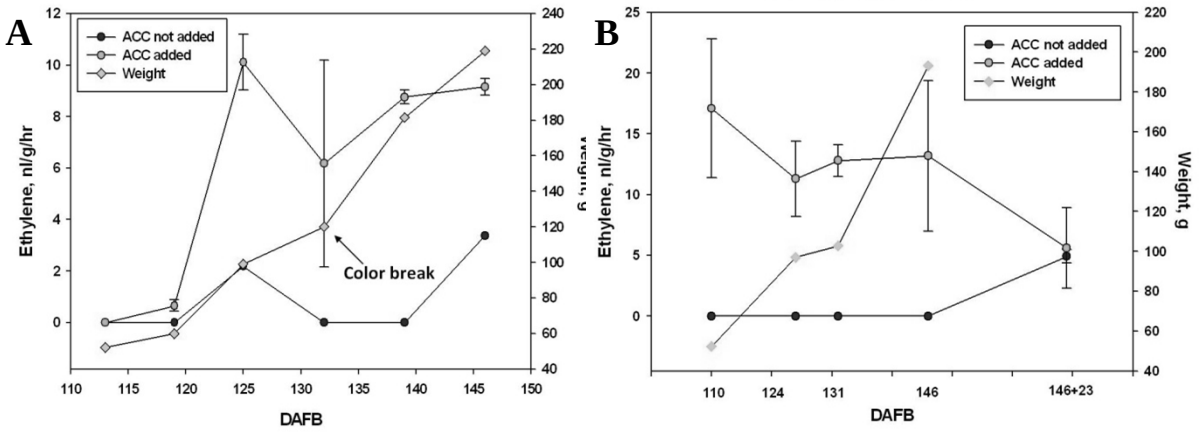


Figure 4. ACO enzyme activity in 'Early Elberta' peach in (A) 2010, and (B) 2011. Assay points in 2010 were measured on 3 samples for +ACC while –ACC was performed on 1 sample. Assay points in 2011 were for 3 samples for both +ACC and –ACC.

A second part of the study aimed at "physiological assays to assign cherry cultivars to ethylene physiology categories" consisted of measuring the ACO enzyme activity in 9 sweet cherry varieties from fruit set until harvest (stylized in Fig. 5, complete results in Fig. 6). The *in vivo* assay was performed similar to studies in year one for cherry and year one and two for peach (Fig. 3 and 4). The measurement of ACC as +ACC revealed many patterns among varieties. The latest season sweet cherry variety 'Regina' had the overall highest ACO activity at nearly 16.0 nl/g/hr (Fig. 5). Also, 'Regina' had the latest peak in ACO activity as +ACC ending at 64 DAFB (Fig. 5). 'Glacier' had the lowest ACO activity among all varieties (4.0 nl/g/hr), and 'Lapins' ACO activity ended the soonest after full bloom, at about 52 DAFB (Fig. 5). A unique observation of the ACO activity as +ACC was that the early color break point (highlighted in red) occurred before the last peak in ACO (Fig. 5). However, two varieties, 'Chelan' and 'Rainer' showed color break at the last peak in ACO activity as +ACC (Fig. 5). This result was noted in year one of the ACO study on three varieties, 'Chelan', 'Bing', and 'Skeena' (Fig. 1). The difference in year one being the peaks in ACO activity seemed to associate with –ACC fed assays. The current year data does not show appreciable peaks in ACO activity as –ACC near color break for any of the 9 varieties (Fig. 5).

In work closely related, various regulators and biochemicals were applied to sweet cherries at several maturities (Hartman, 1992). Ethephon does not stimulate anthocyanin synthesis, moreover, applications of ABA do stimulate anthocyanin production at 8 weeks after full bloom (Hartman, 1992).

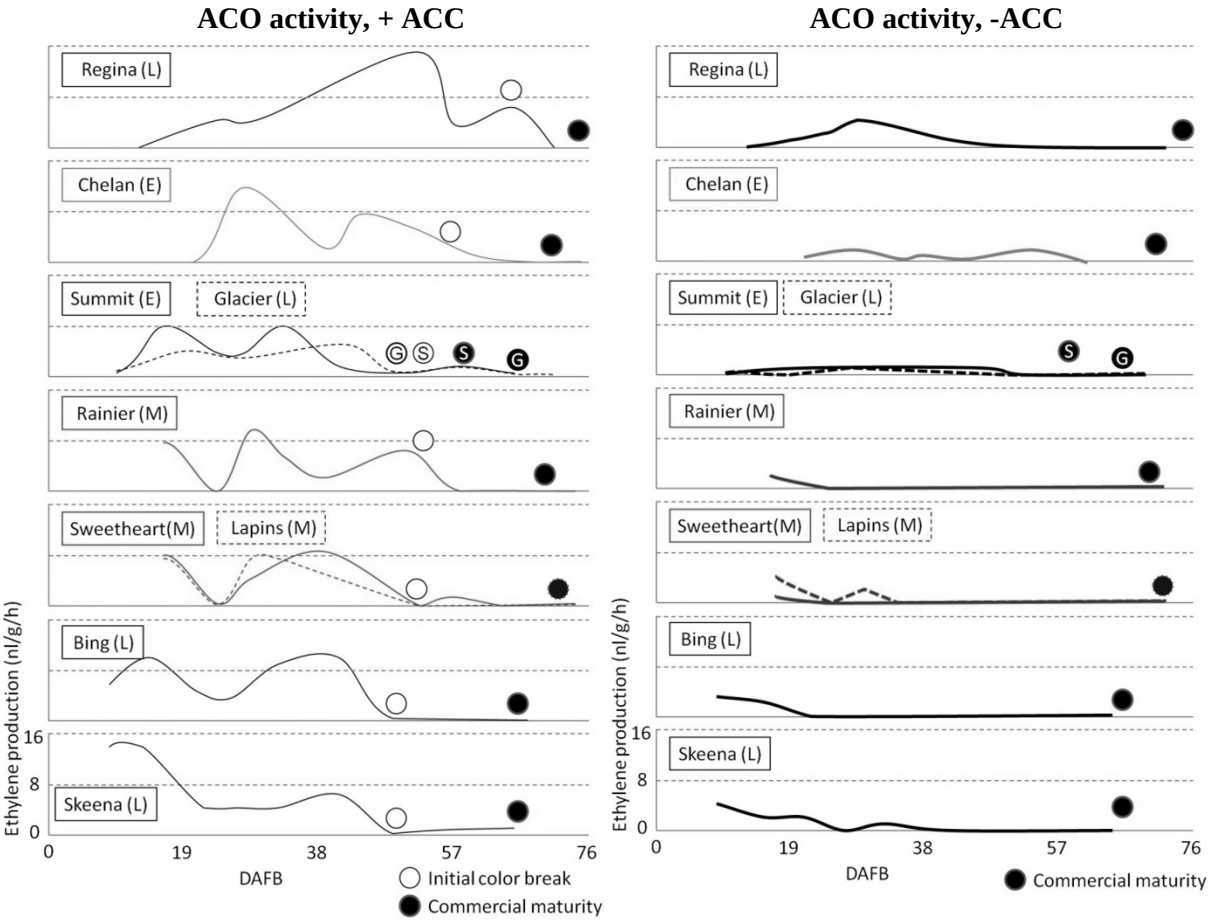
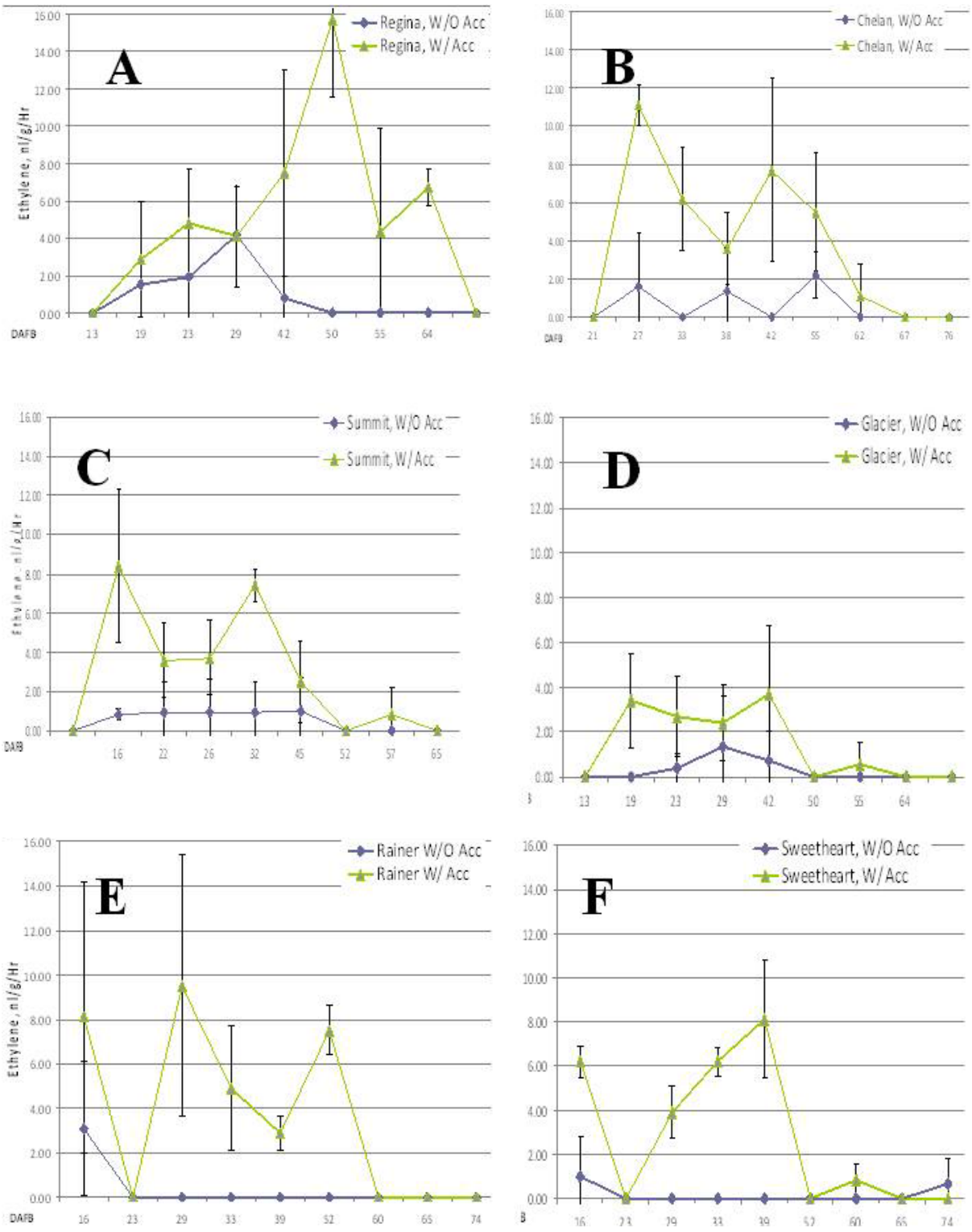


Figure 5. Summary of patterns of ACO enzyme activity from *in vivo* assays on nine sweet cherry cultivars through 76 days after full bloom (DAFB) during the 2011 growing season. ACO assays are displayed as +ACC (addition of the enzyme substrate, ACC) or –ACC (no ACC added).

Figure 6 (next pages). ACO activity from *in vivo* assays in sweet cherry fruit, (A) 'Regina', (B) 'Chelan', (C) 'Summit', (D) 'Glacier', (E) 'Rainer', (F) 'Sweetheart', (G) 'Lapins', (H) 'Bing', and (I) 'Skeena' (n=3 for each time point, +ACC or –ACC).


A
Regina, W/O Acc
Regina, W/ Acc
Ethylene, nl/g/Hr
DAFB
13 19 23 29 42 50 55 64
B
Chelan, W/O Acc
Chelan, W/ Acc
DAFB
21 27 33 38 42 55 62 67 76
C
Summit, W/O Acc
Summit, W/ Acc
DAFB
16 22 26 32 45 52 57 65
D
Glacier, W/O Acc
Glacier, W/ Acc
13 19 23 29 42 50 55 64
E
Rainer W/O Acc
Rainer W/ Acc
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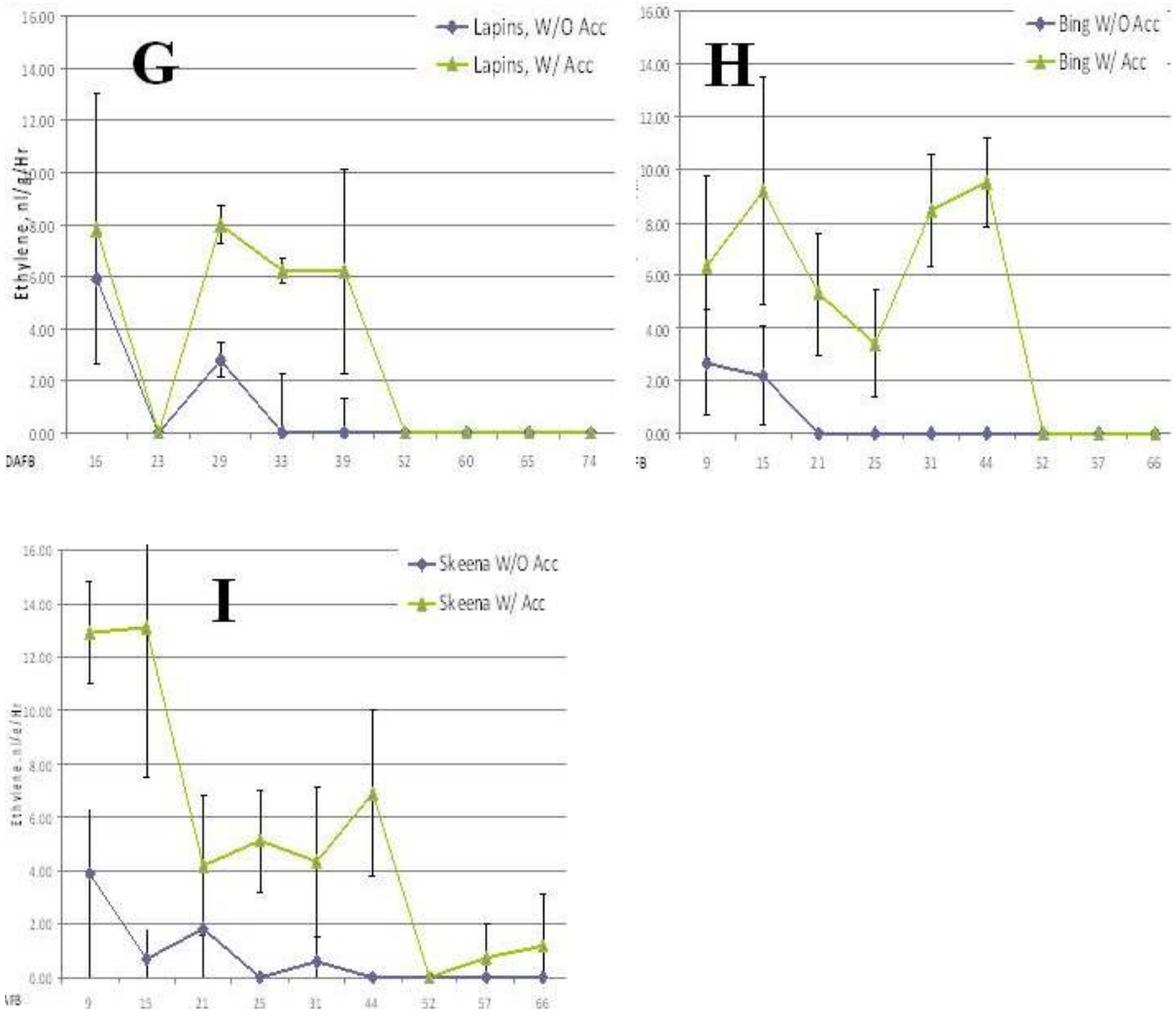

Fruit quality evaluation and association with ethylene physiology categories*:* Fruit quality was determined for nine sweet cherry cultivars grown at IAREC in Prosser, WA, at 1 day after harvest (at commercial maturity) and after a 7-day storage regime of 32°F, 95% RH. Fruit weight at day 1 varied among cultivars between 7.0 and 11.2 grams (data not shown). Firmness (deflection force) increased between day 1 and day 7 for all cultivars except 'Sweetheart' (Table 1). Sweetness (°Brix) increased between day 1 and 7 for 'Regina', 'Chelan', 'Sweetheart, and 'Skeena', and slightly decreased for the other five cultivars (Table 1). Acidity (% TA) decreased for all varieties, except for 'Skeena' which had no change (Table 1). Change in skin color (a+ and Hue) between day 1 and day 7 was variable among cultivars, tending to increase toward redness for 'Lapins' and 'Skeena' and losing redness for 'Chelan', 'Glacier', and 'Rainer' (Table 1). We were not able to find in the literature a more detailed dataset as this on comparative fruit quality after storage for PNW sweet cherry cultivars. Cultivars were grouped in various ways according to ACO activity patterns (Fig. 5), but no consistent statistical differences in any fruit quality attribute was found among groups. Therefore, although ACO activity during sweet cherry fruit development appears to vary among cultivars, these differences do not appear to be associated with differences in fruit quality at harvest or after a week of storage.

Table 1. Fruit quality for nine sweet cherry cultivars evaluated in 2011 at one day after harvest and after 7 days of cold storage. Presented are average values for 10-15 fruit, except for acidity which was an average of 4 readings from 7-8 fruit per sample.

Cultivar	Harvest date	Harvest weight	Firmness (g/mm)		Sweetness (^{o}Brix)		Acidity (%TA)		Color (a+)	
			day1	change	day1	change	day1	change	day1	change
Bing	7 Jul	7.4 g	356	+49	18.4	-0.7	1.16	-0.05	30.2	+1.3
Chelan	28 Jun	7.0 g	443	+71	19.9	+0.2	1.25	-0.08	22.3	-6.5
Glacier	7 Jul	10.3 g	397	+19	23.0	-1.8	1.32	-0.08	25.3	-1.6
Lapins	7 Jul	9.2 g	337	+56	16.5	-0.5	1.01	-0.10	27.9	+6.9
Rainier	7 Jul	10.1 g	298	+103	19.3	-1.9	1.14	-0.06	39.5	-8.6
Regina	21 Jul	10.3 g	425	+2	18.3	+0.4	1.13	-0.11	21.3	+2.1
Skeena	7 Jul	11.2 g	463	+21	17.5	+0.2	1.24	0.00	32.7	+7.8
Summit	28 Jun	9.2 g	394	+16	12.1	-0.5	0.83	-0.08	48.2	+2.4
S'heart	7 Jul	9.3 g	475	-15	14.7	+0.5	1.52	-0.30	43.9	+1.5

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EXECUTIVE SUMMARY

Our overall goal is to improve market life of sweet cherry cultivars through an understanding of the ethylene genetic control in this non-climacteric crop. Specific objectives were to:

1. Characterize differences in the ethylene biosynthetic pathway between sweet cherry and peach—*why do they ripen differently?*
2. Identify ethylene physiology categories of cherry cultivars that simplify development of strategies to extend market life—*does increased synthesis and/or sensitivity to ethylene determine ultimate postharvest response?*
3. Provide specific selection criteria in breeding for improved market life based on ethylene biosynthesis and response—*can a simple high-throughput physiological or genetic assay help predict market life?*

Answers to these above questions are:

1. *Differences between non-climacteric sweet cherry and its climacteric relative peach are either in ethylene synthesis over-regulation and/or in the ethylene signal response pathway such as the absence of critical response element.*
2. *Probably not.*
3. *Possibly, but we haven't found it yet.*

Significant findings and conclusions:

- Sweet cherry is confirmed as a non-climacteric fruit.
- Mature ripe sweet cherry fruit did not respond to ethylene treatment by any changes in respiration or ethylene production.
- An early peak in ethylene production in tiny fruitlets subsided well before, just before, or soon after color break (depending on the cultivar) and became undetectable upon further maturation.
- Differences in several *ACS* genes were observed among three cherry cultivars over six sampling times throughout fruit development
- Activity of ACO (the "ethylene forming enzyme") in sweet cherry fruit declined to zero with advancing fruit development. In stark contrast, ACO activity in peach was high in early season fruit, leveled off, then had a medium activity in mature ripe fruit.
- ACO activity patterns for nine sweet cherry cultivars throughout fruit development showed several patterns. However, no significant associations of these patterns with fruit quality traits were detected, including after a 1-week storage regime.
- The lack of endogenous ethylene production or ethylene response indicates that there are no off-tree quality improvements to be realized for sweet cherry fruit beyond color break.
- These results lead us to believe that the difference between cherry and its climacteric relatives is due to synthesis over-regulation and/or is in the ethylene signal response pathway such as the absence of critical response elements.

Future directions:

- Systematically examine gene expression for all members of the ethylene biosynthetic and signal transduction pathway to identify critical differences between sweet cherry and peach, and key differences among sweet cherry cultivars. Examine whether differences in gene expression patterns among cultivars are associated with differences in market life.
- Comprehensively evaluate many cultivars across the season for their fruit quality attributes at harvest and over extended storage regimes and extended room temperature ripening. Use such a detailed market life performance dataset to better understand market life differences among cultivars. Such a dataset can also be used to seek associations with gene expression differences in the ethylene pathway or other likely fruit quality biochemical pathways.

FINAL PROJECT REPORT

Project Title: Systemic acquired resistance to bacterial canker of sweet cherry

PI: Ken Johnson
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State/Zip OR 97331-2902

Cooperators: Bob Spotts, MAREC, Oregon State University, Hood River

Other funding sources: None

Total Project Funding: $39,200

Budget History:

Item	Year 1 of 2	Year 2 of 2	(type additional year if relevant)
Salaries FRA 3mo	10,000	10,300	
Benefits OPE 63%	6,300	6,489	
Wages			
Benefits			
Equipment			
Supplies	2,000	2,111	
Travel local	500	500	
Miscellaneous plot fee	500	500	
Total	19,300	19,900	

Footnotes: Annually**:** FRA 3 mo plus fringe, 2K M&S, 1K plot fee, 3% inflation

OBJECTIVES

1) Evaluate use of root drenches and trunk paints of the systemic acquired resistance inducer (SAR), acibenzolar-*S* methyl (ASM), for protection of young cherry trees from bacterial canker.

2) In conjunction with SAR treatment, evaluate treatments for protection of heading cuts on young sweet cherry trees from bacterial canker.

MODIFIED OBJECTIVE 2:

2) Examine the relationship between length of the orphan stub* and initiation of bacterial canker in the trunk tissue below the heading cut.

* The 'orphan stub' is the unsupported trunk tissue below the heading cut but above the topmost lateral (unsupported because there is no potential for further vascular flow through it).

SIGNIFICANT FINDINGS

- Based on June 2011 observations of 2010 plantings, a second heading cut made in May on April-planted cherry trees reduced the amount of dead, shrunken trunk tissue (canker) that developed below the heading cut. Larger cankers developed on those trees headed only once compared to those headed a second time. Moreover, canker lengths were positively correlated with lengths of orphan stubs.

- Spring trunk paint and root dip treatments with the SAR inducer, acibenzolar-*S* methyl (ASM), onto trees plants in 2010 did not suppress bacterial canker. In fact, trunk paint treatments were phytotoxic to cherry and exacerbated canker symptoms. Additional fall treatments of ASM to 2010 trees did not provide a response a response in 2011.

- In a 2011 planting, long orphan stubs increased the incidence of gummosis associated with the heading cut by three fold compared to short orphan stubs. Heading the trees a second time, however, did not reduce gummosis or initial canker development.

- Drench and spray treatments of ASM were applied to the 2011 planting in September and October. These trees will be evaluated in summer 2012.

METHODS

2010. Parallel 200-tree plantings of sweet cherry cv. 'Bing' on 'Mazzard' rootstock were established in Hood River and Corvallis. Trees with ¾" trunk diameters were planted in Corvallis; trees with 5/8" trunk diameters were planted in Hood River.

The experimental design is shown in Table 2 on the next page. The timetable of plot treatment activity was as follows:

Table 1. 2010 plantings.

Experimental Activity	Corvallis	Hood River
Planting & ASM root dip	7 Apr	13 Apr
ASM trunk paint	15 Apr	26 Apr
First heading& pathogen inoculation	19 Apr	26 Apr
Fertilize (50 g $(NH_4)_2SO_4$ per tree)	19 Apr	11 May
Second heading & wound sealant	28 May	11 May
Drench ASM	12 Aug & 14 Sept	13 Sept
Spray ASM	13 Oct	
Fall pathogen inoculation	29 Oct	
Tree Measurements	5 Sept 27 June 2011	14 Sept 27 June 2011

Table 2. 2010 treatments in randomized completed block with 4 replications.

Primary Spring Treatment	Heading cut treatment applied to each primary treatment
1) ASM Hi Root Dip 10 g/L in 1% Pentrabark 2) ASM Lo Root Dip 5 g/L in 1% Pentrabark 3) ASM Hi Trunk Paint 30 g/L in 2% Pentrabark 4) ASM Lo Trunk Paint 15 g/L in 2% Pentrabark 5) Untreated control	**a)** Heading near planting - inoculated cut with *Pseudomonas syringae* (sprayed 10^6 cfu/ml immediately after heading) **b)** Second heading 10-12 cm below the first - cut treated with a mix of kasugamycin (100 ppm) and oxytetracycline (200 ppm), then sealed with TreeKote® tree wound dressing. This cut was made at the beginning of a warm, dry period of weather (3 days at Corvallis, 4 days at Hood River)

Table 3. Additional late summer/fall ASM treatments

Primary Spring Treatment	Additional late summer/fall ASM treatments
1) ASM Hi Root Spray 10 g/L in 1% Pentrabark	Per tree: 300 mg ASM in 250 ml crown drench; 13 Sept Hood River, 14 Sept Corvallis
2) ASM Lo Root Spray 5 g/L in 1% Pentrabark	Per tree: 200 mg ASM in 250 ml crown drench; 13 Sept Hood River, 14 Sept Corvallis. Additionally, in Corvallis only, trees were sprayed with ASM (1 g/L) in 0.5% PentraBark on 13 Oct.[#]
4) ASM Lo Trunk Paint 15 g/L in 2% Pentrabark	Per tree: In Corvallis only, 200 mg ASM in 250 ml drench on both Aug. 12 and Sept 14.

[#] **This treatment is based similar spray we applied to lilac in fall 2009 that significantly reduced Pseudomonas blight in spring of 2010.**

2011. A third 240-tree planting of sweet cherry cv. 'Bing' (¾" caliper) on 'Mazzard' rootstock was established at Corvallis. The timetable of plot treatment activity design were as follows:

Table 4. 2011 planting.

Experimental Activity	Corvallis
Planting	28 Apr
First heading& pathogen inoculation	7 May
Second heading (70% EtOH on cuts)	19 May
Fertilize (50 g $(NH_4)_2SO_4$ per tree)	6 June
Drench ASM	19 September
Spray ASM	19 Oct
Fall pathogen inoculation	21 Oct
Tree Measurements	15 Sept

Table 5. 2011 treatments in randomized completed block with 10 replications

Primary Spring Treatment Secondary fall treatment	Heading cut treatment applied to each primary treatment
1) Long orphan stub Half the trees received ASM as drench (Actigard 1 g/tree) in September followed by ASM spray (Actigard 0. 3 g /L to runoff) in October 2) Short orphan stub Half the trees received ASM as above.	**a)** Headed near planting - inoculated cut with *Pseudomonas syringae* (sprayed 10^6 cfu/ml immediately after heading) **b)** Second heading 10-12 cm below the first - cut treated with 70% ethanol. This cut was made at the beginning of a warm, dry period of weather (6 days in length).

RESULTS -- 2010 plantings.

Tree establishment and growth. All trees received from the nursery established and developed healthy shoots. Although the trees at Hood River were smaller caliper than at Corvallis, initial budbreak and shoot growth was faster at the Hood River site. However, as the seasons progressed, total new shoot growth at Corvallis (6 meters per tree) was superior to Hood River (2.5 meters per tree) (Fig 1. A, B, C, D). Initial tree caliper was probably a reason for this difference, but at Hood River, moderate deer damage in outer rows of the plot area and planting the trees into soil that had hosted cherry previously also may have contributed to poorer growth at this site.

Overall, applying a second heading cut to the trees had no significant effect on number of shoots per tree and total shoot length **(**Fig 1. A, B, C, D).

Effect of spring ASM treatments. Spring-applied ASM treatments were not beneficial to tree growth (Fig 1. A, B, C, D), nor did they suppress the initial development of bacterial canker (Fig 1. E, F). In fact, at both Corvallis and Hood River, painting the trunk with ASM in 2% PentraBark was apparently phytotoxic, resulting in larger cankers associated with the heading cut compared to trees that received the other treatments (Fig 1. E, F). At Hood River, the largest cankers developed on the trees painted with ASM and headed only once (Fig. 1F). At both sites, the distribution of lateral shoots on ASM-painted trees were generally skewed lower on the trunk compared to both untreated and root dip treated trees (data not shown).

The ASM root dip treatments reduced total shoot length and number of shoots per tree by 5-15%, compared to the untreated control (Fig 1. A, B, C, D) but the trees showed no apparent symptoms of phytoxicity. At Hood River, canker development on ASM root-dipped trees was similar to that observed on the untreated controls (Fig 1. E, F), but at Corvallis, root-dipped trees headed only once developed larger cankers relative to the untreated controls.

Effect of the second heading cut on canker development. In general, application of the second heading cut reduced the amount of dead, shrunken trunk tissue that developed immediately adjacent to the cut (what we are calling the 'initial canker'). The treatment effect was most dramatic for the trees the received the ASM-paint treatment at Hood River, but consistent reductions in the initial canker size also were observed for double-headed trees of the other treatments (Fig 1. E, F).

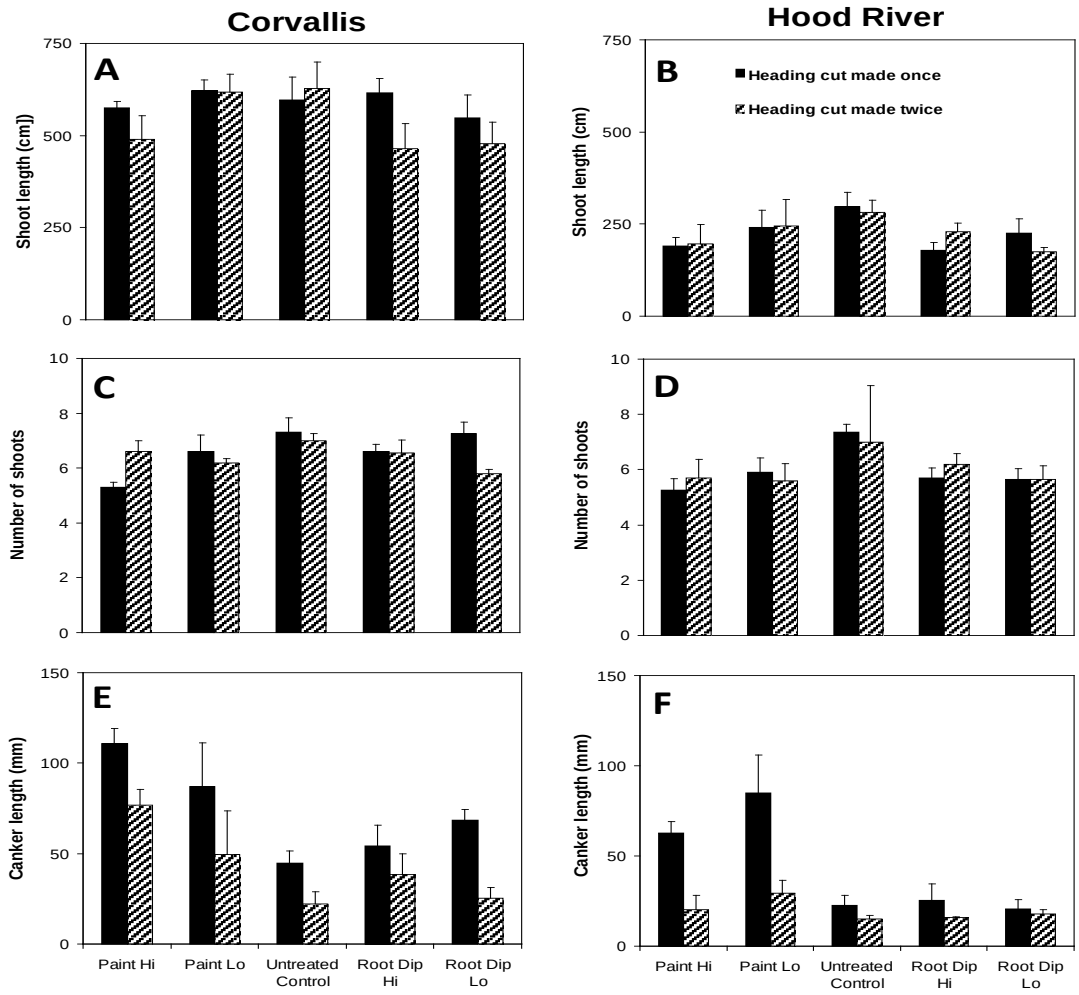

Fig. 1. Effects of spring- and fall applied root dips, paints, drenches and sprays of the systemic acquired resistance inducer (SAR), acibenzolar-*S* methyl (ASM), on total shoot length, number of shoots, and length of the dead, shrunken trunk tissue (canker) that developed immediately below the heading cut on trees of sweet cherry cv. 'Bing' (Mazzard rootstock) planted at Corvallis and Hood River, OR. After planting in April 2010, trees were headed once near the day of planting (black bar), or headed a second time two (Hood River) to five (Corvallis) weeks after the first heading (hatched bar). The surface of the first heading cut was inoculated with *Pseudomonas syringae*, whereas the surface of the second heading cut was treated with antibiotics and sealed with TreeKote® tree wound dressing. Measurements were made mid- to late June 2011. Lines at tops of bars are ± one standard error of the mean; experimental design was RCB with 5-tree plots replicated four times.

Effect of the orphan stub length on canker development. The amount of dead, shrunken trunk tissue that developed immediately adjacent to the heading cut (the 'initial canker') was positively correlated with the length of the orphan stub (Fig. 2 A-F). Longer stubs occurred on trees that received ASM, which resulted in longer lengths of necrotic cankers. In Fig. 2 A, B, C. D, nearly all of the points above the regression lines represent trees that received a paint treatment of ASM.

For control trees that did not receive an ASM treatment (Fig. 2 E, F), trees headed a second time had smaller cankers than those head once. Furthermore, trees headed a second time with a short orphan stub generally developed a small cankers; this relationship was less consistent for trees headed only once.

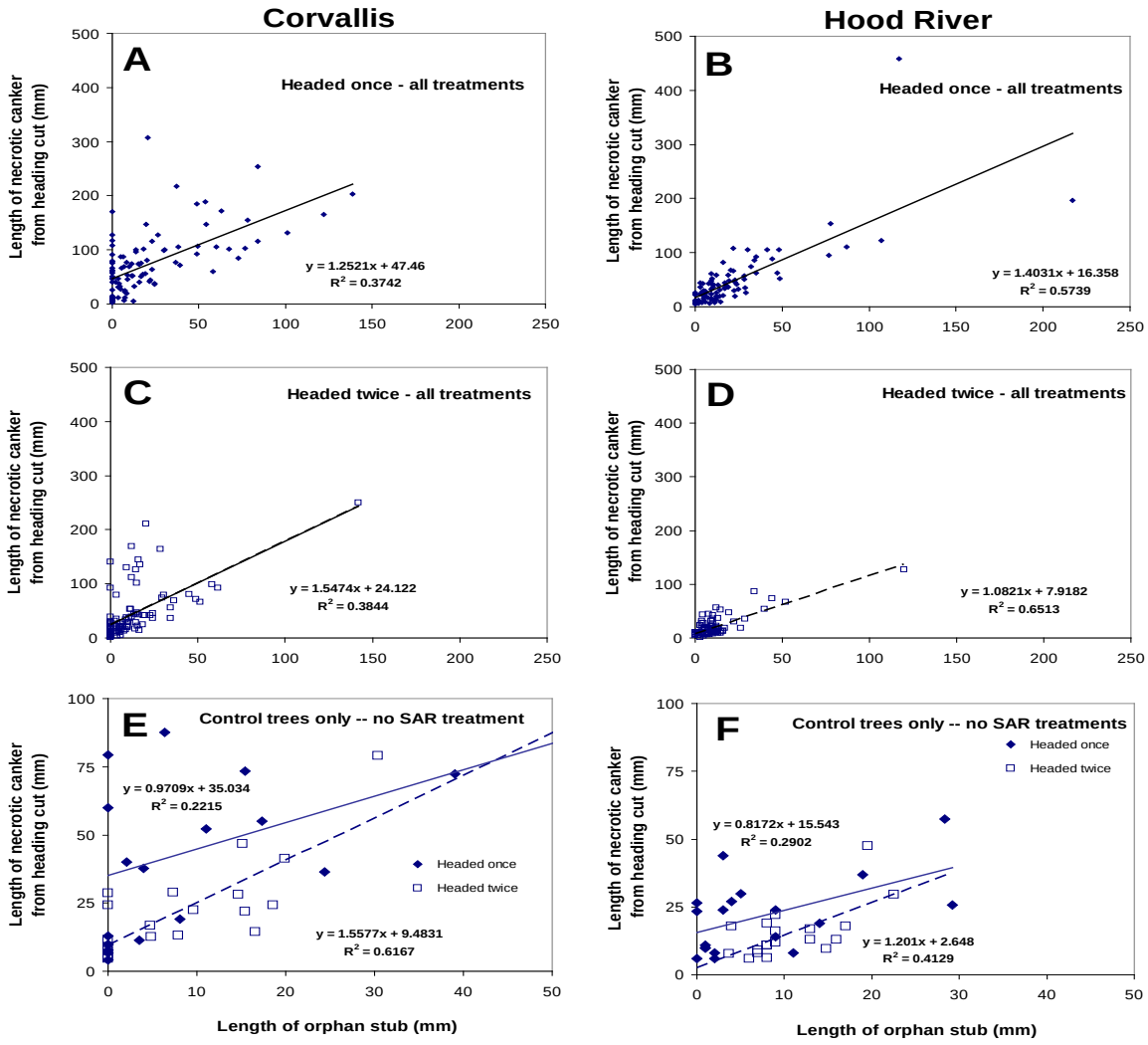

Fig. 2. Regression of 'length of necrotic canker from heading cut' on 'length of the orphan stub'. Panels A-D includes all trees in the experiment including those that received spring and fall treatments of the systemic acquired resistance inducer, acibenzolar-*S* methyl (ASM); panels E and F are trees in the plots of the untreated control. After planting in April 2010 , trees were either headed once near the day of planting (solid diamond in panels A, B, E, F)), or headed a second time two (Hood River) to five (Corvallis) weeks after the first heading (open square in panels C, D, E, F). The surface of the first heading cut was inoculated with *Pseudomonas syringae*, whereas the surface of the second heading cut was treated with antibiotics and sealed with TreeKote® tree wound dressing. Measurements were made mid- to late June 2011.

2011 planting.

Tree establishment and growth. All trees received from the nursery established and developed healthy shoots. Total new shoot growth was intermediate (Fig. 2 A) to that observed in the first year of the 2010 Corvallis (highest) and Hood River (lowest) plantings (see last year's report). Initial tree caliper, although purchased as ¾", also was intermediate to the ¾ (Corvallis) and 5/8 caliper (Hood River) trees planted in 2010.

Long or short orphan stubs, and heading once or twice had no consistent effect on number of new shoots per tree (Fig. 3 B), but total shoot length on trees with short orphan stubs was reduced ~15-20% compared to trees with long stubs regardless if the tree was headed once or twice (Fig. 3 A).

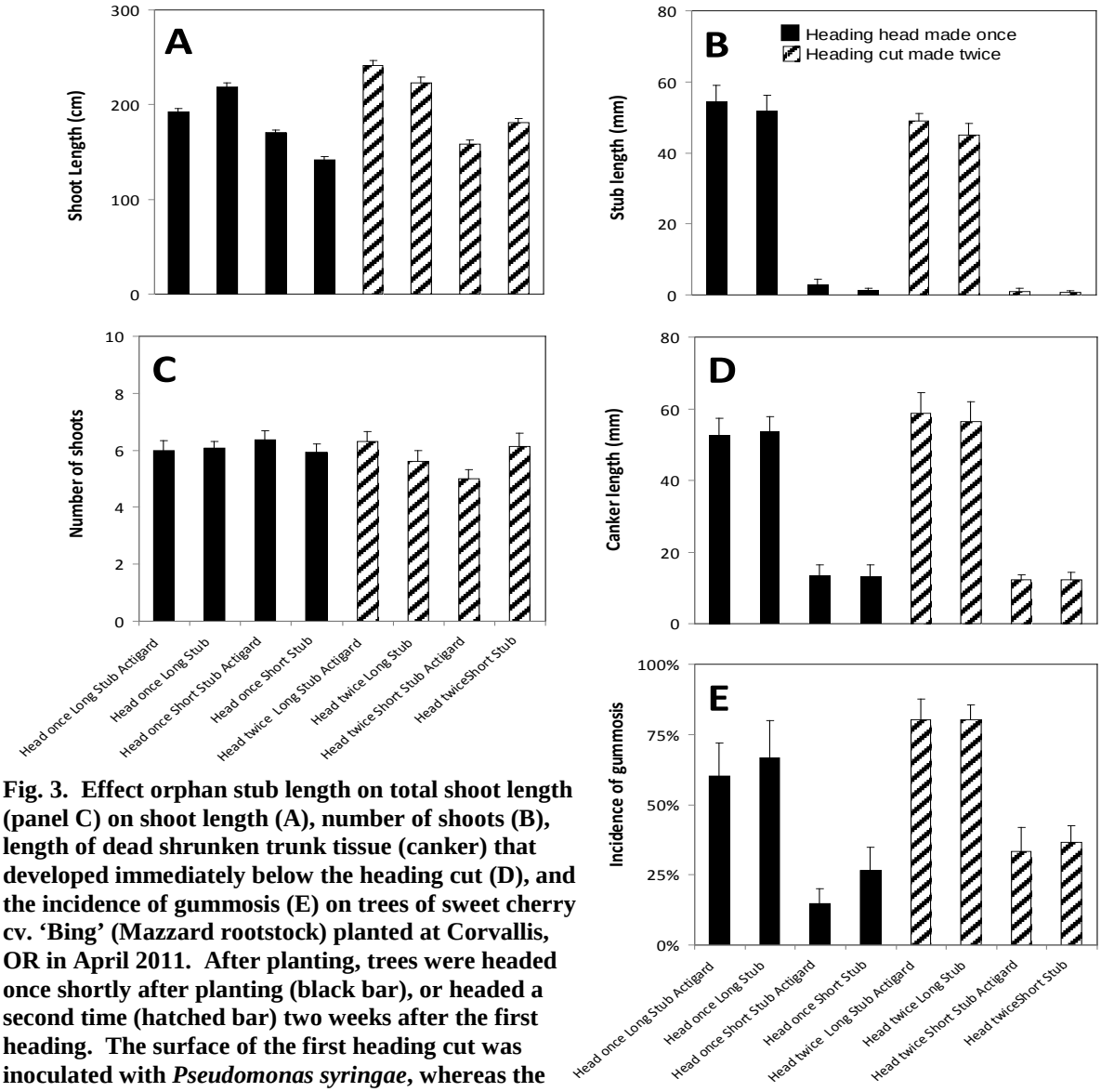

Fig. 3. Effect orphan stub length on total shoot length (panel C) on shoot length (A), number of shoots (B), length of dead shrunken trunk tissue (canker) that developed immediately below the heading cut (D), and the incidence of gummosis (E) on trees of sweet cherry cv. 'Bing' (Mazzard rootstock) planted at Corvallis, OR in April 2011. After planting, trees were headed once shortly after planting (black bar), or headed a second time (hatched bar) two weeks after the first heading. The surface of the first heading cut was inoculated with *Pseudomonas syringae*, whereas the surface of the second heading cut was treated 70% ethanol. Measurements were made mid-September 2011. Lines at tops of bars are ± one standard error of the mean; experimental design was an RCB with 3-tree plots replicated 10 times. Labels below the panels that include 'Actigard' had not yet received this treatment at the time of measurement (it was applied late-Sept and Oct 2011).

Effect of orphan stubs on necrotic canker development. As the season progressed, trunk tissue associated with the orphan stub became necrotic with canker lengths and stub lengths having similar values at the time of measurement in September (Fig. 3 D). Unlike 2010, the length of necrotic tissue was not influenced by the number of times the trees were headed. Similarly, the incidence of gummosis associated with the orphan stub was not affect by the number of times the trees were headed (Fig. 3 E), but long stubs showed an incidence of gummosis that was 2.5 times greater than observed on trees with short stubs (long stubs, 70% incidence of gummosis, versus short stubs, 30%).

DISCUSSION

Systemic acquired resistance. The rationale for this project was based on research in citrus that has shown systemic acquired resistance (SAR) inducers applied to the root zone protects trees from canker caused by *Xanthomonas citri* spp. *citri.* The citrus industry in Florida has begun to utilize this technology for canker control in commercial orchards. Moreover, in our pear and apple research, we are continuing to observe significant effects of ASM on fire blight canker expansion in pear and apple rootstock protection from the fire blight pathogen.

In cherry, however, we did not see a benefit from spring application of ASM, and in fact, some treatments were phytotoxic. Moreover, fall ASM treatments in 2010 to onto trees that received the spring treatments provided no apparent response. Fall treatments also were applied to the 2011 planting, and these will be evaluated in summer 2012; at this point in time (October 2011), we have no expectation that these treatments will show an effect.

Orphan stubs. The rationale for the study of orphan stubs in association with the heading cut was based on results of Spotts et al. (2010) who found that *P. syringae* infections initiated in the heading cut resulted in large cankers and frequent death of the newly planted sweet cherry trees. In our plots, we had only a few trees die, of which all received a phytotoxic paint treatment of ASM. One difference in our methods relative to Spotts et al. (2010) was inoculum dose sprayed onto heading cut wounds. Spotts et al. (2010) inoculated heading cuts with suspensions of *P. syringae* prepared at 10^8 CFU/ml whereas we used a concentration that was 100-fold smaller (10^6 CFU/ml). Our reason for the smaller dose was that we believed it would more realistically represent what the typical tree in a commercial orchard was likely to experience. In hindsight, a higher inoculum dose may have helped to clarify our data.

Bacterial canker of cherry is most severe in trees/tissues that received some kind of stress (Kennelly & Sundin 2009). The tissues associated with the orphan stub are stressed because they are unsupported by the vascular system of the tree. Orphan stubs typically dieback to the point of the topmost lateral regardless of whether *P. syringae* is present in the necrotic tissue. Nonetheless, a stress pathogen like *P. syringae* readily colonizes the unsupported stub tissues, which results in an exacerbated rate of necrosis and canker expansion. The pathogen also can utilize the colonized stubs an energy source to further invade healthier portions of the trunk. Our results indicate that newly planted cherry will remain healthier if the length of orphan stubs is minimized. The best results (i. e. smallest cankers) were observed when a heading cut was made ~5 mm above the emerging bud of (what will become) the topmost lateral. Our heading cuts were angled downward 45° from just above the emerging topmost lateral toward the distal side of the trunk.

Double heading. The rational for double heading is that cherry trees are planted in March or April when weather is showery, and mature cherry, pear and apple are flowering. With warmer

temperatures and the abundance of flowers and new leaves, epiphytic *P. syringae* populations in the surrounding environment increase very rapidly. Just after planting, the orchardist heads the newly planted tree, and the large, perhaps horizontally-oriented, slowly-healing wound becomes contaminated with *P. syringae* being dispersed in showery spring weather (the *P. syringae* also could come from the tree itself). This initiates bacterial canker at the heading cut. The second heading cut 2 to 5 weeks after the first is made going into a period of warmer and dry weather, such that the wound has a chance to heal before it gets wet again.

Results from 2010 plantings indicated that a second heading cut in dry weather 2 to 5 weeks after the heading cut made near planting reduced the size of the canker associated with the orphan stub, but the results from the 2011 plantings showed no benefit from this second heading. Based on theses conflicting results, we recommended that growers consider the second heading (especially if the first was made in wet weather) or alternatively, delay the first heading until it can be made going into a period of warm dry weather. Based on the data, producing a short orphan stub and avoiding wet weather when making heading cuts are likely more important than whether the tree is headed a second time.

Clarification on what is a 'stub'. Researchers at Cornell University (Carroll et al. 2011) are recommending leaving a 'stub' when pruning laterals on sweet cherry. The objective in their research was to determine if by leaving pruning stubs, trunk and scaffold branch cankers caused by *P. syringae* could be reduced. Their data demonstrated that leaving lateral stubs protected against bacterial canker by distancing the main trunk and scaffolds from the pruning wounds where invasion by *P. syringae* occurred; lateral stubs also tended to increased the formation of new laterals by leaving existing buds (on the stubbed branch) on the tree. Compared to this study, their use of the term stub means 'it is the length of branch left on the tree, but potential new laterals are (ideally) distributed along this length'. What we are calling the 'orphan stub' is the 'unsupported trunk tissue above the topmost lateral on the trunk of the newly planted tree'; unsupported because there is no potential for further (future) vascular flow through this tissue.

Existing plots. This 640 trees planted in is this study are still in the ground. The experimental trees will be measured again in summer 2012; 2010 plantings will be removed at that time. The 2011 will be maintained and measured again in summer 2013.

LITERATURE CITED

Carroll, J. E., Burr, T. J., Robinson, T. L., Hoying, S. A., and Cox, K. D., 2011. Evaluation of pruning techniques and bactericides for managing bacterial canker of sweet cherry. (Abstract) Phytopathology 101:S27

Kennelly, M. M., Cazorla, F. M., de Vicente, A., Ramos, C., Sundin, G. W. 2007. *Pseudomonas syringae* diseases of fruit trees: Progress toward understanding and control. Plant Dis. 91:4-17

Spotts, R. A., Wallis, K. M., Serdani, M., and Azarenko, A. N. 2010. Bacterial canker of sweet cherry in Oregon: Infection of horticultural and natural wounds, and resistance of cultivar and rootstock combinations. Plant Dis. 94:345-350.

EXECUTIVE SUMMARY

The purpose of the study was to evaluate practices with potential to reduce the development of bacterial canker associated with heading cuts on newly-planted sweet cherry.

Specific objectives evaluated:

Use of root drenches and trunk paints of the systemic acquired resistance inducer (SAR), acibenzolar-*S* methyl (ASM), for protection of young cherry trees from bacterial canker.

and, the relationship between single or double heading and the resulting length of the orphan stub* on initiation of bacterial canker in the trunk tissue below the heading cut.

*The 'orphan stub' is the unsupported trunk tissue below the heading cut but above the topmost lateral (unsupported because there is no potential for further vascular flow through it).

Results:

- Spring trunk paint and root dip treatments with ASM in 2010 did not suppress bacterial canker. In fact, trunk paint treatments were phytotoxic to cherry and exacerbated canker symptoms.
- A second heading cut made in May on April-planted cherry trees reduced the amount of dead, shrunken trunk tissue (canker) that developed below the heading cut. Larger cankers developed on those trees headed only once compared to those headed a second time.
- Canker lengths were positively correlated with lengths of orphan stubs.
- Long orphan stubs increased the incidence of gummosis associated with the heading cut compared to short orphan stubs.

Recommendation to orchardists:

Growers should consider a second heading curt in dry weather 2 to 5 weeks after the first heading cut, or alternatively, delay the first heading until it can be made going into a period of dry weather. Whichever the case, the final heading should strive to achieve a short orphan stub by cutting ~5 mm above the emerging bud of (what will become) the topmost lateral angled downward 45° toward the distal side of the trunk. Based on the data, producing a short orphan stub and avoiding wet weather wet weather when making heading cuts are likely more important than whether or not the tree is headed a second time.

Future directions:

The current consensus among researchers is management/prevention of bacterial canker of sweet cherry is best approached through horticultural practices and/or host resistance. Resistance in the pathogen to copper is widespread, rendering copper fungicides ineffective for suppression of this disease. Sprays of other chemicals (e.g., antibiotics) are cost prohibitive because they are not persistent enough to suppress *P. syringae* for long periods of time. Further understanding of bacterial canker and its suppression needs to continue to focus on how and when wounds are made and the causes of stress in host tissues.

FINAL PROJECT REPORT

Project Title: Improving biological control of insect pests of cherry

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State/Zip: WA 98801

Total Project Request: Year 1: $38,802 Year 2: $40,683

Other funding sources

Agency Name: USDA/CSREES Specialty Crop Research Initiative
Amt. awarded: $2.24 million
Notes: Enhancing biological control to stabilize western orchard IPM systems. $2.24 million awarded to WSU. 2008-2013. V. P. Jones PI, P. W. Shearer Co-PI.

WTFRC Collaborative expenses: none

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

Item	Year 1	Year 2
Salaries[1]	9,126	9,582
Benefits[2]	5,476	5,845
Wages[3]	2,000	2,100
Benefits[4]	160	168
Equipment	0	0
Supplies[5]	2,400	2,520
Travel[6]	325	341
Total	19,487	20,556

Footnotes:
[1] **25% FTE Technician**
[2] **benefits at 60% yr 1, 61% yr 2**
[3] **student (time slip) summer help**
[4] **benefits at 8%**
[5] **includes traps, chemicals, field supplies**
[6] **within state travel**

Budget 2

Organization: WSU-TFREC **Contract Administrator:** Mary Lou Bricker, Kevin Larson
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KL 509-663-8181 x221 **Email:** kevin_larson@wsu.edu

Item	Year 1	Year 2
Salaries[1]	9,434	9,811
Benefits[2]	3,585	3,728
Wages[3]	2,000	2,163
Benefits[4]	296	308
Equipment	0	0
Supplies[5]	2,400	2,520
Travel[6]	1,600	1,680
Total	19,315	20,127

Footnotes:

[1] **25% FTE Technician**

[2] **benefits at 38%**

[3] **student (time slip) summer help**

[4] **benefits at 14.8%**

[5] **includes traps, chemicals, field supplies**

[6] **within state travel**

Objectives:

1. Assess natural enemy complex of cherry arthropod pests from representative OR and WA cherry orchards using herbivore-induced plant volatiles (HIPV), visual inspections, and beating tray sampling.
2. Determine phenology of key natural enemies in OR and WA cherry orchards.
3. Validate predictive emergence models for key natural enemies that occur in OR and WA cherry orchards.

Significant Findings:

- Herbivore-induced plant volatiles (HIPV), when used as natural enemy attractants, are providing us with new information about which natural enemies are present in WA and OR cherry orchards.
- HIPVs are useful to determine what time of year natural enemies are present or absent from these orchards.
- HIPVs provide additional information in natural enemies that are difficult to sample with beating trays and when used together provide a more complete understanding of which species and life stages of various natural enemies are present at a particular point in time.
- It appears that natural enemy populations in cherry orchards are found at levels lower than apple and pear.
- We can use these attractants and levels of natural enemy abundance to help understand the impacts of spray programs on these and other beneficial insects.
- We are finding similar trends in natural enemy phenology in apple and pear orchards that increases the likelihood that we will be able to develop natural enemy phenology models for PNW orchard crops.

Results and Discussion:

Objective 1. Assess natural enemy complex of cherry arthropod pests from representative OR and WA cherry orchards using herbivore-induced plant volatiles (HIPV), visual inspections, and beating tray sampling.

We used the same three orchards (six total) in both Oregon and Washington that were used last year. In Oregon, all three orchards were managed conventionally. Lat year in Washington, the Orondo and Malaga orchards were conventionally managed and the Quincy orchard was organic; this year the Quincy orchard transitioned back to conventional management. Each orchard was sampled weekly using beating trays and three different natural enemy attractants (geraniol + methyl salicylate + 2-phenylethanol [=GMP], squalene [SQ], and 2 phenylethanol + methyl salicylate [PE+MS]) paired with white (SQ, PE+MS) or yellow panel traps (GMP). In year 1, we used all lures with white delta traps, but studies in our labs suggested that we would have fewer undesirable non-target insects on the panel traps.

All of the beating tray data for year 1 has been processed, but not completely analyzed. We have not yet completed processing all of this years' beating tray data but preliminary results for 2011 from Oregon indicate that HIPVs capture more *Deraeocoris, syrphids, and adult lacewings (Brown, Chrysopa nigricornis* and *Chrysoperla plorabunda*) than beating trays (Table 1). This is partially due to the fact that beating trays are a snapshot measurement conducted once per week while the HIPV traps can catch insects continually on a week-by-week basis. More importantly, though, the above insects, especially syrphids and lacewings, don't readily fall on to beating trays so aren't readily captured or identified. However, immature insects don't have wings and can't fly to these traps thus, these stages of insects and spiders are more readily captured with beating trays.

We hope to have all the cherry beat tray specimens identified by early winter and would be happy to provide that information at meetings next year. We have not yet received the spray records for some of the orchards, but hope to have that information by Thanksgiving.

Table 1. Comparisons of insects captured with beating trays versus HIPV traps

	Total number found in Oregon sites	
	Sampling Method	
Natural Enemy	Beating Tray	HIPV
Coccinellid adults	141	138
Deraeocoris brevis adults	24	341
Spiders	754	N/A
Syrphids	N/A	250
Lacewing larvae	61	N/A
Brown Lacewing adults	17	120
Chrysopa nigricornis adults	N/A[1]	3319
Chrysoperla plorabunda adults	N/A[1]	911

[1]Green lacewing adults (*C. nigricornis* and *C. plorabunda*) were recorded using beating trays but the adults could not be identified to species using this sampling method. The total number of green lacewings recorded from beating trays was 20 adults.

All the insects have been identified on the HIPV traps for years 1 & 2. The diversity of natural enemies was much greater in year 2, probably because the panel traps are more efficient and incorporate a visual component as well as the chemical lure. Year two also had significantly more black cherry aphids at all Washington locations but they were still absent for the most part in Oregon orchards with the exception of the Mosier orchard. The presence of black cherry aphids likely contributed to the greater diversity of aphid specific predators and parasitoids we observed and was correlated with the presence of *Deraeocoris brevis* in the Mosier, OR orchard early to mid-May (Fig. 1).

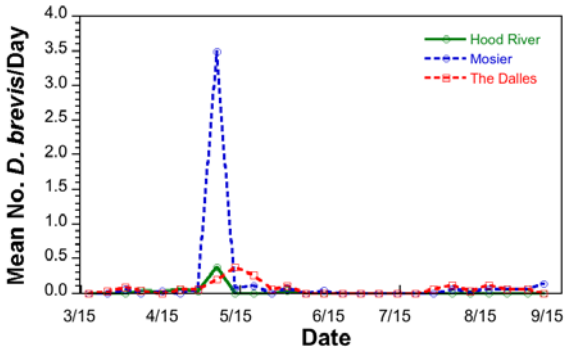

Figure 1. Abundance of *D. brevis* captured on HIPV traps in OR, 2011.

In 2011, we identified over 33 different species of natural enemies from the Oregon sites. The most common were (in order) lacewings including *Chrysopa nigricornis* (Fig. 2), *Chrysoperla plorabunda* (Fig. 3), and brown lacewings; an unknown ichnuemonid wasp; over 15 species of syrphids (Fig. 4), many of which are predacious against aphids including two Platycherus spp., *Eupeodes fumipennis*, and *Syrphus opinator*; over 7 coccinellid species (=lady bird beetles) which feed on aphids and motes including *Coccinella transversa*, *Adalia bipunctata*, and *Stethorus* (= spider mite destroyer) (Fig. 5). In Washington, the most common NE groups (in order) included two green lacewings, *Chrysopa nigricornis* (Fig. 6) and *Chrysoperla plorabunda*, an ichnuemonid wasp (still being identified), the ladybird beetle *Stethorus*,

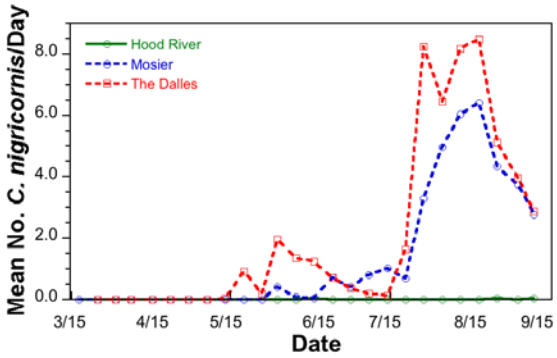

Figure 2. Abundance of *C. nigricornis* captured on HIPV traps in OR, 2011

a mixture of syrphid flies that prey on aphids (*Syrphus* spp., *E. americanis*, *E. fumipennis*, *E. volucris*, *Scaeva pyrastri*, + small numbers of another 4 species) (Fig. 7), the parasitoid *Ceranius menes* (a parasitoid of western flower thrips), *D, brevis*, *Trichogramma* spp. (egg parasitoid of moths), and a mymarid parasitoid of leafhoppers. In addition, there were an additional 50 species of natural enemies found in at least one of the three orchards, but generally in small numbers. Overall, natural enemy abundance appeared to be greater in the Washington orchards compared with those found in Oregon.

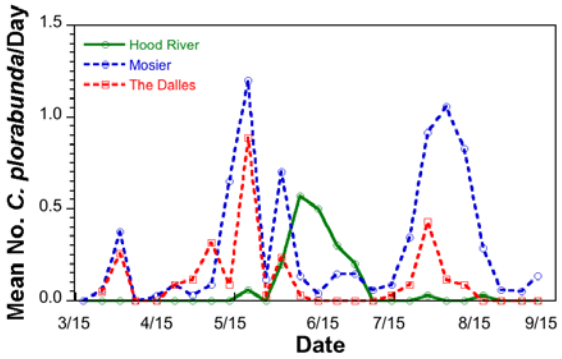

Figure 3. Abundance of *C. plorabunda* captured on HIPV traps in OR, 2011

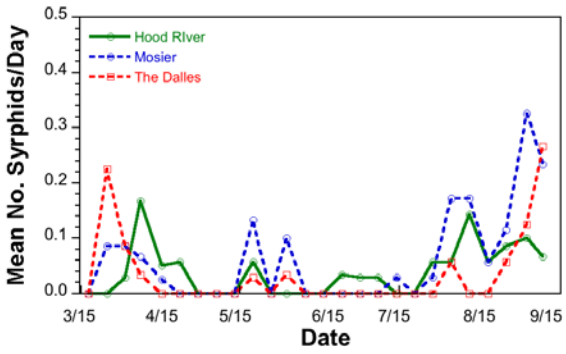

Figure 4. Abundance of syrphids captured on HIPV traps in OR, 2011

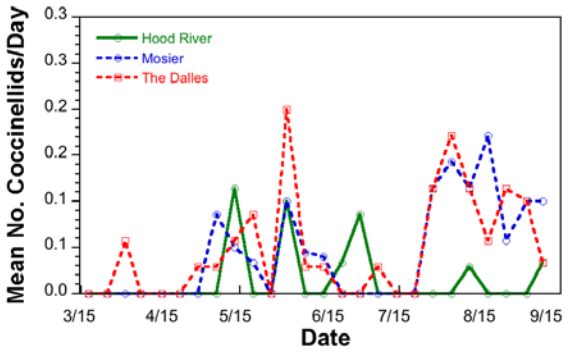

Figure 5. Abundance of Coccinellids captured on HIPV traps in OR, 2011

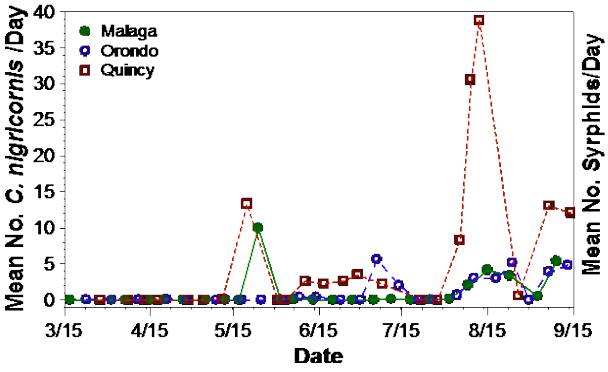

Figure 6. Abundance of *C. nigricornis* captured on HIPV traps in WA, 2011

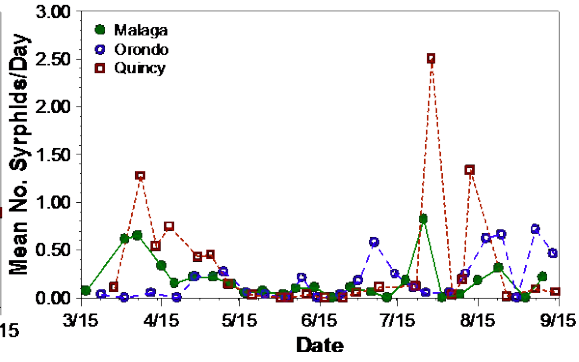

Figure 7. Abundance of syrphids captured on HIPV traps in WA, 2011

Objective 2. Determine phenology of key natural enemies in OR and WA cherry orchards.

As with last year, the natural enemy abundance was extremely low during the period from mid-May until harvest. This is likely a result of pesticide applications to suppress black cherry aphid, powdery mildew, western cherry fruit fly and spotted wing drosophila. For example, the complex of syrphid flies emerge early, but nearly disappeared during the mid-May to harvest period, likely because of either the pesticides or suppression of black cherry aphid. As with last year, it appears that the first

generation of the most abundant lacewing, *C. nigricornis*, is nearly completely suppressed during the same period, but after harvest, the second generation moves in from other locations. We also can say with certainty that the lack of *C. nigricornis* in the Hood River orchard from mid-July on was a result of two applications of Danitol applied for SWD and CFF (Fig. 4). This product appears to have a long lasting effect.

Objective 3. Validate predictive emergence models for key natural enemies that occur in OR and WA cherry orchards.

The development of phenology models for the lacewings and potentially for the more common syrphid flies is underway using the larger data sets from the SCRI project which includes apple, pear, and walnut information. To date, we have shown that the cherry data fits well for the lacewing *Chrysopa nigricornis* with the exception of the "missing" first generation in cherry. The model for *Chrysoperla plorabunda* is being developed this fall and will use the cherry data as well as data from apple, pear, and walnut. The other models will be developed over the next year and will be reported back as soon as they are completed and evaluation is completed.

Executive Summary

Project Title: Improving Biological Control of Insect Pests of Cherry

The results of this study add to our knowledge about the usefulness of using herbivore induced plant volatiles (HIPVs) to monitor natural enemy populations in orchards. These chemicals, when combined with sticky traps, allow us to determine which of several natural enemies are present in orchards, evaluate the impacts of IPM programs on natural enemy abundance and are providing information for developing predictive models that simulate when these beneficial insects may be present in an orchard. Ultimately this information will benefit pest managers by providing them with new tools to for making pest management decisions that incorporate natural enemies in their IPM programs.

We are conducting similar research in other cropping systems including pears, apples and walnuts as part of a western SCRI grant. Information from both grants will enhance our knowledge about natural enemies which should enable end-users to have more information on natural enemy impacts. This information will be applicable to other cropping systems.

We are fine-tuning HIPV-baited traps that selectively capture key natural enemies. We are narrowing down the list of useful HIPVs and trap types in order to develop a few combinations that will be useful to pest managers. We are investigating ways to provide information to the fruit industry on how to conserve natural enemies and use and interpret HIPV-baited traps. We are developing biological control short courses using information from this grant and the SCRI grant that will be offered to the industry in WA, OR, and CA early next year. Among other things, these short cources will train participants on how to identify of natural enemies, present information on the importance of incorporating biological control into IPM programs, the costs associated with using or disrupting natural enemies in orchard systems and how to use HIPVs. For more information on the short course and about these two projects, please see the following website: http://enhancedbc.tfrec.wsu.edu/

FINAL PROJECT REPORT

Project Title: Spotted Wing Drosophila management on sweet cherry

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Bob Van Steenwyk, UC Berkeley, Berkeley, CA
Howard Thistlewood, Agriculture and AgriFood Canada, Summerland, BC

Other funding sources

Agency Name: NIFA-SCRI
Amt. awarded: $95,726 (ca. $20,000/year)
Notes: Walton et al. 2010. Funded 5-year project, 9/1/2010 through 8/31/2015

Total Project Funding: $125,000

Budget History:

Item	2011
Salaries	20,000
Benefits	7,500
Wages	38,400
Benefits	29,472
Equipment	0
Supplies	7,628
Travel	22,000
Miscellaneous	**0**
Total	**125,000**

Objectives

1) Determine seasonal phenology in the fruit-growing areas of eastern Washington.
2) Compare trapping systems for optimal use in large-scale monitoring.
3) Determine stage at which cherries are susceptible to attack.
4) Determine effectiveness of pesticides for SWD on cherry.

Significant Findings

- As of mid-October, trap catches are considerably lower in 2011 than in 2010. In 2010, the highest numbers were found north of I-90; in 2011, higher numbers are being caught south of I-90.
- Of the six SWD trap types tested, the Haviland trap (hardware cloth top) caught significantly more SWD than all other types; the modified Haviland (holes in sides) caught much less than the standard Haviland, indicating the large diffusion area may be responsible. However, this trap was also the least selective for other *Drosophila* and Diptera.
- Spinosad insecticides are highly toxic to SWD, however, activity drops off with decreasing rate; the 1.25 oz rate of Entrust was less effective than the maximum label rate. Using spinosad as a toxicant, several other sugar-based baits caused similar (high) levels of mortality as GF-120. Success, Entrust, Warrior and Endigo caused high levels of mortality, but Delegate allowed more survival in a field-lab bioassay.
- GF-120 reduced fruit infestation by SWD in comparison to an untreated check, whereas Entrust eliminated it completely.

Results and Discussion

SWD Trapping Program. Meetings with fieldmen were held in Brewster and Yakima in April of 2011 to determine optimal trap locations. Traps were deployed in April, and regular weekly checks began in early May. All traps were barcode labeled, and a barcode scanner was used in the lab when the samples were processed, and checked against the known list of trapcodes. Each trap had a duplicate (either a duplicate trap for the Contech, or a sample cup for the other trap types) with matching barcodes; one trap was in the field, and on in lab at any given time. The duplicate trap was cleaned out and re-baited at the end of the week in preparation for deployment the following week.

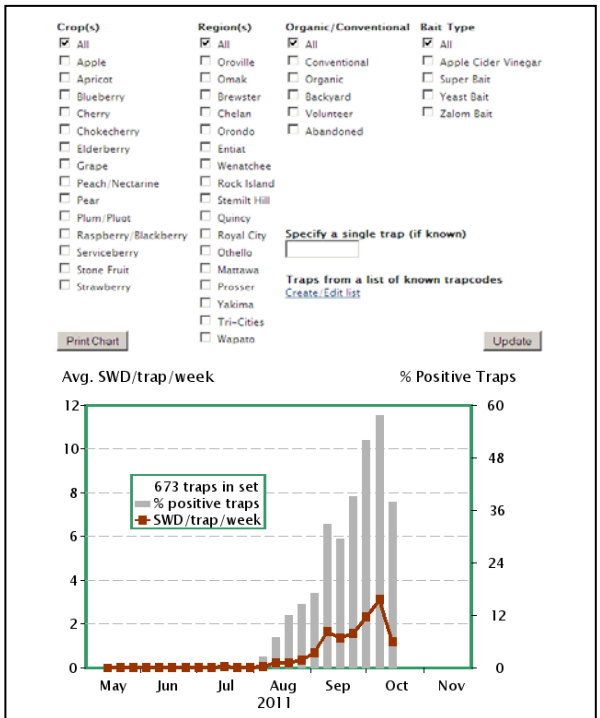

Fig. 1. Filter selection and sample graph from SWD website

The contents of the traps were counted in the laboratory using a binocular microscope. SWD males and females were recorded separately, and other drosophila were counted in order to assess trap selectivity. The counts were recorded directly into a computer, and uploaded at the end of each working day to the WSU SWD website (http://extension.wsu.edu/swd/). The table on the front page gave a list of growing regions in eastern Washington, and the alert status of each region (whether the first fly had been caught in that region). First catches were posted on the day the sample was processed, and an update was added to the blog at the end of the week.

In addition to the alert table, website users could graph the contents of the database, either using the entire database, or by filtering the contents by crop, region, or growing regime (Fig. 1). This facility allowed access to the database information while preserving the anonymity of individual operations. Users could also create custom lists of their own traps using individual trap codes.

Overall, trap catches were considerably lower in 2011 than in 2010 (Fig. 2). The reasons are not known, but the cold snap in late November of 2010 may have drastically reduced the overwintering population, and the delayed development of fruit trees due to cold weather may also have negatively affected development of SWD. Unlike 2010, trap catches south of I-90 are double on the average of those north of I-90 (0.30 v. 0.61/trap/week).

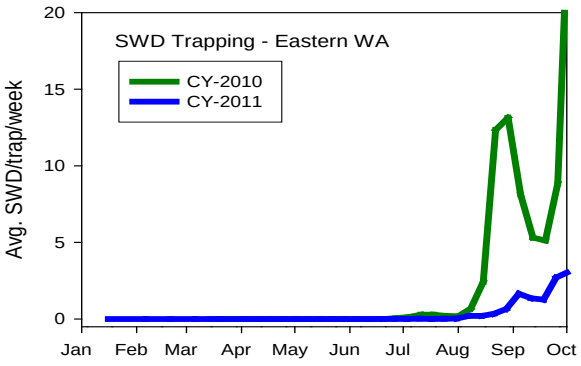

Fig. 2. SWD trap catches (2010 versus 2011).

Trap Type Comparison.

Six standardized trap types were compared in six replicate orchards in eastern Washington. The same traps were also tested by other SCRI participants in other regions and crops. The trap types consisted of different sizes, shapes, and colors of containers, and varying numbers and positions of holes for diffusion of the bait odor and entrance of flies (Fig. 3). All traps had 150 ml bait load, with the exception of the Contech, which had 50 ml. An additional treatment consisted of the Contech with the different brand of apple cider vinegar. The replicate orchards (all cherry) had a history of high trap catches in 2010. Traps were deployed in mid-July when captures began to increase across the region, and contents were counted and bait replaced weekly.

The Haviland trap had significantly higher trap captures than the other traps; the clear deli cup and the Van Steenwyk trap had the next highest capture (Fig. 4). The modified Haviland, red cup, and Contech (with Western Family Apple Cider Vinegar) had the lowest captures, with the exception of the Contech with Heinz apple cider vinegar, which caught the fewest.

Haviland trap mesh top, cover	Modified Haviland trap (10 holes in sides, no cover)	Van Steenwyk trap, 1 qt ice cream, mesh top, cover
Deli cup (1 qt, 10 holes in sides)	Red cup (1 qt, 10 holes in sides)	Contech (2 holes in sides)

Fig. 3. Traps used in standardized trap comparison, eastern Washington, 2011.

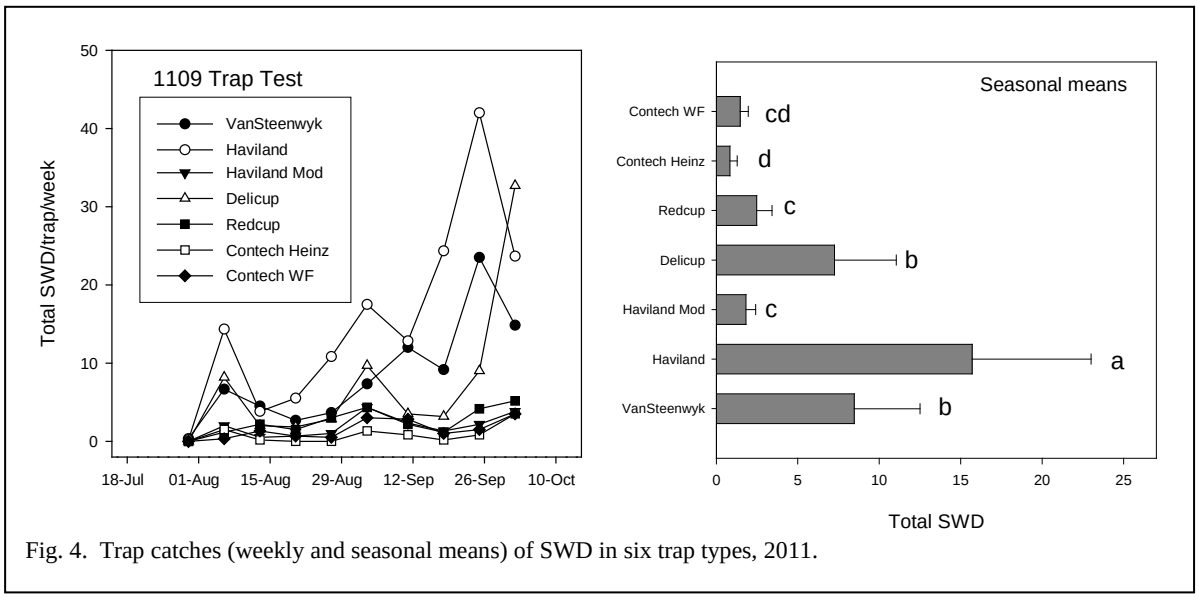

Fig. 4. Trap catches (weekly and seasonal means) of SWD in six trap types, 2011.

Bait Comparison. A comparison of bait and trap type/bait combinations was tested in eight replicate orchards. The highest total captures were made in the deli cup/ACV trap, followed by the Contech/Superbait and Contech/ACV, which is consistent with the 2010 trapping program. The yeast bait was the least attractive of the three baits tested (Fig. 5).

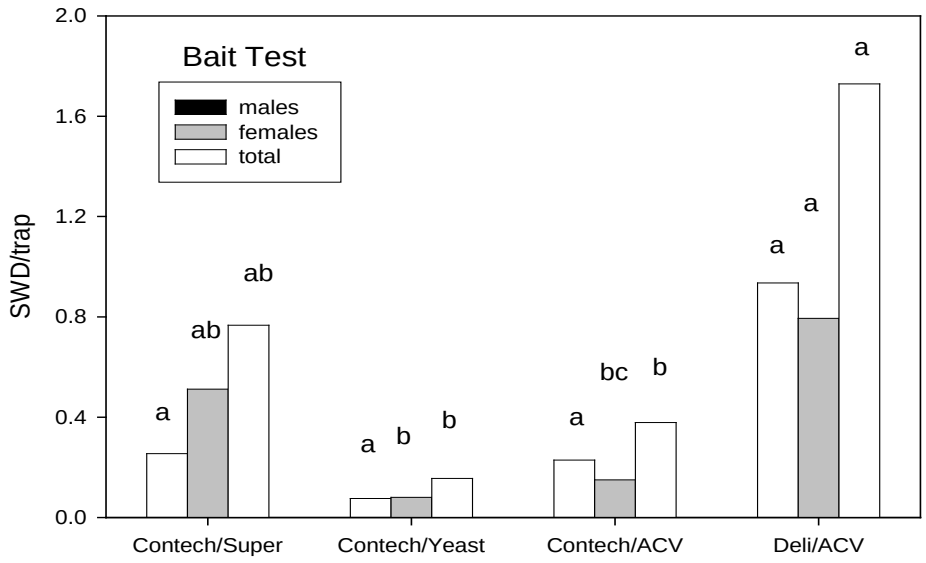

Fig. 5. Three baits in Contech traps compared ACV in a deli cup trap.

Scentry Lure Test. A series of dry lures from Scentry was tested in July-October. The lures consisted of dry plugs or fibers impregnated with various fruit odors. The compounds tested included damascenone (plug); apple cider vinegar (fiber); cis-2-Penten-1-ol (plug); 2-(diethylamino)ethanol (plug), red raspberry vinegar (fiber), and 2-methylbutyic acid (plug). The lures were placed in a small plastic basket with large holes for scent diffusion, and suspended above 100 ml water plus surfactant in a 1 qt clear plastic glass jar with a screw top. The lures were compared to 100 ml liquid apple cider vinegar in the same size container. All traps had 10 3/16th in holes drilled around the upper edge for scent diffusion; the holes were about at the same level as the lure basket.

None of the compounds tested provided significant attraction of SWD, or for that matter, other insects (Fig. 6). Only liquid apple cider vinegar caught appreciable numbers of SWD. After it became

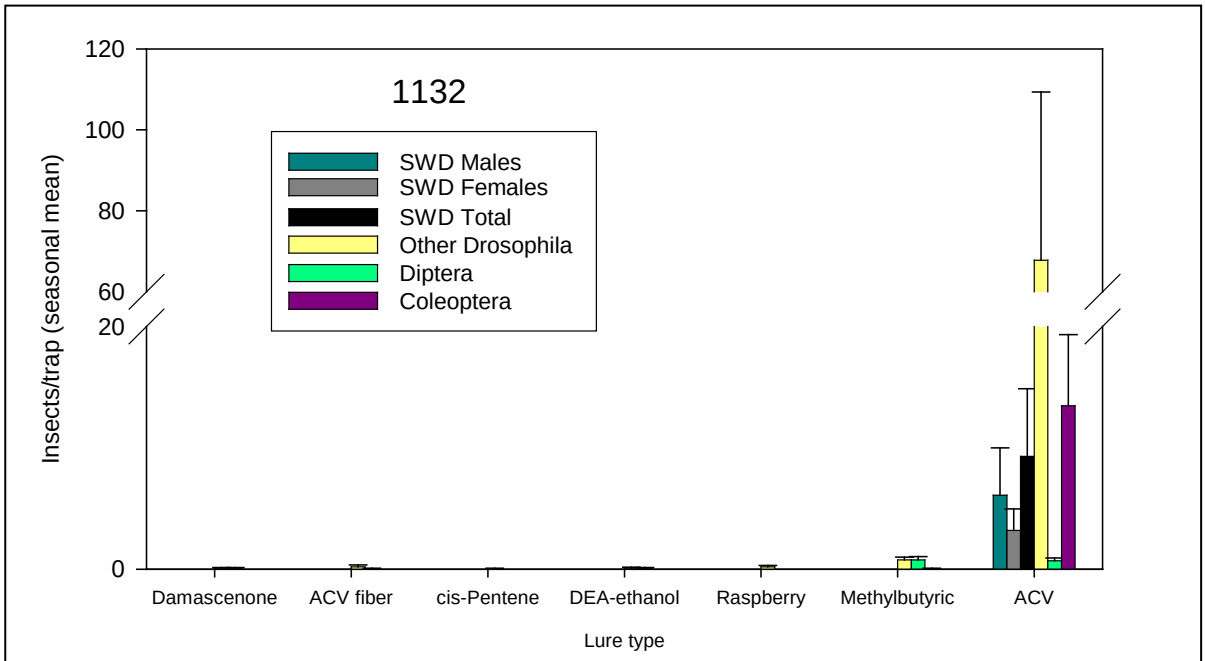

Fig. 6. Capture of SWD and other trap contaminants with various fruit-scented dry lures in comparison with apple cider vinegar bait, 2011.

apparent that no catch was occurring in the dry lure traps, sticky cards were added to the trap to ensure that lack of capture was not due to the fluid used (water vs. ACV). However, even with the addition of sticky cards, there was no capture of SWD with the dry lures.

Cherry Fruit Susceptibility. These data are still being analyzed, however, preliminary analysis indicates that in laboratory bioassays, both ripe and unripe fruit were susceptible to attack by SWD in no-choice tests. Fruit were not attacked until after commercial harvest time in field tests, although this was likely a result of the method of exposure (sleeve cages) rather than a true reflection of susceptibility. A full report will be posted on the SWD website when it becomes available.

Pesticide Efficacy. Twenty-one bioassays of varying types were performed with SWD during 2011 (tests still ongoing); selected tests are shown in the following tables. Early bioassays used a plastic portion cup provisioned with honey/water and drosophila medium; later bioassays used cherry leaf-lined 16 oz deli cups, similarly provisioned. A variation was the addition of fruit to the bioassay arena, which allowed us to evaluate fruit damage (oviposition punctures) as well as mortality. These bioassays provide an initial screening of some toxicants, and more detailed rate effects on those known to be effective. Early bioassays clearly indicated that female SWD were more difficult to kill than males; since females are also the damaging stage, only females were used in later tests.

Among the new materials, tolfenpyrad shows promise as a topical material for SWD (Table 1). An organically approved compound, EF300 (a mixture of ground herbs and spices) had no effect on mortality (Table 2). Pyganic 1.4EC caused a moderate amount of mortality at the highest label rate of 64 fl oz, but none of the lower rates tested were significantly different than the check. Interestingly, Pyganic 5EC (Table 2) had even poorer activity, although it was tested at the maximum rate per acre and the highest concentration allowed by the label.

Table 1. Mortality of male and female SWD with tolfenpyrad and Delegate, 2011

1101-02, 48 h mortality, portion cup, contact+oral					
		Females		Males	
Treatment	Rate/100 gal	% Mortality		% Mortality	
Tolfenpyrad 15SC	27 fl oz	73.84	b	90.00	b
Delegate 25WG	7 oz	100.00	a	100.00	a
Check	-----	0.00	c	4.00	c
1101-03, 48 h mortality, portion cup, contact only					
		Females		Males	
Treatment	Rate/100 gal	% Mortality		% Mortality	
Tolfenpyrad 15SC	27 fl oz	34.00	b	84.00	b
Delegate 25WG	7 oz	100.00	a	100.00	a
Check	-----	1.00	c	16.00	c

Table 2. Mortality of male and female SWD with EF-300 and Pyganic, 2011

1101-11, 48 h mortality, contact, portion cup,					
		Female		Male	
Treatment	Rate (% v/v)	% Mortality		% Mortality	
EF300 1.25%	1.25%	17	a	14	a
EF300 0.75%	0.75%	11	a	3	a
Check	---	13	a	6	a

1101-12, contact, 48 h mortality, females only			
		Female	
Treatment	Rate/100 gal	% Mortality	
PyGanic 1.4EC	64 fl oz	51	a
PyGanic 1.4EC	32 fl oz	16	b
PyGanic 1.4EC	16 fl oz	13	b
Check		1	b

1101-15, contact, 48 h mortality, females only			
		Female	
Treatment	Rate/volume	% Mortality	
PyGanic 5EC	18 fl oz/20 gal	29.26	a
PyGanic 5EC	18 fl oz/50 gal	9.00	ab
PyGanic 5EC	18 fl oz/100 gal	0.00	c
Check		4.00	bc

Entrust showed a significant rate effect in two separate bioassays (Table 3). The two higher rates (>1.8 oz/100 gal) had high (97-100%) levels of mortality, but the lower rate (1-1.25 oz/100 gal) caused significantly less mortality (about 70% for females).

Table 3. Mortality of male and female SWD with Entrust, 2011

1101-09, 48 h mortality, contact, portion cup					
		Female		Male	
Treatment	Rate/100 gal	% Mortality		% Mortality	
Entrust 80W	3 oz	100	a	100	a
Entrust 80W	2 oz	100	a	100	a
Entrust 80W	1 oz	69	b	90	b
Check	-----	2	c	1	c

1101-10, 48 h mortality, contact, portion cup					
		Female		Male	
Treatment	Rate/100 gal	% Mortality		% Mortality	
Entrust 80W	2.25 oz	100	a	100	a
Entrust 80W	1.80 oz	97	a	100	a
Entrust 80W	1.25 oz	71	b	89	b
Check	-----	0	c	0	c

Fruit dip bioassays provided further information on whether materials could, in addition to killing adults, protect fruit from damage (Table 4). Either all or half of cherry fruits were dipped in solutions of Entrust (2.25 or 3 oz) or Provado (8 fl oz). Entrust at 3 oz provided the highest level of fruit protection (complete suppression of oviposition), regardless of whether whole or half fruits were treated. The 2.25 oz rate of Entrust provided significant protection, but some oviposition occurred. Provado suppressed fruit damage, although when only half the fruit was treated, the damage was not different than the check.

Table 4. Oviposition damage and mortality following exposure to treated cherries, 2011

1101-07, 48 h oviposition, cherries whole or half fruit dipped

Treatment	Rate/100 gal	Fruit part treated	Ovip/fruit	
Entrust 80W	2.25 oz	whole	2.80	bc
Entrust 80W	2.25 oz	half	0.40	bc
Entrust 80W	3.0 oz	whole	0.00	c
Entrust 80W	3.0 oz	half	0.00	c
Provado 1.6F	8 fl oz	whole	0.60	bc
Provado 1.6F	8 fl oz	half	4.20	ab
Check	-----		11.40	a

1101-05, 24 h mortality, cherries, whole or half fruit dipped

Treatment	Rate/100 gal	Fruit part treated	% Mortality	
Delegate 25WG	7 oz	whole	96	a
Delegate 25WG	7 oz	half	96	a
Tolfenpyrad 15SC	27 fl oz	whole	44	b
Tolfenpyrad 15SC	27 fl oz	half	12	c
Check	-----	---	8	c

Delegate caused high levels of mortality when either whole or half fruit were dipped; tolfenpyrad, however, caused only moderate amounts of mortality when whole fruit were treated, and nonsignificant levels when only half fruits were treated (Table 4).

Table 5. SWD mortality exposed to GF-120, 2011

1101-06, 48 h mortality, portion cup

Treatment	Dilution	% Mortality	
GF-120 1:1	(undiluted)	100	a
GF-120 1:5	1:5	100	a
Check	-----	20	b

When adults were exposed to droplets of GF-10, all dilutions tested caused 100% mortality (Table 5). An additional series of bioassays tested (Fig. 7) various combinations of baits and toxicants, using the same ppm of spinosad as GF-120. All combinations tested provided similar levels of mortality (Fig. 7) to GF-120; the addition of the bait to the toxicant always improved mortality over the toxicant alone. This provides preliminary evidence that other baits may be used to enhance control of SWD. It should be noted that tests in bioassay arenas are essentially no-choice tests, and that results cannot be extrapolated to field situations without additional testing.

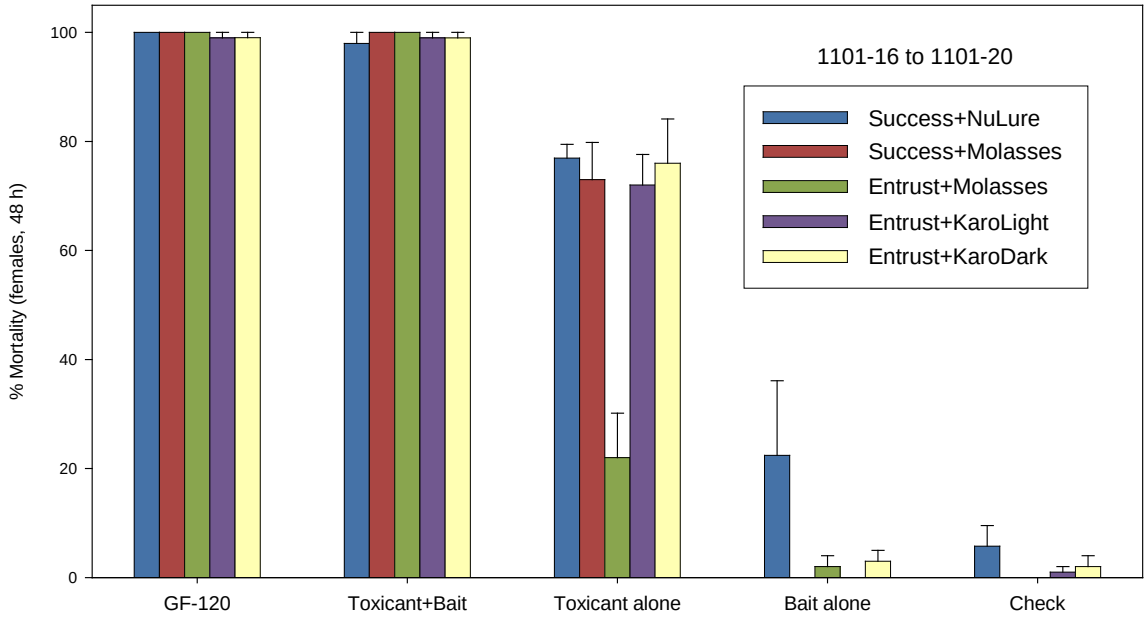

Fig. 7. Mortality of SWD exposed to droplets of bait+toxicant (spinosad), 2011.

A further extension of this concept was tested using the same bioassay arena, but adding three cherry fruits to each and evaluating both mortality and oviposition (fruit protection) (Table 6). All treatments containing a toxicant had high levels of mortality in both males and females, and decreased the oviposition damage to the fruit. However, only the Entrust+NuLure treatment significantly reduced the number of emerged adults in relation to the check.

Table 6. Mortality, oviposition, and adult emergence of SWD exposed to bait+toxicant droplets, 2011

Treatment	n	% Mortality (Males)	x	% Mortality (Females)	x	Total oviposition punctures/ 3 fruit		Emerged adults	x
GF-120	5	100	a	100	a	31.80	b	14.60	ab
Entrust+NuLure	5	90	a	96	a	12.20	b	10.40	b
Entrust alone	5	94	a	96	a	49.80	b	25.80	a
NuLure alone	5	10	b	2	b	131.40	a	38.40	a
Check	5	0	b	4	b	137.40	a	39.20	a

[x]Data transformed arcsine(sqrt(x/100) (percentage data) or log(x+0.5) (continuous).

GF-120 Field Trial. An unreplicated large block trial was performed to test the efficacy of GF-120 against SWD. A 0.4 acre research cherry orchard was divided into three plots of 3-4 rows each. Treatments consisted of 4 weekly applications of 1) GF-120, applied with an ATV-mounted sprayer, 2) Entrust applied with a mist blower, or 3) untreated check. Fruits were harvested on three dates to determine infestation (Fig. 8).

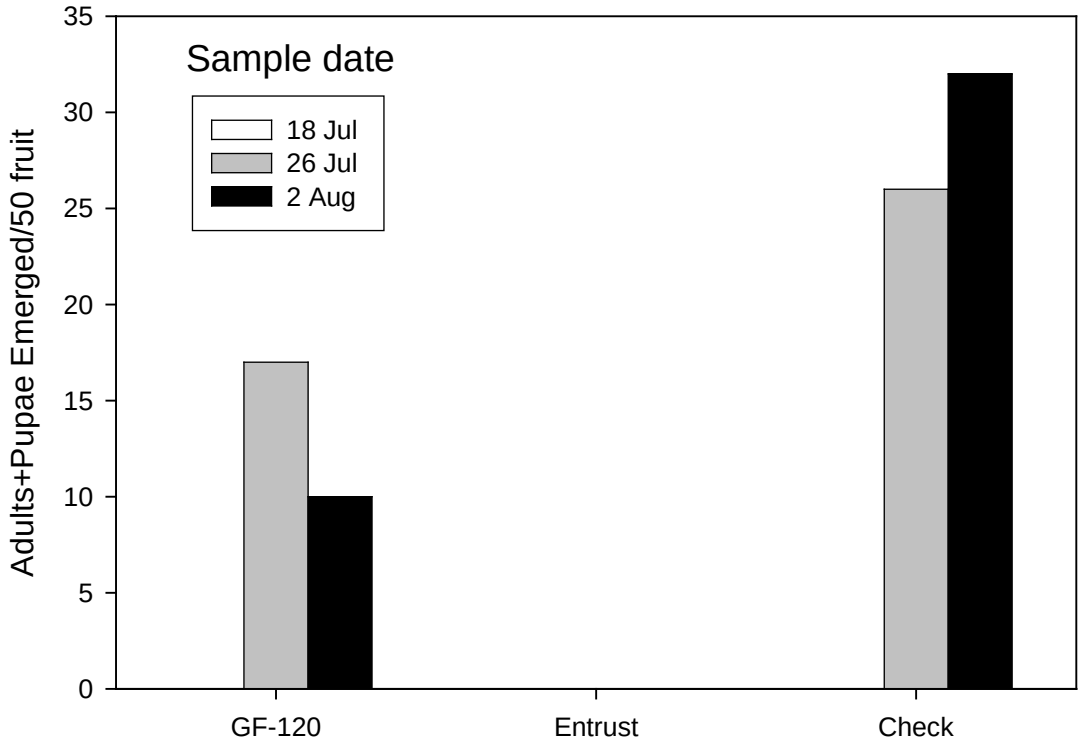

Fig. 8. Unreplicated field trial of GF-120, 2011.

Entrust provided excellent control of SWD infestation, with no fruit damage evident (Fig. 8). GF-120 appeared to suppress infestation levels relative to the untreated check. While GF-120 may not provide stand-alone control of SWD, this technique merits further evaluation in organic orchards as a supplementary measure, especially within the preharvest interval for Entrust, or when airblast equipment can no longer go through the orchard.

Replicated Field Trial. A replicated field trial was performed to test various combinations and timings of materials for control of GF-120. The test was conducted at the Sunrise orchard near Rock Island, WA. Plot size was 3 rows x 4 trees, with 6 treatments replicated 4 times.

No natural infestation was evident (likely due the late season), so residues on fruit and leaves were challenged with lab-reared flies to determine the efficacy of the residues (Fig. 9). All treatments except Delegate provided excellent prevention of oviposition damage, which allowed a moderate level of oviposition. All treatments caused high levels of mortality (data not shown).

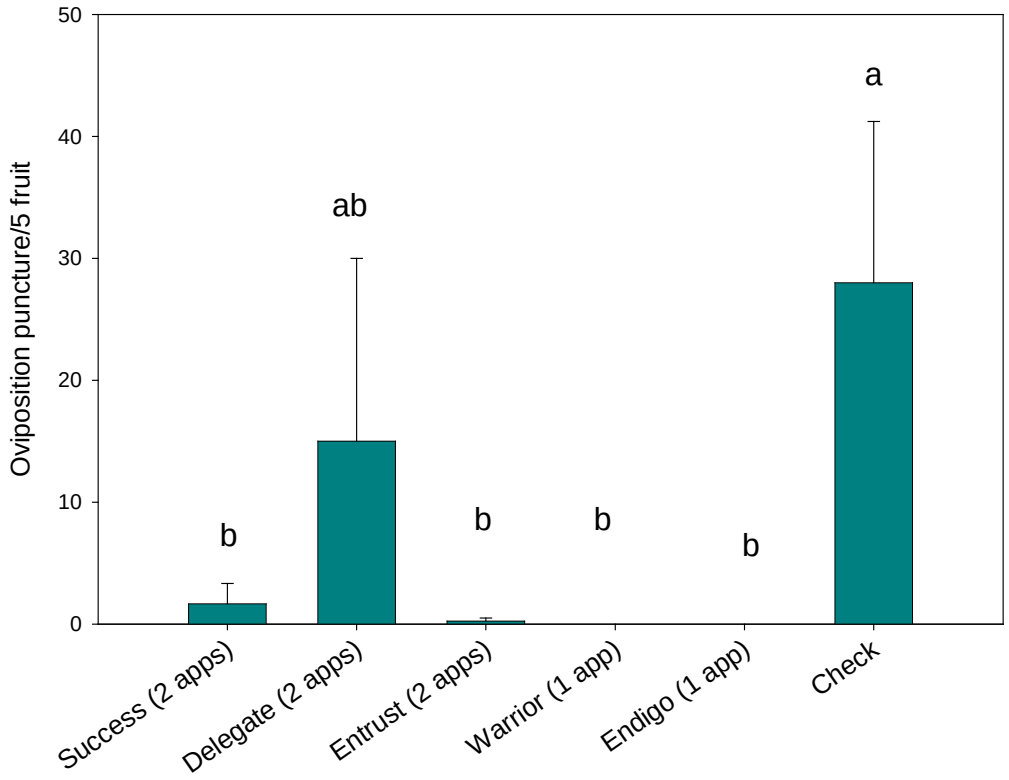

Fig. 9. Fruit protection from field treatment of 'Sweetheart' cherries treated with various pesticide regimes, 2011.

Executive Summary

The eastern Washington trapping program and website/email alert system provided consultants and growers with reliable and accessible information on trap catch in the various fruit-growing regions in 2011. This information served as a guide to begin crop protection measures. The populations in 2011 were considerably lower than in the previous year; some orchards were harvested before the first trap catch occurred in that region. However, significant questions still remain on the interpretation of trap catch, and specifically its value as a predictor of crop risk. Part of the question hinges on the type of trap and lure used; information from a trap type test (all using apple cider vinegar) indicates that more SWD are caught in a trap with a large bait surface and diffusion area. However, this trap type requires a shield to keep rain and irrigation from diluting the contents, and is more difficult to manufacture and maintain than a closed-top style. Alternative baits (wine- and yeast-based) are alternatives to ACV; wine baits appear to improve capture over SWD, while yeast baits had lower capture. Both alternatives are more labor intensive (in the sense they currently must be custom mixed), and more expensive to produce. Dry lures present a low-maintenance alternative to wet bait traps, but to date, none of the lures tested were attractive to SWD.

Pesticide choices are still being refined for SWD. Initial work indicated spinosyns, organophosphates, and pyrethroids as materials with efficacy against SWD. My work has helped refine rates for some of the more important materials, as well as screen new compounds for efficacy. I have given emphasis to organically approved materials, with the understanding that control in organic orchards is a greater challenge than in conventional orchards.

FINAL PROJECT REPORT

Project Title: Development of residue degradation curves for insecticides against SWD

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Project Funding
Agency Name: Project was jointly funded by the California Cherry Advisory Board, the Oregon Sweet Cherry Commission, the Washington Tree Fruit Research Commission, and the Okanagan Kootenay Cherry Growers Association, British Colombia.

Total Project Funding: $30,000 (year 1 of 1)

Budget Distribution:

Item	CCAB	WTFRC	OSCC	OKCGA
Salaries	2,089	3,161	1,875	1,498
Benefits	911	1,378	817	644
Equipment	0	0	0	0
Supplies (Residue analysis)	12,000		4,300	
Travel		460		307
Indirect costs				551
Total	15,000	4,999	7,001	3,000

INTRODUCTION AND OBJECTIVES

Maximum Residue Limits (MRLs) are a measurement of the maximum level of pesticide residues that are allowed on a commodity for human consumption. These levels, commonly referred to as tolerances, are dictated by government organizations in their efforts to ensure that food products are safe to eat.

All countries have the right to establish their own MRLs, leading to discrepancies in the amount of residues that are tolerated on food imported from other countries (Table 1, Fig. 1). These differences arise from the use of different datasets and criteria while establishing tolerances, and from different policies about allowable residue levels prior to when an MRL can be established. Thus, a commodity with a legal amount of residue in the country in which it was produced may have an illegal residue in a country to which it is exported. If detected, the shipment will be rejected, and unless an alternative market is found rapidly, may result in a complete economic loss for the exporter.

The recent introduction of spotted wing drosophila (SWD) into cherry-producing areas of the western United States has heightened concerns regarding MRLs for countries that import U.S. cherries. Current management programs for SWD require one or more insecticide treatments within the last few weeks of harvest. The problem is that these treatments, though considered safe according to the U.S. and California Environmental Protection Agencies, have the potential to cause fruit to be rejected when it is shipped to countries where tolerances for residues are not established or are established at levels lower than those in the US.

The purpose of this project is to address this issue through two objectives. The first was to improve our understanding of the in-field degradation rates of six insecticides used for SWD. The second was to evaluate the effects of post-harvest washing on residue levels. The overall goal once these objectives were accomplished was to propose treatment programs that would not only be effective, but that could also still allow for the exportation of fruit.

SIGNIFICANT FINDINGS

During the spring of 2011 we established two experimental orchards and evaluated the use of six insecticides (Table 2) when used approximately 21 days before harvest and four insecticides when used approximately 7 days before harvest. These orchards were used to evaluate degradation curves for each insecticide at each location. Results of the experiments were used in conjunction with current international maximum residue levels to determine the relative risk associated with exporting fruit that has been treated with each insecticide. This information was combined with existing knowledge about the biology of spotted wing drosophila, its relationship with cherries as a host, and information regarding pesticide efficacy to propose an insecticide program that would be effective but still allow for the export of fruit to all major export markets. Based on this information we proposed a three-spray program based on Warrior II approximately 21 days before harvest, followed by an application of Success approximately 7-14 days before harvest, followed by an application of a low rate of Malathion approximately 3-7 days to harvest. In cases where only two applications are needed we proposed the substitution of a single application of Success or Malathion approximately 7 days to harvest in place of using each product individually as in the three-spray program. Following these programs should allow cherry growers to effectively control spotted wing drosophila while still maintaining the ability to export fruit.

RESULTS

Applications of the spinosyns Delegate and Success resulted in relatively low residue levels that degraded quickly (Fig. 2a-b). When applied 21 days before harvest, residue levels for both insecticides ranged from 0.06 to 0.19 ppm during the evaluations at 0 and 3 DAT, and at or below the limit of detection of 0.05 ppm thereafter. The 21 DAT sample was omitted due to the minimal to non-detectable residue levels during the previous two samples. When applications of Delegate and Success were made 7 days before harvest, similar results were found with residue levels ranging from non-detectable to 0.09 ppm through 3 DAT, followed by levels below the minimum detection level for both products at both sites by the preharvest interval of seven days.

Residue levels for pyrethroids (Fig. 2c-e) were more variable among products than for spinosyns and remained higher for a longer period of time. Applications of fenpropathrin produced the highest residue levels and had the slowest degradation. When applied 21 or 7 days before harvest, Danitol residue levels 3 DAT (the US preharvest interval) ranged from 0.89 to 2.93 ppm. These numbers are well within the U.S. and Japanese MRLs for fenpropathrin (5 ppm), but exceed tolerances for Canada, Korea, Taiwan and the EU (0.01 – 0.5 ppm) (Table 1). Residue levels on both cultivars remained above the MRLs for the latter countries even at 21 DAT.

Applications of lambda-cyhalothrin at 21 days before harvest resulted in residue levels ranging from 0.10 to 0.31 ppm from the time of application through 7 DAT (Fig. 2d). At 14 DAT (the U.S. preharvest interval), residue levels ranged from 0.08 to 0.11 ppm. These levels were approximately one-half to one-fifth lower than the MRLs for all major export markets (0.20 to 0.50 ppm) (Table 1).

Applications of zeta-cypermethrin at 21 days before harvest resulted in residue levels ranging from 0.08 to 0.23 ppm at 0 through 7 DAT (Fig. 2e). At the preharvest interval of 14 days residue levels ranged from 0.09 to 0.11 ppm. This is within the U.S., Japan and EU MRLs (1.0 to 2.0 ppm), but is about equivalent to the Canada MRL of 0.1 ppm and above the Australian MRL of 0.01 ppm (Table 1). Korea and Taiwan do not have MRLs established for zeta-cypermethrin, thus any residue would cause fruit to be rejected. By 21 DAT residue levels ranged from 0.02 to 0.05 ppm, which would have qualified fruit for export to Canada, Japan and the EU (0.1 to 2.0 ppm), but would still result in the rejection of fruit in Australia, Korea and Taiwan (0.00 to 0.01 ppm).

Applications of the organophosphate Malathion at 21 and 7 days before harvest at the 1,754 ml/ha (1.5 pt/acre) rate (which is lower than the maximum label rate due to risk of phytotoxicity) resulted in residue levels that ranged from non-detectable to 0.12 ppm through 2 DAT and from non-detectable to 0.06 ppm at the preharvest interval of 3 DAT (Fig. 2f). These levels were below the MRLs for all countries (0.50 to 8.0 ppm) except the EU (0.02 ppm); the extremely low MRL for the EU meant that some of the residues found would be unacceptable (Table 1). By 7 DAT residue levels for Malathion ranged from non-detectable to 0.02 ppm.

Evaluations of the effects of simulated post-harvest processing had variable results on residue levels. Of the six pyrethroid samples tested thirteen days after application, Danitol residues were decreased by an average of 22.0%, Warrior residues were decreased by an average of 15.7% and Mustang levels were increased by an average of 5.6%. Simulated processing two days after application of Danitol resulted in an average reduction of just over half of the residues (51.7%) whereas changes in residue levels for Delegate, Success and Malathion could not be determined due to one or both residue levels being below the minimum detection levels of 0.01 (Malathion) or 0.05 (Delegate and Success). These results suggest that cherry producers can make a general assumption that post-harvest processing is going to likely help reduce pesticide residues, especially when residues are initially high. However, the high variability in the results of this study suggest that making predictions about

residue reductions will be sufficiently complex that growers should rule out post-harvest reductions as a reliable method for ensuring that fruit does not exceed residue tolerances. Growers need to continue ensuring that residue levels are below MRLs for intended markets at the time of picking and prior to processing.

DISCUSSION

Current management programs for SWD are based on three general types of treatments. These are long-residual products with preharvest intervals of ≥14 days, mid-range products with a 7-14 day preharvest interval, and products for use close to harvest (1-3 day preharvest interval). Long-residual products are those that are typically applied at the initiation of the straw stage of development when fruit becomes susceptible to attack by *D. suzukii*. Of the products tested the pyrethroids Danitol, Mustang and Warrior II all had relatively long residuals. Of these, Warrior II has the best overall profile as a long-residual product whose application resulted in residue levels in this study that were below the MRLs of all major export markets for cherries. These data also suggest that growers who export fruit should avoid the use of Danitol; Mustang use should be avoided on fruit that is for export to Canada, Korea and Taiwan.

Of the middle-range products for use 7 to 10 days before harvest, Delegate and Success both produced residue levels below the lower detection limit of 0.05 ppm at the preharvest interval of seven days. This suggests that either insecticide is equally valuable for use. However, between these two products Success has a better MRL profile of 0.05 to 1.00 ppm for major export markets whereas MRLs for Delegate include a default MRL in Canada of 0.01 ppm while for Taiwan no MRLs have been established such that any detection would disqualify fruit.

Malathion and Danitol are the only two insecticides in our study that have preharvest intervals of three days or less. At a use rate of 1.75 liters/ha (1.5 pt/acre) residue levels for Malathion in our studies were low enough to allow for the export of fruit to all major export markets with the exception of the EU, which has an exceptionally low MRL for this product. Growers planning on shipping fruit to the EU should probably avoid Malathion because residue levels in our trials, even at 7 DAT with a below-maximum labeled rate, were still close to the EU MRL of 0.02 ppm. In weighing their options these growers might also consider the use of permethrin or pyrethrin, which are considered to have very short residuals, but were not tested as part of this project.

When all things are considered, data from this project can be used to outline potential spray programs that should be effective for *D. suzukii* and still allow for the export of fruit. For example, areas requiring three insecticide applications could consider using Warrior II at the initiation of straw, followed by an application of Success 7 to 14 days before harvest, and followed by an application of Malathion 3 to 7 days before harvest. This should allow fruit to be shipped to all major export markets with the possible exception of the EU (depending on how quickly Malathion residues degrade). In areas where only two applications are needed, the second and third applications described above could be combined into one application of either Success or Malathion around 7 days before harvest (with the same potential concern for Malathion in the EU). As needed, additional applications of Malathion and or permethrin or pyrethrin (not tested) would be the most likely candidates for treatments between harvests.

Another variation would be the use of spinosad close to harvest. The results of this study were used to support a Special Local Needs SLN label for Entrust (the organic formulation of spinosad) in Washington [and Oregon] which allows a preharvest interval of three days on sweet cherry. In addition, IR-4 studies are underway nationwide which test a preharvest interval of one day for this product on cherries, which would provide even greater flexibility to producers near or during harvest, with minimal risk of violating export MRLs.

When organized in the manner described above growers should be able to successfully treat for *D. suzukii* in a manner that is effective, that utilizes multiple modes of action as part of a resistance management program, and that allows fruit to qualify for export. However, because of the

complexity of treatment programs for *D. suzukii* and the potential for residue-based export restrictions of fruit, growers should develop plans for management well before harvest. Plans should be made only after consulting with representatives of the packing house and should include multiple options for control programs depending on where the fruit will be shipped. They should also be flexible enough to account for one or more treatments based on in-field monitoring programs.

Growers should also be conservative while estimating how data from this project relate to their individual orchards. Residue levels are dependent on many factors such as equipment type, application type, water volume, drive speed, rate used, tree size, canopy density, exposure to sunlight, precipitation, etc. Despite the fact that this project was conducted under typical commercial field conditions, it is important to remember that this project only represents two orchards in Kern County, CA during the 2011 harvest season, and results are expected to vary among locations throughout the western United States.

Table 1. Maximum residue limits (MRLs) of major international importers of cherries for six insecticide active ingredients commonly used for control of *D. suzukii*. MRLs are current as of May 2011[1].

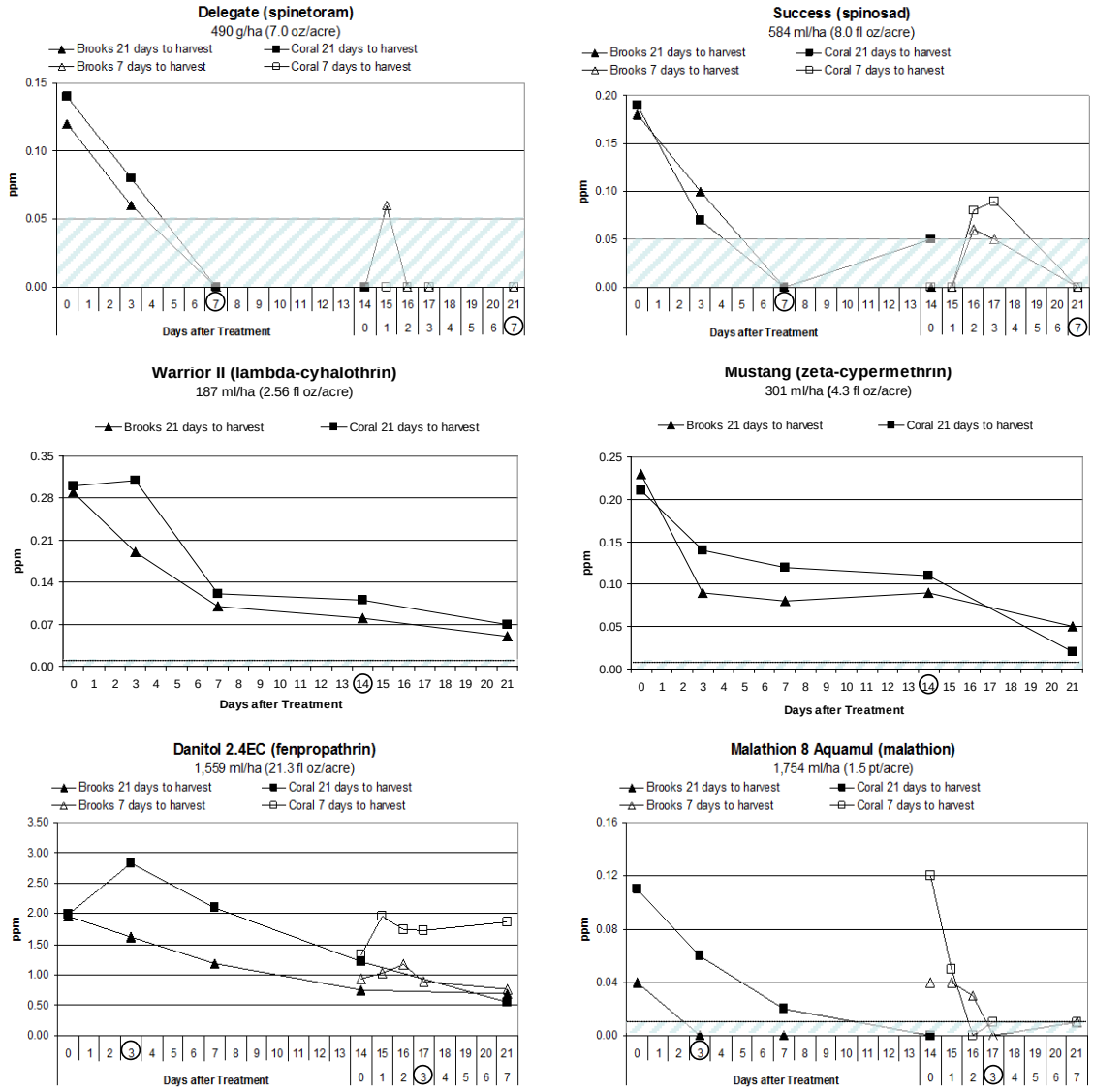
Active Ingredient	Lower Detection Level[2] (ppm)	MRL (ppm)						
		US	Canada	Japan	South	Taiwan	EU	Aust-ralia
Fenpropathrin	0.01	5.00	0.10	5.00	0.50	0.50	0.01	-
Spinetoram	0.05	0.20	0.20	0.01	0.10	-	0.05	0.20
Malathion	0.01	8.00	6.00	6.00	0.50	0.50	0.02	2.00
Zeta-cypermethrin	0.01	1.00	0.10	2.00	-	-	2.00	0.01
Spinosad	0.05	0.20	0.20	0.20	0.05	0.20	1.00	1.00
Lambda cyhalothrin	0.01	0.50	0.20	0.50	0.50	0.40	0.30	0.50

[1]Source: Based on the California Cherry Advisory Board's Online Export Manual, May 2011 (http://www.calcherry.com/industry). Since MRLs change frequently be sure to check for updated and current MRLs prior to shipping fruit to export markets.
[2]Minimum level at which residues can be detected.

Table 2. Names, manufacturers, use rates and preharvest intervals for insecticides that were tested for residues.

Product and formulation	Manu-facturer	Active ingredient	Rate form. product[1]		Preharvest interval[2] (days)
			per ha	per acre	
Danitol® 2.4 EC	Valent	Fenpropathrin	1,559 ml	21.3 fl oz	3
Delegate™ 25 WG	Dow	Spinetoram	490 g	7 oz	7
Malathion 8 Aq	Loveland	Malathion	1,754 ml	1.5 pt	3
Mustang® 1.5 EW	FMC	Zeta-cypermethrin	301 g	4.3 oz	14
Success® 2 SC	Dow	Spinosad	584 ml	8 fl oz	7
Warrior II 2 CS	Syngenta	λ-cyhalothrin	187 ml	2.56 fl oz	14

[1]With the exception of Malathion, application rates were defined as the highest rate allowable per the pesticide label. Due to the risk of phytotoxicity, the Malathion rate was lowered to a level that is generally considered to be effective on *D. suzukii*, but that minimizes the risk of damaging the leaves and fruit.



Figs. 1 (a-f). Residue levels of a) Delegate, b) Success, c) Warrior II, d) Mustang, e) Danitol, and f) Malathion following applications at 21 and or 7 days before harvest. Residue levels of non-detectable are reported as zero residues even though actual residue levels may be anywhere between 0.0 ppm and the minimum detection threshold of 0.05 ppm (for Delegate and Success) or 0.01 ppm (for Warrior II, Mustang, Danitol and Malathion), indicated by the shaded areas. Circled dates indicate the preharvest interval for California in 2011

EXECUTIVE SUMMARY

The recent introduction of spotted wing drosophila (SWD) into cherry-producing orchards of the western United States has resulted in the need for insecticide-based management programs close to harvest. These treatments have become problematic due to inconsistencies among export markets regarding maximum residue levels (MRLs) that are allowed on imported fruit. As a result, fruit that was treated and harvested in a safe manner according to the U.S. Environmental Protection Agency may or may not qualify for export to countries that have lower MRLs, or in some cases no MRLs at all.

This project addressed this issue by evaluating the degradation curves of six insecticides when applied at 21 days to harvest and four insecticides when applied at 7 days to harvest. Results were used to propose three-spray treatment programs based on the use of Warrior II at 21 days before harvest, Success at 7-14 days before harvest, and a low rate of Malathion at 3-7 days before harvest that would be effective, would allow for the export of fruit, and would incorporate the rotation of chemistries as part of a resistance management program. An alternate two-spray program was also proposed for orchards with lower pressure by spotted wing drosophila that combines the latter two treatments in to a single application of either Success or Malathion approximately 7 days before harvest.

Data from this project also documented the effects of simulated post-harvest washing on residue levels on fruit at harvest. Generally speaking, residue levels on fruit that were processed had lower residues than fruit harvested directly off of the tree. However, these reductions were not consistent, predicable or significant enough to recommend that growers rely on washing as part of a residue reduction program.

FINAL PROJECT REPORT

Project Title: Use of methyl bromide to eliminate SWD

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Other funding sources: None

Total Project Funding:

Budget History:

Item	Year 1:	Year 2:	Year 3:
Salaries	10,920		
Benefits			
Wages			
Benefits			
Equipment			
Supplies	2,600		
Travel			
Miscellaneous			
Total	$13,520		

Objectives. The purpose of this study was to demonstrate the insecticidal efficacy of postharvest methyl bromide fumigation toward spotted wing drosophila in sweet cherries, including the exports to Australia, which are treated for 2-h with a 64 mg/L dose at 43 < T < 54°F, a 48 mg/L dose at 54 < T < 63°F, and a 40 mg/L dose at 63 < T < 72°F.

Significant Findings. At 54 ± 0.5 ºF, "CT" concentration – time cross products > 88 mg h/L have resulted in complete mortality of ~55,000 internal feeding large larvae (ca. 96-120 h old at fumigation), the most tolerant life stage of SWD. Between 43-51 ºF, the individual and interactive effect(s) of temperature, time, and methyl bromide (MB) dose on the survivability of the most tolerant SWD life stage was quantitatively delineated; a multifactorial experiment was generated and the results were analyzed using Design Expert 7.0 (Stat-Ease, Inc.). The mathematical model generated in this study can be used as a predictive tool for ensuring that targeted mortality is achieved during individual fumigation events, such as when "Probit 9-level" control of insects (i.e., ≥ 99.9986% mortality) is required by trading partners.

Results & Discussion.

Most MB-tolerant life stage. Direct and indirect analytical methods were used to identify 48-96 h old larvae (60-108 at fumigation) as the life stage of SWD that is most tolerant toward MB. Data support a conclusion that this is a result of larvae burrowing into commodity to feed internally, where fumigant concentrations are relatively lower than on the surface of the fruit. We often observe this larger and older larval life stage completely submerged, including spiracles. On the other hand, eggs, pupae, and younger larvae are exclusively associated with the fruit periphery and an external "feeding" behavior where they have a relatively uniform expose to fumigant concentrations measured in chamber headspace (i.e. external scenario).

Strawberries (▲), cherries (♦), and grapes (■) were infested with SWD over a 48 h period, removed for 48 h, tempered for 12 h, and held as a fumigation control. Adult emergence from fruit obeyed Gaussian distribution over ~ 50h, which was centered 14 day after the initial infestation when incubated at 27 °C and 80% RH. Derivatives of emergence provide a useful illustration, with the slope of the intersects being directly related to uniformity for a particular fruit. One strawberry, two cherries, and three grapes were in each cage; total emerged adults were respectively 1436, 1189, and 2034.

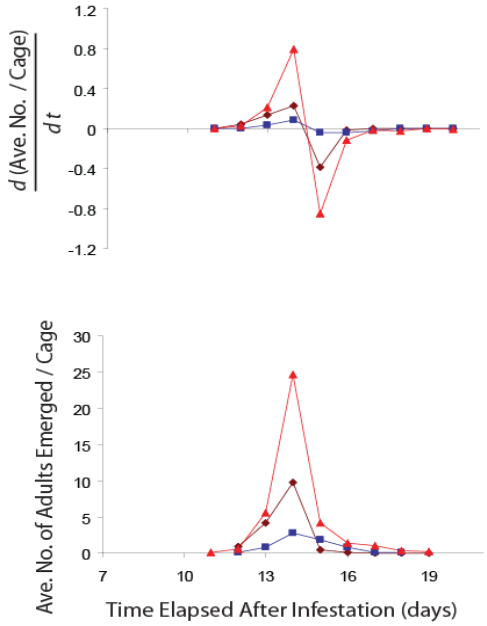

Figure 1. Emergence of adult SWD from fruit can be accurately predicted and occurs with uniform synchronization.

To indirectly diagnose "large larvae" as the most tolerant life stage, strawberries (▲), cherries (♦), and grapes (■) were infested with SWD over a 96 h period, tempered for 12 h, and fumigated with MB exposures of 10.7 mg-h/L at 60°F. The emergence of adults after incubation is at a maximum 14 d after infestation, indicating that "large" larvae (~ 60-108 h-old at time of fumigation) are the most MB-tolerant SWD larval life stage. Numbers of specimens treated are estimated by control emergence of 4,321 in nonfumigated controls.

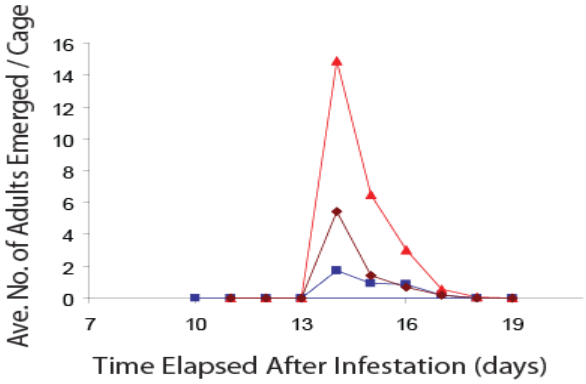

Figure 2. The uniform synchronization of emergence allowed the most tolerant life stage to be determined indirectly.

Dose-mortality data on segregated developmental life stages was used to directly diagnose “large” larvae as the most MB-tolerant SWD life stage. Larvae and eggs specimens were obtained from “natural infestation”, while pupae and adults, which are only encountered on the periphery of the fruit unlike larvae, were treated in cages (Table2).

Table 2. Survivability of SWD dvelopmental stages exposed to MB for 2h at 60F/15.6C

MB Dose	(Stages in order from most (left) to least (right) tolerant)									
	Lg. larvae (48-96h)		Sm. larvae (0-48h)		egg (0-24h)		Pupae		Adult	
(mg/L)	% Survival	% Mortality	% Survival	% Mortality	% Survival	% Mortality	% Survival	% Mortality (corrected) *	% Survival	% Mortality (corrected) *
0 (CG)	-	-	-	-	-	-	90.8	-	93.6	-
16	72.8	29.2	43.6	56.4	26.4	73.6	10.2	88.8	0.0	100.0
32	13.6	86.4	1.9	98.1	2.7	97.3	0.0	100.0	0.0	100.0
48	0.4	99.5	0.0	100.0	0.1	99.9	0.0	100.0	0.0	100.0
64	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
n **	4510		4120		3840		3238		1854	

* Mortality correction = ((control % survival - treated % survival)/control % survival) * 100

** n = total number observed in treated groups for each stage; estimated from the number surviving to adult from control groups (CG) for each stage

A paired t-test was used to further differentiate the target stage as being “older and large larvae” and not “younger and smaller” larvae when feeding on fruit; means were converted to probabilities of emergence for graphics. Statistical values for 0-48 h-old larvae vs. 48-96 h-old larvae with MB exposures of 10.2 mg-h / L: t= 3.2, 10df, P = 0.01; 0-48 h-old larvae vs. 48-96 h-old larvae with MB exposures of 22.9 mg-h /L: t = 2.6, 22df, P = 0.018. The tolerance to MB of the “older and larger” SWD larval life stage, ~60-108 h- old at the time of fumigation (12 h temper period prior to fumigation), is likely due to a more pronounced ability to feed completely “submerged” and relocate more quickly within the fruit pulp relative to “younger and smaller” larvae. This behavioral distinction increases the potential for large larvae to be physically separated from MB, which increases in concentration toward the fruit periphery. SWD eggs, pupae, and adults are associated with the external surface of the fruit and are unable to avoid exposure to MB. Percentages of specimens treated are estimated by emergence from untreated controls of 1,499 and 1,572 for 0-48 h-old and 48-96 h-old larvae, respectively.

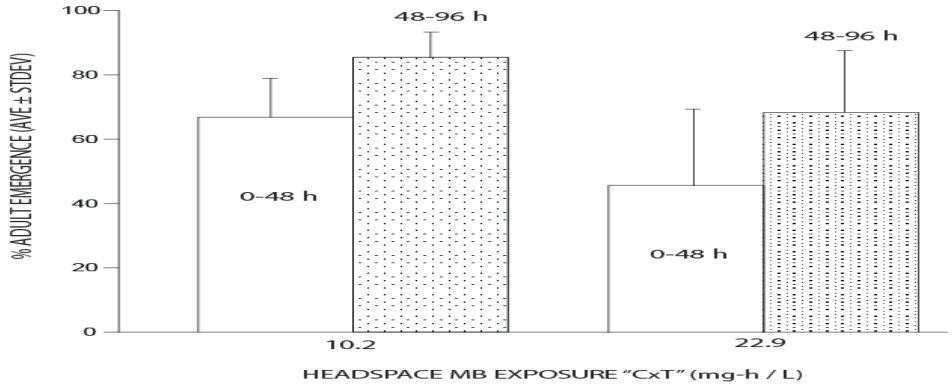

Figure 3. Older and larger larvae are more MB-tolerant than younger and smaller larvae in comparative fumigations of infested grapes at 60 ºF.

Probit Analyses. Dose-mortality regressions were generated using Probit 2007 software; Probit 9 (P9) doses project 99.9968% mortalities. Number of insects specimen treated (n) and regression heterogeneity (H) are noted in Figure 4.

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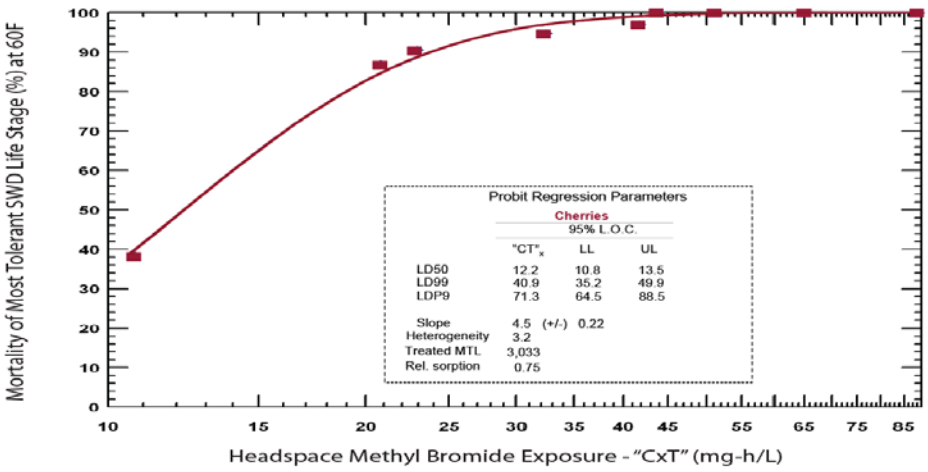

Figure 4. The survivability of the most tolerant SWD life stage(MTL), 60-108 h- old larvae, to MB is directly related to the sorption capacity of the commodity across equivalent 2-h exposures at 60 °F. These data support the conclusion that the "observed tolerance" of this life stage is due, in large part, to internal feeding behavior and physical avoidance of MB, which increases in concentration toward the fruit periphery.

Multivariate Analysis: Negotiating the design space over 43 – 51F. A multifactorial experimental design was generated and the results were analyzed using Design Expert 7.0 (Stat-Ease, Inc.). A three-factor central composite design was employed,[4,5] which contained three levels (-1, 0, 1) of the three factors, x_1–x_3, and six replicates of the center-point. Conditions of dose, temperature, and duration were chosen to accommodate, or span, those applicable to standard industrial practice with respect toward methyl bromide schedules for the import of cherries (APHIS T101-r-1 & T101-s-1) and export of cherries to Japan and Australia (temp-ºF, initial dose-mg/L, time-h): >72, 32, 2; 72-63, 40, 2; 63-54, 48, 2; 54-43, 64, 2. The design involved a total of 34 experiments run in a randomized sequence (Tables 1 and 3), and the modeled response (y) was survivability, which was expressed as a percentage of adult emergence after a treatment relative to estimates of numbers treated based on emergence from non-treated controls.

Table 1. Three factors and three factor levels used in the central composite multivariate experimental design.

Factor (units)	Factor levels		
	-1	0[a]	1
x_1: dose (mg/L)	40	48	56
x_2: temp (°F)	43	47	51
x_3: duration (h)	1	2	3

[a] 0 = center point

A full second-order quadratic expression was fitted to data on insecticidal efficacy of MB versus SWD in cherries; it contained 10 parameters including linear and quadratic dependencies on each factor and all possible two-factor interactions:

$$y = \beta_0 + \beta_1 x_1 + \beta_{11} x_1^2 + \beta_2 x_2 + \beta_{22} x_2^2 + \beta_3 x_3 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3$$

Each parameter of this full second-order model includes a coefficient: β_0, a constant or offset term; β_1, β_2, β_3 estimate the linear effects of the factors; β_{11}, β_{22}, β_{33} estimate the quadratic (curvature) effects of the factors; and β_{12}, β_{13}, β_{23}, estimate the interaction effects between every pair of two factors. Equation 1 represents the optimized model, which was developed to negotiate the design space with greater accuracy between factor levels: 43-47°F, and 47-51°F; it fitted the data with a correlation coefficient (R^2) of = 0.95 (adjusted R^2 = 0.94) and predicted SWD mortality with a correlation coefficient (R^2) of = 0.90 (Table 4 and 5).

$$y = 0.35 - 3.64x_1 + 1.08x_1^2 - 1.59x_2 + 1.38x_2^2 - 6.35x_3 + 4.16x_3^2 + 1.02x_1x_2 + 4.06x_1x_3 + 1.57\,x_2x_3 \quad (1)$$

Table 3. The experimental conditions and modeled response of SWD mortality.

run	dose (mg/L)	Temp (°C)	duration (h)	CT exposure (mg h/L)	treated specimens	survivability (%)
1	40	51	3	97.3	375 ± 146	0.000
2	48	47	2	86.5	341 ± 112	0.293
3	40	47	2	70.7	341 ± 112	2.933
4	48	47	2	86.5	341 ± 112	0.000
5	48	47	2	86.5	341 ± 112	0.293
6	56	47	2	100.9	341 ± 112	0.000
7	40	47	2	70.7	341 ± 112	2.346
8	56	43	3	149.3	794 ± 37	0.000
9	40	43	3	105.1	794 ± 37	0.252
10	40	43	3	105.1	794 ± 37	1.008
11	48	47	3	131.8	341 ± 112	0.000
12	56	51	1	54.1	375 ± 146	4.533
13	56	51	1	54.1	375 ± 146	3.733
14	56	43	3	149.3	794 ± 37	0.000
15	40	51	3	97.3	375 ± 146	0.000
16	48	51	2	91.0	375 ± 146	0.533
17	48	47	1	43.3	341 ± 112	9.091
18	56	47	2	100.9	341 ± 112	0.000
19	48	47	2	86.5	341 ± 112	0.587
20	56	51	3	152.4	375 ± 146	0.000
21	40	43	1	36.3	794 ± 37	24.937
22	56	51	3	152.4	375 ± 146	0.000
23	48	47	1	43.3	341 ± 112	8.504
24	48	47	2	86.5	341 ± 112	1.466
25	40	43	1	36.3	794 ± 37	29.597
26	40	51	1	37.5	375 ± 146	15.733
27	40	51	1	37.5	375 ± 146	18.133
28	56	43	1	53.1	794 ± 37	8.060
29	48	43	2	86.8	794 ± 37	1.511
30	48	47	3	131.8	341 ± 112	0.000
31	48	43	2	86.8	794 ± 37	3.778
32	48	51	2	91.0	794 ± 37	0.630
33	48	47	2	86.5	341 ± 112	0.293
34	56	43	1	53.1	375 ± 146	5.867
					Σ 16,464 ± 634	

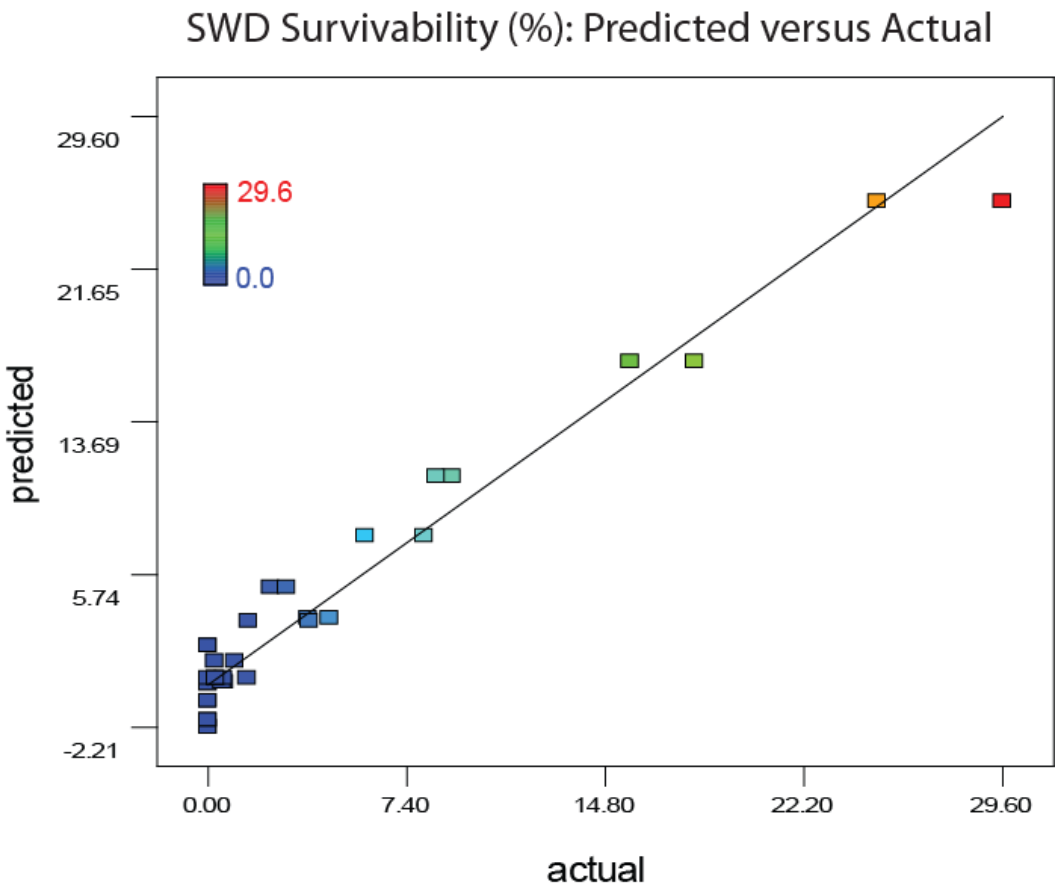

Figure 5. The quadratic model, which was optimized to fit the data on SWD mortality, can also be used for to estimate the success of a fumigation event.

The coefficients (β_x) were tested for significance against the null hypothesis ($\beta_x = 0$), that the factor was unimportant in determining survivability (Table 5). At the 95% level of confidence, SWD survivability depended linearly on the dose (β1), temperature (β2), and duration (β3), interactively on dose-temperature (β12), dose-duration (β13) and temperature-duration (β23), and quadratically on duration-duration (β33).

Table 4. ANOVA statistical analysis of the agreement between the model (equation 2) and the data regarding the survivability of the most tolerant life stage of SWD, the "large" internal feeding larvae (ca. 96-120 h old at time of fumigation).

source	sum of squares[a]	df	mean square[b]	F-value[c]	p-value[d] Prob > F
model	1706.94	9	189.66	56.59	< 0.0001
residual	80.43	24	3.35		
lack of fit	59.44	5	11.89	10.76	< 0.0001

a total for the sum of squares for the terms in the model
b estimate of variance, models sum of squares / model degrees of freedom
c comparison of term variance (mean square) with residual variance (res. mean square)
d probability of seeing observed F value if the null hypothesis is true (no factor effect)

Table 5. ANOVA statistical tests for single parameters of the quadratic model (equation 2) fit to the data on SWD mortality.

parameter coefficient	factor effect	estimate	standard error(1df)	sum of squares [a]	F value [b]	p-Value [c,d] prob > F
β_0	intercept	0.35	0.055			
β_1	dose	-3.64	0.041	7.12	78.95	**< 0.0001**
β_2	temp	-1.59	0.041	1.47	15.01	**< 0.0007**
β_3	duration	-6.35	0.041	36.37	240.37	**< 0.0001**
β_{12}	dose-temp	1.02	0.046	0.18	4.93	**0.0360**
β_{13}	dose-duration	4.06	0.046	4.46	78.67	**< 0.0001**
β_{23}	temp-duration	1.57	0.046	0.44	11.72	**0.0022**
β_{11}	dose-dose	1.08	0.079	0.074	1.87	0.1839
β_{22}	temp-temp	1.38	0.079	0.39	3.03	0.0948
β_{33}	duration-duration	4.16	0.079	3.07	27.69	**< 0.0001**

a *n* of experiments / 4 x squared factor effect
b comparison of term variance (mean square) with residual variance (res. mean square)
c probability of seeing observed F value if the null hypothesis is true (no factor effect)
d "prob > F" values < 0.05 tests as significant at the 95% confidence level

Table 6. Initial doses and "CT" products required in commercial scenarios to achieve Probit 9 control of SWD (right column) were generated based on predictions derived from a multivariate model (left column).

		multivariate prediction		commercial evaluation	
		Dose (± 0.7mg/l	"CT"[a] (mg h/L)	Dose (± 2 mg/L)	"CT"[b] (mg h/L)
51 °F	2 h	48.2	88.1	54.1	88.1
	3 h	35.1	93.3	41.7	93.3
50 °F	2 h	49.6	90.2	55.3	90.2
	3 h	35.5	94.3	42.1	94.3
49 °F	2 h	50.7	92.2	56.6	92.2
	3 h	35.6	94.5	42.2	94.5
48 °F	2 h	51.7	93.9	57.6	93.9
	3 h	35.8	95.1	42.5	95.1
47 °F	2 h	52.5	95.6	58.7	95.6
	3 h	36.0	95.7	42.7	95.7
46 °F	2 h	53.3	97.0	54.6	97.0
	3 h	36.7	97.6	43.6	97.6
45 °F	2 h	54.0	98.2	60.3	98.2
	3 h	37.0	98.4	43.9	98.4
44 °F	2 h	54.6	99.3	60.9	99.3
	3 h	41.3	109.8	49.0	109.8
43 °F	2 h	55.2	100.4	61.8	100.4
	3 h	44.6	118.7	53.0	118.7

a sorption profile (time, % headspace loss of MB): 0.5 h, 7.5%; 1 h, 9.5%; 2 h, 14.5%, 3 h, 17.5%
b sorption profile (time, % headspace loss of MB): 0.5 h, 10.0%; 1 h, 20.0%; 2 h, 34.0%, 3 h, 44.0%

Confirmatory fumigations. MB fumigations were conducted to confirm the mortality of all SWD life stages in cherry export to Australia and Japan The minimum allowable exposure of this schedule between 54-63F, denoted by a shaded box, is a concentration-time "CxT" product of 64.5 $mghL^{-1}$ based on the standard indices used by APHIS to account for percentage fumigant loss through time (75% at 30 min, 63% at 1h, 50% at 2h, and 38% at 3h). These projected losses, which are used to generate the concentration-time cross product "CT" exposure minimum for prescribed fumigations, accommodate chamber leakage and sorption to fruit. The differential sorption of MB between replicate fumigations was used to establish a range of "CxT's" encompassing the target/confirmatory exposure minimum (Table 7). Data indicates that >88 mgh/L exposures of SWD to MB in cherry loads packaged for export resulted in the mortality of all ~ 55,000 test specimens.

Table 7. MB exposures at 54F for each fumigation replicate indexed relative to minimum requirements.

# treated specimens	Applied (mg/L)	1/2 h [MB]	1 h [MB]	2 h [MB]	3 h [MB]	% Sorp.	CxT (mgh/L)	survivors
Min.	48.0	36.0	30.0	24.0	-	50.0	64.5	
3854 ± 210	45.5	36.6	32.8	27.4	-	39.7	68.1	27
3854 ± 210	47.5	37.3	33.3	27.6	-	41.8	69.5	14
4340 ± 275	49.9	38.4	33.3	27.9	-	44.1	70.6	10
4340 ± 275	51.5	39.7	34.2	28.3	-	45.1	72.6	3
4170 ± 468	48.5	43.7	39.4	33.8	-	30.3	80.4	1
3020 ± 324	42.2	35.5	32.5	27.8	25.4	42.2	84.6	4
4170 ± 468	36.8	32.7	30.4	26.3	-	28.6	86.6	1
4256 ± 334	53.3	48.7	43.3	32.4	-	39.2	86.7	0
4470 ± 239	53.8	48.1	44.4	33.2	-	38.3	87.4	0
3020 ± 324	41.5	33.3	30.5	26.1	24.3	41.5	88.1	0
4125 ± 310	55.4	48.4	43.8	37.9	-	31.5	89.9	0
4256 ± 334	57.6	53.4	44.2	34.2	-	40.6	91.4	0
4125 ± 310	57.1	50.4	45.3	36.8	-	35.6	91.9	0
4470 ± 239	57.6	52.9	47.6	36.4	-	36.8	94.8	0
3741 ± 102	38.2	35.3	33.5	29.0	26.3	31.7	94.9	0
4581 ± 467	44.9	39.1	33.9	29.1	25.6	43.7	98.0	0
4581 ± 467	44.7	37.3	34.2	29.8	26.3	41.2	98.4	0
2984 ± 198	62.2	55.4	49.2	41.0	-	34.1	100.7	0
3741 ± 102	41.2	39.8	35.6	31.2	29.1	29.4	102.7	0
3761 ± 273	47.5	41.4	37.7	29.8	25.9	45.5	103.6	0
2984 ± 198	64.1	57.6	51.2	42.2	-	34.0	104.3	0
3761 ± 273	44.3	40.7	37.4	32.2	27.1	38.8	105.2	0

Σ 87,144 ± 1449.6

References.

1) Tebbets, J.S., C.E. Curtis, and R.D. Fries. 1978. Mortality of Immature Stages of the Navel Orangeworm Stored at 3.5 C. J. Econ. Entomol. 71: 875-876.
2) USDA http://www.ars.usda.gov//Main/site_main.htm?docid=18134
3) USDA http://www.ars.usda.gov//Main/site_main.htm?docid=18577
4) Deming, S. N.; Morgan, S. L. Experimental Design: A Chemometric Approach; 2nd ed.; Elseiver Science Publishers B.V.: Amsterdam, 1993.
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6) Finney, D.J. Probit Analysis, 3rd edition,1971, Cambridge University Press

Executive Summary. This research is ongoing and a final report will be sent to WTFRC upon completion.

CONTINUING PROJECT REPORT **YEAR**: 2011 (2 of 3)
WTFRC Project Number: CH-10-105

Project Title: Improved management of powdery mildew of sweet cherry

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

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

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

Footnotes:
[1]postdoctoral research associate
[2]associate in research
[3]reagents for qPCR; field supplies
[4] travel to The Dalles and Hood River to read T/RH dataloggers and to conduct disease incidence and severity evaluations (objective 4).

OBJECTIVES

1) Determine the presence and regional extent of resistance to QoI fungicides in populations of *Podosphaera clandestina* in Eastern Washington.
2) Investigate "early" 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 chasmothecia (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 isolates were collected from the Yakima, Wenatchee, and Columbia Valleys. Most isolates were sensitive to trifloxystrobin at up to 4x labeled rates but several appeared less sensitive at $\geq$ 160 ppm.

- 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) an ascospore release the depletion of the overwintered inoculum source were for the second year inconclusive.

- Full-season fungicide programs (standard preharvest programs + postharvest oil applications) were found to have no significant effects on chasmothecia production.

- Irrigation / fertigation experiments were inconclusive due to low mildew incidence and severity but voluminous amounts of microclimatic data were collected.

- Several experimental fungicides were effective against powdery mildew under high disease pressure

METHODS

Objective 1. *(QoI Resistance Survey).* Both conventional and molecular techniques were developed to study the presence and distribution of *P. clandestina* strains resistant to QoI fungicides. Our efforts in 2011 focused on applying the leaf disk bioassay. Isolates were collected from 1-2 orchards in each of The Prosser, Kiona, Benton City, Mattawa, Wenatchee, and Rock Island growing areas. Mass isolates were used. Infected cherry foliage was collected from each orchard site and conidia used to inoculate 'Sweetheart' leaf disks. Leaf disks were treated with 0, 80, 160, 320, 640, and 1280 ppm trifloxystrobin and then inoculated with a known concentration (10,000 / ml) of conidia of various isolates of *P. clandestina*. Disks were transferred to petri plates containing 1.5% water agar and incubated 14 days at 20 C. Seven leaf disks are inoculated in each of four single-plate replications.

Objective 2 *(inoculum sources)***.** A cv. 'Bing' orchard was used for this study. Rotorod and Burkard cyclonic spore traps were placed within and about 0.1 km downwind of the orchard. Traps were operated continuously beginning at bud burst and continuing through harvest of later varieties in the area. The concentration of inoculum of *P. clandestina* in the air at each sampling location was determined using quantitative PCR and primers developed in the WSU-IAREC pathology laboratory. Daily qPCR signal strengths were compared with actual daily spore counts.

Objective 3 *(inoculum depletion)***.** A 3 acre cv. 'Bing' orchard at WSU-IAREC was used for this portion of the study. The formation and dispersal large numbers of chasmothecia was documented at the site during the late summer and early autumn of 2010. Water was applied by handgun on about April 1, April 8, April 19, and May 10, 2011. At this time chasmothecia/ascospores were mature but there was not yet (aside from April 19 and May 5) much cherry foliage available for infection. The orchard was divided into 4 quadrants. Two quadrants were watered while two were left dry. The air of all quadrants was monitored using Rotorod air samplers. The presence and concentration of *P. clandestina* was determined using the quantitative PCR. The sampling periods compared were 1) 4 hours prior to watering 2) 4 hours after wetting 3) 15 hours during the subsequent evening and 4) 12 hours the following day.

Objective 4 (*irrigation and fertilizer influences*). Studies were conducted in a cv. 'Lapins' orchard near The Dalles, OR. Three fertigation treatments (#1 100 lbs N/acre delivered weekly via injection into irrigation lines, #2 100 lbs N/acre delivered to the ground in a spring split application, #3 60 lbs N/acre delivered weekly via injection into irrigation lines) were superimposed over (microsprinkler) irrigation treatments. Percentage water (irrigation treatments) are based on irrigating the 100% treatment at one acre-inch of water per set (one set per week). Other irrigation treatments include 80%, 60%, and regulated deficit. Treatments were arranged in a randomized complete block design with 5 replications. Each replication consisted of four trees with the center two serving as experimental units. Trees received normal powdery mildew treatments applied by the grower. However, polyethylene bags were used to cover selected branches during fungicide applications and removed immediately afterwards. "Bagged" branches served as untreated foliage and fruit. The incidence and severity of powdery mildew on fruit and foliage was *not* determined at harvest due to insufficient levels of powdery mildew. Temperature and relative humidity was monitored in selected irrigation treatments treatments using Hobo Pro U23-001 data loggers.

Objective 5 (*reduction of carryover inoculum*). A cv. ‘Bing’ orchard near The Dalles, OR was used for this portion of the study. Treatments were arranged in a randomized complete block design with 4 replications. Each replication consisted of 35 cv. 'Bing' trees. Four treatments were compared: 1) non treated control 2) standard preharvest fungicide program 3) standard preharvest program + a single 2% oil application applied two weeks after harvest 4) standard preharvest program + 2% oil

application applied four weeks after harvest and 4) standard preharvest program + two oil treatments applied two and four weeks postharvest. Several weeks after the cessation of fungicide programs 25 leaves were selected at random from each of 5 trees in the center row of each plot, air dried, comminuted in a blender, and the number of cleistothecia per cm^2 of leaf tissue determined using a dissection microscope.

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.

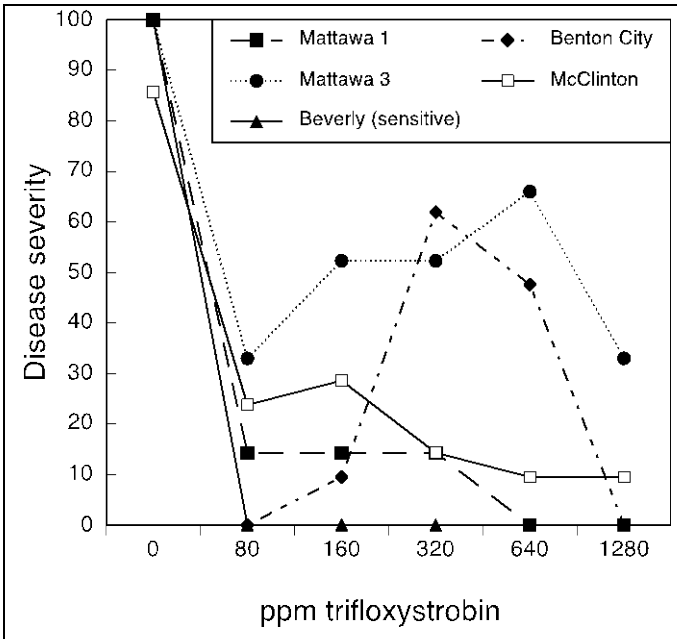

Figure 1. Growth of putative resistant isolates of *P. clandestina* on leaf disks of sweet cherry treated with 0-1280 ppm trifloxystrobin (Flint or Gem). A sensitive isolate (Beverly) is included for comparison.

Objective 2. A new Burkard cyclonic air sampler was evaluated in tandem with rotary impaction (Rotorod) traps for the molecular detection of *P. clandestina* in the orchard air. The new sampler was found to be vastly superior to Rotorod devices 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 correlated (r = -0.65; $P < 0.01$; Figure 2) with daily spore counts taken by volumetric traps. Use of the both volumetric and cyclonic traps confirmed a large increase in aerial spore populations following harvest. Series of these traps will be used during 2012 in further studies on this objective.

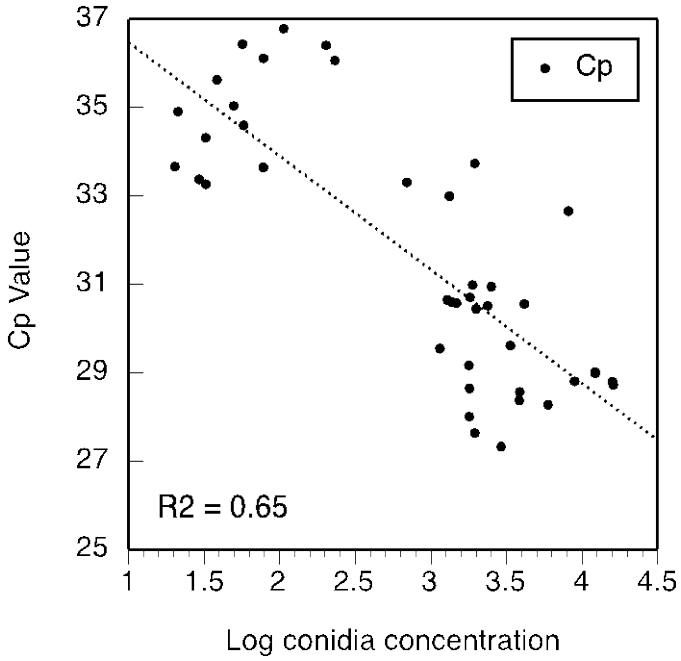

Figure 2. Association of PCR Cp values and daily conidia counts, 2011. Cp values were obtained using species-specific primers to determine the concentration of *P. clandestina* conidia in air samples taken daily. Actual conidia counts were taken using a Burkard volumetric spore trap.

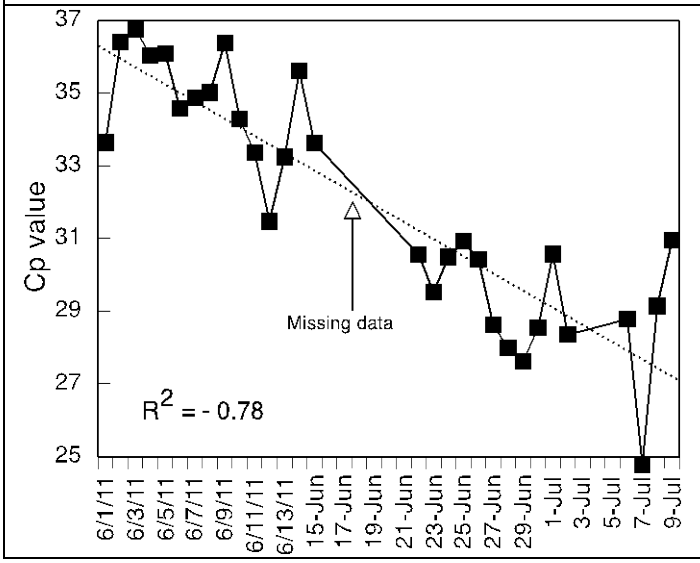

Figure 3. Association of qPCR signal strength and the logarithm of daily conidia during June-early July 2011. Note significant decrease in Cp concurrent with postharvest inoculum increase.

Objective 3. A major component of this research effort is related to the formation, dispersal, and functional or actual eradication/neutralization of chasmothecia, the overwintering propagule of *P. clandestina*. In other pathosystems, the real or functional elimination of such sources has resulted in delayed disease onset and reduced disease severity during the subsequent growing season. The results of early-season irrigation regimes to promote ascospore release were inconclusive (Figure 5). Differences in PCR signal strength were observed but there were no discernible patterns. The experiments will be repeated in 2012 in a larger commercial orchard using the new Burkard cyclonic air sampler.

Cp Values					
Date	**Treatment**	**Period 1**	**Period 2**	**Period 3**	**Comments**
4-1	Wet	35.08/36.71	40.82/na	Na/na	
	No Wet	Na/32.72	Na/33.13	37.4/34.86	
4-8	Wet	Na/na	37.6/37.6	Na/na	
	No Wet	Na/37.14	34.63/36.23	35.49/37.13	
4-19	Wet	35.43/Na	Na/na	40.4/na	
	No Wet	39.68/39.73	35.22/37.54	39.63/35.05	
5-10	Wet	37.64/44.61	36.42/44.19	36.11/37.32	
	No Wet	39.28/39.71	35.85/37.09	34.28/34.5	

Table 1. Quantitative PCR values obtained from air samples taken during the application of water to tree trunks and scaffold branches on April 12. Cp values are inversely proportional to amount of DNA in samples. Consistent differences were not observed during any of the four "watering" periods.

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 2010and 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. Further analysis and mining of this voluminous data set continues.

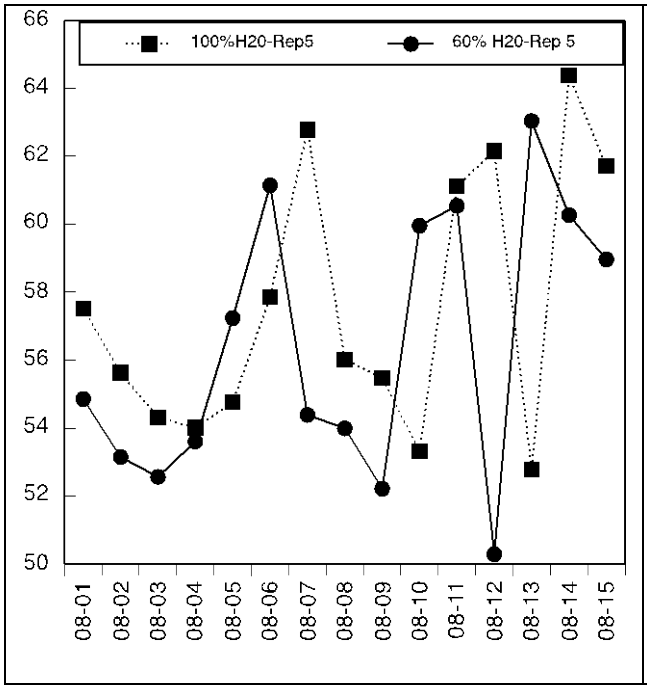

Figure 4. Relative humidity in 100% and 60% irrigation regimes during early August 2011.

Objective 5. The continuation of fungicide programs after harvest could perhaps reduce the number of chasmothecia and therefore reduce potential carryover inoculum. However, the utilization of synthetic fungicides with high potential for the development (DMI, QoI, or quinoline) of resistant populations poses significant risks to the cherry industry. During 2011 we tested a "hybrid" full-season program: a preharvest alternation of synthetics/oils followed by several postharvest oil programs. This particular program resulted in no significant suppression of chasmothecia populations (Figure 5). We will in 2012 investigate various combinations of synthetic and contact compounds for suppression of chasmothecia formation in nursery studies.

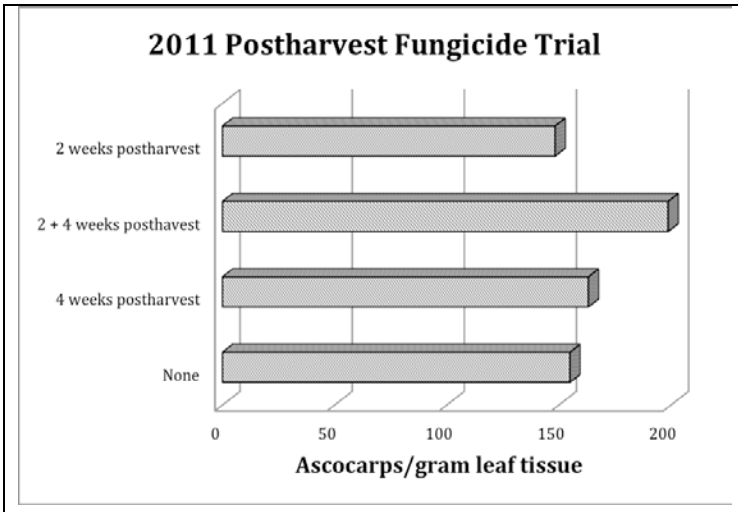

Figure 5 Chasmothecia (ascocarp) production on cv. 'Bing' trees receiving grower-standard preharvest fungicide program versus three full-season programs. There were no significant differences in chasmothecia production at $P < 0.05$

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: CH-10-108

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

PI: Ken Eastwell
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City/State/Zip: Prosser, WA 99350

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

Total Project Request: Year 1: $42,735 Year 2: $44,522 **Year 3: $46,303**

Other funding sources

Agency Name: National Clean Plant Network – Fruit Trees
Amt. requested/awarded: NCPN-FT pays land rental fees and maintenance costs of the virus research block where field experiments are conducted. The estimated cost associated with this project is $42,300 and is a portion of a larger NCPN grant to WSU-Prosser.
Note: WSU is including this information on other funding available for the support of similar research undertaken by the faculty member proposing this research. These resources are listed to identify other support granted for this research and are not included as a commitment of cost-share by the institution.

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

Item	2010	2011	2012
Salaries[1]	$22,537	22,150	23,460[1]
Benefits[2]	8,198	9,711	9,853[2]
Wages		0	0
Benefits		0	0
Equipment		0	0
Supplies[3]	12,000	12,661	12,990[3]
Travel		0	0
Miscellaneous		0	0
Total	$42,735	$44,522	**$46,303**

Footnotes:
1. Salaries: Total of 0.57 FTE Post doctoral researchers for 12 months.
2. Benefits paid at WA State established rates.
3. Supplies: Purchase laboratory reagents and supplies for performing molecular analysis, purchase trees.

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 is being detected in more orchards throughout the state.
- Even in the absence of mixed infections with other viruses, cherry leaf roll virus causes significant reduction of growth of young 'Bing' trees growing on Mazzard rootstock.
- Of the rootstocks evaluated, 'Colt' rootstock offers the most dramatic response to cherry leaf roll virus preventing the transmission of virus from inoculation sites on the rootstock to the scion, and causing inoculated scions to decline quickly. Such reactions would reduce potential field spread of the virus. After 12 months, stunting and premature leaf senescence is observed on trees of 'Bing' growing on Gisela 12 and Gisela 6 rootstock, and the Citation/Z-stem interstem combination.
- Diseases of the rusty mottle group are caused by closely related members of the Betaflexiviridae family of viruses.

METHODS:

'Bing' scions growing on various rootstocks were established in the orchard; either the rootstock or the scion was then inoculated by chip grafting with buds of a source tree infected with cherry leaf roll virus. The source tree was previously virus tested to insure there were no other viruses in the inoculum. In parallel, a duplicate set of trees was inoculated with an isolate of cherry raspleaf virus.

An experiment initiated earlier to determine the role of pollen transmission to virus-free trees was concluded. A new trial was begun to determine if the presence of a second virus would alter the result. Source trees for cherry leaf roll virus-infected pollen including the cultivars 'Lapin', 'Rainer' and 'Sweetheart' are being grown in the research orchard. Pollen from these trees will be collected and used to pollinate 'Bing' trees that are already established and infected with the common ilarviruses: prune dwarf virus and/or *Prunus* necrotic ringspot virus.

Potential sources of the rusty mottle group of viruses were identified in orchards throughout the western U.S. Buds from these sources were chip budded onto reference trees for preservation and also onto a set of greenhouse woody indicators that are used to categorize the virus-like agents associated with cherry diseases. Tissue from the source trees were also subjected to a full analysis of the virus content to determine the population(s) of viruses associated with the disease symptoms.

RESULTS AND DISCUSSION:

As cherry leaf roll virus is detected in more orchards of the PNW, its impact on sweet cherry production continues to increase. It has been shown that cherry leaf roll virus is transmitted between trees when roots naturally graft. In addition to spreading to adjacent trees, cherry leaf roll virus also infects single trees located long distances from known virus sources. Therefore, an aerial route is suspected. Virus testing and tree removal is an effective solution to stop the spread of disease in orchards when a very few trees are involved. However, in areas where larger numbers of trees are involved, a different strategy may be necessary to effectively maintain production of quality fruit. Selection of rootstocks is being investigated as a means to help facilitate disease management. When

a virus infected root of a susceptible rootstock contacts a hypersensitive rootstock, the virus will solicit cell death in the zone immediately surrounding the point of contact, and thus prevent the virus from moving into the tree with the hypersensitive rootstock. Similarly, if the scion becomes infected through an aerial means, a hypersensitive reaction will develop at the graft union leading to the rapid decline of the infected scion. A hypersensitive rootstock thus acts as a barrier to root transmission and eliminates the shedding of virus-infected pollen from the declining scion. A tree on a hypersensitive rootstock is prevented from serving as a reservoir of infection for the rest of the orchard. Of the rootstocks evaluated, 'Colt' rootstock offers the best "protection". This rootstock resulted in the quick decline of trees within one year of inoculation of the scion, and prevented the virus from moving from inoculated rootstock into the scion. Other rootstocks including 'Gisela 12' and 'Gisela 6', and the 'Citation'/Z-stem interstem combination are showing signs of decline after 12 months, but the rate of decline is not as rapid as that observed with 'Colt'.

Previous observations in commercial orchards indicate that severe symptoms appear when trees are infected with cherry leaf roll virus plus prune dwarf virus and/or *Prunus* necrotic ringspot virus. However, it was observed this year that young trees growing on Mazzard rootstock are dramatically impacted by cherry leaf roll virus alone; growth is significantly impaired.

A parallel study was initiated to identify rootstocks that may offer resistance to the nematode transmitted cherry raspleaf virus. Several known locations of cherry raspleaf virus were surveyed to identify a source of inoculum for this study. However, all trees identified with cherry raspleaf virus were also infected with either prune dwarf virus or *Prunus* necrotic ringspot virus. A source tree containing a mixed infection of cherry raspleaf virus and prune dwarf virus was used to inoculate 'Bing' trees growing on various rootstocks. Twelve months after inoculation, all 'Bing' growing on Krymsk 6 are dead regardless of whether the scion or rootstock was inoculated. Krymsk 6 is sensitive to prune dwarf virus so it is unknown which virus elicited this response. Trees on Gisela 12 rootstock on which the rootstock was inoculated exhibit extreme leaf rolling, an indication of severe stress. Gisela 12 is not sensitive to prune dwarf virus. For future research, the process was started to obtain a source of cherry raspleaf virus that is free of contaminating ilarviruses. To that end, an isolate of cherry raspleaf virus was inoculated onto *Chenopodium amaranticolor*, a host for cherry raspleaf virus but not for prune dwarf virus. The chenopodium plants were allowed to grow for approximately 4 weeks at which time they were approach grafted to each of three virus-free 'Bing' trees. After one month, the 'Bing' trees were tested for cherry raspleaf virus and prune dwarf virus. One tree was not infected with either virus, but two trees were infected with cherry raspleaf virus alone, and not with prune dwarf virus. The two infected trees will provide the inoculum to repeat the cherry raspleaf virus rootstock trial on Krymsk 6 and Gisela 12.

The pollen from trees infected with cherry leaf roll virus is heavily laden with the virus. This rich virus source may provide one source of infection for further spread of virus. Previous studies indicated that although the virus from pollen enters the fruiting structure and the fruit stems, it does not enter the tree bearing the fruit, or if it does, it is very infrequent. Trees remain virus-free even after nearly 10,000 blossoms were pollinated with cherry leaf roll virus-infected pollen. These data suggest that the abscission layered between the cherry stem and the spur may provide an effective barrier to virus transmission. This experiment was conducted with trees and pollen where cherry leaf roll virus is the only virus present. However, in other plant virus systems, it has been demonstrated that the presence of one virus may facilitate infection and movement of another virus by suppressing the plant's innate immune response. To pursue the concept that a second virus is necessary for the pollen transmission of cherry leaf roll virus, a research block was established with trees that are infected with cherry leaf roll virus and whose pollen is compatible with 'Bing' trees. 'Bing' trees infected with prune virus and/or *Prunus* necrotic ringspot virus will be pollinated in 2012 with the compatible pollen and the recipient trees monitored for cherry leaf roll virus infection.

In commercial orchards, cherry leaf roll virus-laden pollen is frequently found in association with gutation emitted by emerging leaf buds. A small experiment was conducted to see if this might be a route by which the virus can enter the tree. Two young trees were forced in the greenhouse and grown under conditions to encourage periodic formation and re-absorption of gutation. Cherry leaf roll virus-infected pollen was dusted liberally onto emerging leaf buds. After growing through the summer, the trees were tested for infection for cherry leaf roll virus but none was detected.

Sweet cherry is affected by a number of virus-like diseases whose etiologies are not known, and several of these diseases are thought to have originated in native vegetation of western North America. These diseases of regional importance are being characterized so that they can be used to evaluate symptom expression of new commercial cultivars; this will assist in disease diagnosis and orchard management.

The rusty mottle disease group is a collective term for several different diseases. Many common cherry cultivars affected by rusty mottle exhibit chlorotic spots while the remaining part of the leaf develops into bright yellow to brown or orange as the season progresses. Many symptomatic leaves are cast early in the summer, leaving a sparse tree canopy. A different group of diseases is represented by cherry twisted leaf characterized by abrupt kinking of the midrib or petiole, thereby causing the leaves of the affected trees to be twisted. Both of these disease groups cause diminished fruit quality. In this study, the viruses associated with rusty mottle and twisted leaf diseases of sweet cherry were characterized. In order to achieve this goal, six isolates of rusty mottle disease and four isolates of twisted leaf disease were graft inoculated onto woody indicator trees (*Prunus avium* cv. Bing and Sam, and *P. serrulata* cv. Kwanzan) and symptoms catalogued. Isolates of cherry necrotic rusty mottle virus and cherry green ring mottle virus were also included. In general, symptoms expressed by the indicators were in agreement with those anticipated based on descriptions in the literature. All isolates of rusty mottle induced mottle symptoms in both *P. avium* cv. Bing and Sam; the twisted leaf isolates caused typical twisted leaf symptoms on *P. avium* cv. Bing and mild mottle symptoms in *P. avium* cv. Sam. On the indicator *P. serrulata* cv. Kwanzan, twisted leaf isolates induced both chlorotic rings and severe epinasty symptoms while the rusty mottle isolates caused symptoms ranging from chlorotic rings, chlorotic mottle to no symptoms at all. As anticipated, cherry necrotic rusty mottle virus induced typical necrotic mottle symptoms on *P. avium* cv. Sam and cherry green mottle virus induced severe epinasty on *P. serrulata* cv. Kwanzan.

The coat protein gene sequences obtained from each diseased tree were determined. Subsequent analysis revealed four distinct clades (virus groups believed to have a common ancestry), each of which is unique and appears to represent four different virus populations. Thus, in addition to cherry green ring mottle virus and cherry necrotic rusty mottle virus, cherry twisted leaf disease and cherry rusty mottle disease are associated with specific well-defined viruses. Full genome sequencing of the viruses associated with six isolates of cherry rusty mottle and four isolates of cherry twisted leaf is nearing completion. When finished, this will allow comparison of the entire genomes of the viruses to support differentiation of the disease causing agents into discrete virus species.

Analysis of these disease isolates lead to the development of a well characterized panel of graft transmissible diseases. Representatives from this collection will be used to inoculate recently released cherry cultivars. Observations will be documented to aid in improved recognition of these diseases in commercial orchards. The abundant sequence information that has been gathered also permits the development of more precise diagnostic methods that can be used to aid growers in the identification of elements that may be affected cherry production.

Acknowledgement: Mr. Dan Villamor performed the genetic analysis of the Betaflexiviridae as part of his doctoral research project.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number: CH-11-104

Project Title: Developing a management strategy for little cherry disease

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Cooperators: Elizabeth "Betsy" Beers, Entomologist, WSU-TFREC, Wenatchee, WA
David James, Entomologist, WSU-IAREC, Prosser, WA
Doug Walsh, Entomologist, WSU-IAREC, Prosser, WA
Grower cooperators

Total Project Request: **Year 1:** $28,119 **Year 2: $27,844**

Other funding sources: None

Organization Name:Washington State University Contract Administrator: Carrie Johnston
Telephone: 509-335-4564 Email address: carriej@wsu.edu

Item	**2011**	**2012**	
Salaries	13,464	13,056[1]	
Benefits	5,655	5,484[2]	
Wages	- - -	- - -	
Benefits	- - -	- - -	
Equipment	- - -	- - -	
Supplies	9,000	9,344[3]	
Travel	- - -	- - -	
Miscellaneous	- - -	- - -	
Total	$28,119	**$27,884**	

Footnotes:
1. Salary: Post doctoral research fellow (0.32 FTE)
2. Benefits paid at WA State established rates
3. Molecular biology reagents for cloning virus sequences and for virus diagnosis, tissue culture supplies for developing serological reagents.

OBJECTIVES:

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**. Specific sub-objectives are:

1. An integrated program is required to help slow the spread of little cherry disease. This disease spreads naturally in the orchard, so a coordinated effort is required that includes **identification of potential insect vectors**.
2. **Develop diagnostic capacity to detect viruses associated with little cherry disease**. Current diagnosis can only be accomplished in research facilities. This practice is prohibitively expensive and is not sustainable. Translational research to develop an assay system to be used in a service center environment can provide a critical asset to the future control of this disease.
3. **Develop an educational program to alert growers to existence of little cherry disease in the Washington sweet cherry industry and control measures available to them**.

SIGNIFICANT FINDINGS:

- Little cherry virus 2 was detected in additional orchards, predominantly in the Wenatchee area.
- Preliminary data suggest that grape mealybug is efficient in transmitting Little cherry virus 2 between sweet cherry trees.
- The gene encoding the coat protein of Little cherry virus 1 is highly variable:
  - Comparison of Little cherry virus 1 isolates reveal that the nucleotide sequences encoding the coat protein vary by 30% and the amino acid sequences of the coat proteins vary by 26%.
  - The coat protein of Little cherry virus 1 has been successfully expressed in transgenic mouse cells. This synthesis of the antigen is the critical first step in the production of antibodies for the routine detection of the virus in grower samples.
- The coat protein gene sequences of Little cherry virus 2 are less variable than those of Little cherry virus 1; the prospects of developing diagnostic reagents for Little cherry virus 2 that detect all isolates are good.
  - Nucleotide identity ranges from a minimum of 88% and similarities of the amino acid sequence range from a minimum of 91%.

METHODS:

Research focused on developing reagents suitable for routine testing of orchard samples for the presence of viruses associated with little cherry disease. Existing diagnostic techniques are expensive and cumbersome for routine testing. Two separate strategies were pursued in an effort to circumvent these limitations: 1) partner with a private company to acquire access to new technology for cost effective molecular testing, and 2) develop serological reagents that can be used in a standard ELISA format. Isolates of the two viruses associated with little cherry disease (Little cherry virus 1 and Little cherry virus 2) were identified and the region of the genome that encodes the coat protein was cloned and sequenced. This region of the virus genome was targeted because it serves a dual purpose of being incorporated into molecular assays and utilized for the synthesis of proteins that will be used in the development of serological reagents.

Despite the challenges of detecting little cherry viruses through existing technology, we tested samples either collected from orchards or submitted by growers. The results provide both epidemiological data and also sequence information for developing the assays described above.

Transmission tests were performed in growth chambers to determine if grape mealybug is capable of transmitting Little cherry virus 2 from one sweet cherry tree to another.

RESULTS & DISCUSSION:

Developing an appropriate industry response to the threat of little cherry disease in the western U.S. is a decision making process based on best available information. Key elements of this knowledge base need to be: the ability to correctly identify the underlying cause of small fruit size on a case-by-case basis (i.e. biotic versus abiotic factors), knowledge of the pathogen(s) causing disease, and the way(s) in which the pathogens are moving into and within orchards.

In the 1970s, the apple mealybug (*Phenacoccus aceris* Signoret) was established as the major insect vector of Little cherry virus 2. Since then, the population of apple mealybug in stone fruit orchards has declined dramatically, and has largely been replaced by grape mealybug (*Pseudococcus maritimus* (Ehrhorn)). Therefore, it is critical to determine whether this relatively new pest in stone fruit orchards is also capable of transmitting a virus that causes little cherry disease. A colony of grape mealybug on *Prunus* spp. was identified. This provided a source of insects for transmission experiments. In a growth chamber, crawlers were placed on shoots cut from a field cherry tree known to be infected with a North American isolate of Little cherry virus 2. After an acquisition period of 7 days, approximately 50 crawlers were transferred to each potted virus-free sweet cherry tree. After one week, trees were treated with pesticide to eliminate the mealybugs. This process was repeated on two separate groups of trees to yield a total of 21 young cherry trees that were exposed to potentially viruliferous mealybugs. Two to four months after the inoculation period, leaves were collected from each of the recipient trees and tested by RT-PCR for the presence of the virus. Of the total 21 trees tested, 18 yielded positive results for Little cherry virus 2. It is possible that the positive reaction in the RT-PCR was the result of virus trapped by mealybug debris on the leaf surface but not transmitted. Therefore, the trees will be allowed to continue to grow in the greenhouse so that new growth can be tested at intervals to verify that the positive diagnostic reaction was the result of plant infection and not residual inoculum on the leaf surface. Notwithstanding this concern, the preliminary data strongly suggests that grape mealybug is an efficient vector of little cherry disease. The apparent transmission by grape mealybug of Little cherry virus 2 is very significant. Grape mealybug populations are an increasing concern in the tree fruit industry because they are difficult to control in established orchards. The presence of infected orchards that serve as reservoirs of Little cherry virus 2 along with this abundant insect pest creates a menacing combination. A similar trial to test the mealybug transmission of Little cherry virus 1 is also warranted.

To assist in the future management of this disease, access to efficient diagnostic methods is required. This essential function will allow growers to differentiate trees affected by virus-induced little cherry disease from those that are producing small fruit because of other factors such as winter damage or poor horticultural conditions. The basic strategy to confirm little cherry disease diagnosis is straight forward, but the processes to achieve that goal are technically challenging. In summary, a simple test for little cherry disease viruses would be based upon the ELISA serological technique. For that, animals are needed to produce antibodies to the little cherry virus coat protein. However, it is nearly impossible to purify enough little cherry virus to immunize an animal. Thus, bacteria are used to produce virus protein based upon the genetic code of little cherry viruses. These proteins can then be injected into the animal (usually a mouse) for antibody production. However, antibodies produced in this fashion frequently have limited use in ELISA. To obtain superior antibodies, the virus gene sequences that were inserted into bacteria are being modified and incorporated into the genetic code of animal cells. Once these cells are transplanted into a mouse, the animal will then not only produce proteins of little cherry virus but will also produce the antibodies needed to detect the virus in an ELISA test. Due to protein configurations, the antibodies against proteins produced in the animal should be superior to those produced in reaction to the bacterially produced protein.

To initiate development of reliable testing methods, a database was established with gene sequences encoding the coat proteins of Little cherry virus 1 and Little cherry virus 2. At the nucleotide level, analysis of 25 clones of Little cherry virus 2 coat protein gene sequences (1,080 nucleotides each)

reveals 88% to 100% identity between isolates. When these nucleotide sequences are translated to protein sequences, amino acid similarity ranges from 91 to 100%. When only isolates from Washington State are considered, the degree of similarity is even higher at 94%. This relatively high degree of sequence identity suggests that diagnostic reagents targeting the coat protein region of the Little cherry virus 2 genome will detect a wide range if not all isolates detected in the state. The nucleotide sequence of the Little cherry virus 2 coat protein gene shares only 2% identity with the analogous region of the Little cherry virus 1 genome. As previously reported for other regions of the genome, sequences of Little cherry virus 1 are highly variable. Comparison of the coat protein sequences from 47 clones of Little cherry virus 1 reveals as little as 70% nucleotide identity between clones. Amino acid sequence similarity is slightly higher ranging from 74% to 100%.

Efforts to develop serological assays for Little cherry virus 1 and Little cherry virus 2 are proceeding in parallel. With combined funding from the Washington Tree Fruit Research Commission and the USDA-ARS in 2004, we generated an antibody based on bacterially expressed protein encoded by the genomic sequence of Little cherry virus 1. The resulting antibody was only effective in Western blot analysis for virus proteins and not suitable for routine detection of virus in grower samples. This is a common fate of antibodies produced against bacterially expressed proteins. However, because of this earlier study, current research on Little cherry virus 1 was greatly accelerated. Access to this antibody allowed us to quickly confirm by Western blot analysis that the mouse cell lines are producing Little cherry virus 1 coat protein. In the absence of a similar tool for Little cherry virus 2, validating the expression of the coat protein of this virus in mouse cells is unconfirmed. Synthesis of messenger RNA has been confirmed in at least seven animal cell lines but it is not known if they are efficiently translated into protein. Production of antibodies as the basis of serological assays such as ELISA will continue.

We also partnered with a private firm to access a relatively new technology for nucleic acid analysis. This assay is based on the unique RNA sequence of the virus genome, but unlike the polymerase chain reaction (PCR) assay format, little or no prior processing of the sample is required. It can also be adapted to operate in a field office. It is dependent on knowledge of the sequences that occur in the virus genome. The database of coat protein sequences described above was a valuable asset in new assay development. Based on sequence information obtained by our team, we developed primer and probe sequences that meet basic assay criteria and this information was forwarded to the company for synthesis of diagnostic reagents. They will be available in early November 2011 for preliminary evaluation.

While the enhanced diagnostic methods are being developed, grower blocks continue to be sampled and tested for the presence of Little cherry virus 1 and/or Little cherry virus 2. Test results are pooled based on three general regions of cherry production: Yakima-Benton-Franklin Counties, Grant County and Chelan-Douglas Counties. From this small sample population, it appears that most of the little cherry disease is centered in Chelan-Douglas Counties (Table 1). However, it must be remembered that this was not random sampling but a targeted sampling so the numbers do not reliably reflect the distribution of the disease throughout the state. Nevertheless, a significant presence of little cherry disease was revealed.

To increase the awareness of growers to the issue of little cherry disease, Tim Smith made a presentation entitled “Little Cherry Virus- an old enemy returns as a serious threat to the local cherry industry” at Stone Fruit Day (January 20, 2011) attended by a large and diverse group of growers. Ken Eastwell made a presentation at a field man’s breakfast (May 17, 2011). A fact sheet describing current concerns about little cherry disease was prepared and distributed at both events and made available on the Clean Plant Center (NW) web site.

Table 1. Samples were collected and tested for the viruses associated with little cherry disease. Orchards were identified based on communication with growers and field men[1].

Counties	Orchards sampled	Little cherry virus 1			Little cherry virus 2		
		Orchards with LChV1	Samples tested	Samples with LChV1	Orchards with LChV2	Samples tested	Samples with LChV2
2010"							
Yakima-Benton-Franklin	3	0	22	0	1	22	2
Grant	2	1	17	5	0	12	0
Chelan-Douglas	17	4	72	9	12	74	60
2011:							
Yakima-Benton-Franklin	3	0	13	0	0	13	0
Grant	1	1	7	1	0	7	0
Chelan-Douglas	6	2	28	2	6	28	21
2-year total	32	8	159	17	19	156	83
% infected		25%		11%	59%		53%

1. The data presented does not include studies from 2005 that indentified an additional orchard in Chelan-Douglas counties with Little cherry virus 1 and 2 infected trees, and two additional orchards in Yakima-Benton-Franklin counties infected with Little cherry virus 1.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number: CH-11-103

Project Title: Understanding genetic basis of powdery mildew resistance in cherries

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Cooperators: Dorrie Main, Murali Bellamkonda, Tyson Koepke, Amy Iezzoni, Dean Glawe, Ines Hanrahan, Tom Auvil, Jim McFerson, Fred Bliss

Total Project Request: **Year 1:** $40,008 **Year 2: $22,289**

Other funding sources

Agency Name: USDA-CSREES Specialty Crops Research Initiative
Amt. 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-PIs include Dhingra and Oraguzie

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

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

Agency name: WTFRC/OSCC
Amount awarded: $62K from 2011-2012
Notes: Understanding the genetic basis of powdery mildew resistance in sweet cherry: PI: Oraguzie with Peace, Dhingra and Grove as Co-PIs

Budget 1

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

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Item	2011	**2012**	
Salaries			
Benefits			
Wages	20,518	**6,490**	
Benefits	1,798	**624**	
Equipment	8,000		
Supplies	8,490	**12,175**	
Travel	3,000	**3,000**	
Miscellaneous			
Total	40,008	**22,289**	

Footnotes: Wages are for the equivalent of 5 temporary employees assisting with pollen collection, hand pollinations, harvest, seed collection and seedling establishment in 2011. One temporary employee will assist Dr Dhingra with gene discovery efforts. In 2012, there will be one temporary employee in the breeding program assisting with data collection and another in Dr Peace's lab assisting with DNA marker development.

Equipment includes a microscope for visualization and characterization of PM leaf symptoms following infection in the greenhouses and artificial inoculations.

Supplies include propagated trees of resistant and susceptible genotypes for *P. clandestina* inoculations, chemicals and other lab supplies in the breeding program and Dhingra's lab in 2011. In 2012, supplies will include tags, fertilizers and other greenhouse supplies as well as chemicals and reagents for use in Drs Dhingra and Peace's labs. Travel includes trips to Pullman, commercial orchards in WA and to the National Clonal Prunus Germplasm collection in Davis, CA to collect inoculum and find new sources of resistance.

JUSTIFICATION:
Powdery mildew (PM), caused by the fungus *Podosphaera clandestina*, is one of the most serious sweet cherry diseases in the Pacific Northwest. Incorporation of natural resistance into elite sweet cherry cultivars would be an effective way to reduce reliance on fungicide and pesticide use and facilitate the transition to sustainable production systems with resultant increase in industry profitability. Currently, most commercial sweet cherry cultivars are susceptible to PM. PMR-1, an open-pollinated seedling of unknown origin with below average fruit quality, has been identified as immune to the disease (Toyama et al. 1993). The PM resistance in PMR-1 and its progeny appears to be controlled by a single locus which has been putatively named *Pmr1* (Olmstead and Lang 2002). Other cultivars identified to date with putative resistance to PM are 'Chelan', 'Hedelfingen', 'Venus' and 'Moreau' (Olmstead et al. 2000), of which only 'Chelan' has barely sufficient fruit quality for early season fresh marketing and is widely cultivated in Washington. Many elite commercial cultivars including 'Bing', 'Rainier', and 'Sweetheart' have been crossed with these parents, but nothing is known about the gene(s) underlying the resistance(s). A major constraint to verifying and characterizing the resistance(s) of known sources and identifying additional sources of resistance or immunity, including understanding genetic control to enable manipulation in breeding, is lack of an effective procedure to characterize PM disease responses. With multiple sources of resistance that have different genetic control it may be possible to develop more durable PM resistance.

OBJECTIVES:
- Develop an effective phenotypic screen to accurately characterize powdery mildew disease response to *Podosphaera clandestina*.
- Identify new sources of disease resistance to powdery mildew in sweet cherry germplasm.
- Identify genes and genetic mechanisms underlying resistances to *Podosphaera clandestina* in the different sources.

SIGNIFICANT FINDINGS AND ACCOMPLISHMENTS

- Crosses made with powdery mildew resistant accssions/genotypes generated a total of 845 seeds across 44 families. Seedlings raised from these crosses will be used in the greenhouses and field to test the utility of the proposed screening method.
- Four trees each of 4 resistant cultivars/selections and 12 susceptible accessions have been grafted and are being used for the study of plant-pathogen interaction and to generate gene expression data for identification of candidate genes associated with powdery mildew resistance.

METHODS

1. **Develop an effective phenotypic screen to accurately characterize powdery mildew disease response to *Podosphaera clandestina.***

Development of an accurate rating scale is critical for understanding the genetic basis for disease symptoms. Presently, visual assessment of PM symptoms in sweet cherry is based on presence and/or absence of infection. Although this binary type data has been used to determine the genetic basis for PM resistance from PMR-1, it does not account for disease severity nor does it capture the different symptoms. Data collected in the breeding program on foliar PM infection over the past three years indicate that disease severity varies both within and among families from year to year. Developing a rating scale other than scoring for presence or absence of infection becomes more expedient as new sources of PM resistance different from *pmr* are being introduced in the

breeding program. In apple, peach and other Rosaceae species, phenotypic assessment of PM infection is based on a rating scale (Evans and James 2003) which accounts for both incidence and severity of infection irrespective of genetic control. We plan to adopt a six-point scale (0-5) (see Table 1) where, 0 = no symptoms and 5=leaves completely infected or very high infection on leaves with most shoots completely damaged), for both greenhouse and field assessment of PM symptoms in sweet cherry. This approach will also facilitate standardized phenotyping within Rosaceae and comparison of symptoms across crops.

We will perform specific crosses in the spring of 2011 using standard PM susceptible cultivars including Bing, Rainier, Sweetheart and Van as seed parents and PM resistant genotypes including Chelan, Hedelfingen, Moreau, Venus, and Mildew Immune Mazzard (MIM) series accessions from the National Clonal Prunus repository in Davis, CA, including MIM 3, MIM 13, MIM 17, MIM 20 and MIM 23, as pollen parents. Crosses using PMR-1 as pollen parent will also be carried out. A target seedling number of 300 per cross will be necessary to obtain useful segregation data. Fruit will be harvested in June and July of 2011, the pits cracked immediately after harvest and seeds treated with 4 ppm of GA3 before cold stratification to ensure early germination (Oraguzie unpublished). Seedlings will be assessed in the greenhouse for PM infection between Mar-May 2012 and between July-Sept 2012 in the field following natural infection using the rating scale in Table 1. We will create an environment conducive for PM infection in the greenhouses including a temperature of ~75 F and 80-100% RH. We expect high correlations between field data and greenhouse data (Evans and James 2003) which is necessary to adopt the field scoring system for field data collection when all the seedlings in the greenhouses would have been planted in the field.

Note that we are mainly focusing on foliar infection in this project since we are already collecting data on fruit PM infection in the breeding program. Correlation results will determine if individuals with foliar resistance will also have fruit free of PM infection.

2. Identify new sources of disease resistance to powdery mildew in sweet cherry germplasm.

The development of fungal resistance to primary chemical control tactics, coupled with the increasing interest of growers in reducing chemical input during production necessitates the use of genetic solutions based on natural in-built resistances for PM control. Presently, there is only one source of PM resistance in sweet cherry that has been characterized. This resistance from PMR-1 is believed to be controlled by a single major gene, *pmr* (Olmstead et al., 2002). Although this single gene will be relatively easy to introgress into new and existing cultivars the longevity of this resistance remains to be determined, since breakdown of resistance conferred by single genes has been reported (Fischer et al. 1994; Parisi et al 1993). Moreover, the presence of the sexual cycle of the causal organism, *P. clandestina* warrants identification and accumulation of additional sources of resistance. Our initial goal in this objective will be to characterize and dissect the genetics of other sources of resistance including Chelan, Hedelfingen, Venus, Moreau and MIM series accessions. This will be done by field screening using the method proposed in Table 1, of breeding populations where these individuals as well as PMR-1 have been used as parents, beginning July 2012. Frequency plots and chi square analyses will provide clues about gene action, including whether resistances are conditioned by one, few or many genes.

At the same time, we will continue to look for new sources of PM resistance while polygenic sources are highly desired as they appear to be more durable than single gene resistances. Our medium term goal which is beyond the scope of this project is to combine multiple sources of resistance into one individual to prevent easy breakdown by new virulent strains of *P.*

clandestina. The ultimate goal is to develop cultivars with durable resistances that are amenable to sustainable production systems by bringing together all sources of pest and disease resistance into these genotypes.

3. **Identify genes and genetic mechanisms underlying resistances to *Podosphaera clandestina* in the different sources.**

No genes and/or DNA marker tools linked to PM resistance in sweet cherry have been identified as yet. Our aim is to develop DNA marker tools that can be used for marker-assisted breeding (MAB) for PM resistance. Beginning April 2011, we plan to carry out blast searches to identify orthologues of PM resistance genes in sweet cherry. Also, the existing resistant varieties/accessions including PMR-1, Chelan, Venus, Hedelfingen, Moreau, MIM 3, MIM 13, MIM 17, MIM 20 MIM 23 and susceptible varieties including Bing, Rainier, Van and Sweetheart, will be challenged with *P. clandestina* inoculum in the greenhouses and the first order genomic responses of the genotypes will be captured at the transcriptional level. This will be achieved by collecting the pathogen-challenged leaf material from the selected genotypes at four defined time points to capture the repertoire of genes that are activated. This will be followed with gene expression profiling using 454 sequencing with the resolution close to a single transcript targeted. The gene expression profiles of resistant and susceptible varieties will then be compared to identify the suite of genes potentially involved in plant-pathogen interactions. Gene-based polymorphisms or alleles are also expected to be identified using the methods described above. Candidate genes will be screened over a diverse array of cultivars to establish allelic diversity based on which markers will be developed for favorable alleles. These gene-based markers along with markers linked to PM resistance in other Rosaceae crops published in the literature (see www.rosbreed.org for a summary of marker locus trait (MLT) associations in Rosaceae compiled by Dr Oraguzie and Dr Richard Bell (USDA)), will be used to screen breeding parents and populations already phenotyped in objectives 1 & 2, to identify linkage to PM resistance in sweet cherry. Genetic linkage maps will be constructed for single gene resistances to locate markers on a map while QTLs will be identified and mapped in the case of quantitative resistances. Once useful marker trait locus (MLT) associations are developed, these will be moved into 'RosBreed', a multi-million dollar, multi-institutional and trans-disciplinary program funded by USDA (which Drs Oraguzie and Peace are Co-PIs on) aimed at establishing the MAB pipeline for fruit quality traits in Rosaceae crops, to establish a MAB pipeline for PM resistance in sweet cherry. The long term goal is to use deploy utile markers in the sweet cherry breeding program for background selection (i.e., selecting for fruit quality) and foreground selection (i.e., selecting for the donor gene for resistance) to facilitate reduction of timeline for the development of new cherry cultivars with combined powdery mildew resistance and superior fruit quality.

Table 1. Proposed scale for evaluation of powdery mildew resistance in the greenhouse and the field

Greenhouse			Field	
Class	Leaf area covered (%)	Description	Class	Description
0	0	No symptoms	0	No visible symptoms
1	1-10	Leaves very slightly infected	1	Very slight infection on leaves
2	11-20	Leaves slightly infected	2	Slight infection on leaves, occasionally on shoots
3	21-35	Leaves moderately infected	3	Moderate infection on leaves and/or shoots
4	36-75	Leaves severely infected	4	Heavy infection on leaves and/or shoots
5	>75	Leaves completely infected	5	Very high infection on leaves, most shoots damaged

LITERATURE CITED

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RESULTS AND DISCUSSION

1. Develop an effective phenotypic screen to accurately characterize powdery mildew disease response to *Podosphaera clandestina.*

Crosses were performed in the spring of 2011 using putative powdery mildew resistant sources including ‘Hedelfingen’, ‘Chelan’, ‘Venus’, ‘Moreau’, ‘Sylvia’, ‘DD’, ‘JJ’, ‘GG’, ‘PMR-1’, ‘MIM3’, ‘MIM13’, ‘MIM17’, ‘MIM20’ and ‘MIM23’ crossed on to susceptible cultivars including ‘Selah’, ‘Rainier’, ‘Sweetheart’ and ‘Lapins’. Due to unusually cold weather during pollination fruit set was poor with 845 seeds collected from a total of 44 crosses. These seeds have been cleaned and stored at 0 to 5ºC for cold stratification to induce germination. Germination will start in November 2011 and by May 2012 the seedlings will be screened using the proposed rating scale. When transplanted in the field, these seedlings will be augmented with those from

similar crosses performed in the previous years to achieve sufficient numbers for screening to determine utility of the rating scale by comparison with greenhouse data. We were able to use the rating scale to score for powdery mildew incidence in the field in 2011.

2. **Identify new sources of disease resistance to powdery mildew in sweet cherry germplasm.**

Apart from 'Sylvia' and 'Regina', we have not identified any other sources of powdery mildew resistance despite the numerous trips to orchards in the eastern part of Washington.
We plan to cover more orchards and other sites in the coming year.

3. **Identify genes and genetic mechanisms underlying resistances to *Podosphaera clandestina* in the different sources.**

Five trees each of sweet cherry cultivars such as 'Chelan', 'Bing', 'Stella' and 'Sweetheart' grafted onto 'Gisela 6' were ordered from Willow Drive Nursery while powdery mildew resistant cultivars/accessions including 'PMR-1', 'Venus', 'Hedelfingen', 'DD', 'GG', 'MIM 3', 'MIM13', 'MIM17', 'MIM20', 'MIM23' as well as 'JJ' (a susceptible advanced selection) were budded on Gisela 6 in the sweet cherry breeding program. Bud wood from the MIM series accessions were provided by the *Prunus* clonal germplasm repository, Davis, CA. All the propagated trees were raised in the green houses with periodic pruning and training to promote emergence of young leaves for powdery mildew inoculation. Infestation of the trees in August 2011 by spider mites delayed inoculation for 3 weeks. Recording fungal growth within the leaf tissue of different genotypes following inoculation including spore germination, germ tube development, appressoria formation and haustoria establishment is useful to establish time points for leaf collection for transcriptomic studies. However, we could not observe these stages of fungal growth under simple light microscopy and have initiated another study of plant-pathogen interaction using fluorescent microscope. We plan to collect leaves from each genotype at each stage of fungal development for RNA extraction so we can make sense of the huge gene expression profile data we will obtain from each individual. We will complete this study in January 2012 and the expected outcome is identification of genes that are either up or down-regulated in each genotype. This information will be useful for identification of candidate genes that underpin the different resistance mechanisms. The next step will be development of molecular markers from the candidate genes for use in exploring association with powdery mildew resistance.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: CH-10-110

Project Title: Start-up funds and support for a full time technician

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Total Project Request: **Year 1:** $25,000 **Year 2:** $26,579 **Year 3: 27,643**

Other funding sources

Agency Name: USDA-CSREES Specialty Crops Research Initiative
Amt. 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. 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

Budget
Organization Name: **WSU-Prosser** **Contract Administrator:** Carrie Johnston
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Item	2010	2011	2012
Salaries	16,340	17,372	**18,067**
Benefits	8,660	9,207	**9,576**
Wages			
Travel			
Total	25,000	26,579	**27,643**

Footnotes: Salary and benefits are for Andrea Young.

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**

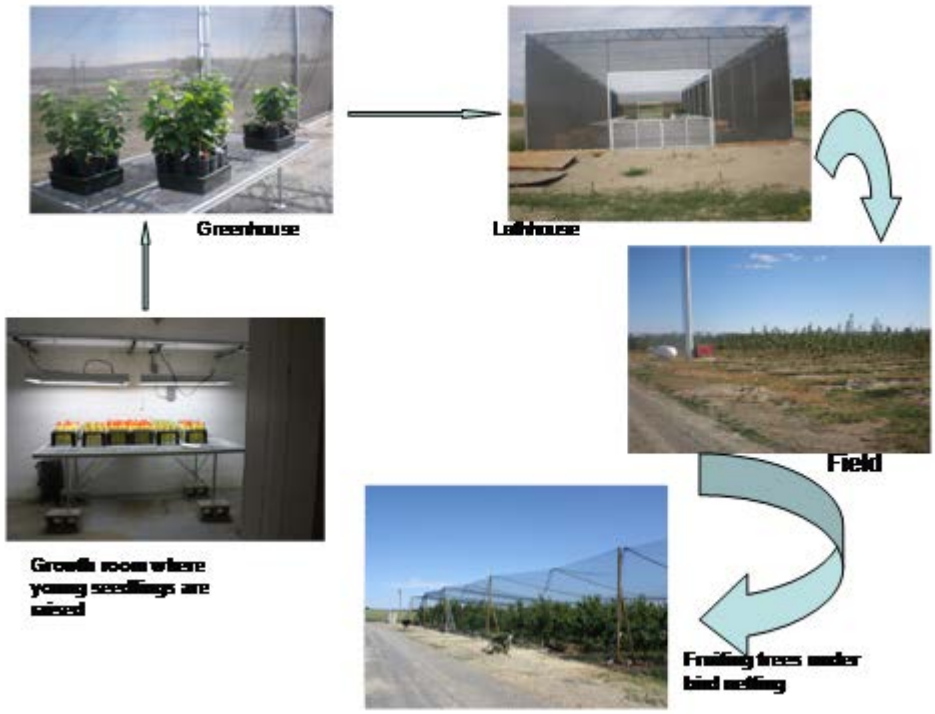
The leadership of the WSU sweet cherry breeding program changed last year with the appointment of a permanent stone fruit breeder. This was followed by active recruitment of staff and infrastructure development to make the program more viable. We have made a lot progress in this direction over the past 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.

Fig. 1: A schematic diagram of seedling development in the breeding program.



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 to selection criteria. 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 2, we will be developing guidelines on field plot techniques, tree maintenance, phenotyping protocols and selection criteria to ensure that only superior genotypes are identified and moved to Phase 3.

SIGNIFICANT FINDINGS AND ACCOMPLISHMENTS

- A new technician, Andrea Young, has been appointed to replace Addie Dahl, who left the program in December for maternity leave. Andrea joined the breeding team on the 1st of June 2011.
- Hand pollinations this year concluded in late April which is 2 weeks longer than usual. The crosses performed focused around powdery mildew resistance, pistil doubling, large fruit size and extended ripening. ‘Bing’, ‘Tieton’, ‘Attika’ and ‘Summit’ had no bloom at WSU Roza orchards due to frost injury. Trees of ‘Tieton’ and ‘Bing’ were sourced from a nearby commercial orchard and used for hand pollinations. About 70,000 flowers were hand pollinated and fruit set was high two weeks after pollination. However, the prolonged rainy and cold weather caused majority of the fruit to abort.
- A total of 5000 seeds were collected from 2010 crosses of which 2610 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. Due to the accelerated seedling growth we had 2 field plantings this year with a total of 776 seedlings planted following marker-assisted seedling selection (MASS).
- MASS was implemented in 2010 which reduced by half the number of seedlings planted in the field. Genotypes which had undesirable alleles for fruit size, firmness and self fertility were eliminated while genotypes which had favorable alleles for fruit size and firmness (including alleles 255 and 237 from’BPPCT034’ and 190 and 204 from ‘CPPCT038’) combined with the S4´ allele for self fertility were field planted.
- Thirty three cultivars and advance selections were planted in the parental crossing block in 2011. This brings the number of parents planted in this block to date to 66. The parents planted in 2009 fruited for the first time this year and these will be used for hand pollination in 2012. We will be able to harvest fruit from ‘Early Robin’ next year for comparison with advance selections in that target market class.
- Of a total of 12 genotypes identified in 2009 for Phase 2 planting, 5 were discontinued because they had fruit firmness, size, soluble solids content and titratable acidity below the

threshold values of 250 g/mm, 10 g, 20% and 0.5%, respectively. Three of these advance selections have been planted into Phase 2 trials at WSU Prosser, OSU MCAREC Hood River and OSU at The Dalles. Two late advance selections were planted at Norm Gutzwiler's orchard in N Wenatchee. The remaining 4 advance selections will be planted in the spring of 2012.

- Of the 13 advanced selections identified in 2010, 8 have been chosen for advancement to Phase 2. The five genotypes proposed to discard have either the '223' allele from 'BPPCT034' associated with small fruit or low firmness. Three of these selections also tested positive for PNRSV and PDV. The chosen genotypes will be planted into Phase trials in the spring of 2012 at WSU Prosser, OSU MCAREC Hood River and The Dalles, as well as in two grower trials.
- Following 2011 fruit evaluation, 20 genotypes were identified for Phase 2 testing and bud wood was sent to Willow Drive Nursery for propagation in summer. Several of these genotypes have been evaluated since 2009 while all 20 will be tested again in 2012 before those that out-perform the market leading cultivars will be identified for field planting into Phase 2 trials.
- Bird netting has been extended to cover ~8 acres of the seedling block. More acres will be covered as the trees come into production.
- Approximately 1.0 acre of the seedling block at the Roza research station including crosses made in 2004 and 2005 have been pulled out following completion of fruit evaluation and identification of advance selections from those blocks.
- A new block of virus clean advance selections have been planted at WSU pear acre orchards. This block has a minimum of 3 trees each from 18 advance selections.

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

Project Title: Establishment and testing of MSU sweet cherry rootstocks

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Cooperators: Tom Auvil**,** Bryce Molesworth, and Travis Schoenwald / Gebbers Farms, Bill Howell

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: Grower-Cooperator Costs[1]

Item	2011	2012	2013
Plot cost: Mosier, Ore.	0	0	0
Plot cost: Manson, Wash.	0	0	0
Tree cost for PNW	0	0	0
Total	0	0	0

Footnotes:
[1]This budget was for Objective 3. This objective was not initiated due to problems with tree health.

Budget 3: 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 4: 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).

Budget 5: Todd Einhorn

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

Item	2011	2012	2013
Salaries[1]	0	0	0
Benefits[2]	0	0	0
Wages			
Benefits			
Equipment			
Supplies			
Travel[3]	0	0	0
Miscellaneous			
Total	0	0	0

Footnotes
[1]This budget was for Objective 3. This objective was not initiated due to problems with tree health.

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. Establish three new trials of the MSU rootstock candidates in spring of 2011 at Mosier, Ore., Manson, Wash., and Clarksville, Mich.
4. Test the nine MSU candidate rootstocks at the NCPN for viruses and other infectious agents to provide a source of commercial propagation material.
5. Conduct DNA fingerprinting to assure that the genetic identity of the rootstock selections is correct.

SIGNIFICANT FINDINGS:

- (Obj. 1) All 9 MSU candidate rootstocks that were planted in Clarksville, Mich. in 2001 – 2004 continue to show no signs of graft incompatibility.
- (Obj. 2) Compared to Gi5 and Gi6, four MSU rootstocks conferred a higher number of flowering spurs with Bing scion based on data collected in April 2011 from the plot at the WSU Roza Station. These four MSU rootstock selections with are CASS, CLARE, CLINTON, and LAKE.
- (Obj. 3) In Fall 2010, virus symptoms were detected on the nursery trees designated for test plantings in Mosier, Ore., Manson, Wash. and Clarksville (MSU Research Station), Mich. Virus tests confirmed the widespread presence of Cherry Virus A, presumably due to the use of virus infected budwood. Due to this virus infection, the trees were destroyed and the plots scheduled to be planted in spring 2011 were canceled.
- (Obj. 4) Virus testing of the nine MSU rootstock candidates that were established at the National Clean Plant Network was completed. Virus negative material is available for seven of these rootstocks. Funding to eliminate the virus in CASS through the use of heat therapy is requested in an accompanying proposal.
- (Obj. 4) Due to the need to destroy the plant material targeted for 2011 plantings, the process of increasing liners from virus-certified and genetically-verified plant material was accelerated. MTAs were signed between MSU and seven liner nurseries and budwood of LAKE, CLINTON, and CLARE, was sent to these nurseries in early September.
- (Obj. 5) DNA diagnostics conducted for the MSU candidate rootstock selections at the NCPN confirmed that the identity of the rootstocks is correct.

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 Tom

Auvil and a crew from the WTFRC. M. Whiting, T. Auvil, A. Iezzoni and WSU farm manager Clint Graf will meet at least once a year at the plot to review pruning and training plans. Trunk circumference will be measured on every tree at 20 cm above the graft union each spring. Prior to bloom, the flower buds on a maximum of 15 spurs will be recorded and the mean number of buds per spur will be calculated. Unfortunately in 2011, spring freeze damage greatly diminished the crop. Therefore no fruit data was collected. However, in 2012 and 2013, the 'Bing' and 'Sweetheart' trees will be harvested at optimum maturity and the individual tree yields (kg/tree) will be recorded in the field. Subsamples will be randomly selected and analyzed in the lab for fruit quality. Fruit evaluations will be done with a goal of 100 fruit per tree; however, this number may not be achieved in year 1 for all rootstocks. Fruit evaluations will include individual fruit weight (g), diameter (mm), row size, firmness and soluble solids. Crop value per tree will be calculated from fruit yield and size relationships using values that represent average grower returns for fresh market 'Bing' and 'Sweetheart' sweet cherries. Plot tours will be conducted in 2012 and 2013 (the 4th and 5th leaf) prior to 'Bing' harvest.

3. *Experimental plots in Mosier, Ore., Manson, Wash., and Clarksville, Mich.*: This objective was eliminated due to problems with tree health.

4. *Virus testing*: All nine MSU candidate rootstocks were completely tested at the National Clean Plant Network to meet all the requirements for certification. The testing procedures are outlined at: www.nrsp5.prosser.wsu.edu. Three selections had virus, CASS, KENT and GARFIELD. Budwood of these three selections from the mother block at MSU was sent in an attempt to find virus clean wood. Budwood of CASS was also sent from the original tree, and budwood of KENT was sent from a sucker located in the Clarksville, Mich. planting. These selections were tested once again at the National Clean Plant Network to identify virus negative budwood. When testing is completed and if no infections are found, the nurseries can be given certified plant material. Due to the cancelation of objective 3, we moved ahead quickly with the virus testing and distribution to commercial nurseries for pilot propagation trials.

5. *Conduct DNA fingerprinting to assure that the genetic identity of the rootstock selections is correct:* DNA testing has already confirmed the correct identity of the six MSU candidate rootstocks that tested virus negative at NCPN. DNA fingerprinting was also done to assure correct clonal identity of the three additional rootstock candidates that were shipped to the NCPN in December 2010. In addition, DNA diagnostic support will be provided for any nursery that seeks to establish any of the MSU rootstock candidates during the three years of this proposed project. 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)].

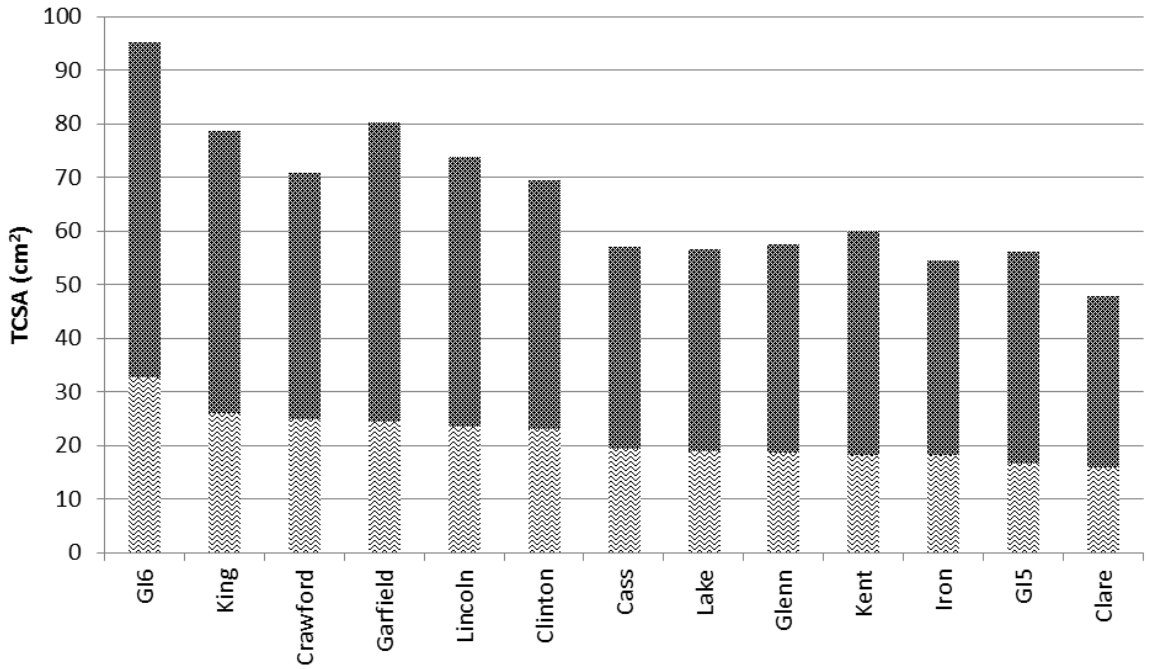
RESULTS and DISCUSSION:

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, and CRAWFORD was also discontinued due to symptoms of graft incompatibility in the original planting in Michigan. 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 2011 the leaders were headed back by 1/3 and side limbs 40 to 50%.

The trunk cross sectional area (TCSA; cm^2) of all 9 MSU candidate rootstocks is less than that for 'Bing' on Gi6, and half of the MSU candidate rootstocks have TCSAs similar to that of 'Bing' on Gi5 (Fig. 1).

Fig 1. Trunk cross sectional area (TCSA; cm^2) of 'Bing' trees grafted on 10 MSU rootstock candidates, Gi 5, and Gi 6 for trees planted in 2009 at WSU-Prosser Roza Experiment Station. The top bar indicates TCSA recorded on 28 September 2011. The bottom bar is the TCSA measurement on 13 October 2010. Therefore the top bar indicates the TCSA increase from 2010 to 2011.



In 2011, three of the MSU candidate rootstocks had significantly more flowering spurs than 'Bing' on Gi5 or Gi6. For example, on average trees on LAKE, CASS, and CLINTON had 79, 74, and 54 flowering spurs/tree compared to 33 and 29 spurs per tree for Gi6 and Gi5 respectively (Fig. 2) . One MSU candidate rootstock, CLARE had on average 34 flowering spurs/tree which is similar to Gi5 and Gi6. The average number of flowers per spur ranged from 3.2 for CLINTON to 2.2 for GARFIELD (Fig. 3). LAKE, CASS and CLARE had on average significantly more flowers per spur than five of the other MSU candidate rootstocks.

Unfortunately due to a spring freeze, there was significant flower death and therefore yield data could not be obtained. Therefore, fruit size and quality and yield data to be collected in 2012 and 2013 will be critical to determining the potential commercial value of any of these rootstocks. However, taken together, the floral display exhibited in the 3rd leaf for LAKE, CASS, CLARE and CLINTON was sufficiently promising in comparison to Gi5 and Gi6 (Fig. 4) that these four selections were chosen for propagation trials and liner production.

Fig 2. Average number of flowering spurs of 'Bing' trees grafted on 10 MSU rootstock candidates, Gi 5, and Gi 6 for trees planted in 2009 at WSU-Prosser Roza Experiment Station. Data was recorded in May, 2011.

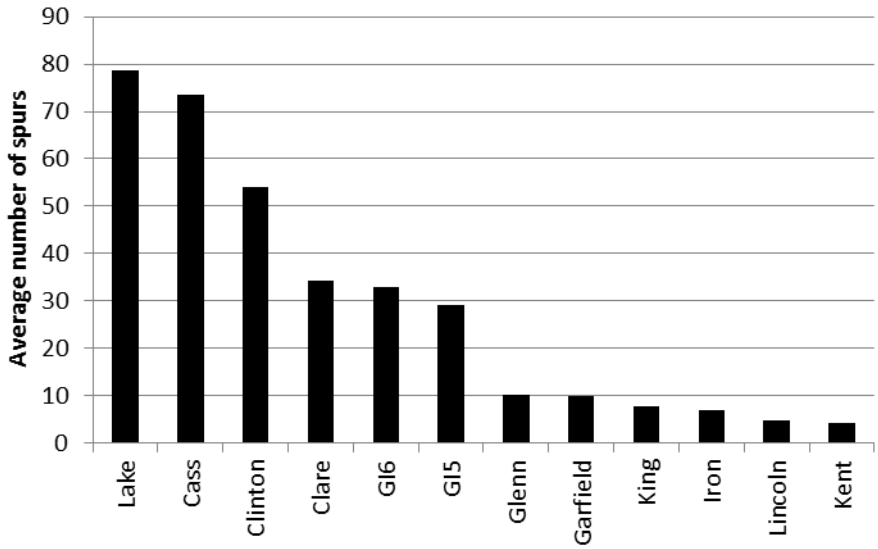
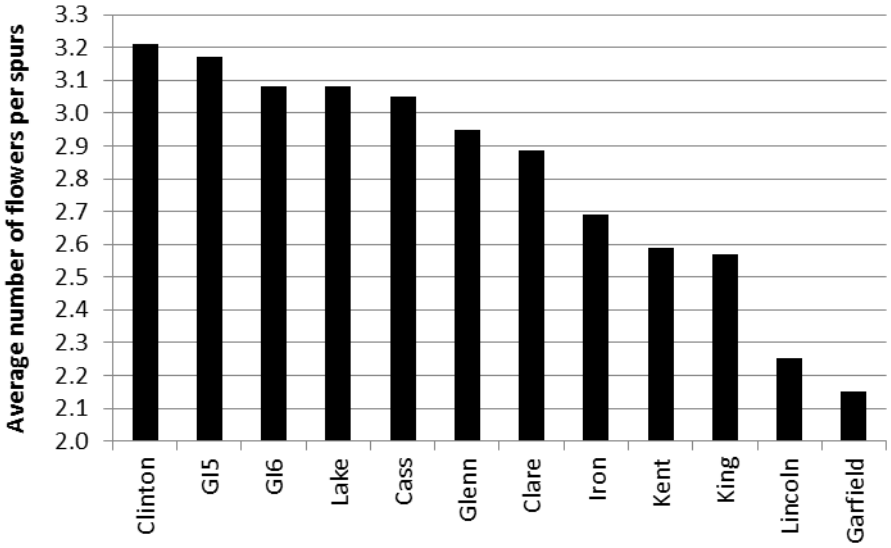


Fig 3. Average number of flowers per spur of 'Bing' trees grafted on 10 MSU rootstock candidates, Gi 5, and Gi 6 for trees planted in 2009 at WSU-Prosser Roza Experiment Station. Data was recorded in May, 2011.

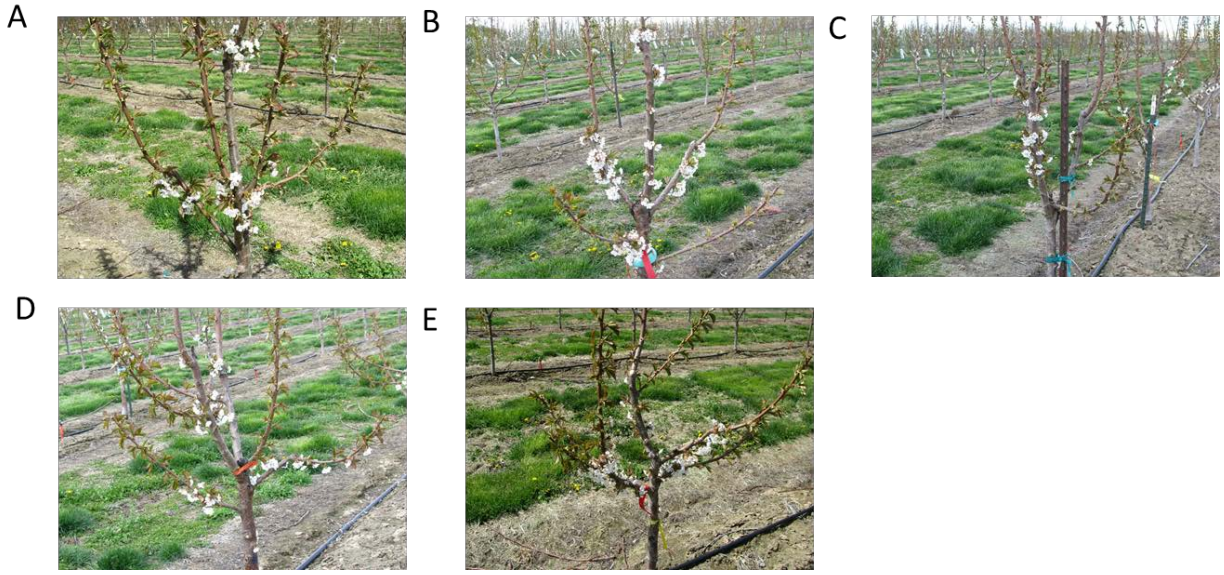


Cancelation of the year 2011 plantings of the MSU rootstock candidates planned for Mosier, Ore., Manson, Wash. and Clarksville, Mich. In Fall 2010, virus symptoms were detected on the nursery trees designated for test plantings in Mosier, Ore., Manson, Wash. and Clarksville (MSU Research Station), Mich in spring of 2011. Virus tests done by Ken Eastwell confirmed the widespread presence a flexivirus, presumably due to the use of virus infected budwood. Due to this virus

infection, the trees were destroyed and the plots scheduled to be planted in spring 2011 were canceled.

Note: The only trees that were not virus infected were trees with 'Montmorency' scion. Therefore, these trees (replicated with 25 trees per rootstock), were planted at MSU's Northwest Michigan Horticultural Research Station, Traverse City, Mich. on 3 May 2011 to assess the potential of these rootstocks for an over-the-row tart cherry production system. The tree and plot costs are and will be paid for by funds from the Northwest Michigan Horticultural Research Foundation.

Fig 4. Flowering trees of Bing/Gi6 (A), Bing/CASS (B), Bing/CLARE (C), Bing/CLINTON (D) and Bing/LAKE (E) planted at the WSU Roza Station. The photographs were taken in April 2011. The trees were planted in spring of 2009.



Generation of virus-certified genetically-verified rootstock budwood for the MSU candidate rootstocks. All 9 MSU candidate rootstocks were established at the NCPN in 2010 and virus testing was completed in 2011. The following rootstock selections tested negative for all viruses: LAKE, KING, CLINTON, CLARE, GLENN and LINCOLN. Three tested virus positive: CASS, KENT, and GARFIELD. To locate a PDV negative KENT selection, budwood from a KENT sucker from tree 28 19 (19) in the Clarksville trial was sent to the NCPN. This KENT sucker tested negative for PDV. DNA diagnostics were also performed in summer 2011 and confirmed that the KENT sucker was genetically correct. Unfortunately additional plant material of GARFIELD tested positive of Cherry Virus A and CASS tested positive for *Hop Stunt Viroid* (HSVd). Testing was done for the CASS in the mother block and the original tree as it was still available. All CASS selections contained HSVd suggesting that the original seedling was infected.

Distribution of rootstock budwood for pilot propagation trials and limited liner production. Due to the need to destroy the trees targeted for the 2011 spring plantings, it was necessary to develop an alternate mechanism to generate trees for future trials and possible commercialization. Therefore the distribution of the most promising rootstocks that were already virus certified, e.g. CLINTON, CLARE, and LAKE, was accelerated. The goal is for the collaborating nurseries to (1) gain experience and optimize protocols for propagating the MSU candidate rootstocks, (2) generate

rootstock liners that can be used to make trees for future trials, and (3) have an established inventory of virus-certified and genetically-verified stock trees that can be immediately used for commercialization if that decision is made. The steps accomplished to date are as follows.

On 7 March 2011, Amy Iezzoni and Jim McFerson met with Tom Herlache from MSU's Intellectual Property Rights Office to discuss a template MTA for the MSU candidate rootstocks. A final MTA was developed that includes the following conditions. MSU requires the nurseries to raise the test rootstocks in blocks that are virus certified by their respective states. From this material, it allows the nurseries to propagate up to an inventory of 1000 liners per selection. These nurseries would provide MSU with these liners as needed to make trees for future rootstock trials. In addition, these nurseries shall allow MSU to conduct yearly visual and genetic tests to verify the genetic identity of the plant materials.

Nurseries that generate liners via rooted cuttings and/or tissue cultivar were contacted by Amy Iezzoni in August 2011 regarding their interest in collaborating. The rootstocks were offered to all the major liner nurseries serving the cherry industry. All seven nurseries signed the MTA with Mich. State Univ. (see list below) and budwood was sent in early September 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)

Equal quantities of budwood of CASS, CLINTON and LAKE were sent to each nursery. In addition budwood was sent to Willow Drive Nursery to make a set of stock trees to be sent to Amy Iezzoni. In 2012, the establishment of these three rootstocks at these six nurseries will be assessed and morphological and genetic checks will be initiated to assure the genetic identity of the candidate rootstocks. The genetic tests will continue to be conducted by the Iezzoni laboratory. CASS will be added to this fast-tracked set of candidate cherry rootstocks once virus certified material is generated (see accompanying proposal).

CONTINUING PROJECT REPORT **YEAR**: 2011

Project Title: Prediction and mitigation of rain induced cherry cracking

PI: Ines Hanrahan
Organization: WTFRC
Telephone: 509 669 0267
Email: hanrahan@treefruitresearch.com
Address: 104 N 1st St., Suite 204
City: Yakima
State/Zip: WA, 98901

Cooperators:

- Internal program staff: Felipe Castillo, Tory Schmidt, Tom Auvil, James McFerson
- Grower collaborators: Jim Kelly, Mike Duim, John Verbrugge, Jaime Reyes, Dave Poirier, Ray Wolverton

Other funding sources: Pace International, Pacific Biocontrol

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

Item	2010-11
Salaries	7,822
Benefits	2,425
Wages	55,800
Benefits	17,298
Equipment	0
Supplies	192
Travel	116
Stemilt RCA lease	360
Revenue	12,450
Total	**71,563**

Comments: All numbers are based on the fiscal year July 2010-June 2011. Salaries are calculated based on actual time spent for Schmidt, Castillo, Hanrahan.

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: Three of eight trials demonstrated significant field cracking (≥9%) of fruit. RainGard and SureSeal, reduced cracking incidence by approximately half. Fruit quality and storage performance was largely unaffected. No effect of combined GA and RainGard programs on cracking development was found, but fruit pitting was reduced.

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 8 cherry cracking trial sites utilizing 4 cultivars (Bing, Rainier, Tieton, Skeena) and 4 products or product combinations (Table 1). Trial designs were typically randomized complete blocks with 4 replications. In another set of 3 trials, GA and RainGard were applied alone or in combination to Bing or Skeena trees (Table 2). All materials were applied by a) grower cooperators or b) WTFRC staff with an AccuTec 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 2011.

	Spray schedule Wks before harvest				Concentration	Active ingredient(s)
Material	4[z]	3	2[y]	1		
Calcium nitrate	x	x	x	x	1% solution	Osmotic salt
RainGard	x	x	x	x[w]	.8 gal/acre	Natural fatty acids
RainGard+Calcium nitrate	x	x	x	x	same as above	
VaporGard	x		x		1 gal/acre	Di-1-p-menthene
SureSeal[v]	x		x		1% solution	Copolymer: stearic acid, cellulose and calcium

[z] equals light green; [y] equals early pink; [w] only under strong rain pressure; [v] 'Widespread' surfactant was added @ 13oz/acre

Table 1: Material, concentration, and timing of gibberellic acid (GA) and RainGard (RG)

Material	Concentration	Timing
GA	10 ppm	1 x (week 1)[z]
GA	10 ppm	2 x (week 1, 2)
GA	20 ppm	1 x (week 1)
GA + RG	10 ppm+ 0.8 gal/acre	1 x (GA week 1, RG week 1 - 3)
GA + RG	10 ppm+ 0.8 gal/acre	2 x (week 1, 2; RG week 1-3)
GA + RG	20 ppm+ 0.8 gal/acre	1 x (GA week 1, RG week 1 - 3)
RG	0.8 gal/acre	Weekly (3 times)
UTC	-	-

[z] refers to approximately 3 weeks before harvest

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

An artificial cracking test (modified after Christensen, 1972) was employed to assay cracking susceptibility under extreme osmotic gradients. Cherries were immersed in distilled water for up to five hours. After each hour, fruit that had split during that time period was removed and the numbers recorded. A 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 x 5	n_2 x 4	n_3 x 3	n_4 x 2	n_5 x 1
Total weighted value					$\sum$ (Nc x F)
Maximum possible value					100*
Cracking index:				CI (%) = $\frac{\sum(Nc \times F)}{100} \times 100$	

* 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 has. 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, 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

Three out of eight trials demonstrated significant field cracking (>9%) of fruit (Table 3). RainGard and SureSeal, a new product developed by Clive Kaiser, OSU, reduced cracking incidence significantly once and twice respectively by approximately 50%. Most other quality parameters remained unaffected at harvest and after 14 days in cold storage (Table 3). SureSeal decreased row size significantly in 2 of 6 trials (Table 3). RainGard sometimes increased fruit weight at harvest and total soluble solids concentration (Table 3). No other treatments affected fruit quality (data not shown).

Both tested materials performed well in reducing the incidence of cracking in cherries in 2011. In WTFRC trials from 2009-11, RainGard consistently reduced field cracking by up to 50% in trials with significant cracking pressure. SureSeal performed well in 2011, but has proven to be difficult to use under real orchard scenarios: only the recommended sticker (Widespread) will guarantee sufficient spreading of the material on the tree, the tank has to be extremely clean (triple rinsing recommended) to avoid adverse reactions of the spray solution with other chemical residues, and a high volume of water (at least 200gal/acre) is needed to achieve good performance. Although we have not observed any phytotoxicity in the field trials, we did induce fruit damage when immersing single fruit in spray solutions of SureSeal.

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 tonnes/acre) or expect high returns (>$1.50) it is always worth using such materials.

Table 3: Cracking severity and at harvest fruit quality of cherry. WTFRC 2011.

Treatment	Weight	Acids	Sugars	Firmness	Diameter	Row Size	Color	Cracking*
	(g)	(% malic acid)	(% Brix)	(g/mm)	(mm)		(1-7)	(%)
Pasco 'Tieton'								
RainGard	13.7 ns	0.78 a	22.0 ns	348 ns	30.8 ns	8.9 ns	4.4 ns	10 b
SureSeal	12.4	0.77 ab	20.6	352	30.7	9.0	4.6	9 b
UTC	13.4	0.68 b	20.9	322	30.5	9.0	5.4	16 a
Wenatchee Heights 'Bing'								
RainGard	10.0 ns	0.71 a	18.5 a	354 ns	28.9 ns	9.5 ns	4.4 ns	9 a
SureSeal	9.2	0.64 ab	16.0 ab	319	27.5	10.0	3.9	4 b
UTC	9.8	0.62 b	14.4 b	328	28.1	9.8	4.3	9 a
Wenatchee Heights 'Skeena'								
RainGard	10.5 a	0.59 a	17.6 a	341 b	28.2 b	9.7 a	3.8 ns	13 a
SureSeal	9.6 ab	0.56 c	16.5 b	385 a	28.0 b	9.8 a	3.9	6 b
UTC	9.5 b	0.58 b	15.7 b	366 ab	29.8 a	9.3 b	3.8	11 ab

*on tree reading based on 400 frt./rep

Influence of GA on cherry cracking susceptibility: In 2011 we performed 3 trials to determine the combined effect of GA and RainGard applications on cracking development. Cracking ranged from 3-60% in untreated controls and no product showed a significant treatment effect. Harvest quality parameters were not affected (data not shown). After two weeks in cold storage, 13-26% of untreated

fruit was pitted, while GA applied alone or with RainGard increased the amount of clean fruit by 4-30% (Figure 1).

GA is typically applied to promote fruit firmness at harvest, but some growers are concerned that GA applications could influence cracking susceptibility of cherries. Results from our trials did not demonstrate either effect.

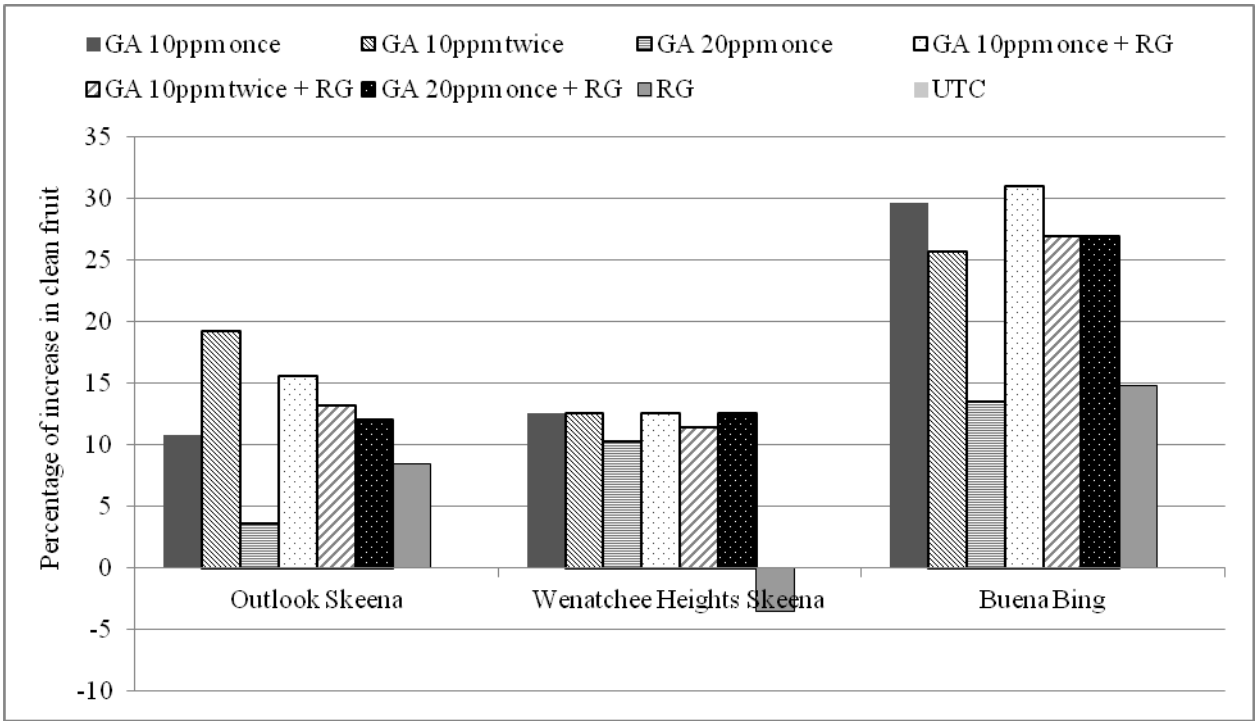


Figure 1: Percentage increase of fruit without pitting after two weeks of cold storage at 33F. Influence of gibberellic acid (GA) and RainGard (RG) on cherry quality. WTFRC 2011.

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

We observed 17 blocks during the month before harvest in 2011 (4 Bing, 3 Tieton, 2 Skeena, 1 each Sweetheart, 8011-3, Regina, Santina, Van, Early Robin, Lapins, Rainier). 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 started to crack in bench top assays 14-23 days before harvest with rapidly increasing susceptibility, including sustained CI levels in excess of 20 for the final week before harvest. (Table 4)

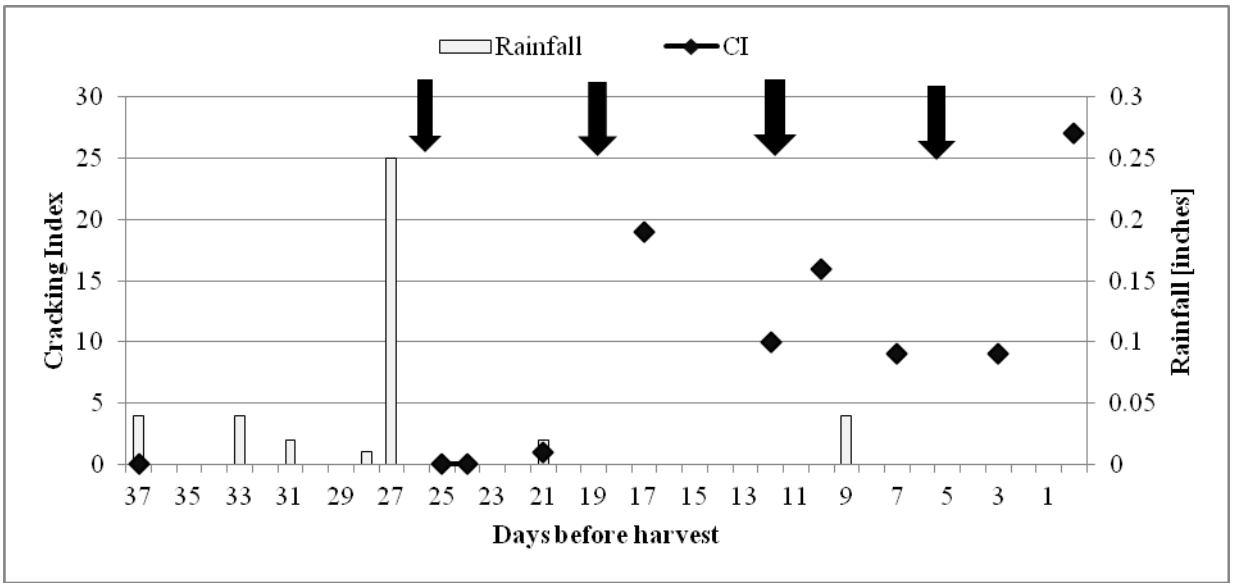
Of the 3 Tieton blocks observed, one (Pasco) started to crack 6 days before harvest at low levels (CI=5); the remaining 2 blocks started to crack 22 and 26 days before harvest respectively, without ever reaching high cracking potentials (Table 4 and Figure 2). Tieton cherries behaved differently in 2011 than in previous years, never reaching high cracking potentials as in 2009 and 2010, when blocks were highly susceptible for up to 13 days (Table 4). In most cases, CI levels correlated well to the actual cracking observed in the field: the block in Sawyer had natural fruit cracking levels below 5% at harvest, where the bench-top test indicated no need to apply additional protectants (Figure 2).

Rainier, Santina, Early Robin, Van, and Skeena all had prolonged periods of cracking susceptibility, with 1-2.5 weeks of hightened cracking potential (Table 4). This data corresponds well with

industry's experience with these varieties. Conversely, Lapins and 8011-3 would be grouped as having close to zero cracking potential based on 2011 data. While field observations on Lapins correspond well with this assertion, 8011-3 is generally believed to be of moderate cracking potential. This inconsistency 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 4: Days of susceptibility (DOS; CI > 0), days of high susceptibility (DOHS; CI ≥ 20) and maximum cracking index (max CI) for cherry orchards in Washington from 2009-11.

Variety	Location	Year	DOS	DOHS	Max CI
Bing	Zillah	2009	16	9	62
		2010	3	3	25
		2011	23	7	47
Bing	Sawyer	2011	18	8	49
Bing	Pasco	2011	14	0	9
Bing	Outlook	2009	19	12	40
		2010	22	5	37
Bing	Buena	2010	23	0	7
Rainier	Zillah	2009	13	10	26
		2011	29	8	31
Tieton	Sawyer	2009	8	6	52
		2010	24	12	38
		2011	22	0	19
Tieton	Outlook	2010	28	13	36
		2011	26	1	58
Tieton	Pasco	2011	6	0	5
Santina	Buena	2011	35	10	50
Early Robin	Buena	2010	19	17	71
		2011	16	16	63
Skeena	Outlook	2010	24	3	42
		2011	30	12	66
Regina	Buena	2011	1	0	1
Van	Buena	2011	15	9	41
Lapins	Buena	2011	28	1	40
8011-3	Buena	2011	16	0	17



*arrows indicate applications dates in trial site, CI was determined on untreated fruit

Figure 2: Development of cracking potential (cracking index), timing of rainfall and application of rain-cracking materials for Tieton/Sawyer. WTFRC 2011.

Literature cited

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

Outreach

2010: Programs to reduce rain induced cherry cracking. Cherry Institute. Yakima. Talk
WTFRC internal program. WSU Horticulture seminar. Pullman. Talk
Rain-induced cherry cracking: what we learned in 2010. WSHA 106th Annual Mtg, Yakima. Talk

2011 Cherry anti-splitting product research. BC-Hort Symposium, Kelowna, Talk
Cracking susceptibility varies. Good Fruit Grower. Article by M. Hansen
Prevention of rain-induced cherry cracking. WSU Cherry Field Day. Prosser. Talk
Cherry anti-splitting product research. NCWFA. Wenatchee. Talk
How to prevent cherry cracking. 2 chilean tour groups. Talk

CONTINUING PROJECT REPORT **YEAR**: 2 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

- '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 results were sustained through the remainder of the season, and at harvest (heavy croploads reduced fruit size by ~ 15%).
- Heavy croploads limited pit size (endocarp). Pit growth ceased by 38 days after full bloom, and was positively correlated with final fruit size (i.e., a larger pit results in a larger fruit).
- Roughly half of the cells comprising an individual fruit at harvest are already present at full bloom. This finding suggests that we may be capable of adding cells (i.e., increasing final fruit size) by stimulating cell division cycles the season prior, in late summer/early fall. We will investigate the effects of application rates and timings of plant growth regulators postharvest, 2012.
- 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.
- We have documented spatial and temporal changes in cell shape and size of cells comprising the mesocarp tissue (flesh). 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. Cropload effects were most obvious in the outer mesocarp during final fruit swelling.
- 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.
- We confirmed our findings in 2010, that the majority of the nuclei of 'Chelan', 'Bing' and 'Sweetheart' fruit become polyploid (i.e., cells possess multiple copies of chromosomes). However, leaf tissue and flowers at full bloom showed no polyploidy confirming the diploid status of sweet cherry. After full bloom, polyploidy in fruit increased rapidly to > 60% by 7-10 days, and to 75% at harvest time. Flowers that were bagged to prevent pollination did not show polyploidy indicating that 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 the magnitude or timing of polyploidy during fruit development. However, the small-fruited, wild-type *Prunus virginiana* (Chokecherry) had < 10% polyploidy in fruit cells. We will investigate whether differences in the level of polyploidy, and its relationship to fruit size, exist between large-fruited (ex., Selah) and small-fruited cultivars (ex., NY 54, and wild type cherries). Results may inform breeding efforts focused on developing large-fruited genotypes.

2. GA Experiments

- Low-moderate rates (0, 20, 30, 40, 60 ppm) of GA did not statistically, negatively influence return bloom of Skeena when applied in the prior season during stages II and III of fruit growth.
- The largest differences among all tested GA concentrations (0, 10, 20, 30, 40, 60 ppm) and timings (pit hardening and midway through Stage III) were observed between 0 and 10 ppm. The quality attribute most affected was fruit firmness (higher when provided GA, but not consistently with rates beyond 10 ppm).

- Skin color (darkening) was delayed with the application of GA; however, beyond the 10 ppm rate, the effects were highly variable, and difficult to qualify with our current experimental design.
- The severity of fruit cracking of Skeena due to several rain events (~2 inches) occurring within the week prior to commercial harvest, was not influenced by GA application or rate (i.e., all treatments had cracking levels ~60 %).

Methods

Fruit development studies: Methods were described in the 2010 report. Changes in 2011 are described below. 'Bing', and 'Sweetheart' were investigated in both Washington and Oregon (The Dalles); 'Chelan' bloom was frozen in WA, and subsequently only used in Oregon. 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 uniformly, 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 fruit from un-tagged fruit on similar 2 and 3 year old wood were stored in fixative. This second sampling has revealed that our "flower set" method greatly improved the uniformity of the tagged fruit.

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 volume an ellipsoid. This method avoided the difficulty of fresh weight and caliper measurements, especially in very small fruit, and allowed evaluation of 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.

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.

***GA Experiments*:** Methods were similar to those described in 2010, with the exception of the addition of a new 'Sweetheart' site, on account of spring frost injury and high infection rates of bacterial canker sustained at the original site. Additionally, we did not continue work on the Staccato site. In the 'Sweetheart' orchard, our control (0 ppm) limbs were inadvertently given the commercial application rate of 20 ppm; therefore, in the 'Sweetheart' plot we did not have rates lower than 20 ppm.

Results and Discussion: 1. Fruit growth, cell number and size

Ontogeny: Better uniformity in the timing of pollination and a greatly increased number of experimentally "set" fruit were obtained by our modified procedures. 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 and will be measured to establish the validity of this method. Preliminary results confirm that better uniformity in fruit size was obtained throughout the growing season compared to the crop average.

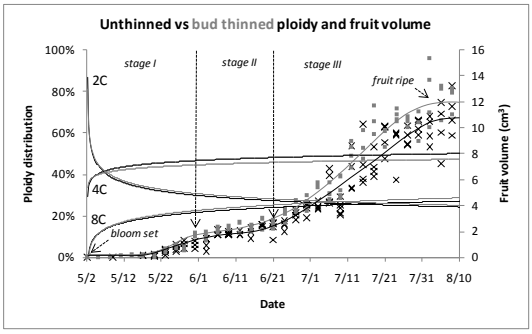

Figure 1. Growth curves and the distribution of nuclei according to ploidy level for 'Sweetheart' mesocarp of unthinned (high cropload, black) and bud-thinned (low cropload, light grey). (2C [diploid]; 4C [tetraplolid]; or 8C [ocataploid]) Fruit growth stages are provided at top of figure.

Fruit growth of 'Sweetheart' was observed to follow the classic growth pattern of stone fruit (Fig. 1). The limiting effect of high fruit density on fruit growth was observed early in fruit development, and sustained until harvest, resulting in a ~ 15% reduction in fruit size, relative to the light cropload fruit.

Heightened cell activity in 'Sweetheart' fruit can be observed immediately following bloom (decreasing 2C/increasing 4C and 8C), irrespective of cropload level (Fig. 1). 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 (Fig. 1). When plant cells become polyploid they typically cease cell division. If this process, termed endoreduplication, could be delayed, then more cell divisions could occur resulting in larger fruit. Further, increased polyploidy in fruit was positively correlated with fruit size when comparing two strains of 'Gala' apple (Peter Hirst, personal communication). Though we did not observe significant differences in ploidy distribution among the cultivars that we investigated ['Chelan', 'Bing' and 'Sweetheart'] (data not shown), leaves of these cultivars, and leaf and fruit tissue of the small-fruited wild type *Prunus virginiana* (Chokecherry) were nearly entirely diploid [2C] (data not shown). We will 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 may improve our understanding of the

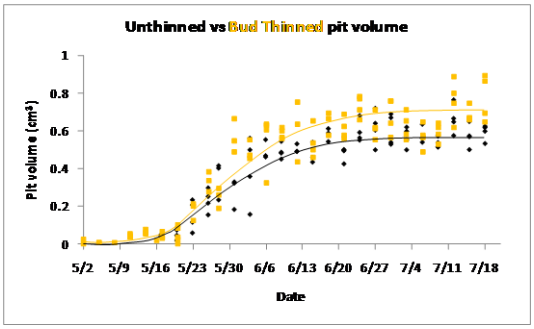

Figure 2. Effect of cropload level on pit size in 'Sweetheart'. Unthinned (high cropload, black and bud-thinned (low cropload grey) fruit.

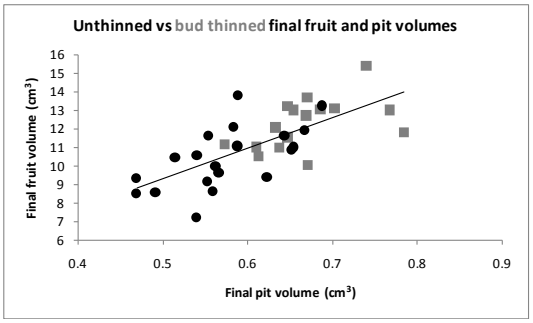

Figure 3. Relationship of fruit and pit volumes at harvest in 'Sweetheart'. Unthinned (high cropload, black) and bud-thinned (low cropload, light grey).

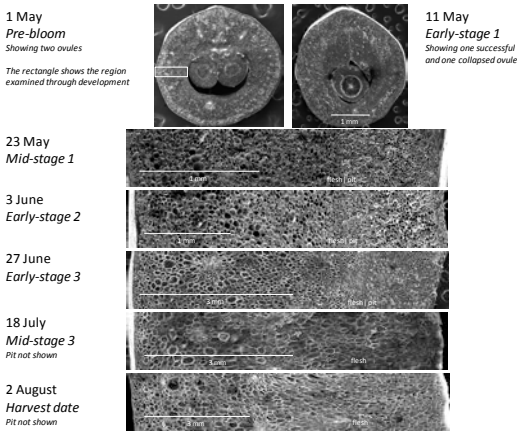

Figure 4. SEM images for 'Sweetheart' cell number and size measurements.

contribution of polyploidy to sweet cherry fruit size, and potentially inform breeding efforts focused on developing large-fruited progeny.

Growth of the endocarp (pit) was completed by June 9 [38 days after full bloom], and was negatively influenced by cropload level (Fig. 2). Differences in pit volume between heavy and light cropload treatments were observed by ~ May 20 (18 days after full bloom) (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.

SEM: High quality images were obtained using standard sample preparation techniques (Fig. 4). 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 and plot these measurements in relation to their position in the fruit (Fig. 5). 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 (Fig. 6). 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 (Fig. 7).

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; although flow cytometry data (Fig. 1) do not indicate a cropload effect on the timing of cell division. SEM data for all cultivars will be completed as we process all the materials that have been collected.

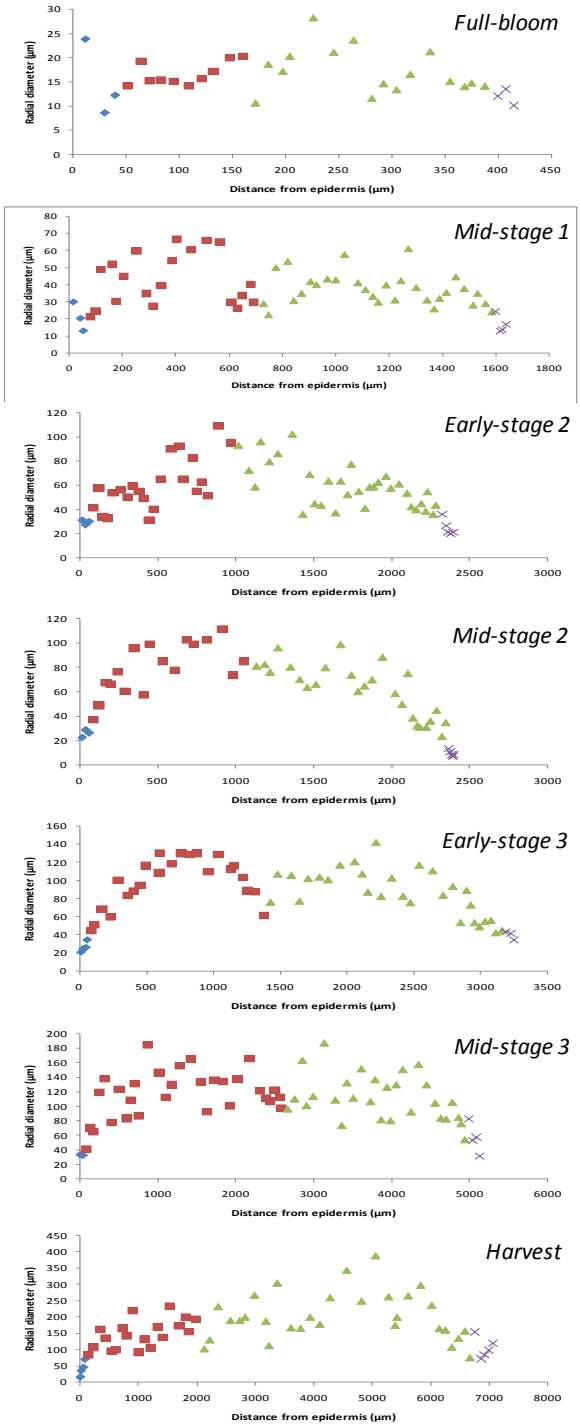

Figure 5. Examples of cell radial diameter measurements from SEM photographs during the growing season. Individual cell diameters were plotted in relation to their position within the fruit. Only 'Sweetheart' is shown (hypodermis, diamonds; outer mesocarp, squares; inner mesocarp, triangles; pit boundary cells, x's).

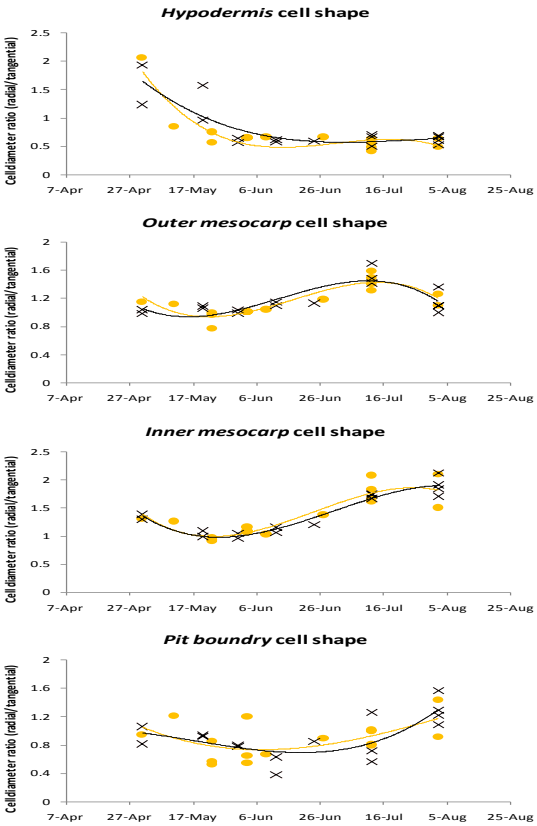

Figure 6. Cell shape is estimated by the ratio of the radial to tangential diameters. Values >1 indicate a radially elongated cell. Only 'Sweetheart' is shown (unthinned, black; bud-thinned, grey.

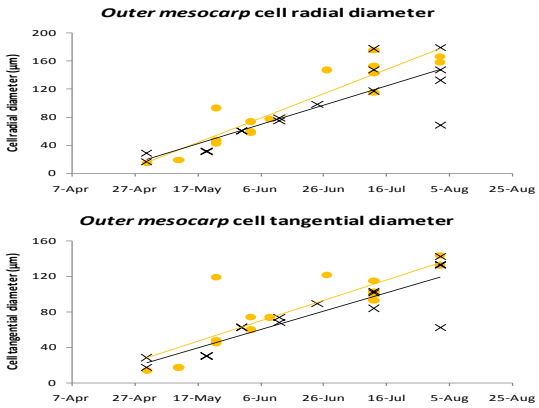

Figure 7. Averages of the radial and tangential cell diameters of the outer mesocarp during the growing season. The greatest differences were found in the outer mesocarp. Only 'Sweetheart' is shown (unthinned, black; bud thinned, grey).

GA Experiments. Previous season application rates between 0-40 ppm did not adversely affect return bloom of Skeena (data not shown). Rates of 60 ppm did not affect the number of return flower buds per spur, but reduced the number of flowers per bud by ~10% (data not shown). However, we were only able to measure return bloom dynamics in one orchard due to the combination of high bacterial canker infection and flower bud injury resulting from early spring frosts. We will compile these measurements from the current season applications in 2012. Both 'Skeena' and 'Sweetheart' trees responded similarly to 2011 GA rate and timing treatments (data only shown for Skeena for space considerations). No added benefits were observed when the same GA rates were applied in multiple applications. The most notable and quantifiable effects of GA on 'Skeena' fruit were an increase in fruit firmness, and a decrease in skin color (Table 1); however, both of these attributes ceased to change with increasing rates of GA (i.e., beyond 10 ppm) (Table 1). These results were similarly observed in 2010, though the minimum rate applied in those studies was 20 ppm. Average fruit size was seen to have improved in 2010 between the 0 and 20 ppm rates, and only slight, non-significant changes were observed with higher rates (data not shown). In 2011, we observed no appreciable changes in fruit size in response to GA (Table 1). These results may have been due to the overall low croploads in 2011 (roughly 250 fruit per scaffold). Stem retention force was lowest for the 0 ppm/no surfactant treatment, but values for all treatments were extremely low (Table 1). The combination of high elevation, a cool spring, and higher commercial GA applications delayed the 2011 harvest (August 23). Otherwise, fruit quality was considered excellent.

Table 1. Yield, cropload (number of fruit per cm^2 of limb cross-sectional area), and fruit quality attributes (fruit size, soluble solids, fruit firmness, CTIFL [skin color score]), for 7th leaf 'Skeena' / 'Gi6' limbs receiving different rates and timings of GA, on thinned or unthinned limbs. Data are means of 6 replicate limbs (n= 100 for fruit size, FF, CTIFL; n=2 for SS; n=1 for yield and cropload).

GA Treatment (ppm)	Yield per limb (lbs)	FF (g/mm)	Avg fruit diam. (mm)	Avg fruit wt (g)	SS (%)	TA (%)	Stem retention force (g)	Skin color CTIFL (1-7)
0 (no surfactant)	4.6	316	30.1	11.7	20.7	0.5	180	6
0 + surfactant	5.5	336	30.7	12	20.6	0.6	230	6
10	6	370	31	12.6	20.9	0.5	210	5
20	6.2	373	30.7	12.2	20.8	0.6	220	5
30	6.2	377	30.6	11.9	20.7	0.5	280	5
40	5.7	390	30.2	11.9	20.4	0.5	250	5
40 (20 stageII, 20 stageIII)	3.8	383	31	12.7	22.3	0.6	380	5
60	6.4	404	30.6	12.1	18.2	0.5	260	5
60 (20 stageII, 40 stageIII)	6.8	373	30.5	11.9	20.6	0.6	220	5

Timings for GA rates 20/10, 20/20, 20/40, 30/10 were applied at straw/mid Stage III. Single rates were applied at straw.

In a second 'Skeena' site, rain events occurring the week of harvest limited our ability to find differences in fruit quality attributes among GA treatments. Interestingly, GA rate did not have any measurable impacts on the percentage of total cracking; all treatments, including the 0 ppm controls, had ~60% cracking (100 fruit analyzed per replicate limb [6 replications]; data not shown).

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

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

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Cooperators: WA Producers

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

Budget 1
Organization Name: WA State University **Contract Administrator:** Carrie Johnston
Telephone: 509.335.4564 **Email address:** carriej@wsu.edu

Item	2010	2011	**2012**
Salaries	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	45,378	46,443	**7,000**

Footnotes: The travel in the requested 3rd year of funding is used to conduct field tests/demonstration all over the state.

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 singe 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. The tree training systems on which the device being tested included UFO, Steep Leader and Spanish Bush systems at different bloom stages. Major findings include:

1. Pre- and post-thinning blossom counts indicated 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, those 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. No noticeable difference on thinning efficiency was observed on strip materials when using plastic or plastic covered wires.
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 for the device as a whole) 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 third prototype, the battery was detached from the thinner and a battery with bigger capacity was connected to it 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 to be solved.
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, 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 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.

For all the spindle configurations designed and investigated, the followings were the major findings obtained from the field validation tests:

- Three different trimmer line configurations were selected and tested for prototype-one: two lines of 180° installation, three lines of 120° installation, and four lines of 90° installations;
- Three types of strip materials, 0.1" diameter plastic strip, 0.14" diameter plastic covered wires and 0.15" diameter rubber, were selected and tested on their suitability for this particular application; and
- Different string lengths from 3 to 8 inches were designed and tested in field operations.

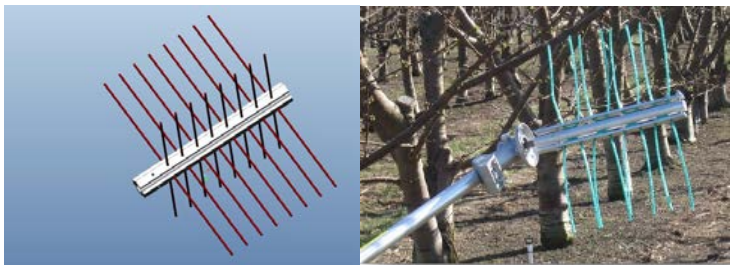

Figure 2. Examples of thinning spindle design: A 3D sketch (left) with 4 trimmer lines, eight 6 inches long strings in each line; and a thinning head (right) with 2 trimmer lines of 6 inches long plastic covered wire

Table 1 Thinning spindle configurations

Parameter	configuration
Number of trimmer lines	2, 3, and 4
Trimming strip materials	Plastic, Plastic covered wires, Rubber
Length of trimming strip	From 3 to 8 inches
Number of trimming strips	4, 6, and 8

First Prototype Development

This prototype was fabricated by replacing the trimming head with the specially designed blossom thinning spindle (detailed in previous section) on a commercially available gasoline-powered hand-held grass trimmer (STIHL Inc, Model FS 90 R). The weight of the developed prototype was about 13 lbs and it could be extended to 5 feet in length (Figure 3).

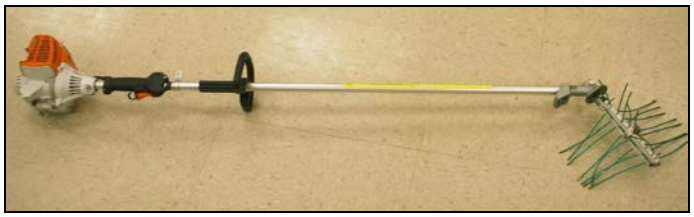

Figure 3. Prototype-1: gasoline-powered hand-held mechanical blossom thinner

Since the spindling force played an important role in removing flowers, the rotary speed control was essential for thinning operation, especially when we wanted to control the removal percentage of the flowers. To add this speed control function to the device, a finger-tip speed control system was designed to control the engine throttle on the gasoline-powered prototype. As shown in Figure 4, five holes were drilled in the trigger and the users could use a pin to restrain the position to regulate the spindle operation speed.

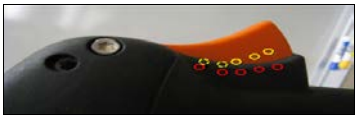

Figure 4. The speed control device designed for the gasoline-powered prototype

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 5). 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.

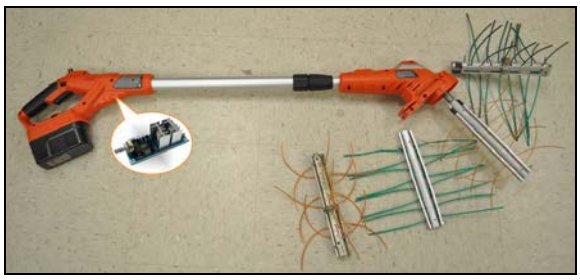

Figure 5. Prototype-2: DC motor-powered hand-held mechanical blossom thinner with four spindles

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 7. Both configurations could support thinning operations for an 8 hour + work schedule.

Figure 7. Single-person prototype with backpack (left) and two-person prototype with platform carried batteries

Experimental design

Cherries Field tests were designed to evaluate the functionality and performance of 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. 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.

Apricot One large commercial trial was conducted on 'Robada' apricots in 2011. Treatments included Darwin thinned, hand held thinned and green fruit thinned. Pre harvest data included: time to apply thinning treatment, time required for follow up green fruit thinning, pre and post bloom counts, and final fruit set. Fruit was segregated at harvest and were packed on a commercial line.

Apple One trial was conducted at the WSU Sunrise orchard. Varieties included Fuji, Gala, Golden Delicious and Jonagold. Treatments included each of the following with and without a follow up BA application; hand held device, Bonner string thinner and Darwin string thinner. Standard protocol for data collection is being followed.

RESULTS AND DISCUSSION

Configurations Effects

Cherries 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 (Table 1). The results indicate that the designed thinning spindle could effective remove cherry blossom to different percentage by controlling spindle speed.

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

Spindle speed range	Removal percentage
Low	18.0%
Medium	30.8%
High	61.6%

Results obtained from our Chilean collaboration in 2010 season illustrated that the number of fruits was significantly reduced by thinning operations. The percentage of fruit set was decreased from 48.6% to 25.5%. However, no significant difference could be found between different speed settings. The reason for this result could be that operators may not employ the same thinning strategy in each treatment. Figure 8 shows the number of flowers and fruits counted previous to treatment and at pre-harvest respectively; and the percentage of fruit set upon different treatments is shown as Figure 9. Both figures show the 2010 data.

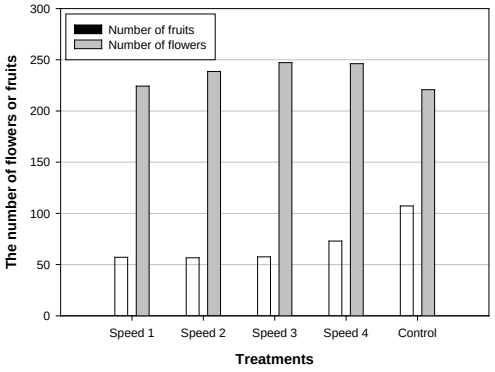

Figure 8. The number of flowers counted previous to treatment and the number of fruits counted at pre-harvest

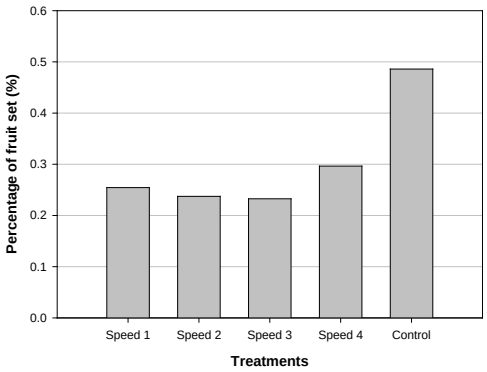

Figure 9. The percentage of fruit set upon different treatments

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 such a thinning operation on a tree was over 300 seconds.

In testing the effect of different string lengths, the results obtained so far was insufficient to conclude that there was a significant difference among the strings being tested. However, it was visually observed that (1) shorter strings (3 or 4 inches long) 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 (7 or 8 inches long) 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. Two-spine design proved efficient in cherries.

Data obtained from almost all field tests indicated that there was a significant difference between average fruit weight of the samples from thinned and non-thinned branches. The average weight of sample fruit from configurations A, B, C, and D are 10.6, 10.4, 10.3, and 10.0 g respectively, compared to average 8.4 g to control (non-thinning). Where, configuration A means 2 trimmer lines with wire string material; B means 3 trimmer lines with wire string material; C means 2 trimmer lines with plastic string material; and D means 3 trimmer lines with plastic string material. Figure 10 shows the comparison of the average weight of samples thinned with different configurations of the

thinner head and control. The data did not indicate any significant difference of sample weight between different string materials. There was no significant difference among different numbers of trimmer lines.

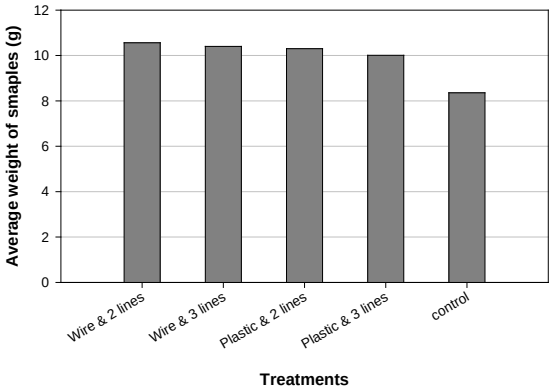

Figure 10. Average weight of fruit samples thinned with different configurations of thinner head

'Lapin' trials – In Washington, clipped green fruit treatment resulted in a greater percentage of fruit in size 10 and larger when compared to hand held blossom thinned. Time to apply hand held treatment was several seconds compared to a minute or longer for green fruit clipping. In PA, observations reported included impressive improvement in fruit size.

'Sweetheart' on Mazzard trials – No significant difference in surviving flower numbers across bloom stage and head / spine treatments. Row 10 and larger was best achieved by hand fruitlet thinning (100%) and hand flower thinning at 20-40% full bloom (92%). Of the mechanical treatments, two-spine at 20-40% yielded 76% fruit in row 10 or larger.
In the one trial that compared degrees of thinning, the percentage of 10 row and larger was highest in the heavy thinning treatment, closely followed by the fast thinning treatment. There was no significant difference between light thinning and control.

Apricot 'Robada' apricot responded favorably to the hand held device. Hand held treated fruit peaked on sizes 64 and 72. Darwin thinned fruit peaked on 72 and 96. Green fruit thinned only peaked on size 96 and 80. First pick volume was largest on Darwin thinned rows – followed by hand held thinned rows. Based on percent fruit – 32% of fruit that was thinned by hand held packed out in top 3 grades, compared to 26% for both Darwin thinned and green fruit thinned. When looking at top four grades, it evens out to 49% for hand held, 44% Darwin and 46% for green fruit thinned.

Overall Effectiveness

Obtained results also indicated that the designed thinning spindle could effectively remove cherry blossom, and the spindle rotating speed could noticeably impact the amount of blossom being removed. The users could control the percentage of blossom removal within a cluster by controlling spindle speed and could control total blossom removal by the strategic placement of the device on a tree. The configuration of thinning head could also affect the blossom thinning efficiency, but had less impact when compared to spindle speed.

Collaborative trials are underway in Chile with both the WSU hand held prototype and the commercially available Electro'flor. 2010-2011results will be presented at Cherry Institute in January.