2010 NW Cherry Research Review
Red Lion Hotel at Yakima Center
Thursday, November 12

Page	PI	Project Title
		Final Reports
2	Elfving	Branch induction in young sweet cherry trees without injury to bark
7	Elfving	Bioregulators, fruit loosening, mech harvest of sweet cherry ***(No '09 funds)***
12	Whiting	Efficient production of superlative fruit ***(teleconference)***
24	Dhingra	Sweet cherry regeneration and transformation system
32	Peace	Establishing the marker-assisted breeding pipeline for sweet cherry
44	Bliss	Consulting for the NW cherry improvement project ***(Final & new report)***
49	Iezzoni	Consulting for the PNW sweet cherry breeding program ***(Final & new report)***
58	Eastwell	Managing virus diseases detrimental to cherry production
67	Yang	Chemical genomics ***(written report only)***
		Continuing Reports: 10:45 - 11:45
77	Einhorn	Impact of harvest timing on fruit quality of sweet cherry cultivars
85	Einhorn	Irrigation and fertilization for optimal cherry fruit quality
92	Azarenko	Horticulture management systems for fresh & brine cherries
99	Iezzoni	Establishment of test plots for MSU sweet cherry rootstock candidates
108	Oraguzie	Breeding & genetics program for PNW sweet cherries
117	Hanrahan	WTFRC Internal research: programs to increase yields of target fruit in cherries

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	Culbertson	California update
		Break
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	McFerson	WTFRC technology projects ***(see reports in appendix)***
	Hanrahan	WTFRC Internal research: programs to increase yields of target fruit in cherries

FINAL PROJECT REPORT

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

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Cooperators: Matt Whiting, WSU Prosser; Eugene Kupferman, Dwayne Visser, WSU Wenatchee

Other funding sources: None

Total Project Funding: **Year 1**: 12,266 **Year 2:** 13,441

Budget History:

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

Objectives:

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

Significant findings 2008:

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

Significant findings 2009:

1. Pro-Vide (GA_{4+7}, Valent Biosciences, Walnut Creek, CA) at 5,000 ppm supplemented with 0.1% v/v Regulaid and applied to nicking cuts on one-year-old wood of 'Selah'/G.6 sweet cherry successfully induced lateral branching on treated shoots. Pro-Vide at the same concentration

supplemented with 2, 4 or 6% v/v Pentra-bark surfactant and applied as similar bands to non-nicked one-year-old wood did not result in lateral branch development in 2009. Temperatures following treatment were considered as suitable for the formation of lateral branches.

2. An identical set of treatments was applied to two-year-old wood of 'Selah'/G.6 trees; in this case the cut treatment involved scoring cuts made every 6 inches down the woody stem with a linoleum knife. No treatment resulted in branching, but fruiting buds on scored and painted two-year-old sections showed strongly elongated fruit pedicels, indicating that the GA did enter the bark tissues and did translocate.

Results and Discussion:

Research in 2008 confirmed observations in 2007 that appropriate surfactants can substitute for cutting the bark in assuring that branch-inducing bioregulator products penetrate into living tissues in shoots. Several questions remain to be explored; perhaps the most important of those has to do with the relative importance of surfactant type vs. applied concentration. It may be that a variety of commonly-used surfactants will work if applied in high enough concentration. In 2009 we tested surfactant concentrations up to 6% v/v, but the treatments were unsuccessful. Difficulties in test solution preparation may have contributed to the lack of results in 2009.

Gibberellic acid alone again proved effective for branch induction in one-year-old wood. In addition, GA was effective without the need for bark-cutting when either GA_4 (Novagib) or GA_{4+7} (ProVide) was combined with an effective surfactant, in this case Pentra-bark (2% v/v). In 2007, we showed that GA_3 (Pro-Gibb) was not a very effective inducer of lateral branching in sweet cherry; we have discontinued work with this formulation. We also observed that the cytokinins thidiazuron (TDZ) and forchlorfenuron (CPPU) were ineffective when applied at 5,000 ppm with a surfactant but without GA. In 2008, PR as the undiluted formulation (20,000 ppm) banded without the benefit of either surfactant or nicking was not impressive; this treatment induced branching in only one of five trials. In 2009, GA had no effect on branching in two-year-old wood, even when applied in conjunction with scoring. The observation that scored two-year-old branch sections treated with GA produced fruits with greatly elongated pedicels indicates that the GA entered living tissues under the scoring cut and also translocated a short distance to developing flowers. Unlike one-year-old wood, however, even high concentrations of GA did not induce bud activity and branch development.

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

References:

D.C. Elfving and D.B. Visser. 2009. Stimulation of lateral branch development in young sweet cherry trees in the orchard without bark injury. **Int=l. J. Fruit Sci. 9:166-175.**

D.C. Elfving, T.D. Auvil, F. Castillo, S.R. Drake, H. Künzel, E.M. Kupferman, B. Lorenz, J.R. McFerson, A.N. Reed, C. Sater, T.R. Schmidt and D.B. Visser. 2009. Effects of preharvest applications of ethephon and ethylene antagonists to sweet cherry trees on fruit loosening for mechanical harvest and on fruit quality. **J. Amer. Pomol. Soc. 63:84-100.**

Schrader, L.E., J.G. Zhang, J.S. Sun, J.Z. Xu, D.C. Elfving and C. Kahn. 2009. Changes in internal fruit quality with sunburn browning. **J. Amer. Soc. Hort. Sci. 134:148-155.**

EXECUTIVE SUMMARY

Research in 2008 confirmed observations in 2007 that appropriate surfactants can substitute for cutting the bark in assuring that branch-inducing bioregulator products penetrate into living tissues in shoots. Difficulties in test solution preparation may have contributed to the lack of results in 2009.

Gibberellic acid alone again proved effective for branch induction in one-year-old wood. In addition, GA was effective without the need for bark-cutting when either GA_4 (Novagib) or GA_{4+7} (ProVide) was combined with an effective surfactant, in this case Pentra-bark (2% v/v). In 2007 GA_3 (Pro-Gibb) was not a very effective inducer of lateral branching in sweet cherry and is no longer being tested. We also observed that the cytokinins thidiazuron (TDZ) and forchlorfenuron (CPPU) were ineffective when applied at 5,000 ppm with a surfactant but without GA. In 2008, PR as the undiluted formulation (20,000 ppm) banded without the benefit of either surfactant or nicking was not impressive; this treatment induced branching in only one of five trials. In 2009, GA had no effect on branching in two-year-old wood, even when applied in conjunction with scoring. The observation that scored two-year-old branch sections treated with GA produced fruits with greatly elongated pedicels indicates that the GA entered living tissues under the scoring cut and also translocated a short distance to developing flowers. Unlike two-year-old wood, however, even high concentrations of GA did not induce bud activity and branch development.

Temperature regimes following branching applications on UFO-trained trees in Buena were quite different; extended daytime cold temperatures and freezing overnight temperatures prevailed for the ten days following the first application date. Branch development in this trial was poor. For the comparable interval after the second set of applications, nighttime minima were not a great deal different from the same interval after the first application date. However, the daytime maxima averaged about 17°F (10C) higher than during the first interval, with the highest daytime maximum reaching 78°F (25C). In this case branching was good. Waiting for optimum temperatures must be tempered with the knowledge that if trees advance much beyond the green-tip growth stage, they become insensitive to branch-inducing bioregulator treatments.

FINAL PROJECT REPORT

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

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Cooperators: Matt Whiting, WSU Prosser; Eugene Kupferman, Dwayne Visser, WSU Wenatchee

Other funding sources: None

WTFRC Collaborative expenses:

Item	2007	2009
Stemilt RCA room rental		
Crew labor	840	900
Shipping		
Supplies		
Travel	520	600
Miscellaneous		
Total	**1360**	**1500**

Total Project Funding: **Year 1:** 15,518 **Year 2:** 17,723 **Year 3:** 19,427

Budget History:

Item	**Year 1:** 2007	**Year 2:** 2008	**Year 3:** 2009
Salaries	7,000	7,500	8,000
Benefits	2,380	2,550	2,720
Wages	1,200	1,500	1,800
Benefits	138	173	207
Equipment	0	0	0
Supplies	800	1,000	1,200
Travel	4,000	5,000	5,500
Miscellaneous	0	0	0
Total	15,518	17,723	19,427

Objectives:

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

Significant findings 2007:

1. Ethephon again effectively loosened 'Bing' cherries when applied approximately 14 days before harvest. However, only the lower concentration of ethephon (150 mg/liter a.i. or 0.5 pint/100 gallons) combined with "Pentra-Bark" penetrant reduced flesh firmness significantly. The factors that influence the relation between fruit loosening and firmness loss are unknown. No visible effects on defoliation were observed with any ethephon treatment.
2. Two known auxin transport inhibitors, cyclanilide and diflufenzopyr (DFFP), were tested on limbs of 'Bing' cherries for efficacy in loosening and effects on flesh firmness loss.
3. Cyclanilide at 500 mg/liter destroyed the crop due to phytotoxicity; this product does not appear promising.
4. The potent auxin transport inhibitor DFFP at 0.5-5 mg/liter a.i. did not induce fruit loosening, flesh firmness loss nor defoliation.
5. Methyl jasmonate (MJ) has been proposed as a possible fruit loosener for sweet cherries. At 1000 mg/liter a.i., MJ did not loosen fruit, stimulate flesh firmness loss or induce defoliation.
6. Applications of GA_3 and GA_7 to 'Rainier'/G.5 trees in 2006 produced a small reduction in flower buds per spur in 2007. In addition, GA_3 reduced flowers per bud in proportion to concentration, while GA_7 did not.
7. GA treatments on 'Rainier'/G.5 trees in 2006 did not produce significant effects on mean fruit size, brix, percent red color or percent of crop in fruit-size classes in 2007 at any of three harvests. Compensating fruit set on differential bloom may have accounted for this observation.
8. GA_3 at up to 75 mg/liter improved mean fruit size but had no effect on fruit firmness or total yield when applied in 2007 to 'Sweetheart'/G.5 trees. Bloom and crop characteristics data will be taken in 2008.
9. Cytokinin products applied to 'Bing'/G.1 trees 6 days after full bloom (fruit diameter 4.8±0.1 mm) failed to improve fruit size. The cytokinins were thidiazuron (TDZ, Dropp, Bayer Crop Science), forchlorfenuron (CPPU, Kim-C1 Co.), and 6-benzyladenine (BA, Maxcel, Valent Biosciences), each applied at either 10 or 50 mg/liter. These concentrations may not have been high enough to stimulate cell division in sweet cherry fruit.
10. The high rate of TDZ reduced fruit red color rating at harvest. The other cytokinin treatments had little effect. There were no significant effects of any treatment on fruit firmness at harvest.

Significant findings 2008:

3. 2008 was a difficult year for cherry growers. Extensive early cold and frost conditions compromised crop loads and crop quality in many orchards. No trials were carried out in 2008.
4. A GA trial on 'Sweetheart' cherry applied in 2007 to examine effects on crop load and fruit size in 2008 was damaged by spring cold temperatures in 2008 and was not evaluated.

Significant findings 2009:

5. Glycine-betaine [GB, the active ingredient in "Blue-Stim" (Monterey Ag. Resources, Fresno, CA)] had little effect on fruit removal force or any fruit-quality parameter at harvest in 'Bing' sweet cherries when applied on one of two dates (3 weeks before harvest or 1.5 weeks before harvest) or on both dates.
6. GB did NOT alter flesh firmness, but slightly darkened flesh red color.
7. Soil-applied Sil-Matrix (K-silicate, PQ Corp., Valley Forge, PA) drenched around the trunks of 'Bing' sweet cherry trees 6 and 3 WBH did not affect either fruit removal force or flesh firmness.
8. Sil-Matrix retarded external fruit color development when applied alone, but this effect was totally offset when ethephon was applied after Sil-Matrix had been applied.
9. Sil-Matrix had no effect on total soluble solids or titratable acidity, but reduced Sugar/Acid Ratio (SAR) when ethephon was also applied.
10. Sure-seal (Ca acetate hydrate, Agros Organics, Fair Lawn, NJ) is reported to control fruit cracking and may also favorably affect fruit flesh firmness or other fruit-quality parameters.
11. Sure-seal had no effect on fruit removal force but appeared to reduce flesh firmness nearly as much as, but not in addition to, ethephon.
12. Limited evidence suggests Sure-seal may have reduced fruit size, internal red flesh color, TSS and TA, but did not affect SAR.
13. In the three trials conducted in 2009, ethephon applied 2 WBH at 3 pt/acre consistently reduced fruit removal force from around 700 g to about 300 g, a level satisfactory for mechanically harvested fruit removal.
14. Ethephon also consistently reduced flesh firmness, improved both external and internal fruit color, reduced both TSS and TA, but had little effect on SAR.

Results and Discussion:

Since the repeated absence of beneficial effects of sprayable MCP (e.g., "Harvista") on control of fruit firmness loss in ethephon treated cherries (2003-2006), we have been exploring other possible options for loosening fruit. A main direction in 2007 was the examination of auxin-transport inhibitors, based on the physiological principle that auxin transport to an abscission zone keeps that group of cells healthy, preventing abscission. Once auxin flow is reduced, or eliminated, the abscission zone begins to deteriorate, which ultimately should lead to the separation of the fruit from the tree.

In 2007 we tested methyl jasmonate, diflufenzopyr (a powerful anti-auxin from Chemtura) and cyclanilide (another anti-auxin from Bayer) in comparison to ethephon alone or supplemented with the cytokinin forchlorfenuron (CPPU). Ethephon produced the same results as it has every year since 2001, namely, loosening of fruit with, in the case of 2007, little effect on flesh firmness except where the penetrant "Pentra-bark" was combined with ethephon. The factors that influence the relation between fruit loosening and firmness loss in ethephon treated fruit are unknown. At the concentrations used, the anti-auxins were ineffective for loosening fruit. Cyclanilide at 500 mg/liter produced substantial phytotoxicity to both leaves and fruit, but no loosening. The physiological activity in sweet cherry trees of methyl jasmonate is not understood, but it was also ineffective for loosening fruit. Since, with the notable exception of cyclanilide, there were no symptoms of any kind of phytotoxicity or defoliation due to any other treatment, it is possible that product concentrations were too low to produce abscission in sweet cherry trees.

In 2009, three products were tested either alone or along with a standard ethephon application (3 pt/acre 2 weeks before commercial harvest) to evaluate possible effects on fruit quality. Glycine-betaine (Blue-Stim), potassium silicate (Sil-matrix) and calcium acetate hydrate (Sure-seal) showed limited effects on fruit quality parameters at harvest, but in no case was the ethephon-mediated loss of

flesh firmness beneficially affected. In 2009 the ethephon effect on fruit was similar to its effects in the seven previous seasons in which it has been tested.

GA trials oriented toward managing crop load have so far not proven to produce as dramatic results as we had hoped. Because GA applications affect the current season's fruit maturity, as well as bloom formation for the next year, the concentration range must be chosen such that neither goal is unfavorably affected. So far, our results have been inconsistent, which may be a reflection of the differential effects of one season vs. another on factors that influence flower-bud induction and formation. This lack of predictable results, along with the increasingly severe effects of higher GA concentrations on fruit maturation, do not encourage further work at this time.

References:

D.C. Elfving and D.B. Visser. 2009. Stimulation of lateral branch development in young sweet cherry trees in the orchard without bark injury. **Int=l. J. Fruit Sci. 9:166-175.**

D.C. Elfving, T.D. Auvil, F. Castillo, S.R. Drake, H. Künzel, E.M. Kupferman, B. Lorenz, J.R. McFerson, A.N. Reed, C. Sater, T.R. Schmidt and D.B. Visser. 2009. Effects of preharvest applications of ethephon and ethylene antagonists to sweet cherry trees on fruit loosening for mechanical harvest and on fruit quality. **J. Amer. Pomol. Soc. 63:84-100.**

Schrader, L.E., J.G. Zhang, J.S. Sun, J.Z. Xu, D.C. Elfving and C. Kahn. 2009. Changes in internal fruit quality with sunburn browning. **J. Amer. Soc. Hort. Sci. 134:148-155.**

EXECUTIVE SUMMARY

Over the three-year period 2007-2009, we explored other possible options for loosening fruit besides ethephon. In 2007 we tested methyl jasmonate, diflufenzopyr (a powerful anti-auxin from Chemtura) and cyclanilide (another anti-auxin from Bayer) in comparison to ethephon alone or supplemented with the cytokinin forchlorfenuron (CPPU). Ethephon produced the same results as it has every year since 2001, namely, loosening of fruit with, in the case of 2007, little effect on flesh firmness except where the penetrant "Pentra-bark" was combined with ethephon. The factors that influence the relation between fruit loosening and firmness loss in ethephon treated fruit are unknown. At the concentrations used, the anti-auxins were ineffective for loosening fruit. Cyclanilide at 500 mg/liter produced substantial phytotoxicity to both leaves and fruit, but no loosening. The physiological activity in sweet cherry trees of methyl jasmonate is not understood, but it was also ineffective for loosening fruit.

In 2009, three products were tested either alone or along with a standard ethephon application (3 pt/acre 2 weeks before commercial harvest) to evaluate possible effects on fruit quality. Glycine-betaine (Blue-Stim), potassium silicate (Sil-matrix) and calcium acetate hydrate (Sure-seal) showed limited effects on fruit quality parameters at harvest, but in no case was the ethephon-mediated loss of flesh firmness beneficially affected. In 2009 the ethephon effect on fruit was similar to its effects in the seven previous seasons in which it has been tested. Planned trials for 2008 were terminated due to excessive fruit damage due to severe frost incidence that year.

GA trials oriented toward managing crop load did not produce as dramatic results as had been hoped for. Because GA applications affect the current season's fruit maturity as well as bloom formation for the next year, the concentration range must be chosen such that neither goal is unfavorably affected. So far, our results have been inconsistent, which may be a reflection of the differential effects of one season vs. another on factors that influence flower-bud induction and formation. This lack of predictable results, along with the increasingly severe effects of higher GA concentrations on fruit maturation, do not encourage further work at this time.

FINAL PROJECT REPORT

Project Title: Efficient production of superlative fruit

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Other funding sources: None

Total Project Funding:

Budget History:

Item	**Year 1:** 2007	**Year 2:** 2008	**Year 3:** 2009
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel			
Panel Testing			
Miscellaneous			
Total	136,138	137,386	140,766

OBJECTIVES

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

SIGNIFICANT FINDINGS (2009 only)

ORCHARD SYSTEMS

- In WSU and collaborator orchards, a novel architecture dubbed U.F.O. (upright fruiting offshoots) shows great potential for improving input efficiency and incorporating mechanization
- Key issues for establishing and maintaining the UFO architecture are 1) promoting uniform, well-spaced uprights in years 1 & 2, and 2) tree density (i.e., uprights/tree) for uniform, balanced growth of uprights.
- Industry collaboration has been significant, we estimate >25 acres of UFO plantings are established with UFO plantings also established in Chile, Argentina, and Australia
- 3rd-leaf yields of UFO orchards were between ca. 1.5 and 5 tons/acre
- Summer pruning vigorous uprights is effective for reducing vigor
- Key issues for further research are: 1) potential for sleeping eye or fall planting systems; 2) cost:benefit analyses of fruiting wall systems
- Funding for continued development of the UFO training system was secured via the Specialty Crop Research Initiative

FRUIT SET/CROP LOAD MANAGEMENT

- Pollen germination was similar on the stigmas of cultivars with low fruit set (Benton, Regina) and high fruit set (Bing, Sweetheart)
- Temperature affects pollen germination and pollen tube growth rate
- Pollen tube growth is similar in high and low fruit set cultivars
- Ovule longevity appears to limit fruit set in many low set cultivars.
- Paternal elements do not appear to limit fruit set.
- The combination of GA_3 or $GA_{4/7}$ (30 ml l^{-1}) with Prohexadione-Ca at 150 mg l^{-1} (PCa, Apogee®) applied at the onset of endocarp lignification of fruit (30 days after full bloom) increased fruit weight and firmness significantly and similarly, by about 15%.
- 'Bing' fruit treated with PCa+GA_3 or PCa+$GA_{4/7}$ exhibited delayed fruit maturity of ca. 7 days compared to untreated control.
- Treatment with PCa + $GA_{4/7}$ resulted in 35-40% of fruit in ≤9 row size compared with only 20% of untreated fruit in the same size class. PCa + GA_3 however increased yield of similar, premium size fruit to 80%, regardless of application timing.
- Following 30 days in 4C storage only 5% of fruit were marketable from untreated trees whereas 50 – 30% fruit were marketable from PCa+GA_3 treatment
- The effect of a single GA_3 spray on sweet cherry fruit size is sensitive to the timing of application – applications earlier than straw appear to be beneficial

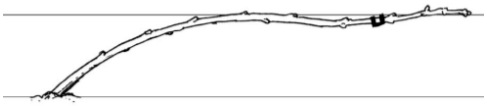
SENSORY STUDIES

- Overall, consumers have difficulty identifying differences in fruit firmness, particularly at high firmness levels

- When evaluating ‘Chelan’ fruit firmness, consumers were able to distinguish between low and high firmness, and low and intermediate firmness cherry groups.
- For ‘Bing’, consumers were able to distinguish only between low and high firmness cherry groups.
- For both harvest times, panelists did not distinguish between cherries from the same firmness group, or between intermediate and high firmness cherries.
- Consumer acceptance of cherry appearance, flavor, juiciness and firmness significantly influenced the overall acceptance of the cherry. Overall acceptance was not as influenced by appearance as it was by flavor.
- Acceptance of cherry firmness and juiciness significantly differed between early and late harvest cherries, with the early harvest cherries having a higher acceptance based on both of these attributes.

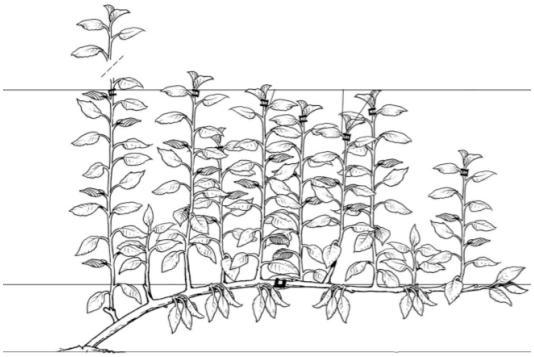
RESULTS AND DISCUSSION

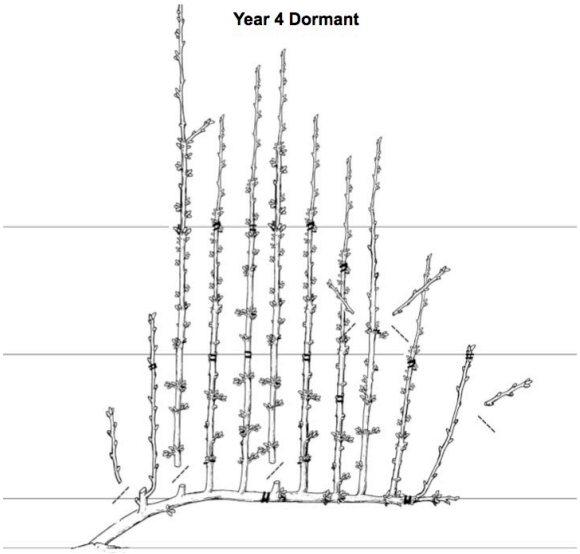
ORCHARD SYSTEMS We continued our collaborative development of planar fruiting wall architecture for high efficiency sweet cherry orchards – the UFO system. We currently are working with an illustrator to finalize a one-page (two-sided) handout for industry, illustrating the key training steps. This information will be posted also online at: http://fruit.prosser.wsu.edu/UFO.html. Third-leaf yields in a collaborating orchard ranged from 1.5 tons/ac (‘Early Robin’/’Gisela®6’ and ‘Kiona’/’Gisela®5’) to about 5 tons/ac for ‘Cowiche/’Gisela®5’. Yield of 3rd-leaf ‘Selah’/’Gisela®6’ was estimated at 4.5 tons/ac.



Unheaded whips are planted at an angle of 45° and brought horizontal gradually. Height of first wire should be 18 - 22". Recommended spacing: 8 – 10' x 6 – 8' (vertical walls), 13-15' x 3 – 4' (angled walls). Size-controlling, precocious rootstocks are recommended. Goal is to fill between-tree space at planting.

First growing season – develop wall of well-spaced uprights (6" apart), summer prune vigorous uprights. Remove uprights originating near base of scaffold and those that can’t be trained vertical. Ideal vertical growth is 20 - 30"/upright. Goal is to grow abundant, uniform upright shoots, establishing the canopy. Upright growth in year 1 = precocity.





Dormant, yearly (4 yr old illustrated) – prune lateral growth with thinning cuts leaving unbranched uprights. Renew uprights with stub cuts (i.e., leaving multiple renewal growing points). Regrowth from renewal pruning is thinned to a single upright.

FRUIT SET/POLLINATION Our investigations of environmental factors affecting fruit set continued in 2009. We have utilized four model cultivars: 'Bing' (high natural fruit set, self-sterile), 'Tieton' (low fruit set, self-sterile), 'Benton' (low fruit set, self-fertile), and 'Sweetheart' (high fruit set, self-fertile). We are combining field studies with others in temperature controlled growth chambers. Pollen germination, pollen tube growth rate, ovule viability, and stigma receptivity are each being assessed. This report will focus on pollen tube growth assessments.

From manual pollinations of recently opened flowers in temperature controlled growth chambers, we observed pollen germination as early as 2 HR post pollination, in Sweetheart at high temperature (Table 1). The growth of pollen occurred at a similar rate among cultivars and was inhibited at low temperature. At low temperature, initial pollen germination and growth did not occur until 24 to 32 HR post pollination. There was no apparent inhibition of pollen germination or pollen tube growth in the low productivity cultivars Benton and Regina, compared to the high productivity cultivars, Bing and Sweetheart. Similarly, there was no apparent difference among self-fertile and self-sterile cultivars. Temperature was the most influential factor. Overall, there were only subtle differences in pollen tube growth between medium and high temperatures. We observed comparable pollen germination and tube growth at medium and high temperature, irrespective of cultivar (Table 1). At medium and high temperature regimes, pollen tubes had reached the base of the style or grown beyond by 48 HR post pollination, again, irrespective of cultivar. Hand pollinations were made also in the field and samples were collected at similar intervals. These samples are being analyzed currently for comparison with growth chamber results – preliminary sampling shows pollen tube growth to the base of styles in every cultivar by 24 HR post pollination.

Table 1. Effects of temperature on pollen germination and pollen tube growth rate in four model cultivars. Pollinations were manual with NY54 (S_2S_6). L = 38/50F day/night; M = 8/18F; H = 12/24F. 0 – no pollen germination; 1 – germination but no penetration of style; 2 – pollen tube growth to ¼ way to stylar base; 3 – halfway to stylar base; 4 – three quarters way to stylar base; and 5 – to or beyond stylar base

Hours post pollination	Bing			Benton			Regina			Sweetheart		
	L	M	H	L	M	H	L	M	H	L	M	H
2	0	0	0	0	0	0	0	0	0	0	0	1
4	0	0	0	0	1	1	0	0	1	0	1	1
8	0	1	2	0	1	2	0	2	2	0	3	2
24	0	3	3	1	2	3	0	2	3	0	3	3
32	2	4	4	2	4	4	3	4	4	2	4	4
48	3	5	5	4	5	5	5	5	5	3		
72	4	being analyzed										
96	being analyzed											

In 2009 we collaborated with Dr. Dave Rudell to assess flower nectar quantity and quality of our four model cultivars. It was hypothesized that variability in pollinator activity, vis-à-vis nectar reward, might account for differences in fruit set among cultivars. 'Regina' flowers had the greatest volume of nectar volume whether sampled from the field or the controlled environment chamber. 'Benton' and 'Bing' were similar, and 'Sweetheart' flowers had the smallest volume. Qualitatively

there were no significant differences. Analysis showed that sucrose accounted for ca. 50% of nectar sugar weight in all varieties. 'Regina' and 'Benton' have more fructose and glucose than 'Bing' and 'Sweetheart', and no difference in sorbitol concentration was observed among four cultivars. From this preliminary investigation it appears that floral nectar quantity and quality do not account for differences in natural fruit set. However, we believe the role of pollinators should be investigated further, in relation to flower nectar and pollen reward.

We took the best performers from our PGR trials in 2008 and repeated trials in 2009. Treatments were made to 'Bing', 'Tieton', 'PC8011-3', and 'Regina' trees to improve fruit set. 'Bing' flowers treated with 4-chlorophenoxyacetic acid (CPA, 30 ppm) exhibited 70 – 100% higher fruit set than untreated in both small (i.e., limb) and large (i.e., whole-tree) trials. This treatment also improved fruit size by ca. 10% over untreated trees. We are particularly interested in further trials with CPA and have initiated trials in Tasmania on 'Regina' and 'Kordia'. Most of the cytokinins tested had no effect on fruit set though CPPU-treated trees had 10 – 25% greater set than untreated. Interestingly, another cytokinin, Topolin exhibited efficacy as a thinner, reducing fruit set by about 10 – 20%. Of the gibberellins tested, GA_1 was ineffective while $GA_3 + GA_{4+7}$ increased fruit set by 30 – 50%. In 'Tieton' both CPA and GA_{4+7} significantly increase fruit set but the latter led to greater improvements in fruit size and is recommended for further testing. We also tested polyamines (e.g., putrescine, spermine) and Harvista (1-MCP, in collaboration with Dr. Dana Faubion) in 'Tieton' and 'Regina'. In 'Tieton', fruit set was increased by 8% compared to control but we found no improvements in 'Regina' fruit set. Natural fruit set in 'Regina' in 2009 was about 4% - likely related to rapid ovule senescence in during warm weather. Flower samples were collected post-application for assessment of pollen tube growth. No PGR treatment increased growth rate of pollen tubes. This suggests that improvements in fruit set may have been due to extending the viability of the ovules (an approach that we intend to pursue for improving fruit set). In our current proposal, we outline large-scale field trials with the most promising treatments. If successful, we may be able to partially overcome the low fruit set/crop load issue with certain cultivars.

In 2009 we followed up limb trials from 2008 with whole-tree applications of the most promising PGR treatments for fruit quality. Combinations of Apogee (PCa) and gibberellins were effective at improving quality of 'Bing' when applied 30 or 37 days after full bloom. Analyses of fruit weight distribution showed that, the percent of fruit that were 9-row and larger was ca. 20%, 37%, and 80% for control, PCa + $GA_{4/7}$, and PCa + GA_3, respectively (Figure 6). In addition, both first and second spray of PCa alone resulted in a 15% increase in the ≤ 9 row size category than the control. Further analyses of fruit yield vs. fruit size relationships from plotting crop yield/tree vs. yield of premium size class fruit (≤ 9 row) showed that both PCa+ GA_3 and PCa + $GA_{4/7}$ treatments have potential to improve crop yield and fruit size in 'Bing' sweet cherry (data not shown).

The ability of two ostensibly counteracting PGRs to affect fruit quality as reported herein is intriguing, and worthy of further investigation. Prohexadione-Ca (PCa), is an inhibitor of GA biosynthesis, that can reduce vegetative extension growth in apple and sweet cherry (Elfving et al, 2005). We hypothesized initially that we could improve fruit quality by reducing competition between vegetative sinks and fruit growth with PCa applications during rapid shoot growth. Our results of shoot growth (data not shown) show significant reductions in shoot growth rate beginning ca. 2 weeks after application. The benefits from combinations of GA and PCa are greater than from PCa alone. We attribute this to the additional benefit of increasing sink strength of the fruit and therefore, improved canopy source-sink relations to allow greater carbon partitioning to fruit growth. PCa + GA treatments are being tested in Tasmania in large-scale field trials in the current season. Results will inform efficacy in another environment/season and on additional cultivars ('Regina' and 'Sweetheart') for further testing in WA for 2010.

A separate trial studied the incidence of sweet cherry flowers with protruding pistils (i.e., stigma and portion of style extended beyond the unopened corolla) and whether fruit set was affected by this condition (Fig. 1). In 2008 we observed significant incidence under field conditions. In 2009 we recorded the incidence in 19 cultivars, fruit set of flowers with protruding pistils, and studied the

role of temperature on incidence and floral organ characteristics in growth chambers. The natural incidence of protruding pistil flowers in 2009 varied from 0% to ca. 23%. 'Sweetheart' (11%), 'Lapins' (23%), and 'Rainer' (9%) had the highest percentage of flowers with protruding pistil flowers. We did not observe protruding pistils in 'Olympus', 'Attika', 'Chelan', 'Blackgold', 'Regina', 'Selah', or 'Benton' flowers. Interestingly, our investigations of fruit set showed that flowers with prematurely exposed stigmas had similar fruit set potential to normal flowers. Growth chamber studies revealed the incidence of flowers with protruding pistils flowers is greater at low temperatures. Flowers opening in Low temperature treatment induced the formulation of stigma exsertion, and 'Sweetheart' (14%) and 'Regina' (21%) had higher ratio of stigma exertion than 'Bing' (1%) and 'Benton (0%)'. The length of the exposed stylar varied among cultivars from 0.02 mm to 2.5 mm among cultivars, and 'Sweetheart' and 'Lapins' have the longest ones.This is likely due to growth of petals and pedicels being more sensitive to cold temperature than pistil growth. Low temperature (2/10°C, day/night) reduced petal size but not stylar length. However, after the flowers open, the anthers were sufficiently long to reach or extend beyond the stigma in most cultivars and be suitable for pollination by bees visiting, which indicates that the cherry flowers have a compensation mechanism in the reproductive process for the achievement of pollination and set fruit under adverse weather conditions.

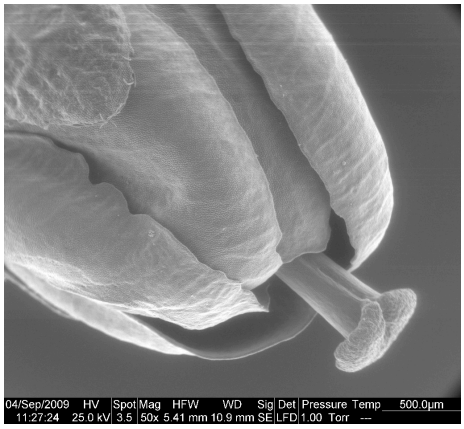


Figure 1. Scanning electron micrograph of flower with a protruding pistil.

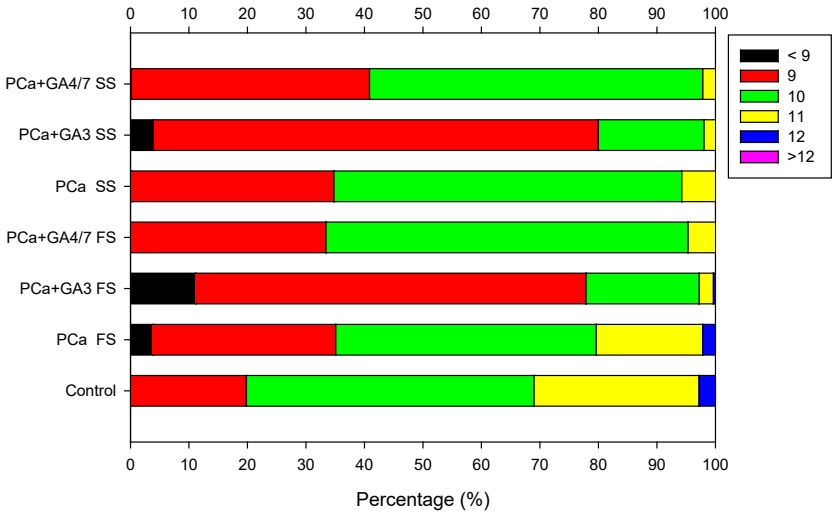


Fig. 6 Effect of PCa and GA isomers (GA_3, $GA_{4/7}$) alone or theire combination applied at 30 (first spray, FS) and 37 (second spray, SS) days after anthesis on cherries fruit row size distribution of 'Bing'., 2009.

CROP LOAD MANAGEMENT

Trials in 2009 addressed bloom thinning, post-bloom thinning, timing of thinning, and a novel approach for delivery of hormone treatments to developing fruit. We continued investigating the potential for post-bloom thinning of sweet cherry in 2009. Overall, our attempts to induce premature abscission of fruit were unsuccessful. No treatment reduced fruit set compared to unthinned control (Table 2). Each thinner was applied at 14 and 21 days after full bloom. Lack of thinning may be due to the ineffective of hormones tested, timing, or rate. We conducted a pilot study to develop a method for delivery of hormones to abscission zones in situ, using hormone solutions and thread. This method shows promise and will be pursued in the future and a means of screening hormones for their effect on fruit abscission. The challenge of inducing abscission in pollinated, developing fruit is novel for sweet cherry (perhaps stone fruit in general) and will require concurrent and complementary avenues of research.

Treatment	Fruit set (%)

Table 2. Thinning efficacy of 6-benzyladenine (BA), ethephon, and naphthalene acetic acid (NAA) applied at either 14 or 21 days after full bloom to 12-year-old 'Bing'/'Gisela®5' sweet cherry trees.

	14 DAFB	21 DAFB
Control	22%	31%
BA	29%	32%
Ethephon	27%	25%
NAA	34%	34%

In a separate post-bloom thinning trial with 'Bing', we tested ABA, BA, NAA, and Topolin. Each PGR was applied alone and in combination with PCa at 30 days after anthesis. PCa + NAA and PCa + ABA showed efficacy potential for bloom thinning with fruit set being ca. 56%, compared to 85% for untreated control. These results suggest that timing of application is important since earlier applications of NAA were ineffective. We hope to pursue these combinations at more timings to better understand the timing vs. efficacy relationship. Interestingly, PCa + BA exhibited potential for increasing fruit size (treated fruit were 20% larger than untreated) without decreasing in fruit set – another treatment worthy of further, larger scale testing.

We also studied the role of timing of thinning on fruit quality and yield in 'Bing' and 'Sweetheart'. On each thinning date, the entire crop was reduced by 50% by removing half of the flowers/fruit on every spur. Regardless of timing of thinning, fruit yield was reduced, however, not all thinning timings improved fruit quality. It appears that thinning at full bloom is significantly better than later thinning (Fig. 2), irrespective of cultivar. Thinning at straw and later (i.e., during stage III) did not improve fruit quality in either cultivar, despite significant reductions in yield. Recent research has highlighted the importance of mesocarp cell size (i.e., stage III of fruit development) in final fruit size, diminishing the role of cell number (i.e., stage I of fruit development). The current data contradicts this by showing greater benefits to fruit size with earlier thinning. Earlier thinning also improved soluble solids compared to later thinning and unthinned fruit. Firmness was not affected. Further, fruit from trees thinned at full bloom were subtly advanced in maturity (ca. 2 days), based on fruit exocarp color data (not shown).

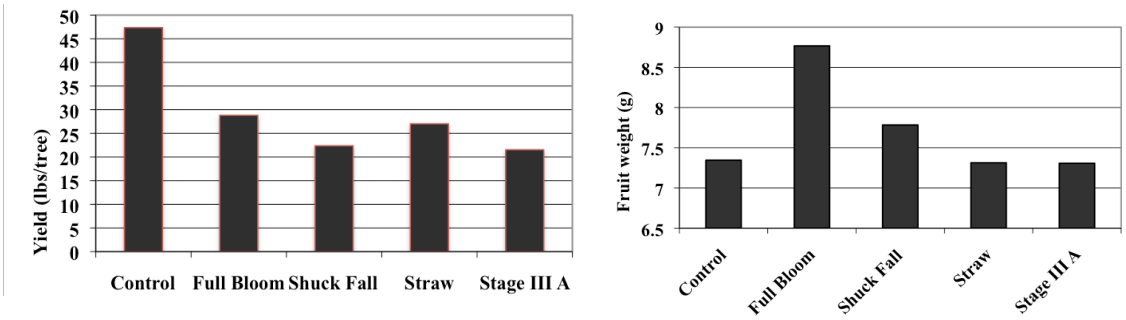


Figure 2. The effect of timing of thinning to 50% natural fruit density on fruit yield and weight of 'Bing' and 'Sweetheart' trees.

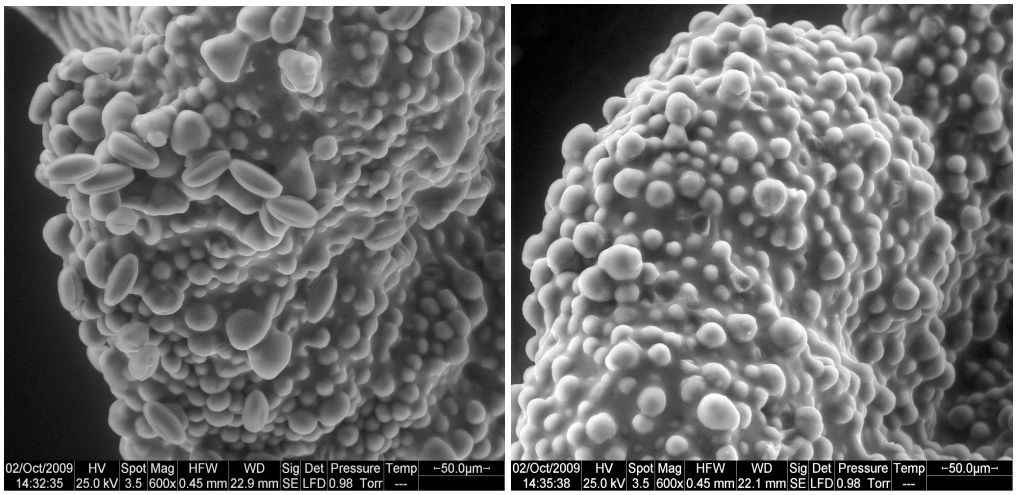


Figure 3. Scanning electron micrographs of portions of 'Sweetheart' stigmas (600x) captured at Central Science Lab, University of Tasmania. A – treated with 2% ATS; B – untreated. Images were captured 4 hr after application from recently opened flowers.

Previous work in our lab documented significant variability in individual fruit quality among fruit within a limb, and spur (data not shown). We recorded greater than two-fold variation in fruit weight and firmness among fruit on a spur and hypothesized that these differences were due to date of anthesis/pollination. In 2009 we flagged individual flowers on their day of anthesis (i.e., accessible to bee) and evaluated fruit quality individually at commercial harvest maturity. There is a clear negative relationship between day of anthesis and fruit quality potential (Fig. 4). This preliminary result suggests that effort should be made to pollinate the early-opening flowers to maximize fruit quality. It may be prudent to eliminate late-blooming flowers with chemical means, or remove pollinators from the orchard early (i.e., before full bloom).

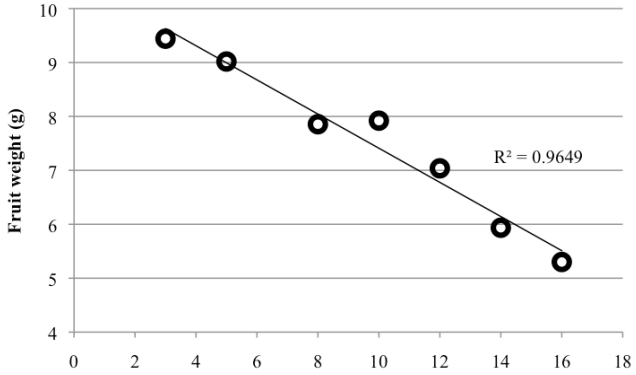


Figure 4. Relationship between mean final fruit weight at commercial harvest and relative day of anthesis in 'Bing'.

In 2009, we began assessments of stigmatic anatomy over time, in relation to receptivity, using scanning electron microscopy. Two lines of research were undertaken in 2009, both in Tasmania and in Washington. First, observations of stigmatic surface ultrastructure were made over time, on flowers marked for their day of anthesis. Flowers were harvested and assessed at 24 hour intervals post-anthesis. Second, we collected flowers within 4 hours of 2% ATS application to assess the effects of this thinner on stigma structure and receptivity. We observed maximum stigmatic exudate (and presumably receptivity – to be confirmed) just prior to anthesis/first opening, irrespective of cultivar. There were no apparent anatomical differences between cultivars with high fruit set and low fruit set (e.g., 'Sweetheart' vs. 'Regina'). Interestingly, flowers treated with ATS were structurally similar to those untreated but had significantly more pollen adhered to the stigmatic surface (Fig. 3). It appears that the airblast application has inadvertantly transferred pollen to the

stigma. This preliminary finding of pollen transfer during chemical bloom thinning suggests that thinning applications in self-fertile cultivars may not be as effective as in self-sterile. In reviewing previous chemical bloom thinning trials, we found that we typically under-thinned self-fertile cultivars. This hypothesis is one we propose to test further.

Field and growth chamber studies of ovule longevity and stigma receptivity supported our previous season's findings. 'Benton' and 'Bing' had the longest ovule viability compared to 'Regina' and 'Sweetheart'. Stigmatic receptivity, as evaluated the by perex test, was high in 'Regina' so it appears that rapid ovule senescence contributes to poor fruit set in 'Regina'. We propose to investigate use of PGRs to extend ovule longevity and increase fruit set.

SENSORY STUDIES

Consumer firmness evaluation of three cherry firmness groups

In 2009 our studies of sweet cherry fruit quality were focused on consumers' perceptions and preferences for fruit firmness. Using a Firmtech we evaluated firmness of > 1000 individual fruit. We used these data to develop cherry firmness groupings. This approach was followed for two cultivars, grown commercially, 'Chelan' and 'Bing'. For 'Chelan', low firmness corresponded to a firmness value of <225 g/mm (>20th percentile), intermediate to a value of 240-275 g/mm (40-60th percentile) and high firmness to a value of >290g/mm (<80th percentile). For 'Bing', low, intermediate and high corresponded to firmness values of <169 (20th percentile), 189-198 (40-60th percentile) and >219 g/mm (80th percentile), respectively. These values were similar to those reported in the previous year for 'Selah' cherries. Consumers were presented with cherry samples from two firmness groupings and asked whether there was a difference in firmness between them and which sample they preferred the firmness of (Figure 5).

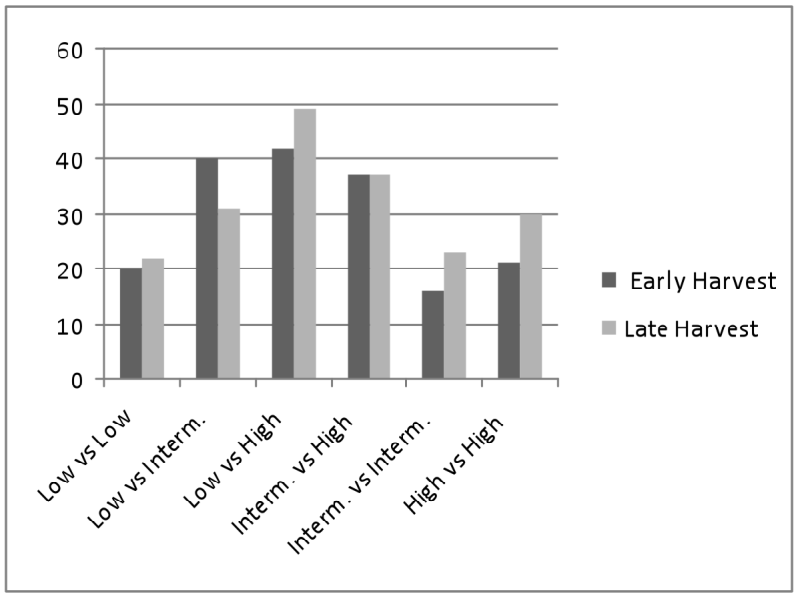


Figure 5. Consumer evaluation of 'Chelan' (early harvest) and 'Bing' (late harvest) cherries of different firmness levels (n= 65). Data are presented as frequency of selection for the analytically firmer sample using a directional paired comparison test. The test was a non-forced choice test, with the question "Which sample is firmer?"

Results showed no significant differences in firmness between cherries from the same firmness grouping. For both cultivars, no significant differences in firmness were found when intermediate firmness cherries were compared to high firmness cherries. Further, for both cultivars, significant differences in firmness were perceived when comparing the low and high cherry firmness groupings, however this did not exceed 50% at any time. When comparing low to intermediate firmness cherries, significant differences were observed for 'Chelan' but these groupings were not significantly distinguished in 'Bing'.

Consumer firmness evaluation of cherries of specific firmness levels

The influence of the acceptance of appearance, firmness, flavor and juiciness on overall acceptance was examined also (Table 3).

Table 3. ANOVA table of the influence of appearance, flavor, firmness and juiciness acceptance on the overall acceptability of early and late harvest cherries.

Attribute	df	F	p-value
Appearance	6	3.99	0.001
Flavor	6	211.72	<0.0001
Firmness	6	33.30	<0.0001
Juiciness	6	37.6	<0.0001

Results indicated that the acceptance of the four individual attributes (appearance, flavor, firmness and juiciness) significantly influenced overall acceptance of the cherries. Of these attributes, appearance had less of an impact on overall acceptance compared to the other attributes while flavor acceptance had a greater impact on overall acceptance. The influence of the analytical measurement of firmness and cultivar ('Chelan' vs. 'Bing') on cherry acceptance was evaluated also (Table 4). Cultivar had a significant impact on the acceptance of firmness and juiciness. Analytical firmness, as measured using the FirmTech, significantly impacted the acceptance of firmness, appearance, juiciness and overall acceptance of the cherries.

Table 4. ANOVA table showing the influence of cultivar and analytical firmness evaluation on the acceptance of firmness, appearance, flavor, juiciness and overall acceptance. The p-value indicates the strength of influence of that particular attribute with * indicating significance at p<0.001.

	Sensory Attribute				
Attribute	Firmness	Appearance	Flavor	Juiciness	Overall Acceptance
Cultivar	0.0018*	0.1229	0.4230	0.0066*	0.3180
Analytical Firmness	0.0000*	0.0063*	0.3301	0.0064*	0.0004*

In examining the specific differences between harvest times, firmness and juiciness acceptance were significantly higher in 'Chelan' cherries compared to 'Bing' (Table 4). Even though the acceptance of these texture attributes was significantly higher in 'Chelan' cherries, there was no significant difference in the overall acceptance of the cherries when compared to 'Bing'.

Table 4. Mean values of overall acceptance and sensory acceptance of firmness, appearance, flavor and juiciness. All attributes were evaluated along a 7-pt acceptance scale. Significant differences (p<0.0001) between harvest times are indicated by *.

	Firmness	Appearance	Flavor	Juiciness	Overall Acceptance
'Chelan'	5.86*	6.14	5.67	6.07*	5.78
'Bing'	4.64	5.82	5.53	5.69	5.46

EXECUTIVE SUMMARY

This research and outreach program has achieved, over three years, what it set out to do. Towards developing high efficiency orchard systems that are well-suited to mechanization and automation, we have worked with industry in the creation of a novel training system, the UFO. This training system, designed for creating upright or angled fruiting walls, has been planted and tested throughout the Northwest, and preliminary results are encouraging. We have partnered fully with growers in the development and evaluation of the system – many of the training principles originated from grower innovations. Summer and dormant tours of grower-collaborator orchards have successfully engaged the industry and been effective outreach models. The UFO system is now being planted around the world and included in national training system trials. Further, research funding orchard systems research and mechanical harvest was critical to a successful proposal to the USDA Specialty Crop Research Initiative. We leveraged WA/OR funding into a 4-year, $3.9 million project.

Our research into fruit set and pollination has taken a systematic approach to investigating the role of key factors affecting fruit set:

1. pollen viability
2. pollen growth rates
3. pollinator activity
4. stigma receptivity
5. ovule longevity

Our work has implicated maternal factors as causal to low fruit set. Items 1 & 2 do not appear to limit fruit set in sweet cherry. There is little reason that pollinator activity should limit fruit set as long as hives are available for hire (unless we experience an unusually cold spring and bee flight is negligible). In short, the work over the past few years has yielded new information on factors limiting yield and revealed several promising new avenues for research. Further, we have good preliminary data that can be used for better understanding effective pollination period in sweet cherry, and the role of temperature. Knowing that low fruit set is caused by maternal factors (i.e., stigma receptivity, ovule longevity) rather than by pollen related steps informs the development of potential ameliorative programs. However, it appears that low fruit set may be due to either poor stigma receptivity ('Benton') or rapid ovule senescence ('Regina'). Pollen race trials showed similar fruit set potential whether a cross is "fully" compatible (i.e., both S alleles are distinct from the maternal cultivar) or "partially" compatible (i.e., only one of the two S alleles is compatible). However, we recorded significantly lower fruit set in self-fertile cultivars when using self pollen vs. foreign pollen. We also showed that wind has a negative effect on fruit set. Field trials proved that even low velocity wind reduced fruit set more than high temperature. We've also evaluated many PGRs for their ability to affect fruit set (increase or decrease). Several promising programs are recommended for larger scale trials.

Crop load management trials studied potential to balance fruit number with whole-tree carbon supplies throughout the 15-month fruiting timeline. Trials with gibberellins showed that fruit bud initiation can be reduced in a rate-dependent manner when applied at early straw. This approach may have application for late-maturing, highly productive cultivars where harvest delay with higher rates of GA may also be beneficial. The most consistent blossom thinning program evaluated is ATS at 2%, applied at both 20% and 80% of full bloom. Our research has now begun revealing ATS mode of action to develop more effective protocols. Thinning efficacy is greatest on open, unpollinated flowers. This research has helped focus questions for further research. In this project we have also begun testing efficacy of various PGRs as post-bloom thinners. We've evaluated caustics and hormonal thinners and developed a method for more targeted screening of potential thinners. Further, we have begun to understand the optimum timing for thinning in sweet cherry by thinning whole trees at key fruit growth stages and evaluating fruit yield and quality relationships. This work has shown benefits to early thinning vs. late thinning and therefore, the importance of reducing competition among fruit for carbohydrate resources in early stages of development. This has prompted us to

rethink the relative importance of mesocarp cell number and size in determining final fruit size – we intend to study seasonal cell division and expansion cycles in a new proposal. Lastly, we have shown the ability to manipulate canopy source-sink relations and improve fruit quality with timely application of PGRs. We recommend larger trials of PCa (150 ppm) plus GA_3 (30 ppm) at ca 30 days after full bloom. Perhaps as important as the results from individual experiments, we have evolved the process of fruit set/crop load management investigation. The work reported herein has focused the questions for further, integrative study of yield and quality components in sweet cherry (e.g., bud/flower hierarchy, timing of flowering, mechanical pollination, thinner mode of action, flower populations, etc.).

Our investigations into consumers' preference for sweet cherry attributes and the potential for assigning cultivars to flavor groupings has helped redefine what fruit 'quality' is. This research has underscored the importance of overall flavor, and sweetness in particular, while revealing an inability of consumers (and trained panelists) to discern firmness differences during consumption. For example, only 40% of consumers polled detected a difference in firmness between samples that were < 225 g/mm and > 290 g/mm. Despite their difficulty reconciling different gradations of firmness measure by the Firmtech, consumers do rate fruit firmness and juiciness as important towards eating quality of the fruit.

FINAL PROJECT REPORT

Project Title: Sweet cherry regeneration and transformation system

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Other funding sources: None

Total Project Funding: $30,000

Budget History:

Item	Year 1: 2007	Year 2: 2008	Year 3: 2009
Salaries			
Benefits			
Wages	2250	1729	1729
Benefits		271	271
Equipment			
Supplies	7000	7000	7000
Travel	750	1000	1000
Miscellaneous			
Total	10,000	10,000	10,000

Objectives:

A. We had proposed to employ a three-pronged approach to establish an efficient regeneration system in sweet cherry. Parts of cherry plant to be tested in tissue culture included leaf, internodes and liners. Our specific objectives included:

1. **Identify the best media formulation for each variety being tested**. Test of different explants derived from two sweet cherry varieties in different media combinations. Initially we proposed to test Bing and Rainier cherry explants on media established for Fragaria by one of the PIs. Previously published media will also be tested for their effectiveness.

2. **Identify the best line for regeneration**. We will bring the breeding experiment to a Petri dish. Explants derived from selfed or cross progeny obtained from elite sweet cherry cultivars will be tested in selected media combinations. Our goal is to sample elite material with a wide range of genetic backgrounds as the ability to regenerate easily in culture is likely to require a unique complement of genes. Samples will be made from within the 1,712 existing seedlings available in the breeding program and from the seedlings expected to be germinated from the 17,848 seeds currently in stratification.

3. **Monitor the progression of regeneration using known markers of regeneration**. Expression of regeneration linked genes like *knotted*-1 and *Leaf cotyledon -1* (LCE-1) will be tested to assess and direct progression of regeneration. Sequence information for *knotted*-1 is already available from *Malus* on the GenBank (Accession no. Z71981). This sequence will be used to derive Rosaceae specific primers to be used in our experiments.

B. **Establishment of an efficient transformation system**. Availability of an efficient regeneration system will pave the way for devising Agrobacterium- and particle gun-mediated transformation system for whole tissue explants and PEG-mediated transformation for protoplast cultures.

Significant Findings:

Our final goal is to establish both regeneration and micropropagation capacity in sweet cherry. *Regeneration means developing multiple shoots from individual cells from any part of an existing variety. Micropropagation means propagation from liners or stems with multiple internodes in tissue culture conditions.*

It is an established fact that sweet cherry, peach, plum and other stone fruits are highly recalcitrant in tissue culture. Several protocols have been published on sweet cherry regeneration however; there is rarely a repeat report from the same laboratory. One method that uses seeds as a starting material is well established at Kearneysville Agriculture Research Station. However, the original variety is lost if seeds are used for this process. Thus, this method is not of extensive utility for our goals for the sweet cherry breeding program at WSU and the nursery industry in the PNW.

Recognizing this major resource gap in stone fruit research, three years ago we initiated our experiments in sweet cherry where our aim was to establish a leaf or axillary meristem or liner based regeneration and micropropagation. Some of the significant findings of the project are:

1. Sweet cherry buds and leaf tissues have a heavy pathogen load that is hard to get rid of with bleach treatment alone. We have established a method for decontaminating the explants for successful establishment in tissue culture.

2. The time of collecting the cherry buds determines the survival of the explant and its progression in tissue culture.
3. The effect of GA is unique on sweet cherry tissue culture. It supports development of somatic embryos.

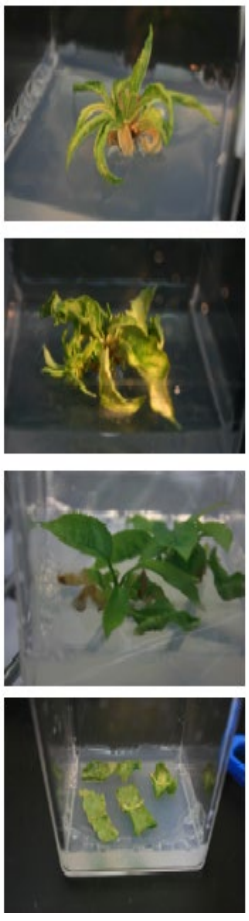

Figure 1: Panel A.
Top – Bing
Second – Lapin shoot regeneration
Third – Rainier
Fourth – Sweetheart forming callus

4. Glucose instead of sucrose is required for sweet cherry explants to respond.

Figure 1: Panel B.
Top – Gisela 5
Second – Gisela 6
Third – Gisela 12
Fourth – Krympsk

5. A particle gun can be used for introducing foreign genes in sweet cherry leaf tissues.

Results and Discussion

Objective A1, A2 and A3

Summary: Objectives A1 and A2 were aimed at identifying the best media formulations for each variety and we had proposed to identify the best line for regeneration from the breeding population. We tested 89 different media formulations defined by previous publications and our own formulations based on observations of sweet cherry biology in tissue culture. We have now identified few media types that support vegetative growth of 5 scions and 4 rootstocks in tissue culture. The procedures with these 9 genotypes have been extremely labor-intensive which precluded us from testing accessions from breeding populations. Looking back the objective A2 seems ambitious and would require an independent project.

Sweet cherry as a tissue culture system is extremely different from apple and pear. Objective A3 was aimed at monitoring regeneration using known genes involved in regeneration. We were unable to reach this stage in this project.

Details
Over the past three years following varieties have been successfully established in tissue culture:

5 Scions: Bing, Rainier, Lapins, Sweetheart, Kiona
4 Rootstocks: Krymsk6, Gisela5, Gisela6, Gisela12
Please see Figure 1 Panels A and B.

Explants:

Vegetative Buds: These were taken at various points during the year with the best results coming from September/October and March/April collections. The spring collections responded much quicker to the media as they were not dormant but the fall collection has not shown significant detrimental effects.

Leaves: mostly younger non-fully expanded leaves

Explant Sterilization

The first bottleneck in establishing a tissue culture/micropropagation system is the preparation of explants to prevent fungal and bacterial contamination. A standard 10 percent bleach solution was used initially and was shown ineffective. The explants were contaminated over various exposure times to the bleach through the point where all of the explants were killed from the bleach while the contamination persisted. Anti-fungal and anti-bacterial additives were added to the media though they had minimal beneficial aspects and as were later determined to also effect explants growth. After further experiments, a combination protocol of ethanol, bleach and mercuric chloride with bud scale removal proved to be effective for decontaminating bud tissues. Although a prolonged procedure, this protocol has provided clean explants that have been maintained in culture for over 1 year and has been used multiple times with tissues from multiple sites. Ethanol followed by 0.1% mercuric chloride treatment for 10-15 minutes are sufficient for cleaning leaf explants of sweet cherry rootstocks and scions while the combination is toxic to pear leaves.

Organogenesis/Embryogenesis

Leaf tissues have been shown in other systems to give rise to adventitious shoots and callus. Our experiments have shown that sweet cherry leaves respond to tissue culture more effectively when the leaves are placed adaxial (top) side down onto the media.

Callus formation

Cutting the leaves into sections approximately 1cm across when measuring along the midrib has provided sections that produce callus at the cut sites along the vasculature. Two weeks of initiation of callus in low light produced multiple callus per leaf while high light and no light produced very few. The media we are using for callus formation was initially discovered after a Bing leaf produced callus and has been repeated with success in Sweetheart.

Callus Maintenance/Proliferation

We have identified a combination of Kinetin, IBA, and GA3 that is promoting growth of callus tissues from the Bing callus. We are experimenting to optimize the growth rate of the callus. The callus from Sweetheart will be moved into these media in after a couple more weeks of initiation.

Shoot Initiation

After callusing, the undifferentiated cells must be given the proper signal to grow into a shoot. Experiments identifying a media for this step are underway since sufficient callus has been generated for experimentation. From the information we gained from sweet cherry to this point we have identified a few combinations of hormones that we expect to lead us toward completion of this aspect of a callus regeneration system.

Shoot Elongation

Post cleaning of bud tissues and shoot initiation of leaf tissues, elongation of the shoot is next important step. Several media have been tested to obtain elongation with modifications to: carbon source, osmolality, photoperiod, incubation temperature, auxins, cytokinins, and gibberellic acid levels. Recently, a modification to media has resulted small amounts of elongation though the effect

needs to be further isolated and optimized and could possibly be variety specific. The stem elongation was not a lasting effect, however, which expected to be the result of slow loss of GA3 to levels ideal for growth and continued loss until the effect was removed again. This has lead to a trial of multiple levels of GA3 at very low concentrations to reproduce this phenotypic change.

Micropropagation

Once elongated shoots are grown in axenic conditions, we expect that they can be propagated using a method similar to that used with pear and apple where the shoots are laid horizontally to stimulate axial bud growth by reduction apical dominance. The axial buds will produce shoots that can be separated, elongated and used to produce more shoots cyclically.

Rooting/Acclimatization

This is a step that we have yet to explore; however, grafting of the materials we produce in tissue culture could provide an intermediate step between tissue culture and common propagation techniques where losses during rooting can be circumvented. We plan to explore this possibility with NNII to help them in establishing a method in obtaining virus free cherry stocks and scions.

Following media combinations were tested during the course of this project. These media formulations were derived from published work as well base on our observations. A total of 89 media types were tested.

1. Basal nutrition media after Murashige and Skoog (27 variations)

We used various intensities and combinations of the following hormones.

Ascorbic Acid	None, 100-200mg/L
BAP	1-3 mg/L
TDZ	None
GA3	None
GA4+7	None, .5-1, 2, 3 mg/L
IBA	None, .1mg/L
PPM	1-2 ml/L

2. Woody Propagation Media (7 variations)

We used various intensities and combinations of the following hormones.

Charcoal	500 mg/L
Ascorbic Acid	None
BAP	None, 1, 7 mg/L
TDZ	.25, 1 mg/L
GA3	None, 1 mg/L
GA4+7	1 mg/L
IBA	.1mg/L
PPM	2 ml/L

3. Variation of basal nutrition media– **Sucrose** (21 variations)

We used various intensities and combinations of the following hormones.

TC Agar	5.6, 6 g
Ascorbic Acid	None

BAP	None, .25-1mg/L
TDZ	None
GA3	None, 1,10 mg/L
GA4+7	None, .5-1 mg/L
IBA	None, .1mg/L
PPM	None
Kinetin	2, 4, 8 mg/L
DDT (1 Sample)	150 mg/L

4. Basic nutrition media – **Combination of Glucose, Sorbitol, Fructose** (20 variations)

We used various intensities and combinations of the following hormones.

TC Agar	6 g
Ascorbic Acid	None
BAP	None
TDZ	None, 1 mg/L
GA3	None, 125, 500 microgram/L
GA4+7	None
IBA	5 microgram/L, .1mg/L, 4 mg/L
PPM	None
Kinetin	4, 8, 12, 16 mg/L
DDT	None

5. Basic nutrition media with different nitrogen source – **Glucose** (14 variations)

We used various intensities and combinations of the following hormones.

TC Agar	6, 7 g
Ascorbic Acid	None
BAP	None, 1mg/L
TDZ	None, 125 micro, .2, .5mg/L
GA3	None, .1, .2, .4 mg/L
GA4+7	None, .1, 1, 2, 4, 8mg/L
IBA	None, .1-.4 mg/L
PPM	None
Kinetin	None, 2, 4, 8 mg/L
DDT	None

Objective B

In order to establish a sweet cherry transformation system we needed to test the method for DNA delivery into leaf or other explants. We preferred to use the particle gun method. The rationale behind using this method is that we don't have to worry about the specificity of the Agrobacterium strain for sweet cherry.

Leaf explants were used for standardizing DNA delivery into sweet cherry leaf material. Different parameters like distance between target and microcarrier launch assembly and pressure were used to test the DNA delivery system. A schematic of the particle gun is shown in Figure 2A. A cell expressing the red fluorescent protein in sweet cherry leaf cell is shown in Figure 2B.

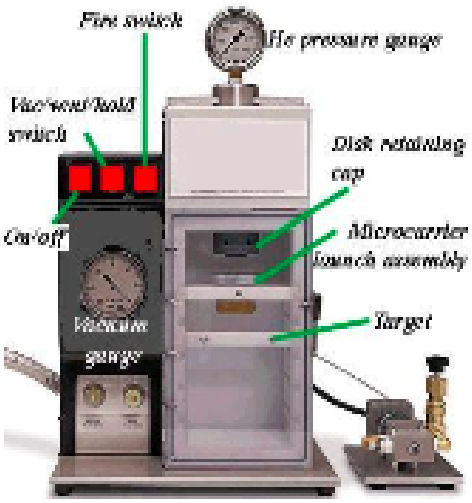

Figure: 2A. Particle Gun used for standardizing DNA delivery in to sweet cherry explants.

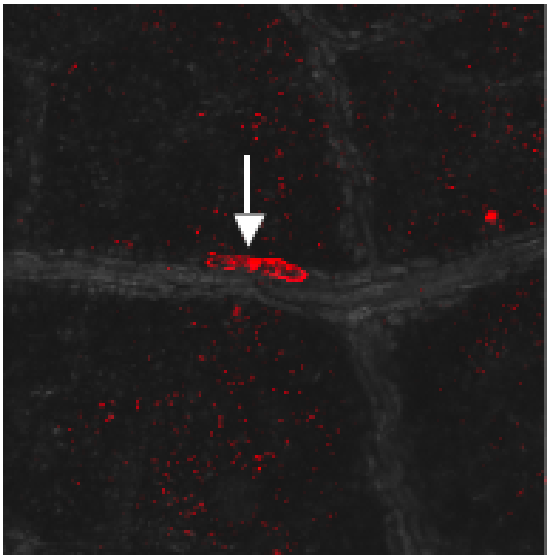

Figure: 2B. Arrow indicates a fluorescent sweet cherry cell expressing red protein

Thus we have a good system for DNA delivery that we can now utilize for creating targeted mutations or controlled sports induction for improving certain aspects of existing varieties. Our efforts continue to fine-tune the regeneration system. A combination of good regeneration system and DNA delivery approaches can greatly benefit the sweet cherry research.

Executive Summary

The time involved in propagation of tree species is a major factor controlling the amount of time between variety release and widespread adoption of that variety. In other tree fruits, apple and pear, we have used micropropagation to successfully increase the propagation rate, thereby decreasing the amount of time invested to provide growers with adequate amounts of material. Sweet cherry is a member of the Prunus family where attempts at tissue culture and micropropagation have resulted in minimal success.

Sweet cherry has a narrow genetic diversity therefore extensive crosses will not yield large diversity in traits. The methods developed in this project are expected to enable us in creating random and directed sports generation thereby increasing the diversity. Mutations are a safe way of improving existing variety or creating novel varieties. These are not considered GMOs.

After extensive experimentation we have developed media for 9 genotypes – 5 scions and 4 rootstocks. We have also established preliminary methods for introducing DNA into sweet cherry using particle gun.

One overarching observations is that sweet cherry is unique compared to apple and pear. In tissue culture there are not many successful reports available for either regeneration or micropropagation. Here we have created a large repository of media formulations for enabling these techniques, which undoubtedly require further work.

Broader impact of our methods developed here will be its integration with Clean Plant Network activities coordinated by Bill Howell. Our methods can be used to clean scions and stocks of viruses. This project was carried out as part of Ph.D. work of Tyson Koepke who has been supported by an NIH protein Biotech training program, ARCS fellowship and now USDA-SCRI support. The project provided an opportunity for the training of three undergraduate students – Cory Druffel, Ashley Koepke and Matt Allan. These students have been successful in obtaining undergraduate research fellowships from CAHNRS and Matt has recently been awarded the Auvil Fellowship. The work done with support of this project has been presented at several national and international forums in the form of poster presentations.

FINAL PROJECT REPORT

Project Title: Establishing the marker-assisted breeding pipeline for sweet cherry

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Other funding sources

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

Total Project Funding: $45,000

Budget History:

Item	Year 1: 2009	Year 2:	Year 3:
Salaries	$ 4,000		
Benefits	$ 1,805		
Wages	$16,675		
Benefits	$ 1,325		
Equipment			
Supplies	$11,195		
Travel	$ 5,000		
Miscellaneous[a]	$ 5,000		
Total	$45,000		

[a] Miscellaneous – development, hosting, and publicizing of a participatory field day to demonstrate MAB methodology and workshops to be held in conjunction with the 2009 WTFRC cherry research review and the 2010 Cherry Institute.

ORIGINAL OBJECTIVES

1) Establish individual components of the MAB Pipeline not yet in place for the PNW sweet cherry breeding program, particularly the final stages of Cost Efficiency and Trial Use, to ensure that planned MAB efforts confer costs and/or time savings to breeding, and to put theory into practice.
2) Formalize and continue the process of Prioritization, Marker Improvement, Validation, and Utility assessment of new marker-trait associations for cherry as they are discovered and reported.
3) Demonstrate the MAB Pipeline to the PNW sweet cherry producer community through outreach activities, using high impact markers for self-fertility and fruit size.

SIGNIFICANT FINDINGS

- Modern genetic screening capability integrated with traditional routine breeding operations is now enabled for the PNW sweet cherry breeding program (PNWSCBP), with the establishment of the **Marker-Assisted Breeding (MAB) Pipeline** for this program. From 2010, DNA information can routinely augment crossing decisions to result in a greater proportion of superior seedlings, can routinely support seedling selection decisions as cost-efficient early selection tools to cull inferior seedlings, and can be routinely used in genetic potential descriptions of new cultivars to facilitate industry planting decisions. The infrastructure is now established to efficiently pipeline genetics and genomics advances into routine breeding operations.

- The MAB Pipeline was refined in the last year during preparations for the multi-million dollar federally funded **RosBREED** project, including the previous seven stages being increased to eight. This MAB Pipeline (Figure 1) is to be adopted by numerous U.S. Rosaceae breeding programs, allowing collaborative development of powerful infrastructure and implementation for tremendous benefit to the PNWSCBP and the PNW sweet cherry industry.

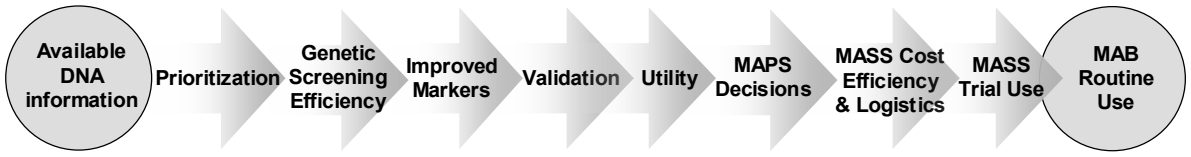

Figure 1. The MAB Pipeline

- The first six stages of the MAB Pipeline were formalized for the PNWSCBP in 2009 (addressing **Objective 2**), although even greater formalization will be undertaken in the next four years within RosBREED due to the establishment of powerful infrastructure for each stage. New opportunities for applying DNA information to augment the breeding program were progressed through the Pipeline, and future new genetics and genomics discoveries can be readily channeled in through this Pipeline.

- The final two stages of the MAB Pipeline were successfully implemented for the PNWSCBP (addressing **Objective 1**), using genetic tests for fruit size and *S*-alleles including self-fertility. Cost-efficient and logistically feasible high-throughput genetic screening schemes were identified and successfully trialed, completing the connection between genomics research and routine breeding operations.

RESULTS & DISCUSSION

The MAB Pipeline (Figure 1) represents a series of practical stages to convert genomics research into breeding application. This is not the only approach that could be taken, but such a focus on individual stages addresses important considerations that otherwise could impede the efficient use of modern genetics and genomics knowledge and tools to enhance breeding. Indeed, the failure to address considerations such as trait priorities, availability of high-throughput genetic screening services, cost-efficiency, and on-the-ground logistics, has restricted tree fruit marker-assisted breeding to very few examples around the world. The value of the MAB Pipeline approach was recognized by the U.S. Rosaceae genomics, genetics, and breeding community, with stakeholder and international support, in the coordinated development of the first "RosBREED" proposal to the Specialty Crops Research Initiative in August 2008 – led by Dr. Iezzoni. Soon after, the same approach was proposed for this WTFRC-funded cherry project, and in parallel efforts for the Washington apple breeding program. While unsuccessful in the first round, the RosBREED proposal, with the MAB Pipeline approach retained, refined, and reinvigorated, was resubmitted in April 2009 and proved successful in obtaining and directing more than $14 million to targeted application of genomics and socio-economics knowledge for accelerated and streamlined fruit breeding. RosBREED will run for four years from September 2009. In the meantime, with WTFRC funding support in the present project, we have forged ahead with establishing the Pipeline for the PNWSCBP.

The MAB Pipeline consists of eight stages:
(1) Prioritization of reported marker-locus-trait associations is essential to sift through the volumes of available genomics information. Not all genomics discoveries are created equal, and their impact on crop improvement varies by value of a trait to breeding, industry, and consumers, and the strength of association and effect on performance of the tagged controlling genes. Marker-locus-trait associations are specific genetic markers with a known position (locus) in the genome that tag a specific trait of interest.
(2) Genetic Screening Efficiency is identified by locating and testing efficient and logistically feasible methodologies for high-throughput genetic screening (sampling, DNA extraction, genotyping, and timely provision of data to breeder) that suit the idiosyncratic routine operations of breeding programs.
(3) Improved Markers are developed to ensure robustness and amenability to use in the high-throughput pipeline needed for genetic testing of thousands of seedlings.
(4) Validation of robust marker-locus-trait associations is performed in the wider germplasm pool of a crop, beyond the experimental material in which they are usuaully first discovered.
(5) Utility assessment of validated markers is conducted to determine their potential application specifically within a breeding program, detecting the maintenance of marker-locus-trait associations in breeding program germplasm and describing functional marker variants (favorable or not) in each potential breeding parent.
(6) MAPS (marker-assisted parent selection) Decisions are enabled, where the information gained from the previous stage is used to guide crossing decisions by a better understanding of breeding value.
(7) MASS (marker-assisted seedling selection) Cost Efficiency and Logistics calculations and considerations are made to identify optimal seedling selection schemes that integrate available robust, validated, utile genetic tests for some traits into routine breeding operations with phenotypic selection for other traits.
(8) MASS Trial Use is conducted in a high-throughput manner on a subset of breeding program seedlings to transform the pipeline into reality, comparing theory with practice to optimize MASS implementation.

Progress in establishing and implementing each of these stages in 2009 is described below.

1. Prioritization

To facilitate the prioritization of marker-locus-trait associations for application in breeding, and to help direct future marker development research, traits of interest to the PNWSCBP have been placed into the groups of Market-defining, Primary, Secondary, Preferred, and Lineage-specific (Table 1). Traits of highest priority (Primary traits) for MAB in this breeding program are currently fruit size and firmness. Therefore, we need to direct greatest effort toward developing and pipelining marker-locus-trait association for these two traits.

Table 1. Assignment of marker-locus-trait associations for application in the PNWSCBP according to trait groups. Within each group, traits are treated equally and simultaneously, and available DNA information should be combined for decision-making.

Trait groups	Traits in group	DNA information available?[a]	MAB approach
Market-defining	Harvest date Self-fertility Fruit color PM resistance	(Yes) Yes (Yes) No	MAPS[b]. Used on parents to predict target market class(es) of resulting seedlings.
Primary	Fruit size Firmness	Yes ([Yes])	MAPS & MASS[c]. Seedlings must perform above threshold for each.
Secondary	Sweetness Acidity Taste Low astringency Low bitterness	(Yes) (Yes) (as above) (Yes) No	MAPS & MASS. Seedlings sought above threshold for each, but weighed together – lower values tolerated.
Preferred	Fruit cracking resistant Fruit doubling resistant Bacterial canker resistant Self-fertile PM resistant Precocious Freestone Mechanical harvestability	No No No Yes No (Yes) ([Yes]) [Yes]	MAPS. Aim for increasing proportion of seedlings to have any of these. Parents and seedlings with rank higher than those without.
Lineage-specific	e.g. Super-sweet	No	MAI[d]. Use for parent and seedling selection only in certain lineages.

[a] Marker-locus-trait associations in published reports (unpublished research of PIs) [promising leads from related crops]

[b] MAPS = marker-assisted parent selection (using DNA information of parents to aid crossing decisions)

[c] MASS = marker-assisted seedling selection (using high-throughput genotyping of seedlings to cull those inferior)

[d] MAI = marker-assisted introgression (introducing new traits from unusual sources, usually requiring several generations to combine into elite backgrounds)

RosBREED will apply greater objectivity to the Prioritization process by establishing a method of quantifying the economic value of each trait (with surveys of trait and market segment values and preferences of producers/processors, marketing groups, trade organizations, and consumers), and weighing economic values by the degree to which a breeder can genetically improve the trait.

2. Genetic Screening Efficiency

The four components of high-throughput genetic screening have been developed to a working system, although further refinements will continue to be applied. A successful high-throughput DNA extraction protocol was developed, which is the Silica Bead Method (SBM) as used for the Washington apple breeding program but with minor modifications (i.e., the addition of PVPP to the initial extraction buffer to reduce interfering polysaccharides in cherry leaves, and tripling the amount of template DNA in PCR reactions due to lower extracted yields). SBM involves a simple greenhouse/field tissue sampling method without the laborious step of freeze-drying, and unexpectedly but fortuitously the method is effective for older leaves (unavoidable from mid summer to fall) as well as for DNA-rich young leaves that are usually only available in spring and early summer. To date, this extraction method has been used to extract >1000 samples with >95% success.

The Pacific Northwest Tree Fruit Genotyping Laboratory (PNWTFGL) was established in Pullman in 2009 with the purchase of an ABI 3730xl DNA Analyzer with $100K funding support from the WTFRC and Washington Wheat Commission (WWC), additional equipment provided by the WSU Agricultural Research Center (ARC) support totaling another $100K, and the recent addition of a $90K Laboratory Automated Workstation (a DNA handling "robot") funded by the WWC, ARC, and Dr. Deven See (USDA-ARS Pullman). The PNWSCGL was established to service the PNWSCBP and the Washington apple breeding program as well as supporting research, and is run by Dr. Peace in close collaboration with Dr. Deven See who manages the Western Regional Small Grains Genotyping Laboratory. Despite the availability of such equipment and appropriate technical expertise, successful routine genotyping of sweet cherry on the ABI3730xl eludes us for now (whereas apple works just fine). We continue to troubleshoot, and expect success by the end of 2009. In the meantime, we have used the fallback of large polyacrylamide gels, which are effectively medium-throughput (130-370 data points per day) utilizing the technical expertise currently in the lab. This genotyping system is being used for *S*-genotyping and fruit size genotyping of hundreds to thousands of seedlings in 2009 (described below in Trial Use).

While we continue to develop a streamlined data handling system for the many thousands of datapoints to be collected and provided to the breeder in subsequent seasons, we have already had success in providing data in a suitable and simple format: a color print-out of *S*-genotypes of '04 seedlings allowed the breeder and consultants to cross-reference field performance with parentage while walking the breeding rows during the 2009 fruiting season. By the end of 2009 we expect to have a system of genotypic data provision that can be readily used by breeding personnel to cross-reference marker genotypes with close-packed seedlings in the greenhouse or lath house for ease of culling inferior plants. RosBREED will expand on such efforts for the PNWSCBP.

3. Improved Markers

An efficient genetic test was developed for *S*-genotyping that includes identification of self-fertility in addition to common *S*-alleles. This test is now routinely performed in the lab. The "universal" *S-RNase* gene primers (Tao et al. 1999) are multiplexed with our new S_4'-specific marker, "Pav-S4-indel" (forward primer: TGCGAAAAATTGACTTCTGG; reverse primer: TCAAGAACTTGCTTGGATTCG). Standard PCR conditions are used, and alleles are resolved on large polyacrylamide gels. Pav-S4-indel generates a 194 bp fragment for the *S4* allele and 190 bp for the S_4' allele imparting self-fertility.

For fruit size, we are using MSU-developed markers that flank two QTLs for fruit size components (cell number on G2 and pit size on G6) discovered in the 2005-2008 NRI project of Dr. Iezzoni. However, we changed one of the G6 markers for a new one, "Pp-ACS3-SSR". This is a marker for a texture candidate gene that just happens to be located at the pit size QTL, and we are taking advantage of the greater allelic diversity offered by Pp-ACS3-SSR which may help identify new functional pit size alleles.

For other traits, we are creating new markers for reported fruit gene markers, facilitating greater refinement of functional effects and development of predictive markers for use with PNWSCBP germplasm. Examples include the *MYB1* gene associated with cherry fruit color for which we developed a new microsatellite-based marker, the *Pp-ACS3* gene that is the equivalent gene to *Md-ACS1* influencing softening in storage of apple, and the *Pp-PG1* gene (which we call "*PG4*") that is equivalent to the *Md-PG1* gene associated with softening during room temperature ripening of apple and putatively involved in creating air pockets around the stone of peach.

4. Validation

The Parent Set and Diversity Set, which covers most of the currently used and near-future diversity of the PNWSCBP, acts as our Validation material. The Parent Set was slightly refined to include some additional ancestors of important PNW cultivars. In 2009, fruit quality data were collected for many of these parents and ancestors to help establish baseline performance predictions of progeny. A greater level of validation will be achieved in RosBREED, where 480 pedigree-linked representing the U.S. cherry breeding germplasm (the cherry Crop Reference Set) will be comprehensively genotyped and phenotyped.

Markers for *S*-alleles including self-fertility were already validated by the scientific community and in use worldwide. We used our improved multiplex genetic test to screen the Parent Set and thereby confirm *S*-alleles for many cultivars and ancestors, and obtained *S*-genotypes for some cultivars that were previously unknown.

Markers for fruit size were screened on the Parent Set. Fruit size alleles were traced through the generations of the Parent Set, and marker combinations were identified that appear to predict larger and smaller fruit based on the genotypes and fruit size of these parents and ancestors. For example, 'Glacier', 'Tieton', and 'Kiona' have an allele for BPPCT034 observed only in these large-fruited cultivars and their parent/grandparent 'Burlat' (allele 237).

Chloroplast genotyping was used to group cultivars into three lineages. Lineage "B" is the most prevalent in PNWSCBP germplasm carried by 'Van', 'Bing', and daughter cultivars. Lineage "C" is also common, introduced through 'Stella' and 'PMR-1'. Lineage "A" is rare, with no representation in locally grown modern cultivars. A diagram depicting these lineages was provided at the 2009 Cherry Field Day in June so that industry members could see the pedigree relationships of many cultivars and appreciate the power of DNA information such as the chloroplast markers to define genetic groups. However, unlike fruit size markers, chloroplast markers are not known to be trait-associated – instead helping define ancestral genetic relationships among cultivars through the maternal line and ensuring the Parent and Diversity Sets (-> Crop Reference Set) are comprehensive.

5. Utility

Utility assessment requires a pedigree-linked set of germplasm representing the breeding program with enough individuals to achieve sufficient statistical power. While such germplasm does not need to be physically separate from the breeding program, separation allows the trees to survive for longer for extra seasons of performance data to be collected. A separate germplasm planting does not (yet) exist for the PNWSCBP, but the '04 crosses – 245 seedlings from 22 crosses made in 2004, planted in 2006, and with 70% fruiting in 2009 – is a suitable set for current purposes.

The '04 seedlings, and 50 of '05 crosses, were phenotyped in the 2009 season for a range of traits within the routine operations of the breeding program and with funding support from this project. In addition to its value for evaluating performance of seedlings for breeding selection decisions, the comprehensive dataset is very valuable for determining utility of markers that have progressed this far through the MAB Pipeline.

S-genotyping was used to determine parentage of '04 seedlings and to identify self-fertile seedlings. Many unintended outcrosses, selfs, and incorrect assignments were revealed. Some of the seedlings with correct *S*-alleles for their intended cross may still have arisen from outcrossing, which additional marker genotyping can be used to identify. In fact, additional DNA information (from four fruit size markers) refined seedling parentage verification (Table 2). 104 self-fertile seedlings, carrying the S_4' allele, were observed. Results were discussed at the 2009 Cherry Field Day in June (Prosser, WA), presented at the ISHS Symposium on Molecular Markers in Horticulture (Corvallis, OR), and written in a submitted paper for the journal Acta Horticulturae (Haldar et al. 2009). *S*-genotyping provides an excellent example of many MAB applications:

- Parent and cross choice (e.g. to avoid incompatible crosses)
- Evaluating crossing success (Table 2)
- Marker-assisted seedling selection (MASS; to select for self-fertile seedlings)
- Characterizing advanced selections and new cultivars (*S*-alleles to assign to compatibility groups and thereby speed adoption of new cultivars)

Table 2. Crossing success for seedlings of '04 crosses of the PNWSCBP, as initially determined by *S*-genotyping and then refined by four additional markers.

Parentage	According to *S*-genotypes	Proportion by *S*-alleles	According to 4 more markers	Proportion by all markers
Intended	143	59%	166	68%
Self	55	23%	24	10%
Outcross	28	12%	28	11%
Does not belong	17	7%	27	11%
Total	243	100%	245	100%

For fruit size genotyping, the four markers for the G2 and G6 QTLs were tested on all '04 seedlings (and '05 seedling genotyping is underway – currently ¼ complete). Statistical analyses are still underway, and '04 data will be added to the MSU dataset to even better define allelic effects across cherry and identify specific utility in the PNWSCBP, in time to inform spring 2010crossing decisions.

In the meantime, interesting and confirmatory results are being achieved, with an example shown in Figure 2. Conclusion: The genetic markers for fruit size developed at MSU in recent years with federal and WTFRC funding support will indeed be valuable for increasing breeding efficiency for large fruit in the PNWSCBP.

The recommended next step is expanding analyses from the 245 '04 seedlings to the '05 and '06 seedlings fruiting in the next couple of years. These 5000-6000 seedlings would represent the "training population" for verifying and characterizing utility of fruit size markers. We can then confidently answer questions of how to most efficiently improve fruit size, namely: how can the breeding program produce and plant a greater proportion of large-fruited seedlings, and what is the effect of genotypic selection for fruit size on other traits of importance (especially firmness and flavor)? Incorporating self-fertility into MASS considerations, we wonder: what is the effect of selecting for self-fertility on achieving enough seedlings with large fruit? According to '04 seedling results, we predict that early culling of self-incompatible seedlings would not much reduce the ability to obtain large-fruited seedlings from which to select additional traits of interest (Figure 3).

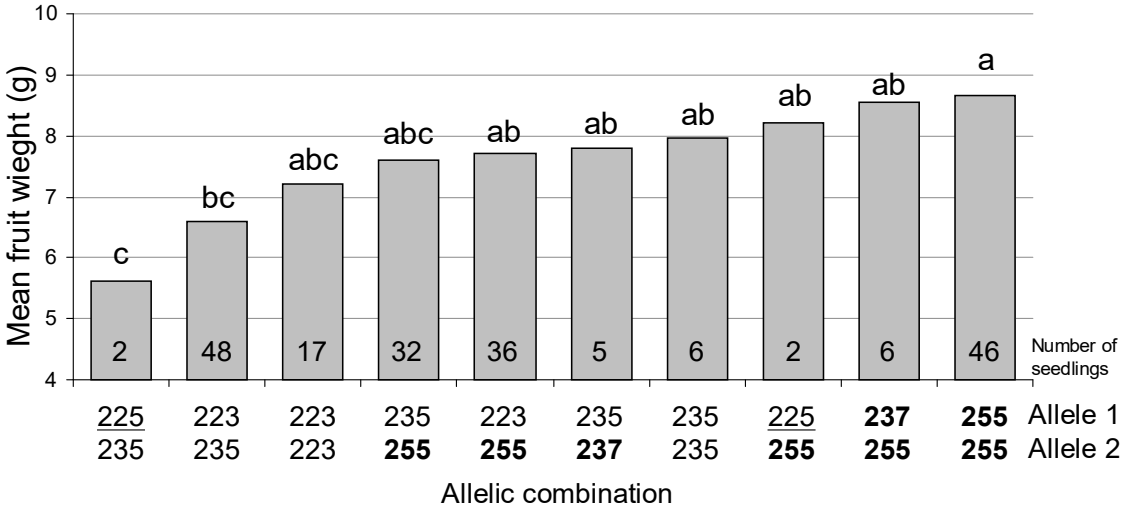

Figure 2. Fruit size in the 2009 season of '04 seedlings of the PNWSCBP according to allelic combinations of BPPCT034, a G2 fruit size QTL marker for cell number. Alleles in **bold** (**255** and **237**) were those predicted from their presence in large-fruited Parent Set cultivars to be associated with large fruit in seedlings. The allele underlined was predicted to be associated with small fruit. The **255** allele was also the one associated with large fruit, and 225 with small fruit, in the NY x EF experimental population (Zhang et al. 2009).

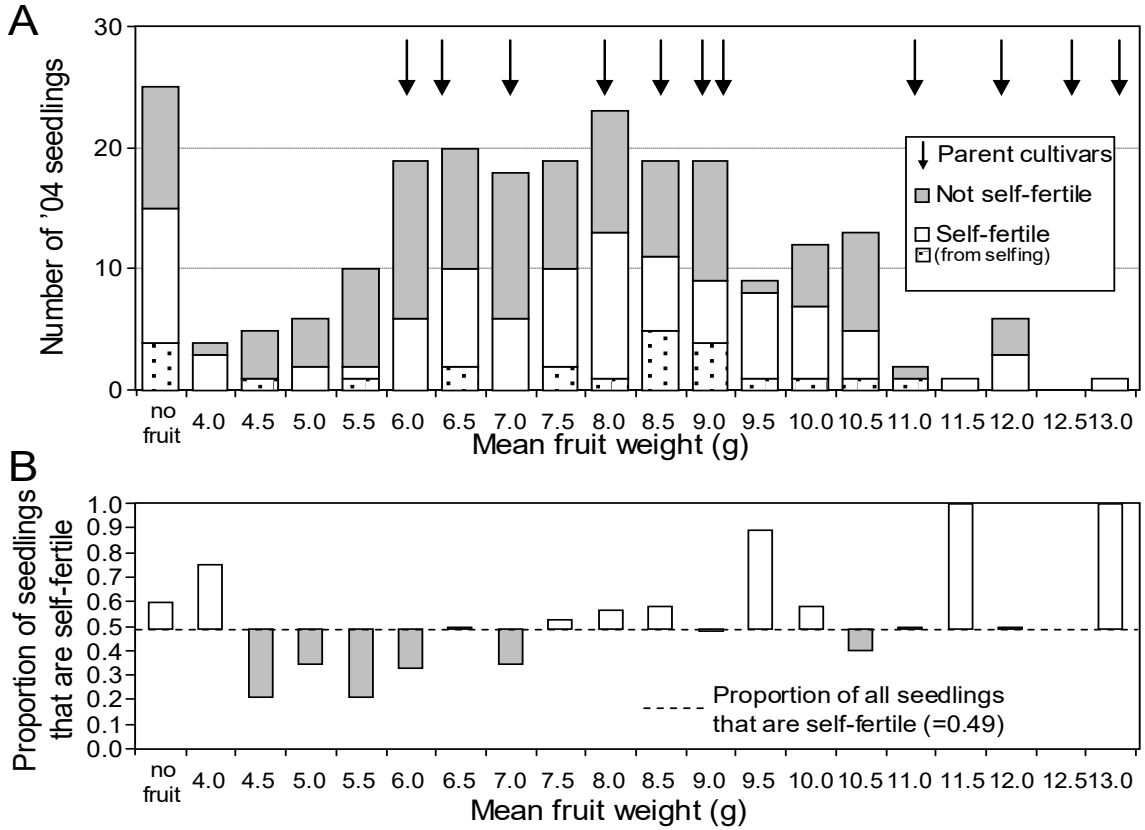

Figure 3. Fruit weight distributions observed in the 2009 season for '04 seedlings of the PNWSCBP. (A) Mean fruit weight distributions for self-fertile (including those derived from selfing) and self-incompatible seedlings. (B) Fruit weight distribution of proportions of seedlings that are self-fertile.

6. MAPS Decisions

Using DNA information to aid crossing between pairs of sweet cherry parents that result in a greater proportion of superior seedlings is a very efficient MAB application, even more so than seedling selection. Therefore, particular attention is being paid to obtaining such information and making it available in a format and timely manner to maximize use in crossing decisions. Marker information will be particularly informative for new parents to be used in the breeding program where such information has not previously been available – such as some of the best-performing '04 seedlings.

S-genotypes are being used to refine crossing decisions, by avoiding incompatible crosses and by preferring crosses that result in a high proportion of self-fertile seedlings. Selfing as a crossing strategy, which enables efficient doubling up of desirable alleles in seedlings but has been mostly avoided to date, is being reassessed with the observation from 2009 *S*-genotype data that field-planted selfs may perform as well as any other tree (i.e., without exhibiting inbreeding depression in the field). However, in future work, assessment is required of whether selfing results in lower seed set, reduced germination, and/or fewer vigorous seedlings.

Fruit size DNA information will be used from spring 2010 to support the development of families predicted to result in large-fruited combinations (e.g., families with a high proportion of seedlings with two copies of the 255 allele for BPPCT, according to the results shown in Figure 2). If we are able, fruit color markers will be used to predict proportions of fruit color types (mahogany vs. blush, and others) in each cross produced (see second last paragraph of New Marker Identification below).

7. MASS Cost-Efficiency and Logistics

We have used the MASS decision support tool to identify efficient MASS schemes for the PNWSCBP. The tool predicts that the best stage for genotyping will usually be while seedlings are in the lath house in early spring before being field-planted, although MASS instead at the preceding greenhouse stage should usually confer at least 90% of those savings. Culling after trees are already planted also appears worthwhile, – e.g., it typically confers ~75% savings of the best stage if done during the first year in the field, down to 20% or less by the fifth year. As routine field maintenance and fruit evaluation costs are greater than for the apple breeding program, potential savings with MASS are even greater when selecting for traits not expressed until trees reach reproductive maturity.

8. MASS Trial Use

High-throughput genetic screening is underway with '04, '05, '07, and '08 seedlings, to gain practical experience in MASS in the PNWSCBP. We will continue the Trial Use through the winter of 2009, especially for the >1000 seedlings of '05 crosses. We've found so far that:

- Genetic screening at the greenhouse stage will be the most logistically feasible, especially with the use of seedling pots arranged in 8 x 12 arrays to streamline information transfer from genetic screening to culling activities.
- DNA should be discarded following seedling phenotyping, as decisions will be immediately made on information obtained. Any further marker data desired on remained seedlings after planting (e.g., with new useful markers coming pipelined in future years) will be for descriptive purposes only and too late for culling, and thus can be gained by re-extracting and genotyping the small number of target seedlings. This approach eliminates the complication of ensuring individual labels on seedlings are maintained from greenhouse to field, that DNA is kept for an indefinite period, and that DNA samples are sorted to remove culled seedlings.
- Our high-throughput DNA extraction system is effective for older as well as young leaves.
- While the high-throughput genotyping system is still being optimized for cherry, the med-throughput system (large polyacrylamide gels) is a less-efficient, but successful fall-back with the current numbers of seedlings in the breeding program. Greater than a few thousand seedlings to be genotyped in any year will certainly require the high-throughput ABI platform.

- Routine implementation of MAB in the PNWSCBP can be implemented immediately in collaboration with the PNWTFGL in Pullman. While further refinements of logistics will be useful to streamline the process, and new markers are expected to enter the system over time, we can and are using MAB now in this breeding program, and have the opportunity to become one of the first tree fruit breeding programs in the world to routinely conduct high-throughput MASS.

Future routine MAB in the PNWSCBP

Partial support of a dedicated Genetic Screening Technician is recommended to conduct the genetic screening component of future MAB in the PNWSCBP (not to be confused with research assistance). Funds for this position from within operating costs of the PNWSCBP and the WABP, or separately funded to allow breeding programs to continue field operations and field evaluations at current capacity, will ensure the availability of the labor component of the genetic screening service in Pullman. Dr. Peace's research and development program, supported by federal and WTFRC grants and WSU infrastructure, is aimed at establishing the MAB Pipeline and developing new markers for the program. However, this program will not fund a routine genetic screening service through such research grants. Supporting research does not fall under the breeding program's operating budget, but rather under separate research projects such as those led by Dr. Peace (WTFRC-funded) or Dr. Iezzoni (federally funded).

The expectation is that the Cost-Efficiency calculator will identify MAB schemes that provide a *savings* to the breeding program even after the cost of genetic screening is taken into account. Therefore, routine cost-efficient MAB (compared to breeding by traditional phenotypic selection alone) will not only allow genetic screening via the PNWTFGL at no additional cost, but it will also provide a savings to the breeding program – arising from not having to plant, maintain, and evaluate genetically inferior seedlings. We will not implement routine MAB schemes that do not provide a net savings to the breeding program. Understanding this concept of MAB being a net savings and not a cost is critical for all involved.

New Marker Identification

New markers for flavor components of sweetness (overall and individual sugars), acidity, and stringency are under development using MSU data on the NY x EF population and combined with WTFRC-funded research on flavor candidate genes. As expected, the genetic components of sweetness remain difficult to pin down, as this trait is highly affected by non-genetic influences (e.g., maturity and water content). However, using data collected at MSU in 2008 (SSC) 2006-2008 (individual sugars of glucose, fructose, and sorbitol) in collaboration with Dr. Wayne Loescher, we have identified several possible genomic locations of sweetness-related traits, especially for proportions of individual sugars. The sugar profile of cherry fruit, as defined by such sugar proportions and predicted by DNA markers, may be a very important breeding selection criterion. Drs. Jim Olmstead and Dave Rudell reported (at the Plant & Animal Genome Conference in January, San Diego, CA) relationships between individual sugar proportions and SSC/TSS (total soluble solids – total of individual sugars) for ~70 cherry accessions (34 sweets, 19 tarts, and 12 related species) grown at the Davis Repository, CA. Interestingly, while proportions of glucose and fructose remain fairly stable across cultivars from low to high SSC/TSS, sorbitol is extremely associated with SSC/TSS, increasing in proportion almost linearly as SSC/TSS increases – for example, an increase of 1° Brix is associated in sweets and tarts with +1.4% increase in sorbitol on average, at the expense of glucose (-0.5%), fructose (-0.5%), and the minor sugar, sucrose (-0.3%). While sorbitol contributes as much as any sugar to a refractometer reading of SSC, it contributes to perceived (tasted) sweetness only 2/3 as much as glucose, 1/2 as much as sucrose, and 1/3 as much as fructose, according to sugar sensory science. Therefore, to develop cultivars with a pleasant sweet taste, breeding should target a relatively high fructose to sorbitol ratio (F:S) rather than relying only on SSC. Based on these

findings, using SSC as the primary selection criterion for sweetness is predicted to result almost invariably in the development of high sorbitol cultivars if the only parents used are from current elite germplasm. According to the Davis germplasm study, parent material with high F:S and a high SSC is very rare. However, the study of individual sugars of the NY x EF population at MSU indicates that NY54 is one of those rare high SSC + high F:S individuals, and NY x EF seedlings exist that have this sweetness attribute and medium (rather than tiny) fruit size. Such individuals are being used as parents in the PNWSCBP based on this knowledge. Furthermore, the collection of this phenotypic data in the genetically mapped NY x EF population provides the opportunity to dissect the genetic control of F:S and other sweetness attributes and develop predictive markers. Some markers for sugar proportions and ratios, not yet validated as being robust, were identified from QTL analyses of NY x EF and may be useful to track the introgression of high F:S from NY54 into breeding populations and ultimately new cultivars with unique and desirable sugar profiles.

QTLs for acidity and SSC were identified in 2008, but require further analyses to see if the marker-locus-trait associations were maintained in 2009. Phenotypic data were collected again in 2009, and QTL analyses will be conducted in late 2009.

The genetics of fruit skin, blush, and flesh color is being dissected by recent work conducted at MSU using the NY x EF population. The gene that is largely responsible for detecting mahogany vs. blush fruit types appears to be identified, and we are currently developing DNA markers for fruit color prediction. Such markers will likely be used in MAPS rather than MASS in the PNWSCBP. Both of the major fruit color types are desirable and so there is no purpose in culling one or the other at the seedling stage. However, because fruit color type defines the target market class of a potential new cultivar, and each target market class will have specific thresholds for other traits such as size and firmness, prediction of the proportions of seedlings that will fall into each color type for any given cross would be a valuable tool.

Using the genetic map based on the NY x EF population, markers for further traits can be pursued if the traits are genetically variable in the population and if they are measured. Therefore, additional traits measured in 2009 were astringency (0-2 scale, also measured in 2008), freestone (1-5 scale), and scar (tear or dry) Traits measured by the MSU team since around 2006 include harvest date (as well as fruit size and color previously mentioned). QTL analyses will be conducted for such traits in winter 2009. New useful marker-locus-trait associations for entering the MAB Pipeline are expected.

Outreach Activities

With the departure of Dr. Jim Olmstead, the outreach component of the project has not been conducted as planned, and allocated funds, thus far, remain unspent. Participation at the Cherry Field Day, using S-genotyping as examples for various MAB applications, somewhat addressed the outreach objective. We will participate in the 2009 WSHA Annual Meeting (Wenatchee, WA) to provide a poster and props to demonstrate MAB, involving technicians, graduate students, PIs, and the breeder, Dr. Oraguzie.

References:

Haldar S, Haendiges S, Edge-Garza D, Oraguzie N, Olmstead J, Iezzoni A, Peace C (2009). Applying genetic markers for self-fertility in the WSU sweet cherry breeding program. Acta Horticulturae (submitted)

Tao R, Yamane H, Sugiura A, Murayama H, Sassa H, Mori H (1999). Molecular typing of *S*-alleles through identification, characterization and cDNA cloning for S-RNases in sweet cherry. Journal of the American Society for Horticultural Science 124:224-233

Zhang G, Sebolt AM, Sooriyapathirana SS, Wang D, Bink MCAM, Olmstead JW, Iezzoni AF (2009). Fruit size QTL analysis of an F_1 population derived from a cross between a domesticated sweet cherry cultivar and a wild forest sweet cherry. Tree Genetics & Genomes, DOI: 10.1007/s11295-009-0225-x (online)

EXECUTIVE SUMMARY

Goals and Outcomes

The main goal of this 2009 project was to establish the marker-assisted breeding (MAB) Pipeline approach for the PNW sweet cherry breeding program (PNWSCBP). A further objective was to channel promising new markers into the Pipeline. We have successfully achieved these goals, made some interesting research discoveries, and secured substantial federal funding for the future enhancement and sustainability of this Pipeline for the PNWSCBP.

The third objective was to demonstrate the Pipeline to the sweet cherry producer community. The departure of Dr. Jim Olmstead, MAB outreach coordinator, hampered our activities in this area, but some efforts in 2009 were and will be made nevertheless.

Summary of Findings

- To integrate the MASS approach into the PNWSCBP, cost-efficient and logistically feasible stages for conducting genetic screening were identified. These are the greenhouse and lath house stages, in the months prior to field-planting. Potential savings with MASS are highest for traits not expressed until trees reach reproductive maturity (such as all fruit quality attributes), and even more so than for the Washington apple breeding program (WABP).

- The genetic markers for fruit size developed at MSU in recent years with federal and WTFRC funding support will indeed be valuable for increasing breeding efficiency for large fruit in the PNWSCBP.

- S-genotyping of 245 ’04 cross seedlings determined that about 70% of seedlings had intended parentage. 104 self-fertile seedlings were identified.

- A genetic marker for self-fertility is available, but its use in early seedling selection is pending investigation of the opportunity cost to other important traits.

Recommendations

The recommended next breeding step is to incorporate DNA information gained on parents, selections, and seedlings into breeding decisions in the PNWSCBP, and to consider the use of existing genetic tests for reducing the number of inferior seedlings to be field-planted from 2010.

The recommended next research step is to expand analyses from ‘04 seedlings to the ‘05 and ‘06 cross seedlings fruiting in the next couple of years. With fruit quality evaluations, these 5000-6000 seedlings would represent a powerful “training population” for verifying and characterizing utility of fruit size markers, the self-fertility marker, and selfing as a crossing strategy, to deliver efficient MAB schemes.

We recommend support of a dedicated Genetic Screening Technician, using funds from routine breeding program operating costs, to ensure labor availability for conducting the DNA marker screening component of future high-throughput seedlings selection in the WSU sweet cherry and apple breeding programs. We will not implement routine MAB schemes that do not provide a net savings to the breeding program, after accounting for Technician support and other molecular screening costs. Our concept of routine MAB is to reduce traditional operating costs, and recommend reinvestment of savings into breeding operations for increased efficiency of producing superior new cultivars for the PNW sweet cherry industry.

FINAL PROJECT REPORT

Project Title: Consulting for the northwest cherry improvement project

PI: Fredrick A. Bliss
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Cooperators: Jim McFerson, Nnadozie Oraguzie, Amy Iezzoni, Cameron Peace, Amit Dhingra, Matt Whiting, Jim Olmstead, Yanmin Zhu

Other funding sources: None

Budget History:

Item	**2009**		
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel	$4,000		
Miscellaneous	5,500		
Total	**$9,500**		

ORIGINAL OBJECTIVES:

- Coordinate and lead monthly conference calls among members of the cherry team to facilitate discussion of key issues related to sweet cherry improvement.

- Facilitate collaboration among team members and key resources in the public and private sectors in the PNW and externally.

- Work with N. Oraguzie the breeding program leader to continue development and implementation of an efficient breeding program for developing commercial sweet cherry cultivars suited for the PNW.

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

SIGNIFICANT ACTIVITIES and FINDINGS:

- Coordinated conference calls with members of the cherry team
  - Eight conference calls during the year to discuss issues relevant to sweet cherry improvement. Participants included Jim McFerson, Amy Iezzoni, Cameron Peace, Amit Dhingra, Matt Whiting, Jim Olmstead, Yanmin Zhu, David Rudell, Dorrie Main and Nnadozie Oraguzie

- Reviewed and critiqued research proposals from cherry team members.
  - Individual submissions to the NRI competitive grants program
  - Presentations at the 2009 Molecular Markers in Horticulture Symposium
  - Group proposals for the RosBreed and RosTrait submissions
  - Various proposals to the WTFRC

- Interacted with key members of the sweet cherry improvement team about important issues relating to cherry improvement research
  - Nnadozie Oraguzie and Amy Iezzoni about breeding program activities and a Best Practices document
  - Cameron Peace and other team members about optimal use of molecular markers for cherry breeding

- Participated in the 2009 Molecular Markers in Horticulture Symposium at Prosser, OR July 2009.
  - Made a keynote presentation
  - Interacted with international scientists and breeders about use of molecular markers for breeding tree fruits.

- Facilitated interaction among breeders and scientists.
  - Discussed Prunus germplasm activities at Clonal Repository in Davis with Nnadozie Oraguzie relative to his trip to California
  - Continued interactions with molecular scientists at Kearney Agric. Center, Parlier, CA and with UC Davis scientists relative to peach rootstocks.
  - Participated in GRIN-Global process review as member of the oversight committee.

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

- Submitted invoices for expenditures on a quarterly basis.

RESULTS & DISCUSSION:

The monthly (except during the summer busy season) conference calls provide a good forum for members of the cherry team to discuss important issues relative to cherry improvement. Although all members are not able to participate in all calls, most have been consistent and provide good ideas and constructive discussion. These are regularly scheduled meetings where issues such as trait importance, testing sites, marker-locus-trait associations and similar topics were discussed.

Members of the cherry team continue to do constructive, interactive and inter-dependent work on cherry improvement. They exchange ideas and provide critical analysis each others' ideas. There is a lot of sharing of ideas to promote synergy without redundancy and duplicated effort. I am available and have read a good number of proposals during preparation which I believe contributes to a higher likelihood of acceptance and funding success. Several members of the team submitted research proposals that were accepted and funded by various competitive grants program. Many members of the cherry team contributed substantial time and effort to preparing the RosBreed proposal, which was funded. Amy is to be congratulated for her excellent guidance and leadership of the RosBreed Team. Other members are leaders of the various sub-programs in that proposal.

There are an exceptional number of outstanding scientists in the PNW (WSU, OSU, ARS, WTFRC and perhaps others) devoting significant effort to improving the quality and competitiveness of the tree fruit industries. They continue to secure significant funding from outside sources that adds measurably to the support provided by the Commission. Through continued collaboration these efforts can result in significant new cultivars, better production and handling methods, improved consumer acceptance, and ultimately sustainability of growers. Continued interaction among the researchers is critical to continued support and success.

I feel the Northwest Sweet Cherry Breeding Program led by Dr. Oraguzie has made significant progress during the past year toward meeting goals that were established. He has hired professional assistants to aid with key program activities and others that can provide day to day breeding work and horticultural maintenance that is needed. Pollination, seed handling, germination, seedling growing and tree management are all essential but difficult processes for sweet cherry. The cherry team is continuing to learn how to manage the plant materials, and development and use of a 'Best Practices' document is proving helpful in that regard. He has successfully guided the upgrading of research facilities at Prosser which was badly needed. The number of crosses and seeds produced in 2009 were on target with germination results yet to be seen.

Field plantings and plant maintenance have been improved and the first seedling selections for possible 2nd stage testing in 2011 were made and are to be propagated this fall. The facilities for evaluating fruit-related traits are in place and were used for data collection in 2009. The extensive data being collected in the breeding program points to the need for establishing a good electronic data base, which will be an important activity in the coming year.

Good progress has been made by the team toward identifying additional marker-locus-trait associations that can be used for selection in the breeding program. The development of a program by Dr. Peace and associates to assess how markers can best be used for practical selection provides an objective means for decision making. Seedling screening using markers for compatibility/incompatibility has provided not only an indication of self fertile plants but also which seedlings result from hybridization as opposed to selfing or unintended outcrossing. This information can be used to increase information about populations to be used for not only breeding but also genetic and genomic studies. Collaborative work with Dr. Iezzoni for analyzing populations for markers linked to genes that control fruit size and quality factors is an important resource that will be

available shortly for use in targeted selection for these important traits. Dr. Dhingra is providing significant collaboration on tissue culture and is leading the studies on sequencing the cherry genome to provide basic information for future studies. Exploration of pathways involved in important quality traits is being actively pursued by Dr. Rudell. The leadership of Dr. Main for database management and support for the Rosaceae is an important resource for the breeding program. This is a brief mention, and there is likely other, of some key support available and flowing to the breeding program. The challenge is how best to use this effectively to meet breeding objectives.

EXECUTIVE SUMMARY
Title: Consulting for the Northwest Cherry Improvement Project
PI: Fredrick A. Bliss
WTFRC Funding: $9,500.

I have continued as a consultant to the Northwest Cherry Improvement Project which is an integrated project focused on development of new cultivars through classical breeding and application of applied genomics technology to improve breeding efficiency. The long-term nature of tree fruit breeding requires efficient use of resources and plant materials and well-integrated activities that will minimize the time required to develop and release new cultivars that fit the needs of the N.W. cherry industry.

I continued working with other researchers, cooperators and members of the industry to provide expertise and knowledge about fruit breeding.. I provide insight and ideas for identifying and applying appropriate technology to facilitate cultivar development in a minimum timeframe. My role is to support the efforts of the breeders and researchers working on this project and provide information and feedback to Jim McFerson and Board members about progress toward breeding and other research objectives.

Objectives this year were to: 1) Coordinate and lead monthly conference calls among members of the cherry team to facilitate discussion of key issues related to sweet cherry improvement., 2) facilitate collaboration among team members and key resources in the public and private sectors in the PNW and externally, 3) work with N. Oraguzie the breeding program leader to continue development and implementation of an efficient breeding program for developing commercial sweet cherry cultivars suited for the PNW, and 4) provide analysis and critique of reports and proposals for competitive funding of research and development related to cherry improvement.

The objectives were met through activities conducted from my home office in Davis, CA such as telephone conference calls, electronic communication, and participation in the Cherry research review. Activities included: 1) Coordinated conference calls with members of the cherry team that included Jim McFerson, Amy Iezzoni, Cameron Peace, Amit Dhingra, Matt Whiting, Jim Olmstead, Yanmin Zhu, David Rudell, Dorrie Main and Nnadozie Oraguzie; 2) review and critique of research proposals from cherry team members, 3) interaction with key members of the sweet cherry improvement team about important issues relating to cherry improvement research; 4) participation in the 2009 Molecular Markers in Horticulture Symposium at Prosser, OR July 2009; 5) facilitating interaction among breeders and scientists; and 6) alerting cherry team members to key references for breeding and genetics of sweet cherry.

The scientists in the PNW (WSU, OSU, ARS, WTFRC and perhaps others are devoting significant effort to improving the vitality and competitiveness of the tree fruit industries. Through collaboration these efforts can result in significant new cultivars, better production and handling methods, improved consumer acceptance, and ultimately sustainability of growers. Good progress continues in the breeding program and other research programs that provide supporting information and technology for efficient breeding efforts.

FINAL PROJECT REPORT

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

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

Other funding sources

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

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

Total Project Funding: $13,000

Budget History:

Item	Year 1: 2009	Year 2:	Year 3:
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel & expenses	$ 3,000		
Consulting fee	$ 10,000[a]		
Miscellaneous			
Total	**13,000**		

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

OBJECTIVES:

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

SIGNIFICANT FINDINGS/ACCOMPLISHMENTS:

- Reviewed and contributed to the crossing plan.
- Traveled to Prosser at the beginning of the bloom season to provide organizational and technical assistance to help the crossing team.
- Traveled to Prosser during the growing season to see the seedlings and review horticultural practices.
- Developed a photo-illustrated document outlining seedling growth benchmarks that can be used to implement a seedling growth tracking system so that potential problems can be identified and corrected in a timely manner.
- Provided specific information on the genetic control of fruit size and cherry skin and flesh color to C. Peace for validation in the breeding populations
- Provided C. Peace and N. Oraguzie with a database of genetic and phenotypic data that will be the cornerstone used to determine the genetic control of important phenotypic traits in the cherry breeding program.

RESULTS and DISCUSSION:

Assist in generating breeding populations & provide horticultural guidance.

In April I visited Prosser and assisted N. Oraguzie organize for spring crossing. This included review of the Best Management Practices, the crossing scheme, pollen viability testing, seeds in stratification and seedlings in the field. Numerous specific recommendations were made. In addition, I spent several hours with the entire pollinating crew at the Roza Farm where I demonstrated the best management practices for crossing activities and discussed the rationale for the various strategies.

In July, I visited Prosser to tour the seedlings in the field and the greenhouse. The seedlings in the field were growing nicely; however, survival of newly germinating seedlings continues to be problematic. To help address this problem, I developed a photo-illustrated document outlining seedling growth benchmarks that can be used to implement a seedling growth tracking systems (Fig. 1). I have suggested to N. Oraguzie that his team record seedling growth according to these benchmarks and make the data available to me on a weekly basis. This would make it possible for me to diagnose and help solve problems in a timely manner.

Provide genetic expertise

My cherry genetics team is currently developing the genetic infra-structure for the PNW sweet cherry breeding program in collaboration with C. Peace to include the generation of molecular markers and genotyping of many of the parents used in the program. This work is funded by USDA grants. Specific deliverables in 2009 include:

- A database (called FlexQTL™) containing genetic and phenotypic characterizations for the majority of the parental germplasm used in the breeding program, plus populations from MSU (NY x EF), WSU (PMR x Rainier) and France (Regina x Lapins)(Fig. 2).
- Knowledge of genomic regions controlling fruit size and skin and flesh color in cherry (Fig. 3)(Zhang et al., 2009; Sooriyapathirana et al, 2009). This information was shared with C. Peace for validation in the PNW sweet cherry breeding program.
- Identification of DNA marker polymorphisms in six parental selections used in the breeding program. This information is being used to design a high-throughput genotyping platform for sweet cherry with state-of-the art markers by spring 2010.

Collectively, these efforts provide the building blocks that will allow the cherry team to implement marker assisted breeding to increase the efficiency and success of the breeding program.

EXECUTIVE SUMMARY:

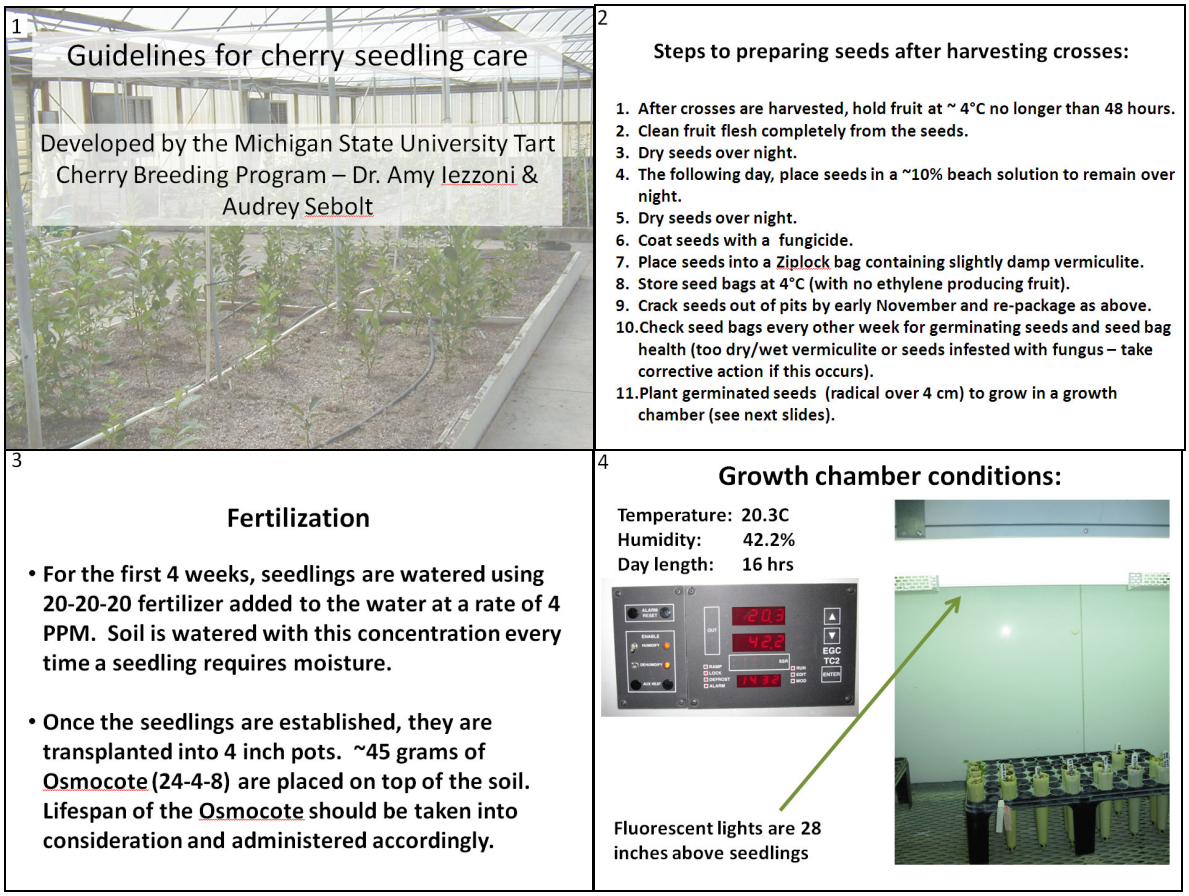
The building blocks of a successful breeding program include the use of diverse germplasm, generation of large numbers of progeny populations for evaluation, appropriate horticultural management of the breeding materials, the ability to identify and commercialize superior cultivar candidates, and judicious use of genetics knowledge. The goal of my consultancy with the PNW sweet cherry breeding program is to assist in our ability to excel at all of these objectives so that we can deliver superior sweet cherry cultivars to the Oregon and Washington industries as quickly as possible. In 2009, I provided knowledge and recommendations regarding breeding and horticultural practices, and advances made this year in cherry genetics. In addition, a photo-illustrated document outlining seedling growth benchmarks was developed to address problems with seedling survival. This document can be used to implement a seedling growth tracking system so that potential problems can be identified and corrected in a timely manner. In addition a core genetic database was developed and used to elucidate the genetic control of fruit size and color. This knowledge was provided to C. Peace for further validation within the sweet cherry breeding populations. Continued collaboration, whereby I contribute my time and knowledge of cherry germplasm, breeding and genetics, will help us achieve our collective vision of a cost-effective aggressive and successful sweet cherry breeding program.

LITERATURE CITED:

Sooriyapathirana SS, Khan A, Sebolt AM, Wang D, Bushakra JM, Lin-Wang K, Allan AC, Gardiner SE, Chagne D, Iezzoni AF. 200x. QTL analysis and candidate gene mapping for skin and flesh color in sweet cherry fruit (*Prunus avium* L.). Tree Genet Genomes (in review).

Zhang G, Sebolt AM, Sooriyapathirana SS, Wang D, Bink MCAM, Olmstead JW, Iezzoni AF. 2009. Fruit size QTL analysis in an F_1 population derived from a cross between a domesticated sweet cherry cultivar and a wild forest sweet cherry. Tree Genet. Genomes (in press).

Fig. 1. Guidelines for cherry seedling care.



5

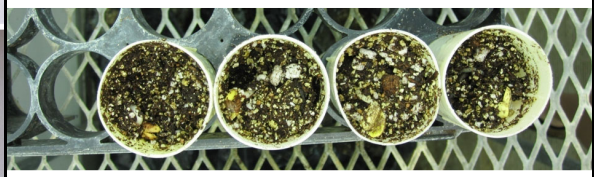
Seedling health in seed germination bags:

This an example of a healthy seedling and one that is ideal in length to plant. Radical is between 4-7 cm in length and appears to be healthy.

This is an example of a seedling that is beginning to show signs of poor health, as the end of the radical is brown. Reasons for poor health include: too much/little moisture in the seedling bags or not enough vermiculite in the seedling bags. Once the problem is identified, action should immediately be taken to ensure that seedlings are healthy. In this case, there was not enough vermiculite in the bag. Action: vermiculite was added as well as water.

6

Day 1: After seedlings are planted, they are watered until fully saturated with fertilizer/water. After watering, seedlings are examined and care is taken to be sure cotyledons are above the soil line.

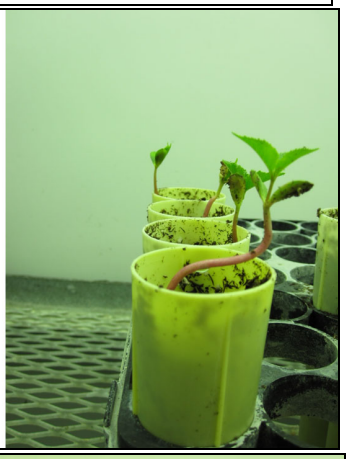


7

Day 2: Soil is still damp at the top and bottom of the cone-tainer. Seedlings were not watered.

8

Day 3: Soil is just beginning to dry out. Added ~ 50 ml of fertilizer/water.



9

Day 6: Soil is still moist on the surface and at the bottom of the cone. ~ 14 ml of fertilizer/water was added.

10

Day 10: Soil is still moist on the surface and at the bottom of the cone. No water was added.

11

Day 15: Soil is still moist on the surface and at the bottom of the cone. No water was added.

12

Day 21: Seedlings were watered (if dry) with fertilizer/water until soil was fully saturated (water dripping out of the bottom of the cone-tainer). The second seedling from the left required less frequent watering as it is much slower in growth. Continued care for the taller seedlings will involve watering every other day until soil is fully saturated. For the second seedling from the left, only as needed (much less frequently and less volume of water). Approximately on Day 28, seedlings will be transferred to 4 inch pots.

NOTE: These pictures and this timeline are for tart cherry. Sweet cherry seedlings will grow with a lot more vigor and apical dominance than tart cherry. So achieving these size measurements by Day 28 should not be a problem.

13

Suggested seedling watering schedule

Day 1 Watered soil until fully saturated	Day 2 No water added	Day 3 ~ 50 ml of water	Day 4 No water added	Day 5 No water added	Day 6 ~ 14 ml of water	Day 7 No water added
Day 8 No water added	Day 9 No water added	Day 10 No water added	Day 11 Watered soil until fully saturated*	Day 12 No water added	Day 13 No water added	Day 14 No water added
Day 15 No water added	Day 16 Watered soil until fully saturated*	Day 17 No water added	Day 18 ~ 50 ml of water	Day 19 No water added	Day 20 No water added	Day 21 Watered soil until fully saturated*

*Seedlings that were less vigorous were watered less frequently.

14

Seedling growth progression over the course of three weeks

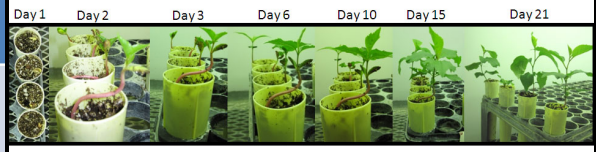


Fig. 2.B. FlexQTL genome coverage where the NYxEF linkage map is used as the backbone. Black boxes represent the locations of the genetic markers on the 8 *Prunus* linkage groups.

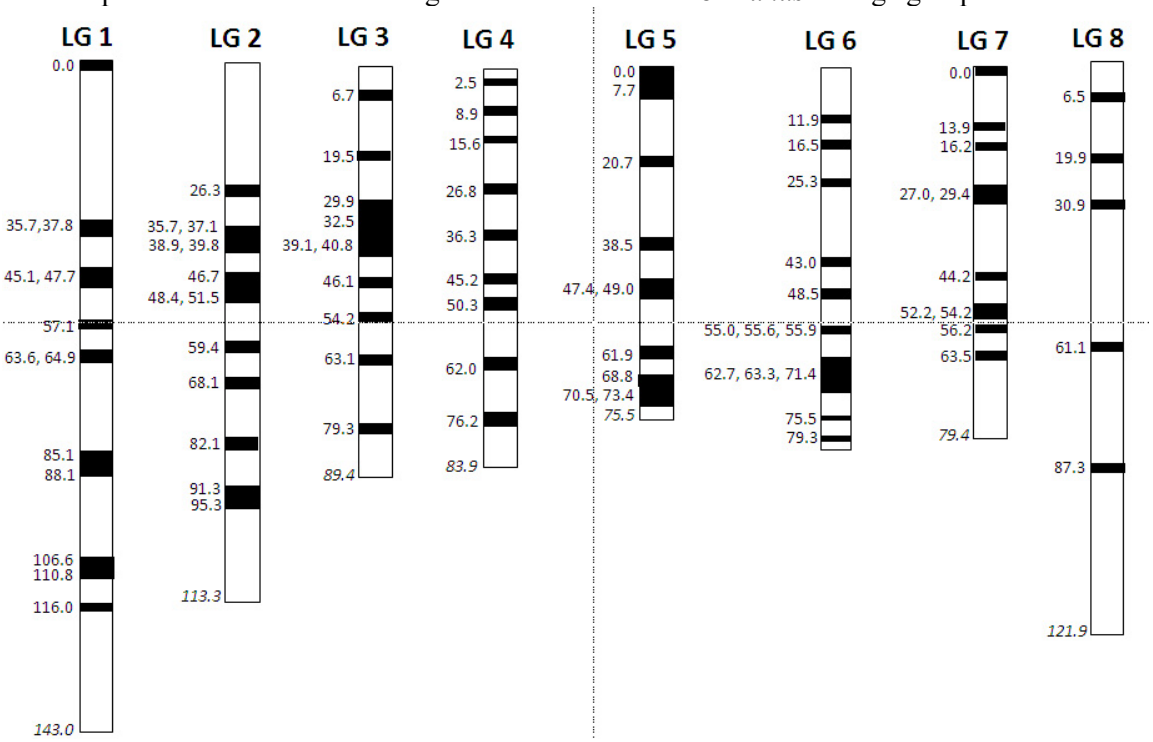


Fig. 3.A. Range of skin & flesh colors used in the genetic analysis. Skin Color Indexes are a modified version of the MSU Sweet Cherry Maturity Index. The flesh color index is the Washington State University Sweet Cherry Flesh Color Index. Skin color 1 and skin color 2 refer to bluch color and ground color, respectively.

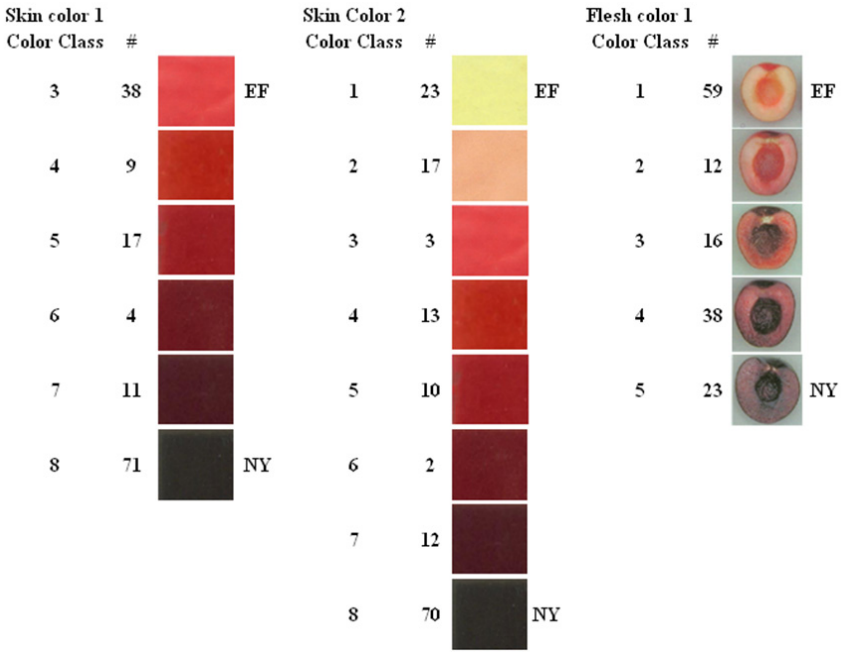


Fig. 3.B. Progeny distribution for skin color. Color scale (3-8) is for skin color 1 as defined in Fig. 3.A.

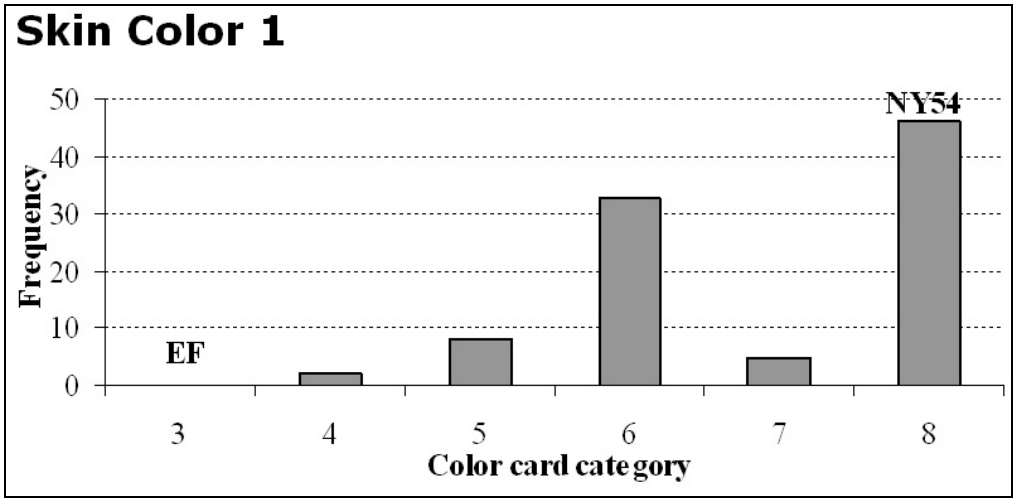
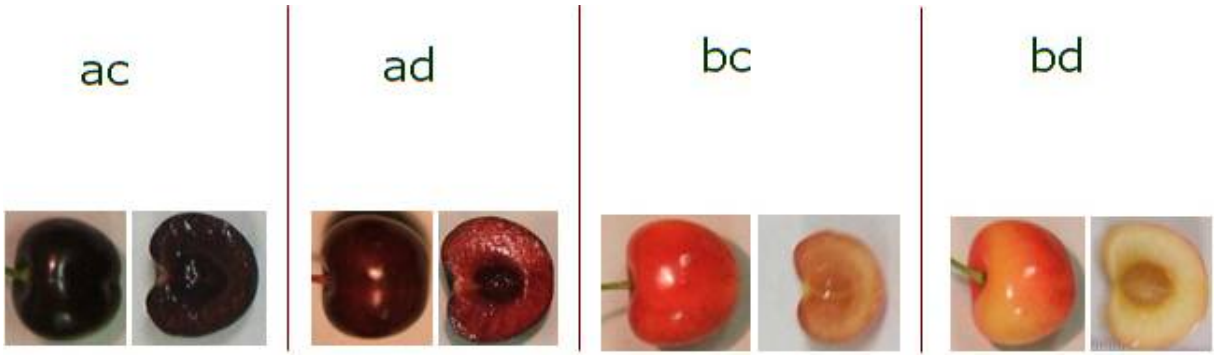


Fig. 3.C. Genetic markers on cherry linkage group 3, simplified by letters a, b, c, d, illustrate the contribution of this genomic region to the genetic control of flesh color. Only those progeny individuals that have the genetic markers termed "a", have dark skin and red flesh.



FINAL PROJECT REPORT

Project Title: Managing virus diseases detrimental to cherry production

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Other funding sources

Agency Name: California Cherry Advisory Board
Amount awarded: $5,000 in 2008 and $10,000 in 2009

Agency Name: ANLA/HRI
Amount awarded: $132,000 (project ended Sept 30, 2008)
Notes: Objectives of the ANLA/HRI project partially overlapped with the characterization of the rusty mottle group of cherry viruses.

Total Project Funding:$100,680 from the WTFRC plus $15,000 from the CCAB

Budget History:

Item	**Year 1:** 2007	**Year 2:** 2008	**Year 3:** 2009
Salaries	$ 5,618	$10,722	$13,498
Wages	$ 3,275	$ 1,776	$ 4,706
Benefits	$ 2,212	$ 4,076	$ 4,576
Equipment	$ 0	$ 0	$ 0
Supplies	$31,635	$ 5,261	$ 9,016
Travel	$ 0	$ 40	$ 0
Miscellaneous			
Total Expended	$42,740	$21,875	$31,796[1]
Total WTFRC Funded	$36,938	$33,823	**$29,919**

1. Expenditures as of October 9, 2009. Remaining project expenses for salaries and benefits will be derived from the balance of WFTRC and CCAB funds.

OBJECTIVES:

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

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

SIGNIFICANT FINDINGS:

- Root grafting is the major means by which cherry leafroll virus spreads within an orchard.
- Rootstock selection offers the potential of minimizing transmission of viruses by root grafting.
- Pollen transmission of cherry leafroll virus is suspected, but if it does occur, it is very inefficient.
- Viruses of the rusty mottle group can be dispersed through propagation and planting of symptomless carriers. Symptom expression is cultivar dependent and infected trees can be juxtaposed with other sweet cherry cultivars that express severe symptoms with resulting crop loss.
- Isolates of Little cherry virus 1 are genetically diverse; conserved regions of the genome were identified that permitted development of a reliable molecular assay.
- Little cherry virus 1 is present in western cherry producing regions.
- Broad spectrum molecular assays assist in detecting deleterious viruses where they are the cause of poor production in cherry orchards.

RESULTS AND DISCUSSION:

Objective 1: Development of alternative methods of managing viruses.

Cherry leafroll virus (CLRV) poses a serious risk to sweet cherry production in the Pacific Northwest. Our program performed many assays by request that demonstrated that the geographic distribution of known CLRV infections has expanded to new production regions of Washington State. As an aid to determining factors that affect the spread of CLRV, six plots in commercial blocks in the Yakima Valley were tested annually for CLRV over a period of ten years. Two distinct patterns of virus spread were observed. Dispersion from an infected tree to trees immediately adjacent to the original infection site was relatively fast. This rate of virus movement appears to be dependent on orchard architecture and is particularly rapid in sprinkler irrigated and older orchards. There was also a much less frequent transmission to new sites areas previously free of disease. These new foci of infection occurred within or outside of the original cherry block. The existence of these two scenarios points to the existence of two modes by which CLRV is transmitted from one tree to another.

Our research on the epidemiology of CLRV demonstrated that transmission through root grafts is an important route for tree-to-tree spread within an orchard. Consequently, genetically diverse rootstocks are being evaluated for their ability to provide field resistance to CLRV that could minimize or even eliminate this significant route of infection. A small on-farm trial was established in 2000 to test the influence of rootstock on this mechanism of virus transmission and was completed in 2008. Twenty trees of 'Bing' on 'Colt' (*P. avium* × *P. pseudocerasus*) rootstock and 20 trees of 'Bing' on 'Mazzard' (*P. avium*) rootstock were planted in plots in three separate orchards. Each year they were monitored for CLRV. It was suggested that pollen-borne virus may play a role in CLRV transmission, so any flower buds that developed during the first four years of this study were removed from the subject trees. During the first 7 years of testing, five of the 20 trees on 'Mazzard' rootstock

became infected, some within the second growing season. None of the trees on 'Colt' rootstock became infected with CLRV. During this same period, many replacement trees planted by growers on 'Mazzard' rootstock at these locations also become infected. This limited trial suggested that 'Colt' offers protection from the root grafting transmission of CLRV. In parallel studies conducted by others, 'Colt' rootstock has been shown to be effective in controlling the spread of *Tomato ringspot virus* (ToRSV), a nematode transmitted virus, in cherry trees. 'Colt' reacts to ToRSV with a hypersensitive reaction and thus prevented movement of virus away from the site of nematode inoculation. Both CLRV and ToRSV are members of the same genus of viruses, but there is no evidence that a nematode vector of CLRV exists in North America. ToRSV does, however, provide an example where rootstock selection can be used effectively to control virus transmission.

In the final year of this on-farm trial, one of the trees on 'Colt' became infected with CLRV. The tree declined rapidly and by mid-summer, the canopy was collapsing. Further examination revealed the development of necrotic tissue at the graft union. CLRV was detected by serological (ELISA) (Table 1) and molecular (RT-PCR) (data not shown) methods in two of the four leaders. None of the suckers emerging from the rootstock below the graft union contained detectable CLRV. This distribution of virus suggests that infection of the young tree had occurred through an aerial route. The appearance of necrotic tissue (dark discoloration) at the graft union suggests that 'Colt' rootstock responded to CLRV infection by development of a hypersensitive reaction leading to death of plant tissue adjacent to the infected 'Bing' scion. Development of necrotic tissue at the graft union restricts movement of nutrients and water to the scion leading to decline of the scion. This same relationship was subsequently observed in a mature orchard planted on 'Colt' rootstock where natural infection by CLRV had occurred. A zone of necrotic tissue developed at the graft union and the scions quickly declined. This dramatic response of 'Colt' rootstock leads to loss of the infected tree, and mimics the pattern of CLRV infection of walnut trees where pollen transmission of the virus leads to "black line" disease and death of walnut trees planted on northern California black walnut or Paradox rootstock. However, from a disease management perspective, the rapid decline of sweet cherry scions on 'Colt' rootstock quickly eliminates sources of virus-laden pollen from the orchard that would otherwise sustain the continued spread of the disease. Since CLRV has a distinct negative impact on fruit production and quality, the rapid decline and removal of an infected tree significantly minimizes the long term economic impact of CLRV infection in the orchard.

Table 1. Portions of a tree ('Bing' scion on 'Colt' rootstock) were tested by ELISA to determine the distribution of cherry leafroll virus after natural infection.

Tree position	ELISA Absorbance values (interpretation)	
South-east leaders	0.319 (+)	0.315 (+)
North-west leaders	0.002 (-)	0.053 (-)
Lower branch from main trunk	0.319 (+)	0.367 (+)
Suckers from 'Colt' rootstock	0.001 (-)	0.001 (-)

The above results indicate that 'Colt' rootstock offers important disease management options to mitigate the spread of CLRV: the rootstock prevents systemic movement of the virus from root grafts, and quickly eliminates sources of virus-infected pollen that would support secondary spread of infection to other trees. While 'Colt' rootstock confers good horticultural properties in sandy and/or rocky soils, trees on 'Colt' rootstock produce excessive vegetative growth and lack precocity when planted in rich deep soils (Perry *et al*., 1997). Therefore, other rootstocks and rootstock/interstock combinations are being evaluated for their potential to offer similar protection from the ingress of CLRV, but offering greater desirable horticultural characteristics in a wider array of settings. To investigate the potential of rootstocks to provide field resistance to soil-borne viruses, 132 trees on

rootstocks and rootstock/interstock combinations were propagated and planted. Rootstocks include 'Colt', 'Krymsk 5', 'Krymsk 7', 'Gisela 5', 'Gisela 6', and 'Gisela 12'. Zee-stem interstocks are reported to offer size control and precocity to cherry trees, so 'Zee-stem' interstocks on 'Citation 'and 'Myrobalan 29C' rootstock were included in this study. All rootstocks and rootstock/interstock combinations were grafted with a virus-free clone of 'Bing'. Finished trees of 'Zee-stem' interstocks on 'Colt' rootstock were also prepared, but the graft union of this combination was particularly fragile and impractical for further consideration during this trial. Finished trees were established in the orchard and graft-inoculated in June 2009 with 'Bing' infected with CLRV. The source of CLRV inoculum was tested by ELISA to ensure freedom from other common viruses. The inoculating chips were grafted onto trees of each combination either directly onto the rootstock or onto the scion. At the end of the first growing season after inoculation, the most overtly visible reaction to CLRV was observed where infected buds were placed directly on 'Krymsk 5' (*Prunus fruticosa* × *P. lannesiana*) rootstock. A severe hypersensitive reaction characterized by prolific gumming around the inoculation site is evident. This rootstock is also known to be sensitive to the ilarviruses *Prunus necrotic ringspot virus* (PNRSV) and *Prune dwarf virus* (PDV). 'Krymsk 7' (*Prunus lannesiana*) has not produced any reaction to direct budding of CLRV onto the rootstock, nor is this rootstock known to be sensitive to PNRSV and PDV. 'Krymsk 6' (*Prunus cerasus (Lyubskaya)* × *Cerapadus Michyunin* (*P. cerasus* × *P. maackii*)) is sensitive to infection by the ilarviruses but has a different genetic background so the response to CLRV cannot be predicted; 'Krymsk 6' was not included in this first trial. Of the Gisela series used in this study, initial observations suggest that 'Gisela 12' (*Prunus canescens* × *Prunus cerasus*) is responding adversely to inoculation by CLRV. There is no evidence of a hypersensitive reaction at the site where the infected chip is grafted directly to the rootstock. However, when the scion is inoculated, there is a proliferation of suckers from the rootstock which is suggestive of an adverse reaction at the graft union. 'Gisela 12' rootstock is not sensitive to the two ilarviruses PNRSV or PDV. Final interpretation of these grafting experiments cannot be made until trees are sacrificed and the graft union examined for the appearance of abnormalities; this will occur after subsequent growing seasons. The preliminary observation of a hypersensitive reaction from 'Colt', 'Krymsk 5' and potentially 'Gisela 12' suggests that there are multiple sources of genetic resistance to CLRV.

Serological tests (ELISA) by our program confirmed that pollen derived from cherry trees infected with CLRV carries a large amount of virus particles, and mechanical inoculations confirmed that the virus associated with the pollen is infectious. In an effort to obtain a measure of the risk of pollen transmission, clusters of flower buds were surrounded by organza cages before the blossoms opened. The organza cages prevent the introduction of pollen from other sources and limit movement of Western flower thrips and other insects. In control cages where no pollen was introduced, there was no fruit set. This demonstrated that the organza cages successfully exclude sources of compatible pollen. In May 2006, 800 blossoms of a virus-free tree were pollinated with CLRV-infected pollen. As previously reported, at the time of shuck fall, 50% of the pedicels tested contained CLRV detectable by RT-PCR. In this and all subsequent pollen trials, the branches and spurs exposed to virus-laden pollen are tagged with tree marking paint to ensure they are not removed during routine orchard pruning operations. In spring 2007, leaves adjacent to each of the spurs that had been pollinated with infected pollen were collected and tested for CLRV by RT-PCR. No samples yielded positive results. In 2008, adjacent leaves were again tested by ELISA and all were negative.

In 2007, pollination experiments were repeated on a different set of trees and 800 flowers of 'Van' were hand pollinated with 'Bing' pollen collected from CLRV-infected trees. In June of 2007, fruit was harvested from each cage and the pedicels extracted and tested by RT-PCR. Overall, 20% of the pedicels from cages into which CLRV-infected pollen was introduced yielded positive results by RT-PCR. In contrast to the 2006 experiment, most fruit was carried to maturity. When leaves adjacent to spurs of fruit formed in 2007 were sampled in spring 2008, no samples yielded positive results. This was consistent with results from the pollination experiments begun the previous season. Thus, in

each of two years, although CLRV is present in the pedicels of fruit after blossoms are pollinated with virus-infected pollen, the virus has not replicated to detectable levels in the adjacent vegetative tissue.

In 2009, caged blossoms of 'Van' trees were pollinated with pollen from CLRV-infected 'Bing' pollen; a total of 8,679 blossoms were exposed to the virus infected pollen. Trees from all experiments will continue to be monitored for the presence of CLRV.

To validate methods used for surveys and pollen transmission experiments, it is important to estimate the rate at which the virus is able to replicate to detectable levels and move through susceptible cultivars. Previous observations of naturally infected trees suggested that based on visual assessment of symptoms and supported by ELISA data, it may take 3 to 4 years for CLRV to become fully detectable throughout the tree. To verify, each of two major scaffold limbs of four trees were T-grafted with a single bark patch from an infected 'Bing' tree in August 2006. In spring 2007, CLRV could be detected only in the shoots immediately adjacent to the buds. In spring 2008, leaves from the base of each scaffold limb were assayed by ELISA for CLRV (Table 2). After one full growing season, CLRV had not moved and replicated to a level detectable by ELISA in all parts of the tree.

Table 2. One-year old shoots of two major scaffold limbs of each tree were inoculated with cherry leafroll virus-infected bark patches in the autumn of 2006. In spring 2008, leaves at the base of each scaffold limb were assayed by ELISA for cherry leafroll virus.

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

The amount of inoculum introduced by bark patch inoculations is much greater than that potentially introduced through pollination. This suggests that additional periods of observation are warranted to determine if CLRV had been successfully transmitted from infected pollen during the experiments initiated in 2006 thru 2009. In studies of CLRV pollen transmission in Europe, trees were monitored for four years after pollination in order to assess the rate of pollen transmission in other perennial species.

Impact and economic benefits: CLRV, like so many other viruses that infect sweet cherry, reduce the size and quality of fruit, and hence their marketability. This program identified this virus in the western US and alerted the industry to its presence so that measures could be implemented to reduce further encroachment by the virus and the diseases that it causes. Greater grower awareness has resulted in the identification and elimination of many infected and unproductive trees, thus reducing sources of inoculum that would foster further spread. Knowledge gained from our project has impacted industry-wide operations in an effort to control this virus. The nursery certification program implemented more stringent standards to reduce the entry of the virus into plantings through Washington State Certified cherry trees. One county has introduced and sustained control measures. Additionally, awareness of the potential aerial transmission of this virus led some pollen companies to engage the WSU ELISA Testing Service Center in a program to ensure that their products are free of CLRV. Root grafting appears to be the major route by which CLRV spreads to adjacent trees. It has been demonstrated that minimizing transmission via this process through rootstock selection dramatically slows the spread of the virus within infested orchards. Further studies are required to identify horticulturally beneficial rootstocks that bestow this same ability to reduce virus transmission via root grafting.

Objective 2. Development of laboratory tests to increase accessibility to rapid virus diagnosis.
Many of the viruses that significantly diminish fruit size and quality are members of the genus *Foveavirus*; these viruses are frequently referred to as the rusty mottle group of viruses and are encountered in many cherry production areas. Several are likely native to wild *Prunus* species of western North America. At least some of these diseases spread naturally via an aerial transmission route, but vectors of the diseases are not known. Green ring mottle virus and cherry necrotic rusty mottle virus are the best characterized members of this group. Green ring mottle virus does not induce recognizable symptoms on sweet cherry, but does on sour cherry and ornamental flowering cherry trees whereas the remaining viruses of the rusty mottle group have been defined by the range of symptoms that are induced on select sweet cherry cultivars. Diseases caused by putative members of this group include cherry rusty mottle, cherry necrotic rusty mottle, cherry twisted leaf, cherry stem pitting and Montmorency stem pitting. The diversity of viruses and the varied responses of different cultivars renders diagnosis based on visual observations very difficult. Moreover, symptoms of infection often resemble those of adverse physiological conditions, chemical injury, bacterial or fungal infections. The ability to confirm the presence or absence of foveaviruses and their identity would greatly aid growers in properly ascertaining underlying causes of poor tree productivity and associated symptoms before initiating a response to declining trees. During the course of this project, techniques developed in our laboratory were used to obtain sequence information from approximately 13% of the genome from each of 26 foveavirus isolates associated with distinct disease symptoms in cherry, plus seven isolates of green ring mottle virus that are symptomless in sweet cherry. This process confirmed the ability of "universal" foveavirus primers to reliably screen for members of this virus genus, and further analyses of the data provided a strong footing on which further studies are based. Further refinement is necessary to allow the assay system to distinguish between green ring mottle virus, which is symptomless in sweet cherry, and other foveaviruses that cause disease. Expanding the database of sequences representing regional isolates improves the ability to develop assays specific for pathogenic virus strains. In an effort to increase accessibility and affordability of diagnosis to growers, a serological assay was also sought. We characterized the genes that encode the structural proteins of the foveaviruses found in cherry and applied this information to develop polyclonal antibodies for use in ELISA. The resulting serological assay has the desirable characteristic of not detecting green ring mottle virus, while still detecting a large number of pathogenic viruses of the rusty mottle group. Approximately 50% of the pathogenic viruses in this group are recognized by the assay so further enhancement is needed. Success with the antibodies produced in this manner provides great optimism for the potential to provide future refinement in robust serological assays for the foveaviruses of cherry. Additionally, trials are underway to adapt ELISA procedural parameters so additional strains of the viruses are detected by these new antibodies.

Another molecular assay that detects foveaviruses (Foissac *et al*., 2005) has received increasing acceptance internationally. This is a polymerase chain reaction-based system that uses "TriFoCap" primers, and is capable of detecting members of multiple virus genera including trichoviruses, foveaviruses and capilloviruses. Thus, this single test would detect a wide range of viruses of concern to the fruit tree industry. Fortunately, there has been excellent agreement between the new assay formats ("TriFoCap" and "universal" foveavirus molecular assays) and the traditional greenhouse indexing. Unfortunately, the broader scope of viruses recognized by the "TriFoCap" primers can complicate real world interpretation. Our "TriFoCap" assay detected two previously unreported viruses in *Prunus* spp. samples. Although samples with these viruses were positive by woody indexing in the greenhouse, they were negative by the "universal" foveavirus assay. Sequence analysis indicated that the two new viruses are closely related to, but distinct from known foveaviruses and appear to be closely related to viruses that infect citrus and remain unclassified members of the *Flexiviridae* family. These observations highlight the advantages of non-specific

molecular assays for virus detection with the necessity of using tests with narrow specificities to define the pathogenic agent for the particular orchard disease of concern to a grower. Efforts are underway to refine the "TriFoCap" assay such that more information can be obtained about the nature of the viruses contained in the sample under analysis. This would add the desired specificity to the broad spectrum "TriFoCap" assay.

Detection methods for viruses associated with little cherry disease were also addressed. This disease is now known to be associated with two related viruses that are distinct and belong to different genera within the family *Closteroviridae*. Serological assays for Little cherry virus 2 (LChV2) are not routinely available, but molecular assays for LChV2 were developed many years ago (Eastwell & Bernardy, 2001). The development of reliable detection methods for Little cherry virus 1 (LChV1) have been evasive. Through this project, we revealed the diverse nature of the genomes of North American isolates of LChV1 relative to Eurasian isolates (Figure 2). With this knowledge, the first reliable molecular assays were developed (Bajet *et al*., 2008). Again, serological assays remain a very desirable objective for future development. The critical need for reliable detection of LChV1 has become much more evident in recent years. Coordinated efforts between this program and similar programs in Canada and Germany have confirmed that current biological methods for detection are unreliable. Furthermore, it has become evident that the host range of LChV1extends beyond sweet cherry. Several other *Prunus* spp. (peach, almond and plum) are now known to be symptomless carriers of the virus (Matic *et al.,* 2009). The latency of the virus in several fruit tree hosts and the difficulty of detection combine to create the potential for this virus to continue to make inroads into major cherry production regions. The mechanism(s) by which LChV1 is transmitted in the field, other than through the use of infected propagation material, remains unknown.

Orchards with poor fruit production were inspected for signs of little cherry disease (small, light colored fruit and late ripening). A small number of representative samples were collected from these orchards and tested by the above methods for the presence of the viruses known to be associated with

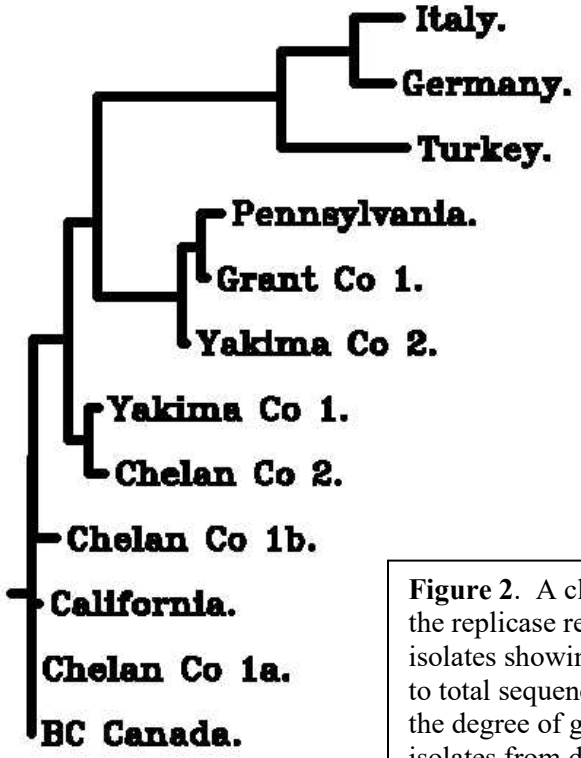

Figure 2. A cladogram of sequences from the replicase region of *Little cherry virus 1* isolates showing branch lengths in proportion to total sequence difference. This illustrates the degree of genetic variability of virus isolates from different geographic locations.

little cherry disease. From these data (Table 3), it is evident that LChV1 has become established in western North America. Because the symptoms expressed by infected trees are less severe than those

Table 3. Incidence of the viruses associated with little cherry disease in orchards with poor production.

Orchard designation and county	Trees with		
	LChV-1 only	LChV-2 only	Both LChV-1 + LChV-2
Yakima Co., orchard 1	4/4	0/4	0/4
Yakima Co. , orchard 2	3/13	6/13	0/13
Chelan Co., orchard 1	2/10	3/10	1/10
Chelan Co., orchard 2	0/3	0/3	1/3
Grant Co., orchard 1	10/18	0/18	0/18
Total Positives/Total Assayed	**19/48**	**9/48**	**2/48**

symptoms induced by LChV2, there is a greater tendency to assume that poor tree performance is the result of horticultural practices. The data generated in this project increases our ability to discern the underlying cause of the poor yields and to address that cause appropriately.

Impact and economic benefits: All of the virus diseases studied in this project are present in cherry production areas of western North America. The diseases associated with these viruses often resemble physiological conditions or symptoms induced by other pathogens. Therefore, it is critical that the grower has the tools to discriminate between potential underlying causes of poor cherry production and tree growth. The incorrect diagnosis would result in ineffective and frequently costly investments in remedial treatments with little or no relief from poor production.

The viruses from the rusty mottle group of viruses and those associated with little cherry disease directly impact fruit quality to different degrees. Trees infected with one or more of these viruses can display an extremely diverse array of symptoms that, in many cases, can only be distinguished from symptoms caused by other pathogens or agricultural practices with great difficulty. By increasing awareness of these viruses in the grower community and by providing the diagnostic tools for them, we hope to increase the ability with which diseased trees are identified. Viruses diseases do not respect property boundaries so these concerns are an industry issue. As the frequency of on-farm propagation increases, so does the need to ensure that these trees are free of deleterious viruses.

References cited in this report:

Bajet NB, Unruh TR, Druffel KL, Eastwell KC. 2008. Occurrence of two little cherry viruses in sweet cherries in Washington State. Plant Disease 92:234-238.

Eastwell KC, Bernardy MG. 2001. Partial characterization of a closterovirus associated with apple mealybug-transmitted little cherry disease in North America. Phytopathology 91:268-273.

Foissac X, Svanella-Dumas L, Gentit P, Dulucq M-J, Marais A, Candresse T. 2005. Polyvalent degenerate oligonucleotides reverse transcription-polymerase chain reaction: A polyvalent detection and characterization tool for trichoviruses, capilloviruses, and foveaviruses. Phytopathology 95:617-625.

Matic S, Minafra A, Sanchez-Navarro J, Pallas V, Myrta A, Martelli, GP. 2009. 'Kwanzan stunting' syndrome: detection and molecular characterization of an Italian isolate of Little cherry virus 1. Virus Research 143:61-67.

EXECUTIVE SUMMARY:

Cherry leafroll virus continues to encroach into cherry production regions of the Northwest. Grower access to diagnostic tests at WSU-Prosser for identifying infected trees is helping to reduce potential sources of inoculum. Root grafting is a major route of virus transmission and the judicious selection of appropriate rootstocks may be very helpful in minimizing the damage inflicted by cherry leafroll virus to our industry. At least one rootstock has been identified that responds to cherry leafroll virus with a hypersensitive reaction; this may be the foundation of one strategy to reduce the spread of disease. Pollen of infected trees is a rich source of infectious virus particles, and it has been assumed that pollen is at the center of long distance spread of cherry leafroll virus. Our data suggest that the virus does enter fruiting structures from infected pollen, but this translates into a new tree infection relatively infrequently, if ever. This lack of frequency makes the possibility of scientifically monitoring the migration of virus past the abscission layer between the pedicel and the fruiting spur impractical. As a consequence, studies should now be aimed at alternate means of aerial transmission.

The rusty mottle group of viruses causes several serious diseases in Pacific Northwest cherry orchards. The group consists of a complex array of virus genotypes that induce many symptoms that differ in appearance and severity. Viruses in this group appear to express symptoms that are very dependent on the specific variety that is infected, with symptoms ranging from none to severe. This enables the viruses to be distributed in varieties that act as symptomless carriers. Once planted in juxtaposition to other varieties, the viruses then infect sensitive cultivars with the possibility of causing severe crop loss. These viruses appear to spread naturally in orchard settings, but it is not clearly understood how members of this group of viruses are transmitted other than by propagation. Research has validated broad spectrum tests that can reliably detect viruses of this group. However, further refinement is needed in order to discriminate between those viruses that may be symptomless from those that can cause significant reduction in tree productivity. The first attempt to produce antibodies that can be used as the basis for a serological assay for these diverse viruses was very encouraging. Using the reagents developed in this study, a single ELISA will detect approximately one-half of the virus isolates that cause disease in the western states. Modifications to this initial process should be implemented to expand the range of viruses that can be detected. At the very least, future work to improve serological assays could produce complementary assays to expand the range of virus isolates detected.

Increased knowledge of Little cherry virus 1 is leading to greater concern about its role in cherry production. The genetic variability of this virus created great difficulties in developing accurate diagnostic methods. We identified well conserved portions of the genome that permitted the development of molecular assays that will detect all known strains. As our ability to detect and confirm the presence of the virus increased, it became apparent that the virus is already entrenched in major sweet cherry production areas. Concomitant with this observation, other programs have identified several additional *Prunus* species as symptomless carriers of Little cherry virus 1. Therefore, the potential exists for this virus to increase in its importance in the cherry industry. The means by which this virus is transmitted other than through the use of infected propagation material is unknown.

FINAL PROJECT REPORT

Project Title: Chemical genomics

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Other funding sources: None

Total Project Funding: 60,000

RECAP ORIGINAL OBJECTIVES:

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

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

Our specific objectives were:

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

SIGNIFICANT FINDINGS

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

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

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

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

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

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

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

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

RESULTS & DISCUSSION

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

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

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

Figure 5 shows that six chemicals can be separated into two groups, negative group (No. 2, 3) and positive group (No. 1, 4, 5, 6) based on their effects on the fruit weight. They all increased fruit color as compared with GA control. As for fruit firmness, No. 4, 5, 6 had no significant difference as compared with GA control. Among six chemicals, No. 4 showed the best in all measurements. Figure 6 are the photos exhibiting the effects of No. 4 chemicals on fruit ripening. In the same tree, the fruits sprayed with No. 4 chemical were ripen a week to 10 days later than no spray fruits in the same tree. The fruit weight in sprayed fruits was significantly improved (~40% increase). It also had better effects on fruit weight, skin color than GA control. However, the fruit firmness was comparable with GA control. We made efforts on isolation of RNAs from cherry fruits treated with different chemicals, but the quality of RNA was not very good to proceed the subtraction cloning.

In conclusion, we have identified a few powerful chemicals which affect sweet cherry fruit quality and flower numbers. The tests on different locations and different varieties in different years indicate that these chemicals are more effective than GA. These chemicals may also have the potential for other tree fruits such as apple and pear.

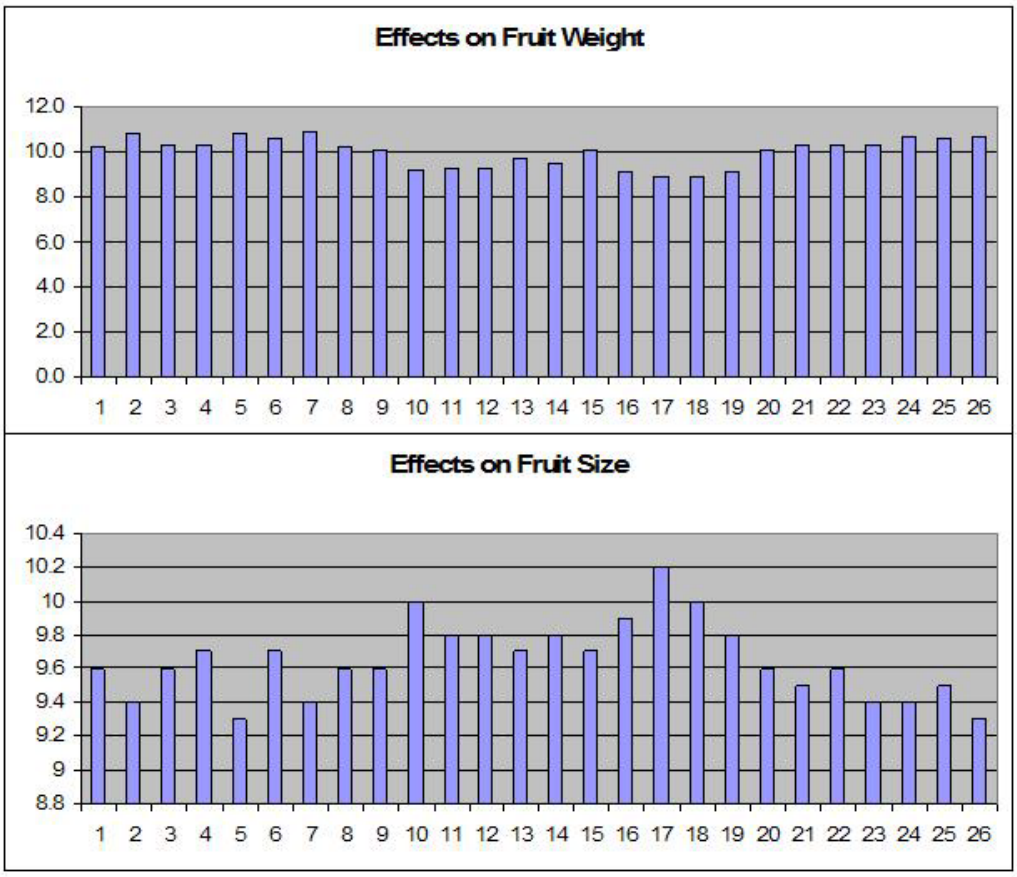

Figure 1. The effect of 25 selected compounds on the fruit weight and size. No. 26 represents the control which was treated with GA3. (Prosser, WA, 2007)

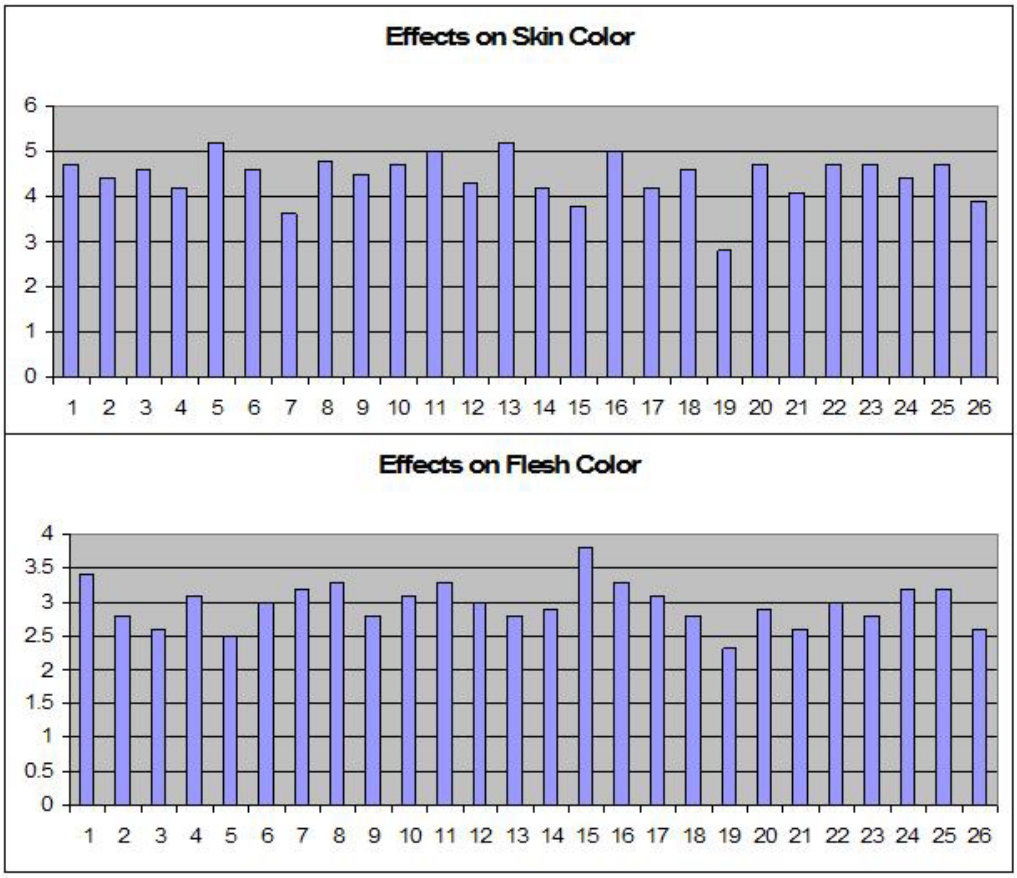

Figure 2. The effect of 25 selected compounds on skin color and flesh color. No. 26 represents the control which was treated with GA3. (Prosser, WA, 2007)

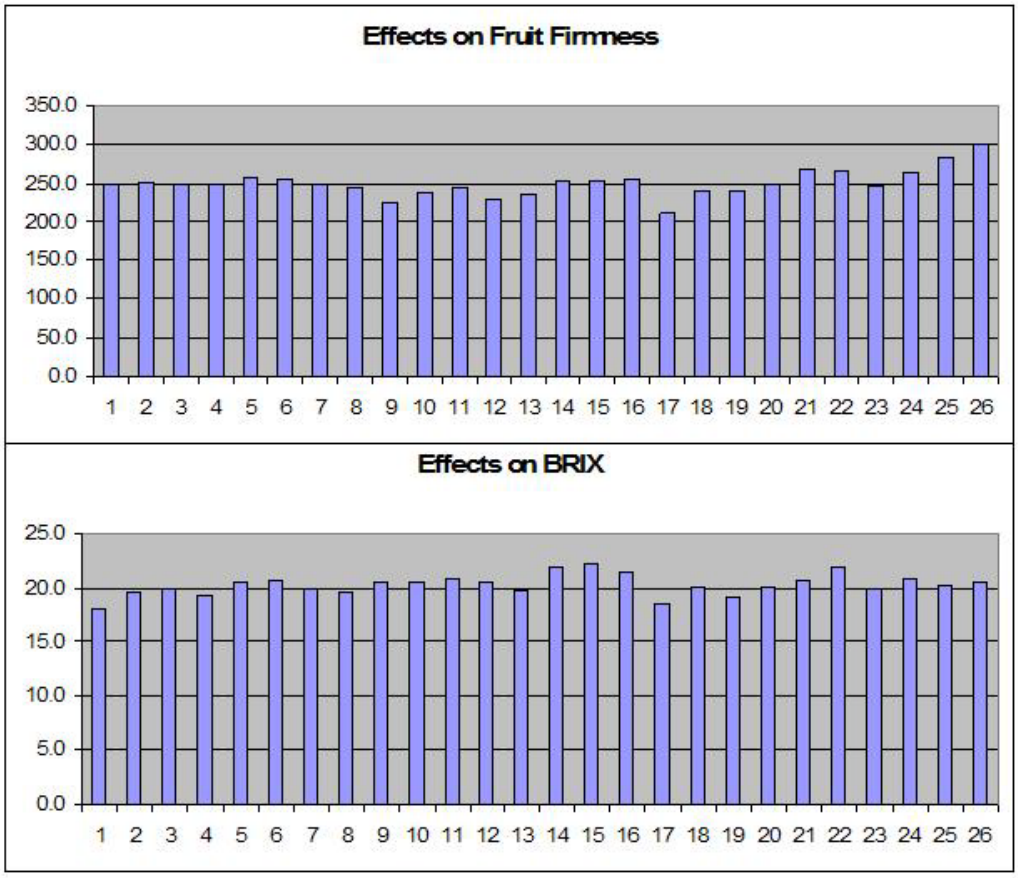

Figure 3. The effect of 25 selected compounds on fruit firmness and Brix. No. 26 represents the control which was treated with GA3. (Prosser, WA, 2007)

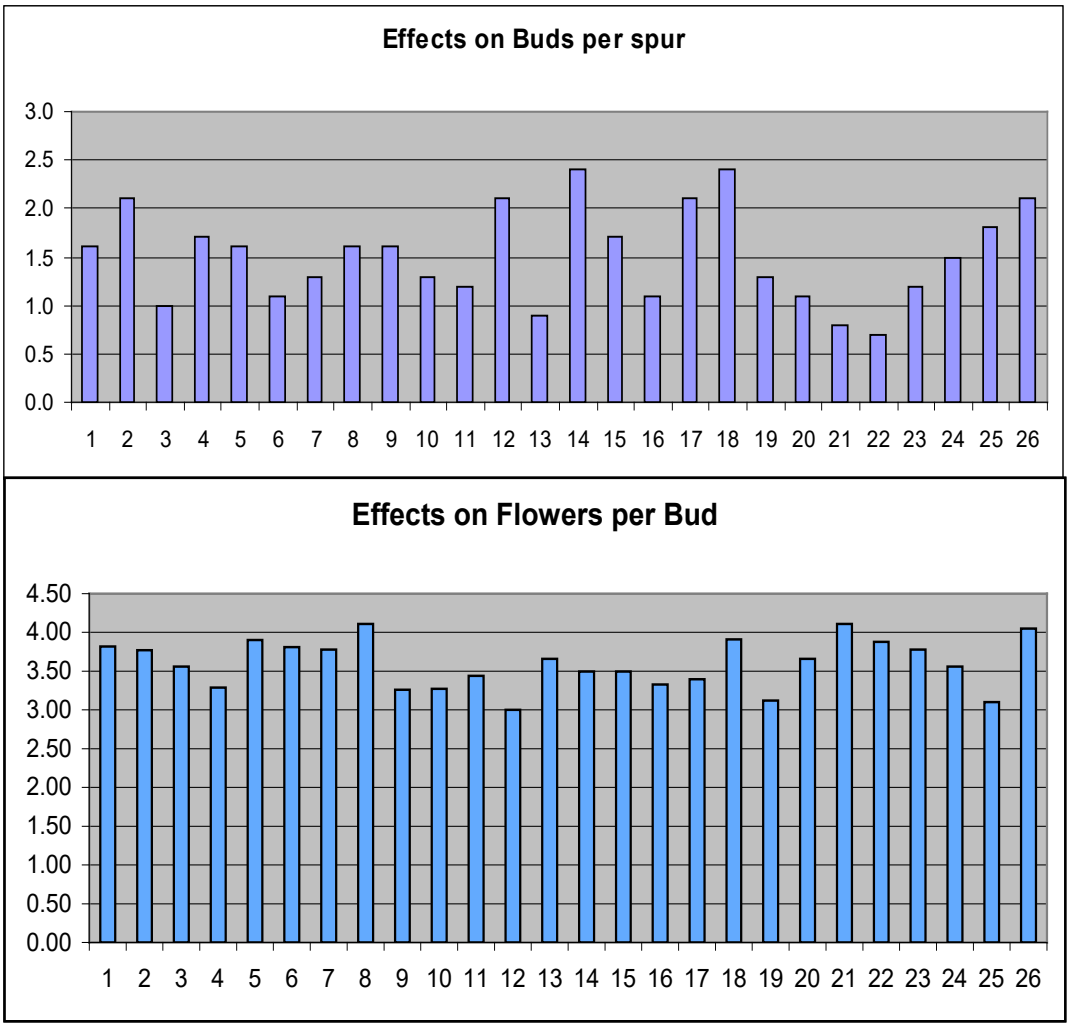

Figure 4. The effect of 25 selected compounds on bud numbers and flower numbers. No. 26 represents the control which was treated with GA3. (Prosser, WA, 2007)

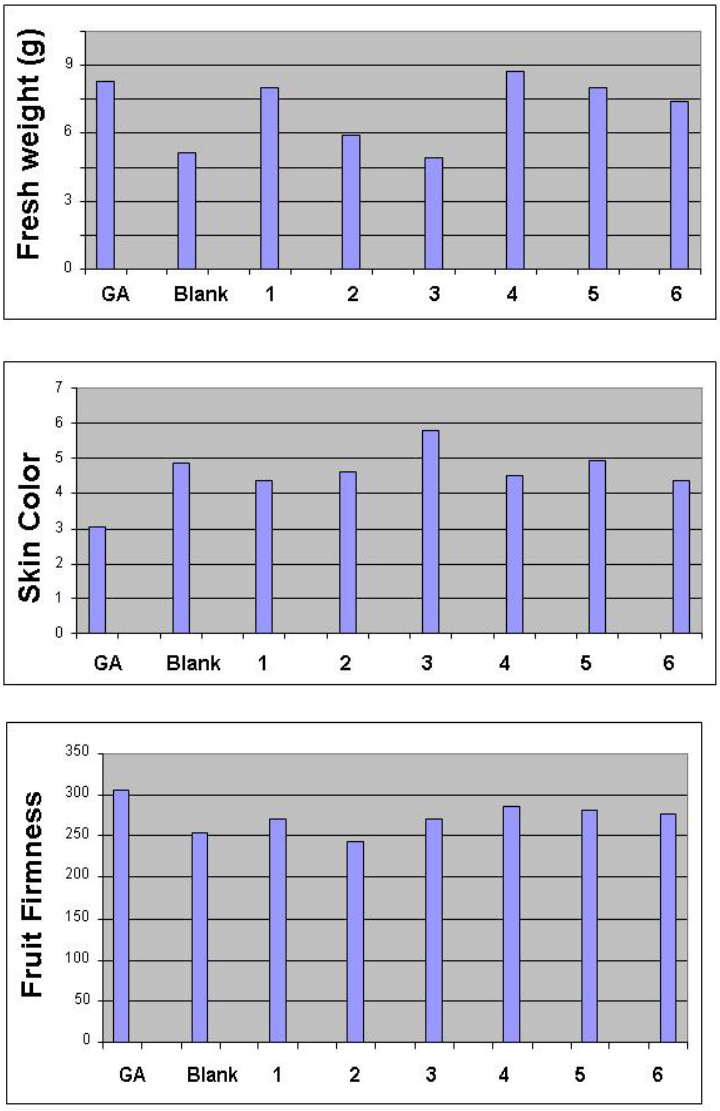

Figure 5. The effect of 6 selected compounds on the fruit weight, fruit size and fruit firmness. (Pullman, WA, 2008)

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

EXECUTIVE SUMMARY

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

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

To sum up, we have identified a few very powerful chemicals which affect sweet cherry fruit quality and flower numbers. These chemicals are more effective than GA. Besides, these chemicals which are effective in sweet cherry may work in other tree fruits such as apple and pear, too. Our results indicate that using chemical genomics approach can save time and money for tree fruits gene disco very and crop improvement.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number:

Project Title: Impact of harvest timing on fruit quality of sweet cherry cultivars

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Total project funding request: **Year 1**: $24,226 **Year 2**: $25,079

Other funding sources: None

WTFRC Collaborative expenses: None

Budget 1 Todd Einhorn
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Item	2009	2010	
Salaries	3,947	4105	
Benefits	2,053	2,199	
Wages			
Benefits			
Equipment			
Supplies	500	500	
Travel	1,500	1,500	
Miscellaneous			
Total	8,000	**8,304**	

Footnotes: Salaries include ¼ time Associate in Research to organize field sites, follow crop phenology, manage and collect data at harvest and throughout cold storage. Travel is for regional orchard monitoring to determine bloom dates, follow development and harvest.

Budget 2 Lynn Long
Organization Name: Wasco County Extension **Contract Administrator: Dorothy Beaton**
Telephone: 541-737-3228 **Email address: dorothy.beaton@oregonstate.edu**

Item	2009	2010	
Salaries			
Benefits			
Wages[1]	4,500	4,680	
Benefits (10%)	450	468	
Equipment			
Supplies			
Travel[2]	1,000	1,000	
Miscellaneous			
Total	5,950	**6,148**	

Footnotes:
[1] Time-slip assistance for harvest, data collection, and fruit quality analyses
[2] Travel to plots and cold storage

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

Item	2009	2010	
Salaries			
Benefits			
Wages	8,000	8,320	
Benefits	776	807	
Equipment			
Supplies	500	500	
Travel	1,000	1,000	
Miscellaneous			
Total	10,276	**10,627**	

Footnotes:

Objectives

1) Quantify and identify changes in quality attributes of ten cultivars, ranging from early to late season ripening, throughout final fruit growth and ripening in OR (Einhorn/Long) and WA (Whiting).
2) Determine the effect of harvest timing on quality throughout storage, including pitting incidence (Einhorn, Whiting, Long).
3) Determine the value of growing degree day models to predict growth, development and maturity of cherry varieties growing at different sites (Einhorn, Whiting, Long).
4) Develop extension materials (e.g., color charts) for identifying optimum harvest timing for new cultivars (Long).

Significant Findings

- Variability in cherry fruit quality is high
- Skin color darkened with advancing harvest dates, though the degree and rate of darkening were cultivar dependent
- Although early, mid and late harvests of individual cultivars occurred when a pre-determined average fruit color was attained, a wide range of color classes was observed within each harvest date
- Not all fruit quality attributes consistently changed with skin color. Firmness and stem retention force typically decreased, while weight, soluble solids, and mesocarp color increased
- Fruit quality attributes were not necessarily consistent when fruit of a given cultivar and skin color (same CTIFL score) were harvested at different dates
- Firmness tended to increase with storage time, stem retention force decreased, and acids and sugars were inconsistent
- Weight loss was minimal throughout storage
- Pitting severity of sweetheart was not related to harvest timing
- Pitting severity of Bing and Lapins slightly increased with time in storage, and skin color

Methods

Objective 1: Ten culitavrs were evaluated: 'Chelan', 'Tieton', 'Benton', 'Cowiche', 'Bing', 'Lapins', 'Skeena', 'Regina', 'Selah', and 'Sweetheart'. Two sites were used for each cultivar (one in WA and one in OR), with the exception of 'Cowiche', due to a lack of bearing trees identified in OR. For each cultivar, three similar trees were identified as representative of the general state of the orchard. The first of three successive harvest dates began when fruit entered the very early end of the commercial range. On each harvest date, occurring several days apart and determined by the rate that skin color changed, one entire tree was strip-picked, fruit were brought to the lab, and each fruit was classified according to its skin color using a CTIFL color scale (1, light red-7, black). Within each CTIFL class, five replicates of five fruit (25 total fruit) each were randomly selected for evaluation of quality attributes. Quality attributes assayed were: titratable acidity (TA), soluble solids (SS), fruit firmness (FF), stem retention force, weight, diameter, row size, and mesocarp fruit color.

Objective 2: For the methods outlined above, 200 fruit were chosen randomly from each CTIFL class of each harvest date, for all cultivars. Fruit were placed in poly liners, boxed and held at 1° C. Beginning one week from the harvest date, 25 fruit were chosen from each CTIFL class and analyzed, as discussed above, weekly for a one month period (10 cultivars* 3 harvest dates * 4 post-harvest sampling dates * 2 sites for each = 240 sampling periods, each comprised of multiple CTIFL classes).

Two methods for evaluating pitting incidence and severity were employed: 1) artificial pitting using a tool ('Bing' and 'Lapins'), and 2) a commercial packing line ('Sweetheart'). For Bing and Lapins pits were induced opposite the suture side on the equatorial region of fruit using an instrument (developed and provided by F. Kappel) designed to mimic the occurrence of impact injury by dropping a 10 gram steel ball on the fruit surface. Following the mid harvest timing, fruit was immediately cooled to 4° C, separated into CTIFL classes (125 fruit for each class), pitted, then placed in 1° C storage and evaluated weekly for one month. Pits were classified according to the diameter of the pit. A four point scale was used to report pit severity (4 = severe, 1= no pitting), based on a previously published correlation between visual assessment of pit severity and pit diameter (Toivonen et al., 2004). For 'Sweetheart', whole bins of fruit harvested at four harvest timings, chosen when trees reached a pre-determined average CTIFL, were handled commercially, run over a packing line, place in lined boxes, and held at 1° C until evaluated. At three and four weeks postharvest, entire contents of 20 lb cherry boxes were analyzed for pitting. Briefly, total fruit per box were divided into CTIFL color classes, and pits were counted on all fruit within each color class. Further, twenty-five fruit were then chosen from each pit severity category, and each pit was measured (diameter).

Objective 3: Full bloom and harvest dates were recorded for each cultivar. Orchards with meteorological stations present, or nearby were selected. Growing degree day models will be constructed this fall/early winter.

Objective 4: Digital images were taken of fruit, and will be compiled for extension education materials.

Results and Discussion

Harvest timing: Table 1 shows fruit quality attributes averaged across all fruit for each of three harvest dates per cultivar. Although skin color was shown to advance with later harvests, fruit quality attributes did not necessarily respond in a consistent manner. In fact, inconsistencies such as those occurring for fruit size (diameter, row size and weight) for Bing, Lapins and Sweetheart (Table 1), are largely influenced by factors other than advancing maturity, such as cropolad. Some attributes such as firmness and stem retention force declined with advancing maturity, while others (sugars) typically increased, albeit dissimilarly, and in some cases opposite responses were observed. Table 1 means are derived from all skin color classes represented at each harvest. The general relationships between individual fruit quality attributes and the timing of harvest is not strong, and supports frequent observations of high variability of sweet cherry fruit. Further, it underscores the need to examine the contributors to this variability.

Although maturity advanced with successive harvesting, when averaging skin color of all fruit of an entire canopy, by the very nature of averaging, the ability to link an individual fruit's color with a quality attribute is diminished. Therefore, at each harvest date all fruit were classified according to their skin color. An example of the frequency distributions for skin colors comprising each of the three successive harvest dates is provided for Chelan (Fig 1). Marked variation of skin color within harvest date was evident, though as color advances a narrower range of classes contributes to the overall CTIFL. Between three and five color classes were typically associated with each harvest event, irrespective of cultivar. The exception was Regina, which advanced very uniformly, over a fairly long time span. Data are still being analyzed, however preliminary results indicate that significant tree to tree variability may be responsible for some of the poor coupling observed among attributes and color class, and thus requires the testing of replicate trees for each harvest date. Subsequently, we will evaluate this on several cultivars in year two. Of the fruit quality attributes

analyzed, stem retention force typically declined with advancing color, and with the exception of Selah (100-200g), most varieties maintained adequate retention force, even at darker CTIFL scores. For the third harvest timing, CTIFL 6, Selah had values as low as 51 g. In fact, all color classes (4-6) of Selah harvested at the late timing had a large proportion of cherries arrive in the stemless condition. Fruit firmness, total acids, and weight all appeared to increase with color. One surprising finding is that quality attributes of a given color class for a specific cultivar, can be markedly different on different harvest dates. This suggests that degrees of maturity and/or ripening can exist in the absence of observable changes in surface color. Statistical analyses are ongoing and advanced models are currently being explored to determine the strength of preliminary relationships.

Pitting: There was a weak positive relationship between pitting severity and color for Bing and Lapins sampled from the mid harvest timing. Pitting slightly increased with storage duration, and was higher for advanced maturity fruit (CTIFL 5 for Lapins, and CTIFL 5-7 for Bing). This is in agreement with work performed by Zoffoli et al. (personal communication). For Sweetheart, fruit were harvested over four levels of maturity. The frequency distribution in skin color for each harvest date is shown in Figure 2. All fruit had some degree of pitting (based on pit number/fruit), with proportionately more fruit registering 4-6 pits, irrespective of harvest timing (Figure 3). Diameter of all pits on fruit was measured and grouped into three classes (0-2mm, 2-4 mm, >4 mm). Data is still being analyzed, but it is evident that the highest proportion of pits fell into the 2-4 mm range, followed by >4 mm, then 1-3 mm, and this does not appear to be influenced by fruit maturity.

Storage: As with harvest timing, inconsistent responses of Fruit firmness values were consistently higher with increasing time in storage. Soluble solids were largely unrelated to storage time, as were acids. Total acids have previously been shown to reduce with increasing storage time (Drake and Elfving, 2002; Proebsting and Mills, 1981), though these studies were limited to Bing and Lapins. Water loss, determined indirectly through weight change, did not appear to be significant.

Data analyses are presently underway to determine the interactions among storage time, color class, harvest timing and quality attributes, for each cultivar. Multiple regression analysis is currently being utilized, and it is expected that this type of analysis will greatly improve our ability to determine relationships among factors, both within and between sites (OR and WA).

Table 1. Effect of harvest timing on fruit quality attributes (FF= fruit firmness, SS=soluble solids, TA=total acids) for nine cultivars harvested from different sites in Oregon. Data are averages of the total CTIFL class means represented at each harvest.

Cultivar	Harvest	Avg. Skin Color	Fruit wt.	Stem Retn.Force	FF	Fruit Dia.	Row Sz.	Mesocarp Color	SS	TA
		CTIFL	(g)	(g)	(g/mm)	(mm)		CTIFL	(%)	(%)
Bing	Early	4.5	10.0	629.2	213.0	27.2	10.0	4.5	22.2	1.1
	Mid	5.0	8.1	504.8	215.3	25.5	11.0	4.0	19.6	0.6
	Late	5.4	8.4	427.2	240.7	26.5	10.5	4.4	20.5	0.6
Benton	Early	3.8	10.7	612.4	285.9	29.2	9.5	2.9	19.3	0.9
	Mid	4.2	11.1	766.8	272.5	29.2	9.5	2.1	19.4	0.8
	Late	4.5	11.8	682.6	227.6	29.7	9.5	3.6	19.7	0.7
Sweetheart	Early	4.4	9.4	666.7	326.7	27.8	10.0	2.8	19.0	0.8
	Mid	4.5	10.2	566.4	289.6	28.7	10.0	2.9	19.8	0.8
	Late	4.9	8.5	716.3	264.0	26.4	10.5	3.3	20.2	0.6
Skeena	Early	4.3	7.3	558.0	304.2	26.1	10.5	3.3	16.7	0.4
	Mid	5.9	10.5	423.7	263.7	29.3	9.5	4.8	21.4	0.5
	Late	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Selah	Early	3.5	13.7	308.5	291.5	32.1	9.0	2.1	21.0	0.6
	Mid	3.7	12.6	464.9	209.5	31.0	9.0	2.2	17.3	0.6
	Late	4.8	11.9	100.5	164.9	29.2	9.5	3.7	21.2	0.8
Regina	Early	5.4	11.5	788.8	194.7	28.2	10.0	4.3	21.5	0.6
	Mid	5.7	12.3	825.7	190.0	28.9	10.0	4.5	23.0	0.7
	Late	6.3	12.3	771.9	202.3	28.7	10.0	4.9	23.2	0.8
Lapins	Early	4.3	11.2	726.2	246.9	30.2	9.0	3.0	17.5	0.6
	Mid	4.5	8.6	386.3	238.5	26.2	10.5	3.5	17.5	0.6
	Late	4.9	11.0	379.9	196.6	28.3	10.0	4.0	19.5	0.7
Tieton	Early	3.2	9.3	854.7	268.8	27.4	10.0	2.9	16.9	1.2
	Mid	5.4	9.9	560.3	235.5	27.8	10.0	5.0	19.7	0.7
	Late	6.4	14.2	658.5	266.4	32.2	8.5	6.0	22.5	0.9
Chelan	Early	4.9	6.9	474.5	268.5	24.8	11.0	4.1	16.7	1.1
	Mid	6.0	7.9	408.0	278.2	25.9	11.0	5.7	21.4	1.0
	Late	6.4	9.6	340.2	284.7	27.6	10.0	6.0	21.2	1.0

n.d.= no data.

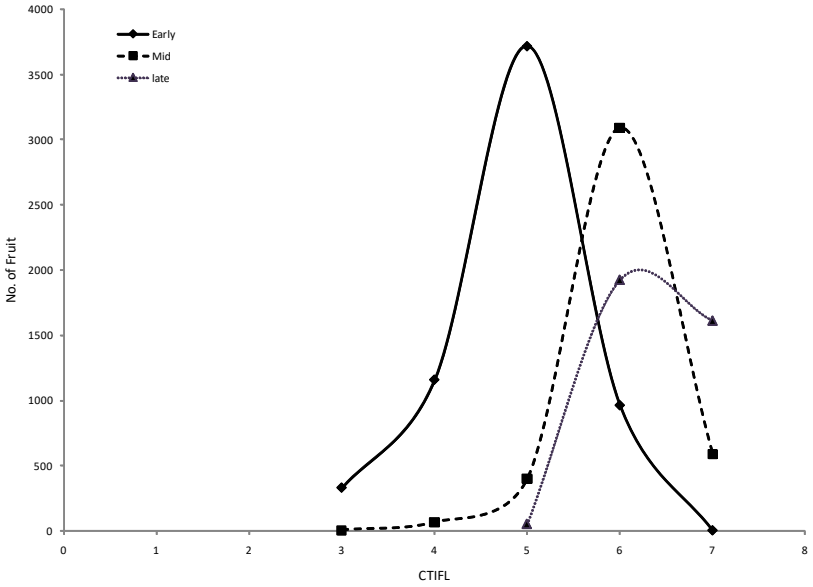

Fig 1. Frequency distribution of 'Chelan' skin color using the CTIFL color scale (3-red, 7-black), for three separate harvest dates (early, mid and late). On each date one whole tree was strip-picked, fruit were brought to the lab, and each fruit was classified according to its skin color. Total fruit per harvest was 6,165, 4,155 and 3,585 for the early, mid and late harvests, respectively. All cultivars and harvest dates received the same protocol.

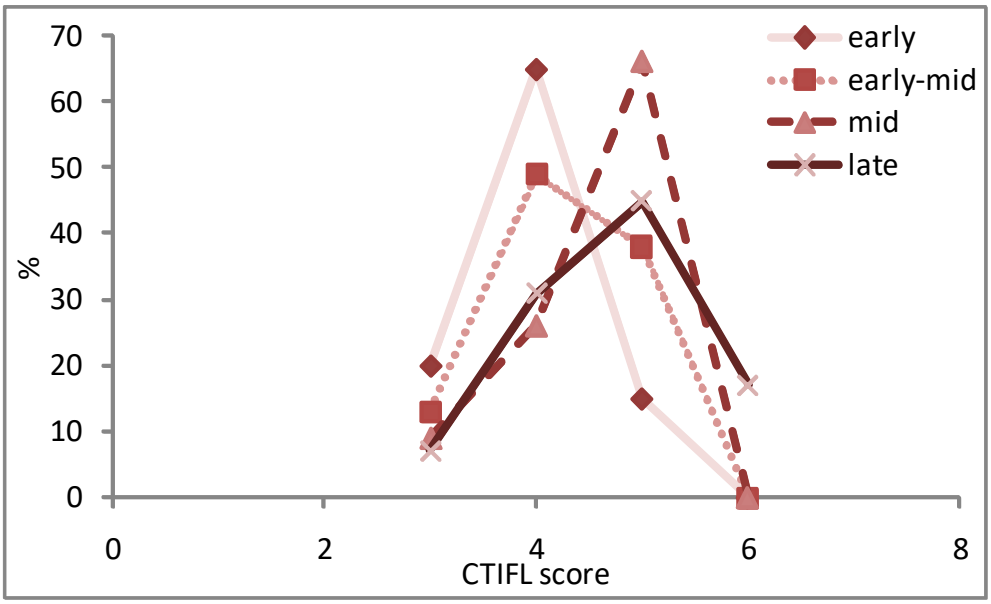

Fig 2. Frequency distribution of 'Sweetheart' fruit skin color (CTIFL score: 2-light red, 7- Black) for total fruit in 20 lb boxes picked on four separate harvest dates. Fruit was harvested, run over a packing line, and held at 1° C for one month.

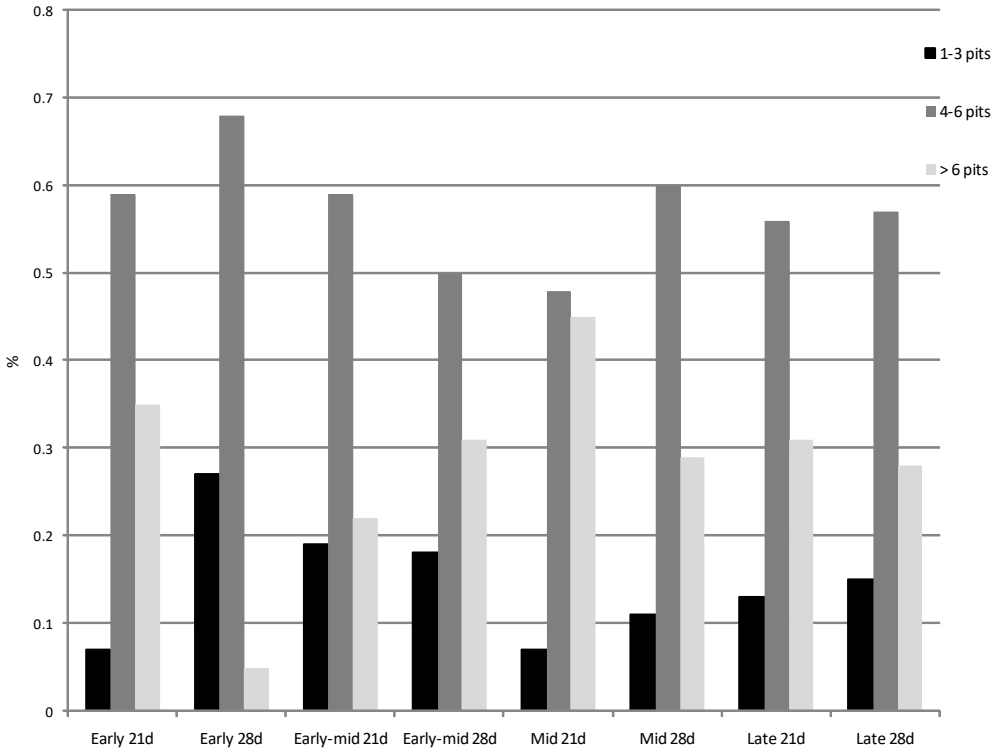

Fig 3. Effect of harvest timing (Early, Early-mid, Mid and Late) on pitting frequency, reported as percent of fruit with either 1-3, 4-6, or >6 pits. Analysis was performed on entire contents of 20 lb cherry boxes, on 21 and 28 days post-harvest. Frequency distribution of color classes within each harvest timing is provided above (Fig 2).

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

Project Title: Irrigation and fertilization for optimal cherry fruit quality

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Cooperators: Jac le Roux, Don Fesler

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

Other funding sources: None

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

- Reducing irrigation water by 20 and 40 % of Control levels, did not negatively affect yield, fruit size, or quality (soluble solids, total acids, firmness) at harvest, and following four weeks postharvest storage, of drip irrigated 'Tieton'/'Mazzard' trees
- Reducing irrigation water by 20 and 40 % of Control levels, did not negatively affect yield or quality of micro-sprinkler irrigated 'Lapins'/'Mazzard' trees, though fruit size was slightly higher for the control and RDI treatments
- Stem water potential declined with increasing % of water withholding, albeit nonsignificantly
- Trees provided 20 and 40 % less water than controls, utilized significantly more water from deeper profiles than control trees to meet their evaporative demand. This utilization ameliorated water stress and explains the lack of any observable adverse effects on yield and fruit quality from deficit irrigated treatments
- Photosynthesis, transpiration and stomatal conductance were slightly, but not significantly, reduced by degree of water withholding
- Annual per acre water savings for 'Lapins' 80 % of control, 60 % of control, and RDI treatments was 116,160 gallons, 232,320 gallons, and 69,696 gallons, respectively
- Annual per acre water savings for drip irrigated 'Tieton' 80 % of control, 60 % of control, and RDI treatments was 41,817 gallons, 83,635 gallons and 55,757 gallons, respectively
- Seasonal trunk cross-sectional area increase was slightly greater for Control irrigated trees, at both sites
- Frequency of irrigation (replacement of tree-water-use every other day, or once per week) for drip irrigated 'Tieton'/'Mazzard' did not significantly affect trunk growth, yield or fruit quality at harvest or following 4 weeks postharvest storage
- Nitrogen delivery method (fertigation or broadcast) and concentration (100 lbs/a, 60 lbs/a) did not significantly influence yield, fruit quality attributes or trunk growth, although effects of N would likely not be realized until the season following application

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, in 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 (12 hour sets) via microsprinklers, and were: 1) Control [100 %], 2) 80 % of control, 3) 60 % of control, and 4) regulated deficit irrigation (RDI), in which trees received an identical rate as the 60 % treatment, from bloom through pit-hardening, control levels from the end of pit hardening until harvest, and then 60 % for the remainder of the postharverst period. 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 week from full bloom, or provided through the irrigation system (fertigation). Ferigation 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 a clock. Rates were 100, 100, and 60 lbs/a, for the broadcast, fertigation-high, and fertigation-moderate treatments, respectively.

Soil moisture was measured at three sites per replicate to a depth of 3 feet, in 6 inch intervals using a neutron probe.

Stem water potential was performed using a pressure bomb every 7-10 days, to study plant water status. Briefly, shoot leaves were selected in the mid portion of one-year-old growth, bagged, and allowed to equilibrate for no less than 20 minutes prior to measurement. Leaves were bagged roughly 1 hour prior to solar noon so measurements could bracket solar noon (+/- 1 hr).

Photosynthesis and stomatal conductance was measured using an infra-red gas analyzer. Measurements were taken throughout the season on one tree per replicate (four fully developed leaves on current-season shoot growth per tree).

Trunk cross-sectional area was measured 20 cm above the graft union prior to bloom, at harvest, and again near leaf drop. At harvest individual tree yield was taken, and 100 fruit subsamples were collected from each treatment tree for evaluation of fruit quality attributes at harvest, and again following four weeks of storage at 1° C.

Objectives 2 and 3: A nine-year-old '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 (same treatments as described in experiment 1) were applied to replace tree water use via drip irrigation either once weekly (12 hour set), or every other day. Irrigation volume was controlled at the head of each row by mechanical shutoff flow meters.

Nitrogen was applied to all treatments through the dripline (fertigation) at a rate of 12.5 lbs/week once weekly over an eight week period (100 lbs/acre).

All measurements were performed as outline in experiment 1, with the exception of soil moisture, which did not begin until late summer.

Results and Discussion

For two separate sweet cherry orchards (Lapins and Tieton) significant water savings were achieved without sacrificing yield, fruit size or quality. Much of these results can largely be explained from a combination of stem water potential and soil moisture measurements. Stem water potential is a sensitive indicator of the hydraulic status of the plant. Under increasing water stress stem water potential values will be lower (more negative). Additionally, as the season progresses higher temperatures and lower relative humidity (in semi-arid regions) increase the vapor pressure deficit (VPD). These conditions increase the evaporative demand, and result in lower water potential values, even though plants may have ample soil moisture available. This is attributed to the fact that resistance to water flow occurs throughout the plant's hydraulic pathway, and as a consequence, plant

water uptake lags behind the increased evaporative demand at the leaf surface. In the present study water potential of both 'Lapins' and 'Tieton' trees receiving 80 and 60 % of irrigation replacement water, were lower than controls, albeit not significantly (Fig 1). Further, for the most part all plants responded similarly to changing VPD values (i.e., declining stem water potential with increasing VPD) (Fig 1).

Early in the spring from bud-break through pit-hardening ample water exists in the soil profile due to accumulation from winter/early spring precipitation events. Consequently, treatments that supplied limited irrigation during this period did not result in plant stress (Fig 1). This was the rationale for providing the RDI treatment trees with 60 % of control irrigation volumes prior to the cell expansion phase of fruit growth. However, as the season progresses evaporative demand increases and soil moisture reserves start to become depleted. In the microsprinkler 'Lapins' orchard this becomes apparent in the slight decrease in water potential for trees receiving 80 and 60 % of control irrigation on 69 days after full bloom (DAFB) (Fig 1A), and for the 'Tieton' orchard roughly 75-80 DAFB (Fig 1B-C). The fact that stem water potential values were not significantly lower for the 80, and certainly 60 % treatments, at either site, is somewhat surprising. Gas exchange (photosynthesis, transpiration and stomatal conductance) of deficit treated trees was slightly decreased relative to control, though not significantly (data not shown).

Seasonal trends in soil moisture taken at the 'Lapins' site is provided in Fig 2. Soil moisture in the top foot of soil is roughly 0.2 inches/foot lower in the 0.8 and 0.6 treatments as compared to control and RDI treatments, and equates to ~ 20 % moisture by volume (Fig 2A). In the two and three foot deep soil profiles, soil moisture is markedly reduced for the deficit irrigated treatments, by 0.3-0.6 inches per foot (Fig 2B,C), and extraction of water in the deeper soil profile increases with time (Fig 2 B,C). These data indicate that deeper roots were active in supplying the necessary water to meet an increasing evaporative demand and limiting soil moisture supply in the top foot of soil (Fig 2 A-C). Per irrigation event, 0.8 and 0.6 treatment trees received 36 and 72 gallons fewer than control trees, respectively. Assuming that control treatment trees are being supplied with the appropriate amount of water to meet their needs, it would take ~ four weeks for the 0.8 treatment to reach a deficit of 160 gallons, although ample water existed in the soil profile early in the season to lengthen this time period. Using the 15 x 18 foot Lapins spacing, a difference of an inch of available soil moisture (total from the three foot profile) is roughly equivalent to 157 gallons of water per tree, and therefore provides an explanation for the unobservable water stress in deficit treatments. The 60 % treatment would have used the equivalent amount of water in half the time, and these trees responded by extracting a greater amount of soil moisture a deeper depths (possibly below the measured 3 foot depth) (Fig 2 B,C). In addition, because photosynthesis decreases following harvest, transpirational water loss is lessened, and this further limits the rapid development of severe stress as the season progresses.

Yield was not affected by irrigation volume, frequency, nutrition amount or delivery (Tables 1 and 2), though in the first year this would be highly unlikely since fruit set has a proportionately greater influence over yield than individual cherry fruit size, and soil moisture, and nitrogen status, were likely non-limiting during the set period. However, 2010 fruit set and yield will be much more dependent upon treatments imposed this season. Given the source limited nature of sweet cherry in the pre-harvest interval, combined with high croploads, it is plausible that carbohydrate reserves may be further depleted in deficit treated trees. Trunk cross sectional area increase of the heavily cropped Lapins trees was < 4 % in the preharvest interval, and only ~ 3 % in the postharvest period, irrespective of treatment, as compared to a 5 and 17 % increase for Tieton trees, for the same periods. The inherent differences in productivity level between 'Lapins' and 'Tieton' can be seen for yield data (Tables 1 and 2). Lapins fruit size was slightly greater for Control treated trees, however this was a < 5 % increase, and when expressed as row size, all treatments peaked on 9.5 row. Sugars,

acids and firmness were not significantly influenced by irrigation or nutrition treatments, with the exception of SS for 'Tieton', measured following four weeks cold storage.

In year two, we will continue with all measurements, but will include weekly measurements of soil moisture in the 'Tieton' block. In addition, soil and leaf nutrient status will be quantified in the nutrient experiment, and more frequent gas exchange measurements will be performed. We will continue to look at the potential of ET modeling of whole canopy water use.

Tables and Figures

Table 1. Yield and fruit quality attributes (Fruit dia.= average fruit size; FF= firmness; SS=soluble solids; TA= total acids) at harvest and 4 weeks postharvest (PH) for 'Tieton'/'Mazzard' trees receiving different levels of irrigation amount, and frequency.

Treatment	Yield	Fruit dia.	FF	SS	TA	FF-PH	SS-PH	TA-PH
	(Kg tree^{-1})	(mm)	(g/mm)	(%)	(%)	(g/mm)	(%)	(%)
Irrigation Amount								
100%	42.4	29	240.6	18.2b	0.56	266.7	16 b	0.57
80%	41.8	28.6	227.5	18.2b	0.53	255.6	17.5a	0.59
60%	38.3	28.4	231.4	18.6ab	0.58	253.9	17.2a	0.58
RDI	40.5	28.9	225.1	18.8a	0.59	260	17.4a	0.58
Frequency								
High	41	28.8	230.1	18.3	0.59	258.5	17	0.57
Low	40.6	28.7	232.2	18.5	0.54	259	17	0.59
Pr > F								
Frequency	0.892	0.681	0.513	0.249	0.412	0.757	0.9	0.345
Irrigation	0.726	0.365	0.09	0.075	0.829	0.355	0.03	0.911
F x I	0.913	0.253	0.473	0.769	0.919	0.02	0.71	0.583

Table 2. Yield and fruit quality attributes (Fruit dia.= average fruit size; FF= firmness; SS=soluble solids; TA= total acids) at harvest and 4 weeks postharvest (PH) for 'Lapins'/'Mazzard' trees receiving different levels of irrigation, and nutrients.

Treatment	Yield	Fruit dia.	FF	SS	TA	FF-PH	SS-PH	TA-PH
	(Kg tree^{-1})	(mm)	(g/mm)	(%)	(%)	(g/mm)	(%)	(%)
Irrigation Amount								
100%	82.1	30.8a	278.8	18.3	0.78	314.2	17.4	0.71
80%	80.6	30b	274.4	18.3	0.78	311.3	17.7	0.72
60%	84.2	29.7b	274	18.2	0.75	311.8	17.5	0.7
RDI	78.4	30.3ab	268.8	18.4	0.74	305.6	17.8	0.72
Fertilization								
Fertigation-High	81	30.3	277	18	0.75	314.9	17.4	0.73
Fertigation-Mod.	79.7	30	276.3	18.3	0.79	310	17.5	0.72
Broadcast-High	83.3	30.3	268.7	18.6	0.75	307.8	17.8	0.7
Pr > F								
Fertilization	0.563	0.44	0.136	0.153	0.29	0.445	0.178	0.233
Irrigation	0.517	0.024	0.554	0.961	0.568	0.326	0.5	0.28
F x I	0.768	0.385	0.524	0.178	0.958	0.382	0.465	0.636

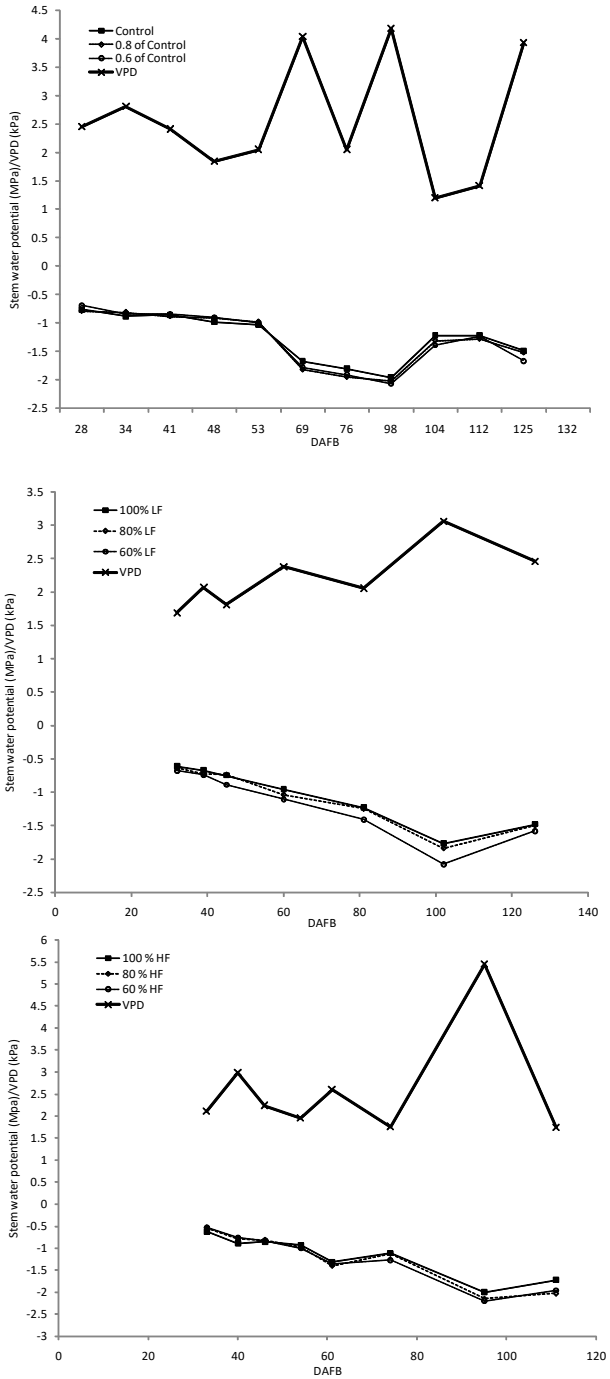

Fig 1. Effect of irrigation level (100, 80, 60 %) on stem water potential (MPa) for 'Lapins'/'Mazzard' (A), 'Tieton'/'Mazzard' irrigated once per week [LF] (B), and 'Tieton'/'Mazzard' irrigated every other day [HF] (C). Measurements were taken between 12:00-15:00 PST, on bagged leaves located in the middle portion of one-year branches. Vapor pressure deficit (VPD) values are the means of 20 min. measurements taken during the sampling periods. Water potential data points are means of 5 replicates (n=4). RDI treatment omitted for purposes of clarity.

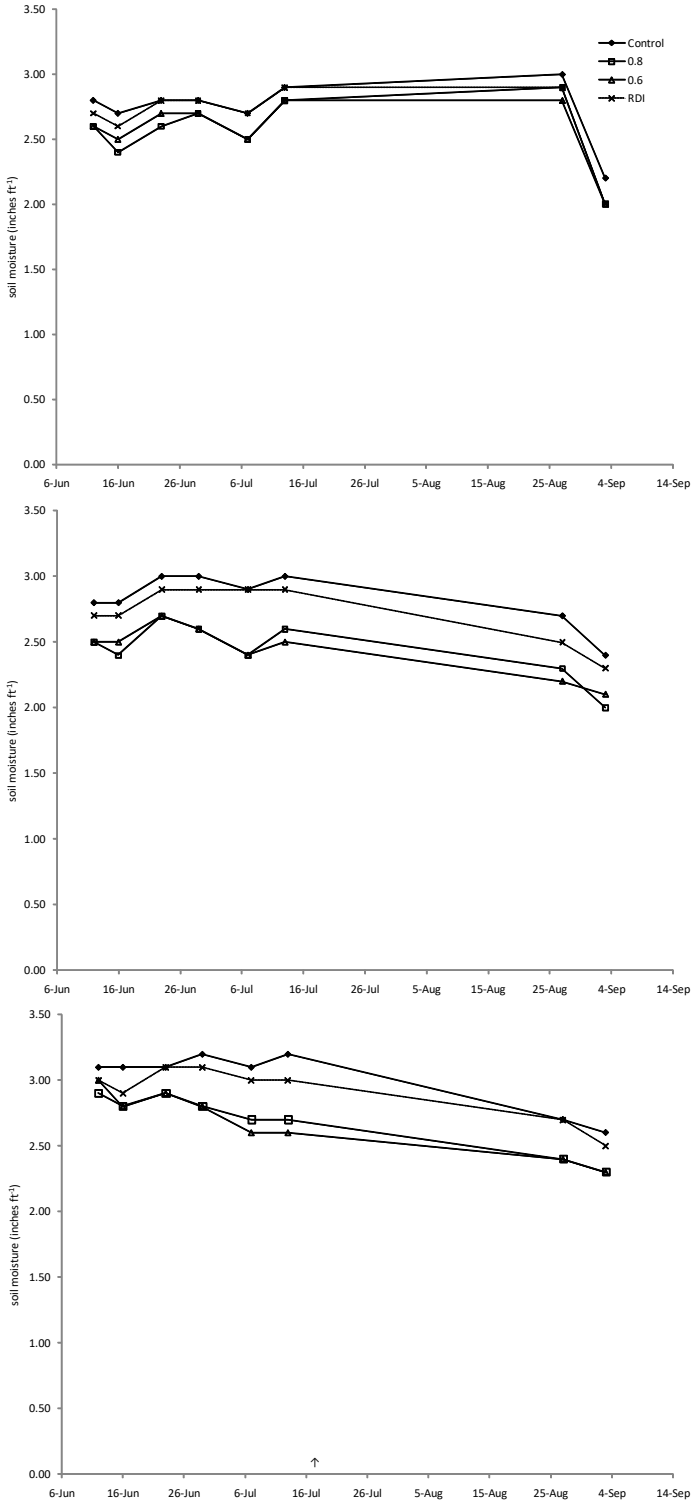

Fig 2. Effect of irrigation level (100, 80, 60 % or RDI) on volumetric soil moisture content (inches per foot) of the soil profile at (A) 0-30, (B) 30-60, and (C) 60-90 cm depths for 'Lapins'/'Mazzard'. Arrow indicates harvest date. Each data point is the mean of 5 replicates (n=3).

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

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

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

Total project funding request: **Year 1**: $56,785 **Year 2**: $55,905

Other funding sources: None

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

Item	**(2009)**	**(2010)**	
Salaries	29,500	29,500	
Benefits	18,585	18,585	
Wages	4,000	4,000	
Benefits			
Equipment			
Supplies			
Travel	500	2,000	
Misc.(plot charges)	4,200	1,820	
Total	56,785	**55,905**	

Footnotes:

Continuing Proposal to the Agricultural Research Foundation
Oregon Sweet Cherry Commission

Project title: Horticultural management systems for high value fresh and brine cherries
Objectives (for 2010**):**

1. Identify and evaluate cherry cultivars, rootstocks and training systems suitable for the sweet cherry industry especially for the wetter districts in the Pacific Northwest.
2. Refine and test a growing degree hour model for fruit growth in 'Bing', 'Sweetheart' and 'Regina' sweet cherry for the PNW. Expand to include other commercial cultivars in 2010.
3. Evaluate Stimplex and plant growth regulators (MaxCel or Prestige™, Harvista™, NAA) for stem retention on 'Skeena' and 'Selah' at two timings of application, green-straw and color break.
4. Propagate MxM 39, MxM46 and Giessen 196-4 rootstocks and distribute to nurseries.

Significant findings and results

1. *Rootstock and varieties*

a. *2002 'Sweetheart'/MxM trial* –MxM46 had the highest yields and moderate tree size (Table 1 and Fig.1). MxM60 and 2 have the greatest TCSA. Yield efficiency was highest for MxM 39 and 46. Very little bacterial canker was observed in this trial. MxM 39 and 46 are currently not in the nursery trade. Because of their ability to reduce vigor, have similar yields to the larger MxM's and their performance throughout the duration of the study, these rootstocks should be propagated and made available for further distribution to the sweet cherry industry. Low-budded trees are currently being evaluated at the Lewis Brown Research Farm and by Tim Dahle.

b. *2003 'Skeena'/Krymsk rootstock trial* - Krymsk 6 bore more fruit (33kg) than either Gisela 6 (19 kg) or Mazzard (17 kg). Unlike Krymsk5 with 80% tree loss, mortality in Krymsk6 is only 10% in its 6th leaf. Fruit size remains good on Krymsk 6 and it is a suitable alternative rootstock for low-budded trees in the Willamette Valley.

c. *2006 NY blush and variety trial on Gisela 6* –Results from only one of three sites for 2009 are summarized in Table 2 (The Dalles- Cooper). Growers were most interested in NY213 since the harvest window would fill a critical niche between 'Bing' and 'Regina' harvest. Fruit size was generally good to very good for most selections. Firmness was more variable and highlighted genotypes were of concern. NY7679 has been named 'Radiance Pearl'. It matures 4 days after 'Rainier' but shattered from the tree with the stem attached. NY 113 was difficult to harvest and had very long, thin stems with signs of browning at maturity. Stem pull force was generally above our 600g threshold for all genotypes.

d. *2006 Dark cherry cultivar and rootstock trial* – Results from only one of three sites for 2009 are summarized in Table 3 (The Dalles- Cooper). Giessen 196-4 trees generally had a similar or slightly greater TCSA than Gisela 6. Yields were similar or less on Giessen 196-4 trees than Gisela 6 trees. This slightly higher vigor and delay in production may be of benefit for certain precocious cherry cultivars to reduce blindwood and runting out. 'Bing', 'Rainier' and 'Sweetheart' trees produced the highest yields while all cultivars on Mazzard rootstock produced almost no fruit. 13N 07-39 and 'Tieton' were the only selections with fruit cracking. Some cultivars had marginally acceptable or low fruit firmness (<250g/mm).

e. *2008 'Regina' rootstock trial* – 'Regina' trees low-budded onto Gisela 6, Giessen 196-4, MxM14, MxM39 and MxM46 and trained to a central leader have good vigor and tree structure. No bacterial canker symptoms have been observed. There were significantly more scaffold spurs forming on the two-year wood of MxM14 trees in their second leaf (MxM14 – 6.8, all others <5.0).

2. *Training systems*

a. *2006 On-farm training systems trial* – 'Early Robin' trees on Gisela 6 rootstock (The Dalles, Carter), trained to a central leader, had the highest yield. Fruit from central leader trees had the best color, firmness, size, and soluble solids concentration and the least amount of cracked fruit compared to multiple and steep leader trees (Table 4). 'Rainier'/Gisela 6 trees were harvested early for brine at the second on-farm trial, therefore no yield or fruit quality data could be collected. Steep leader trees are most vigorous, with reducing vigor for central, then even less for multiple leader trees.

b. *2003 Training systems and rootstock trial trees* – 'Sweetheart' produced its highest yields on Gisela 6 for both central and multiple leader training systems. MxM14 was the most vigorous rootstock followed by Mazzard, and Gisela 6 and Giessen 196-4, for all varieties. The modified Tatura trellis system does not fare well in the Willamette Valley and probably also not in high *Pseudomonas* pressure growing regions primarily due to the incidence of bacterial canker that occurs where wires rub the trunk (54% tree mortality). Low-budded Giessen 196-4 had highest mortality in this trial, followed by Gisela 6, Mazzard and MxM14. MxM14 had little to no mortality (Table 5).

3. *Alternative cropping systems*

2005 Alternative orchard floor and fertility management – After five growing seasons, there was a 21% reduction in organic matter in the plots where landscape cloth was used (Table 6). OM increased by 24% in the systems management plots. Estimated N release, mineralizable N, NO_3, K, Zn, and B levels were higher in plots receiving organic amendments.

4. *Growing degree hour model* –GDH can be used to predict time of GA application and harvest for sweet cherry cultivars (Tables 7 and 8). Grower data collected over 4 years for 'Bing', 'Regina', and 'Sweetheart' demonstrate the shift in time of application to greater congruence with the end of pit hardening and the green to straw color change (Figure 2). We suspect that the timing of GA application for 'Early Robin' was too late based on our knowledge that earlier ripening cherries have a shorter duration from bloom until the end of pit hardening. GA sprays are often applied later than the time of transition to final swell (green/straw color change).

Materials and Methods:

- Train trees, maintain orchard plots and obtain data on yield, fruit size, tree vigor, bacterial canker tolerance and other relevant data from the existing cherry trials (0.65 ha) which include:

  Lewis-Brown Farm Trials
  2002 'Sweetheart'/MxM rootstock trial (0.09 ha)
  2006 Variety and rootstock trial (0.15 ha)
  2006 NY blush and dark cherry cultivar trial (0.20 ha)
  2008 'Regina' Rootstock Trial (0.17 ha)
  2010 'Skeena' stem retention trial (0.04 ha)
  On-Farm Trials
  2006 Variety and rootstock trial- Omeg and Cooper
  2006 NY blush and dark cherry cultivar trial- Omeg and Cooper
  2005 'Early Robin' and 'Rainier' training systems trials- Morgan and Carter
  2009 'Skeena' and 'Selah' stem retention – to be determined
- *Stem Retention Trial* – Apply Stimplex, NAA (Stopdrop), MCP (Harvista), MaxCel or CPPU (Prestige) in combination with GA (ProGibb) on 'Skeena' or 'Selah' trees at green/straw and color break stages to determine if stem retention can be enhanced.

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

Table 1. Effect of rootstock on TCSA, yield and yield efficiency in 2009 of 'Sweetheart' trees topworked onto five MxM rootstocks.

MxM Rootstock [z]	Yield (kg/tree)	TCSA (cm^2)	Yield efficiency (kg/cm^2)	Yield (tons/acre)
14	20.3 c	171 b	.12 bc	3.1 c
46	42.3 a	202 b	.21 a	6.4 a
2	35.1 ab	281 a	.13 b	5.3 ab
39	28.7 bc	206 b	.14 b	4.3 bc
60	28.3 bc	330 a	.09 c	4.3 bc
MSD[y]	11.2	65	.04	1.7

[z]Rootstocks were planted in 2000 at a 18' x 18' spacing in a completely randomized design with 6 replicate trees and top-worked in 2001.
[y]Means separation is by the Waller Duncan k-ratio t-test, k-ratio=100.

Figure 1. Cumulative yields (tons/acre) and cumulative yield efficiency (kg/cm^2) from 2005-2009 for five MxM rootstocks top-worked with 'Sweetheart' in 2001.

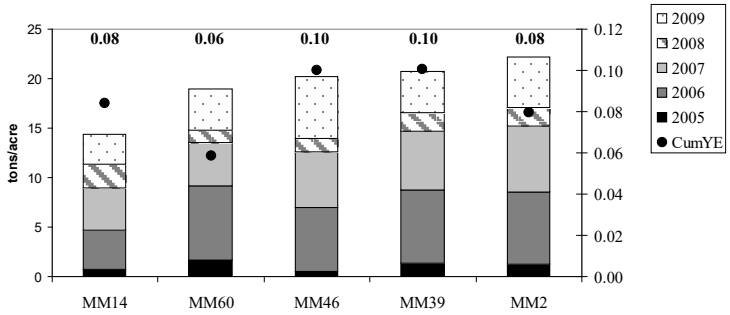


Table 2. 2009 performance of NY blush and dark cherry selections on Gisela 6 rootstock planted in 2006 in The Dalles, OR (Cooper) in their fourth leaf at one of three research trials.

Selection[z]	Yield (kg)[y]	TCSA (cm^2)	Yield efficiency (kg/cm^2)	Size (mm)	SSC (°Brix)	Firmness (g/mm)	Pullforce (g)
Mid season (2 July)							
'Rainier'	16.5 a	72.4 bc	.24 a	28.1 bcd	18.7 h	246 cdef	1063 ab
NY288	7.0 cdefg	60.9 c	.12 cd	27.8 cd	26.3 a	211 efg	1029 bc
NY113	1.9 efg	79.9 abc	.02 ef	31.0 a	25.2 ab	290 c	759 ef
NY2068	1.3 fg	96.5 ab	.01 ef	29.8 abc	24.0 bcd	255 cde	906 d
Mid-season (6-10 July)							
'Radiance Pearl'	16.4 a	81.4 abc	.20 ab	29.9 ab	19.4 gh	222 efg	638 gh
NY8039	11.4 abc	98.1 ab	.14 bc	31.3 a	20.5 fgh	241 def	880 d
NY252	10.5 abc	75.8 abc	.14 bc	27.2 d	22.6 cde	242 def	851 de
NY132	9.2 bcd	90.2 abc	.10 cd	28.3 bcd	25.3 ab	216 efg	1036 ab
NY8033	8.5 bcd	78.2 abc	.10 cd	31.3 a	22.0 def	256 cde	1026 bc
'Skeena'	7.4 cdef	93.1 ab	.09 cde	30.0 ab	19.3 gh	363 b	1153 a
NY1913	4.1 defg	75.0 bc	.05 def	26.2 d	25.3 ab	436 a	912 cd
NY213	1.1 g	105.2 a	.01 f	29.6 abc	21.3 efg	405 ab	851 de
Late season harvest (23-31 July)							
NY9116	13.4 ab	85.9 abc	.16 bc	26.4 d	21.5 efg	209 fg	520 h
Regina'	.	94.3 ab	.	28.2 bcd	24.4 abc	190 g	713 fg
MSD[y]	6.0	29.4	0.08	2.1	2.2	46	119

[z]This planting is in 3 locations with 3 replications per selection per location.
[y]Means separation is by the Waller Duncan k-ratio t-test, k-ratio=100. Grey highlighted numbers are of concern.

Table 3. 2009 performance of several fresh cultivars in The Dalles (Cooper) in their fourth leaf on Giessen 196-4, Gisela 6 and Mazzard rootstocks, from one of three research trials.

Selection	Rootstock	Yield (kg)[z]	TCSA (cm^2)	Yield efficiency (kg/cm^2)	Size (mm)	SSC (°Brix)	Firmness (g/mm)	Pullforce (g)
'Tieton'	Giessen 196-4	0.9 b	110.8 a	.01 b	31.4	19.0	291 a	1104
S^3S^9	Gisela 6	2.8 a	75.4 b	.04 a	30.8	19.2	222 b	949
	Mazzard	0.5 b	115.5 a	.003 b	30.8	17.5	334 a	1093
'Early Robin'	Giessen 196-4	1.0 ab	77.8 b	.02 ab	29.8	20.6	407	913
S^1S^3	Gisela 6	3.5 a	75.6 b	.05 a	.	.	.	.
	Mazzard	0.3 b	91.2 a	.003 b	28.2	18.8	363	761
13N 07-39	Giessen 196-4	.	69.6	.	.	.	.	.
$S^1S^{4'}$	Gisela 6	3.4	72.3	.05	29.5	24.9	338	1495
	Mazzard	1.0	87.3	.011	27.3	25.5	398	1396
'Benton'	Giessen 196-4	1.1 b	113.2	.01 b	30.0	23.9 a	230	1050
$S^3S^{4'}$	Gisela 6	2.6 a	123.3	.02 a	29.8	23.4 a	263	1079
	Mazzard	0.2 c	146.6	.002 c	29.9	21.0 b	283	967
'Sylvia'	Giessen 196-4	6.8	67.2 b	.10	29.7	18.7 a	260	776 ab
S^1S^4	Gisela 6	7.6	87.5 ab	.09	29.3	17.8 a	257	693 b
	Mazzard	1.3	104.6 a	.017	28.9	15.8 b	292	938 a
'Bing'	Giessen 196-4	.	.	.	.	.	.	.
S^3S^4	Gisela 6	14.4	74.1	.20	28.4	22.0	219	813
	Mazzard	.5	97.5	.005	27.1	23.7	284	735
'Rainier'	Giessen 196-4	14.6 a	97.7	.22 a	29.4	21.0 a	279 b	1090
S^1S^4	Gisela 6	16.5 a	79.0	.21 a	28.1	18.7 b	246 c	1063
	Mazzard	1.0 b	80.5	.013 b	28.8	20.4 ab	367 a	1154
'Skeena'	Giessen 196-4	1.8 b	94.4	.02 b	30.1 a	20.3	352	1094
$S^1S^{4'}$	Gisela 6	7.3 a	97.1	.08 a	30.0 a	19.3	363	1153
	Mazzard	0.8 b	98.2	.008 b	28.4 b	18.8	353	1008
'Regina'	Giessen 196-4	2.5	92.4	.03	27.2	23.3	280	756
S^1S^3	Gisela 6	2.2	94.2	.02	28.2	24.4	190	713
	Mazzard	0.1	92.3	.001	Inadequate sample size			
'Sweetheart'	Giessen 196-4	16.7 a	74.4	.22 a	27.6	23.2	231	609
$S^3S^{4'}$	Gisela 6	18.6 a	69.5	.27 a	27.3	24.1	221	653
	Mazzard	0.5 b	88.0	.005 b	28.2	23.4	224	559

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

Table 4. 2009 performance of 'Early Robin' trees on Gisela 6 rootstock planted in 2006 (The Dalles, Carter) and trained to three training systems.

	TCSA (cm^2)	Yield (kg)	Color (%)	Firmness (g/mm)	Cracks (%)	Pullforce (g)
Steep Leader	90.8 a	15.0	55.3 b	295.3	10.4 a	918 b
Central Leader	85.6 b	17.2	63.2 a	306.5	1.6 b	795 c
Multiple Leader	78.1 c	14.0	46.8 c	303.2	8.0 a	1016 a
MSD[z]	4.4	ns	5.7	ns	5.7	63

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

Table 5. Cumulative mortality due to bacterial canker beginning in the third leaf, 2006.

Cultivar / Rootstock	2006	2007	2008	2009
'Stardust'				
MXM14	0	1	removed	
Mazzard	Not in trial	Not in trial	removed	
Gisela 6	3	15	removed	
Giessen 196-4	6	24	removed	
'Royal Ann'				
Gisela 6	2	5	9	removed
Giessen 196-4	1	1	9	removed
MXM14	0	0	0	removed
Mazzard	1	1	1	removed
'Sweetheart'				
MXM14	0	0	1	4
Mazzard	1	2	2	8
Gisela 6	0	1	2	10
Giessen 196-4	2	9	12	20

Table 6. Effect of five years of alternative orchard floor management systems on chemical soil constituents. (No Am= landscape cloth; Or Am = mulch/compost)

	pH	CEC	OM	Estimated N release	NO_3-N	Incubation N	Mineralizable N	NH_4-N
Begin 2005	**5.9**	**21**	**4.2**	**115**	**18**	**.**	**.**	**.**
No Am	6.3	32	3.4	98	8	24.1	21.1	3.02
Or Am	6.8	32	5.7	144	27	61.4	58.5	2.93
Pr > t	*.44*	*.9868*	*.0006*	*.0005*	*.0122*	*0.0002*	*0.0001*	*0.9021*

	P1	K	Mg	Ca	SO_4-S	Zn	Mn	Fe	Cu	B
Begin 2005	**31**	**261**	**614**	**2246**	**24**	**2.7**	**25**	**72**	**3.4**	**0.3**
No Am	29	213	1011	3837	12	1.4	11	50	4.1	0.38
Or Am	43	556	878	3781	23	4.3	13	56	2.8	0.73
Pr > t	*.0279*	*.0003*	*.403*	*.9269*	*.0494*	*.0061*	*.1901*	*.2651*	*.0896*	*.0006*

Table 7. Growing Degree Hour (GDH) accumulation from peak bloom (80%) to first GA spray and to harvest for eight varieties reported by several growers in The Dalles, OR.

	'Chelan'	'Early Robin'	'Bing'	'Rainier'	'Lapins'	'Skeena'	'Regina'	'Sweetheart'
GDH accumulated from peak bloom to GA spray								
2008	10720	.	10972	.	12615	11510	12324	13181
2009	9337	10787	9692	.	10746	10873	10893	11178
Average	**10029**	**10787**	**10332**	**.**	**11681**	**11192**	**11609**	**12180**
GDH accumulated from peak bloom to Harvest								
2008	14388	15070	17644	18205	20153	21093	20426	21404
2009	15698	15096	17859	17161	21888	19091	21516	23065
Average	**15043**	**15083**	**17752**	**17683**	**21021**	**20092**	**20971**	**22235**

Cumulative GDHs were calculated from peak bloom. To determine GDH accumulation at temperatures between 4 and 25°C (base and optimum) the following formula was applied:

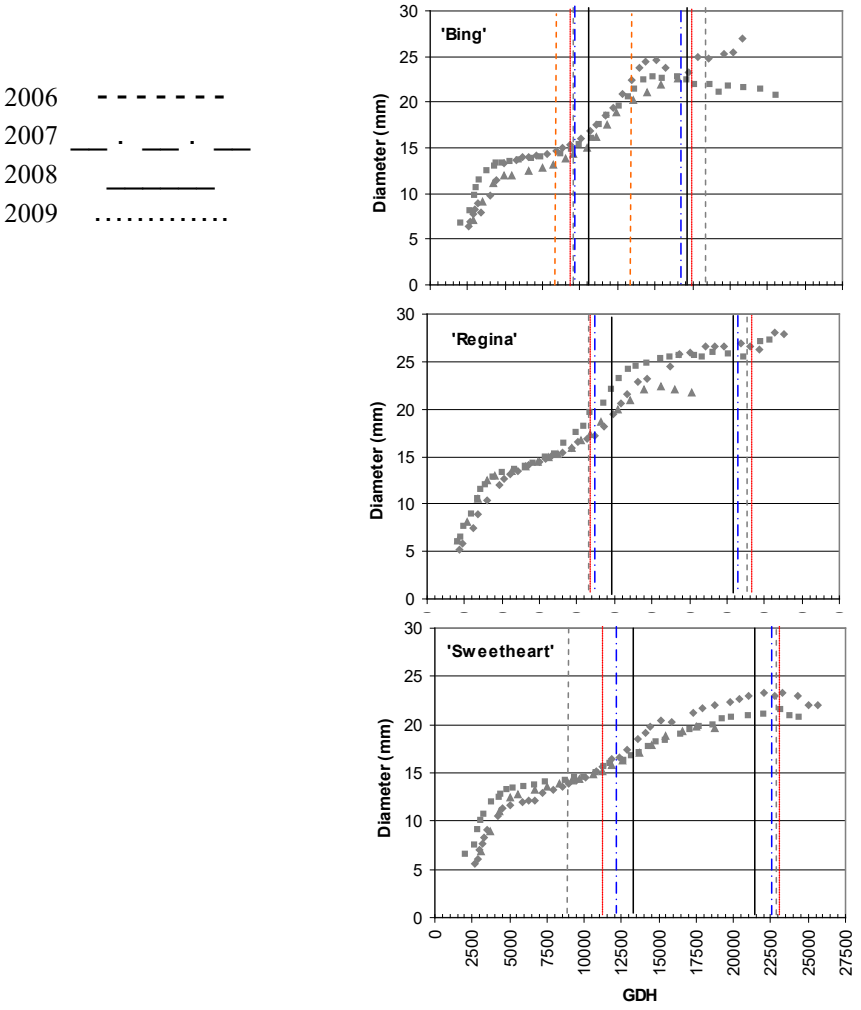
GDH=[(25-4)/2](1+cos(π+π(hourly T°-4)/(25-4))). At temperatures above optimum a second formula (Anderson, et al., 1986) was applied incorporating the critical temperature for fruit trees (36°C):

GDH=(25-4)(1+cos(π/2 +π/2 (hourly T°- 25)/(critical T°- 25))).

Table 8. Average number of GDH for 'Bing', 'Regina', and 'Sweetheart' based on reported dates from growers of time of GA application and harvest.

	2006	2007	2008	2009	2009 GDH/day	Approx. no. of days in 2009
'Bing'						
GA	9881	9949	10972	9692	306	1.9
Harvest	18874	17140	17644	17859	299	2.4
'Regina'						
GA	10754	11146	12324	10893	317	2.3
Harvest	21436	20636	20426	21516	258	2.1
'Sweetheart'						
GA	8095	12018	13181	11178	312	3.2
Harvest	22808	22645	21404	23065	261	2.8

Figure 2. Growth curve data was collected over 3 years; 2001, 2003 and 2004. Dates when orchardists applied GA and harvested were collected in 2006-2009. GDH were calculated from peak bloom, averaged across producers and plotted as vertical lines at the time of the GA spray and at harvest.



CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: CH-08-801

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

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Cooperators: Tom Auvil**,** Bryce Molesworth

Total project funding request (Fall 2007): Yr 1: $40,974; **Yr 2**: $19,120; **Yr 3**: $17,416

Revised project funding request (Fall 2008): Yr 1: $14,574; **Yr 2:** $24,589 **Yr 3:** $33,011

- The trees from Duarte Nursery were anticipated to be available for spring 2009 planting. However, due to a longer length of time for liner production, they will now be available for spring 2010 planting. This delay resulted in a shift of funds from year 1 to years 2 and 3.

Revised project funding request (Fall 2009): Yr 1: $14,574; **Yr 2:** $24,589 **Yr 3:** $35,141

- Todd Einhorn was added as a Co-PI with a new budget line to replace Jim Olmstead who had assumed responsibilities for the rootstock plots prior to his departure from WSU.

Other funding sources: None

Budget 1: Tree and plot costs

Item	2008	2009	2010
Plot costs	$ 0	$ 5,310[1,2]	$ 4,420[2]
Tree cost for PNW	$ 0	$ 7,209[1]	**$ 14,070[3]**
Total	$ 0	$ 12,519	**$ 18,490**

Footnotes:

[1]Funds were reallocated between these two budget categories in 2009 to reflect the establishment of only one grower/cooperator plot in Washington and the unanticipated cost of the un-budded liners from Willow Drive Nursery.

[2]Plot cost for the sites in Mosier, OR and Chelan, WA. Plot costs are based on $6.50/tree for plot establishment in 2009 which covers site prep, fumigation and irrigation supplies; $3.50/tree in 2010 for planting, and first year general farming, water, taxes. A portion of the 2009 plot expenses may be claimed in 2010. The budget is based on 973 trees which includes the confirmed tree numbers for the Mosier and Chelan plots plus an additional projected 5% increase in tree numbers from sleeping eye budding done n fall of 2009.

[3]Tree cost is based on the original agreement of 2,345 trees from Duarte Nursery @ $6/tree. This includes the cost of producing liners that did not result in a final tree due to failed bud take and shipping.

These costs were previously identified under "WTFRC collaborative expenses".

Budget 2: Amy Iezzoni

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

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

Footnotes:

[1] This freight fee has been encumbered to cover the cost of tree delivery in 2010.

[2] The 2009 request is reduced and the 2010 request is increased as tree planting has been delayed until 2010.

[3]Total cost of the 750 Gisela liners @ $1.60 per liner (no royalty fee).

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

Item	2008	2009	2010
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel			
Plot charges	$3,500[1]	0	$2,500[2]
Miscellaneous			
Total	$3,500[1]	0	**$2,500[2]**

Footnotes:
[1] Due to the delay in planting, no funds were expended in 2008. These funds have been encumbered and year 2 and 3 requests have been reduced by $3,500.
[2] This budget line was increased $1,000 due to increased expenses at the Roza farm that were not anticipated last year when J. Olmstead was the Washington project leader.

Budget 4: Todd Einhorn
Organization Name: OSU-MCAREC **Contract Administrator:** Dorothy Beaton
Telephone: 541 737-3228 **Email address:** dorothy.beaton@oregonstate.edu

Item	2008	2009	2010[1]
Salaries[1]			$1,500
Benefits[2]			$885
Wages			
Benefits			
Equipment			
Supplies			
Travel[3]			$100
Miscellaneous			
Total			**$2,485**

Footnotes
[1] Salary is calculated for 2 weeks of a Full Time Technician's salary, for oversight of planting, mapping, plant measurements, and data management.
[2] Benefits are calculated according to actual OPE rate of 59 %.
[3] Travel is based on a rate of 50.5 cents/mile, and includes visits to OR orchard site for data collection and grower support.

OBJECTIVES

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

Specific Objectives for 2010:

1. Evaluate the existing trees of the 10 rootstock candidates to determine if they continue to show commercial promise.
    - All the rootstock candidates are currently under evaluation in the original planting at MSU's Clarksville Horticultural Experiment Station.
    - Monitor growth of trees in newly planted trials to include data on terminal bud set and trunk cross sectional area.
2. Conduct DNA fingerprinting to assure that the genetic identity of the rootstock selections is correct.
    - This fingerprinting would specifically be done to assure that the rootstock selections transferred to NRSP5 for virus certification would be the correct identity.
3. Plot establishment to include site preparation and tree purchase, tree planting, and cultural management of the plots to assure the ability to assess rootstock performance.
    - The first rootstock second test plot was planted at WSU-Prosser in spring 2009. In spring of 2010, four more plots will be planted at the following locations: Prosser WA, Chelan WA, Mosier OR, and Clarksville MI. Due to a reduction in anticipated bud take from the April 2009 budding, only two test plots will be planted in Washington instead of the three originally proposed.
    - Based on conversations with the Advisory Committee in January 2009, 'Chelan' was included as another scion due to its unique incompatibility with mahaleb rootstock.

SIGNIFICANT FINDINGS AND ACTIVITIES

- The overall goal of this three year project was to establish the second test plots of the 10 MSU rootstocks. We will achieve this objective with the planting of four test plots in spring 2010. Unfortunately reduced tree numbers resulting from budding in April 2009 at Duarte Nursery required us to redesign our plots and eliminate a second grower cooperator site in Washington. The four sites to be planted in 2010 are WSU-Prosser WA, Mosier OR, Chelan WA and MSU-Clarksville, MI.
- Based on discussions with the Advisory Committee in January 2009, 'Chelan' was added as a scion because of its incompatibility with mahaleb rootstock. The 'Chelan' trees will be planted at the WSU-Prosser location to complement the 'Bing' planting established in 2009. 'Bing' will be the scion for the plot in Mosier, 'Bing' and 'Sweetheart' will be the scions for the Chelan plot, and 'Rainier' will be the scion for the Clarksville plot. Mazzard, Gisela® (Gi) 5, Gi 6 and Gi 12 are included as controls.
- In spring 2009 the rootstock test trees budded at Willow Drive Nursery were planted at WSU-Prosser. Due to unequal liner numbers, the rootstock candidates are represented in this plot by a minimum and maximum of 5 and 30 trees, respectively. All rootstock candidates have 'Bing' scion and Kent also has 'Sweetheart' scion due to the large number of liners and excellent percentage bud take for this rootstock. Gi 5 and Gi 6 are included as controls.
- The approximate dates of terminal bud set for the newly planted trees in the WSU-Prosser plot were recorded as the cessation of terminal meristem growth is known to be the major factor resulting in the scion size difference on Gi 5 versus Gi 6. As expected, terminal bud set for 'Bing' occurred earlier when grafted on Gi 5 compared to Gi 6. Interestingly, terminal bud set for 'Bing' grafted on the MSU rootstocks preceded that of Gi 6 for all the rootstocks except Garfield.

- All 10 MSU rootstocks planted at Clarksville, MI in 2001 – 2004 are showing no signs of graft incompatibility with 'Hedelfingen', and in some cases 'Bing' scion, however, continued testing is warranted.
- At Clarksville, Mich., -22.6°C on January 16th resulted in some spur and flower bud death, thus influencing the evaluations for these production traits. However, in general the MSU rootstocks tended to have a lower flower bud density than Gi 6 when both spur number and flower buds per spur are considered together.

METHODS BY OBJECTIVE

1. Evaluate the existing trees of the 10 rootstock candidates to determine if they continue to show commercial promise.
    - All 10 of the MSU rootstock candidates are currently planted at MSU's Clarksville Horticultural Experiment Station (CHES). It is critical that these rootstocks continue to be evaluated as four of the selected rootstock candidates were only planted at CHES in 2004. Therefore continued monitoring of tree performance is necessary. Additionally, these trees are the oldest representatives of the MSU rootstock selections and therefore provide valuable data on tree size potential and tree longevity. These trees will be evaluated for the following parameters: tree health, structure, trunk cross-sectional area, visual estimates of bloom density and crop load, number of flower buds and fruit per spur, fruit weight, and annual growth of terminal and lateral shoots.
    - The tree growth in the newly planted trials will be evaluated to include date of terminal bud set and trunk cross-sectional area.

2. Conduct DNA fingerprinting to assure that the genetic identity of the rootstock selections is correct.
    - In anticipation of providing budwood of the MSU rootstock candidates to NRSP5 for virus certification, DNA fingerprinting will be done to assure correct clonal identity.

3. Plot establishment to include: site preparation and tree purchase, tree planting, and cultural management of the plots to assure the ability to assess rootstock performance.
    - In spring of 2010, the rootstock trees produced at Duarte Nursery will be planted in test blocks at WSU-Prosser, Chelan WA, Mosier OR, and Clarksville MI. Mazzard, Gi 5, Gi 6 and Gi 12 are included as controls. See summary details in Table 1. These trees will be planted at a spacing of 8 ft × 15 ft. The trees will be managed (e.g. irrigation, pest control, etc.) following standard commercial practices. Trees will be trained to a multiple-leader, open-center canopy architecture.
    - In fall of 2009, sleeping eye buds will be placed in additional MSU rootstocks to provide trees for those rootstock/scion combinations that fell short of desired numbers.

Table 1. Plant material propagated at Duarte Nursery in April 2009 for the MSU rootstock trials to be planted in spring of 2010 (See Table 5 for more details).

Location	No. of MSU rootstocks tested	Scions utilized	Maximum no. of replications	Total Tree No.[1]
Prosser WA	9	'Chelan'	5 reps of 5 trees each	352
Chelan WA	10 & 7	'Bing' & 'Sweetheart'	5 reps of 5 trees each	562
Mosier OR	10	'Bing'	5 reps of 5 trees each	365
Clarksville MI	10	'Rainier'	1 rep of 5 trees each	133

[1] This number includes the addition of the following rootstock controls: Gi 5, Gi 6, Gi 12 and mazzard, plus guard row trees. It does not include any sleeping eye trees that may result from the fall 2009 budding.

RESULTS AND DISCUSSION

Rootstock performance: All 10 MSU rootstocks planted at Clarksville, Mich. in 2001 – 2004 are showing no signs of graft incompatibility with 'Hedelfingen', and in some cases 'Bing' scion, although continued testing is warranted. Unfortunately, temperatures of -22.6°C on January 16th resulted in some spur and flower bud death, thus influencing the evaluations for these production traits. However, in general the MSU rootstocks tended to have a lower flower bud density than Gi 6 when both spur number and flower buds per spur are considered together (Table 2). Mean fruit size for the 'Hedelfingen'/Gi6 control ranged from 7.1 g to 6.1 g with an average of 5.4 and 6.3 fruit/spur, respectively. Mean fruit size for the MSU rootstocks was comparable and ranged from 5.7 g to 8 g.

Rootstock genetic check: DNA fingerprinting of the MSU rootstocks at Duarte continued to verify that the rootstocks used to provide the trees for the text plots are the correct identity. This diagnostic test involves the use of DNA markers to distinguish among the 10 rootstock candidates.

Rootstock test plot establishment: In spring 2009 the rootstock test trees budded at Willow Drive Nursery were planted at WSU-Prosser. Due to unequal liner numbers, the rootstock candidates are represented in this plot by a minimum and maximum of 5 and 30 trees, respectively (Table 3, Fig. 1). All rootstock candidates have 'Bing' scion and Kent also has 'Sweetheart' scion due to the large number of liners and excellent percentage bud take. Gi 5 and Gi 6 were included as controls. The approximate dates of terminal bud set for these test trees were recorded as the cessation of terminal meristem growth is known to be the major factor contributing to the difference in scion size between Gi 5 and Gi 6 (Prassinos et al. 2009 Tree Physiology). As expected terminal bud set for 'Bing' occurred earlier when grafted on Gi 5 compared to Gi 6 (Table 4). Interestingly, terminal bud set for 'Bing' grafted on the MSU rootstocks preceded that of Gi 6 for all the rootstocks except Garfield.

In January 2009, A. Iezzoni visited Duarte Nursery to review the status of the MSU rootstocks and to become familiar with their unique pot culture tree production strategy (Fig. 2). The liners are budded in April, and the finished cherry trees reach approximately 3 feet in height (Fig. 2A). Normally the trees are shipped for June planting, but for our project we requested to receive dormant plants in January/February 2010 in preparation for spring planting.

At the time of my visit to Duarte Nursery, an excess number of liners were available to achieve our desired plot plans. As a result, after consultation with the Advisory Committee, 'Chelan' scion was included in the trials as its unique incompatibility on mahaleb rootstock would provide a valuable test of the universal compatibility of the MSU rootstocks. Unfortunately reduced tree numbers resulting from budding in April 2009 at Duarte Nursery required us to redesign our plots and eliminate a second grower cooperator site in Washington. Therefore the sites to be planted in 2010 now are WSU-Prosser, Mosier OR, Chelan WA and Clarksville, MI (Table 5). The site in Mosier, OR, will have 'Bing' scion, while the site in Chelan, WA will have both 'Bing' and 'Sweetheart' scion. The plot at WSU-Prosser will have 'Chelan' scion to complement the existing 'Bing' plot planted in 2009. The plot at MSU-Clarksville will have 'Rainier' scion, however, the number of replications will be limited.

After discussions with John Duarte, we decided to put "sleeping eye" buds in the MSU rootstocks in Fall 2009 to provide trees of those scion/rootstock combinations that fell short of the desired numbers. However, as the percentage bud take is unsure, the plot plans summarized in Tables 1 and 5 just represent confirmed tree numbers. An additional 18 and 28 sleeping eye trees are projected for the Mosier and Chelan sites and used to calculate the plot costs. The planting of these test plots will achieve our overarching project goal of establishing multiple plots to test the commercial potential of the MSU rootstocks.

Table 2. 2009 data for total number of spurs, mean number of flower buds/spur, mean number of fruit/spur, fruit size for 'Hedelfingen', trunk cross-sectional area (TCSA), and % size of the Gi 6 control for the 10 MSU rootstock selections planted in Clarksville, MI.

Rootstock	Total no. of spurs[1]	Mean no. of flower buds/spur[2]	Mean no. of fruit/spur	Mean fruit size (g)[2]	TCSA (cm^2)[2]	Vigor (% of GI6)[3]
			2001[4]			
Lake	4.1a[5]	4	5.5b	6.6	87	70
Gi 6	6.8b	4	5.4b	7.1	125	100
Iron	5.1a	3	4.1a	7.2	127	102
			2002			
King	5.8a	3	5.0ab	6.3	87	73
Garfield	9.0b	3	4.4a	5.7	115	97
Glenn	6.4ab	3	5.1ab	6.4	115	97
Gi 6	7.0ab	4	6.3b	6.1	119	100
Lincoln	5.5a	3	4.5a	7.1	123	104
Kent	6.5ab	3	5.0ab	7.1	148	124
			2004			
Clare	8.0ab	4	4.9b	8.0	42	-
Clinton	6.8a	4	2.0a	8.0	45	-
Cass	9.8b	4	2.8a	7.5	52	-

[1] Data represents the number of spurs on two branches for second and third year wood. Live and dead spurs were counted.
[2] Unable to determine significant differences due to small sample size.
[3] Calculated from TCSA.
[4] Year in which the rootstock selections were planted.
[5] Means denoted by same letters within the column are not significantly different at $P < 0.05$.

Table 3. Tree numbers for the 10 MSU rootstock candidates and Gi 5 and Gi 6 control trees grown at Willow Drive Nursery that were planted at WSU-Prosser in spring of 2009.

Rootstock selection	Scion	No. of trees planted
Cass	Bing	5
Clare	Bing	25
Clinton	Bing	25
Garfield	Bing	10
Glenn	Bing	5
Iron	Bing	10
Kent	Bing	25
Kent	Sweetheart	30
King	Bing	10
Lake	Bing	5
Lincoln	Bing	25
Gisela 5	Bing	15
Gisela 6	Bing	20
Gisela 6	Sweetheart	20
Guard	Bing & SwHt	34
TOTAL		264

Fig. 1. 'Bing' and 'Sweetheart' trees growing at WSU-IARDC Roza farm in summer 2009.

Table 4. Percentage 'Bing' and 'Sweetheart' shoots that had set terminal bud by three dates in September 2009 for trees grafted on MSU rootstocks. The trees were produced at Willow Drive Nursery and planted at WSU-Prosser Roza Farm in April 2009.

		Percentage of shoots that had set terminal bud[1]		
Scion	Rootstock	Sept 14	Sept 30	After Sept 30[2]
Bing	Cass	0%	100%	0%
Bing	Clare	28%	68%	4%
Bing	Clinton	0%	72%	28%
Bing	Crawford	10%	80%	10%
Bing	Garfield	0%	40%	60%
Bing	Glenn	0%	80%	20%
Bing	Iron	0%	80%	20%
Bing	Kent	16%	56%	28%
Bing	King	0%	90%	10%
Bing	Lake	0%	80%	20%
Bing	Lincoln	12%	72%	16%
Bing	Gi5	7%	80%	13%
Bing	Gi6	0%	40%	60%
Sweetheart	Kent	43%	30%	27%
Sweetheart	Gi6	5%	60%	35%

[1] Percent based on totals of 100%.
[2] Shoots represented in this column set terminal bud after Sept 30.

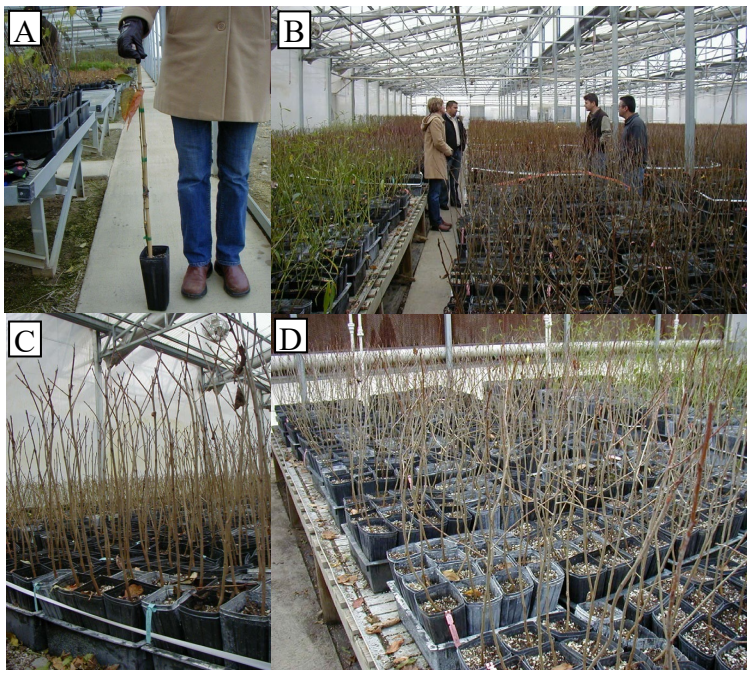
Table 5. Plant material produced at Duarte Nursery from April 2009 budding for the test plots to be planted in 2010[1]. [Sweetheart = Swthrt]

Rootstock	Scion (tree no.) Prosser, WA	Scion (tree no.) Chelan, WA	Scion (tree no.) Mosier, OR	Scion (tree no.) Michigan
Cass	Chelan (25)	Bing (25), Swthrt (20)	Bing (25)	Rainier (5)
Clare	Chelan (4)	Bing (5)	Bing (10)	Rainier (5)
Clinton	Chelan (15)	Bing (25), Swthrt (25)	Bing (25)	Rainier (5)
Garfield	Chelan (25)	Bing (10), Swthrt (25)	Bing (10)	Rainier (5)
Glenn	Chelan (25)	Bing (24), Swthrt (25)	Bing (25)	Rainier (5)
Iron	-	Bing (10), Swthrt (5)	Bing (10)	Rainier (5)
Kent	Chelan (25)	Bing (25), Swthrt (25)	Bing (25)	Rainier (5)
King	Chelan (25)	Bing (25), Swthrt (14)	Bing (25)	Rainier (5)
Lake	Chelan (10)	Bing (25)	Bing (25)	Rainier (5)
Lincoln	Chelan (25)	Bing (5)	Bing (10)	Rainier (5)
Mazzard	Chelan (25)	Bing (25), Swthrt (25)	Bing (25)	Rainier (5)
Gisela 5	Chelan (25)[2]	Bing (10), Swthrt (25)	Bing (25)	Rainier (5)
Gisela 6	-	Bing (9), Swthrt (25)	Bing (10)	Rainier (5)
Gisela 12	Chelan (25)[2]	Bing (4), Swthrt (25)	Bing (4)	Rainier (5)
Pollinator & guard trees	Swthrt & Bing (98)	Bing, Swthrt & Chelan (96)	Swthrt & Chelan (111)	Various (63)
Total tree no. per plot	352	562	365	133

[1] Sleeping eye trees are being made at Duarte Nursery to fill in those rootstock/scion treatments where the tree numbers are below 25 for the Prosser, Chelan and Mosier sites.

[2] The 'Chelan', Gi 5, and Gi 12 controls were purchased from Willow Drive Nursery. 'Chelan' on Gi 6 was not available.

Fig. 2. (A) An example of a finished cherry tree at Duarte Nursery. (B-D) MSU cherry rootstocks as unbudded dormant liners [Duarte Nursery, January 2009].



CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: CH-09-902

Project Title: Breeding and genetics program for PNW sweet cherries

PI: Nnadozie Oraguzie
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Cooperators: Matt Whiting**,** Amit Dhingra, Cameron Peace, Jim Olmstead, Amy Iezzoni, Fred Bliss, Todd Einhorn, Tom Auvil, Jim McFerson

Total project funding request: **Year 1**: 89,405 **Year 2**: 96,279 **Year 3**: 101,054

Other funding sources

Agency Name: WSU
Amt. requested/awarded: $150,000 start-up package plus $35,000 per year for 3 years to hire an associate-in-research

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.

SIGNIFICANT FINDINGS AND ACCOMPLISHMENTS

- The first draft of a best management practice (BMP) document for sweet cherry breeding has been completed. This document provides guidelines on breeding program operations from seed germination to raising seedlings in the nursery, planting trees in the field, hand pollinations, fruit quality evaluations, etc., to ensure that plant materials of adequate size, vigor and health are generated and managed accordingly.
- 1.5FTE technicians have been hired to assist with breeding program operations. There is also a vineyard and orchard manager hired by WSU-ARC who spends 33% of his time assisting with horticultural manipulations and general orchard management in the breeding program. Jan Burgess, with 20 years experience working for the NCPN program has been hired for an hour per day by the breeding program to advise on seedling development in the greenhouses and lathhouse.
- The linkages established with other researchers during the year resulted in successful SCRI grants including "RosBREED" with Dr Iezzoni as lead PI, Cameron Peace, Kate Evans, Dr Doree Main and Nnadozie Oraguzie; ''TfGDR'' with Doree Main as lead PI, Cameron Peace, Kate Evans and Nnadozie Oraguzie, and ''Stem-free cherry production and marketing'' with Dr Matt Whiting as lead PI, Amit Dhingra, Lynn Long, Todd Einhorn and Nnadozie Oraguzie.
- Four small green houses and a lathhouse (that can accommodate 8000-10000 seedlings at a time) have been completed and micro-irrigation and frost protection facilities installed in breeding selection plots. Installation of bird netting was instrumental in getting fruit to evaluate in 2009. The re-modeling of the postharvest fruit quality lab and installation of four commercial walk-in coolers finished just in time for fruit evaluations.
- The last lot of seedlings (960 in total) cold-cycled from crosses made in 2006 were planted in the field in the spring of 2009, thus bringing the total planted acreage of the seedling block to ~7.0.
- Crosses were made in the spring of 2009 based on a combination of genetic and phenology information that included specific crosses to increase fruit size, soluble solids content, low cracking incidence, combined powdery mildew and bacterial canker resistances and for introgression of novel self fertility alleles. A total of 7007 seeds were obtained from these crosses out of which 5875 viable ones (based on a floatation test) have been processed for germination.
- Fruit from most of the 2004 crosses and ~5% of 2005 crosses were evaluated for the first time in summer '09 resulting in the selection of 12 trees that fit into 4 of the 6 target cultivar market groups and that have the freestone character also. These trees were propagated in fall '09 at Willow Drive Nurseries and will be available for planting in 2011 for more advanced tests both on-station and on-farm.
- The powdery mildew resistant advanced selections planted in grower trials in Washington and Oregon including DD 9816-104, GG 9817-97, AA 9816-67 and JJ 9816-96 were evaluated again this year for their resistance status. 'DD', 'GG' and 'AA' showed no symptoms of disease even in unsprayed plots at the Prosser experimental station while 'JJ' had mild symptoms as expected. We are currently collating fruit quality data on these selections to inform release decisions.
- Another powdery mildew resistant selection,Pc-9819-31, from a cross between "PMR-1" and "Van" made in 1998, has been identified in summer '09 and propagated for more advanced tests.
- A parental crossing block has been established at the Roza experimental station in Prosser with 30 cultivars planted in spring '09. Forty nine (49) more cultivars have been propagated for planting into this plot in 2011.

METHODS

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

RESULTS AND DISCUSSION

Establishment and implementation of Best Management Practices

Following change in the leadership of the sweet cherry breeding program, it became necessary to develop best management practice (BMP) guidelines to assist the breeder and his entirely new crew to get up to speed with breeding program operations. A work in-progress BMP document that included seed germination and green house seedling development guidelines was initially put together by Drs Olmstead and Iezzoni. As the breeding program personnel got acquainted with the sequence of activities in the breeding program over the past year, this BMP was developed further into a more comprehensive document with input from Dr Iezzoni, the breeding program consultant. The current document provides practical guidelines on breeding program operations such as seed collection and handling, seedling maintenance in the greenhouse and the lathhouse, tree planting and maintenance in the field, horticultural manipulations to encourage bloom and fruiting, fruit sampling and evaluation to pollen collection and artificial pollination. This document will continue to be updated as the breeding program evolves and more information becomes available.

Physical and Human Resources

Presently, the breeding staff includes 1.5FTE technicians. One of these, Blessing Athanson (1 FTE) is funded by WSU-ARC for three years as part of the start-up support for the breeder while Addie Dahl, the other assistant, is funded part-time by WTFRC/OSCC. The vineyard and orchard manager, Clint Graf, was hired by WSU to support 3 programs including Viticulture (PI-Dr Markus Keller), Tree physiology (PI-Dr Matt Whiting) and Sweet cherry breeding (PI-Dr Nnadozie Oraguzie). Clint spends 33% of his time in the breeding program assisting with general orchard management and horticultural manipulations. Jan Burgess, with 20 years experience raising cherries in the NCPN program works an hour/day in the breeding program advising on seedling development in the greenhouse and lathhouse. Dr Amy Iezzoni works with the breeder to ensure that milestones are delivered in a timely and efficient manner. The breeding program has also made a lot of advances in infrastructure development with the completion of four small greenhouses, acquisition of a lathhouse that can accommodate 8000-10000 seedlings at a time, installation of micro irrigation and frost protection equipment around seedling blocks and bird netting in the units of the seedling block with fruiting seedlings. The bird netting in particular, was instrumental for getting sufficient numbers of fruit for evaluation in 2009 which was not possible the year before. We plan to continue to extend micro-irrigation and frost protection facilities to new plots in the coming years and install bird netting in more plots as the trees come into fruiting.

Linkages were established during the year with other researchers including Drs Amit Dhingra, Amy Iezzoni, Cameron Peace, Todd Einhorn, Lynn Long, Matt Whiting, and Doree Main. These collaborations resulted in successful SCRI grants including “RosBREED” with Dr Iezzoni as lead PI, Cameron Peace, Kate Evans, Dr Doree Main and Nnadozie Oraguzie; ‘’TfGDR’’ with Doree

Main as lead PI, Cameron Peace, Kate Evans and Nnadozie Oraguzie, and ''Stem-free cherry production and marketing'' with Dr Matt Whiting as lead PI, Amit Dhingra, Lynn Long, Todd Einhorn and Nnadozie Oraguzie. The collaboration with Drs Iezzoni and Peace's programs will be crucial for getting a larger proportion of the breeding population segregating for large fruit size using marker-assisted breeding (MAB) strategies. At the moment, less than 5% of the breeding populations have fruit size ≥ 10 g (see Table 3 and Figure 1).

The last lot of trees (960 in total) from crosses made in 2006 that went through three cold-cycles were planted in the field in the spring of 2009 along with parent cultivars in randomized incomplete blocks. The idea is to use parents that are similar in age as the breeding selections and that are reared in the same environment as performance comparison standards. This latest planting brings the total planted acreage of the sweet cherry breeding selection block to 7.0. We plan to start a renovation cycle in 2011 when we would have completed fruit valuation on all trees from crosses made in 2004.

Approximately 1500 seedlings from crosses made in 2007 did not make it to the field in 2009 due to death from cold cycling treatment the year before (Table 1). The reasons for the mortality are unclear. It may well be that the seedlings were too young when cold-cycled and therefore too weak to survive the treatment. But we have decided to eliminate cold cycling as a seedling management strategy since it does not appear to have any special advantage over conventional seedling management. In fact, our field observations on cold-cycled trees planted later in the year and non-cold-cycled trees in the breeding plots to date suggest that non-cold cycled trees have more vigor and come into bearing much quicker than cold-cycled ones planted later in the year. It would appear then that given appropriate horticultural manipulations including use of growth tubes, timely and appropriate watering, fertilization, pruning and training, some trees raised conventionally would come into bearing in their 4th leaf. This was the case with trees from 2004 crosses that flowered for the first time in 2008 although we were not able to harvest the fruit then due to poor weather. We took into account these dead seedlings when we designed the crosses for the 2009.

Table 1. Summary of seedling material developed during 2004-2009 in the sweet cherry breeding program.

	Crossing Year					
	2004	2005	2006	2007	2008	2009
No. of new parents used	19	21	14	9	4	17
No. of crosses made	61	90	109	49	24	62
No. seed	4,466	7,349	14,848	6,827	1248	5875
% germination	5%	20%	34%	25%	35%.	n.a
No. of seedlings	250	1,460	5,120	1672*	101	n.a
No. of seedlings in field	243	1,088	4,329	34^{Y}	101^{Z}	n.a.
No. of Full-Sib families >9 individuals	7	43	59	n.a.	2	n.a.

n.a.-not available. $^{Y, Z}$ Seedlings will be planted in the field in the spring of 2010
* Of this number, 1522 did not grow to sufficient size to survive cold-cycling.

We obtained 101 seedlings crosses made in 2008 crosses (which had 1248 seeds in total) and 34 from left-over seeds from 2007 crosses although germination percentage was comparable to previous years. Apart from 263 open pollinated (op) seeds that failed to germinate in embryo culture experiments done in collaboration with Dr Dhingra's lab, as well as, wrinkled/split seeds thrown out, the percentage germination for seeds from 2008 crosses was ~35%. However, seed germination occurred later than in previous years (started 7-8 months later) and happened after the seeds had been subjected to alternate cycles of warm and cold temperatures. When germinated seeds were planted in the greenhouses they displayed unusual weakness and some died from heat stress. Transferring the

young seedlings to another facility supplied with 24 hours of lighting and maintained at 26 °C and 44% RH improved the seedling survival rate. We have since incorporated guidelines developed in Dr Iezzoni's lab in our seedling management strategy to ensure that healthy sprouts are raised which also develop into mature seedlings. We have also taken adequate care to process the 5875 seeds from 2009 crosses and acquired cold storage facilities for their stratification. Furthermore, we have initiated some germination experiments using OP seeds from early, mid-season and late cultivars to see if we can improve on the timing and uniformity of germination using GA. We will also be taking records of albinos and runts to identify specific crosses and/or cultivars that are more prone to producing higher numbers of these off types to inform pollination strategy development. With the protocols we have in place currently combined with better infrastructure, we believe that seed germination and seedling development will be much greatly improved.

Artificial Hybridizations

Hand pollinations were carried out this year over a period of ten days starting on the 11th of April. Approximately 30,000 flowers were pollinated from a total of 62 crosses with 16 female parents. About 7000 flowers set fruit out of which ~5875 viable seeds were generated (based on a floatation test) for stratification and germination. Cultivars/selections were chosen for use as parents with the following objectives in mind, to; 1) develop new cultivars from hybrid populations following one generation of selection, 2) increase fruit size, 3) introduce novel flavors, 4) introduce novel self fertility alleles, 5) combine powdery mildew and bacterial canker resistances, and 6) minimize cracking incidence. Therefore, the objective was two-pronged: to achieve a short term goal of developing new cultivars and a medium-longer term goal of concentrating useful alleles in breeding progenies to facilitate more comprehensive genetic improvement.

Phenotyping and identification of seedlings for propagation for the next phase

There are six target cultivar market groups including ESB, ESM, E-MSM, LSB, LSM and MSM which formed the basis for breeding objectives and priority traits identified by the breeding program in consultation with the sweet cherry advisory committee (see Table 2 for explanation of the acronyms). These objectives were the focal point for the fruit evaluations carried out this year. Fruit picking started on the 16th of June, with most trees from crosses made in 2004 and 5% of trees from crosses made in 2005 picked and evaluated for skin color, flesh color, stem length, fruit weight, fruit width, fruit length, fruit shape, firmness, soluble solids, titratable acidity (TA), and pH. Fruit were also scored for overall flavor, astringency, bitterness and freestone tendency.

The number of fruit assessed ranged from 5-50 depending upon the number of fruit a tree produced. Fruit were assessed immediately after harvest and following 5 days storage at 0-4 C. Because we did not know the exact maturity date for the breeding selections and skin color not being a very reliable indicator of maturity, fruit from most trees were picked at least twice. Fruit were also evaluated from many named cultivars and parent cultivars for performance comparisons, as well as, from crosses made in 1998 including 'PMR-1' x 'Rainier', 'Rainier' x 'PMR-1', 'PMR-1' x 'Van', 'PMR-1 x 'Bing', and from advanced selections including AA 9816-96, DD 9816-104, GG 9817-97and JJ 9816-96 planted at the Roza experimental station WSU-Prosser. We also obtained fruit of 'DD' and 'GG' for evaluation from Hanrahan and Allan bros orchards, respectively.

Based on the evaluations, we identified 12 trees that fit into 4 of the 6 target cultivar market groups, and for the freestone tendency and fruit shape (Table 3) for advancement to the next phase. Photos of representative selections are shown in Figure 2. Except for the individual selected for the ESM target market group which had ~8 g fruit, other individuals had fruit weight ranging from 10-13 g (Table 3). These selections form the majority of individuals located at the right tail of the frequency histogram in Figure 1. Pearson's correlations between pairs of fruit quality traits (Table 4) showed that fruit weight had negligible correlations with other quality traits but a high correlation (r=0.85) with fruit width. These results highlight two things: 1) the rarity of large fruit size in sweet cherry breeding populations, and 2) the marginal genetic advance that has been made in breeding and

selection for large fruit size. The selected trees have been propagated by Willow Drive Nursery in readiness for planting in 2011 into combined on-station and on-farm trials. Note that the decision of whether to plant the selections into more advanced trials will be based on cummulative data from next season as well. Therefore, we will continue to evaluate the mother trees in the original test plots. One of the reasons for this fast-track strategy is to get input from growers to inform decision on selections to discard or go ahead with much earlier in the evaluation phase. In addition, we plan to plant the 'cultivars to beat' and some standard cultivars (or checks) alongside the test selections to facilitate performance comparison and easier separation of winners from discards. We will continue to work with Tom Auvil to identify grower test sites that fit into 'hot early' and 'cool late' climates.

Table 2. Sweet cherry commercial target market groups for the PNW.

Commercial target market group designation	Current leading cultivar	Target Market Group Description
E-MSM	Selah, others?	Early-mid-late-season, self-fertile, mahogany, suitable for mechanical harvest
ESB	Early Robin	Early-season, self-fertile, blush
ESM	Chelan	Early-season, self-fertile, mahogany, larger fruit size than Chelan
LSB	Rainier	Late-season, self-fertile, blush, powdery mildew resistant
LSM	Sweetheart	Late-season, self-fertile, mahogany, powdery mildew resistant
MSM	Bing	Mid-season, self-fertile, mahogany, larger fruit size than Bing

E-MSM = early-, mid-season, self-fertile, mahogany, mechanical harvest; ESB= early-season, self-fertile, blush; ESM= early-season, self-fertile, mahogany; LSB=late-season, self-fertile, blush; LSM= late-season, self-fertile, mahogany; MSM= mid-season, self-fertile, mahogany.

Table 3: Breeding seedlings identified for propagation following fruit quality evaluations in 2009, their target cultivar market groups and harvest dates. Note that harvest dates are only estimates, pH and TA values above 4.0 and 1.0 respectively, could be due to machine error. Data will be validated in future assessments.

Target market category	Selection	Harvest date (2009)	Mean frt wt (g)	Mean Firmness (Kgf)	Brix (%)	pH	TA (%)
Freestone	4.18.15-10	July	10.15	328.7	20.6	-	0.52
Freestone	4.3.1-2	22 June	12.10	209.2	21.0	3.68	0.62
LSM	4.18.15-47	9 July	11.15	209.4	24.5	2.83	1.12
LSM	4.18.15-48	2 July	13.13	249.9		3.84	
LSM	4.18.15-42	14 July	11.72	226.2	20.5	3.73	1.07
LSM	4.18.15-39	1 July	10.00	194.0	25.9	3.87	0.70
LSB	4.14.17-1	6 July	10.01	233.3	19.6	2.94	0.85
LSB	4.18.12-5	1 July	11.88	176.5	21.4	-	-
LSB	4.10.15-1	1 July	10.63	253.4	22.1	3.94	0.59
ESB	4.18.12-5	12 June	11.65	314.3	16.9	4.25	0.93
ESM	4.10.5-34	18 June	7.96	239.4	22.3	3.88	0.88
Valentine[X]	4.3.1-5	23 June	12.03	195.7	15.7	3.59	0.59

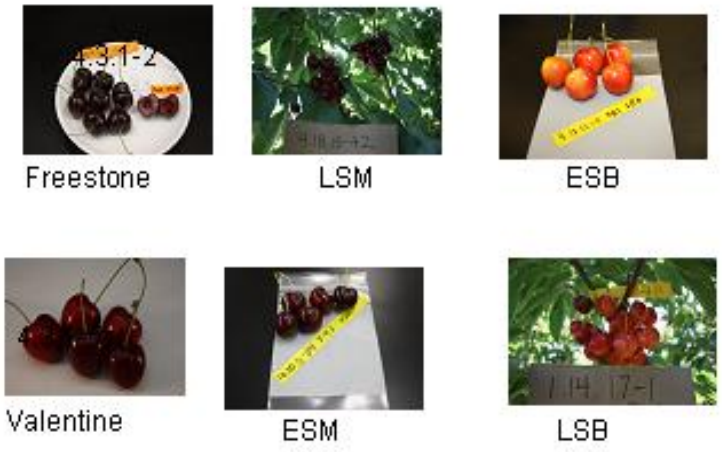
[X] Large heart-shaped dark cherry.
Refer to Table 2 for more information on target cultivar market groups; TA=Titratable acidity.

Fig. 1: Frequency distribution of fruit weight in seedlings evaluated from 2004 and some 2005 crosses in 2009.

Table 4: Phenotypic correlations between pairs of fruit quality traits following 2009 fruit evaluations.

Fruit trait pairs	**Correlation coefficient**
Fruit Weight-TA	-0.03
Fruit Weight-Brix	-0.17
Fruit Weight-Firmness	-0.31
Fruit Length-Width	0.65
Fruit Weight-width	0.85
Firmness-Brix	-0.17
Brix-TA	0.14

Fig. 2: Photos of representative sweet cherry selections made in 2009 that fall into different target market groups.



CONTINUING PROJECT REPORT **YEAR**: 2009
WTFRC Project Number:

Project Title: Programs to increase yields of target fruit in cherries

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Budget 1
Organization Name: WTFRC **Contract Administrator:** Kathy Schmidt
Telephone: 509 665 8271 **Email address:** Kathy@treefruitresearch.com

Item	2008	2009	2010
Salaries	49,822	43,771	43,771
Benefits	15,780	11,241	11,241
Wages	8,182	20,481	22,551
Benefits	3,389	5,461	6,008
Equipment	424	601	1,755
Supplies	4,255	2,322	2,809
Travel	2,598	2,277	2,241
Stemilt RCA lease		610	671
Revenue	16,500	25,300	19,300
Total	67,950	61,464	**71,747**

Comments: All numbers are based on the fiscal year July 2008-June 2009. Salaries are based on a general 15.6% proportion of full time staff: Auvil, Schmidt, Castillo, Hanrahan.
Auvil rootstock and variety testing costs included but not reported. Wages include a portion of collaborative costs for Karen Lewis mechanical thinning project approx. $1,000 (April-July).

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

OBJECTIVES

1. Investigate chemical blossom thinners to manage crop load in sweet cherry.

2. Investigate rain cracking susceptibility and management strategies.

    2.1.Evaluate spray programs to reduce rain-induced cherry cracking.
        -on-tree readings
        -at harvest quality
        -postharvest performance evaluation
    2.2. Track rain cracking susceptibility for 3 cultivars during maturation.
        -Tieton, Bing, Rainier
        -induced cracking test in lab
        -correlate to fruit weight and phenology
    2.3. Chart influence of spray programs to reduce rain induced cherry cracking on natural cracking susceptibility during maturation.

SIGNIFICANT FINDINGS

Objective 1: NC99 and ATS reduced final fruit set in Sweethearts and slightly improved fruit size. Dilute applications (200 gal/acre) of the same materials produced better results than concentrated applications (100 gal/acre). No treatment effects were observed from identical treatments in Staccato.

Objective 2: Only one site was affected by rain-induced cracking in 2009. The only material tested on this site (RainGard) reduced field cracking by half.

Cracking susceptibility during maturation was variety dependent: Bing and Rainier became susceptible 3 weeks before harvest, while Tietons were not until 10 days before harvest.

METHODS

Objective 1: Trials were set up in two varieties (Staccato and Sweetheart on Mazzard, 7 years old, 8' x 16' spacing) as randomized complete blocks with four replications of 7 trees/plot. We evaluated four programs for their effectiveness in reducing cherry crop load: ATS (4%) and NC 99 (8%) applied at either 100 or 200 gal/acre at 20 and 80% bloom with an Accutec sprayer. Initial bloom counts and subsequent fruit counts were performed on two branches/plot. Standard harvest parameters including firmness, titratable acidity, sugar content, weight, diameter, defect incidence, and color were measured.

Objective 2: The trial series included 9 cherry cracking trial sites utilizing 3 cultivars (Bing, Rainier, Tieton) and 7 products or product combinations. Trial designs were typically randomized complete blocks with 4 replications. All materials were applied by a) grower cooperators or b) WTFRC staff with an Accutec sprayer according to protocols developed collaboratively with product distributors.

Table 1: List of spray materials used to prevent rain-induced cherry cracking. WTFRC 2009.

Material	Spray schedule Wks before harvest 4[z]	3	2[y]	1	Concentration	Active ingredient(s)
Bluestim	x		x		4lbs+.5pt surfactant[v]	Glycine Betaine
Calcium nitrate		x	x	x	1% solution	Osmotic salt
RainGard		x	x	x	.8gal/acre	natural fatty acids
RainGard+Calcium nitrate		x	x	x	.8gal/acre+1%solution	n/a
VaporGard	x		(x)[w]		1gal/acre	Di-1-p-Menthene
Platina	x		x	(x)	.16gal/acre	L-Tryptophan
SureSeal	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] Monterey Super 7 surfactant

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 (%) =	$\frac{\sum(\text{Nc x F})}{100}$ x 100

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

The artificial cracking test was used on all fruit after harvest and bi-weekly during the last month before harvest in four selected blocks.

RESULTS & DISCUSSION

Objective 1: Chemical blossom thinners

Final fruit set was not reduced for Staccato cherries in 2009 (Table 2). ATS treatments reduced the final fruit set in Sweetheart, with higher amounts of water leading to more pronounced results. Fruit weight and sugars were increased with the most effective chemical treatment (4% ATS with 200 gal of water per acre) and row size was improved for most treatment combinations in Sweetheart. No effect on yield efficiency was noted.

This years results line up with those obtained in the past, they are inconsistent. We can not fully explain the variability of results between the two cultivars tested, since pollination conditions were excellent for both sites (50F + at application time, followed by warm days). The Staccato trial had a longer interval between applications (3 instead of 2 days) and higher maximum temperatures (50-65F vs. 65-80F) which ultimately might have led to more flower pollination.

One notable new finding is the notion that increased wetting might increase chances of thinning success. We plan on doing more carrier volume work and test a variety of wetting agents in 2010.

Further we collaborated with Karen Lewis to test mechanical flower removal as alternative to chemical means using the Darwin and UniBonn string thinners (results will be presented in the technology committee). Initial tests revealed that string thinners work well in modern high density plantings with planar tree structures such as the UFO (upright fruiting objects). Mechanical flower removal was far more effective than chemical thinning where strings did reach flowers and will be a feasible alternative method in orchards suitable to accommodate the machines.

Table 2: WTFRC cherry thinning results. Wenatchee Heights 2009.

TREATMENT	FRUIT SET (%)	FRUIT (g)	SUGARS (% Brix)	ACIDS (% Malic acid)	FIRMNESS (g/mm)	ROW SIZE	YIELD EFFICIENCY (kg/cm² LCSA)
Staccato/Mazzard							
ATS 200 gal/a	32 ab	10.6 ab	19.0 ns	0.688 ns	335 a	9.5 bc	0.34 a
ATS 100 gal/a	23 b	10.8 a	19.7	0.702	362 a	9.5 c	0.20 b
NC99 200 gal/a	30 ab	10.5 ab	19.1	0.735	337 b	9.6 a	0.19 b
NC99 100 gal/a	38 a	10.1 b	18.9	0.678	343 b	9.6 ab	0.35 a
Control	25 b	10.4 ab	19.6	0.696	356 a	9.6 abc	0.23 ab
Sweetheart/Mazzard							
ATS 200 gal/a	20 c	10.3 a	21.5 a	0.879 a	384 ab	9.5 b	0.22 ns
ATS 100 gal/a	27 bc	10.0 ab	19.3 b	0.836 ab	396 a	9.5 b	0.31
NC99 200 gal/a	28 abc	9.8 ab	20.1 ab	0.801 ab	387 ab	9.6 ab	0.23
NC99 100 gal/a	30 ab	9.5 b	19.6 b	0.781 b	381 ab	9.5 b	0.30
Control	37 a	9.3 b	19.2 b	0.810 ab	376 b	9.7 a	0.34

Objective 2: Prevention of rain cracking:

Only one of nine trials demonstrated significant field cracking (>10%) of fruit. In that lone trial, a single RainGard application five days before rain reduced baseline cracking by half (Table 3). No other quality parameter was influenced at harvest or after 14 days in cold storage (Table 4, 5). Fruit quality analysis for the remainder of the trials revealed no consistent effects of any treatment (data not shown).

Table 3: Cracking severity of Tieton/Gisela. Pasco 2006.

	CRACKING		SPLITTING			CRACKING
	(on tree)	CLEAN	TOP	SIDE	BOTTOM	INDEX
TREATMENT	%	%	%	%	%	%
AT HARVEST						
RainGard	15 b	91 a	1 ns	0 ns	9 b	16 ns
Control	31 a	84 b	0	1	15 a	23

Table 4: At harvest quality parameters for Tieton/Gisela 6. Pasco 2009.

	WEIGHT	ACIDS	SUGARS	FIRMNESS	DIAM	ROW SIZE	COLOR
TREATMENT	(g)	(% malic acid)	(% Brix)	(gm / mm)	(mm)		(1-7)
AT HARVEST							
RainGard	12.2 ns	0.691 ns	14.7 ns	277 ns	30.5 ns	9.1 ns	4.2 ns
Control	12.8	0.757	15.7	278	30.9	8.9	4.3
AFTER 14 DAYS OF COLD STORAGE							
RainGard		0.760 ns	14.9 ns	310 ns	30.7 ns	9.0 ns	4.8
Control		0.704	15.8	292	30.3	9.1	5.0

Table 5: Stem browning, pitting and weight loss after 14 day cold storage at 33F for Tieton/Gisela 6. Pasco 2009.

	STEM BROWNING				PITTING			WT LOSS
	0-25	26-50	51-75	76-100	CLEAN	SLIGHT	SEVERE	14 day
TREATMENT	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
AFTER 14 DAYS OF COLD STORAGE								
RainGard	71 ns	17 ns	11 ns	1 ns	96 ns	4 ns	0 ns	2 ns
Control	63	21	12	5	92	7	1	3

We observed 4 blocks during the last month before harvest (2 Bing, 1 Rainier and Tieton each) (Figure 1). Initial fruit weight averaged 4g and color was green to light green (example in Figure 2). Samples for the artificial cracking test were taken bi-weekly. Bing and Rainier cherries started to crack in bench top assays 19 days preharvest; susceptibility rapidly increased for a week before plateauing 10 days before harvest for Rainier (Figure 1). Tieton cherries were crack resistant until 10 days before harvest with susceptibility steadily increasing until harvest. Tieton and Bing fruit reached similar levels of cracking susceptibility at harvest, while Rainier was considerably less prone to cracking when reaching full maturity (Figure 1). We attempted to follow treatment effects on preharvest cracking susceptibility as well, but results were variable (data not shown).

In summary, Tieton cherries demonstrated later cracking susceptibility than Bing and Rainier. As far as we know, this is the first attempt in the Pacific Northwest to a) describe the development of cracking susceptibility during maturation b) consider new varieties such as Tieton. Natural cracking susceptibility of one cultivar may vary greatly from year to year, hence it is necessary to obtain more than one years worth of data. With robust data sets it will be possible to revise current recommended spray programs which start 3-4 weeks preharvest and can become cost prohibitive with multiple applications. The information will also be fed into the cherry cultivar improvement program.

For the 2010 season, we plan to continue testing of spray programs to reduce rain induced cherry cracking with particular emphasis on optimization of application timings. We will continue to investigate the development of cracking susceptibility during maturation.

Figure 1. Development of cracking susceptibility for Bing, Rainier and Tieton cherries. Yakima Valley 2009.

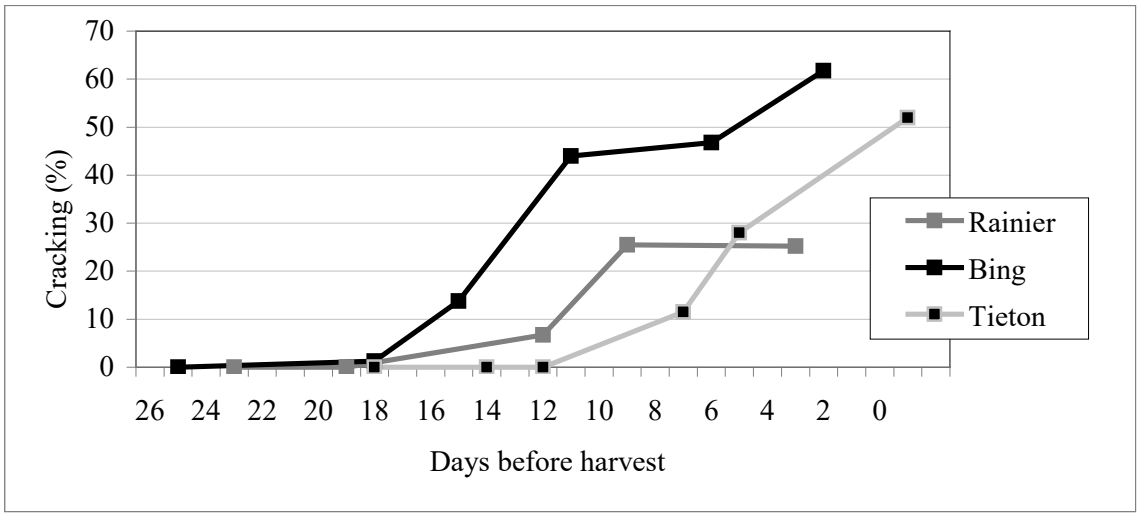
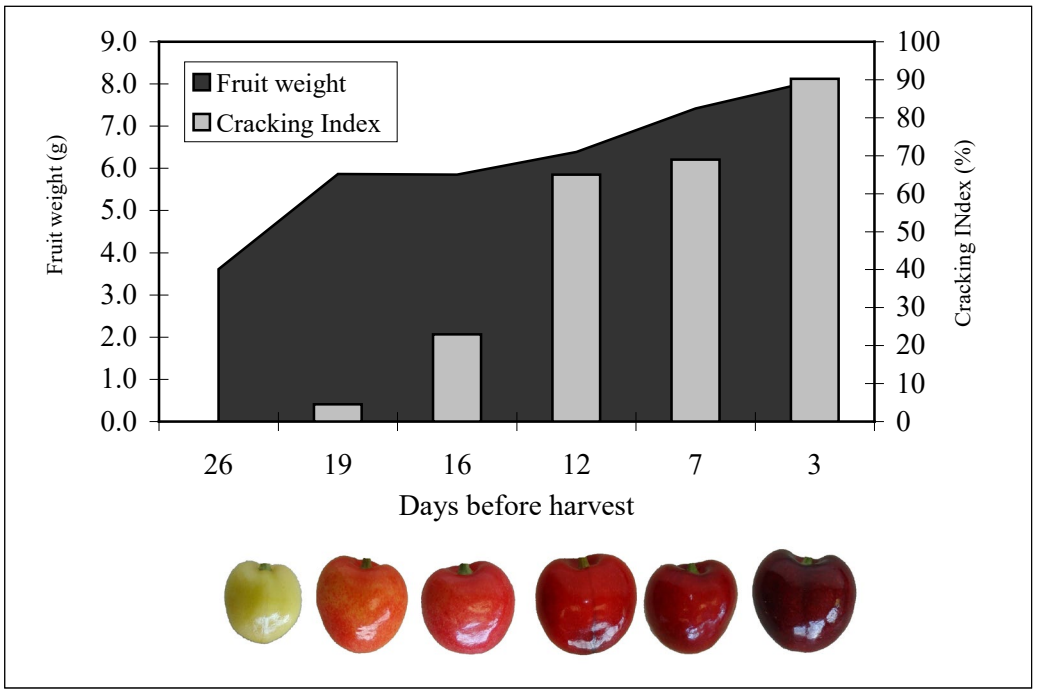


Figure 2: Relationship between fruit growth and cracking susceptibility in Bing/Mazzard. Zillah 2009.



Literature cited

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