2011 NW Cherry Research Review
Confluence Technology Center, Wenatchee, WA

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FINAL PROJECT REPORT **YEAR**: 2010

Project Title: Horticultural mgmt. systems for high value fresh and brine cherries.

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Project Funding History:

2006: $45,000
2007: $50,150
2008: $56,240
2009: $56,785
2010: $11,000

Objectives:

1. Identify cherry cultivars and rootstocks suitable for the sweet cherry industry especially for the wetter districts in the Pacific Northwest.
2. Evaluate the effects of training system, rootstock and variety on tree performance, fruit quality and yield.

Significant findings and impacts

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 while MxM14, 46 and 39 had significantly smaller TCSA's. Very little bacterial canker was observed in this trial, a hopeful observation considering the susceptibility of 'Sweetheart' to this pathogen. MxM39 and 46 had the highest yield efficiency.
Impact: MxM 39 and 46 are currently not in the nursery trade. Because of their ability to reduce vigor and their performance throughout the duration of the study, these rootstocks are being propagated and will be made available for further distribution to the industry. The vigor of trees are reduced when topworked on MxM rootstocks in contrast to low-budded trees. An orchardist in areas with high bacterial canker pressure could consider grafting trees higher than normal to produce a trunk with canker tolerance and reduce vigor of the scion variety.

b. *2003 'Skeena'/Krymsk rootstock trial* - Krymsk6 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 6^{th} leaf.
Impact: The Krymsk rootstocks were only evaluated with Skeena as the scion cultivar. Caution should be taken when planting Krymsk 5 in the Willamette Valley because of its high mortality.

c. *2006 NY blush and variety trial on Gisela 6* –Results from only one of three sites for 2009 and 2010 are summarized in Tables 2a and b (The Dalles- Cooper). Within the table the '☺' symbol was used to identify selections that deserve further testing. 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 but generally met the desired criteria of 225+ g/mm. 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 the 600 g benchmark indicating a likelihood for good stem retention.
Impact: NY288 deserves further evaluation as an early dark cherry. The dark cherry NY213 could fill a critical window between 'Bing' and 'Regina' harvest. Three blush genotypes deserve further evaluation; NY525, NY1913, and NY213. NY 525 and 213 had the least bacterial canker.

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 in 2009. In 2010, the highest yielding cultivars were 'Sylvia', 'Rainier', and 'Sweetheart'.
Impact: Giessen 196-4 deserves further evaluation, especially for precocious cultivars.

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 (Table 4). 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/tree, all others <5.0). The trees in their third leaf grew well and set a handful of fruit. Tree size is not yet substantively different between rootstocks in this trial.
Impact: Clearly 'Regina' is an exceptionally well-suited genotype for the Willamette Valley. In light of the high susceptibility of trees to *Pseudomonas syringae* when grafted onto Gisela 6, the MxM and Gi196-4 rootstocks may be a suitable alternative.

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 yields in 2009 and 2010. Fruit from central leader trees had the best color (Table 5), firmness, size, and soluble solids concentration and the least amount of cracked fruit compared to multiple and steep leader trees in 2009 (data not shown). In 2010, fruit size was slightly smaller on central leader trees (Table 5) with no difference in stem pull force, firmness, or amount of cracking.
Impact: A modified central leader training system was the best choice for this rootstock scion combination. All shoot tips were removed at green tip for three years. Delayed heading cuts (mid-June) were made for three years as well and must be made before floral initiation in order to induce spurring and avoid blindwood formation. Lateral shoots are headed back 25-35% depending on vigor, angle and location of the shoots.

3. *Growing degree hour model* – GDH can be used to predict time of GA application and harvest for sweet cherry cultivars (Tables 6 and 7). 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 (Table 7). GA was likely applied too early to the one 'Rainier' block from which we collected data in 2009. 'Sweetheart' GA sprays were being applied too early when we began this study.
Impact: Time of harvest for different cultivars is largely determined by the duration of Stage II of fruit growth (pit hardening phase) and Stage III (final swell). One can predict end of stage II and, likely, harvest using the fruit growth model by DeJong and Goudriaan (1989) where 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))).**
Based on our findings and our encouragement, the GA applications to 'Sweetheart' fruit have been delayed to a more appropriate timing.

4. *Alternative cropping systems*
2005 Alternative orchard floor and fertility management – After five years of treatment, there was a 21% reduction in organic matter in the plots where landscape cloth or no amendment (NoAM) was used (Table 8). OM increased by 24% in the organic amendment (OrAM) management plots. Nitrates were reduced by half under the NoAM while a 50% increase was observed in the OrAM. plots. Estimated N release, mineralizable N, NO_3, K, Zn, and B levels were higher in plots receiving organic amendments. N-acetyl-b-D-glucosaminidase activity was well correlated with N-mineralization potential (Fig. 3, 4, and 5).
Impact: A commercialization potential exists for rapidly assessing N-mineralization potential via a N-acetyl-b-D-glucosaminidase assay. Singular additions of organic matter can have an immediate influence of N mineralization rates (Fig. 6).

5. *Other notable accomplishments*

- 'Regina' ovules have a shorter ovule longevity than 'Bing' (Fig. 7) requiring special attention be paid to supplying adequate compatible and viable pollen at the beginning of anthesis, good nutrition for enhance floral quality, and careful consideration of pollinizer arrangement.
- 13N7-39 is an exceptional quality, high value blush cherry that also brines well. Oregon Cherry Growers is working with PICO for a license to grow and produce it. Other noteworthy blush genotypes suited for fresh production include 13N07-32, and 2N31-19.
- Five genotypes, NY518, NY8182, NY9295, NY20-11 and 13N07-39 are well suited as brine cherries.
- PiKu rootstocks were not well suited for the Willamette Valley as the mortality rate was very high.
- Burning a notch above the bud created the same effect as notching but without any bacterial canker symptoms.
- Cherry training systems that use wires are not suited for regions with high bacterial canker pressure. The point of attachment serves as an excellent point of entry for the pathogen.

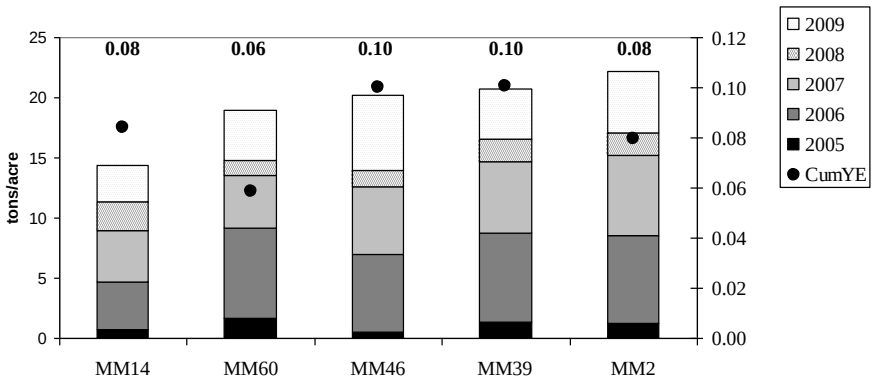
Results: see detailed findings in the following figures and tables.

Table 1. Effect of rootstock on tree performance and fruit quality in 2009 of 'Sweetheart' trees topworked onto MxM rootstocks for mechanical harvest.

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

[z]Rootstocks were planted in 2000 at a 18' x 18' spacing in a completely randomized design with 6 replications 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 yield efficiency for five MxM rootstocks top-worked with 'Sweetheart'.



Tables 2a. 2009 and 2010 performance of NY blush and dark cherry selections on Gisela 6 rootstock in their fourth leaf at an on-farm research trial in The Dalles, OR (Cooper).

Selection[z]	2009 Yield (kg)	2010 Yield (kg)	2010 TCSA (cm^2)	2009 Yield Eff. (kg/cm^2)	2010 Yield Eff. (kg/cm^2)	Bacterial Canker (%)[y] LB Farm Corvallis
			Early season harvest			
☺ ☺ NY288	7.0 cdefg	20.7 a	85 bc	0.12 cd	0.246 a	100
NY113 (Blush)	1.9 efg	0.1 g	117 abc	0.02 ef	0.001 f	67
NY2068	1.3 fg	1.9 efg	150 a	0.01 ef	0.013 def	100
			Mid-season harvest			
'Rainier'	16.5 a	6.1 cde	124 ab	0.24 a	0.060 cd	100
'Radiance Pearl' (NY7679-Bl)	16.4 a	.8 defg	107 abc	0.20 ab	0.026 def	100
NY8039	11.4 abc	0	70 c	0.14 bc	.	80
☺ ☺ NY525 (Blush)	10.5 abc	12.2 b	94 bc	0.14 bc	0.130 b	25
NY132	9.2 bcd	1.0 g	125 ab	0.10 cd	0.009 ef	75
NY8033 (Blush)	8.5 bcd	1.7 fg	120 ab	0.10 cd	0.014 def	50
☺ NY1913 (Blush)	4.1defg	6.3 dc	109 abc	0.05 def	0.058 cde	0
☺ NY213	1.1 g	3.4 defg	147 a	0.01 f	0.024 def	20
			Late season harvest			
☺ NY9116	13.4 ab	5.6cdef	125 ab	0.16 bc	0.048 cdef	100
'Skeena'	7.4 cdef	8.4 bc	151 a	0.09 cde	0.055 cde	100
'Regina'	.	1.7 fg	128 ab	.	0.014 def	0
MSD[z]	6.0	4.3	49	0.08	0.051	

[z] Means separation is by the Waller Duncan k-ratio t-test, k-ratio=100.
[y]Percentage of trees with major scaffold and trunk infestation.

Selection[z]	Fruit Size 2009 (mm)	Fruit Size 2010 (mm)	SSC 2009 (°Brix)	Fruit Firmness 2009 (g/mm)	Fruit Firmness 2010 (g/mm)	Stem Pullforce 2009 (g)	Stem Pullforce 2010 (g)	Comments
				Early season harvest				
☺ ☺ NY288	27.8 cd	27.8 cd	26.3 a	211 efg	234 e	1029 bc	1084 cd	Firmness?
NY113	31.0 a	32.0 a	25.2 ab	290 c	220 e	759 ef	1193 bc	Low yield
NY2068	29.8 abc	31.0 abc	24.0 bcd	255 cde	257 cde	906 d	1032 d	Low yield
				Mid-season harvest				
'Rainier'	28.1 bcd	32.7 a	18.7 h	246 cdef	221 e	1063 ab	726 e	
☺ 'Radiance Pearl' (Bl)	29.9 ab	30.9 abc	19.4 gh	222 efg	330 b	638 gh	1182 bcd	
NY8039	31.3 a	30.9 abc	20.5 fgh	241 def	242 de	880 d	1078 cd	Yield?
☺ ☺ NY525 (Bl)	27.2 d	27.4 ef	22.6 cde	242 def	302 bcd	851 de	1039 cd	Good yield Firm, +pf
NY132	28.3 bcd	29.4 cde	25.3 ab	216 efg	234 e	1036 ab	1461 a	
NY8033 (Bl)	31.3 a	30.0 bcd	22.0 def	256 cde	228 e	1026 bc	1039 cd	
☺ NY1913 (Bl)	26.2 d	25.5 f	25.3 ab	436 a	410 a	912 cd	1037 d	Very firm, fruit size?
☺ NY213	29.6 abc	31.2 abc	21.3 efg	405 ab	314 bc	851 de	703 e	Very firm
				Late season harvest				
☺ NY9116	26.4 d	30.6 abc	21.5 efg	209 fg	305 bc	520 h	1157 cd	Variable results
'Skeena'	30.0 ab	32.1 a	19.3 gh	363 b	307 bc	1153 a	703 e	
'Regina'	28.2 bcd	31.8 ab	24.4 abc	190 g	315 bc	713 fg	1333 ab	
MSD[y]	2.1	2.1	2.2	46	62	119	155	

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

Table 3. 2009 and 2010 performance of several cultivars in their 4th and 5th leaf on Giessen (Gi) 196-4, Gisela 6 and Mazzard rootstocks from one on-farm location in The Dalles (Cooper).

Selection	Rootstock	Yield 2009 (kg)	Yield 2010 (kg)	TCSA 2010 (cm^2)	Yield Eff. 2009 (kg/cm^2)	Yield Eff. 2010 (kg/cm^2)	Size 2009 (mm)	Fruit Firmness 2009 (g/mm)	Stem Pullforce 2009 (g)	N
'Tieton'	Gi 196-4	0.9	2.0	155	.01	.013	30.5	14.5	226	3
S^3S^9	Gisela 6	2.8	3.3	107	.04	.034	30.6	15.5	188	1
	Mazzard	0.5	1.4	149	.003	.011	.	14.2	.	2
'Early Robin'	Gi 196-4	1.0	3.4	100	.02	.035	30.9	17.7	244	2
S^1S^3	Gisela 6	3.5	4.3	102	.05	.042	30.9	16.8	261	1
	Mazzard	0.3	2.0	124	.003	.016	29.3	16.7	241	0
13N 7-39	Gi 196-4	.	0.8	92	.	.008	29.3	28.0	238	2
$S^1S^{4'}$	Gisela 6	3.4	1.9	107	.05	.020	30.8	26.0	348	3
	Mazzard	1.0	0.7	118	.011	.005	29.8	21.0	365	1
'Benton'	Gi 196-4	1.1	.02	199	.01	.000	31.2	17.9	286	1
$S^3S^{4'}$	Gisela 6	2.6	1.4	202	.02	.007	32.0	19.4	279	2
	Mazzard	0.2	.	211	.002	.	.	.	.	2
'Sylvia'	Gi 196-4	6.8	6.3	124	.10	.056	30.5	16.1	238	2
S^1S^4	Gisela 6	7.6	8.6	114	.09	.074	29.5	15.9	235	0
	Mazzard	1.3	0.3	145	.017	.001	28.9	14.1	265	1
'Bing'	Gi 196-4	.	3.0	.	.	.030	.	.	.	0
S^3S^4	Gisela 6	14.4	3.1	107	.20	.037	30.2	18.8	288	1
	Mazzard	.5	.02	126	.005	.002	30.3	19.0	263	1
'Rainier'	Gi 196-4	14.6	5.7	125	.22	.046	32.3	19.8	253	1
S^1S^4	Gisela 6	16.5	4.3	109	.21	.041	32.2	19.1	259	2
	Mazzard	1.0	1.0	116	.013	.008	31.0	18.4	304	3
'Skeena'	Gi 196-4	1.8	1.6	136	.02	.012	31.8	19.0	364	0
$S^1S^{4'}$	Gisela 6	7.3	.	139	.08	.	31.6	18.5	367	1
	Mazzard	0.8	5.5	158	.008	.035	31.2	17.0	384	1
'Regina'	Gi 196-4	2.5	0.4	131	.03	.003	31.2	18.8	319	2
S^1S^3	Gisela 6	2.2	0.4	116	.02	.004	30.3	20.4	275	2
	Mazzard	0.1	0.0	139	.001	.000	.	.	.	3
'Sweetheart'	Gi 196-4	16.7	9.4	99	.22	.098	.	.	.	1
$S^3S^{4'}$	Gisela 6	18.6	16.1	98	.27	.164	.	.	.	1
	Mazzard	0.5	0.7	117	.005	.006	.	.	.	3
'Sunset Bing'	Gi 196-4	.	0.8	121	.	.017	.	.	.	0
S^3S^4	Gisela 6	.	3.3	110	.	.030	28.3	19.5	344	0
	Mazzard	.	0.2	122	.	.001	.	.	.	1

N= Number of trees with no loss primary scaffolds or tree at the LB Farm, Corvallis, OR

Table 4. Early performance in 2010 of a 'Regina' rootstock trial planted in 2008 that were low-budded on MxM rootstocks, Giessen 196-4 and Gisela 6.

	TCSA (cm^2)	Yield (kg)	Fruit Firmness (g/mm)	Fruit Size (mm)	Stem Pullforce (g)	SSC (°Brix)
Gisela 6	34.2	1.04 a	230 a	27.9 a	928 a	20.6
MxM14	37.6	0.73ab	214 a	27.3 ab	818 a	21.5
MxM39	37.3	0.45 b	212 a	27.3 ab	803 a	21.6
Giessen 196-4	35.3	0.43 b	223 a	27.1ab	787 a	22.3
MxM46	37.0	0.20 b	188 b	26.2 b	507 b	22.0
MSD	ns	0.58	23	1.6	178	ns

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

Table 5. 2009 and 2010 performance of 'Early Robin' trees on Gisela 6 rootstock planted in 2006 (The Dalles, Carter) and trained to three training systems.

	TCSA 2010 (cm^2)	Yield 2009 (kg)	Yield 2010 (kg)	Color 2009 (%)	Firmness 2010 (g/mm)	Fruit Size 2010 (mm)	Cracking 2010 (%)	Stem Pullforce 2010 (g)
Steep Leader	122.5	15.0	20.2b	55.3 b	253	29.8 a	8.5	1189
Central Leader	122.9	17.2	25.3a	63.2 a	241	28.8 b	6.7	1212
Multiple Leader	114.7	14.0	16.1c	46.8 c	246	29.2ab	6.4	1109
MSD[z]	0.1	ns	3.6	5.7	ns	0.9	ns	ns

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

Table 6. Average number of growing degree hours (GDH) reported by growers at the time of GA application (end of pit hardening and Stage II) and when fruit were harvested.

	OSU estimate[z]	2006	2007	2008	2009	Mean GDH[y] 2006-09	2009 GDH/ day	Std. Dev. in GDH (days) 2009
'Bing'								
GA applied	8,588	9,881	9,949	10,972	9,692	10,124	306	576(1.9)
Harvest		18,874	17,140	17,644	17,859	17,879	299	728(2.4)
'Regina'								
GA applied	11,014	10,754	11,146	12,324	10,893	11,279	317	715(2.3)
Harvest		21,436	20,636	20,426	21,516	21,004	258	553(2.1)
'Sweetheart'								
GA applied	12,357	8,095	12,018	13,181	11,178	11,118	312	1006(3.2)
Harvest		22,808	22,645	21,404	23,065	22,481	261	738(2.8)

[z] Recommended time of GA application based on end of pit hardening

[y]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 7. Growing Degree Hour[z] (GDH) accumulation from peak bloom (80%) to first GA spray and to harvest for eight varieties reported by several growers in The Dalles, OR in 2009.

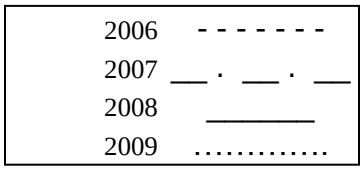
	Chelan	Early Robin	Bing	Rainier	Lapins	Skeena	Regina	Sweetheart
GDH accumulated from peak bloom to *GA spray*								
2008	10720	.	10972	14369	12615	11510	12324	13181
2009	9337	10787	9692	6171	10746	10873	10893	11178
Average	**10029**	**10787**	**10332**	**10270**	**11681**	**11192**	**11609**	**12180**
GDH accumulated from peak bloom until 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**

[z]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))).

Figure 2. A double-sigmoidal curve describes fruit growth as diameter increases with accumulated growing degree hours (GDH) from shuck split onward. Growth curve data was collected over 3 years. The subsequent 4 years, grower dates were recorded and plotted as vertical lines at GA spray and harvest.



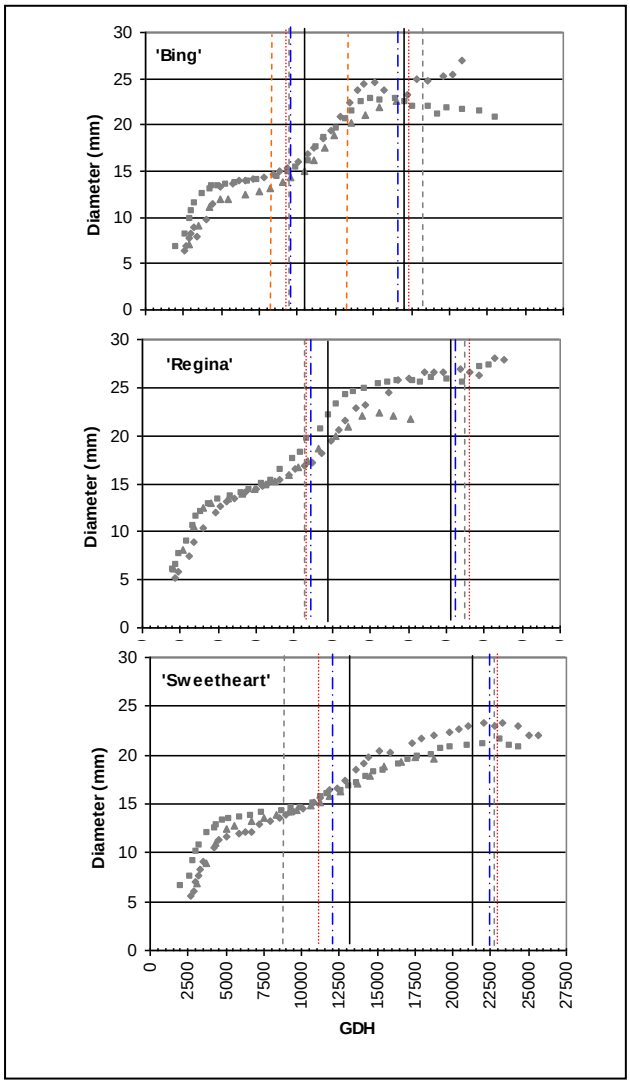


Table 8. Effect of five years of alternative orchard floor management systems on chemical soil constituents. (No Am= landscape cloth; Or Am = mulch/compost)

	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*

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

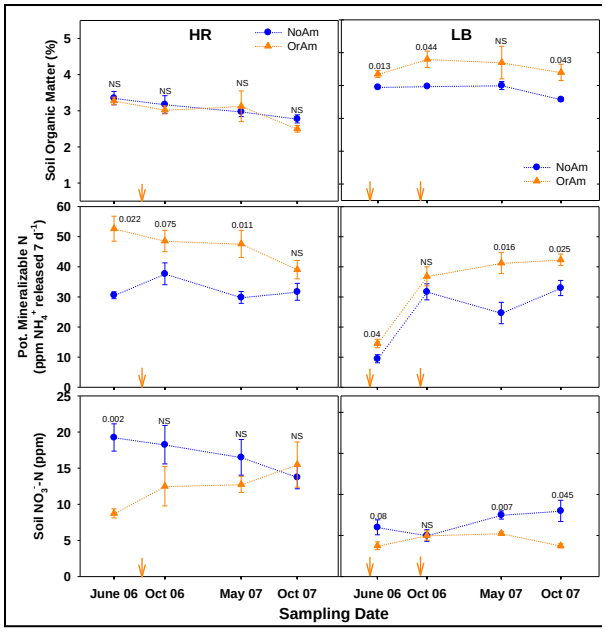


Figure 4. The influence of straw mulch and compost and landscape cloth on N-acetyl-b-D-glucosaminidase activity (µg/g soil) at Hood River (HR) and Corvallis (LB).

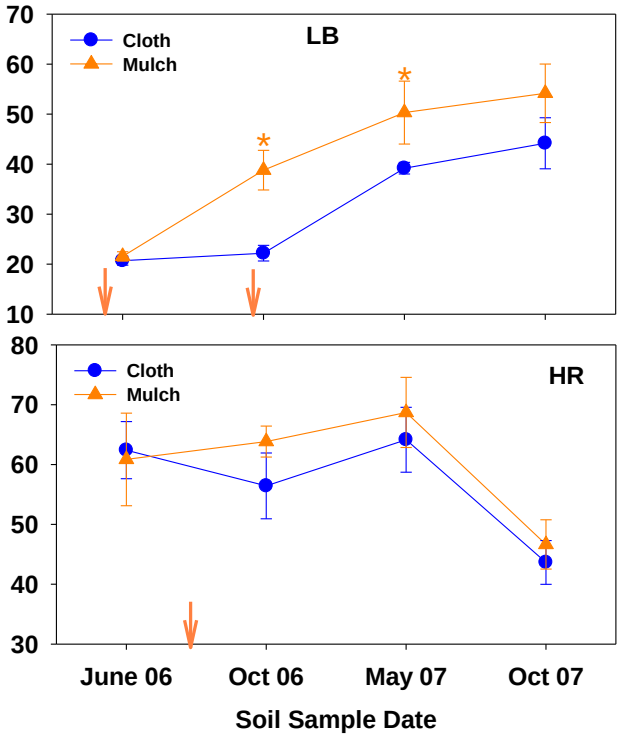


Figure 5. Correlation between N-acetyl-b-D-glucosaminidase activity and N-mineralization.

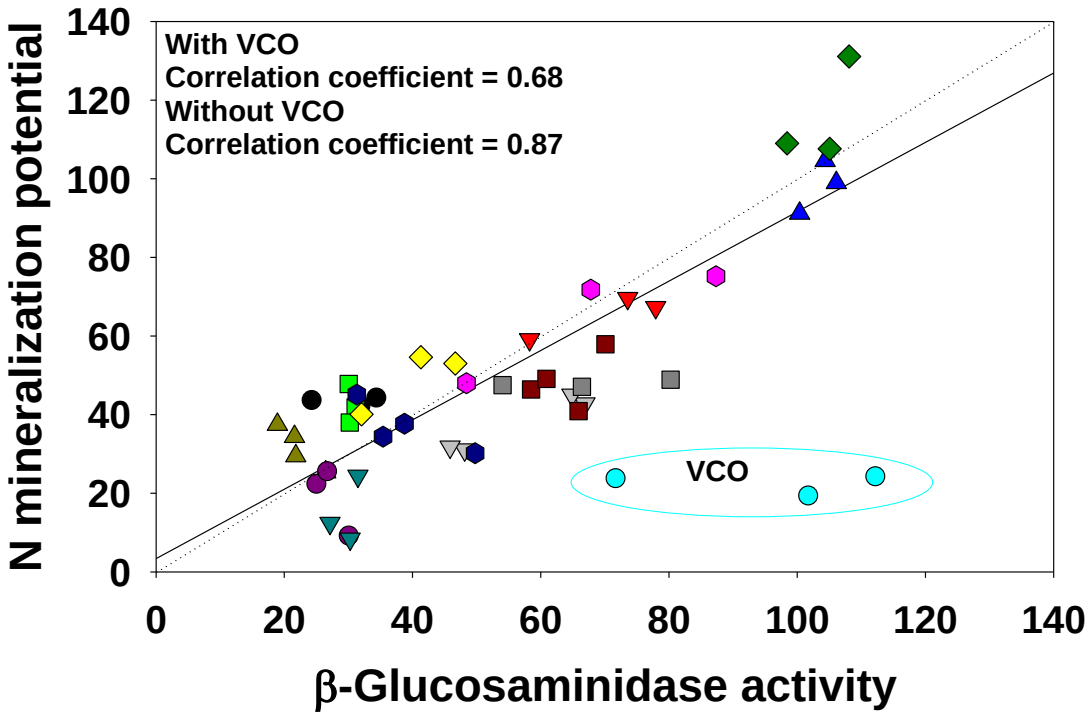


Figure 6. On-farm trials: Influence of organic matter amendments on soil enzyme and N mineralization.

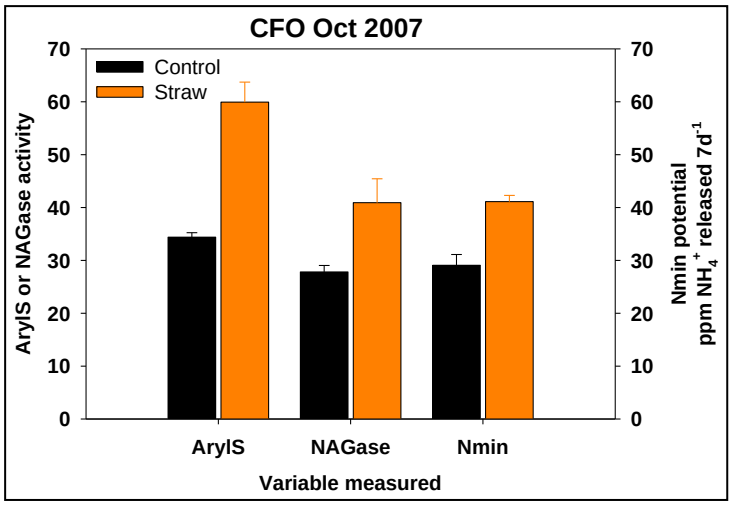
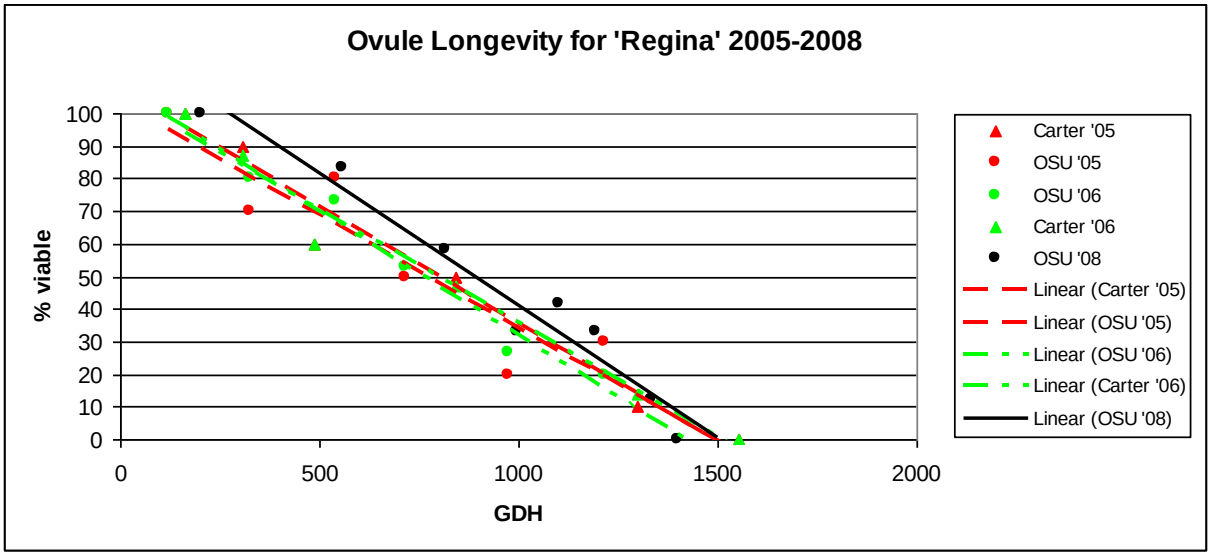


Figure 7. Ovule longevity from 2005-2008 for 'Regina' ovules evaluated in The Dalles and Corvallis, OR.



Executive Summary

ROOTSTOCK AND VARIETIES

MxM 39 and 46 are currently not in the nursery trade. Because of their ability to **reduce vigor** and their performance throughout the duration of the study, these rootstocks are being propagated and will be made available for further distribution to the industry. The vigor of trees are reduced when topworked on MxM rootstocks in contrast to low-budded trees. Trees had virtually **no symptoms of bacterial canker.** An orchardist in areas with high bacterial canker pressure could consider grafting trees higher than normal to produce a trunk with canker tolerance and reduce vigor of the scion variety.

The Krymsk rootstocks were only evaluated with Skeena as the scion cultivar. Caution should be taken when planting **Krymsk 5** in the Willamette Valley because of its **high mortality**.

NY288 deserves further evaluation as an early dark cherry. The dark cherry **NY213** could fill a critical window between 'Bing' and 'Regina' harvest. Three blush genotypes deserve further evaluation; **NY525, NY1913, and NY213**. NY 525 and 213 had the least bacterial canker.

Giessen 196-4 deserves further evaluation, especially for precocious cultivars.

Clearly **'Regina'** is an exceptionally well-suited genotype for the Willamette Valley. 'Regina' ovules have a **shorter ovule longevity** than 'Bing' (Fig. 7) requiring special attention be paid to supplying adequate compatible and **viable pollen** at the **beginning of anthesis**, good nutrition for enhance **floral quality**, and careful consideration of **pollinizer arrangement**.

In light of the high susceptibility of trees to *Pseudomonas syringae* when grafted onto Gisela 6, the **MxM** and **Giessen 196-4** rootstocks may be a suitable alternative.

TRAINING SYSTEMS

A **modified central leader training system** was the best choice for 'Early Robin' on Gisela 6 rootstock. The traditional central leader training system was substantively modified to insure maximum fruiting wood development. All **shoot tips were removed** at green tip for three years. **Delayed heading cuts** (mid-June) were made for three years as well and were made before floral initiation occurred in order to **induce spurring and avoid blindwood formation**. Lateral shoots are headed back 25-35% depending on vigor, angle and location of the shoots. This principle applies not only to central leader training systems but also multiple leader systems. Cherry training systems that use **wires** are not suited for regions with high **bacterial canker** pressure. The point of attachment serves as an excellent point of entry for the pathogen. **Burning** a "notch" above the bud created the **same effect** as **notching** but without any bacterial canker symptoms.

GROWING DEGREE HOUR MODEL

Time of **harvest** for different cultivars is largely determined by the **duration of Stage II** of fruit growth (pit hardening phase) and **Stage III** (final swell). One can predict end of stage II and, likely, harvest using the fruit growth model by DeJong and Goudriaan (1989) where 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:

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

Based on our findings and our encouragement, the GA applications to 'Sweetheart' fruit have been delayed to a more appropriate timing.

ALTERNATIVE ORCHARD FLOOR AND FERTILITY MANAGEMENT

A commercialization potential exists for rapidly assessing **N-mineralization potential** via a **N-acetyl-b-D-glucosaminidase** assay. Singular additions of organic matter can have an immediate influence in increasing N mineralization rates. Estimated N release, mineralizable N, NO_3, K, Zn, and B levels were higher in plots receiving organic amendments.

FINAL PROJECT REPORT **YEAR**: 2 of 2

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

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Cooperators: None

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

Other funding sources: None

WTFRC Collaborative expenses: None

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

Item	**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	$8304	

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

- Significant variability in cherry skin color was observed during mid-harvest timing (commercial harvest) for most cultivars. Variability was greatest at early harvest timing, but quality associated with early harvests was suboptimal
- Skin color darkened with advancing harvest dates. Rate of darkening was cultivar dependent.
- All quality attributes tested (soluble solids, total acids, firmness, stem retention force, fruit size and mesocarp color) were significantly related to skin color (e.g., variability of any given quality attribute could be explained to a high degree by skin color)
- In general, as fruit persist on the tree- skin color, mesocarp color, sugars, and fruit size increase, while firmness, and retention force decrease
- Soluble solids content of fruit increased linearly with darkening skin color.
- Cherry quality attributes were well-maintained for up to 28 days post-harvest
- Cherry fruit firmness typically increased with storage time, irrespective of cultivar
- Sugars and weight did not change appreciably with storage time
- Stem retention force and total acids changed inconsistently
- 2009 and 2010 pitting severity of 'Sweetheart' was unrelated to harvest timing

Results and Discussion

Harvest timing: Several analyses were run to describe the effect of harvest timing on individual quality attributes. Pooling all data (years and cultivars) produced very intriguing results describing general cherry ripening behavior (Table 1).

Table 1. The effects of harvest timing on mean fruit quality attributes, pooled for all cultivars, and both years. Attributes analyzed were: mesocarp color [CTIFL scale (1-light pink, 7-black)], average fruit size (weight and diameter), firmness, stem retention force, soluble solids (SS) and total acids (TA).

Harvest (timing)	Mesocarp color (1-7)	Avg.fruit sz. (g)	Avg.fruit sz. (mm)	Firmness (g/mm)	Retention force (g)	SS (%)	TA (%)
Early	3.1c	10.3c	28.3c	286a	731a	18.6c	0.81a
Mid	3.9b	10.5b	28.4b	275b	618b	19.8b	0.75c
Late	4.4a	11.2a	29a	272b	566c	21.6a	0.79b

Nearly all attributes progressively changed relative to the duration of time that fruit remained on the tree. In fact, one could draw the general conclusion that as cherry harvest timing is delayed, skin color, mesocarp color, size, and sugars all increase, while pedicel retention force and fruit firmness decrease (Table 1). These trends were apparent each year, though the absolute value of given attributes significantly changed from year to year (data not shown). This can be attributed to a

myriad of environmental regulators, of which cropload and climatic factors are dominant. Another interesting observation is the order of magnitude that different attributes change with advancing harvest time (Table 1).

Similar trends are evident for individual quality attributes relative to incremental changes in skin color (years, cultivars and harvests combined) (Table 2); sensible given that cherry skin color darkens

Table 2. The effect of skin color [CTIFL scale (1-light pink, 7-black)] averaged over all cultivars, years and harvest timings on mean fruit quality attributes. Attributes analyzed were: mesocarp color, average fruit size (weight and diameter), firmness, stem retention force, soluble solids (SS) and total acids (TA).

Skin Color (1-7)	Mesocarp color (1-7)	Avg.fruit sz. (g)	Avg.fruit sz. (mm)	Firmness (g/mm)	Retention force (g)	SS (%)	TA (%)
1	1g	7.4d	25.3f	344a	1650a	12.9g	na
2	1.8f	8.9c	26.9e	314b	902b	14.9f	0.76c
3	2.5e	10.2b	28.5bc	296c	704c	17.3e	0.76c
4	3.2d	10.9a	28.8ab	280d	638c	19d	0.77gc
5	4.2c	11.1a	28.9a	277d	611c	20.8c	0.78bc
6	5b	10.8a	28.4c	264e	541c	22.5b	0.8b
7	6a	10.2b	27.7d	229f	541c	24.9a	0.88a

as harvest is delayed. But, only sugars, mesocarp color and firmness (in one out of two years) continued to respond linearly with changes in skin color, while other attributes, such as fruit size and retention force were best explained by a polynomial function (Fig 1). Since harvest timing reflects

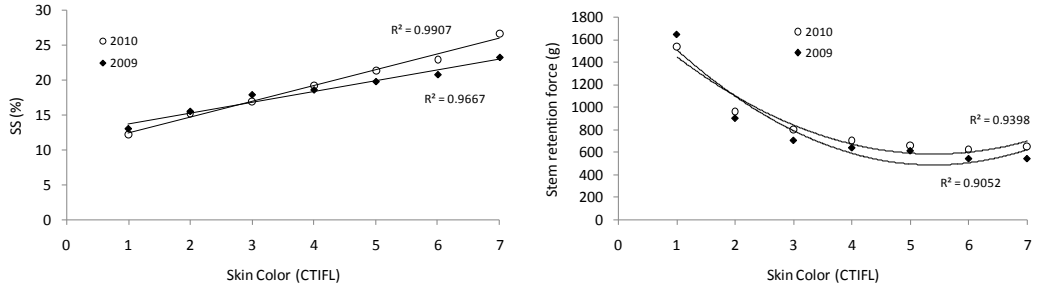


Figure 1. Relationship between soluble solids (left) and stem retention force (right) and cherry skin color (CTIFL). Data are averages of all cultivars, harvest timings and years.

the average skin color, it is less influenced by inclusion of highly under-mature fruit (CTIFL 1 and 2; unmarketable fruit) at early timings, or the presence of fruit which are quite advanced (CTIFL 7) at late timings. When fruit quality attributes are related to CTIFL color class, the extreme ends of the scale tend to deviate from the linearity observed between 3-6 CTIFL, which constitutes the commercial range.

Fruit size increased with each subsequent harvest date to a CTIFL score of 5-6 (Table 1), although, in general, darker fruit (CTIFL 7) were accompanied by a reduction in fruit size. 'Bing', and to a lesser degree 'Regina' and 'Chelan' comprised the entirety of the CTIFL 7 color class, and with the exception of 'Regina' these cultivars were not the largest fruited cultivars tested. Softening in cherry has been suggested to develop at the onset of stage III development. Although cherry is classified as a non-climacteric fruit (primarily due to the poor correlation between ripening, protein synthesis, fruit respiration and generation of internal ethylene), recent research shows several cell wall degrading enzymes to increase in activity during cherry softening. Because fruit softening is predominantly an

enzymatically controlled process it should lend itself to first order kinetic analysis. A model to describe softening using temperature data for the final weeks leading up to harvest might help to explain differences from year to year. For instance, 2009 firmness values were ~20 % lower than those of 2010 [248 and 301 g/mm for 2009 and 2010, respectively] (data not shown). A predictive model could aid in harvest timing decisions when high temperatures are experienced and/or forecasted throughout the harvest period. Mean and maximum temperatures were higher in 2009 than 2010 although the lower firmness values observed in 2009 were likely confounded by the generally higher croploads. In several recent cropload studies on 'Lapins', 'Sweetheart' and 'Skeena' we have observed significantly softer fruit for the heavy treatments relative to the light croploads (Einhorn, unpublished).

Accumulation of soluble solids in cherries coincides with stage III of fruit development, and is a function of translocation of fixed carbohydrates from leaves. Because other competing sinks in cherry are relatively weak during stage III of fruit development, fruit are at an advantage for accumulating sugars. In fact, in terms of soluble solids fruit continued to increase sugars linearly as skin color darkened (Fig 1). Although absolute sugar content varied across genotypes, a significant relationship was observed for all cultivars studied (data not shown). Differences from year to year are likely attributed to differences in cropload. Allowing cherries to persist for longer periods of time on the tree will result in increased sugar content, though this must be balanced against potential adverse effects such as fruit softening and, in specific cultivars, lower stem retention force.

Despite the strong predictive nature of the relationships between skin color and quality attributes, on a whole-canopy basis cherry fruit ripening is inherently variable. Frequency distributions of the number of fruit falling into specific color classes for each of three successive harvest dates is shown for 'Benton' and 'Skeena' (Fig. 2). Similar variability was observed for other cultivars. The mid harvest timing for 'Skeena' alone (concurrent with the commercial harvest of the block) had ~1/3rd of the fruit in each of CTIFL 3, 4, and 5-6 class (Fig. 2). The average CTIFL score of the harvest was 3.9. In terms of internal quality of the fruit comprising the mid 2010 'Skeena' harvest, sugars increased ~ 10 % with each increase in color class between CTIFL 3 and 5 (17.7, 19.7 and 21.5 %), while firmness values all remained > 350 g/mm. Additionally, both skin and mesocarp color darkened to an average CTIFL score of 4.3 for the late timing.

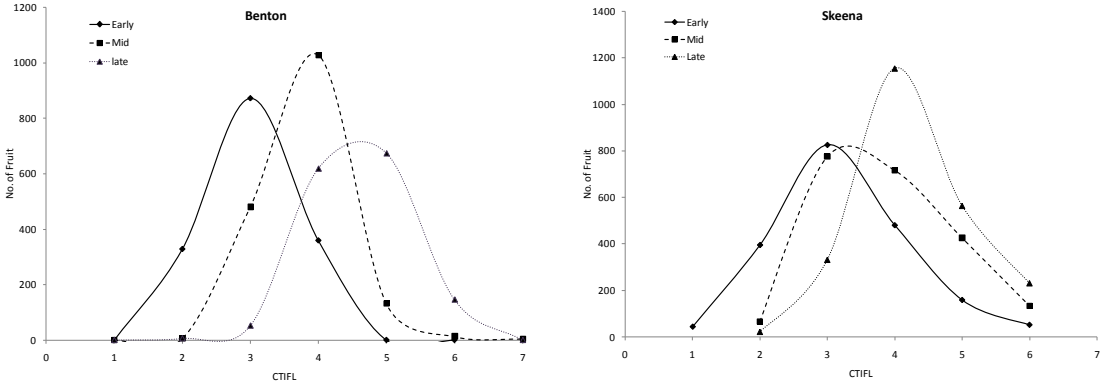
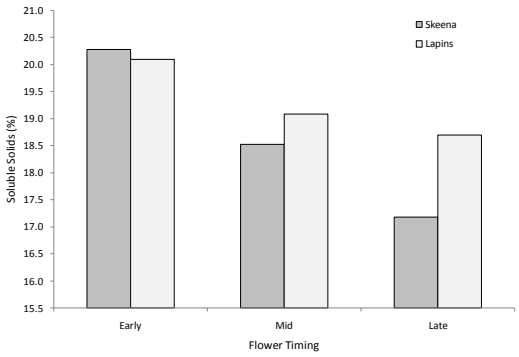


Figure 2. Frequency distribution of 'Benton' (left) and 'Skeena' (right) skin color using the CTIFL color scale (1-light pink, 7-black), for three separate harvest dates (early, mid and late). On each date, one tree representative of the orchard was strip-picked, and each fruit was classified according to its skin color. Total number of fruit analyzed per successive harvest was 1,559, 1,661 and 1,497 for 'Benton', and 1,953, 2,113 and 2,304 for 'Skeena'.

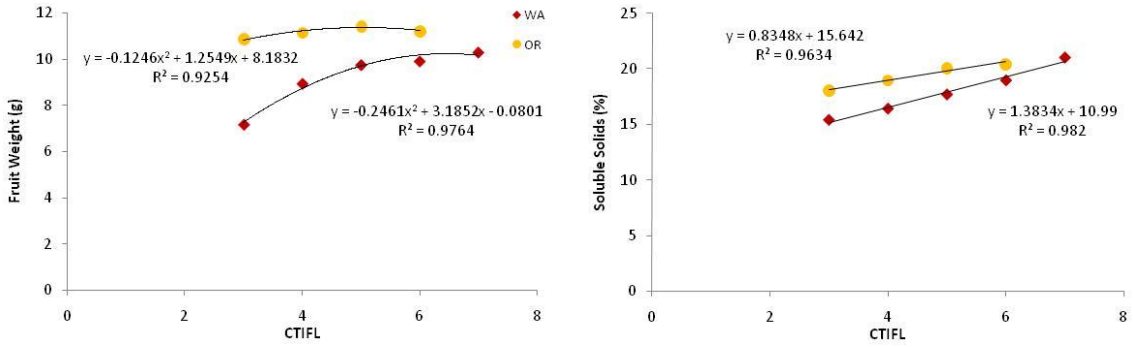


Future identification of sources of variability, and the development of practices which reduce this variability, will be of primary importance if greater consistency is to be gained in fruit quality. Assuming that pests, nutrition and irrigation are well managed, sources of variability of cherry fruit quality include cropload, flower timing [Fig 3], light environment, age of wood, and climatic factors.

Figure 3. Effect of flower timing on soluble solids content of 'Skeena' and 'Lapins' fruit. Fruit were harvested at the commercial harvest date. Sixty flowers were tagged per replicate (4) on each date.

Additionally, there are likely unidentified contributing factors, and we are simultaneously investigating the role of cellular development via microscopy techniques to determine whether we can adequately identify limitations to fruit growth throughout ontogeny. Good horticultural practices (training and pruning to enhance good light penetration and distribution, renewal of fruiting wood, application of PGRs, etc.) likely aid in reducing some of this variability, and as rate of adoption of efficient, planar canopy architectures (such as UFO) increases, it is plausible that a larger portion of this variability will be removed. Furthermore, research efforts aimed at identification of metabolites associated with ripening, and the genetic factors that regulate them, may result in the future development of cultivars which ripen more evenly. Interestingly, for all of the limitations associated with 'Regina' fruit set, ripening occurred over a very narrow range of CTIFL classes in both years of this study (data not shown).

Quality attributes of individual cultivars responded uniquely to harvest timing. Importantly, excellent relationships between skin color and fruit quality attributes were observed, despite the use of multiple sites as is shown for 'Benton' (Fig 4). Although absolute differences were observed between sites (associated with climate, lab instrumentation, etc.), equations and slopes describing the relationships were similar (Fig 4). Data compilation and analysis is ongoing. Recommendations for individual cultivars cannot yet be ascertained until data are analyzed for all years and sites. We will disseminate the results of these analyses, prior to, or during the 2011 NW Cherry research review.



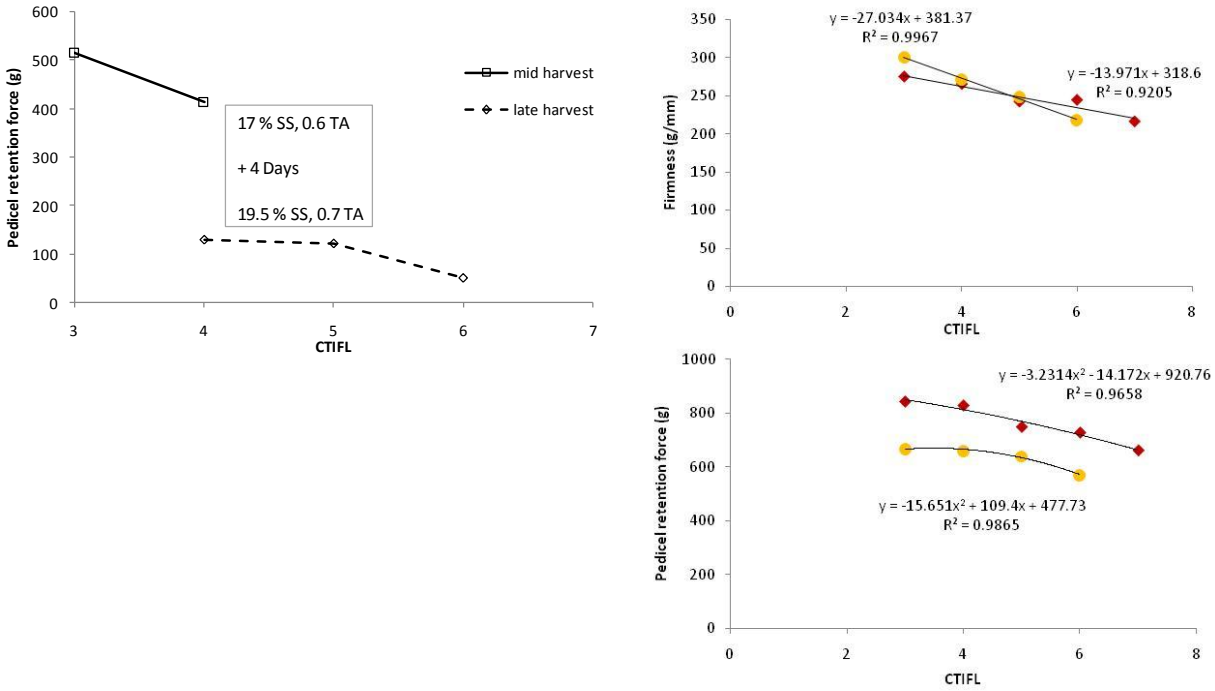


Figure 4. Relationships between skin color (CTIFL; 1-light pink, 7-black) and quality attributes (fruit weight, soluble solids, firmness and retention force) for 'Benton' fruit harvested in OR and WA.

Figure 5. The effect of increasing skin color on 'Selah' stem retention force. The four days between mid and late harvests were characterized by high mean and max temperatures.

In 2009 we reported that quality attributes associated with a given color class and cultivar can be markedly different depending on harvest date. This suggests that maturity and/or ripening can proceed exclusive of changes in surface color, and is likely the case for attributes such as stem retention force, which are highly influenced by temperature (Fig 5).

Storage: Assuming that the fruit evaluated were fair representations of the cultivars, these data will tend toward the optimal end of the post-harvest fruit quality scale, since fruit were spared the hardships of the packing process. As we observed in 2009, fruit firmness either remained constant, or consistently increased up to 28 days of storage. The reason for this phenomenon is not entirely clear. Fruit were allowed to warm to room temperature prior to analysis, since cherry fruit rigidity increases when the temperature of fruit tissue is lowered. This places importance on knowing the firmness values of the fruit upon entry into cold storage. Furthermore, disparity between firmness of fruit entering storage and arrival at destination markets can likely be attributed to temperature fluctuations during transit. In fact, future research could address the relationship between fruit quality and temperature alteration (simulating transportation scenarios) throughout the post-harvest life of the fruit. Soluble solid content was largely unaffected by storage time, and so long as fruit dehydration is kept to a minimal (determined in the present study by changes in fruit weight), levels of soluble solids should not change much from their harvested levels. Total acids typically degraded as storage time lengthened, though this was not consistently observed in all cultivars. A slight darkening of skin color seemed apparent as storage time increased, but this was not documented. We did not qualify stem attributes in this project.

Pitting: For pitting we chose to focus our efforts on 'Sweetheart', given the large area planted to this cultivar, and its documented propensity for pitting. In both 2009 and 2010 'Sweetheart' fruit were

harvested at different levels of maturity in enough volume to run over a commercial packing line. In 2009 four harvest timings were conducted (average CTIFL skin color classes were 3.8, 4, 4.3 and 5, for the early, early-mid, mid and late picks, respectively). Pits were counted for the entire contents of 20 lb cherry boxes. There was no relationship found between pitting incidence and skin color of 'Sweetheart' (data not shown). Two harvest timings in 2010 also yielded poor relationships between color and surface injuries (Table 3).

Table 3. Effect of harvest timing (Early or Late) on skin color (CTIFL scale; 1= pink, 7 =black) and pitting incidence, reported as average number of injuries on fruit for three different classifications of injuries [1-Pits, 2-Creases, and 3-Compressions]. All injuries present on fruit were measured and categorized into three size classes; 0.1-2 mm, 2.1-4 mm, 4.1-6 mm. Fruit were harvested commercially, run over a packing line and packaged into 20 lb cherry boxes (OVF). Two hundred fruit were chosen randomly at 21 days post-harvest (31 °F) from each of three 20 lb cherry boxes per harvest timing.

Treatment		Pit size class			Crease size class			Compression size class		
	CTIFL	0-2 mm	2-4mm	>4mm	0-2 mm	2-4mm	>4mm	0-2 mm	2-4mm	>4mm
Early	**4.1 B**	1.9	1.2	1.1	**0.2 B**	0.9	1.5	0	0.4	1.3
Late	**5.0 A**	2.1	1.1	1.4	**0.4 A**	0.8	1.1	0.2	0.5	1.7
$LSD_{0.05}$	0.84	0.64 (ns)	0.17 (ns)	1.13 (ns)	0.19	0.67 (ns)	1.17 (ns)	0.27 (ns)	0.34 (ns)	1.04 (ns)

Means within columns followed by different letters are significantly different by LSD at *P*=0.05 level
Regression analysis (1,200 fruit) confirmed these observations, yielding a regression coefficient of 0.05 (e.g., can be interpreted as only 5 % of the variability associated with pitting can be explained by skin color). From a practical point of view, these results imply that within the typical skin color range of a commercial harvest of 'Sweetheart' [CTIFL 3.5-5], differences in level of pitting may not be quantifiable, though care should be taken with this interpretation since fruit were only studied at one site.

Methods

Objective 1: Ten cultivars 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, pink-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 (g), fruit weight, fruit 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') [2009], and 2) a commercial packing line ('Sweetheart') [2009-2010]. 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, placed in lined boxes, and held at 1° C until evaluated. Evaluations were performed following 21 days postharvest. Entire contents of 20 lb cherry boxes were analyzed for pitting in 2009. 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). In 2010, 200 fruit were analyzed per replicate box, per treatment.

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 from these data. Data analysis is ongoing.

Objective 4: Digital images were taken of fruit cultivars at each CTIFL score. Images will be compiled for extension education materials.

Executive Summary

A study was initiated to relate skin color (common commercial harvest indicator), harvest timing, and internal fruit quality attributes of ten commercially viable sweet cherry cultivars. Cultivars were studied over a two-year period at multiple sites. In addition, we investigated the effect of duration of cold storage on these quality attributes.

With successive harvest dates, skin color of sweet cherry cultivars darkened. The relationships between skin color and fruit weight, diameter, mesocarp color, soluble solids, firmness and stem retention force, were all highly significant. Total acids did not consistently change with skin color. Fruit firmness and stem retention force declined with advancing skin color, however the rate of change varied from year to year, and in the case of retention force was augmented by high temperatures. Although the averages of individual quality attributes steadily changed relative to harvest timing, individual harvests comprised a wide range of cherry skin colors. Our results concur with previous reports demonstrating inherently high variability in sweet cherry skin color at harvest. We examined the role of flower timing on final fruit quality, and observed an increase in soluble solids and size with early blooming flowers. We have also observed a clear cropload interaction with fruit quality, and are presently investigating the role of cropload on cell division and growth relative to fruit size throughout ontogeny. Future research efforts need to focus on identifying sources of cherry variability.

Individual cultivars yielded excellent relationships between skin color and quality attributes. In fact, despite differences in absolute values between sites (associated with climate, lab instrumentation, etc.), equations and slopes describing the relationships between skin color and quality attributes of a given cultivar were similar. If internal quality is sought to be optimized by lengthening the time that fruit persist on the tree, a balance must be struck between positive (increasing soluble solids and fruit size) and adverse (fruit softening, lower stem retention force) effects. These relationships vary slightly with cultivar. It is evident that these factors are also modulated by environment and we are continuing efforts to explain these changes using growing degree day models.

Storage of fruit at 1 °C, for up to 28 days, did not result in a marked decline in fruit quality. Fruit firmness was consistently observed to increase with storage time, irrespective of cultivar. Total acids typically degraded as storage time increased, but the rate of decline was cultivar dependent, and the response was not observed for all cultivars tested. Sugar content was not influenced by duration of storage, likely due to good humidity control and maintenance of fruit water content (evaluated by fruit weight). We also investigated the relationship between skin color and post-harvest pitting incidence of ‘Sweetheart’. Skin color did not relate to severity of pitting for ‘Sweetheart’ in each of two years of studies.

FINAL PROJECT REPORT

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

PI: Amy Iezzoni
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Cooperators: Tom Auvil**,** Bryce Molesworth

Total project funding: $74,304

Other funding sources: None

WTFRC Collaborative expenses:

Item	2008	2009	2010
Stemilt RCA room rental			
Crew labor			
Shipping			
Supplies			
Travel			
Miscellaneous			
Plot costs	$ 0	$ 5,310[1]	$ 4,420[1]
Tree cost for PNW	$ 0	$ 7,209[2]	$ 14,070[2]
Total	$ 0	$ 12,519	$ 18,490

Footnotes:

[1]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 and 2010 plot expenses may be claimed in 2011 and have not been included in the 2011 budget request.

[2]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. These funds will be used to pay the WDN tree cost in Spring 2011 and therefore this budget item is not included in the 2011 budget request.

Budget 1: Amy Iezzoni

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

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

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

Budget 2: 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 3: 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:
1. Evaluate the existing trees of the 10 rootstock candidates to determine if they continue to show commercial promise.
2. Conduct DNA fingerprinting to assure that the genetic identity of the rootstock selections is correct.
3. Plot establishment to include site preparation and tree purchase, tree planting, and cultural management of the plots to assure the ability to assess rootstock performance.

SIGNIFICANT FINDINGS AND ACTIVITIES

- Of the 11 MSU candidate rootstocks under evaluation at Clarksville, Mich., one showed graft incompatibility and was discontinued for future testing. All other ten MSU rootstocks that were planted in 2001 – 2004 are currently showing no signs of graft incompatibility.
- In general the MSU rootstocks candidates confer a lower flower bud density than Gi 6 when both spur number and flower buds per spur are considered together.
- In spring 2009 the first second test rootstock plot was planted at WSU-Prosser with trees grown at Willow Drive Nursery (WDN). 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 the controls.
- TCSA measurements taken in September 2010 indicated that the majority of the MSU candidate rootstocks result in 'Bing' trees that are smaller than trees on Gi 6 with several rootstocks resulting in trees similar in size to trees on Gi 5.
- Based on discussions with the Advisory Committee in January 2009, 'Chelan' was added as a scion for the trials in the PNW, along with 'Bing and 'Sweetheart', because of its incompatibility with mahaleb rootstock.
- Trees for the other second test plots were produced at Duarte Nursery. Because of extreme difficulty with liner production for Iron, this candidate rootstock was not put forward for virus indexing (see below). Unfortunately reduced tree numbers resulting from budding in April 2009 required us to redesign our plots and eliminate a second grower cooperator site in Washington. The three sites to be planted in 2011 are Mosier, Ore., Manson, Wash. and Clarksville (MSU Research Station), Mich.
- Nine rootstock candidates were established at the National Clean Plant Network (formally NRSP-5) providing the source material for virus testing and the generation of certified plant material.
- DNA diagnostics conducted during critical stages of liner and tree production did not identify any clonal mix-ups.

RESULTS AND DISCUSSION

MSU-Clarksville - Rootstock performance of the originally grafted trees: Of the 11 MSU rootstock candidates planted at Clarksville, Mich. from 2001 to 2004, one rootstock (Crawford) showed signs of graft incompatibility and was discontinued from future testing. The other ten rootstocks are showing no signs of graft incompatibility with 'Hedelfingen', and in some cases 'Bing' scion, although continued testing is warranted. The vigor of seven of the MSU rootstocks could be directly compared to the vigor of Gi 6 (Table 1). Of these seven, four were less vigorous (Lake, Iron, Garfield, King), two were similar (Lincoln, Glenn) and one was significantly more vigorous (Kent). Although the vigor of Clare, Clinton and Cass could not be directly compared to that of Gi 6, all three

rootstocks appear to confer vigor less than Gi 6. This observation is supported by TCSA measurements for the Clare, Clinton, Cass and Gi 6 trees planted at WSU-Prosser Roza Experiment Station (Fig. 1).

In general the MSU rootstocks have a lower flower bud density than Gi 6 when both spur number and flower buds per spur are considered together (Table 1). Mean fruit size was variable, however as the trees were minimally pruned so that the natural growing habit could be evaluated, fruit size measurements may not be predictive of trees that are heavily pruned.

Table 1 Trunk cross-sectional area (TCSA), % size, total number of spurs, mean number of flower buds/spur, mean number of fruit/spur, mean fruit size, total shoot length of the Gi 6 control for the 10 MSU rootstock selections planted in Clarksville, MI. The scion is 'Hedelfingen'.

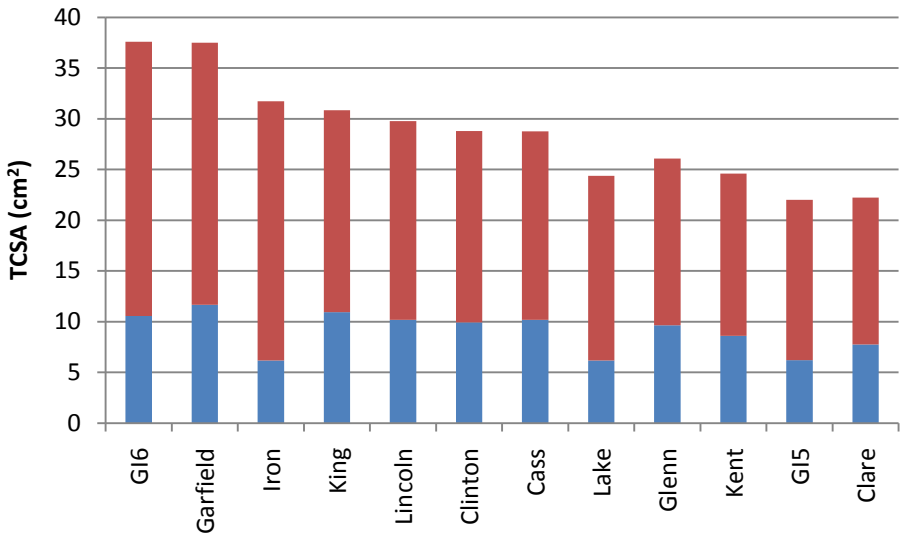
Rootstock	TCSA (cm^2)[1]	Vigor (% of Gi 6)[1]	Total no. of spurs[2,5]	Mean no. of flower buds[2,5]	Mean number of fruit/spur[3]	Mean fruit size (g)[3]	Total shoot length (cm)[1,6]
				2001[7]			
Lake	137	69	5	10	5.5b[4]	6.6	21
Iron	174	88	6	9	4.1a	7.2	24
Gi 6	198	100	7	15	5.4b	7.1	42
				2002			
Garfield	104	61	7	14	4.4a	5.7	35
King	123	71	6	16	5.0ab	6.3	27
Gi 6	171	100	8	15	6.3b	6.1	30
Lincoln	178	104	6	12	4.5a	7.1	26
Glenn	181	106	7	10	5.1ab	6.4	35
Kent	216	126	7	13	5.0ab	7.1	28
				2004			
Clare	58	-	9	16	4.9b	8.0	42
Clinton	47	-	8	23	2.0a	8.0	37
Cass	78	-	8	23	2.8a	7.5	45

[1] Data taken in 2010.
[2] Data taken from 2008 to 2010.
[3] Data taken in 2009.
[4] Means denoted by same letters within the column are not significantly different at $P < 0.05$.
[5] Data represents the number of spurs and flower buds on two branches for second and third year wood.
[6] Data represents the shoot length of two branches for third year wood.
[7] Year in which the rootstock selections were planted.

WSU – Roza Station Plot - Rootstock generation and plant performance: Liners of the MSU rootstock candidates were propagated at MSU in 2006 and planted at Willow Drive Nursery in spring of 2007 (Fig. 2A-C). The 276 trees (including Gi 5 and Gi 6 controls) from these test rootstocks were budded in fall of 2007 and were planted at the WSU-Prosser Roza Station in spring of 2009 (Fig. 2D). Due to unequal liner numbers, the rootstock candidates were represented in this plot by a minimum and maximum of 5 and 46 trees, respectively. All rootstock candidates have 'Bing' scion and Kent also has 'Sweetheart' scion due to a large number of liners and excellent percentage bud take (90%). The trees were trained to 3-5 leaders evenly distributed and tied down if necessary (Fig. 2E, F).

TCSA measurements taken in September 2010 indicated that the majority of the MSU candidate rootstocks result in 'Bing' trees that are smaller than trees on Gi 6 with several rootstocks that are resulting in trees similar in size to trees on Gi 5 (Fig. 1).

Fig 1 Trunk cross-sectional area (TCSA; cm^2) of 'Bing' trees grafted on 10 MSU rootstock candidates, Gi 5, and Gi 6 for trees planted in 2009 at WSU-Prosser Roza Experiment Station. The top of the red bar indicates TCSA recorded on 24 September 2010. The blue bar is the TCSA measurement on 15 March 2010. Therefore the red indicates the TCSA increase in the 2010 growing season.



Duarte Nursery – Tree Production: In 2006, a collaboration was initiated with Duarte Nursery to produce the MSU rootstock liners and trees needed to establish additional second test trials. At the time, it was necessary to increase the liners in tissue culture due to limited plant material. Secondly, having the liners produced at a second location outside of Michigan would assure that any unexpected quarantine on *Prunus* from Michigan would not cripple this project. Plant material of the MSU rootstock selections was sent to Duarte Nursery, established in tissue culture, and liners were grown in their greenhouses (Fig. 3 A-C). Liners of Gi 5, Gi 6, and Gi 12 were purchased from ProTree and sent to Duarte Nursery so that these control trees could be grown using similar horticultural practices as the test trees. Due to unfortunate delays in liner production, budding was delayed until April 2009. In January 2010, A. Iezzoni and T. Auvil visited Duarte Nursery to assess the tree numbers and tree growth. Unfortunately, the trees grown in the Duarte 'pot culture' system were too small to be field planted in spring 2010 (Fig. 3D). Therefore Willow Drive Nursery (WDN) generously agreed to grow out these trees for the 2010 growing season so that the rootstock plots could be established in spring 2011 with trees of excellent quality. In January 2010, the plant material from WDN (including budded trees and extra unbudded liners) were trucked to WDN and stored in their cooler (Fig. 3E). The plant material was planted in the nursery in spring of 2010 (Fig. 3F).

Tree counts made in July 2010 at WDN confirmed that the final tree numbers were only sufficient for the establishment of two large plantings in the PNW (Mosier, Ore. and Manson, Wash.) and one smaller planting in Clarksville, Mich (Table 2). This lower than anticipated tree number was due to poor bud take. A low percentage bud take was also observed for the controls (e.g. mazzard, Gi5, Gi6, and Gi12) indicating that there was not an inherent problem with bud take that was unique to the MSU rootstock candidates.

Table 2. Plant material propagated at Duarte Nursery in April 2009 for the MSU rootstock trials to be planted in spring of 2011.

Location	Scions utilized[1]	Maximum no. of replications	Total Tree No.[2]
Manson WA	'Bing', 'Sweetheart' Chelan', & 'Rainier'	5 reps of 5 trees each	513
Mosier OR	'Bing' & 'Chelan'	5 reps of 5 trees each	371
Clarksville MI	'Rainier'	2 reps of 5 trees each	102

[1]Underline indicates the scion(s) used for comparisons with the other scions included as pollinators and guard trees.
[2]This number includes the addition of the following rootstock controls: Gi 5, Gi 6, Gi 12 and mazzard, plus guard row trees.

Rootstock genetic check & virus indexing: To avoid any future intellectual property and identification issues, fingerprints of the rootstock selections were developed using molecular markers. With the combination of a primer set specific for the self-incompatibility *S-RNase* locus and three SSR markers (PceGA59, PMS40, and PMS67), all ten rootstocks can conclusively be differentiated from each other and Gi5 and Gi6 (Fig. 4A). Fingerprints done on plant material increased at Duarte Nursery did not uncover any clonal mix-ups. Genetic checks were also done on the plant material received from Duarte Nursery and planted in WDN in 2010. As rootstock tissue for DNA diagnostic tests could not be obtained from the grafted trees, leaves were collected in July by A. Iezzoni from a random sample of unbudded liners. I reasoned that if the unbudded liners were labeled correctly, that the liners that were used to produce the grafted trees were also correct. Once again no clonal mix-up was identified (Fig. 4B).

If any of the MSU rootstocks are of sufficient interest to warrant the generation of commercial plant material by nurseries, it would be necessary to have certified plant material available. As this process can frequently be a bottleneck in rootstock commercialization, the initial steps in this certification process were undertaken for nine of the 10 rootstock selections (Fig. 5). Iron was not included in the indexing as it was extremely difficult to generate liners from this selection. For the remaining nine rootstock selections, dormant liners were taken by A. Iezzoni from the plant material in the WDN cooler and planted at the National Clean Plant Network (NCPN, Prosser, Wash.) in April 2010. Some of these liners had sleeping eyes that were removed prior to planting at the NCPN. All 9 rootstocks were tested for four viruses (PDV, PNRSV, CLRV and PPV). Three of the selections tested positive to PDV. We suspect that the inserted sleeping eye bud that we removed prior to planting may have carried this virus. The other six selections tested negative for the four viruses. As these six selections are now poised to enter the testing required for certification, leaf samples were taken in July 2010 and diagnostic DNA tests were conducted. These rootstocks were determined to be true-to-type based on the results from four DNA tests (Fig. 4C). Budwood of the remaining three rootstock candidates will be shipped to the NPCN from the mother block in Clarksville, Mich. in December 2010.

EXECUTIVE SUMMARY

The long term objective of this project is to identify dwarfing precocious rootstocks that increase the profitability of sweet cherry production in the PNW through the establishment of test plots. At the start of this three year period, 11 MSU rootstocks were under consideration; however, one was subsequently eliminated due to graph incompatibility and a second one is of less interest due to extremely poor propagation performance. The remaining nine MSU rootstock candidates confer a range of tree sizes with the smallest trees having TCSA that are ~ 60% the size of trees on Gi 6. In 2009, one rootstock test plot was established at WSU-Prosser Roza Station. The trees are performing well and data on TCSA indicates that the trees will exhibit a range in vigor. Liners and trees for the other test plots were produced at Duarte Nursery. Due to delays in liner production and poor bud take (poor bud take was also exhibited by the control rootstocks), tree planting was unfortunately delayed until 2011. Reduced tree numbers resulting from budding in April 2009 also required us to redesign our plots and eliminate a second grower cooperator site in Washington. The three sites to be planted in 2011 are two large plots with one in Mosier, Ore. and the second in Manson, Wash., and a smaller plot in Clarksville (MSU Research Station), Mich. As problems with the availability of virus certified genetically correct plant material can cause a bottle neck in the potential commercialization of a rootstock candidate, we initiated the process of establishing the MSU rootstock candidates at the National Clean Plant Network (formally NRSP-5) so they can be available for virus screening. DNA diagnostics conducted during critical stages of this project did not identify any clonal mix-ups.

Fig. 2. Flow Diagram of Activities
Plot of MSU Rootstock candidates at the WSU-Prosser Roza Station.

2006 Liners were propagated from softwood cuttings at MSU (Fig. 2A).

↓

2007 Liners were shipped from Michigan and planted at WDN for August budding (Fig. 2B).

↓

2008 Trees grew at Willow Drive Nursery (Fig 2C).

↓

2009 Trees were planted and trained at WSU – Prosser, Roza Station (Fig. 2D).

↓

2010 Tree Training, spring (Fig. 2E) and tree growth, summer (Fig. 2F).

Fig. 3. Flow Diagram of Activities
Generation of liners and budded trees for the MSU rootstock plots to be planted in Mosier, Ore., Manson, Wash, and Clarksville, Mich. in 2011.

2006 Fall	Budwood of the MSU rootstock liners was sent to Duarte Nursery for budding on mahaleb rootstock.
↓	
2007	Duarte Nursery – Meristem cultures were established using shoot tips from the MSU selections (Fig. 3A).
↓	
2007 – 2009 May	Duarte Nursery – Generation of MSU rootstock liners (Fig. 3B,C). MSU - DNA diagnostic fingerprinting was carried out at various times during the propagation process (Fig. 4A).
↓	
2009 April	Duarte Nursery – The liners were budded
↓	
2010 Jan	The trees and extra unbudded liners were prepared for shipment from Duarte Nursery to Willow Drive Nursery (Fig. 3D). The plants were transported from Duarte Nursery to Willow Drive Nursery.
↓	
2010 Jan-Apr	Willow Drive Nursery – the plant material was stored in the cooler (Fig. 3E). Willow Drive Nursery – the budded trees and unbudded liners were planted in the nursery (Fig. 3F). DNA diagnostic fingerprinting was conducted for a random sample of unbudded liners planted at Willow Drive Nursery (Fig. 4B).
↓	
2010 July	Willow Drive Nursery – final tree counts were taken and used to design plots for 2011 planting.

Fig. 3 (cont.)

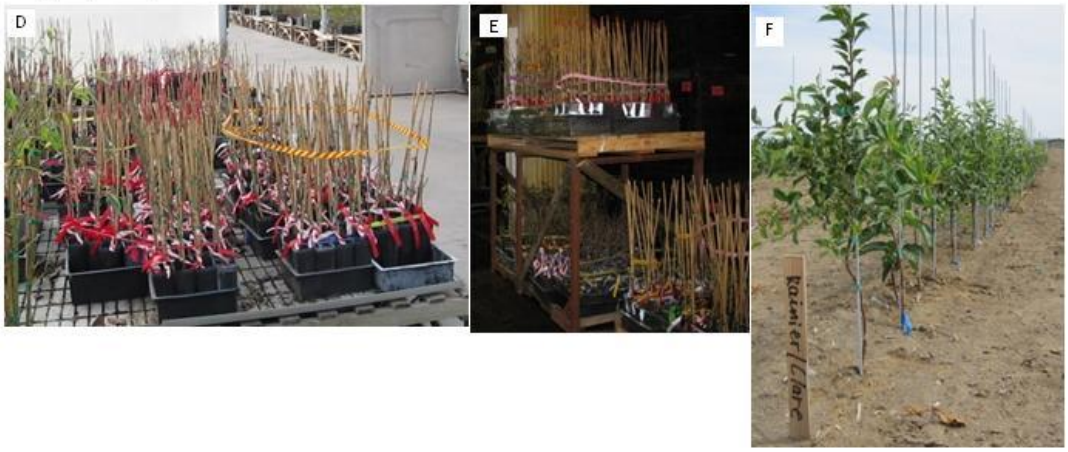


Fig. 4: DNA diagnostics illustrating the use of the S-allele primer pair: (A) Marker polymorphisms for the rootstock selections, (B) Fingerprints from the plant material at WDN, (C) Fingerprinting of the plant material at NCPN.

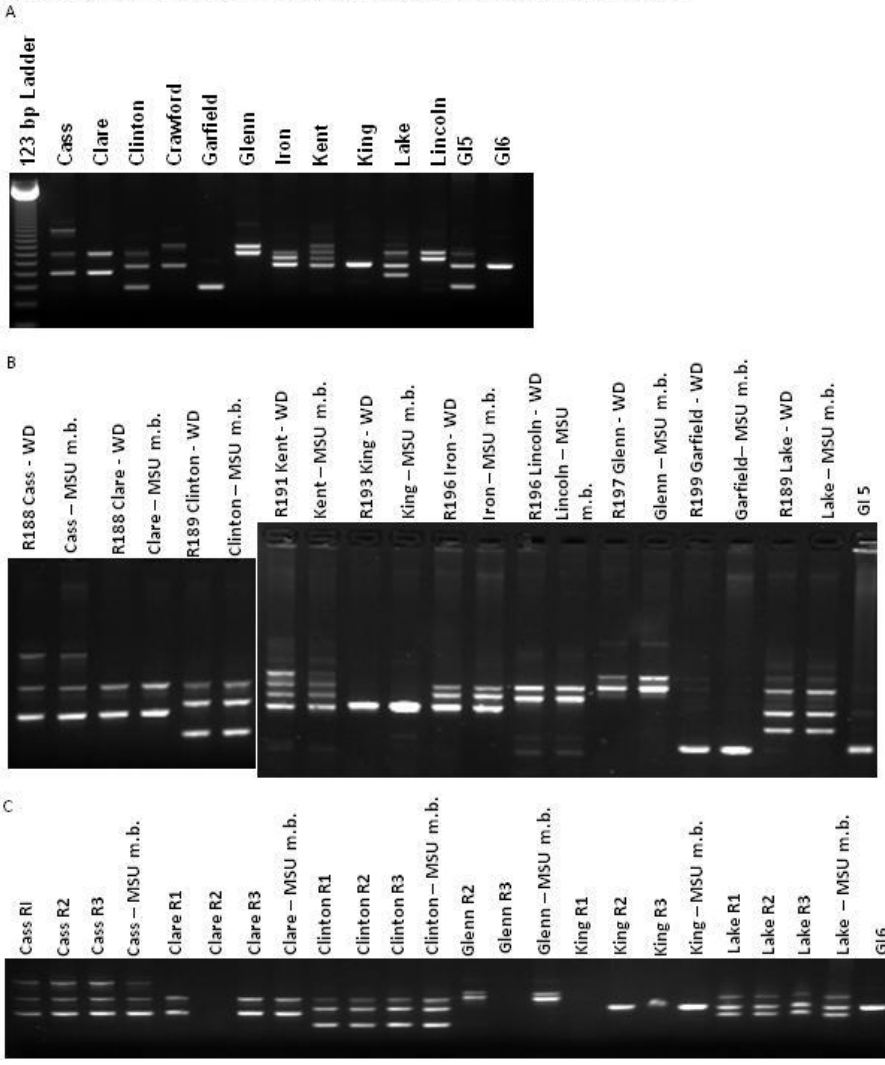


Fig. 5. Flow Diagram of Activities
Virus testing and confirmation

2010 Apr	Three rootstocks per selection were taken from the supply at Willow Drive Nursery and planted at the National Clean Plant Network (NCPN) Greenhouse in collaboration with Bill Howell (see image below).

↓

2010 Summer	Six of the nine MSU rootstocks tested free of PNV, PNRSV and PPV

↓

2010 Summer	DNA diagnostic fingerprinting confirmed that these six rootstocks are true to type (Fig. 4B).

↓

2010 Summer	A Michigan Department of Agriculture Permit was obtained for the shipment of budwood of the three candidate rootstocks that tested virus positive from the Mother Block at MSU's Clarksville Station to the NCPN in Dec. 2010.

FINAL PROJECT REPORT

Project Title: Cherry genome project

PI:	Amit Dhingra	**Co-PI**:	Matthew Whiting
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State/Zip: WA 99350

Cooperators: Ananth Kalyanaraman, WSU; Herman Silva, Lee Meisel, Chile; Cameron Peace, WSU; Amy Iezzoni, MSU; Todd Einhorn, OSU

Other funding sources

Agency Name: USDA – CSREES Specialty Crops Research Initiative
Amt. awarded: $3.8 million plus equal matching, Sep 2009 – Aug 2013
Notes: "A total systems approach to developing a sustainable, stem-free sweet cherry production, processing and marketing system". PIs – Whiting, Dhingra and Oraguzie.

Agency Name: USDA – CSREES Specialty Crops Research Initiative
Amt. awarded: $7.2 million plus equal matching, Sep 2009 – Aug 2014
Notes: RosBREED. The cherry genome data will serve as a scaffold for identifying SNPs using various sequencing technologies. PIs – Iezzoni, Peace and Oraguzie. Collaborator – Dhingra.

Agency Name: USDA – CSREES National Research Initiative
Amt. awarded: $224,000
Notes: Scaffold sequencing and data de-convolution method development to assemble the apple genome. An Apple Genome Sequencing Initiative. PIs – Dhingra and Kalyanaraman.

Agency Name: Universidad Andrés Bello (Herman Silva and Lee Meisel)
Amt. awarded: $27,000
Notes: Collaborative arrangement with Dhingra on the cherry genome and transcriptome project.

Agency Name: Dhingra and Oraguzie Start-up funds
Amt. awarded: $30,000
Notes: Obtained the initial data on cherry genome sequences

Agency Name: Roche Inc.
Amount awarded: $30,000
Notes: Funds being used at 454 to generate scaffold DNA libraries and sequencing to enable efficient assembly of the genome.

Total Project Funding: $48,000

Budget History:

Item	**2010**		
Salary			
Benefits			
Wages			
Benefits			
Supplies	44,000		
Travel	4,000		
Miscellaneous			
Total	**48,000**		

ORIGINAL OBJECTIVE

1. Develop a Genomic BAC library: Entire sweet cherry genome or DNA from Stella (self-fertile progenitor of many new varieties) will be captured in manageable parts of small size using established methods (with Amplicon Express as collaborators).

2. Establish a scaffold to reconstruct the sweet cherry genome: By utilizing a proprietary method of sequencing using techniques developed for apple genome, rapidly generate a scaffold to reconstruct the sweet cherry genome.

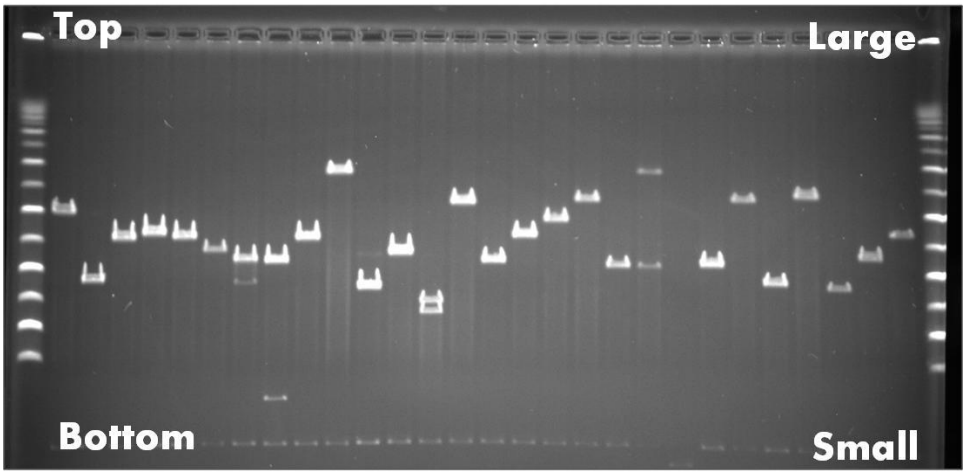
SIGNIFICANT FINDINGS

Objective 1: BAC Library

Definition: BAC Library – It is a method of capturing the large mass of the genome or DNA from any cell and breaking it into small pieces. This needs to be done so that the genome can be studied in manageable pieces.

The basic aim of this project was to expand on the information already generated with funding from Dhingra, Oraguzie and the Chile group and physical resources for cherry genome research in our lab. Support from this project enabled us to construct a high quality BAC DNA library that represents a collection of large pieces of genomic DNA captured in a way that we can multiply them individually, thus making it easy to work with sections of the genome. DNA is made of four letters, A, T, G and C called bases. The average size of the genomic section in the library is 120,000 bases (Fig. 1). The library consist of over 20,000 such pieces representing the genome over 5 times.

Figure 1: This image represents a hard proof to scientists that we have successfully captured the genomic DNA from sweet cherry. The figure is that of a gel in which DNA is separated based on its size. The smaller DNA is present near the bottom of the gel.



The BAC library was prepared as scheduled by June 2010.

Objective 2: Scaffold Sequencing

We had proposed to accomplish this by December 2010. However, to enable this aspect of the project the DNA from over 20,000 BAC fragments needs to be isolated and pooled. This work is underway at Amplicon Express and we expect to commence sequencing in January. The timeline to obtaining all data and final assembly will thus be completed between April – June 2011.

RESULTS & DISCUSSION

To summarize, the BAC library is ready and is currently being processed to move ahead with Objective 2 of performing sequencing. The second step will enable us to generate a scaffold on which genomic sequences can be "hung" to generate the map of the genome.

Impact of Sequence Information: Having a genome sequence does not instantly produce a new variety or solution that the farmers can implement directly in their fields in the coming season. It is the foundational information that once established will be used from here on to serve as a basis of physiological solutions in the near-term and novel varieties in the longer-term.

The cherry GGB group envisions that the genome sequence will be utilized in three broad applications for cherry improvement.

1. Physiological solutions – Near term
Target – Existing varieties and New varieties
All physiological processes in plants are under the control of genes. Prior to the utilization of genome sequence for this purpose, it is important to understand which gene or set of genes controls a desirable process. For example, to develop mechanically harvestable cherry there is a need to understand which genes respond to ethephon and make a certain variety mechanically harvestable. This work is currently ongoing in the program supported by the USDA-SCRI grant to Whiting, Dhingra and Oraguzie.

We are in the process of identifying genes related to the abscission process specifically at the junction of fruit and its pedicel. The cherry genome sequence available so far has been screened for potential genes and the differences in these genes across ethephon responsive and non-responsive varieties. This information is expected to be utilized in finding varieties that will respond to ethephon and the gene-based differences will serve as molecular markers to develop new varieties that can be hand-picked or mechanically harvested depending on the needs and capacities of the farmer.

2. Controlled Sport induction, CSI – Mid-term and Long-term
Target – Existing varieties
In collaboration with Dorrie Main's program, we have functionally tested a gene from Peach that provides broad spectrum and long-term resistance to powdery mildew. Same gene is present in sweet cherry as well. We found that by comparing the peach gene with sweet cherry genome. This highlights another utility of the sweet cherry genome. The only caveat to utilizing this gene is that, resistance is observed when the gene becomes non-functional due to a mutation. In our program we have developed a method where we can regenerate thousands of same variety clones using leaf material. This platform can be used to create sports in existing varieties. How many dollars can be saved if Rainer and Bing were naturally resistant to powdery mildew? This project is not funded however; we are working with Nnadozie Oraguzie to explore the possibility of generating such sports.

A long-term advantage of having the sports is that they can be utilized as parents in future sweet cherry crosses. Since the mutation will be known it can be easily tracked using molecular methods.

3. Novel variety development using molecular markers – Near and long term:
Target: New varieties
As the scientific community discovers which genes control what traits or information from other plant systems becomes available, molecular markers can be rapidly generated to be utilized for parental screening. One such example is the ACS/ACO gene in sweet cherry. The ACS gene has a deletion in cherry compared to the gene in apple. This feature has been utilized to develop a marker by Cameron Peace's group. Now the question is if that deletion is absent in some cherry variety. If yes, then does that cherry respond to ethylene? Answering such questions with the help of genome information can enable rapid development of desired varieties for the PNW cherry industry.

The significance of genome information will far outlive the duration of this project. Each economically important trait or desirable quality in the fruit tree is controlled at some level by genes. An accessible genomic blueprint of cherry enables us to pinpoint what gene or group of genes are responsible for agriculturally important traits. This information will guide cherry improvement in both the short and long term future. Another testimony to this fact is that scientists have now discovered the gene underlying skin and lung cancer in humans utilizing human genome information. As in the case of humans, the potential economic benefits to the industry are apparent. With the cherry genome sequence in hand, we can develop unique varieties for the PNW combining all priority traits that can create lucrative economic opportunities ranging from production to post-harvest stages.

BROADER IMPACTS

Presentations: The cherry genome information has been highlighted at several forums over the last year including WSHA meetings. In 2009, the PI was invited to speak at the Hort Show about Enabling Economic Resilience through Genomics Research. Besides that, the work has been shown as poster presentations at annual international meetings like American Society of Plant Biology and Plant and Animal Genome Meeting. The cherry genome will be presented at the 5th Rosaceae Genome Conference in South Africa in 2010.

Training opportunities: This project has been steered by graduate student Tyson Koepke in collaboration with Artemus Harper, a computer scientist in the program supported by USDA-NRI.

EXECUTIVE SUMMARY

Significant progress: The objective of generating a BAC library from sweet cherry has been completed. The second objective of generating scaffolds for the genome is in progress and we expect that it will be completed by June 2011. Collaborations with Roche Inc. and Andres Bello University and our start-up funds have provided extra resources to the tune of $87,000 to develop a much finer assembly of the cherry genome.

Outcomes and summary of finding: Preliminary cherry genome sequence data are available that are being used by our program to identify coordinates and sequence information of important genes linked to desirable traits like abscission (Dhingra) and ethylene responsiveness (Peace). In summary this is just the start of the most efficient way of connecting traits to genes, an emphasis of our fruit crop genomics program.

Future directions: Abscission-related genes will be tested in sweet cherry parental selections and breeding population to ascertain which varieties has the potential of being mechanically harvestable. We have one proposal under review at NSF, and others at various stages of writing to build upon this foundational information. Our programmatic approach is to connect traits with genes using function information. Future projects are aimed at applying this approach in new and novel ways to the improvement of sweet cherry.

FINAL PROJECT REPORT

Project Title: Overwintering survival and management of Spotted Wing Drosophila

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Total Project Request: **Year 1:** $20,662

Other funding sources: None

WTFRC Collaborative expenses: none

Budget 1
Organization: OSU MCAREC **Contract Administrator:** Dorothy Beaton
Telephone: 541-737-4068 **Email:** Dorothy.beaton@oregonstate.edu

Item	Year 1
Salaries[1]	9,126
Benefits[2]	5,476
Wages[3]	2,000
Benefits[4]	160
Equipment	0
Supplies[5]	2,400
Travel[6]	1,500
Total	20,662

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

Objectives:

Objective 1. Assess overwintering survival of the spotted wing drosophila in various cherry producing areas in Oregon.

Objective 2. Conduct insecticide efficacy tests in sweet cherry to start to develop management guidelines or this pest.

Objective 3: Extend information to industries in a coordinated manner

Methods

Objective 1. Assess overwintering survival of the spotted wing drosophila in various cherry producing areas in Oregon

Laboratory studies: Our objectives were to determine survival of *D. suzukii* adults and pupae under two general scenarios. The first scenario simulated constant mild overwintering temperatures. The second scenario, mild temperatures with a seven day freeze period, was designed to mimic conditions found at high-elevation or inland production sites. SWD will be reared to pupal and adult stages. One day old adults and puparia (minimum 100 each) will be used to test survival of *D. suzukii* under five winter temperature conditions found in fruit production areas in the Pacific Northwest. Initially all individuals will be exposed to mild overwintering conditions for 2 mo. at 50°F and 8:16h L:D. After initial exposure, individuals will be subjected to five climates: 36°F (here we will subject flies at 23°F for one week to take into account a major freeze event in the middle of the two month period), 36, 39, 43, 46 and 50°F, and 8:16h L:D for two mo. A third mild two month period will represent early spring conditions (50°F at 12:12 h L:D). Two hundred individuals at each life stage will be subjected to typical average temperature in the fruit production areas.

Field studies: These sites will be located near weather stations that collect air and soil temperatures. Laboratory-reared SWD will be acclimated using the mild fall regime and placed in emergence cages in the Willamette Valley, Hood River, The Dalles and Milton Freewater in commercial cherry orchards during December. Three emergence cages will be placed in each location and each emergence cage will contain 100 adult and 100 pupae SWD. Adults will be placed in semi-protected cages within each emergence cage in order to simulate protected micro-habitats sought by free-living overwintering individuals. Cages containing both life stages will be left in the field until March-April and the number of surviving adults and emerged pupae recorded.

Objective 2. Conduct insecticide efficacy tests in sweet cherry to start to develop management guidelines or this pest. Insecticide efficacy tests will be conducted to evaluate insecticidal management of the spotted wing drosophila. . Insecticides chosen for this study will be based upon several factors including local grower preference of cherry fruit fly products and results from similar trials to be conducted in California during 2010. Each treatment will be replicated 4 times in a RCB.

Objective 3: Extend information to industries in a coordinated manner.
This pest affects a wide variety of fruit crops. Teaching materials, new research information and seasonal observations will be made available via an online SWD workspace maintained by the OSU Horticulture Department.

Significant findings:

- Our work shows that adult flies can survive up to 88 days at temperatures of 50°F constant, with a slight increase in mortality when a 7 day freeze period is included. It is expected that longevity will be shorter at constant temperatures below 50°F.
- When pupae are subjected to similar temperatures, emerged adults can live to 105 days at constant 50°F and 103 days with a seven day freeze period. Lower survival rates are expected at lower temperatures.

- The fact that only female individuals survived to the conclusion of the experiment may have significant implications during the following season. First, pest management scouts will have to adapt early trapping to specifically search for females. Second, it is noted that female individuals that are unmated may still lay eggs but the proportion of viable eggs is unknown.
- The delay in initiating the field overwintering study has allowed other cooperators to participate this winter. This aspect of study will be conducted in OR, WA, and BC.
- Malathion ULV appears to be effective against the spotted wing drosophila.
- Delegate, entrust, malathion, carbaryl and pyrethroids have been demonstrated to be effective against SWD in field-lab studies.
- Our successful outreach program for the Mid-Columbia cherry industry in concert with this research mitigated SWD problems in this area this past growing season.

Results and Discussion:

Objective 1. Laboratory Study: In all four experiments individual SWD survived for the longest periods at 50°F (Figs. 1-4). SWD survival rates were similar at 36°F and 39°F and were significantly lower than survival rates at 43°F and 46°F. Survival was significantly lower for all temperatures compared to the 50°F treatment.

Adults at 50°F gradually died with no freeze (Fig. 1) over a period of 84 days and displayed linear mortality over the experimental period. The simple regression function of adult survival on time at 50°F estimated that adults will survive to 88 days at 50°F. Adults at colder constant temperatures than 50°F survived shorter periods. The majority of flies died within 8 days at 36 and 39 °F and 20 days at 43 and 46°F. Mortality rates for these four temperatures dramatically decreased after 8 and 20 days.

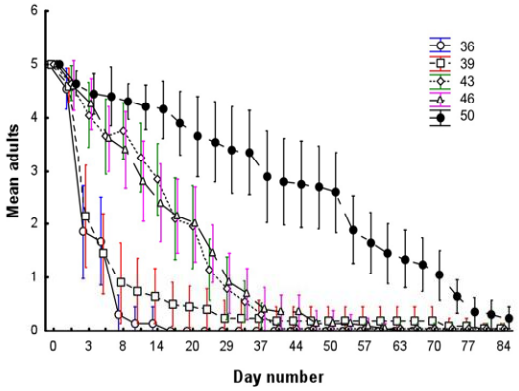

Figure 1. Mean survival of Spotted Wing Drosophila per cup

Adult mortality with the 7 day freeze period (Fig. 2) displayed an approximate straight line over the experimental period. The simple regression function of adult survival on time at 50°F estimated that adults will survive to 85 days at 50°F. Adults at colder constant temperatures than 50°F survived shorter periods. The majority of flies died within 5 days at 36 and 39 °F and 20 days at 43 and 46°F. Mortality rates for all temperatures dramatically increased during the 7 day freeze period, but decreased thereafter (Fig. 2).

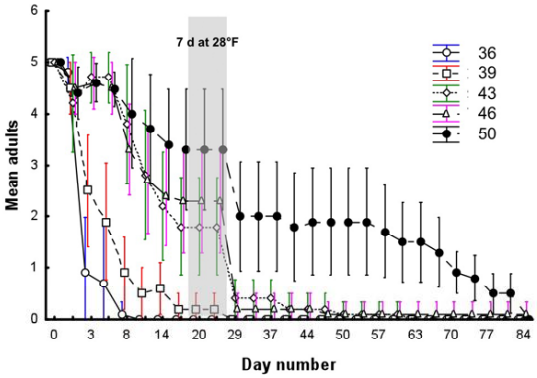

Figure 2. Mean survival of Spotted Wing Drosophila per cup. The freeze period is indicated by the transparent bar.

Pupae with no freeze (Fig. 3) emerged during the first 20 days of the experiment as adult individuals. Subsequently adult mortality after day 20 at 50°F displayed a linear relationship. The simple regression function of pupae developing to adults and subsequent adult survival on time at 50°F estimated that adults emerging from pupae will survive to 105 days at 50°F. Adults emerging from pupae at colder constant temperatures than 50°F survived shorter periods. We believe that the

majority of individuals probably died while in the pupal stage within 8 days at 36 to 46°F. Mortality rates for these four temperatures remained high to 37 days. All individuals died by 60 days at temperatures below 50°F.

In the treatment where pupae were subjected to a 7 day freeze period (Fig. 4), adults emerged during the first 20 days of the experiment until the onset of the 7-day freeze period. No adults emerged after the freeze period. Subsequently adult mortality after day 20 at 50°F displayed an approximate straight line. The simple regression function of adult survival on time at 50°F estimated from this function is that adults will survive to 103 days at 50°F. Adults held at constant temperatures below 50°F survived shorter periods.

The majority of flies died within 8 days at all temperatures. Mortality rates for all temperatures did not show a dramatic increase as seen when adults were subjected to the 7 day freeze period. One notable finding from our work is that the only individuals surviving to the conclusion of the experimental period were females.

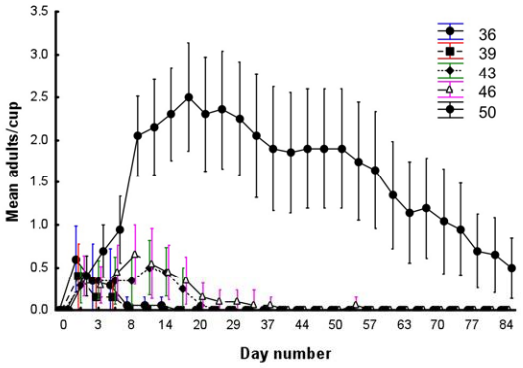

Figure 3. Mean Spotted Wing Drosophila adults started from the pupal stage per cup. The number of adults initially increased as they emerged from the pupal stage, and then decreased due to mortality.

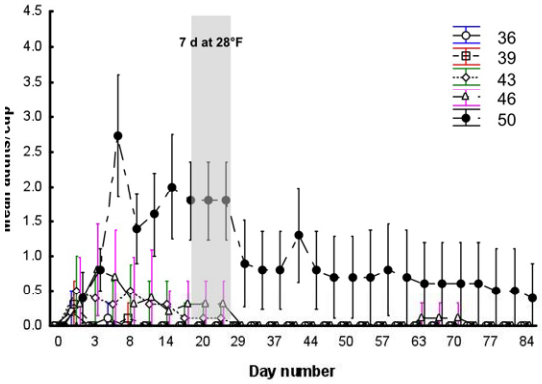

Figure 4. Mean Spotted Wing Drosophila adults started from the pupal stage per cup. The number of adults initially increased as they emerged from the pupal stage, and then decreased due to mortality. The freeze period is indicated by the transparent bar.

Objective 1. Field studies: Overwintering studies were not conducted in 2009/2010 because flies were not available until January. This study will be conducted this winter. We will work with Dr. Elizabeth Beers, WSU TFREC, and Dr. Howard Thistlewood, Agriculture and Agri-Food Canada, Summerland, BC, and we all follow the same procedures. This will greatly expand our study and applicability of our outcomes.

Objective 2. Efficacy trials:
Note: This first test was not originally planned but the opportunity presented itself and we undertook it. On 27 May, 2010, an experiment was conducted to evaluate the potential of aerial applied Malathion ULV against the spotted wing drosophila. Three paired blocks were treated with malathion ULV applied by fixed-wing (Shearer Sprayers) at a rate of 16 oz/A. It was applied approximately at 10:30 AM, with winds at 0.5-1.8 MPH, and 58 F. Before application, we placed in the trees:
1) Oil-sensitive spray cards (8 per tree; 4 along the outside of the canopy at the top, mid, bottom and ground level, and 4 in the inside of the canopy at the top, middle, bottom and ground. One card per location on a tree or ground), 4 trees per site, 12 trees total, 96 cards total,
2) Sticky cards containing live SWD adults glued onto their backs placed around the bottom of the tree canopy and ground (flies on cards were exposed to the aerial treatment), and,

3) Small screened cages containing live SWD adults placed around the bottom of the tree canopy and ground (flies were exposed to treated air but realize that the screen probably acted as a barrier to airflow and/or serve as a surface that captures ULV droplets that may subsequently provide a source of residue for the caged flies to contact).

The plane made 3 passes at 100 ft intervals over each of the 3 test sites. One hour after application, we removed these items plus treated and untreated leaves collected from several areas within the canopy and brought everything back to the entomology laboratory at MCAREC. The leaves were used to line small plastic deli containers that were screened to provide ventilation. Flies were added to these containers to evaluate residual control.

Results and Discussion:
ULV droplets on spray cards. The average number of aerosol malathion droplets recorded on spray cards was no different on cards placed in the inside of tree canopies compared with the number of droplets on cards placed on the outside of tree canopies (Fig. 5). Deposition was relatively uniform from tree top to bottom and ground.
Sticky cards with immobilized flies had 28.5% corrected mortality 22 hours after exposure and 48% corrected mortality at 36 hours indicating that about half of stationary flies received a toxic dose of malathion ULV.
The cages with flies had fly corrected mortality of 71% 22 hours after exposure.
Mortality of flies confined to containers with treated leaves had 12% corrected mortality after 16 hours of exposure, 80.7% corrected mortality after 43 hours of exposure to treated leaves. We observed 12% mortality in the untreated controls at the 43 hr mark.

Based upon laboratory evaluations of malathion ULV applied to cherries by fixed-wing at 16oz/A, it appears that when applied under good conditions to tree canopies that are not completely grown together, malathion ULV could likely be a useful management tool for this insect. Obviously, the only way to say with certainty that malathion ULV will protect fruit from SWD infestation is to apply this product to cherries with populations of SWD present and evaluate fruit for infestation. However, given that we have observed adequate mortality of flies placed in the field and in containers with treated leaves, malathion ULV should be considered as another tool in the SWD arsenal for cherry growers to be combined with ground sprays of products recommended for SWD control.

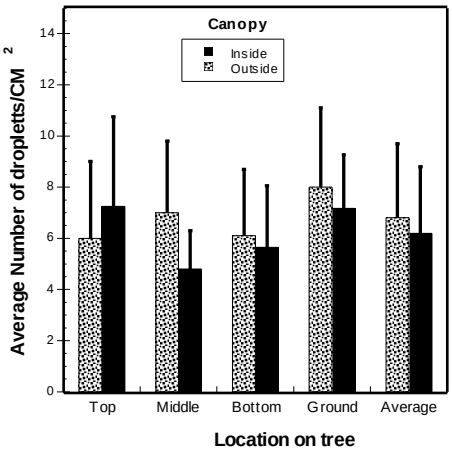

Figure 5. Average number of malathion ULV droplets/cm2 on oil-sensitive spray cards.

Additional efficacy research was conducted at two sites to evaluate promising insecticides for control of spotted wing drosophila (SWD) on sour cherries and sweet cherries. The sour cherry site was a large commercial orchard located near Gaston, OR and the sweet cherry site was located at the Oregon State University Mid-Columbia Agricultural Research & Extension Center (MCAREC), Hood River, OR. Populations of SWD were not present during the test period at these locations so we could not assess insecticidal effects directly in the field. Instead, trees were sprayed with an airblast sprayer and then leaves and fruit were brought back to the laboratory where they were exposed to adult SWD to evaluate adult mortality, egg laying and emergence of adults.

Tests conducted on both types of cherries were conducted using the same protocol. Treatments (Table 1) were applied with an airblast orchard sprayer to individual trees. Each treatment was

replicated 4 times. After treatment, leaves and fruit were collected and brought back to the laboratory. For the leaf assays, leaves were taped to the inside sides and bottoms of plastic deli cups and then 10-15 flies were added to each cup before they were covered with lids (Fig. 6). Both the cups and lids had screen mesh that covered holes added for ventilation. Fruit assays comprised of 5 fruit per tree, 20 per treatment, and each set of five fruit were fastened to the bottom of deli cups with clay (Fig. 7). Ten-fifteen adult SWD were added to the cups. The cups were covered then with lids and mortality was assessed over time. All flies were removed after 2-3 days. Egg-laying was assessed by counting and recording the number of breathing tubes (=spiracles, a visible egg structure) per fruit. Adult emergence was evaluated later from one test.

Figure 6. Example of cup lined with leaves for residual assay.

Figure 7. Example of fruit assay container.

Table 1. Products tested and rate per acre applied.

Active ingredient	Product	Rate/Acre
malathion 56%E	Malathion 5EC	4 pints
acetamiprid 70%WP	Assail 70WP	3.4 oz
carbaryl 43%F	Sevin XLR	2 qts
spinetoram 25%WG	Delegate WG	4.5 oz
spinosad 80%WP	Entrust	2.5 oz
Experimental 1	Cyazypyr	X
imidacloprid 17.4%F	Provado 1.6	6-8 fl oz
lamda-cyhalothrin	Warrior II	2 oz
malathion 79.5%	Malathion 8F	3 pints
Experimental 2	ARY-0556-001	XX
control	untreated	--

Results indicate that several insecticides, including malathion, spinosad, spinoteram, and lamda-cyhalothrin, provide relatively quick mortality of flies placed on treated foliage and fruit (Figs. 8-11).

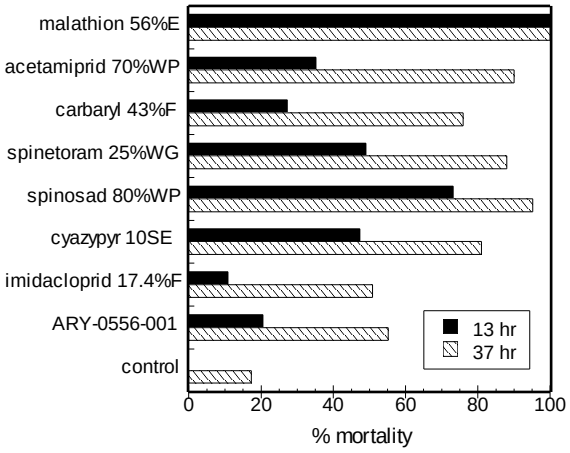

Figure 8. Mortality of adult SWD 13 and 37 hours after being placed on leaves collected from treated trees, Gaston.

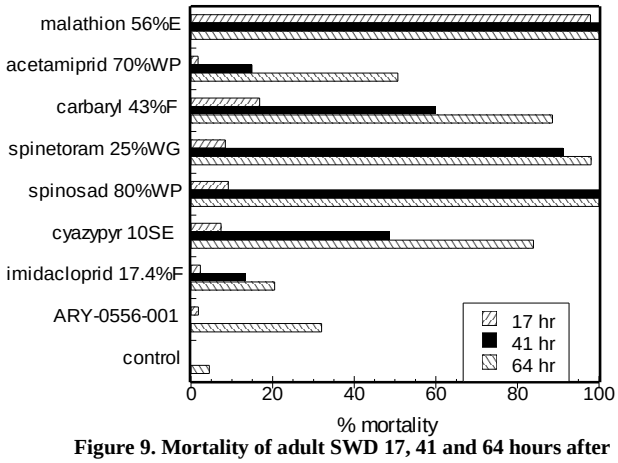

Figure 9. Mortality of adult SWD 17, 41 and 64 hours after being placed on fruit collected from treated trees, Gaston.

Carbaryl is moderately toxic to flies and is slower acting. The neonicitinoids acetamiprid and imidacloprid were not that effective when compared with efficacy of the above products in terms of

mortality and preventing egg-laying (Figs 3-7). However, it appears that acetamiprid, imidacloprid and the Experimental 1 treatments have systemic activity because of the reduction of adult SWD that emerged from treated fruit relative to the number of eggs laid in these fruit (Fig. 12). It is possible that these products may have use as post-harvest cleanup sprays.

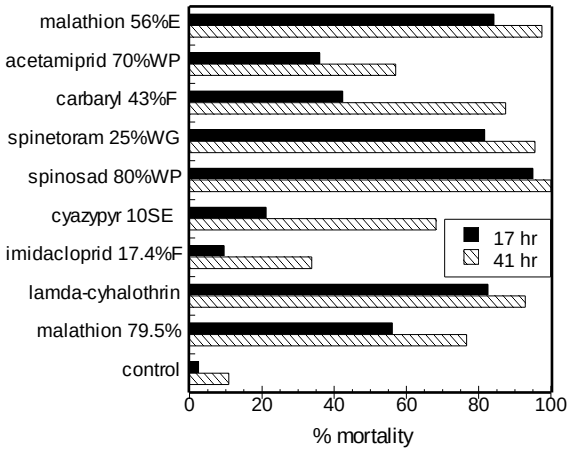

Figure 10. Mortality of adult SWD 17 and 41 hours after being placed on leaves collected from treated trees, MCAREC.

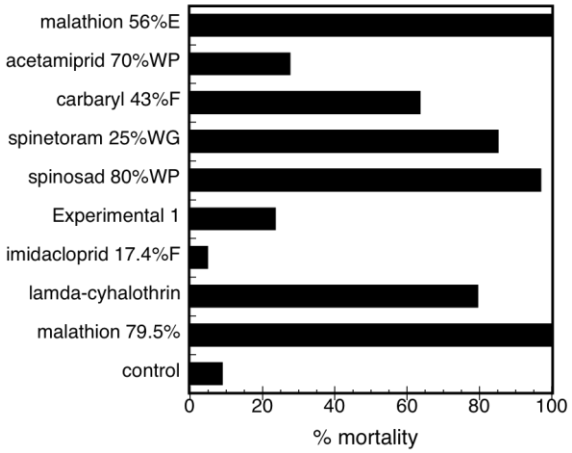

Figure 11. Mortality of adult SWD 24 hours after being placed on fruit collected from treated trees, MCAREC.

It appears that several insecticides, including malathion, spinosad, spinoteram, lamda-cyhalothrin, and carbaryl, which are effective against the western cherry fruit fly, *Rhagoletis indifferens*, will also control SWD. This means that cherry growers currently have a few tools at their disposal until other effective control measures are discovered.

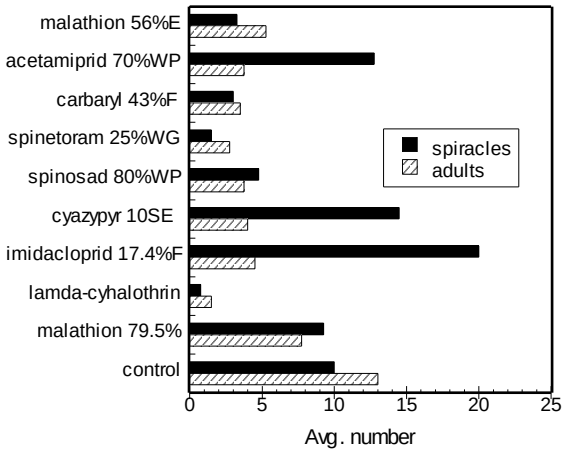

Figure 12. Abundance of eggs laid and adults that emerged from treated fruit, MCAREC.

Objective 3: Extend information to industries in a coordinated manner.

Our outreach efforts including meetings with growers and field men led to the creation of a SWD management plan for Mid-Columbia cherries. We developed list of preferred and emergency materials along with a trapping and monitoring plan that alerted growers when and where flies were found. Growers and field men received this information weekly through updates from our Extension personnel. No SWD-induced load rejections or harvest infestations were reported from the Mid-Columbia cherry district. Teaching materials, new research information and seasonal observations are available via an online at our SWD workspace maintained by the OSU Horticulture Department at: http://swd.hort.oregonstate.edu/

FINAL REPORT

Project Title: Fumigation trials for Spotted Wing Drosophila in sweet cherry

PI: Mike Willett
Organization: Northwest Horticultural Council
Telephone: (509) 453-3193
Email: willett@nwhort.org

Cooperators: Dave Martin, Stemilt Growers, Inc.

Budget: **Year 1**: $10,000.00

Organization Name: NHC
Amount awarded: $10,000.00

Item	**2010**		
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies	$10,000[1]		
Travel			
Miscellaneous			
Total	**$10,000**		

Footnotes: [1] Includes shipping

Justification: The emergence of spotted wing Drosophila (*Drosophila suzukii* or SWD) as a pest of sweet cherry in California led to a requirement by the government of Australia to conduct confirmatory testing to ensure that the current fumigation protocol against western cherry fruit fly (*Rhagoletis indifferens*) is effective against SWD. For 2010, cherries are being exported to Australia under an interim mitigation protocol that requires visual inspection of 600 cherries under 20x magnification following fumigation pending the completion of these confirmatory tests. Earlier rounds of testing to begin to determine the appropriate fumigation temperature at which to conduct the confirmatory fumigation and to ascertain the most resistant life stage were done with cherries donated by California growers. Additional cherries are needed to complete development of the temperature-mortality curve and to conduct the large-scale confirmatory test prior to the end of the season. Cherries are needed beginning on August 2 and again in mid-August, for a total of two 96-carton pallets containing 20 lb. boxes of cherries. Work will be conducted by: Dr. Spencer Walse, San Joaquin Valley Agricultural Sciences Center, USDA/ARS, 9611 South Riverbend Avenue, Parlier, California 93648-9757, (559) 596-2999. This laboratory is the only facility in the western U.S. with the resources and expertise to conduct and evaluate large-scale quarantine treatment studies for SWD and is deeply involved in this work on a number of commodities. Funds will be used to purchase two pallets of sweet cherries and ship them to Parlier, California. Stemilt Growers, Inc. (Mr. Martin) has agreed to provide the cherries at no more than the prevailing market price and arrange for their shipment to Parlier. Estimated cost of the cherries is approximately $42.00 per carton plus about $1.50 per carton for shipping. Invoices for fruit and shipping costs will be sent directly to the Washington Tree Fruit Research Commission for payment, so no interim fund transfer will be necessary. In order to meet Dr. Walse's scheduling requirements, the cherries must be shipped from Wenatchee by Friday, July 30.

Objectives:

- Develop an appropriate understanding of the methyl bromide temperature/time/rate-mortality curve for SWD under the currently fumigation schedule for cherries to Australia.
- Conduct a large-scale test (size of trial currently set at 93,000 insects, all at the most resistant life stage) to confirm the effectiveness of methyl bromide for control of SWD.

Results:

Data analysis is continuing to determine the relative effectiveness of methyl bromide at various rates, times and temperatures. Depending on the outcome of this work additional cherries may be needed, possibly from southern hemisphere sources.

FINAL PROJECT REPORT

Project Title: Powdery mildew workshop

PI:	Gary Grove	**PI:**	Jim McFerson
Organization:	Washington State University	**Organization:**	WTFRC
Telephone	509-786-9283	**Telephone:**	509-665-8271 x1
Email:	grove@wsu.edu	**Email:**	mcferson@treefruitresearch.com

Budget: **Year 1**: $10,000

Justification

Cherry powdery mildew (CPM) is a significant problem for cherry producers, despite development and implementation of new chemical controls and IPM programs in the past decade. The disease received the highest ranking at the 2009 WA-OR cherry priority-setting session and the 2009 crop season highlighted the threat this disease poses, as cherry acreage expands, new cultivars are planted, and market demands for fruit quality escalate. The potential for pathogen resistance to common chemical controls exacerbates the situation. The disease was also identified as a high priority at the 2010 priority session.

The time is right for the PNW cherry industry to work more effectively towards permanently mitigating the impact of this disease. We can no longer rely so strongly on chemical controls, but should expand our efforts to used a more systems-oriented approach, including genetic, chemical, biological, engineering and informational approaches. Extension efforts must be integrated throughout these efforts rather than utilized simply to disseminate information.

We propose to organize a strategic workshop with selected researcher, extension, and industry participants to develop a visionary strategy that includes both short- and long-term goals. We expect to initiate the process with an evening session on Thu 12 Nov at the 2009 OR-WA Cherry Research Review in Yakima to better define specific issues from the industry perspective. We will then organize a one-day workshop, tentatively scheduled for 14 Jan in Yakima, the day before the 2010 Cherry Institute. While the focus will be on CPM, we will attempt to include other crops that also face pressures from PM (apple, grape, hops). This workshop will include significant stakeholder participation, with an emphasis on the development of a strategic roadmap to guide further research and extension activities. Requested funds will be used to conduct the meeting, defray expenses for invited participants, and kick start the new strategic approaches. Funding will also be sought from private sector technology providers.

Objectives

1) Initiate a process to create a visionary strategy to permanently mitigate the impact of powdery mildew on cherries in the PNW

2) Develop a strategic roadmap that:
   a) defines desired outcomes
   b) sets specific research and extension goals
   c) develops performance benchmarks and priorities
   d) explores opportunities for leveraged funding

Methods

The workshop was both educational and strategic, with cherry industry representatives and research and extension scientists from the U.S. In a participatory and facilitated context we:

1) provided fresh perspectives on mildew management by featuring innovative insights on the management of powdery mildews across relevant specialty crops

2) identified cost-effective tools and techniques that could with further research and extension mitigate powdery mildew impacts

3) identified priority areas of research and extension and sources of short- and long-term funding

Morning session: Formal presentations on cherry production in the Western United States provided background on the economic impact of cherry powdery mildew throughout the supply chain. Technical experts reviewed our current understanding of the epidemiology and management of CPM and presented the latest research on powdery mildews of perennial specialty crops. Particular emphasis was placed on the approaches and economics of genetic, cultural, chemical and biological components of disease management across crops. We examined the commercialization of control tactics and technologies, including outreach and implementation of science-based programs, registration of new fungicides, regulatory issues affecting domestic and export markets, and economic parameters.

Afternoon session: The afternoon session featured structured discussion to identify major knowledge gaps, proposed potential research and extension goals, and identified potential sources of funding for further efforts.

Plenary Session: Participants developed a consensus framework for subsequent research and extension activities. A team was formed to ensure effective follow-up.

Participant List
Workshop Organizers:
Gary Grove, WSU Prosser
Jim McFerson, Manager, Washington Tree Fruit Research Commission

Tree Fruit Industry
Denny Hayden, Chair, WTFRC Cherry Committee
Tim Dahle, Chair, OR Sweet Cherry Commission
Brent Milne, WTFRC
Other industry representatives

Grape Industry
Rick Hamman, Mercer Ranches

Hop Industry
Ann George, WA Hops Commission

Plant Pathologists
Gary Grove, WSU Prosser
Disease history, status of current knowledge of CPM epidemiology and management
David Gent, USDA-ARS, Corvallis
Epidemiology of Hop Powdery Mildew
Chang-Lin Xiao, WSU-Wenatchee
Apple powdery mildew
Wayne Wilcox, Cornell University, Geneva
GPM management from the eastern perspective; fungicide resistance
Jim Adaskaveg, University of California Riverside
Powdery mildew of soft fruits

Genetic and Physiology
Markus Keller, WSU-Prosser
viticulture and disease management
Nnadozie Oraguzie, WSU-Prosser
breeding, genetics, and genomics
Todd Einhorn, OSU-Hood River

Extension
Karen Lewis, WSU Extension
Gwen Hoheisel, WSU Extension
Gary Grove, WSU Prosser
Lynn Long, OSU Extension
Clive Kaiser, OSU Extension

Fungicide development, regulations, and usage
Deborah Carter, NHC

Engineering and diagnostics
Qin Zhang, WSU-Prosser

Table 1. Workshop agenda.

Time	Section	Topic	Speaker
9:00	Welcome & Intro		Jim McFerson
9:05	PM Overview		Gary Grove
9:15	**Tree Fruit**/cherry	Cherry industry perspective	Denny Hayden
9:20		Cherry PM state of knowledge/management	Gary Grove
9:40	**Tree Fruit**/soft fruit	California PM perspective (soft fruit)	Jim Adaskaveg
10:00	**Tree Fruit**/apples	Apple industry perspective	Brent Milne
10:05		Apple PM state of knowledge/management	Chang-Lin Xiao
10:20	Discussion	Tree fruit section	
10:30	Break		
10:45	**Grape**	Grape industry perspective	Rick Hamman
10:50		Grape PM state of knowledge/management	Wayne Wilcox
11:20		Viticulture and PM	Markus Keller
	Discussion	Grape section	
11:40	**Hop**	Hop industry perspective	Ann George
11:45		Hop PM state of knowledge/management	Dave Gent
12:15	Discussion	Hop section	
12:30	**Lunch**		
1:15	**Discussion sessions**	Research and extension strategic priorities	
		Cultural practices	
		cropping (training)systems	
		mechanization and sensing	
		irrigation	
		Biological *(epidemiology)*	
		Genetic	
		Economic	
		Chemical	
		current programs	
		chemical pipeline	
		resistance management	
		MRLs	
3:15	**Break**		
3:30	**Plenary session**		
5:00	**Social hour**		

Future Plans. In order to further the effort envisioned at the workshop, the pathology group met at the University of Washington in late May. A table of research priorities was developed (Table 2). The pathology team decided to develop two parallel Specialty Crop Research Initiative (SCRI) proposals, one focusing on mitigating the powdery mildew of grapevines and the second on those of apples, cherries, and hops. Our original plans were to developed a Research and Extension Planning Project but the decision was recently made to develop a Coordinated Agricultural Project (CAP). As of 10/15 the CO-PDs are completing the narrative component for distribution to the entire team by November 1.

Table 2. Research and outreach priorities developed at the Seattle meeting of the pathology team.

	Cherry Mildew	**Hop Mildew**	**Apple Mildew**
Life cycle	chasmothecia	bud perennation	bud perennation (role of chasmothecia poorly understood)
Model	Yes*	Yes*	No*
Automation/Spray Technology	Needs improvement	Needs improvement	Needs improvement
Environmental			
Humidity	Limited	Limited	Limited
Light	No	No	No
Heat / cold	Limited	Limited	Limited
Cultural			
Irrigation	Limited	No	No
Light penetration	No	No	No
Air movement	No	No	No
Intervention**	No	No	No
Molecular detection	Yes	Yes	No (some work in Canada)
Fungicide Resistance	Underway	No	No
Outreach	Needs improvement and modernization	Needs improvement and modernization	Needs improvement and modernization

*** = improvement needed ** = life cycle interruption, "fooling the pathogen", etc.**

Several conference calls have been held since the Seattle meeting and a transdisciplinary team has been formed, stakeholder meetings scheduled, and the proposal preparation process initiated. Current team members are presented in Table 2.

Table 3. Current composition of PM SCRI CAP team.

Person	**Role**	**Organization**	**Expertise/Area**	**Specialty Crops**
Gary Grove	Project leader	WSU	Epidemiology	Cherries, hops
David Gent	CO-PI	USDA-ARS	Epidemiology	Hops
Chang-Lin Xiao	Co-PI	WSU	Epidemiology	Apples
Matt Whiting	Cooperator	WSU	Horticulture	Cherries
Todd Einhorn	Cooperator	OSU	Horticulture/irrigation	Cherries
Clark Seavert	Cooperator	OSU	Economics	
Marcia Ostrom	Cooperator	WSU	Rural Sociology	
Thomas Piento-Nielson	Cooperator	Sierra Nevada Brewing	Sensory analyses	
Clive Kaiser	Cooperator	OSU	Extension/outreach	
Lynn Long	Cooperator	OSU	Extension/outreach	
Gwen Hoheisel	Cooperator	WSU	Extension/outreach	

Executive Summary

Powdery mildew (PM) diseases are problematic on perennial specialty crops (apples, cherries, grapes, hops, and soft fruit) grown in the Western US and in many cases drive the crop-specific IPM systems. Research and outreach groups have historically focused on the disease of specific crops with limited interaction with crop teams. The cherry and hop PM epidemics of 2009, and period problems with PM of apples and grapes, prompted the reevaluation of our research and outreach approaches and presented the opportunity for the formation of a trans disciplinary teams to focus upon the mitigation of these problematic on perennial specialty crops. WTFRC, OSCC, and WSU funded a 1-day powdery mildew workshop that was held in Yakima, WA on January 14, 2010. Industry representatives who put the diseases in historical and economic perspectives developed the workshop focus and discussion frameworks. The morning session also featured presentations by PM experts on specific crops. Presentations included summaries of the current knowledge base and recommendations on areas of future research and outreach. The afternoon session featured structured discussions designed to establish strategic research and outreach priorities related to cultural practices, epidemiology, genetics/breeding, economics, and fungicide resistance and development. The workshop concluded with a plenary session. Workshop pathologists met for a second time in Seattle in late May in order to further develop areas of research and outreach and to plan the development of Specialty Crop Research Initiative Proposals. Participants concluded that the knowledge base on grapevine PM was much further advanced than those on other specialty crops and that the priority SCRI effort should focus on apples, cherries, and hops. A trans disciplinary team was subsequently formed and in a recent conference call decided to develop an SCRI Coordinated Agricultural Project (CAP) instead of a Research and Extension Planning Project. The pathology group is currently developing the project narrative for distribution to the full team by November 1. The first of a series of stakeholder meetings is scheduled for late October.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: CH-10-105

Project Title: Improved management of powdery mildew of sweet cherry

PI:	Gary Grove	**Co-PI (2):**	Todd Einhorn
Organization:	WSU-IAREC	**Organization**:	OSU-MCAREC
Telephone:	509-786-9283	**Telephone**:	541-386-2030
Email:	grove@wsu.edu	**Email**:	todd.einhorn@oregonstate.wdu
Address:	24106 N. Bunn Road	**Address**:	3005 Expermiment Station Dr.
City/State/Zip:	Prosser, WA 99350	**City/State/Zip**:	Hood River, OR 97031

Cooperators: Matthew Whiting

Total Project Request: **Year 1:** $57,280 **Year 2: 59,921** **Year 3:** 61,383

Other funding sources:
Agency Name: Washington State Commission on Pesticide Registration
Amt. requested/awarded: $18,902
Notes: 2009 Funding to support QoI resistance survey

WTFRC Collaborative expenses: None

Budget
Organization Name: WSU-IAREC **Contract Administrator:** ML. Bricker/Lisa Bruce
Telephone: 335-7667/786-2226 **Email address:** mdesros@wsu.edu/lisa-bruce@wsu.edu

Item	2010	2011	2012
Salaries[1]	35,281	**36,692**	38,160
Benefits	14,818	**15,411**	16,027
Wages			
Benefits			
Equipment			
Supplies[2]	5,181	**5,188**	5,196
Travel[3]	2,000	**2,000**	2,000
Total	57,280	**59,291**	61,383

[1] postdoctoral research associate
[2] reagents for qPCR; field supplies
[3] industry-wide travel to collected isolates (objective 1); travel to upper Yakima and Wenatchee Valleys (objective 2); travel to The Dalles and Hood River for to deploy T/RH dataloggers and conduct disease incidence and severity evaluations (objective 4).

Objectives

1) Determine the presence and regional extent of resistance to QoI fungicides in populations of *Podosphaera clandestina* in Eastern Washington.

2) Investigate "early" cherries (e.g. 'Bing') as potential sources of inoculum for infection of later cherry fruit. The initial step in the process will be determining whether there is a large inoculum increase in a cv. 'Bing' orchard once fungicide applications are terminated at harvest.

3) Investigate irrigation sets during late dormancy as a means to deplete overwintered inoculum supplies prior to the availability of susceptible host tissue.

4) Investigate various irrigation regimens and nitrogen fertilizer regimens, on the incidence and severity of powdery mildew on cv. 'Lapins' cherries.

5) Investigate full-season fungicide programs for effectiveness in reducing the production of chasmothecia (cleistothecia) and therefore the amount of potential carryover inoculum.

SIGNIFICANT FINDINGS

- A leaf disc bioassay was developed to screen isolates for QoI resistance and tested against several local isolates of *P. clandestina*
- The mitochondrial cytochrome b gene of *P. clandestina* has been successfully amplified and sequenced. The amino acids at positions 129 and 143 were in agreement with the facts that these four isolates were sensitive to to QoI fungicides. The sequence information of CYTB gene will facilitate the determination of molecular mechanisms for QoI resistance once it arises in *P. clandestina* populations and developing molecular markers for fast identifying resistant isolates.
- A significant increase in airborne inoculum was detected during the month following harvest.
- Experiments designed to force (using irrigation) an ascospore release the depletion of the overwintered inoculum source were inconclusive.
- Chasmothecia were first detected on infected leaves shortly after harvest. Production continued until early autumn. Dispersal by both wind and water occurred sporadically through the July and August and peaked in mid-September in response to precipitation.
- A full-season fungicide program (postharvest oil applications) was found to have no significant effects on chasmothecia production.
- A new Burkard cyclonic air sampler was compared to rotary impaction (RI) traps for the molecular detection of *P. clandestina* in the orchard air. The new sampler was found to be vastly superior to because of improved dependability and the ability to take daily samples with far less labor input and fewer steps in the laboratory components of the assay. PCR Cp values obtained from these air samples were strongly correlated (r = -0.86; $P < 0.001$) with daily spore counts taken by volumetric traps. Use of the cyclonic trap confirmed a large increase in aerial spore populations following harvest.
- Irrigation / fertigation experiments were inconclusive due to lack of mildew

METHODS

Objective 1. *(QoI Resistance Survey).* Both conventional and molecular techniques were developed to study the presence and distribution of *P. clandestina* strains resistant to QoI fungicides. Our efforts in 2010 focused on developing investigative techniques. Beginning in 2011 isolates will be collected from 1-2 orchards in each of The Dalles/Hood River, Prosser-Benton City, Pasco, Upper Yakima

Valley, Mattawa, Wenatchee, Chelan, and Bridgeport/Omak growing areas. Initially, mass isolates will be used. Infected cherry foliage will be collected from each orchard site and conidia transferred to greenhouse-grown cv. 'Bing' or 'Mazzard' foliage in order to increase inoculum. Practical resistance will be investigated using the leaf disk technique described below. The technique involves treating leaf disks with field rates of candidate fungicides and determining the rate and extent of fungal growth following inoculation.

The new leaf disk technique utilized foliage of cv. 'Sweetheart' trees. Immature but fully expanded leaves were excised and refrigerated prior to use. Nine mm diameter leaf disks were prepared using a flame-disinfested cork borer, surface-disinfested for 1 minute in sodium hypochlorite, air-dried, treated with fungicide, and then inoculated with a known concentration (10,000 / ml) of conidia of *P. clandestina*. Disks were transferred to petri plates containing 1.5% water agar and incubated 14 days at 20 C. Flint (trifloxystrobin) was tested at 0, 80, 160, 320, and 640 ppm. Seven leaf disks are inoculated in each of four single-plate replications.

The following steps were involved in developing molecular techniques for monitoring the QoI fungicide resistant populations. Mycelia and conidia of four isolates (two from cherry seedlings grown in the greenhouse and two from cv. 'Bing' cherry orchards where no fungicides had been applied), were collected for DNA isolation. DNA was extracted using an UltraClean Soil DNA isolation kit (MoBio) following the manufacturer's instructions. Quality of fungal DNA for the PCR was evaluated by amplifying the internal transcribed spacer (ITS) region of nuclear ribosomal DNA with primers ITS5 and ITS4. A portion of the CYTB gene was first amplified using degenerate primers from one greenhouse isolate using nested PCR. Primer sets cyt-b1/cytb2rev and cyt-b1/P4-R were isolated in the first round. Amplicons were isolated from gel using a Qiagen extraction kit, cloned into TOPO4 vectors, and sequenced by the WSU Sequencing Core Laboratory. Identity of the amplicon was confirmed as a partial region of the CYTB gene by searching GeneBank with Blastx. An inner specific primer (5'-TCA TTA TGG GGT GCA ACT GTT A-3') designed using Primer3 was used to amplify the rest of the 3' end region combined with primer P4-R. After the amplified 3' end region was sequenced in the manner described above, the complete sequence of the partial CYTB gene was generated. A new forward primer CPM-F2 (5'-CGT GAT GTA ACC GGG TGA-3') was developed to amplify the largest product from the other three isolates in combination with P4-R in second round PCR following the same procedures. The translated protein sequences were analyzed using ClustalX and used to perform homology searches with the BLAST algorithm provided by NCBI with sequence deposited in GeneBank.

Objective 2 *(inoculum sources)***.** A cv. 'Bing' orchard was used for the initial components of this study. Rotorod and Burkard cyclonic spore traps were placed within and about 0.1 km downwind of the orchard. Traps were operated continuously beginning at bud burst and continuing through harvest of later varieties in the area. The concentration of inoculum of *P. clandestina* in the air at each sampling location was determined using quantitative PCR and primers developed in the WSU-IAREC pathology laboratory. Primers were sensitive enough to detect 1 spore in air samples and (after testing for cross-reaction with 23 other powdery mildews in the region) found to be very specific to *P. clandestina*. Daily qPCR signal strength will be compared with actually daily spore counts.

Objective 3 *(inoculum depletion)***.** A three acre cv. 'Bing' orchard at WSU-IAREC was for this portion of the study. Foliar and fruit powdery mildew levels were high in the orchard during 2009. The formation and dispersal large numbers of chasmothecia was documented at the site during the late summer and early autumn of 2009. Water was applied by handgun on about April 12, April 22, and May 6, 2010. At this time chasmothecia/ascospores were mature but there was not yet (aside from May 5) much cherry foliage available for infection. The orchard was divided into 4 quadrants. Two quadrants were watered while two were left dry. The air of all quadrants was monitored using Rotorod air samplers. The presence and concentration of *P. clandestina* was determined using the

quantitative PCR. The sampling periods compared were 1) 4 hours prior to watering 2) 4 hours after wetting 3) 15 hours during the subsequent evening and 4) 9 hours the following day.

Objective 4 (*irrigation and fertilizer influences*). Studies were conducted in orchards near The Dalles, OR. In the 'Lapins' orchard three fertigation treatments (#1 100 lbs N/acre delivered weekly via injection into irrigation lines, #2 100 lbs N/acre delivered to the ground in a spring split application, #3 60 lbs N/acre delivered weekly via injection into irrigation lines) were superimposed over (microsprinkler) irrigation treatments. Percentage water (irrigation treatments) are based on irrigating the 100% treatment at one acre-inch of water per set (one set per week). Other irrigation treatments include 80%, 60%, and regulated deficit.

Treatments were arranged in a randomized complete block design with 5 replications. Each replication consisted of four trees with the center two serving as experimental units. Trees received normal powdery mildew treatments applied by the grower. However, polyethylene bags were used to cover selected branches during fungicide applications and removed immediately afterwards. "Bagged" branches served as untreated foliage and fruit. The incidence and severity of powdery mildew on fruit and foliage was *not* determined at harvest due to lack of mildew. Temperature and relative humidity was monitored in treatments using Hobo Pro U23-001 data loggers.

Objective 5 (*reduction of carryover inoculum*). A cv. 'Bing' orchard near The Dalles, OR was used for this portion of the study. Treatments were arranged in a randomized complete block design with 4 replications. Each replication will consist of individual 'Bing', Sweetheart, and Rainier trees. Four treatments will be compared: 1) non treated control 2) standard preharvest fungicide program 3) standard preharvest program + a single 2% oil application applied two weeks after harvest and 4) standard preharvest program + two oil treatments applied two and four weeks postharvest. Several weeks after the cessation of fungicide programs 25 leaves will be selected at random from each tree/variety and the number of cleistothecia per cm^2 of leaf tissue determined using a dissection microscope.

Results and Discussion

Objective 1. The mitochondrial cytochrome b gene of *P. clandestina* has been successfully amplified and sequenced. The amino acids at positions 129 and 143 were in agreement with the facts that these four isolates were sensitive to to QoI fungicides. The sequence information of CYTB gene will facilitate the determination of molecular mechanisms for QoI resistance once it arises in *P. clandestina* populations and developing molecular markers for fast identifying resistant isolates.

A leaf disk bioassay was developed to study practical resistance of orchard isolates. The technique involves inoculation of "Sweetheart" leaf disks with a known quantity of conidia of *P. clandestina*. Leaf disks are incubated 10-14 days in petri plates containing 1.5% water agar.

Objective 2. A new Burkard cyclonic air sampler was evaluated in tandem with rotary impaction (Rotorod) traps for the molecular detection of *P. clandestina* in the orchard air. The new sampler was found to be vastly superior to Rotorod devices because of improved dependability and the ability to take daily samples with far less labor input and fewer steps in the laboratory components of the assay. PCR Cp values obtained from these air samples were strongly correlated (r = -0.86; $P < 0.001$; Figure 1) with daily spore counts taken by volumetric traps. Use of the cyclonic trap confirmed a large increase in aerial spore populations following harvest. Series of these traps will be used during 2011 and 2012 in further studies on this objective.

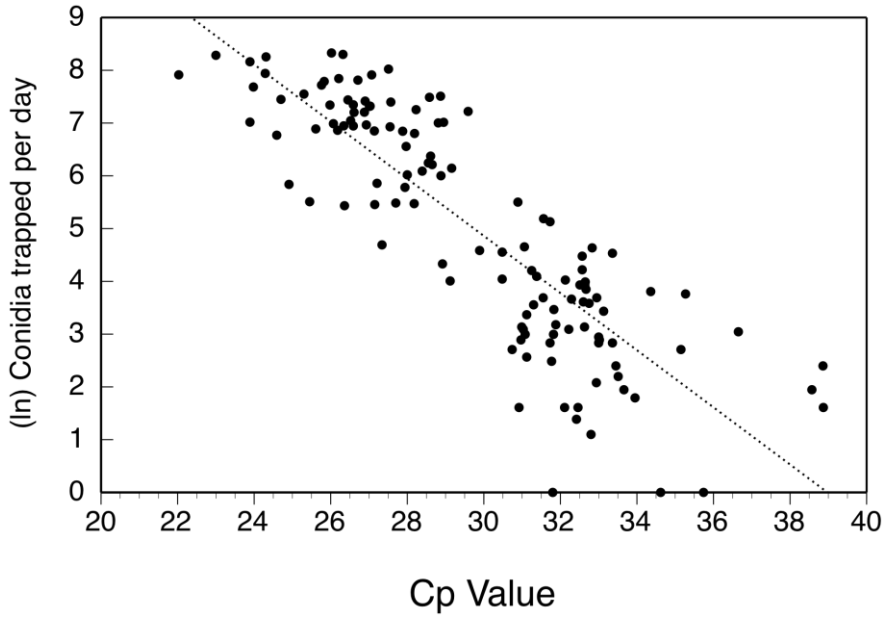

Figure 1. Association of PCR Cp values and daily conidia counts. Cp values were obtained using species-specific primers to determine the concentration of *P. clandestina* conidia in air samples taken daily. Actual conidia counts were taken using a Burkard volumetric spore trap.

Objective 4. Experiments to designed to investigate the influence of irrigation and fertilizer regimens on powdery mildew incidence and severity were unsuccessful due to lack of disease. However, long-term experiments to determine the influence of various irrigation regimens on orchard microclimate were established and will continue over the course of the three-year study.

Objectives 3 and 5. A major component of this research effort is related to the formation, dispersal, and functional or actual eradication/neutralization of chasmothecia, the overwintering propagule of *P. clandestina*. In other pathosystems, the real or functional elimination of such sources has resulted in delayed disease onset and reduced disease severity during the subsequent growing season. Research in 2010 indicated that formation begins several weeks after harvest and continues until early autumn. Ascocarp density was also much higher on cv. 'Bing' than on cv. 'Rainier' grown in the same orchard (Figure 2). Some dispersal occurs whenever ascocarps are present but the majority occurred in late summer following rain events (Figure 3). Two types of dispersal occurred. One type is largely gravitational in nature while the other is wind-driven. The former accounted for the vast majority of ascocarps dispersed. The results of early-season irrigation regimes to promote ascospore release were inconclusive (Figure 5). Differences in PCR signal strength were observed but there were no discernible patterns. The experiments will be repeated in 2011 in a larger orchard using the new Burkard cyclonic air sampler.

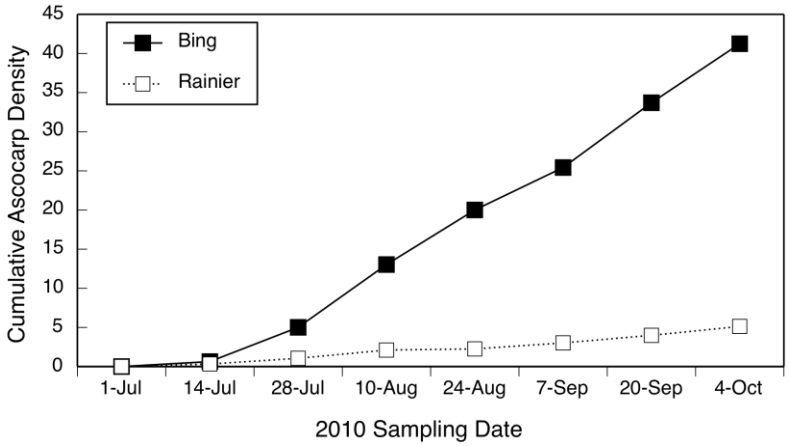

Figure 2. Seasonal formation of chasmothecia *Podosphaera clandestina* on foliage of cv. 'Bing' and 'Rainier) cherries in Prosser, WA 2010. Fruit were harvest about July 1.

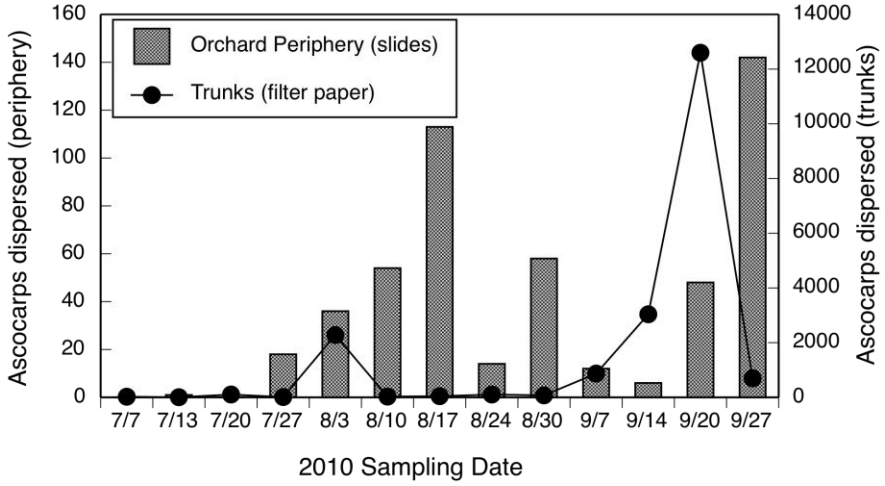

Figure 3. Seasonal dispersal of chasmothecia of *Podosphaera clandestina* in Prosser, WA cherries. Ascocarps were trapped using glass slides affixed to posts at the orchard periphery (indicating dispersal by wind) or on filter paper attached to tree trunks in plastic pots (indicating wind or droplet dispersal).

It is apparent that chasmotheica production begins around harvest, which reinforces the need for season-long disease control. The continuation of fungicide programs after harvest could perhaps reduce the number of chasmthecia and therefore reduce potential carryover inoculum. However, the utilization of synthetic fungicides with high potential for the development (DMI, QoI, or quinoline) of resistant populations poses significant risks to the cherry industry. During 2010 we tested a "hybrid" full-season program: a preharvest alternation of synthetics/oils followed by several postharvest oil programs. This particular program resulted in no significant suppression of chasmothecia populations (Figure 4). However, one significant rain event occurred prior to orchard sampling and may have affected treatment differences. The full season fungicide program (grower standard preharvest program and additional postharvest treatments) failed to suppress the formation of chasmothecia on leaves at upper levels in the tree. Subsequent studies should focus on postharvest programs that include synthetic fungicides and conform to resistance management guidelines developed by the Fungicide Resistance Action Committee.

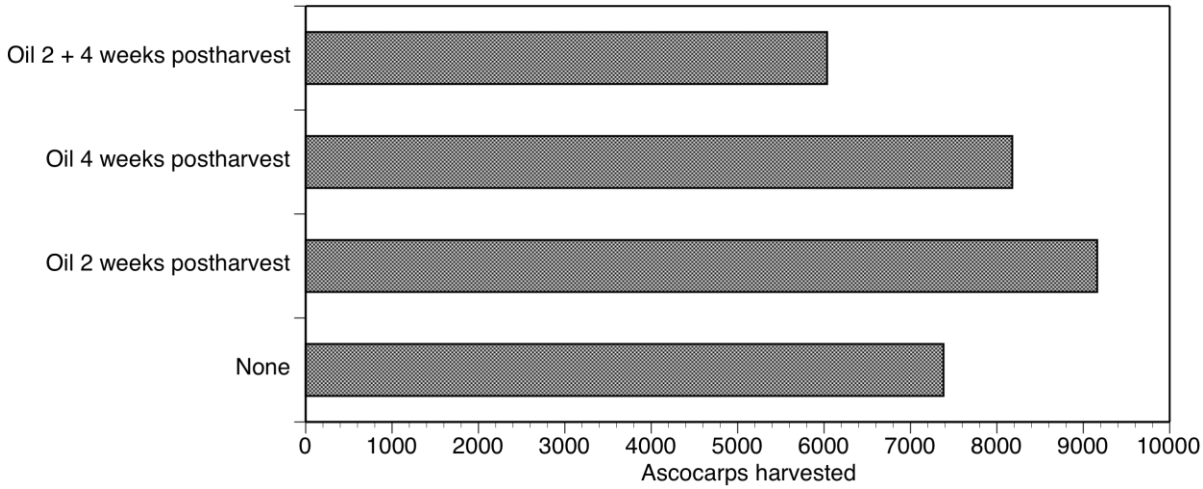

Figure 4. Chasmothecia (ascocarp) production on cv. 'Bing' trees receiving grower-standard preharvest fungicide program versus three full-season programs. There were no significant differences in chasmothecia production at $P < 0.05$.

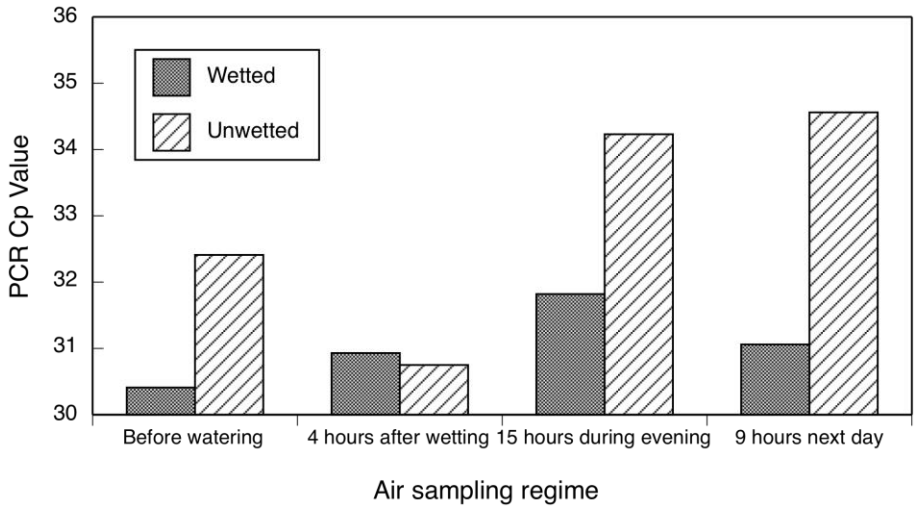

Figure 5. Quantitative PCR values obtained from air samples taken during the application of water to tree trunks and scaffold branches on April 12. Cp values are inversely proportional to amount of DNA in samples. Consistent differences were not observed during any of the three "watering" periods.

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

Project Title: Improving biological control of insect pests of cherry

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

Other funding sources

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

WTFRC Collaborative expenses: none

Budget 1
Organization: OSU MCAREC **Contract Administrator:** Dorothy Beaton
Telephone: 541-737-4068 **Email:** Dorothy.beaton@oregonstate.edu

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

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

Budget 2
Organization: WSU-TFREC **Contract Administrator:** Mary Lou Bricker, Kevin Larson
Telephone: MLB 509-335-7667 **Email:** mdesros@wsu.edu,
KL 509-663-8181 x221 **Email:** kevin_larson@wsu.edu

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

Footnotes:
[1] 25% FTE Technician
[2] benefits at 38%
[3] student (time slip) summer help
[4] benefits at 14.8%
[5] includes traps, chemicals, field supplies
[6] within state travel

Objectives:

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

Significant Findings:

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

Methods

Objectives 1 & 2. We have been testing a variety of natural enemy attractants for the past 3 years on apple and two years ago expanded our testing to pear and walnuts. Based on our work, we have several promising attractants and blends that seem to be highly attractive to different natural enemy groups. We propose to use these compounds to determine the abundance and diversity of the natural enemies present in sweet cherries in Oregon and Washington. In addition, the season-long trapping of the natural enemies when taken in conjunction with weather data will allow us to validate phenology models (Objective 3) already being developed in apple and pear through our other studies.

The field studies are being conducted in large plots, established in three separate cherry orchards per state, using a randomized block design. Within each of the four blocks (per orchard), one trap for each HIPV to be tested will be spaced a minimum of 20 m apart with a minimum of 40 m between blocks. Traps will be placed 1.5-3 m high in the tree canopy. The position of each type of attractant will be randomized among traps within blocks. The traps will be rotated weekly to reduce location effects; during each rotation of the traps, the traps are moved to trees that are offset from the trees previously containing a lure. This rotation prevents contamination of a tree caused by an earlier lure from affecting the performance characteristics of the treatments being rotated. Lures will be replaced as necessary based upon longevity of the different attractants that we have already determined in previous studies. The trials will run between April and late September each year. We will use standard white delta traps with removable sticky liners. Liners are removed each time the traps are checked, covered with plastic wrap, marked with location, block and lure type, and refrigerated until the natural enemies can be identified and counted. Temperature will be recorded at each site for use in modeling of natural enemy phenology (Objective 3).

Objective 3. Phenology models are being developed for several natural enemies found in Washington apple and Oregon pear orchards in the SCRI grant mentioned previously. For those studies, we are collecting temperature data and natural enemy phenology data in several locations to generate models that will allow us to predict their seasonal occurrence. For this grant, we will gather natural enemy phenology data in cherry orchards and use that data to validate the models developed in apple and

pear orchards (validation is easier and cheaper than modeling from scratch). The validation of phenology models is very straightforward with the data being collected in Objectives 1and 2. Observed first occurrence and seasonal capture of natural enemies in cherry orchards will be integrated with temperature data collected in or within 2 km of each orchard site and compared with predicted values from the models we are creating elsewhere. If validation shows marked differences in cherry orchards, we will adjust the models to the target natural enemies found in cherry orchards. Once validated, these models will then be incorporated into the WSU–Decision Aid System so that management recommendations can be modified to maximize natural enemy effectiveness while retaining good management of the key pests.

***Results and Discussion*:**

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

We set up our experiment in 6 different orchards using 3 sites in Washington and 3 in Oregon. The Washington orchards consisted of two conventionally managed orchards (Orondo and Malaga) and one organic orchard (Quincy). All 3 Oregon sites are managed conventionally and located in The Dalles, Mosier, and Hood River, respectively. Each orchard was sampled weekly using beating trays and four different natural enemy attractants (geraniol + methyl salicylate + 2-phenylethanol, acetophenone, squalene, and phenyl-acetaldehyde) and an unbaited control placed in white delta traps that were replicated throughout the block four times (20 traps per week).

All of the beating tray data is stored in ethanol, but has not yet been identified or analyzed. The natural enemy attractant traps have been completely processed up to 11 August for Oregon and 25 August for WA and the results of those analyses is detailed below. The final cherry samples (from 25 Aug to end of Sept) will be analyzed by early November.

The number and diversity of natural enemies captured in our orchards was considerably reduced compared to our experience in apple orchards and pear orchards. The green lacewings *Chrysopa nigricornis* and *Chrysoperla plorabunda,* a syrphid fly (*Eupeodes* spp.), and a small chloropid fly, *Thaumatomyia glabra* are the most common natural enemies captured. The first three species are known to feed on aphids (in general) and are likely predators of black cherry aphid during different parts of the season. *T. glabra* is best known for feeding on sugar beet root aphid and other aphids attacking plant roots. We are unsure of the importance of *T. glabra* in cherry or other tree crops, it has not be previously reported attacking woolly apple aphid, the most serious root aphid pest in apple despite being commonly collected in apple orchards. Regardless, all four species can be considered to be indicators of the potential for biological control, with the first three directly important in BC of black cherry aphid.

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

The lacewing *C. plorabunda* is one of the earliest emerging predators in tree fruits and our WA samples in apples this year found them occurring in the first week of March. They were also found in our earliest samples in cherries, but dropped off to low numbers by the first week in April. In all three orchards, the numbers stayed low until mid-June, then increased in both the Quincy and Malaga sites (Fig. 1). The sharp drop in numbers early in the season at the two conventional sites corresponds to the application of lorsban and oil; the organic site spray records are not yet available. The Quincy site is immediately adjacent to an organic apple block we monitored and it showed a similar decrease at that time, but the numbers in the apple block picked up within 2 weeks and

increased throughout the season. The very low population level from early April to Mid-June corresponds in the conventional orchards to sprays of oil, couraze (imidacloprid), assail, success, and sulfur; all of which may affect natural enemies. We also observed *C. plorabunda* in OR cherry orchards and their population trends were similar to what was observed in WA (Fig. 2). The increase in *C. plorabunda* numbers corresponds to the end of cherry harvest so it's possible their abundance is regulated by insecticides applied against western cherry fruit fly and spotted wing drosophila, *Drosophila suzukii*.

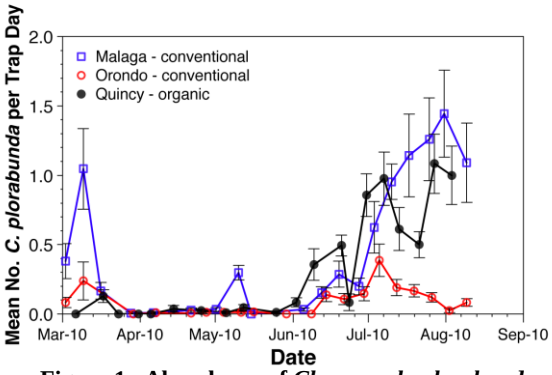

Figure 1. Abundance of *Chrysoperla plorabunda* in 3 WA cherry orchards

The lacewing *C. nigricornis* is one of the most common predators that we have found in our apple and pear plots, and similarly, it is common in cherries as well. In apples and pears, *C. nigricornis* over the past two years has emerged in the middle of May, which also was seen as a slight blip in our cherry plots (Figs. 3&4). However, unlike our data in apples, *C. nigricornis* populations do not increase until late season, typically after harvest and pesticide applications have stopped. The low level of *C. nigricornis* at all sites during the pre-harvest period is troubling and at least suggests that GF-120 in WA has a strong impact on *C. nigricornis*, but a much smaller effect on *C. plorabunda*. Conversely, very little GF-120 was used on OR cherries this year because of concerns of *D. suzukii* so other products, including malathion and delegate that were applied from straw color through harvest may also have negative impacts against this natural enemy.

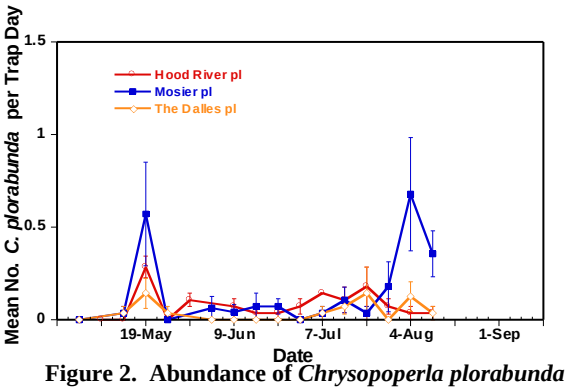

Figure 2. Abundance of *Chrysopoperla plorabunda* in 3 OR cherry orchards

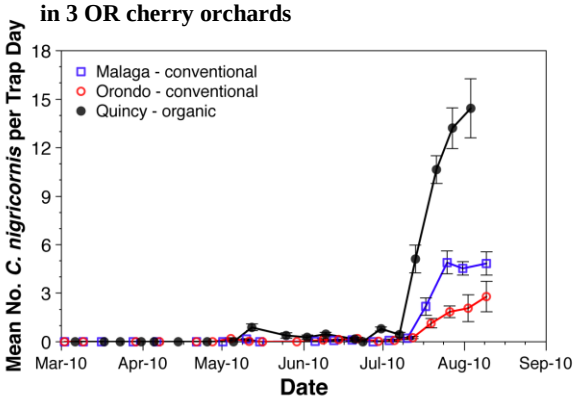

Figure 3. Abundance of *Chrysopa nigricornis* in 3 WA cherry orchards

The syrphid fly (*Eupeodes* spp.) shows a population trend similar to the *C. nigricornis*, but at least in the organic plot seemed to start increasing well before pesticide applications for western cherry fruit fly were likely to have stopped (again, we are waiting for the spray records for this site). Population trends of this fly were high throughout the period from April through mid-June in the organic apple block we monitored that was near this cherry block (Fig. 5). The differences may be related to the differing phenology of the pest aphids in apples versus cherries, or may be related to differing spray programs in the two blocks. Syrphid flies collected on traps in OR have yet to be identified below the family level and are reported here collectively lumped together. These OR syrphids probably include adults of larvae that feed on different diets

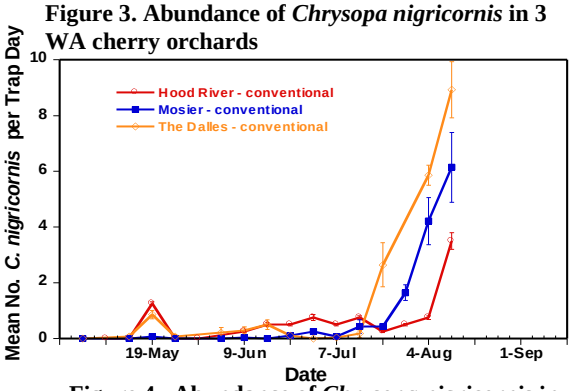

Figure 4. Abundance of *Chrysopa nigricornis* in 3 OR cherry orchards.

including aphids, ants and decaying vegetation. (Fig. 6). In depth identification is ongoing and will be completed this winter

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

We are still processing the trap and beat tray samples and no work on this objective is possible until that data is entered and checked.

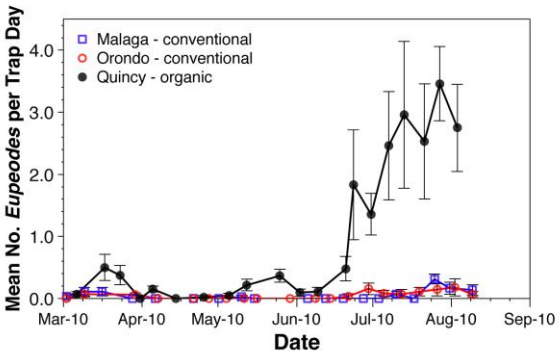

Figure 5. Abundance of *Eupeodes* in WA Cherry orchards

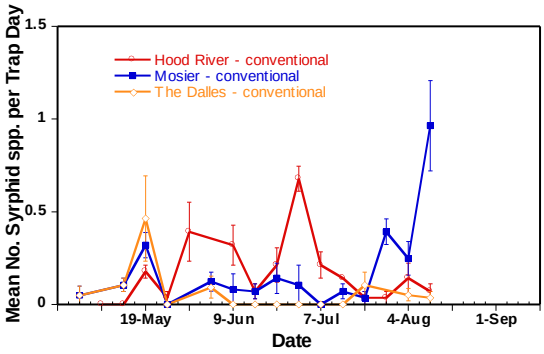

Figure 6. Abundance of various *syrphids* in OR cherry orchards

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: CH-10-108

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

PI: Ken Eastwell
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Cooperators: Dr. Nnadozie Oraguzie and Mr. Bill Howell, WSU-IAREC, Prosser, WA
Mr. Tim Smith, WSU-Extension, Wenatchee, WA
Dr. Tom Unruh and Dr. Wee Yee, USDA-ARS, Wapato
Various growers

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

Other funding sources

Agency Name: National Clean Plant Network – Fruit Trees
Amt. awarded: $30,628 was awarded to support the final year support of a doctoral student investigating Flexiviridae infecting cherry trees in Washington State. This is a portion of a larger NCPN grant to WSU-Prosser.
Notes: WSU is including this information on other funding available for the support of similar research undertaken by the faculty member proposing this research. These resources are listed to identify other support granted for this research and are not included as a commitment of cost-share by the institution.

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

Item	2010	2011	2012
Salaries[1]	22,537	22,150	23,036
Benefits	8,198	9,711	10,100
Wages		0	0
Benefits		0	0
Equipment		0	0
Supplies[2]	12,000	12,661	13,167
Travel		0	0
Miscellaneous		0	0
Total	42,735	**44,522**	46,303

Footnotes:
1. Salaries & benefits:
   0.40 FTE Post doctoral researcher
   0.19 FTE Grounds and nursery services specialist I.
2. Supplies:
   Maintain orchard planting
   Purchase reagents and supplies for performing molecular analysis and antibody development.

Project Objectives:
To identify viruses that cause low quality and quantity fruit production and then develop an understanding of virus biology to ultimately produce effective management methods for growers.

Goal 1: Determine the ability of rootstock and interstock selections to limit the spread of cherry leafroll and related viruses. Expand current field trials to better understand the role that rootstock can play in modifying the rate that viruses are transmitted through root grafting and through aerial routes.

Goal 2: Determine the means of long distance transmission for cherry leafroll virus. Pollen is suspected in the transmission of cherry leafroll virus (CLRV) in cherry trees due to the abundance of virus laden pollen in orchards. However, pollen transmission to trees through blossoms has not been demonstrated. This project explores the role of leaf buds as the point of infection from virus-infected pollen. If confirmed, growers can alter horticultural practices to minimize disease transmission via this mechanism.

Goal 3: Document the responses of new cherry cultivars to viruses. Most studies reporting the response of cherry cultivars to the rusty mottle group of diseases were conducted in the 1970s. As a result, it is not known how these viruses impact contemporary cherry cultivars. A field plot of more recent popular cherry cultivars will be established for inoculation with these viruses once they have been characterized. The virus characterization process also contributes to development of rapid, inexpensive tests that growers can use to identify the cause of tree decline in their orchards.

Significant project findings to date:
- The use of 'Colt', 'Gisela 6', or 'Krmysk 5' rootstocks may be used effectively to limit the spread of cherry leafroll virus. Use of these rootstocks could be integrated into growers' disease management program.
- On Mazzard rootstock, 'Lapins' was the most tolerant cherry variety to cherry leafroll virus as measured by tree growth.
- Related, but distinct, viruses of the family *Flexiviridae* are the cause of the graft-transmissible diseases rusty mottle and cherry twisted leaf. This is the first definitive evidence of an association of these diseases with specific viruses and can provide the basis for diagnostic methods used for growers.
- Single trees are commonly infected by multiple viruses of the rusty mottle group. This 'virus complex' complicates disease diagnosis and management.

Methods:
Goal 1: Based on preliminary data, a field planting of rootstock and rootstock/interstock combinations was established. Selections were based on their potential to confer good horticultural properties (e.g. precocity, reduced tree size), and for potential resistance to transmission of viruses through root grafting in established or replanted orchards. In addition to the rootstocks used in the study started in 2009, this expanded trial will include 'Krymsk 6' (*P. cerasus (Lyubskaya)* × *Cerapadus Michyunin* (*P. cerasus* × *P. maackii*)), a rootstock that was unavailable when the preliminary trial was established. Based on molecular and biological data, isolates of CLRV and cherry raspleaf virus (CRLV) were identified that represent "typical" disease isolates. Trees of each rootstock are inoculated by chip budding with CLRV: four trees are inoculated above the graft union, four trees are inoculated below the graft union, and two trees are reserved as control trees. The same process is repeated on a different set of trees but inoculated with CRLV. Development of hypersensitive reactions as a measure of the ability to resist infection will be monitored over the next two years.

Goal 2: Dormant Mazzard rootstocks are planted in pots and held at 50F (10C) until buds swell. At this point, they are placed under lights with bottom heat 65F (18C) while maintaining an air temperature of 50F (10C). These conditions cause the leaves to "sweat", a process called gutation

where excess water forms droplets along the edges of the leaves at the position of hydathodes. In this experiment, pollen collected from CLRV-infected trees is applied to the droplets. After three hours, trees are moved to a warm 68F (20C) dry location to help the plant re-absorb the water droplets exposed to the virus-infected pollen. After five days, trees are moved to the shade house and then to the field for monitoring of virus infections.

Goal 3: A representative collection of disease isolates of the rusty mottle disease group were assembled from around Washington State and characterized through molecular and biological assays. With defined virus isolates, we can move to the next step of inoculating important cherry cultivars that were not around when the rusty mottle group of diseases was characterized in the 1970s. It is already known that some commercial cultivars may be symptomless carriers of some members of the rusty mottle group. Disease recognition in the orchard will be improved by determining the diversity of symptom expression on contemporary cherry cultivars. This will be augmented by the development of serological testing capacity that will be available to verify virus status of trees in commercial production orchards.

Results & Discussion:

Goal 1: Determine the ability of rootstock and interstock selections to limit the spread of cherry leafroll and related viruses. CLRV is transmitted through root grafting and it is also transmitted via an aerial pathway that may include pollen. Preliminary data from field studies suggested that rootstocks that have species other than sweet cherry in their parentage may provide opportunities for management of CLRV. 'Colt' (*P. avium* × *P. pseudocerasus*) rootstock has already demonstrated durable protection to CLRV transmission through root grafting. Moreover, when scions growing on 'Colt' rootstock became infected, necrosis at the graft union led to the demise of the scion thereby preventing further spread of the virus via aerial vectors. The time delay between infection and the development of the hypersensitive reaction will determine the effectiveness of this strategy. 'Bing' trees on 'Colt' rootstock were inoculated with CLRV in June 2009 by chip budding. By the end of the 2010 growing season, trees inoculated above the graft union had become infected and exhibited indications of stress including premature leaf reddening and leaf rolling. However, the anticipated hypersensitive reaction at the graft union had not yet developed. In contrast, none of the trees inoculated below the graft union showed any adverse symptoms and none of the scions tested positive for CLRV (**Table 1**). Thus, the 'Colt' rootstock has effectively prevented the movement of the virus from the chip-bud into the rootstock. This barrier would also prevent the virus from spreading through root grafting.

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. Therefore, in 2009, field trees of 'Bing' on rootstocks of 'Gisela 5', 'Gisela 6' and 'Gisela 12' (*P. cerasus* Schattenmorelle × P. *canescens*), 'Krymsk 5' (*P. fruticosa* × *P. lannesiana*) and 'Krymsk 7' (*P. lannesiana*) were chip-bud inoculated with CLRV either above or below the graft union. 'Bing' on the Zee-stem interstocks on 'Citation' and 'Myrobalan 29C' rootstocks were also included in this study. With the exception of 'Bing' on 'Myrobalan 29C' rootstock with a Zee-stem interstock, all trees inoculated with CLRV were visibly reduced in stature and vigor to some extent, and expressed signs of stress through premature leaf senescence (**Table 1**). 'Giesela 6', 'Krymsk 5' and 'Krymsk 7' developed a severe necrotic reaction to the introduction of CLRV. In one instance of CLRV-inoculated 'Gisela 6' rootstock, CLRV infected the rootstock and moved into the scion wood. In 'Krymsk 5', severe local necrotic reactions developed around the inoculation sites and prevented the virus from moving from the inoculum into the rootstock. When the 'Bing' scion on 'Krymsk 5' is inoculated, trees are severely stunted but still viable. At the time of leaf drop in the autumn of 2009, there had not been any visible reaction of 'Krymsk 7' to virus inoculation. However, only two trees of 'Bing' on 'Krymsk7' were available at the start of the experiment and neither of the trees survived

into the second season; the reason for this is unclear. Based on the data collected after the first year of this trial, it appears that the use of trees on 'Krymsk 5' or 'Gisela 6' rootstock may slow or even eliminate the progressive movement of CLRV through orchards.

Table 1. 'Bing' trees growing on a variety of rootstocks were monitored for responses to inoculation by cherry leafroll virus. Infected tissue was chip budded either onto the rootstock or onto the scion above the graft union in June 2009 and data collected in September 2010.

Rootstock	Non-inoculated	Rootstock-inoculated	Scion-inoculated
G5	10/10 green; healthy union 0/10 scions CLRV positive	2/5 dead above inoculation 3/5 severe stunting, red leaves, gumming 1/3 scions CLRV positive	5/5 gumming at graft union; red leaves; size reduced 5/5 scions CLRV positive
G6	3/3 green; healthy union 0/3 scions CLRV positive	3/3 stunted; gumming at union; red leaves 1/3 scions CLRV positive	3/3 scion dead
G12	1/1 green leaves; healthy union 0/1 scions CLRV positive	2/2 scion severe decline; gumming; suckering 0/2 scions CLRV positive	1/1 scion declining; red leaves 1/1 scions CLRV positive
Colt	4/4 green leaves; healthy union 0/4 scions CLRV positive	4/4 green leaves; healthy union 0/4 scions CLRV positive	3/3 slight leaf reddening; size reduced 3/3 scions CLRV positive
Krymsk 5	4/4 green leaves; healthy union 0/4 scions CLRV positive	1/3 scion normal; green leaves 2/3 necrosis at inoculation site; suckering below inoculation 0/3 scions CLRV positive	4/4 stunted; stressed; red leaves; size reduced 1/4 gumming on rootstock 4/4 scions CLRV positive
Krymsk 7	----	1/1 dead	1/1 dead
Zstem/myro	1/1 green; healthy union 0/1 scions CLRV positive	1/1 depression around graft area; normal green leaves 0/1 scions CLRV positive	1/1 slight gumming on rootstock; green leaves 1/1 scions CLRV positive
Zstem/citation	6/6 green; healthy union 0/6 scions CLRV positive	5/5 necrosis at inoculation site; leaf reddening & rolling 2/5 scions CLRV positive	5/5 leaf reddening & rolling; size reduced 3/5 scions CLRV positive

In 2005, a trial with greenhouse grown trees was initiated to examine the response of several fruiting varieties to CLRV. These trees were subsequently transplanted into the orchard for further observation. Most finished trees were grown on Mazzard rootstocks with the exception of 'Tieton' which was only available on 'Gisela 5' rootstock and 'Bing' that was on *P. mahaleb* rootstock. Three trees were used for each treatment of seven different scion varieties. Trees were initially virus-free and subsequently inoculated by chip-grafting to introduce CLRV alone, or in combination with prune dwarf virus (PDV) or *Prunus* necrotic ringspot virus (PNRSV). As in the previous experiment, it is evident that CLRV has a negative impact on tree growth and survival. Of the 21 trees in each treatment, 18 of the non-inoculated trees survived one-year in greenhouse culture followed by transplanting to the field. In contrast, 14 trees inoculated with CLRV alone, 12 trees inoculated with CLRV plus PNRSV, and 14 trees inoculated with CLRV plus PDV survived. The cross sectional area of the trees was determined in September 2010 (**Figure 1**). The average area across all cultivars was 37 cm^2 for non-inoculated trees, 21 cm^2 for trees inoculated with CLRV alone, 7.6 cm^2 for trees inoculated with CLRV plus PNRSV and 16 cm^2 for trees inoculated with CLRV plus PDV. These

two sets of data confirm field observations from grower blocks that CLRV is detrimental to tree growth, and that this effect is exacerbated in the presence of either PDV or PNRSV.

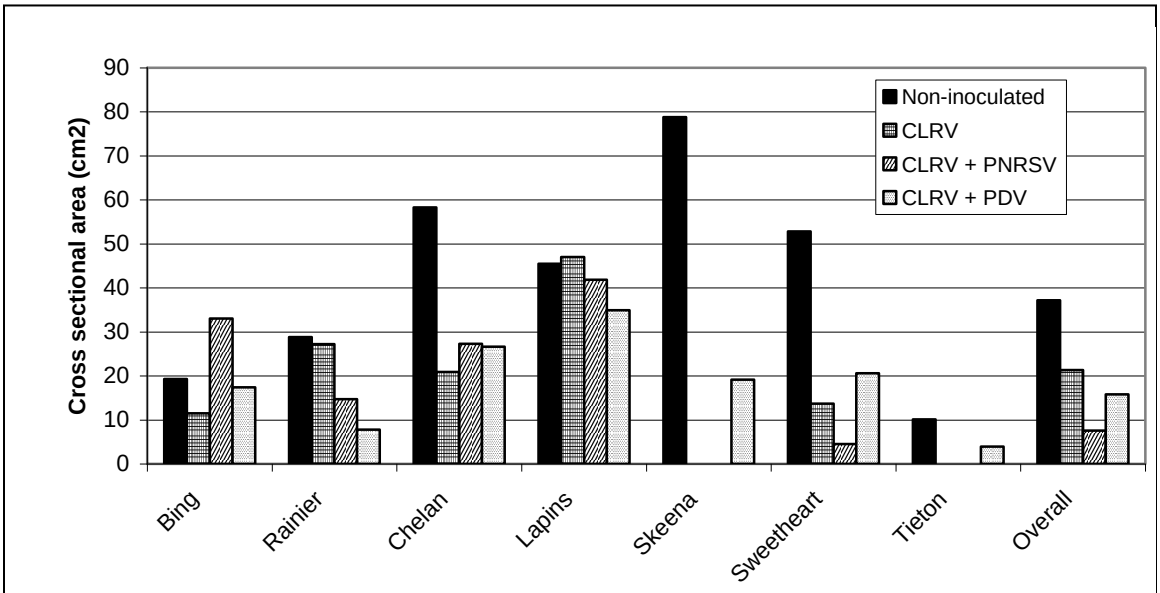

Figure 1. Cross sectional areas of trees were measured after inoculation with cherry leafroll virus (CLRV), CLRV plus *Prunus* necrotic ringspot virus (PNRSV), or CLRV plus prune dwarf virus (PDV). Missing data points indicate treatments in which all of the test trees died.

At the completion of the greenhouse phase of the experiment, 'Tieton' on 'Gisela 5' rootstock appeared to be the most sensitive of the scion/rootstock combinations and exhibited tip dieback during the first year. The combination of 'Tieton' on 'Gisela 5' continued to be the most severely affected by CLRV (**Figure 1**). 'Skeena' was also seriously impacted with most of the trees succumbing to virus infection during the trial period; growth of surviving 'Skeena' trees with CLRV plus PDV was severely suppressed. After the first season in the greenhouse, 'Chelan' was almost symptomless in response to inoculation with virus. However, after extended growth in the field, it is evident that tolerance of 'Chelan' is intermediate with respect to other varieties. 'Lapins' was the most tolerant of the varieties evaluated in this trial.

Goal 2: Determine the means of long distance transmission for cherry leafroll virus. Our research demonstrated that CLRV from virus-laden pollen becomes established in the fruiting structure of the tree (drupe and fruit stems). However, after pollinating 9,479 blossoms since 2007, there is no evidence that CLRV moves from the infected fruiting structures of the tree into the vegetative tissues. Thus, the aerial transmission of this virus by pollen through blossoms is very inefficient, or, there is an alternative method by which the virus is entering trees. During the summer of 2010, trees were potted in the cold room and pollen applied to the gutating leaves as described above. After these trees receive their required chilling requirements, they will be returned to the greenhouse and the new growth tested for the presence of CLRV. Additional trials will be established in 2011 to further challenge this hypothesis.

Goal 3: Document the responses of new cherry cultivars to viruses. Prior to this study, the disease agent(s) associated with the rusty mottle group of diseases were unknown although, because of the graft transmissibility, a virus was suspected. Our research demonstrated that two diseases in the group, cherry rusty mottle and cherry twisted leaf, are indeed caused by viruses. Moreover, our analyses indicate that most infections by the rusty mottle group of viruses are mixtures of several

different virus strains belonging to the family *Flexiviridae*. Therefore, before we could investigate response of cherry cultivars to these diseases, it was necessary to identify and characterize the virus(es) associated with each disease isolate. The virus profile was determined for a total of nine reference isolates and eight samples from grower trees exhibiting necrotic rusty mottle, rusty mottle or twisted leaf disease symptoms. Approximately 1,000 bases of virus genomic sequence were obtained from each isolate; this was repeated ten times for each disease isolate. The sequences used in this analysis include the coat protein gene sequence that will be used for antibody production in the next phase of the project. To correlate sequencing data with symptomatology, bark patches from each tree were chip-budded onto seven woody indicators and symptom expression was recorded 2-3 months after inoculation (**Table 2**). These data reveal the diversity of symptoms elicited by each of these virus isolates on the range of indicator trees and suggests that variability in the response of commercial cultivars might also be expected.

Table 2. Symptoms induced by different isolates of rusty mottle, necrotic rusty mottle and twisted leaf disease on a panel of woody indicators.

Isolate[1]	**Symptoms on woody indicators**[2]						
	P. avium 'Bing'	*P. avium* 'Canindex1'	*P. avium* 'Sam'	*P. serrulata* 'Kwanzan'	*P. persica* GF305	*P. tomentosa*	*Prunus* Shiro plum
CNRM-Flex-01	ns	Mild RM	ns	ns	ns	ns	ns
CTL-Flex-11	severe TL	TL	ns	severe TL	ns	ns	ns
CTL-Flex-10	ns	ns	ns	ns	ns	ns	ns
CTL-Flex-17	ns	ns	ns	ns	ns	ns	ns
CRM-Flex-20	RM	severe RM	RM	chlorotic mottle	ns	ns	ns
CRM-Flex-16	severe RM	severe RM	severe RM	TL	ns	ns	ns
CRM-Flex-18	severe RM	RM	severe RM	chlorotic mottle	ns	ns	ns
CRM-Flex-02	severe RM	RM	RM	ns	ns	ns	ns
CRM-Flex-12	severe RM	severe RM	severe RM	chlorotic mottle	ns	ns	ns

1. Disease isolates: CNRM = cherry necrotic rusty mottle; CTL = cherry twisted leaf; CRM = cherry rusty mottle
2. Symptom descriptions: ns = no symptoms; TL = twisted leaf; RM = rusty mottle

Analysis of the sequences generated from virus profiling revealed four distinct populations, namely, 1) the CGRMV group, 2) the CNRMV group, 3) the cherry twisted leaf virus (CTLV) group and 4) the cherry rusty mottle virus (CRMV) group (**Figure 2**). It appears that grouping of these virus sequences is consistent with symptomatology with few exceptions, and suggests the presence of four distinct but related virus species associated with these diseases. Moreover, the viruses of the four diseases studied herein may define a new genus of viruses within the family *Flexiviridae*.

To make virus detection more accessible for routine application in orchard health monitoring, a clone from each of the four clades has been selected for the production of antibodies for eventual incorporation into serological assays of virus diseases in orchard trees. Polyclonal antibodies elicited against the bacterially expressed coat protein of CRM-Flex-01 have already been produced. A more

efficient strategy is being pursued for cherry twisted leaf, cherry necrotic rusty mottle and cherry green ring mottle viruses by expressing the coat proteins in mice with subsequent production of antibodies. This is done by cloning the coat protein of each virus into pDisplay, a mammalian expression vector. The gene for the coat protein of cherry twisted leaf virus has been successfully cloned into pDisplay in preparation for the next step in antibody production which should commence by January 1, 2011. The genes for the coat proteins of cherry necrotic rusty mottle and cherry green ring mottle have been obtained and are ready to be introduced into the pDisplay vector.

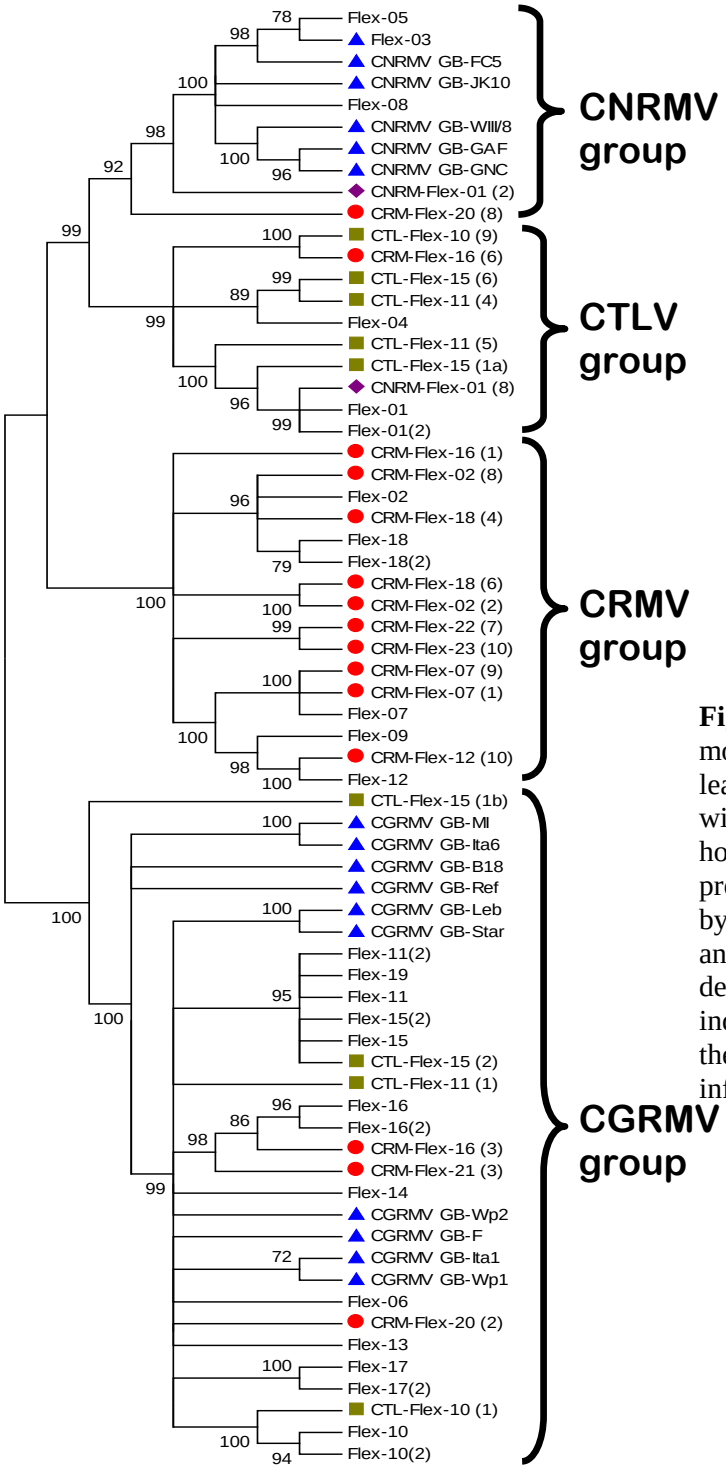

Figure 2. Phylogenetic analysis of rusty mottle, necrotic rusty mottle and twisted leaf virus associated sequences along with Genbank (denoted by ▲) and in-house sequences. The *Flexiviridae* virus profile from rusty mottle (CRM, denoted by ●, twisted leaf (CTL, denoted by ■) and necrotic rusty mottle (CNRM, denoted by ♦) infected trees are indicated. Numbers in parenthesis depict the number of clones obtained from each infected tree.

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

PROJECT TITLE: Systemic acquired resistance to bacterial canker of sweet cherry

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

TOTAL PROJECT REQUEST **Year 1:** $19,300 **Year 2: $19,900**

Other funding sources: None

WTFRC Collaborative expenses: None

BUDGET
Organization Name: OSU Agric. Research Foundation **Contract Administrator:** D. Beaton
Telephone: (541)737-3228 **Email address**: Dorothy.Beaton@oregonstate.edu

Item	**2010**	**2011**	
Salaries FRA 3mo	10,000	10,300	
Benefits OPE 63%	6,300	6,489	
Wages			
Benefits			
Equipment			
Supplies	2,000	2,111	
Travel local	500	500	
Miscellaneous plot fee	500	500	
Total	19,300	**19,900**	

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

OBJECTIVES

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

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

Parallel 200-tree plantings of sweet cherry cv. 'Bing' on Mazzard rootstock were established in Hood River (OSU MCAREC) and Corvallis (OSU BPP Field Lab) to address the objectives. Systemic acquired resistance treatments were made throughout the spring, summer and fall. Evaluations of the plots were made in summer 2010, and will be made again in summer 2011. In addition, a new plot will be established in Corvallis in 2011 to address/repeat significant observations made in the first season.

SIGNIFICANT FINDINGS

- Based on August 2010 observations, a second heading cut made in May on April-planted cherry trees reduced the amount of dead, shrunken trunk tissue (canker) that developed adjacent to the cut.
- To date, spring trunk paint and root dip treatments with the SAR inducer, acibenzolar-*S* methyl, have not provided suppression of developing bacterial canker, and in fact, trunk paint treatments were phytotoxic to cherry and exacerbated canker symptoms.

METHODS

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

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

Table 1.

Spring Treatment Activity	Corvallis	Hood River
Planting & ASM root dip	7 Apr	13 Apr
ASM trunk paint	15 Apr	26 Apr
First heading& pathogen inoculation	19 Apr	26 Apr
Fertilize (50 g $(NH_4)_2SO_4$ per tree)	19 Apr	11 May
Second heading & wound sealant	28 May	11 May
Tree Measurements	5 Sept	14 Sept

Table 2. Spring treatments applied to sweet cherry at or shortly after planting

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

RESULTS

Tree establishment and growth. All trees received from the nursery established and developed healthy shoots. Although the trees at Hood River were smaller caliper than at Corvallis, initial budbreak and shoot growth was faster at the Hood River site.

However, as the season progressed, total new shoot growth at Corvallis (3 meters per tree) was superior to Hood River (2 meters per tree) (Fig 1. A,B,C,D). Initial tree caliper was probably a reason for this difference, but at Hood River, moderate deer damage in outer rows and planting the trees in a plot area that had hosted cherry previously also may have contributed to relatively poorer growth at this site.

Overall, applying a second heading cut to the trees resulted in about a 5% reduction in number of shoots per tree and total shoot length, with larger reductions observed in Corvallis than in Hood River **(**Fig 1. A,B,C,D).

Effect of spring ASM treatments. Spring-applied ASM treatments were not beneficial to tree growth (Fig 1. A,B,C,D), nor did they suppress the initial development of bacterial canker (Fig 1. E,F).

In fact, at both Corvallis and Hood River, painting the trunk with ASM in 2% PentraBark was apparently phytotoxic, resulting in larger cankers associated with the heading cut compared to trees that received the other treatments (Fig 1. E,F). At Hood River, the largest cankers developed on the trees painted with ASM and headed only once (Fig. 1F). At both sites, lateral shoots on ASM-painted trees were generally skewed lower on trunk compared to both untreated and root dip treated trees (data not shown).

The ASM root dip treatments reduced total shoot length and number of shoots per tree by 10-15%, compared to the untreated control (Fig 1. A,B,C,D) but the trees showed no apparent symptoms of phytoxicity. Initial canker development on ASM root-dipped trees was similar to that observed on the untreated controls (Fig 1. E,F).

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

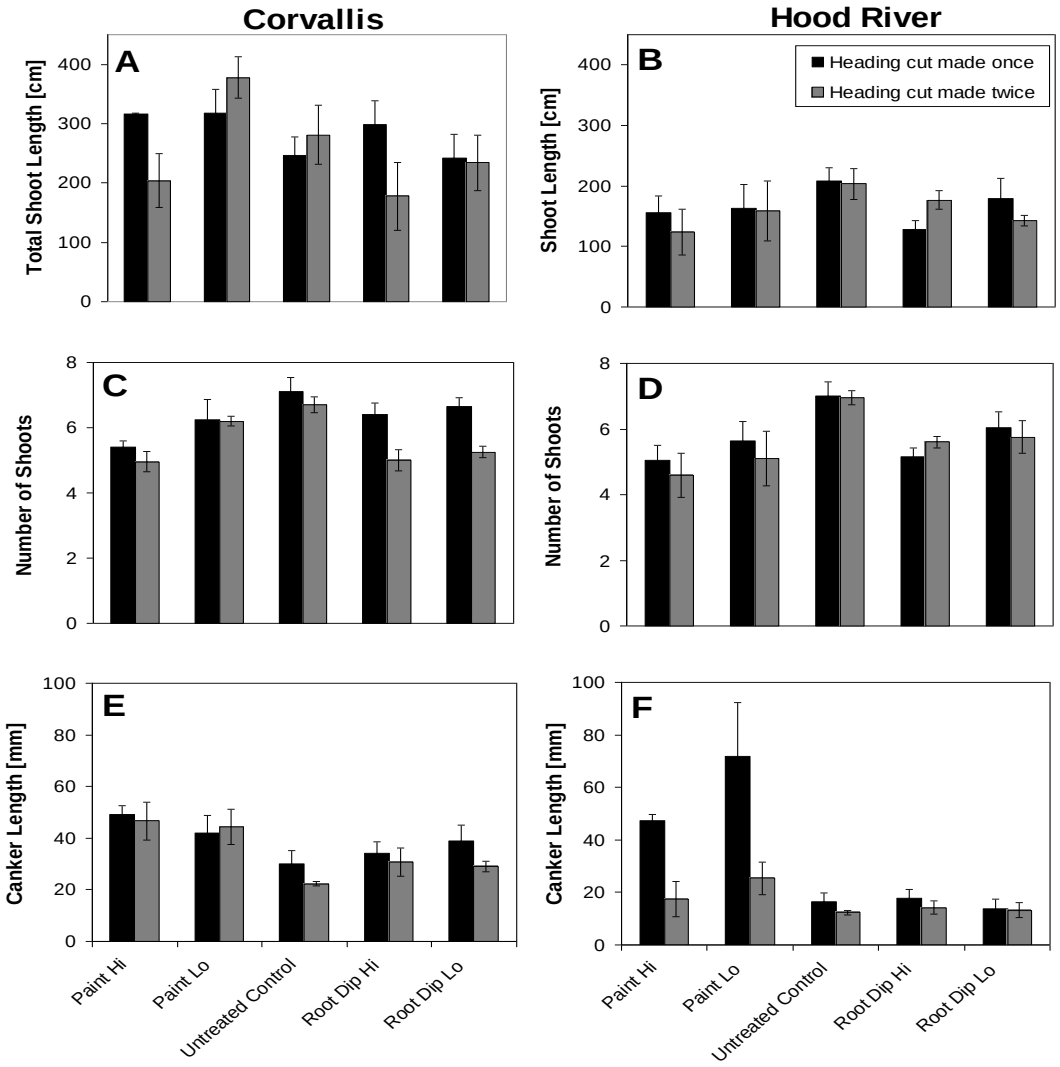

Fig. 1. Effects of spring-applied root drenches and trunk paints of the systemic acquired resistance inducer (SAR), acibenzolar-*S* methyl (ASM), on total shoot length, number of shoots, and length of the dead, shrunken trunk tissue (canker) that developed immediately below the heading cut on trees of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

DISCUSSION

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

In cherry, however, we are not seeing a benefit from spring application of ASM and we will not continue to investigate this treatment timing. We have, however, made late summer/fall treatments to the existing plots, and will plant a new plot next year to follow up on significant observations made in the first season.

Late summer/fall ASM treatments are shown in the table below. Given a lack of response from the spring treatments, our expectations for the autumn treatments are minimal, but we are hypothesizing that fall ASM treatments could offer at least some protection because induction of SAR typically slows/suppresses plant growth, and thus, it may prepare the trees to enter the dormant period in a more resistant state. (We have some evidence from Pseudomonas blight of lilac to support this).

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

* **Existing plots will be sprayed inoculated again with *P. syringae* in late October just before leaf drop.**
[#] **This treatment is based similar spray we applied to lilac in fall 2009 that significantly reduced Pseudomonas blight in spring of 2010.**

The more interesting (and perplexing) results to date have concerned tree response to the heading cuts. While we did observe smaller cankers on the trees headed a second time, the benefit of this cut (with the exception of the phytotoxic ASM paint treatment in Hood River) was not as great as we expected given the first heading cut was inoculated with the pathogen, and the second cut was treated with antibiotic and sealed with TreeKote® tree wound dressing. Confounding this is an uncertainty as to whether the amount of dead, shrunken trunk tissue (canker) that develops immediately below the heading cut is always caused by *P. syringae* (this should begin to resolve itself next spring). For the second heading, we angled the cut downward from just above a strong lateral shoot (i.e., what becomes the new terminal shoot), and over time, many trees developed a small canker at the lowest point of this angled cut (distal to the new terminal shoot). Perhaps this is natural die back (i.e., the tree would do this without the presence of *P. syringae*), and there this also a possibility that the tree wound sealant is phytotoxic and exacerbating the development of dieback/pathogen induced canker. In California, Chuck Ingels (UC Cooperative Extension Farm Advisor) communicated that tree health was worsened in a new cherry orchard (2010) where a phytotoxic dose of copper hydroxide was applied to the heading cuts. In our plots, in contrast to the many double headed trees with a small canker below the second cut, there were a few single heading cuts at both Hood River and Corvallis that developed no symptoms of trunk dieback in spite of being inoculated with the pathogen. On symptomless trees headed only once, a strong lateral shoot was typically positioned very close to the cut (i.e., these trees lacked the blank stub of trunk above the topmost lateral).

Spring treatments in the experiment to be planted 2011 will be mostly concerned with heading cuts: one versus two, inoculation versus no inoculation, length of the blank stub above the topmost lateral, and for the second cut, wound protection versus no protection. If fall ASM treatments from 2010 yield positive benefits, then this treatment will be re-examined in the 2011 experiment.

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number: CH-10-106

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

PI: Donald C. Elfving
Organization: Tree Fruit Research & Extension Center
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City/State/Zip: Wenatchee/WA/98801

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

Total Project Request: **Year 1:** $2,350 **Year 2: $3,525**

Other funding sources: None

WTFRC Collaborative expenses: None

Budget 1
Organization Name: WSU-TFREC **Contract Administrator:** ML Bricker/K. Larson
Telephone: 335-7667/663-8181 **Email address**: mdesros@wsu.edu /kevin_larson@wsu.edu

Item	2010	2011
Salaries[1]	0	**0**
Benefits[1]	0	**0**
Wages[2]	1,000	**1,500**
Benefits[2] **(14.8%)**	150	**225**
Equipment	0	**0**
Supplies[3]	200	**300**
Travel[4]	1,000	**1,500**
Miscellaneous	0	**0**
Total	2,350	**3,525**

[1] No technical help indicated since Technician position no longer exists. Time-slip help is absolutely essential to collect the volume of data needed to set up trials and evaluate growth responses to the various bioregulator applications involved.
[2] Time-slip help substitutes for unfilled Technician position. Time-slip benefit rate is calculated at 15%.
[3] This category includes miscellaneous supplies, non-capital equipment, consumables, repairs, etc. that are needed to carry out the research project. Cell phone charges are allowed under this grant.
[4] Treatment application and data collection at distant sites, all off-station. Includes vehicle lease-to-purchase, operating, repair costs.

Objectives:

1. Test cytokinins (6-BA, CPPU, TDZ) without GA to determine efficacy for stimulation of lateral branch development on two-year-old wood using both cuts and high surfactant concentration additives to evaluate efficacy of cytokinins for bud activation and penetrability of older bark.
2. Assess whether supplementation of treatment solutions with GA produces any beneficial effect on branching of older wood.
3. Evaluate the characteristics of induced branches on older wood and determine follow-up strategies for modification of branch growth habit if needed.
4. Evaluate effects of treatments to older wood on pedicel development of flowers borne on treated wood sections.

Significant findings:

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

Methods:

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

Results and discussion:
One goal of the 2010 program was to determine whether gibberellic acid (GA) alone can induce lateral branching in two-year-old wood of sweet cherry. Previous research has clearly shown that GA alone is about as effective as cytokinin for branch induction in one-year-old wood. One advantage this finding confers is that GA products are OMRI-approved, and thus can be used in organic orchards. They are also a bit cheaper than Promalin. Unfortunately, this year's test of GA vs. cytokinins on two-year-old wood was so damaged by cold injury that no clear conclusions can be drawn, and we need to do this trial again next year, hoping for no fall cold damage in 2010.

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

Few of the surfactant-supplemented treatments showed significant branching activity on either two-year-old or one-year-old wood, but again, the degree to which these observations might have been affected by vascular or bud cold injury is unclear. In the case of the one-year-old wood, killing the terminal portion of those shoots altered the apical dominance situation by producing the equivalent of a heading-back cut. This physiological change resulted in a certain amount of increased branching, thus limiting the degree to which additional branching could be induced by the bioregulator applications themselves.

The question comes up from time to time as to how close scoring cuts should be made to ensure a maximum branching response. In one-year-old wood, we found several years ago that scoring cuts closer than 30 cm (12 Inches) did not increase branching at all. In 2010 one of the experiments was designed to compare several Promalin/surfactant combinations and Promalin/scoring cuts with making scoring cuts or banding bioregulators at 15 cm (6 inches) vs. 30 cm (12 inches) intervals on two-year-old wood. Distance between scores or bands made absolutely no difference in terms of branching on older wood, just as was previously observed on one-year-old wood. Applying treatments every foot (30 cm) along a branch section appears to be quite adequate for branch induction, regardless of the age of the treated wood.

Acknowledgements:
The assistance and support of the following people and organizations is gratefully acknowledged: Dr. Greg Clarke, Dan Flick, Gary Goodman, Jeff Henry, Dr. Lawrence Marais, Dr. Peter Petracek, Ed Schaplow, Richard Scranton, Wm. Stringfellow, Dr. Matt Whiting, Allview Orchards, Goodman Orchards, Monterey Ag Resources, Sunrise Orchard (WSU-TFREC), Quest Products Corp., Valent BioSciences, Washington Tree Fruit Research Commission and the WSU Agricultural Research Center.

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

Project Title: Irrigation and fertilization for optimal cherry fruit quality

PI: Todd Einhorn
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City: Hood River
State/Zip: OR 97031

Cooperators: Jac le Roux, Don Fesler

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

WTFRC collaborative expenses: None

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

- As observed in 2009, 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
- Microsprinkler 'Lapins'/'Mazzard' trees receiving control irrigation had the highest yields, though these did not significantly differ from 60 % of control treatment yields
- Level of irrigation treatment did not affect fruit quality attributes of 'Lapins'/'Mazzard'
- For 'Tieton' and 'Lapins' trials, stem water potential (measure of water stress) declined as the season progressed, irrespective of treatment. No differences were observed among treatments
- As observed in 2009 deficit treatments (except RDI) utilized significantly more water from deeper profiles than control trees to meet their evaporative demand. This utilization lengthened the onset of water stress and explains the lack of any observable adverse effects on yield and fruit quality from deficit irrigated treatments. Additionally, 'Tieton' benefits from an early harvest date, prior to the occurrence of significant soil moisture depletion.
- Percentage of 2010 fruit doubling in 'Tieton' was not related to irrigation level
- Large annual water savings were achieved for deficit treatments
- Frequency of irrigation (replacement of tree-water-use every other day, or once per week) for drip irrigated 'Tieton'/'Mazzard' did not significantly affect yield or fruit quality at harvest, though high frequency treatments had slightly less available soil water than low frequency
- Higher yields were observed for the high nitrogen fertigation treatment [100 lbs/a] as compared to the moderate nitrogen fertigation treatment [60 lbs/a]. Broadcast nitrogen [100 lbs/a] was intermediate. Nitrogen level or delivery did not affect fruit quality attributes

Methods

Objectives 1 and 3: A ten-year-old 'Lapins'/'Mazzard' orchard, located in The Dalles, OR, and trained to a multi-leader system, was used for a fertilization x irrigation experiment. The experimental design was a 2 x 2 factorial, split plot with four levels of irrigation volume and three levels of fertilization. Main plot treatments (irrigation volume) were arranged in an RCBD, with five replicates. Subplot treatments were fertilization. Each replicate comprised of four trees, with the two center trees used for data collection. Four levels of irrigation amount, based on replacement of a percentage of tree water use, were delivered once weekly via microsprinklers, and were: 1) Control (~ one acre-inch of water applied in a 12 hour set), 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). Fertigation events occurred once per week for an eight-week period. For each event, nitrogen was injected over a four hour period during the middle of the irrigation set. The fertigation pump was controlled by a programmer. 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 chamber every 7-10 days, to study plant water status. Briefly, shoot leaves were selected in the mid portion of one-year-old shoot sections, bagged, and allowed to equilibrate for a minimum of 20 minutes prior to measurement. Leaves were bagged roughly 1 hour prior to solar noon so measurements could bracket solar noon (+/- 1 hr).

At harvest, individual tree yields were recorded and 100 fruit subsamples per replicated were collected for evaluation of fruit quality attributes (size, soluble solids, total acids, and firmness), 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 (see 'Lapins' experiment) were applied to replace tree water use via drip irrigation either once weekly (Low frequency- 12 hour set), or every other day (High frequency- 3 hour set; totaling an equivalent amount of weekly irrigation as Low frequency).

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 be explained from a combination of stem water potential and soil moisture data. Stem water potential is a sensitive indicator for detecting plant water stress. Similar to our results in 2009, higher temperatures and lower RH as the season progressed resulted in declining water potential values (increasing stress), irrespective of cultivar, deficit treatment, irrigation systems, and site factors (Fig 1).

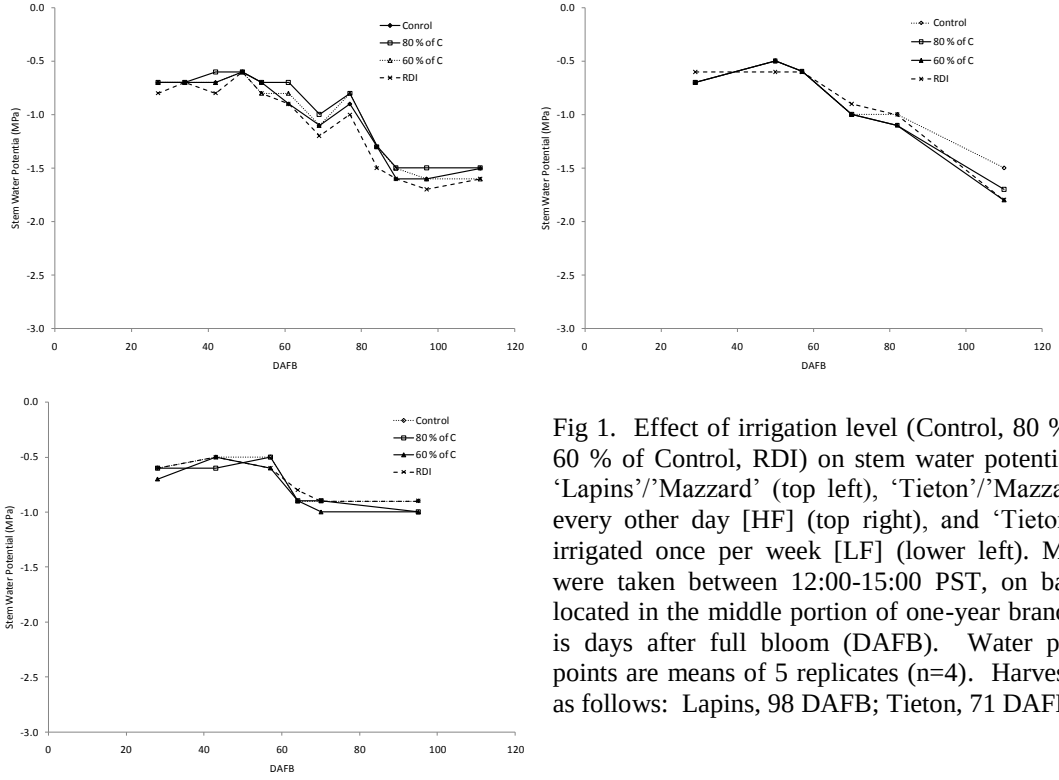

Fig 1. Effect of irrigation level (Control, 80 % of Control, 60 % of Control, RDI) on stem water potential (MPa) for 'Lapins'/'Mazzard' (top left), 'Tieton'/'Mazzard' irrigated every other day [HF] (top right), and 'Tieton'/'Mazzard' irrigated once per week [LF] (lower left). Measurements were taken between 12:00-15:00 PST, on bagged leaves located in the middle portion of one-year branches. X-axis is days after full bloom (DAFB). Water potential data points are means of 5 replicates (n=4). Harvest dates were as follows: Lapins, 98 DAFB; Tieton, 71 DAFB.

For ‘Tieton’ blocks, early in the spring from bud-break through pit-hardening (~30 days after full bloom [DAFB]) ample water exists in the top 60 cm/2ft) of the soil profile due to accumulation from winter/early spring precipitation events (Fig 2), however at 90 cm (3 ft) 2009 deficit treatment effects carried over, and winter precipitation was not adequate to replenish these profiles (Fig 2). ‘Tieton’ has a clear advantage over later season cultivars when imposing deficit irrigation regimes, due to its early harvest date (71 DAFB). Following harvest, soil moisture, and stem water potential (Fig 1), decreased, suggesting that our drip irrigation treatments (including the control), were not compensating for plant transpiration. In fact, the ‘Tieton’ site is located in a water-limited region, and the grower’s irrigation level is at ~ 60 % of ET. By the time water stress develops, shoot growth is complete, and fruit has been harvested. Further, the low productivity of ‘Tieton’ also ameliorates the potential of early soil moisture depletion from limiting fruit size, due to low competition among fruit

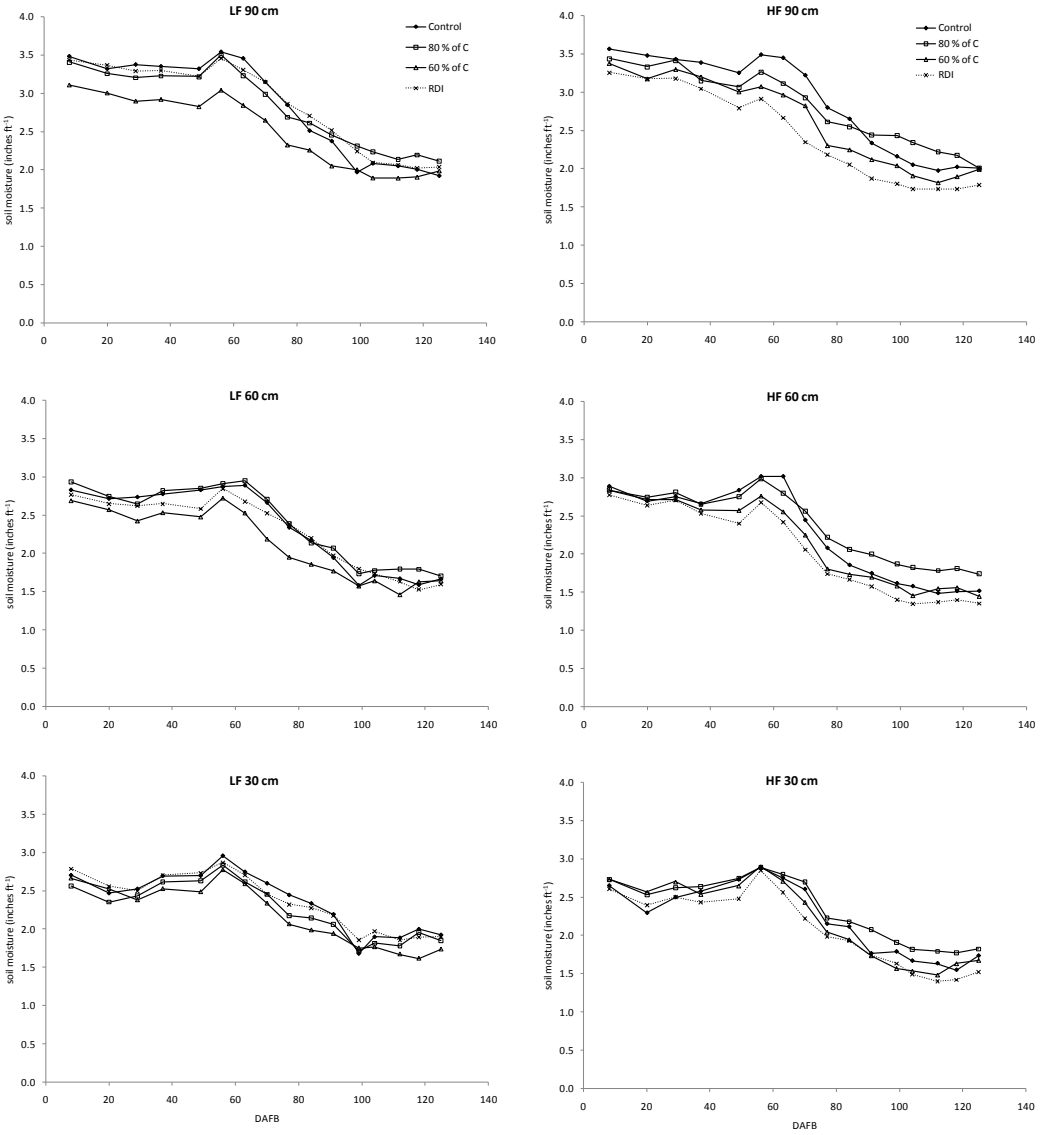

Fig 2. Effect of irrigation level (Control, 80 and 60 % of Control, and RDI) on volumetric soil moisture content (inches per foot) of the soil profile at 3 ft (top), 2 ft (middle), and 1 ft (lower) depths for ‘Tieton’/’Mazzard’. Left figures are low frequency treatments; right are high frequency. Harvest was 71 DAFB. Each data point is the mean of 5 replicates (n=3).

Treatment	Doubling (%)
Irrigation Amount	
Control	5.2a
80% of C	4.5a
60% of C	4.6a
RDI	4.5a
Frequency	
High	5.2a
Low	4.2a

Table 1. Effect of 2009 irrigation amount and interval on 2010 'Tieton' fruit doubling. Doubling evaluations were made on a 350 fruit sample per replicate.

for carbohydrates. One interesting observation was the slight reduction in soil moisture for the high frequency treatment, relative to the low frequency treatment (Fig 2). This can likely be attributed to an increase in evaporative water loss. High frequency treatments are applied 3-4 times per week, vs. the once/week application rate of the low frequency treatment.

One potential adverse effect associated with late-season development of water stress is the disruption of reproductive bud development for next years' crop. M. Whiting has provided direct evidence of a high temperature effect during post-harvest reproductive bud development on 'Tieton' twinning, or doubling. Because water stress reduces plant transpiration and subsequently increases tissue temperatures, water stress might aggravate doubling. We did not observe a 2009 irrigation effect on 2010 doubling (Table 1). One possibility for this might have been our relatively low volume control treatment, however soil moisture differences were evident between the control and 80 % of C treatments, and the lower deficit treatments (RDI and 60 % of C) (Fig 2).

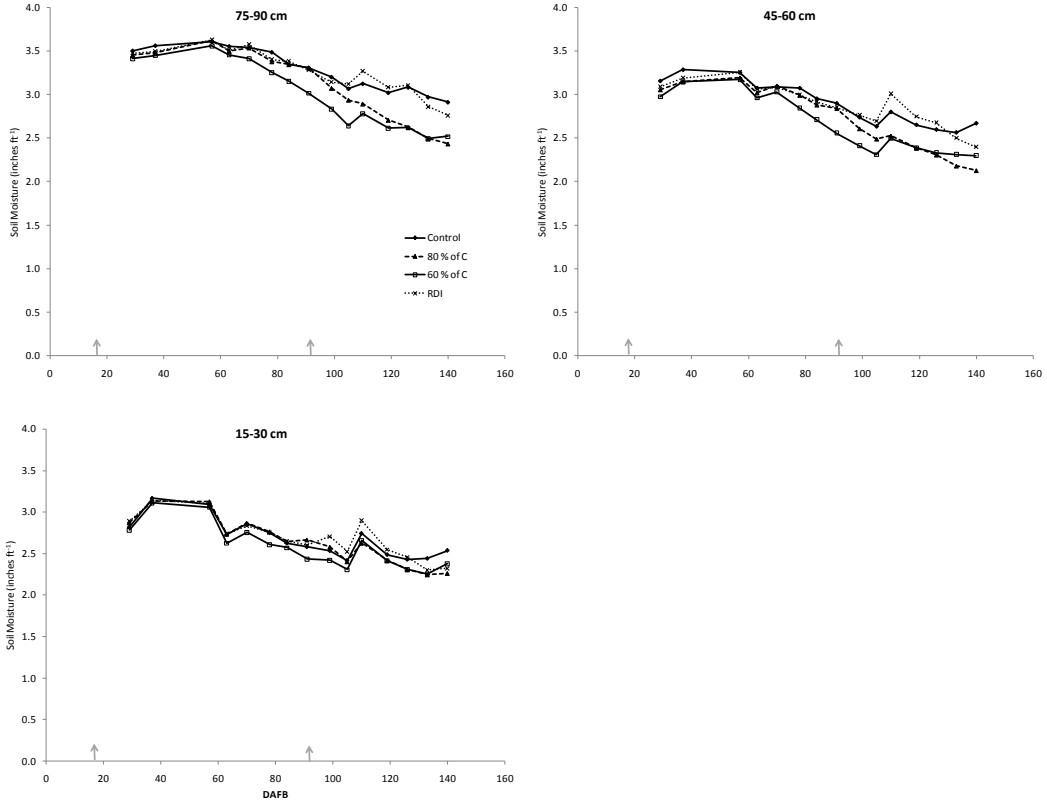

Fig 3. Effect of irrigation level (Control, 80 and 60 % of Control, and RDI) on volumetric soil moisture content (inches per foot) of the soil profile at (upper left) 3 ft, (upper right) 2 ft, and (lower) 1 ft depths for 'Lapins'/ 'Mazzard' trees. Arrow to right signifies the beginning of irrigation treatment. Arrow at left shows harvest date. Each data point is the mean of 5 replicates (n=3).

Soil water depletion in 'Lapins' was not nearly as severe as in the 'Tieton' experiments. Carryover effects from 2009 deficit treatments on early-season soil moisture reserves are not apparent (Fig. 3). As we demonstrated in 2009, significant soil moisture was pulled for the 60 and 80 % of Control

treatments from lower depths in the soil profile (Fig 3). Soil moisture in the top foot of soil is roughly 0.2 inches/foot lower in the 80 and 60 % of C treatments as compared to control and RDI treatments, and equates to ~ 20 % moisture by volume (Fig 3). In the two and three foot deep soil profiles, soil moisture is markedly reduced for the deficit irrigated treatments (~0.3-0.6 inches per foot) (Fig 3), and extraction of water in the deeper soil profile increases with time (Fig 3). These data indicate that deeper roots are active in supplying the necessary water to meet an increasing evaporative demand.

'Tieton' yield was not affected by irrigation volume or frequency of application (Table 2), as observed in 2009. This is not surprising since treatments did not differ in terms of their stem water potential values. Further, no effects on fruit quality were observed (Table 2). Row size peaked on 9.5, irrespective of treatment.

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 'Tieton'/'Mazzard' trees receiving different levels of irrigation amount, and frequency.

Treatment	Yield (Lbs tree^{-1})	Fruit Dia. (mm)	FF (g/mm)	SS (%)	TA (%)
Irrigation Amount					
100%	65a	29.2a	255a	16.8a	0.71a
80%	75a	28.8a	249a	17.5a	0.69a
60%	68a	29.3a	251a	17.4a	0.72a
RDI	77a	28.9a	241a	17.7a	0.75a
Frequency					
High	69a	29.1a	244a	17.5a	0.72a
Low	74a	29a	253a	17.2a	0.72a

The inherent differences in productivity level between 'Lapins' and 'Tieton' were observed (Tables 2 and 3). Treatment yields of 'Lapins' varied significantly, though these data do not appear to be consistent with irrigation treatment (Table 3). Fruit size, firmness, sugars and acids were not significantly affected by irrigation treatment. We also did not detect any differences in fruit growth rate (monitored weekly throughout the season) among irrigation treatments.

Table 3. Yield and fruit quality attributes (Fruit dia.= average fruit size; FF= firmness; SS=soluble solids; TA= total acids) at harvest for 'Lapins'/'Mazzard' trees receiving different levels of irrigation, and nutrients.

Treatment	Yield (Lbs tree^{-1})	Fruit Dia. (mm)	FF (g/mm)	SS (%)	TA (%)
Irrigation Amount					
100%	141a	27.6a	301a	18.3a	0.74a
80%	115b	28.1a	305a	18.7a	0.75a
60%	123ab	28.2a	304a	18.6a	0.75a
RDI	117b	27.9a	307a	18.7a	0.76a
Fertilization					
Fertigation-High	132a	28.2a	308a	18.8a	0.76a
Fertigation-Mod.	117b	27.6a	301a	18.5a	0.74a
Broadcast-High	123ab	28a	304a	18.4a	0.75a

Post-harvest fruit quality was not affected by irrigation treatment (data not shown), though this would not be expected given that harvest fruit quality remained unaffected. There was a weak relationship between nitrogen level and application method, and yield (Table 3).

In summary: 1) Significant water savings were achieved in both orchards, without negative impacts on fruit growth, yield or trunk growth, 2) Drip irrigation practices resulted in greater water savings than micro-sprinkler irrigation on a per acre basis (water applied directly to trees as opposed to dispersed across tree rows and alleyways), though this was enabled by the very short fruit development characteristics of 'Tieton', 4) deep soil moisture reserves served to limit the development of tree water stress in deficit treatments, indicating that significant water savings can be achieved in early spring.

Modifications in 2011: Midway into 2010 we installed atmometers (ET gages) at each site, to provide a measure of real-time ET. We will relate water potential, soil moisture, and ET data generated by these instruments, to gain an improved understanding of cherry tree water use. Additionally, we plan to host a visiting professor of agricultural engineering, presently studying tree fruit irrigation in Spain. He has submitted documentation to arrive in April, 2011 until October, 2011.

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

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

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

Other funding sources: None

WTFRC Collaborative expenses: None

Budget 1 Todd Einhorn
Organization Name: OSU-MCAREC **Contract Administrator:** Dorothy Beaton
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Item	2010	2011	2012
Salaries[1]	37,350	38,844	40,397
Benefits	21,758	22,628	23,533
Wages[2]	1,500	1,560	1,622
Benefits	150	156	162
Equipment			
Supplies[3]	6,500	6,500	6,500
Travel[4]	2,000	2,000	2,000
Miscellaneous			
Total	69,258	**71,688**	74,214

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

Objectives

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

Significant Findings

1. Ontogeny

- We have collected cherry fruit from three different cultivars, each at two cropload levels, and from two separate sites (WA/OR) throughout ontogeny. These samples are preserved in fixative for imaging on an environmental scanning electron microscope (OSU-EM facility). We tested the instrument in spring 2010 and demonstrated its ability to clearly image cells in cherry fruit tissue without requiring extensive tissue preparation.
- We were successful in filling our postdoctoral research associate position with an experienced, highly-qualified candidate; hired October 1, 2010.
- We have developed a protocol using flow cytometry to identify the ploidy level of cherry fruit. The technology will provide direct information pertaining to cellular division periods of cherry fruit during its development throughout the season (ontogeny).
- Significant polyploidy was observed in cherry fruit throughout the 2010 season, irrespective of cultivar or cropload treatments.

2. GA Experiments

- Low-moderate rates (0, 20, 30, 40, 60 ppm) of GA, in combination with two cropload levels (unthinned and heavily thinned) were tested on limbs of 'Skeena' (2 sites) and 'Sweetheart' trees. GA concentrations between 30-40 ppm consistently provided the best results for fruit quality (increased firmness and, for 'Sweetheart', slight size improvements). No significant gains in fruit size or quality were observed at higher application rates.
- Unthinned cropload levels were not extremely high. GA rate and cropload level did not significantly interact, indicating that increasing the application rate of GA as cropload increases from light to moderate does not translate to improvements in fruit quality.
- No advantages were observed for multiple applications of GA (straw color + mid Stage III) vs. a single application at straw color.
- Whole canopies of a pedestrian 'Staccato'/'Mazzard' orchard were treated with moderate rates of GA (0, 20, 30, 40, 60 ppm). GA applied at 30 ppm was the most effective.

3. Fruit Removal Experiments

- Pre-defined percentages of fruit were removed weekly from 'Sweetheart' and 'Skeena' limbs, beginning with spur bud removal ~30 days prior to full bloom through ~80 days after full bloom (DAFB). For 'Sweetheart', pre-bloom thinning resulted in the largest fruit at harvest, compared to an unthinned control.
- Fruit size of 'Skeena' was improved by thinning, relative to the unthinned control. This size advantage was maintained as late as 79 DAFB.

Method Development

1. Ontogeny: Experiments were conducted on 'Chelan', 'Bing', and 'Sweetheart' trees, each occurring at two separate sites (OR/WA). Five replicate trees were selected for each cultivar. Two cropload levels were applied on independent, primary scaffold limbs: Un-thinned, and bud thinned to one reproductive and one vegetative bud per spur, ~ 30 days prior to bloom. At approximately 40 % full bloom, recently opened flowers [< 24 hours old (identified by color intensity of anthers)] were hand pollinated using 'Sam' pollen, prepared by M. Whiting. 'Sam' was chosen for its compatibility with all three cultivars. Hand pollination was performed to minimize differences in the age/maturity that naturally occurs for a given population of fruit, based on the date of fertilization. A minimum of 60 flowers on 60 separate spurs were pollinated per cropload treatment, per replicate, and the pedicel

was tagged to allow for later identification of pollinated fruit. Sampling of fruit occurred every four days throughout the first 30 DAFB. One fruit per replicate limb (5 per treatment) was picked and immediately immersed in fixative for later imaging on a scanning electron microscope (SEM). Sampling intervals were increased to seven days between 30 DAFB and harvest (the more intensive early sampling was designed to thoroughly capture the anticipated cell division period). An additional five fruit per treatment were collected and sent overnight to OSU for analyses using flow cytometry.

A flow cytometer was identified in Dr. Ryan Contreras' lab at OSU. Briefly, fresh cherry fruit mesocarp tissue was finely chopped in buffers that separate and stain cell nuclei. A solution containing these nuclei was then injected into a cytometer and passed through a laser. The stained nuclei fluoresce in the light source, and the amount of fluorescence is proportional to the DNA content of the nuclei. The cytometer reports the number of individual nuclei at each density (equivalent to ploidy level). This information will provide direct insights into the timing and level of cell activity throughout fruit development. We will use this information to understand how cell number and activity change throughout ontogeny, and relate these processes to cherry fruit size. Dr. Contreras agreed to participate in the project, worked collaboratively to develop a cytometry protocol for cherry fruit tissue and ran samples several times weekly for the remainder of the season. He will continue his involvement with the project for the remaining two years.

The EM facility at OSU will be used for imaging cells of cherry fruit tissue using SEM. M.Whiting and T.Einhorn traveled to OSU to evaluate different instruments and techniques which could provide high-quality images of cells comprising cherry fruit. We were able to capture images from samples stored in fixative, without need for extensive sample preparation (embedding techniques) using an environmental SEM. The SEM has been reserved for use beginning in November, 2010.

***2. GA Experiments*:** GA (GA_3 - ProGibb 40 %WSG) treatments were applied at several low to moderate rates (20-60 ppm) to limbs using a compressed CO_2 hand wand applicator. Six replicate limbs (each on a separate tree) per treatment were selected in two 'Skeena'/'Gi6' orchards, and one 'Sweetheart'/'Mazzard' orchard based on uniformity of size (circumference at base of limb), orientation and fruit number. Two cropload levels were established as described above. Control (0 ppm GA) limbs were bagged to exclude grower GA applications from affecting treatments. GA was applied at straw color, and, in several treatments, again during mid Stage III of fruit development. A whole-tree study was performed on 'Staccato' (8 replicate trees) using GA rate treatments only. All harvests coincided with commercial harvest timing. Fruit were analyzed for fruit quality attributes immediately following harvest.

***3. Fruit Removal Experiments*:** In a separate study, fruit was removed from 'Sweetheart' [at two levels, 50 % and 66 %] and 'Skeena' [66 %] from single primary scaffold limbs (selection as described above; five replicates). Fruit adjustment began pre-bloom by removing the above percentages of reproductive buds from spurs, and then at ~weekly intervals up to ~ 80 DAFB. Fruit were harvested at commercial harvest timing and analyzed.

Results and Discussion

1. Ontogeny: We have just begun to analyze cytometry data collected during 2010. We were able to develop a protocol and begin sampling for flow cytometry at 16 DAFB for 'Chelan' and 21 DAFB for 'Bing' and 'Sweetheart'. Significant improvements in the assay were made as sampling intensified, and we are confident in our technique for 2011. Samples were analyzed for 7, 10 and 13 dates for 'Sweetheart', 'Bing', and 'Chelan', respectively. We observed a high degree of polyploidy (> 2n nuclei) for all cultivars tested [roughly 60-70 % of nuclei on most dates] (Fig 1). Data is shown for dates aligning with key phenological stages (Stage I [cell division], Stage II [pit hardening], and Stage

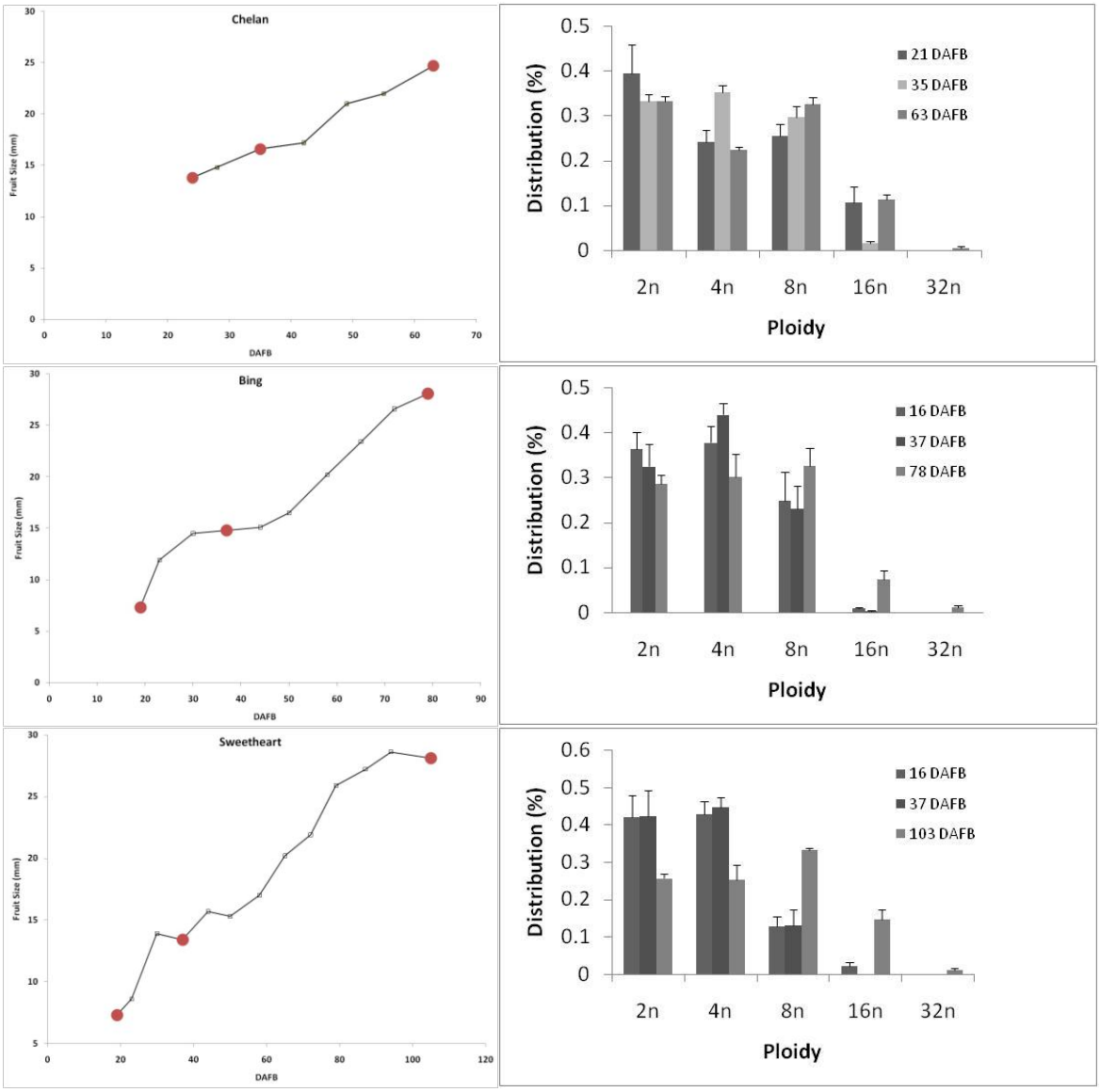

Figure 1. Fruit size (left) of samples collected for ploidy characterization (right) using flow cytometry for 'Chelan' (top), 'Bing' (mid) and 'Sweetheart' (lower), during 2010. Histograms on the right show proportion of nuclei with different ploidy levels at three different sample dates during distinct periods of fruit ontogeny (Stage I, II and III). Sample fruit used for cytometric analyses are represented by the enlarged symbols on fruit growth curves. Data are means of 5 reps.

III [cell expansion]), however, it is plausible that the bulk of the cell division stage is nearly complete by 16-21 DAFB. One process by which cells are borne is termed endoreduplication, and describes a doubling of the DNA in cells, without the accompanying mitotic division. This process may or may not affect cell number via the cell division process, but has been linked to cell size, and ultimately fruit size. In apple, polyploidy was recently associated with fruit size, and partially explained the differences between a large-fruited spontaneous mutant and the cultivar from which it derived. To examine the role that ploidy level plays on cherry fruit size, we will compare small ('NY54') and large fruited cultivars ('Selah' or 'Regina') throughout the 2011 season. Associations between cell number, cell size, ploidy level and fruit size throughout ontogenty will be made. We have not yet determined whether differences exist between cultivars, or relative to cropload level.

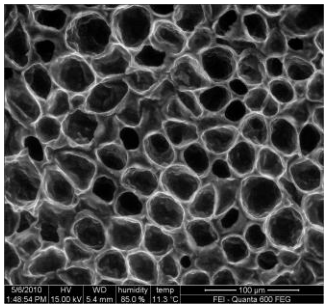

Figure 2. Environmental scanning electron micrograph of a section of 'Sweetheart' mesocarp fruit tissue. Image was captured at the EM facility at OSU. Cherry fruit was removed from a vial of fixative, sectioned freehand and imaged.

Fruit samples have been collected in both OR and WA, and are presently stored in fixative awaiting imaging on the SEM. We have identified an instrument and established a preliminary protocol to capture quality images with very little preparation of the sample (Fig 2). The SEM will provide the means to estimate average cell size and cell number throughout fruit development, since our samples were collected in fairly short intervals. Data generated from SEM will be compared to those from flow cytometry.

***2. GA Experiments*:** Both 'Skeena' and 'Sweetheart' trees responded similarly to GA rate and timing treatments. Application of 30 and 40 ppm GA at straw color resulted in the best overall quality for 'Skeena' (Table 1) and 'Sweetheart' (Table 2), respectively, though these improvements were only slightly greater than those observed at the lower rates. No additional gains in fruit quality were observed when rates were increased to 60 ppm, or when applied in multiple applications. Firmness was the most responsive attribute to increasing GA rate for 'Skeena'.

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

Treatment	Avg. Fruit Sz. (g)	SS (%)	FF (g/mm)	CTIFL	Cropload (fruit no./cm^2)
GA					
0	11.8 B	18.9	371 C	5.3 A	7.2 ABC
20	12.7 A	19.3	405 B	4.8 B	6.0 ABC
30	12.5 AB	19.7	414 AB	4.7 B	7.3 ABC
40	12.1 AB	19.6	443 A	4.3 C	5.8 BC
60	12.5 AB	19.3	447 A	4.2 C	6.3 ABC
20/10	12.2 AB	19.1	440 A	4.3 C	5.4 C
20/20	12.4 AB	19.6	441 A	4.2 C	9.4 A
20/40	12.5 AB	19.3	427 AB	4.3 C	9.3 AB
30/10	12.5 AB	19.2	435 AB	4.3 C	6.2 ABC
Cropload					
Heavy	12.3	18.6 B	408 B	4.4 B	11.2 A
Light	12.4	20.0 A	442 A	4.6 A	2.8 B
GA x Cropload	ns	ns	ns	*	ns

Timings for GA rates 20/10, 20/20, 20/40, 30/10 were applied at straw/mid Stage III. Single rates were applied at straw.
Means within columns followed by different letters are significantly different by LSD at *P*=0.05 level.

Skin color of 'Skeena' (measured with CTIFL color chips [1= light pink and 7= dark mahogany/black]) decreased with increasing rate, up to 40 ppm. Because skin color at the higher GA rate was significantly lighter than lower rate treatments, an alternative approach would be to allow these fruit to remain on the tree until they attain desirable skin color. This could result in improvements in fruit size (based on relationships established in our harvest timing work) due to a longer growing period, and might improve returns by extending the harvest season with late varieties such as 'Skeena' and 'Sweetheart'. We plan to investigate this in 2011. 'Sweetheart' fruit size (when expressed as diameter) increased with GA rate, up to 40 ppm (Table 2). Skin color was less affected by GA rate.

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

Treatment	Avg. Fruit Wt. (g)	Avg. Fruit Diam. (mm)	SS %	FF (g/mm)	CTIFL	Cropload (fruit no./cm^2)
GA						
0	9.4 B	27.4 C	17.4 B	380 B	4.2 A	2.6 AB
20	10.9 A	29.3 B	18.5 A	417 A	3.8 AB	2.4 B
30	10.9 A	29.4 B	18.6 A	416 A	4.0 AB	3.8 A
40	11.2 A	29.7 AB	19.1 A	419 A	4.0 AB	1.9 B
60	11.4 A	29.8 A	18.4 A	417 A	3.8 B	2.0 B
20/10	10.9 A	29.4 B	18.9 A	418 A	3.7 B	2.4 B
20/20	10.9 A	29.4 B	18.8 A	414 A	3.7 B	2.3 B
20/40	11.2 A	29.6 AB	19.1 A	417 A	4.0 AB	3.1 AB
Cropload						
Heavy	11.0	29.4	18.2 B	399 B	4.0	3.5 A
Light	10.6	29.1	19 A	425 A	3.8	1.6 B
GA x Cropload	ns	ns	ns	ns	ns	ns

Timings for GA rates 20/10, 20/20, 20/40 were applied at straw/mid Stage III. Single rates were applied at straw.
Means within columns followed by different letters are significantly different by LSD at *P*=0.05 level.

GA treatments were applied to both unthinned and light croploaded limbs. Cropload reduction was implemented to address the uncertainty surrounding fruit response to a given rate of GA based on the cropload level of the tree (e.g., does increasing GA rate to heavy set trees offset retardation of fruit growth due to the increased competition for carbohydrates?). Although the 'Skeena' heavy cropload treatment had nearly fourfold the cropload as the light treatment (Table 1) these levels were still relatively low, and did not benefit from higher GA rates. This was entirely the case for the 'Sweetheart' study (Table 2). Aside from the obvious differences in cropload, the factors most consistently affected by cropload treatment were soluble solids concentration and firmness.

A GA rate, whole tree study was also carried out in a 'Staccato'/'Mazzard' pedestrian orchard. Improvements in fruit quality were detected up to 30 ppm GA (Table 3). GA trials were aimed at targeting low to moderate application rates (20-60 ppm) on commercially important cultivars other than 'Bing'. The rationale for theses rates was due to previous research showing adverse effects of higher rates (100 ppm) on return bloom characteristics. We will assess return bloom in 2011.

Table 3. Yield and fruit quality attributes (avg. fruit size, soluble solids, fruit firmness, CTIFL [skin color score]), for 7th leaf 'Staccato/'Mazzard' whole canopies receiving different rates of GA. Data are means of 8 replicate trees (n= 100 for fruit size, FF, CTIFL; n=2 for SS; n=1 for yield).

Treatment GA (ppm)	Avg. Fruit Wt. (g)	Avg. Fruit Dia. (mm)	SS (%)	FF (g/mm)	TA (%)	CTIFL	Yield (lbs)
0	8.6 B	26 C	24.1 A	320 B	0.77 B	4.8 A	26.3
20	9.6 A	27 B	23.6 AB	459 A	0.84 A	4.1 AB	22.1
30	9.8 A	27.8 A	24 A	448 A	0.83 A	3.9 B	30.6
40	9.4 A	27.3 AB	22.9 AB	474 A	0.79 AB	3.6 B	28.8
60	9.7 A	27.2 AB	22.1 B	440 A	0.81 AB	4 B	37.0

Means within columns followed by different letters are significantly different by LSD at *P*=0.05 level.

***3. Fruit Removal Experiments*:**

We have chosen to investigate effects of cropload on cell dynamics and their relationship to final fruit size (see ontogeny section) in order to gain insights into factors contributing to large fruit size. Once effective tools are developed for consistent control of cropload, these pieces of information will be

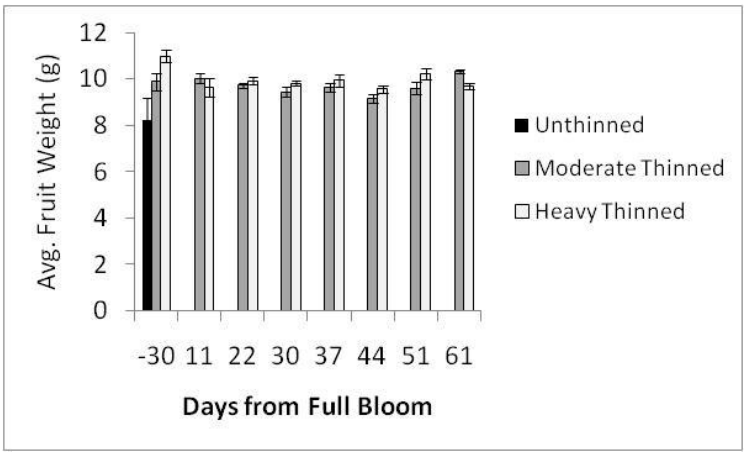

Figure 3. Effect of fruit removal at weekly intervals on final fruit size of 8th leaf 'Sweetheart'/'Mazzard' trees. Fruit was hand-thinned at ~weekly intervals to two levels: 1) Moderate thinned, ~50 % of fruit removed, and 2) Heavy thinned, ~75 % of fruit removed. Each week similar limbs (2° scaffolds) were chosen based on circumference (~10 inches at base), orientation and starting cropload. Each data point is the mean of 5 replicate limbs (n=100 for fruit weight).

assembled into practical strategies to improve fruit size and quality. The timing of fruit removal (thinning) is critical in order to optimize the positive effects of thinning on fruit size. We removed fruit at 7-10 day intervals from both 'Sweetheart' and 'Skeena' trees. Relative to unthinned limbs 'Sweetheart' fruit size was significantly improved by degree of thinning [~ 30 % increase for the aggressive treatment] (Fig 3). In addition to scaffold limbs, entire trees were bud thinned 30 days prior to full bloom, by reproductive bud removal. Tree yields for the unthinned, moderate and heavy thinned treatments were 135, 87 and 60 lbs, respectively. The moderate treatment resulted in best overall balance between fruit quality and yield, averaging 9.5 row, as opposed to 10.5 row for the unthinned treatment (data not shown). Thinning at 11 DAFB resulted in slightly reduced benefits in final fruit size for the heavily thinned treatment, but not for the moderate thinned trees. Surprisingly, the positive effects on fruit size were not compromised by our later thinning dates, and fruit size was significantly greater than unthinned treatments for all thin dates (Fig 3). 'Skeena' appeared to behave differently, showing greater improvements in fruit size over the first few thin dates (Fig 4), though this effect was slight.

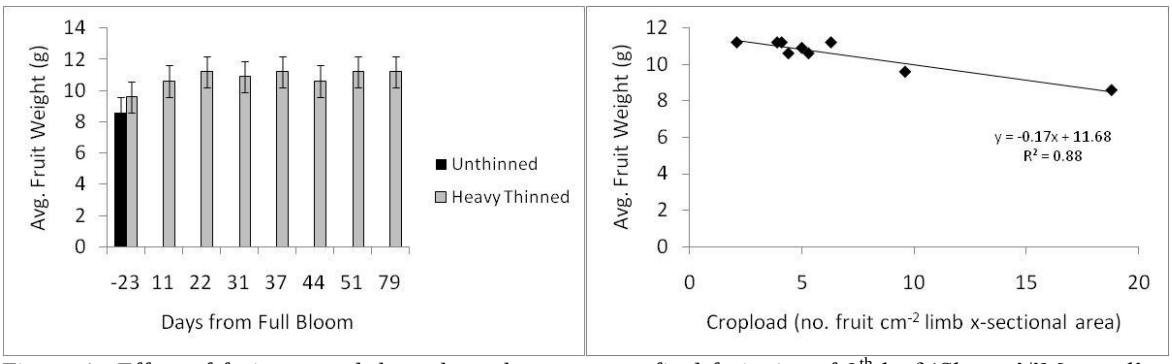

Figure 4. Effect of fruit removal throughout the season on final fruit size of 9th leaf 'Skeena'/'Mazzard' trees (left), and the relationship between final fruit size and cropload of limbs tested (right). Fruit was hand-thinned at ~weekly intervals (~66 % fruit removed). Limb selection was as described in Fig 1. Each data point is the mean of 5 replicate limbs (n=50-100 for fruit weight, depending on number of fruit at harvest).

Although similar sized limbs were selected weekly for thinning, we observed a decrease in yield with advancing thin date, and this relationship was largely responsible for the apparent increase in fruit size, as confirmed by regression analysis (Fig 4). Overall 'Skeena' yields were not high, and it appears that the combination of an aggressive thinning level, and generally low croploads contributed to our results. Results from these two contradict our previous data showing significant decrease in fruit size for heavily cropped 'Sweetheart' trees within the first 30-40 days from full bloom (Einhorn, unpublished). 'These studies will be carried out over the next two years to account for natural variation in cropload

CONTINUING PROJECT REPORT **YEAR**: 1of 3
WTFRC Project Number: CH-10-112

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

PI:	Qin Zhang	**Co-PI(2)**:	Karen Lewis
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City/State/Zip:	Prosser, WA 99350	**City**:	Ephrata, WA 98823

Cooperators: WA Producers

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

Other funding sources: None

WTFRC Collaborative expenses:

Item	2010	2011	2012
Crew labor	0	3,000	0
Shipping	0	0	0
Supplies	0	0	0
Travel	0	800	0
Miscellaneous	0		0
Salaries[1]		1,000	
Total	0	**4,800**	0

Footnotes:[1] **T. Schmidt**

Budget 1
Organization Name: WA State University **Contract Administrator:** M.L. Bricker
Telephone: 509.335.7667 **Email address:** mdesros@wsu.edu

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

Footnotes:[1] **Graduate Assistantship**

OBJECTIVES

To develop a hand-held tool for sweet cherry thinning. Tool would be a good fit in all current sweet cherry production systems, could be used by workers on the ground or on platforms and could be used both on mobile multi-worker platforms and singe worker bucket type platforms.

SIGNIFICANT FINDINGS

In 2010, several field tests were conducted at both Roza Research Orchard and commercial orchards. The tree structures being tested included UFO, Steep Leader and Spanish Bush systems at different blossom stages. Important findings include:

1. The results of pre- and post-thinning blossom counts indicated that the designed rotating thinning spindle could effectively remove cherry blossom, and that spindle rotating speed could noticeably impact the amount of blossom being removed. The users could control the percentage of blossom being removed within a cluster by controlling spindle speed and could control total bloom removed by the strategic placement of the device within a tree.
2. The configuration of thinning head could also affect the blossom thinning efficiency, but had less impact in comparing to spindle speed, especially when four lines of trimming strips were used. Therefore, only configurations of two and three trimmer lines with 6" to 7" strips on them will be further tested on prototype two. The strip materials will be limited using plastic or plastic covered wires for their widely availability.
3. There were some obvious drawbacks of using gasoline power for blossom thinner – the load-based speed control nature. The user uncontrollable feature of the gasoline powered thinning speed is unacceptable for the hand-held device.
4. The relatively heavy weight and the inevitable vibration prevented users from performing the thinning tasks for long periods of time.

METHODS

This project will be conducted in three phases: (1) prototype design; (2) field tests and prototype improvement; and (3) field demonstration & documentation.

To date, we have completed the first prototype design, built and field tested first prototype, completed modification design of second prototype, and sent second prototype to Chile for bloom thinning trials.

Prototype-one Development

Prototype-one development is the first step of this project. In this design, the prototype device was built based on a gasoline-powered hand-held grass trimmer, by replacing the trimming head with a specially designed blossom thinning head and adding a finger-tip speed controller, as shown in Figure 1. Other than the thinning head shown in Figure 1, ***eight*** heads of different configurations were also designed and manufactured.

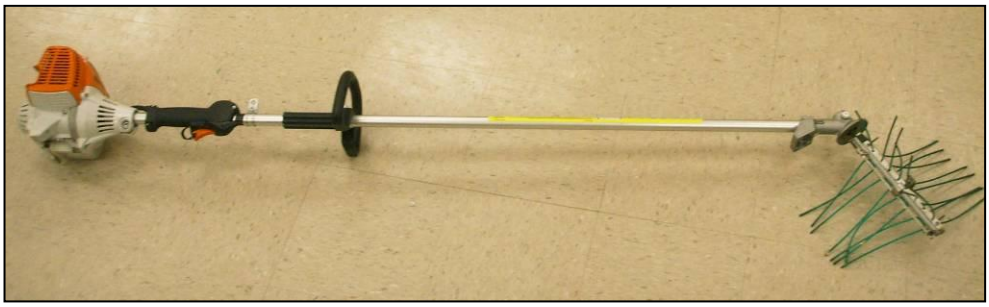

Figure 1. *The 1st prototype of mechanical hand-held device for cherry thinning*

The effectiveness of this hand-held device mainly depends on the functionality of the thinning head. In this prototype, all thinning heads were designed in a form of a rotary spindle with different arrangements and different materials of trimmer lines. Figure 2 shows one example of the thinning head design.

Figure 2. *An example of thinning head design: with 2 trimmer lines of plastic covered wire of 6 inches long, and 8 strips in each line.*

Figure 3 shows the general design of prototype thinning head spindle. In this prototype design, a narrow metal piece was used to fix trimming strips on the spindle to form one trimmer line. Three different trimmer line configurations were selected for prototype-one design: two lines of 180° installation, three lines of 120º installation, and four lines of 90º installation. Three types of strip materials, 0.1" diameter plastic strip, 0.14" diameter plastic covered wires and 0.15" diameter rubber, were selected to test their suitability for this particular application. Table 1 summarizes the configurations prototype-one design.

Figure 3. *General design of thinning head spindle*

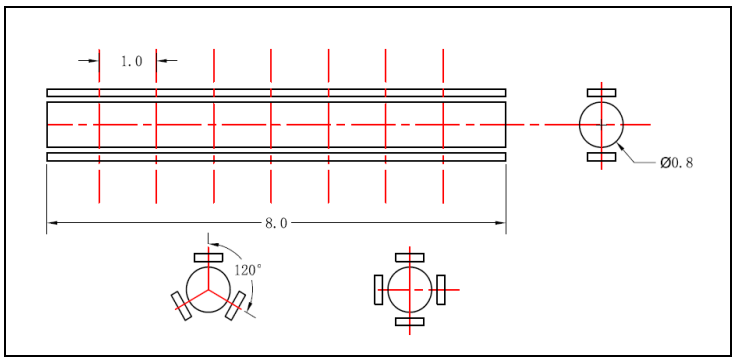

Table 1. *Thinning head spindle configurations*

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

The Improvement of the Prototype

To eliminate the drawback of gasoline power on prototype one, we decided to use a battery powered DC motor as the power source for prototype two. For the same reason as developing the first prototype, this new prototype was also developed based on a commercially available electrical powered grass trimmer as shown in Figure 4. An electronic speed controller was designed and integrated into the device for controlling the DC-motor speed. By supplying driving power from 0 to 24V, this prototype could control thinning spindle speed from zero to the highest speed limited by the DC motor. In addition, the weight and the vibration of the device are remarkably reduced for this prototype. Two different designs of prototype-two have been built, and both devices have shipped to Chile, and identified collaborators in Chile will conduct field test using prototype two. This collaboration will speed up our development process by gaining an additional bloom season.

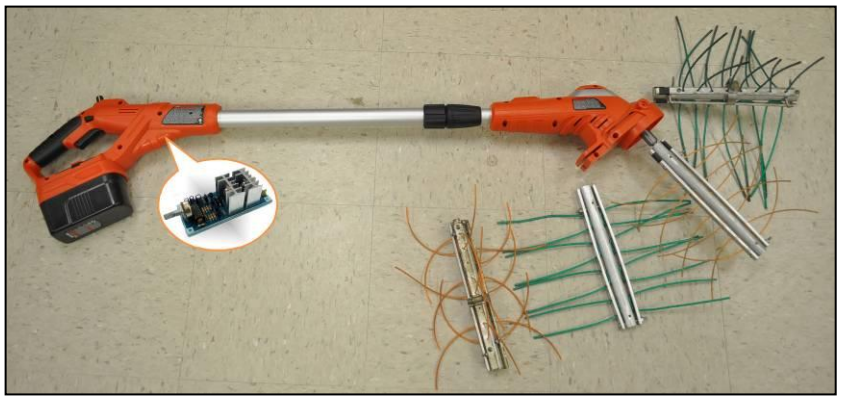

Figure 4. *The second prototype of electrical powered with four tinning spindles and an electronic spindle speed controller*

RESULTS AND DISCUSSION

The results of pre- and post-thinning blossom counts indicate that the current design of a rotating thinning spindle is effective at removing bloom that as suspected, the rotating speed of the spindle could noticeably impact the amount of blossom removed. The operators of the device could control the percentage of bloom removed within a cluster by controlling spindle speed and the percentage of total bloom removed by strategic placement of device within the tree.

The configuration of thinning heads also affected the amount of bloom removed, but had less impact in comparing it to spindle speed, especially when four lines of trimming strips were used. Therefore, only configurations of two and three trimmer lines with 6" to 7" strips on them will be further tested on prototype two. The strip materials will be limited using plastic or plastic covered wires for their widely availability.

The gasoline powered base was replaced with an electric powered base in prototype two. The electric base is lighter, quieter and has zero emissions and allows for greater speed control.

Year 1 of this project yielded many important findings that will allow us to stay on schedule for proposed milestones and planned deliverables. Prototype one was designed, built, demonstrated and tested with continuous input from horticulturists, growers, and orchard managers. Data and input from practitioners contributed to the design and development of prototype two.

Year One Plan and Up-to-Date Accomplishment

Table 2 summarizes year-one project management plan and up-to-date accomplishment.

Table 2. *Year One Plan, Expected Outcomes and Up-to-Date Accomplishments*

No.	Milestone	Time Period	Planned Deliverables	Accomplishments
1	Finish design 1st prototype	1Q, 2010	A prototype of hand-held with several thinning heads ready to be tested	Built a gasoline powered prototype, with 9 heads for 1st round field test
2	Round-one field validation tests	2Q-3Q, 2010	Test prototype one at field thinning at different blossom stages.	Conducted a series field tests at both research and commercial orchards
3	Improve prototype design	3Q, 2010	Design prototype two based on lessons learned from field tests	A battery powered and speed controllable prototype being built
4	Field test in Chile	3Q-4Q, 2010	Prototype two has shipped to Chile	To be done
5	Result analysis & year-two plan development	4Q, 2010	Analyze all data collected from field tests using prototype one & two, make an improvement as needed, develop plan for year-two	To be done

CONTINUING REPORT Year 1 of 3
WTFRC Project number: CH-10—110

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

PI: Nnadozie Oraguzie
Organization: Washington State University
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City/State/Zip: Prosser, WA 99350

Cooperators: Jim McFerson, Amy Iezzoni, Fred Bliss and John Fellman

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

Other funding sources

Agency Name: WTFRC/OSCC
Amt. awarded: $249K for 2009-2010
Notes: Sweet cherry Breeding and Genetics program with Oraguzie as PI

Agency Name: WTFRC/OSCC
Amt. awarded: $78K for 2009-2010
Notes: MAB for fruit size and self fertility in sweet cherry. PI: Peace with Iezzoni and Oraguzie as Co-PIs

Agency Name: USDA-ARS
Amt. awarded: $13.5K for 2010.
Notes: This is for evaluation of fruit from the National Prunus Germplasm Repository. PI: Peace with Iezzoni and Oraguzie as Co-PIs.

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

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

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

Agency Name: WTFRC/OSCC
Amt. awarded: $96, 279 (see WTFRC # CH-09-902)
Notes: This is for the sweet cherry breeding and genetics program with part of the funds used to support a part-time technician (Addie Dahl).

WTFRC Collaborative expenses: None

Budget
Organization Name: WSU-Prosser **Contract Administrator:** Mary Lou-Bricker
Telephone: 5093357667 **Email address:** mdeseros@wsu.edu

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

Footnotes:
Salary and benefits are for Addie Dahl**.**

JUSTIFICATION

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

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

OBJECTIVE:

The objective of this proposal is:

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

METHODS

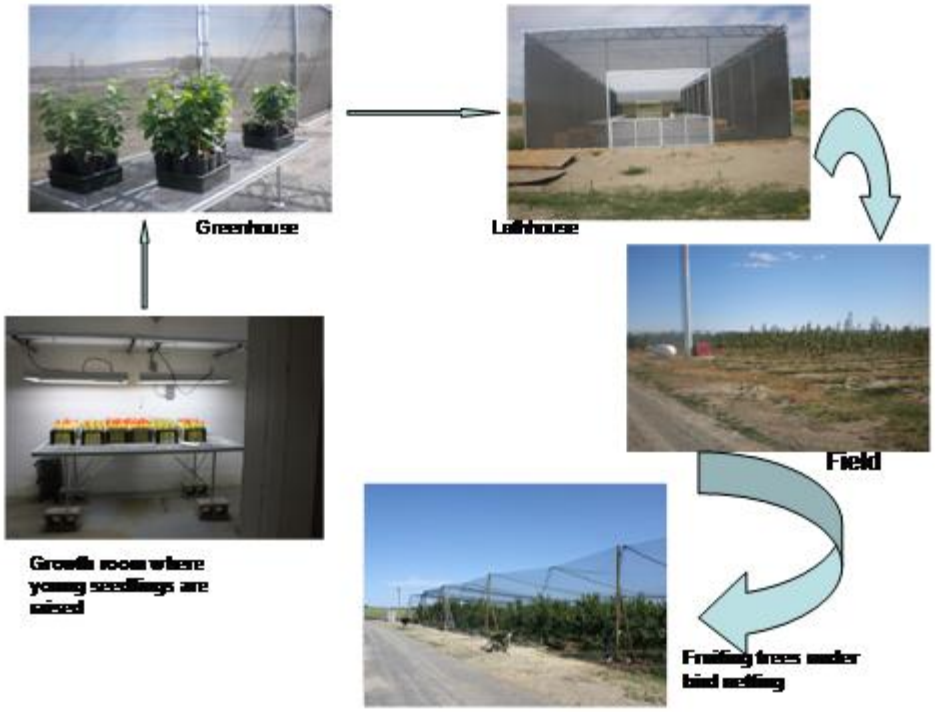
1. **Support for a full-time technician**

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

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

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

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



SIGNIFICANT FINDINGS AND ACCOMPLISHMENTS

- Jan Burgess (who works for the National Clean Plant Network program in WSU) who used to be hired for an hour/day by the breeding program to advise on seedling development in the greenhouse and lath house now works on the basis of 'as and when' needed.
- A total of 7000 seeds were collected from 2009 crosses of which 5000 were viable. Mean seed germination was slightly over 60% which is more than double the germination rate recorded in breeding programs in the past. Seedling survival rate at the baby stage was over 90%.
- We have developed protocols in the greenhouses for generating seedlings over 3 ft tall in less than a year for field planting. Due to the accelerated seedling growth we had 3 field plantings this year with the total number of trees planted slightly over 2500.
- Marker assisted seedling selection (MASS) was used for the first time in the breeding program to cull inferior seedlings so only the best genotypes with potential to yield desired traits could be planted. Of the 845 seedlings submitted for genetic tests before the October 2010 field planting, 341 had favorable genotypes for self fertility and fruit size. Care was also taken in selecting genotypes with a combination of favorable alleles for large fruit size that will also yield firm and sweet fruit. The more than 60% seedlings that were culled had mostly self incompatible genotypes and unfavorable alleles for large fruit size.
- A set of 12 seedlings identified in 2009 from 2004 crosses for propagation were validated using a combination of 2009 and 2010 fruit quality data and DNA marker information for self fertility and fruit size. Five individuals with small fruit and/or firmness below 250 g/mm were dropped while the 7 with fruit over 10 g combined with high values of other quality traits will be advanced to 2nd phase tests in grower trials and on research stations.
- Another set of 13 individuals were identified for propagation for more advanced tests following fruit evaluations this year. We will decide how many of these to move forward with after 2011 fruit evaluations.
- The powdery mildew resistant elite 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 analyzing the huge data set on yield and fruit quality collected by Ines Hanrahan (WTFRC) from Buenos, Naches and Pasco grower trials along with the data collected on these genotypes by the breeding program over the past 3 years to inform release decision.
- A total of 4500 trees from 2006 crosses that have never fruited were sprayed with etherol this summer to promote flowering and fruiting next year. They were also pruned while a majority of them had their limbs tied down to promote open canopy and blooming. Our assessments to date suggest that a large majority of these trees will be flowering next spring.
- We screened over 8000 trees for foliar powdery mildew infection in the greenhouses, lath house and the field this year. Our observations suggest that a rating scale that takes into account disease severity and varied disease symptoms would be more appropriate than visual scores based on presence or absence of infection to more effectively characterize the different sources of powdery mildew resistance being accumulated in the breeding program. Combining of different sources of resistance is necessary to attain durable resistance.

CONTINUING PROJECT REPORT **YEAR**: 2 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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City: Prosser
State/Zip: WA 99350

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

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

Other funding sources

Agency Name: WTFRC/OSCC
Amt. awarded: $249K for 2009-2010
Notes: Sweet cherry breeding and genetics program with Oraguzie as PI

Agency Name: WTFRC/OSCC
Amt. awarded: $78K for 2009-2010
Notes: MAB for fruit size and self fertility in sweet cherry. PI: Peace with Iezzoni and Oraguzie as Co-PIs

Agency Name: USDA-ARS
Amt. awarded: $13.5K for 2010.
Notes: This is for evaluation of fruit from the National Prunus Germplasm Repository. PI: Peace with Iezzoni and Oraguzie as Co-PIs.

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

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

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

WTFRC Collaborative expenses: None

Budget

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

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

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

Footnotes: Salaries include a 1/2 time associate-in-research (2010) responsible for seed collection, raising seedlings in the lathhouse and greenhouse, and tree maintenance in the orchards.

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

OBJECTIVES

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

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

PROPOSED SCHEDULE OF ACCOMPLISHMENTS

End of year 1 (2009)

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

End of year 2 (2010)

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

End of year 3 (2011)

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

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

SIGNIFICANT FINDINGS AND ACCOMPLISHMENTS

- A total of 7000 seeds were collected from 2009 crosses of which 5000 were viable. Mean seed germination was slightly over 60% which is more than double the germination rate recorded in breeding programs in the past. Seedling survival rate at the baby stage was over 90%.
- We have developed protocols in the greenhouses for generating seedlings over 3 ft tall in less than a year for field planting. Due to the accelerated seedling growth we had 3 field plantings this year with the total number of trees planted slightly over 2500.
- Marker assisted seedling selection (MASS) was used for the first time in the breeding program to cull inferior seedlings so only the best genotypes with potential to yield desired traits could be planted. Of the 845 seedlings submitted for genetic tests before the October 2010 field planting, 341 had favorable genotypes for self fertility and fruit size. Care was also taken in selecting genotypes with a combination of favorable alleles for large fruit size that will also yield firm and sweet fruit. The more than 60% seedlings that were culled had mostly self incompatible genotypes and unfavorable alleles for large fruit size.
- A set of 12 seedlings identified in 2009 from 2004 crosses for propagation were validated using a combination of 2009 and 2010 fruit quality data and DNA marker information for self fertility and fruit size. Five individuals with small fruit and/or firmness below 250 g/mm were dropped while the 7 with fruit over 10 g combined with high values of other quality traits will be advanced to 2nd phase tests in grower trials and on research stations.
- Another set of 13 individuals were identified for propagation for more advanced tests following fruit evaluations this year. We will decide how many of these to move to 2nd phase trials after 2011 fruit evaluations.
- The powdery mildew resistant elite 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 analyzing the huge data set on yield and fruit quality collected by Ines Hanrahan (WTFRC) from Buenos, Naches and Pasco grower trials along with the data collected on these genotypes by the breeding program over the past 3 years to inform release decision.

The section below on results and discussion will focus on the milestones for 2010.

1. Update best management protocol

A draft of the best management protocol for sweet cherry breeding was first developed in 2009. This work-in progress document provides practical guidelines on breeding program operations such as seed collection and handling, seedling maintenance in the greenhouse and the lath house, tree planting and maintenance in the field, horticultural manipulations to encourage bloom and fruiting, fruit sampling and evaluation to pollen collection and artificial pollination. Additions to this document this year include new protocols for seed planting and seedling maintenance that guarantee ~95% seedling survival at the baby stage under controlled conditions including a temperature of 76 F, RH of 40-45% and 24 hours lighting supplied by grow lights. In the greenhouses as well, we can now generate trees that are over 3 ft tall within 2 months. This accelerated seedling development necessitated field planting of less than a year old trees that are over 3 ft tall unlike in the past where shorter trees two years old and beyond were field planted. In collaboration with Drs Iezzoni and Peace, standardized phenotyping protocols were developed for both sweet and tart cherry breeding. These methods were used in the sweet cherry program this summer for fruit quality assessment.

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

Approximately 7000 seeds were generated from crosses made in 2009 of which 5000 were viable. Mean seed germination across crosses was over 60% which is more than double the seed germination rate recorded in sweet cherry breeding programs in the past. Over 3000 seedlings were raised in the greenhouses this year. The current main challenge for accelerated seedling development is prolonged seed germination. More than half of the seeds can germinate within six months following cold stratification while about 20-30% depending on cross may not germinate until a year or so later. Experiments are on-going in the breeding labs to achieve more uniform germination to facilitate more streamlined seedling establishment and field planting.

3. Plant ~1000 seedling trees generated from 2008 crosses in the field

Field planting was carried out three times this year in April, June and October with ~2500 trees from 2008 and 2009 crosses planted. 2008 crosses contributed just 250 trees. The staggered nature of the planting was due to a combination of prolonged germination period as mentioned above and accelerated seedling establishment whereby trees grow over 3 ft tall in the greenhouses within 2 months of transplanting. Marker-assisted seedling selection (MASS) was used for the first time in the breeding program in the October planting to cull inferior seedlings. Of 845 seedlings submitted for genetic tests in Dr Peace's lab based on DNA markers for self fertility and large fruit size, 341 trees that had favorable alleles for these traits were selected and planted in the field. In future field plantings MASS will be routinely used to cull inferior seedlings to ensure that only genotypes that have the potential to give the desired phenotypes are taken to the field. Apart from improving selection efficiency this approach will result in reduction of the cost of tree maintenance in the field.

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

Information on DNA markers linked to self fertility and large fruit size were used to select parents and F_1 hybrids to intercross. In particular, cultivars including 'Glacier', 'Tieton', 'Selah', 'Summit' and 'Krupnoplodnaya' were selected and intercrossed to increase the proportion of progeny with large fruit size in the breeding program. Crosses also emphasized early ripening and late ripening to increase the likelihood of obtaining good quality progeny later than 'Sweetheart' and earlier than 'Chelan' to extend the marketing window for PNW cherries. The crossing plan also emphasized novel flavors and skin colors.

Hand pollinations started at the end of March, ~10 days earlier than last year with approximately 50,000 flowers hand pollinated in Prosser and Pullman by a crew of 16 in the rainy and cold weather.

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

We harvested and evaluated fruit from 189 trees from 2004 crosses and 166 from 2005 crosses. There were no fruiting trees from 2006 crosses. Eighty cultivars and advanced selections were also evaluated as well as powdery mildew resistant elite selections including DD, GG and AA. Ines Hanrahan and Tom Auvil (WTFRC) were responsible for collecting yield and fruit quality data on the powdery mildew resistant selections from grower trials planted in Buenos, Naches and Pasco.

We re-evaluated the 12 selections identified last year from 2004 crosses for propagation at Willow Drive Nurseries. Based on combined phenotypic data for 2009 and 2010 and DNA marker information, we dropped off 5 individuals (firmness and fruit size highlighted in red in Table 1) that did not meet the threshold firmness of 250g/mm. In particular, '4.10.5-34', had fruit ~8 g with a firmness of 239 g/mm. This individual also lacks the 237 and 255 fragments from 'BPPTC034' favorable for large fruit size.

Another set of 13 individuals mainly from 2005 crosses were identified for advancement to the next phase following fruit evaluations. As you will notice, some parents and/or standard cultivars have larger fruit size than these selections. The selection criteria focused not only on large fruit size but also on threshold values of other desirable traits as well. Two of the individuals chosen for powdery mildew resistance (from 'PMR-1') have 'Sweetheart' as a common parent. Although these are not as late ripening as 'Sweetheart', they do constitute an important part of our breeding strategy to develop a suite of new mid- and late-season disease resistant 'spray-free' selections alongside 'DD' and 'GG' already in elite selection stage. The individual 'FR2T68', was selected for its low pedicel retention force (PRF) of 1.9N. This PRF is much lower than those of its two parents; 'Sweetheart' and 'Ambrunes', and standard cultivars such as 'Selah' and 'Skeena' known for low PRF. Low PRF will be an important attribute for mechanical harvesting. Finally, 'FR11T59', was selected for higher brix (28%) than those of the two parents including 'Rainier' and 'Regina'. Some fruit from this individual had brix up to 35%.

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

We are still working on our methodology for recording pitting and rain cracking. Rain cracking was a lot easier to record due to the rains during harvest. The highest incidence of pitting (0.4) was recorded in 'FR7T37 ('Sweetheart' x 'CC') while majority of the selections had zero or minimal pitting (Table 2). The incidence of cracking was high and varied with 'FR6T93' ('Sweetheart' x 'CC') showing maximum pitting followed closely by 'FR3T75' ('Selah' op) and 'FR7T37' ('Sweetheart' x 'CC'). 'FR11T59' ('Rainier' x 'Regina') showed no cracking. These results however, should be interpreted with caution since the sample size used for the estimation was small. For example, 'FR3T75' had only 7 fruit while sample size in general ranged from 7-25. More accurate estimation would require larger sample sizes using the same trees on a rootstock. However, progeny from 'Sweetheart' x 'CC' appear to have a high likelihood of showing these disorders and if the trend continues in 2011, we may not move forward with them. We will also endeavor to collect pitting and cracking data from 'CC' and 'Sweetheart' in 2011 to ascertain if these attributes are passed on from one or both parents.

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

Presently the 19 selections reported above have been propagated on Gisela 6 at Willow Drive Nurseries. The October freeze last year which wiped out a large number of trees budded at WDN warranted re-propagation of the 7 seedlings chosen from 2004 crosses.

Table 1. Fruit quality data collected in 2009 and 2010 and DNA marker genotypes for self compatibility/incompatibility and fruit size for F_1 seedlings first identified from 2004 crosses in 2009 for propagation and their parents/standard cultivars. Note that only F_1 hybrids in black print have been chosen for advancement to 2nd phase trials. Individuals that have the S4' allele are self fertile. Allele 255 from BPPTC034 is favorable for large fruit size.

Selection	Cross	S-genotype	Fruit size markers		Harv date		Mean frt wt (g)		Mean Firmness (g/mm)		Mean Brix (%)		Mean TA (%)	
			CPSCT038	BPPCT034	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
4.18.15-10	Swt x Regina	S1S3	190:204	235:255	6-Jul	8-Jul	10.15	11.41	328.7	284.7	20.6	18.8	0.52	0.64
4.3.1-2	Kordia op	S1S3	190	255	22-Jun		12.1		209.2		21		0.62	
4.18.15-47	Swt x Regina	S1S3	190:204	235:255	9-Jul	21-Jul	11.15	10.31	209.4	250.8	24.5	27.2	1.12	0.89
4.18.15-48	Swt x Unknown	S4'S9	190:204	255	2-Jul	21-Jul	13.13	11.57	249.9	293.7		23		0.78
4.18.15-42	Swt x Regina	S1S3	190:204	255	14-Jul	21-Jul	11.72	10.94	226.2	253.7	20.5	21.4	1.07	0.82
4.18.15-39	Swt x Regina	S1S3	190:204	255	1-Jul	8-Jul	10	12.l49	194	203.7	25.9	18.5	0.7	0.62
4.14.17-1	Rainier x Sunburst				6-Jul	15-Jul	10.01	9.16	233.3	328.5	19.6	20.7	0.85	0.63
4.18.12-5	Swt x Unknown	S1S3	190:204	223:255	1-Jul	8-Jul	11.88	12.59	176.5	200.7	21.4	18.6	-	0.71
4.10.15-1	Lapins x Regina	S3S4'	190:204	255	1-Jul	8-Jul	10.63	11.62	253.4	291.8	22.1	21.7	0.59	0.66
4.18.5-2	Swt x Chelan	S4'S9	190:204	225:255	12-Jun	2-Jul	11.65	10.54	314.3	316.5	16.9	20.6	0.93	
4.10.5-34	Lapins x Chelan	S3S4'	192:204	223:235	18-Jun		7.96		239.4		22.3		0.88	
4.3.1-5	Kordia op		190	255	23-Jun		12.03		195.7		15.7		0.59	
Block	**Cultivar**													
A36B	Chelan	S3S9	190:192	223		16-Jun		10		<200		16		0.71
C34	Chelan	S3S9	190:192	223:237	25-Jun	17-Jun	8.69	10	250.9	>250	17.9	18	0.98	0.73
C36	Tieton	S3S9	190		22-Jun	17-Jun	12.88	13	215.2	282	16.5	18.5	0.99	0.73
A36B	Tieton	S3S9	190			17-Jun		13		278		16.5		0.56
B53	Kiona	S4'S9	190			21-Jun		11.5		~200		18.5		0.6
B40	CC	S4S9	190:190	255:255		28-Jun		9.5		271		20		
B40	EE					28-Jun		10		>250		23		
B34	Van	S1S3	190:204	235:255	26-Jun	1-Jul	6.0	10.5	267.1	384	14.2	22.5	0.84	1
A36	Attika	S3S6				1-Jul		12		256		20		0.81
C36	Bing	S3S4	190:204	235:255	24-Jun	1-Jul	10.82	9	266.2	271	22.3	20	0.67	0.73
A37	Selah	S3S4'	190	223:255	7-Jul	8-Jul	11.68	13.5	184.3	219	18.8	20	0.75	
B40	BB	S4S9			25-Jun	12-Jul	7.27	9	183.7	~200	18.7	30		0.78
D38	Rainier	S1S4	190:204	235:255	20-Jul	12-Jul	6.75	10.6	295.2	~300	18.2	23	0.8	0.67
C36	Sweetheart	S3S4'		235:255	17-Jul	14-Jul	7.66	9.71	272.4	297	16.1	18	0.9	0.78
C46E	Regina	S1S3	190:204	223:255	29-Jun	14-Jul	12.42	13	224.9	>300	15.9	15	0.8	0.77
D36	Lapins	S1S4'	204:204	235:255	17-Jul	14-Jul	8.96	14	224.05	258	25.5	19	0.95	0.54
A37	Ambrunes	S3S6	190	223:255	21-Jul	23-Jul	5.62	7.9	356.1	~300	24.8	17	1.09	0.79

Table 2. Fruit quality and powdery mildew resistance/susceptibility data recorded in 2009 in the breeding program for F_1 seedlings from 2005 crosses and their parents/standard cultivars. The 13 F_1selections have been propagated at Willow Drive Nurseries and many of these will be planted into 2nd phase trials if their performance holds up in 2011.

Tree Id	Cross	Harv date	Eval date	Skin color	Mean Fruit wt(g)	%Brix	TA (%)	Firmness(g/mm)	PRF (N)	PM (09)	PM ('10)	Pitting incidence	Cracking incidence
FR3T41	Lapins x Chelan	14-Jun	15-Jun	Mahogany	10.5	20	0.56	~280	13.2	S-L	R	0	0.3
FR1T5	Sweetheart x Kiona	27-Jun	28-Jun	Mahogany	10.5	19	0.74	~280	11.73	S-L	S-M	0	0.2
FR2T72	Selah x Van	29-Jun	30-Jun	Blush	13	25	0.88	~270	9.94	S-L	S-M	0	0.3
FR3T75	Selah op	29-Jun	30-Jun	Mahogany	11	19	0.52	300	8.5	S-M	S-M	0.1	0.9
FR10T51	EE x Lapins	1-Jul	2-Jul	Mahogany	11	20	0.9	>300	11.5	R	S-M		
FR6T93	Sweetheart x CC	1-Jul	2-Jul	Mahogany	12	19	0.68	300	12.2	S-M	S-L	0	1
FR7T37	Sweetheart x CC	1-Jul	2-Jul	Mahogany	11.5	20	0.68	300	11.46	R	R	0.4	0.8
FR6T59	Sweetheart x EE	5-Jul	6-Jul	Mahogany	11.5	19	0.74	>300	13.75	SL	SL	0	0.5
FR6T63	Sweetheart x EE	5-Jul	6-Jul	Mahogany	11.5	22.5	0.85	>300	13.36	R	R	0	0.3
FR11T59	Rainier x Regina	8-Jul	9-Jul	Blush	11	28	0.83	>250	8.9	S-M	S-H	0	0
FR10T23	Lapins x BB	19-Jul	20-Jul	Mahogany	11	20		>270	8.62	S-M	S-H		
FR2T68	Selah x Van	21-Jul	22-Jul	Mahogany	12	19	0.73	>270	4.15	S-L	S-H	0.05	0.3
FR2T38	Sweetheart x Ambrunes	22-Jul	22-Jul	Mahogany	10.5	20	0.61	~220	1.9	S-L	S-L	0.1	0.3
Block	**Cultivar**												
A36B	Chelan	16-Jun	17-Jun	Mahogany	10	16	0.71	<200	10	R	R	0.08	0.08
C34	Chelan	17-Jun	18-Jun	Mahogany	10	18	0.73	>250	11.6	R	R	0.2	0.04
C36	Tieton	17-Jun	18-Jun	Mahogany	13	18.5	0.73	282	14.74	S	S	0.05	0.2
A36B	Tieton	17-Jun	18-Jun	Mahogany	13	16.5	0.56	278	15.8	S	S	0	0.4
B53	Kiona	21-Jun	22-Jun	Mahogany	11.5	18.5	0.6	~200	13.5	S	S	0	0.1
B40	CC	28-Jun	28-Jun	Mahogany	9.5	20		271	14.08	R	R		
B40	EE	28-Jun	15-Jul	Mahogany	10	23		>250	10	R	R	0.2	0.1
B34	Van	1-Jul	2-Jul	Mahogany	10.5	22.5	1	384	11.5	S	S	0	0.2
A36	Attika	1-Jul	2-Jul	Mahogany	12	20	0.81	256	13.6	S	S	0.1	0.3
C36	Bing	1-Jul	2-Jul	Mahogany	9	20	0.73	271	13.5	S	S	0.1	0
A37	Selah	8-Jul	9-Jul	Mahogany	13.5	20		219	8.65	S	S		
B40	BB	12-Jul	12-Jul	Mahogany	9	30	0.78	~200	12	R	R	0.2	0.3
D38	Rainier	12-Jul	12-Jul	Blush	10.6	23	0.67	~300	9.61	S	S	0	0.3
C36	Sweetheart	14-Jul	15-Jul	Mahogany	9.71	18	0.78	297	12.45	S	S		
C46E	Regina	14-Jul	15-Jul	Mahogany	13	15	0.77	>300	10.47	S	S		0.4
D36	Lapins	14-Jul	15-Jul	Mahogany	14	19	0.54	258	5.1	S	S		
A37	Ambrunes	23-Jul	23-Jul	Mahogany	7.9	17	0.79	~300	7.76	S	S	0	0.2

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

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

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

Total Project Request: **Year 1**: $44,000 **Year 2**: **$44,600**

Other funding sources

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

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

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

Agency Name: USDA-CSREES Specialty Crop Research Initiative
Amount awarded: $3.8 mil plus equal matching, Sep 2009 – Aug 2013
Notes: "A total systems approach to developing a sustainable, stem-free sweet cherry production, processing and marketing system". PI: Whiting. Co PI

include Oraguzie. Leveraged with WTFRC/OSCC funding. In addition to developing genomics knowledge on cherry abscission for amenability to mechanical harvesting, includes cell number and size measurements of local cultivars that integrates with this project.

Agency Name: USDA-CSREES Specialty Crop Research Initiative
Amount awarded: $7.2 mil plus equal matching, Sep 2009 – Aug 2013
Notes: "RosBREED: Enabling marker-assisted breeding in Rosaceae". PI: Iezzoni. Co-PIs include Peace and Oraguzie. Broad umbrella project on genetic marker development and application. Leveraged with WTFRC/OSCC funding. Our MAB here for fruit size in Dr. Oraguzie's breeding program is used in RosBREED as an example of the process and use, and is receiving much attention in that project in 2010-2011 particularly for earlier stages of the MAB Pipeline – providing increasingly detailed genetic information.

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

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

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

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

Footnotes:
[1] Technical assistance - molecular work (Pullman): $15,000 (wages and 9.5% benefits) in year 1 and 4% increase in year 2; Technical assistance - phenotyping work (Prosser): $14,000 (wages and 9.5% benefits) each in year 1 and year 2.
[2] Reagents and consumables - molecular work (Pullman): $1500 for DNA extraction of 3700 seedlings, $2000 for S-genotyping 2000 seedlings, $14,500 for fruit size marker genotyping of 1900 (4 markers) and 3700 (8 markers) seedlings, spread evenly over the two years; Consumables for phenotyping (Prosser): $2000 per year
[3] Within-state: Pullman-Prosser for coordination of experimental work: $2000 per year; Interstate: WSU-MSU for coordination among PIs: $2000 per year

OBJECTIVES

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

Specific objectives are to:

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

Year 2 will focus on:

- Continuing and completing genotypic data collection on'05 and '06 seedlings (Oct-Dec 2010).
- Calculating and displaying summary statistics of 2010 season phenotypic data on fruiting '04, '05, and '06 seedlings, and combine with 2009 season data (Oct-Dec 2010).
- Confirming utility of markers for the PNWSCBP using 2010 performance data on '05 and '06 seedlings. Integrate the information with RosBREED's "fast-tracked MAB Pipelining" on fruit size. Use results to inform and impact Dr. Oraguzie's crossing decisions in spring 2011 (Jan-Mar 2011).
- Collecting seedling performance data on '04, '05, and '06 in 2011 season (Jun-Aug 2011).
- Refining predictive power of fruit size markers using 2009-2011 performance data (Sep-Oct 2011).
- Determining the genetic basis for low occurrence of large-fruited seedlings in sweet cherry crosses (Feb-Mar 2011).
- Calculating effects of selfing and devise strategies to exploit selfing (Feb-Mar 2011).
- Developing and implementing markers for other high priority breeding targets (continual).
- Coordination with MSU's NRI project for fruit size marker knowledge, and with the RosBREED project (in both research and outreach) – including using fruit size in sweet cherry as an example of successful MAB (continual).
- Second year of fruit size evaluation in cultivars (May-Jul 2011), determination of genetic potential categories with physiological explanations (Aug-Sep 2011), dissemination of information to industry (Oct-Dec 2011).

Anticipated accomplishments by end of project (as described in original proposal):

- Implementation of MAB strategies for improving fruit size in the PNWSCBP.
- Implementation of MAB strategies that simultaneously improve fruit size and other target traits.
- Implementation of MAB strategies that increase the occurrence of large-fruited seedlings in the PNWSCBP.
- Implementation of MAB strategies that combine large fruit with self-fertility.
- Implementation of MAB strategies that exploit the benefits of selfing while avoiding the pitfalls.
- An active process of pipelining new genomics and genetics information into the PNWSCBP for improved breeding efficiency.
- Strategic positioning of the PNWSCBP to best utilize new investments in Rosaceae genomics, genetics, and breeding.
- Description of genetic categories that define fruit size genetic potential of cultivars of the PNW sweet cherry industry.

SIGNIFICANT FINDINGS

- DNA information impacted decisions in 2010 for parent crossing, seedling selection, and seedling planting. The PNWSCBP is now an official routine user of DNA information.
- ➔ This translates into enhanced efficiency of breeding operations, and is expected to lead to better new cultivars, with predicted genetic potential to inform grower adoption decisions.

- Performance evaluation in 2010, the second year of fruiting for the modern era of the PNWSCBP, confirmed the strong genetic control of fruit size and firmness.
- ➔ Breeding for these high priority traits will continue to be a worthwhile endeavour, and that a set of genetic markers may be developed that accurately predicts fruit size genetic potential of cultivars, selections, and seedlings.

- Coordination with federally funded projects in 2010 was strong and mutually beneficial.
- ➔ We remain strategically positioned to best utilize investments in Rosaceae genomics, genetics, and breeding on the international stage.

METHODS

1. Deliver MAB strategies for both large fruit and self-fertility to the PNWSCBP.
We are using the thousands of fruiting '04-'06 seedling populations of the PNWSCBP as the "training population" on which to calculate the explanatory power of fruit size DNA markers in cultivars of importance to the local industry and in breeding parents and seedling populations, and develop and implement efficient MAB strategies beginning in the 2010 spring crossing season. This involves evaluating seedling performance and obtaining DNA marker information on fruit size, comparing these two data types with Pedigree-Based Analysis to determine the utility of available fruit size DNA markers in Dr. Oraguzie's breeding program, understanding the effects of selfing in the breeding program, and implement efficient MAB strategies that improve fruit size with other target traits including self-fertility, firmness, and flavor.

2. Exploit additional opportunities for key trait improvement in the PNWSCBP using MAB.
Using the MAB Pipeline approach (established in previous projects), we are continuing to develop genetic markers for *Primary* traits such as firmness, *Secondary* traits such as sweetness and acidity, and *Market-defining* traits such as fruit color, and then channel this DNA information into the breeding program to enhance efficiency.

3. Coordinate MAB strategy development, implementation, and outreach in the PNWSCBP with Dr. Iezzoni's NRI project and RosBREED, and Dr. Main's Tree Fruit GDR project.
By remaining integrated with related federally funded projects led by Dr. Iezzoni and Dr. Main, we are ensuring that the PNW sweet cherry industry and the PNWSCBP are strategically positioned to best utilize new investments in Rosaceae genomics, genetics, and breeding.

4. Deliver knowledge on cultivar genetic potential for fruit size to PNW cherry growers.
We are using fruit size markers that have explanatory power in breeding germplasm to categorize cultivars, and then expand our understanding of the physiological basis of fruit size for each category of fruit size genetic potential by characterizing fruit size components. If the genetic markers for fruit size are indeed predictive of fruit size genetic potential, we will disseminate to the PNW sweet cherry industry the prediction of genetic potential of each cultivar (including new cultivars) for achieving large fruit. This outcome would mirror the way that genetic tests for *S*-alleles allow placement of cultivars into cross-compatible groups and identify self-fertility.

RESULTS & DISCUSSION

1. Deliver MAB strategies for both large fruit and self-fertility to the PNWSCBP.
Summary: Strategies were delivered, impacting the efficiency of breeding operations, although further refinement of the strategies is underway.

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

- Using *S*-genotype knowledge of parents to avoid incompatible crosses.
- Using *S*-genotype knowledge of parents to produce families with 25%, 50%, 75%, or 100% of seedlings predicted to be self-fertile (*S*-*S*- x S_4' *S*-, S_4' S_a x S_4' S_a, S_4' *S*- x S_4' *S*-, or S_4' *S*- selfed, respectively).
- Avoiding the use of self-fertile mothers in crosses because they tend to produce a large proportion of selfed seedlings – unless this is desired in some cases, because such seedlings are always self-fertile and other useful genetics from the mother can be concentrated.
- Using fruit size and firmness genotype knowledge of parents to produce families enriched with genetics for large and firm fruit.

Interpretation of seedling performance was supported by DNA information. Parentage of seedlings can now be verified by genetic markers, and was determined in 2009 for all ’04 seedlings. Furthermore, *S*-genotypes and fruit size genotypes of ’04 seedlings are also known. Examples of using this information include:

- Verifying an excellent germplasm source of very firm fruit.
- Deducing the germplasm source of a high degree of freestone.
- Understanding cases of seedlings that don’t perform as predicted from their supposed parents – because their true parentage is different.

Selection of the best-performing seedlings for the last two seasons was supported by DNA information. Examples include:

- Elevation to selection status of several seedlings with large and firm fruit for the last two seasons and with the genotypes predicting such genetic potential.
- When all else is equal, selecting seedlings that are self-fertile (carrying the S_4' allele) rather than self-incompatible.
- Excluding from further consideration an early-season selection that had predicted small fruit size genetics.

Planting decisions for hundreds of seedlings were supported by DNA information:

- 800 seedlings growing in the lath house and destined for fall planting were genotyped with two available predictive markers of genetic potential, and most of those with predicted small to medium fruit size and self-incompatibility were culled. Only 340 seedlings were subsequently planted. This effort is calculated to provide a net savings (in efficiency, via reallocation of resources) of around $25,000 by avoiding costs involved in planting and future maintenance and evaluation of inferior seedlings. The cost of DNA analysis of these seedlings was less than 10% of those savings!

Progress was made toward further refinement of our knowledge of genetic control of fruit size, the effects of selfing, and utility of this knowledge for the PNWSCBP.

- Fruit size performance data was obtained for 355 fruiting seedlings (189 of ’04 and 166 of ’05), using phenotyping protocols standardized with MSU. Traits evaluated were ripening date, fruit firmness, fruit weight, fruit length and width, pit weight, pit length and width, stem length, stem pull force, fruit shape, fruit skin color and fruit flesh color (based on both spectrophotometer reading and visual rating), freestone/clingstone, titratable acidity (TA), pH, and total soluble solids (TSS).

- For the ’04 seedlings which were also phenotyped in the 2009 season, fruit size was fairly consistent between the two seasons (R=0.64 based on each seedling’s maximum observed fruit weight).
- The categories of largest and smallest fruit described by the genotype at a single DNA marker from the 2009 season data were again predictive of fruit size in 2010 (Figure 1).
- Leaf tissue was obtained for several thousand ’05 and ’06 seedlings and DNA extraction is underway.
- Genetic tests of fruit size and self-fertility were prepared for use on our high-throughput genotyping platform.
- The existence of a third genomic region influencing fruit size, for which Regina is the only known source of large size, was verified via coordination with Dr. Iezzoni’s NRI project. The genetic marker for it was successfully tested on our medium-throughput platform.

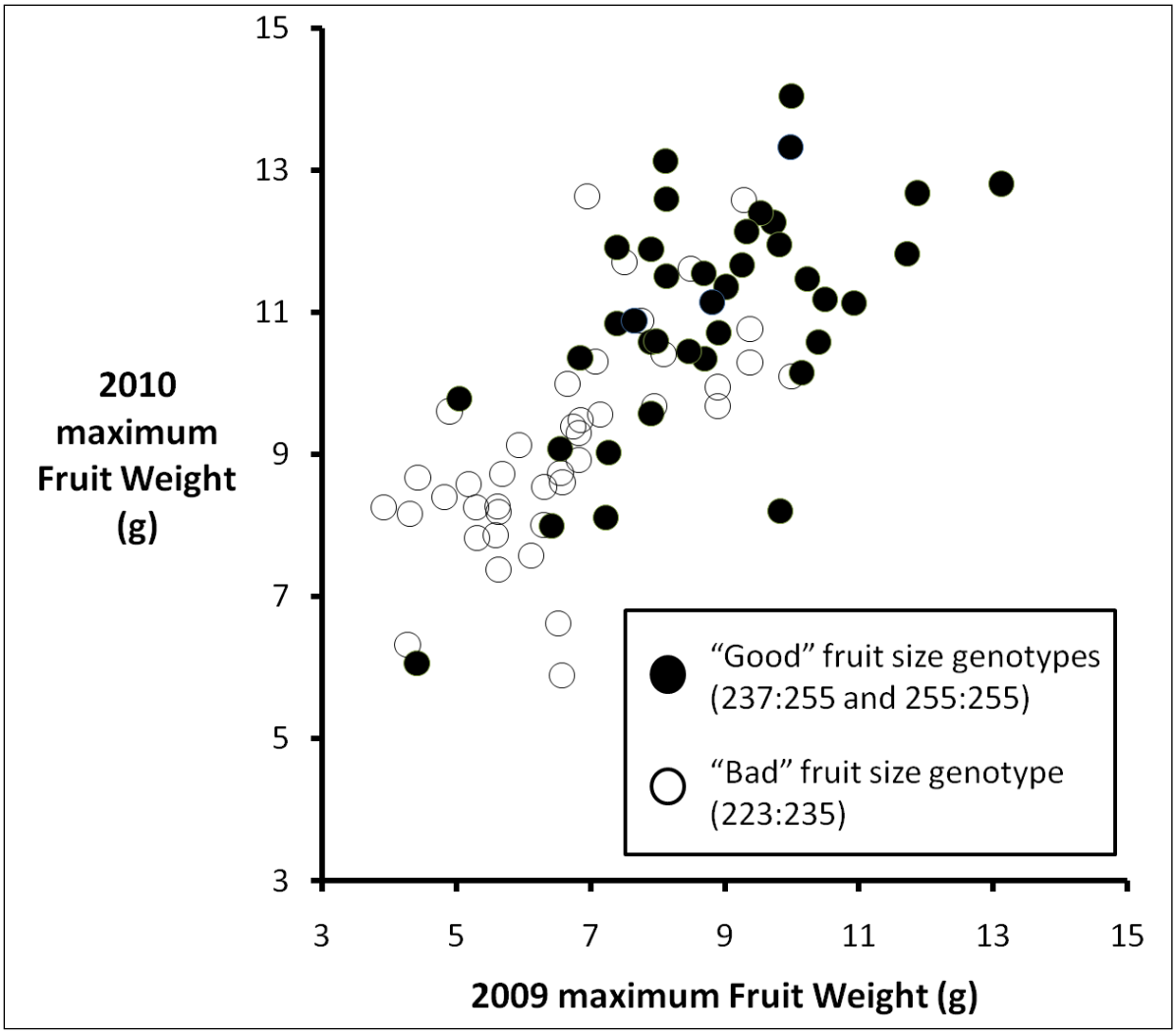

Figure 1: Fruit weight over two years of seedlings from crosses made in 2004, here displaying only the 82 seedlings with extreme fruit size genotypes (for the marker BPPCT034). Not shown here are a further 81 seedlings with intermediate fruit size genotypes and for which fruit data were obtained in both 2009 and 2010.

2. Exploit additional opportunities for key trait improvement in the PNWSCBP using MAB.
A genetic marker for acidity and fruit color was taken further though the MAB Pipeline toward routine use in the PNWSCBP. Firmness and sweetness are associated with the major marker for fruit size on G2, and so as long as those traits are evaluated on seedlings (as they were in 2010), genetic tools for their improvement are also proceeding. Efforts in the RosBREED project are furthering the development of markers for all of the PNWSCBP's Primary traits, Secondary traits, and Market-defining traits.

3. Coordinate MAB strategy development, implementation, and outreach in the PNWSCBP with Dr. Iezzoni's NRI project and RosBREED, and Dr. Main's Tree Fruit GDR project.
Coordination with other projects continued throughout 2010, with information on genetic markers shared among our projects. Our main fruit size genomic region is a target for detailed genomic dissection in both federally funded projects, to find the underlying genes as well as better understand its predictive power. This genetic test was chosen as the major example of impactful MAB in a RosBREED talk by Dr. Peace at the American Society for Horticultural Science annual conference in August (Palm Desert, CA) and similarly at the upcoming International Rosaceae Genomics Conference in November (Stellenbosch, South Africa). A genomic region influencing acidity, first identified in our previous WTFRC-funded cherry MAB projects, is also currently targeted in RosBREED.

4. Deliver knowledge on cultivar genetic potential for fruit size to PNW cherry growers.
The first season of a two-year experiment was conducted. To maximize expression of fruit size genetic potential, whole trees or two large branches were bloom-thinned to one bud per spur for each of the following cultivars and selections: Benton, Bing, Cashmere, Chelan, Cowiche, Rainier, Selah, Skeena, Summit, Tieton, Van, BB, CC, and DD. These cultivars and selections were chosen to cover the available range of our current preliminary idea of fruit size genotype groups, with 1-3 representatives per group. Fruit weight, dimensions, and other components of fruit size were measured for these individuals.

CONTINUING PROJECT REPORT 2010 **YEAR: 1 OF 2**
WTFRC Project Number: CH-10-111

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

PI:	Cameron Peace	**Co-PI (2)**:	Paul Wiersma
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Cooperators: Sanchita Haldar (WSU PhD student, Pullman), Scott Mattinson (WSU, Pullman), Amit Dhingra (WSU, Pullman), GNM Kumar (WSU, Pullman), John Fellman (WSU, Pullman)

Budget: **Year 1**: 28,000 **Year 2**: **28,000**

Other funding sources

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

WTFRC Collaborative expenses: None
Organization: WSU **Contract Administrator:** Mary Lou Bricker
Telephone: (509)335-7667 **Email address:** mdeseros@wsu.edu

Item	2010	2011	
Salary[a]	5,312	**5,524**	
Benefits	2,019	**2,099**	
Wages[b]	4,959	**5,157**	
Benefits	471	**490**	
Supplies[c]	10,739	**10,230**	
Travel[d]	4,500	**4,500**	
Total	28,000	**28,000**	

[a] Salary and benefits for Pullman are for 1.5 months of technical assistance for enzyme assays and GC analyses.
[b] Wages and benefits for Pullman are for summer support of PhD student Sanchita Haldar to conduct fruit quality evaluations. [c] Supplies include all materials (plastic, chemicals, DNA primers, gas cylinders etc.) necessary to conduct biochemical and genotyping studies. [d] Travel includes $2,000 budgeted for in-state travel and $2,500 for reciprocal 1-week visits between Pullman and Summerland for Wiersma and Haldar for coordinating experiments.

OBJECTIVES

Our overall goal is to improve the storage and shelf life of sweet cherry cultivars through an understanding of the ethylene genetic control in this apparently non-climacteric crop. This proposal addresses Fruit Quality Management, which is high on the list of Cherry Research Priorities for the 2010 season. Specific objectives are to:

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

Significant Findings

1. In contrast to climacteric fruits in general, immature (green) stages of cherries are associated with higher rates of respiration and low ethylene production. Respiration and ethylene production declines with fruit development. Ethylene levels were undetectable following 'color break' stage.
2. Cultivars do not differ in the magnitude of ethylene production during immature green stages. However, they differ with respect to the timing of peak ethylene production.
3. The activity of ACC Oxidase (involved in the catalysis of ACC to ethylene), could be detected at the immature green stage. ACC oxidase activity declined with advancing fruit development.
4. A growth stage model was developed for both cherry and peach to compare the equivalent stages for genetic and physiology stages.

Methods

Cherry fruit were collected from WSU, IAREC, Prosser twice per week begininng full bloom (April 2010) until the commercial harvest (July 2010). Three cultivars 'Bing', 'Chelan' and 'Skeena' with differential abscission characteristics, and abscission is a related to ethylene levels, were included in the present study. Respiration and ethylene evolution was monitored continuously for 120 h following harvest using a flow-through system on two replicates (500g fruit per replicate) as described by Patterson and Apel (1984). CO_2 and ethylene levels in the out-flow were measured by gas chromatography and detected via thermal conductivity (GC-TCD) and flame ionization (GC-FID) detectors, respectively. Peach fruit were collected from Wilson Banner Ranch, Clarkston. A late season cultivar, 'Golden Elberta' was used for the purpose. Respiration and external ethylene was measured in a similar fashion as cherry. ACO activity was detected by following ethylene produced by peach/cherry tissue in response to exogenous ACC in sealed vials. Fresh tissue collected in the summer of 2010 fruiting season, was frozen in liquid nitrogen and stored at -80C for subsequent analysis of transcripts of genes from the ethylene biosynthetic pathway. Expression of transcripts during the climacteric rise of peach will form the basis to examine the molecular aspects of ripening regulation in cherry with a hope to detect traits that determine postharvest life of cherries. Real time PCR will be employed to quantify relative expression of transcripts.

The result gathered from objective 1 has the potential benefit of developing assays to dissect physiological differences, if any, in the ethylene biosynthesis/response among cherry cultivars. The assays developed will then serve to categorize commercial cherry cultivars into three types namely 'Bing type', Chelan type' and 'Skeena type'. At least 20 major industry cherry cultivars will be screened and classified. We now know that cultivar differences in ethylene production exists (Fig. 5), hence strategies to improve fruit quality and extend market life will be devised from the information gained.

If objective 2 succeeds in identifying meaningful ethylene physiology categories, simpler phenotypic assays will be developed that can be used in breeding to help predict and describe fruit quality of seedlings, selections, and parents of the PNW sweet cherry breeding program (PNWSCBP). If possible, DNA-based tests to distinguish ethylene physiology categories will also be devised.

Results and discussion

The regulation of (1-Aminocyclopropane-1-Carboxylate Synthase) ACS and ACO during fruit growth and development of cherry was compared to that of peach. While both cherry and peach follow a typical *Prunus* growth curve (early cell division, mid lag in growth, and final rapid cell enlargement), the climacteric respiratory rise in peach occurs at the later stage of development. In view of the differences in respiratory and ethylene climacteric bursts between cherry and peach, gene expression studies will be carried out at the different developmental stages but center at the equivalent stages of cherry fruit. A comprehensive sampling of fruit across development was done in the summer 2010 fruiting season. Cherry fruit was collected on a bi-weekly basis from just after anthesis until fully ripe, with careful analysis of fruit mass, diameter, respiration, and ethylene production. Changes in color and timing of pit hardening were also recorded. Three cherry cultivars with known differences in ethylene response were used in this study ('Chelan', early maturing; 'Bing', mid-season and industry standard; and 'Skeena', late maturing). 500g fruit samples were analyzed in respiration chambers for 120 hours (5 days) post harvest for CO_2 evolution and ethylene levels. Peach was sampled in a similar fashion; a late-season cultivar 'Golden Elberta' was used for comparative purposes (Fig. 2). The amount of carbon dioxide produced by the fruit per hour is known as the rate of respiration, which was high in younger cherry fruit and as the fruit mature respiration rate decreased (Fig. 1A). The external ethylene produced was higher in the green fruit and decreased to zero as the fruit reached maturity and began ripening (Fig. 1B). Another interesting finding of our 2009 and 2010 season was the detectable amount of ethylene produced in the ripening fruit, which has been sporadically reported in the literature (data not included). The highest level of ethylene was detected in 'Bing', 9.5 µl/kg/hr harvested 10 days post full bloom, followed by 'Skeena' which produced 4.8 µl/kg/hr by samples harvested after 3 days post full bloom and Chelan produced 3 µl/kg/hr from the samples harvested 32 days post full bloom. Gene expression studies for the later stages of the fruit should reveal the genetic reason for this late ethylene burst. The ACO assay is also in accordance with the cherry ethylene emission levels, the enzyme is functional in the early green fruit and becomes non-functional as the fruit enters or exits the color break stage (Fig. 4). Upon comparisons among the three cultivars, which were different in their abscission pattern, it was apparent that there are three different categories of ACO activity between 40-60 days post full bloom. The second ethylene peak appeared at 35 days in 'Skeena', 40 days in 'Bing' and 45 days 'Chelan' (Fig. 5) which corresponds with the measured ACO activity thus allowing classification into ACO activity categories. These key differences will be used to categorize at least 20 more commercial cherry cultivars in the 2011 fruiting season.

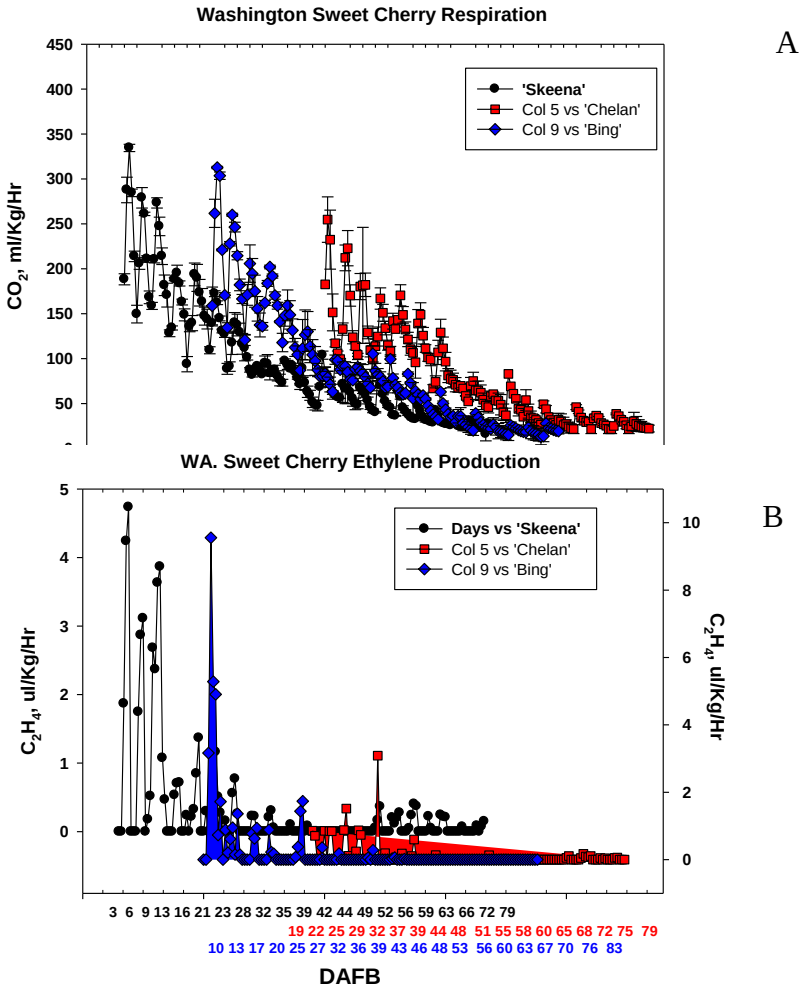


Figure 1. Washington sweet cherry respiration (A) and ethylene production (B) from three commercially grown cultivars.

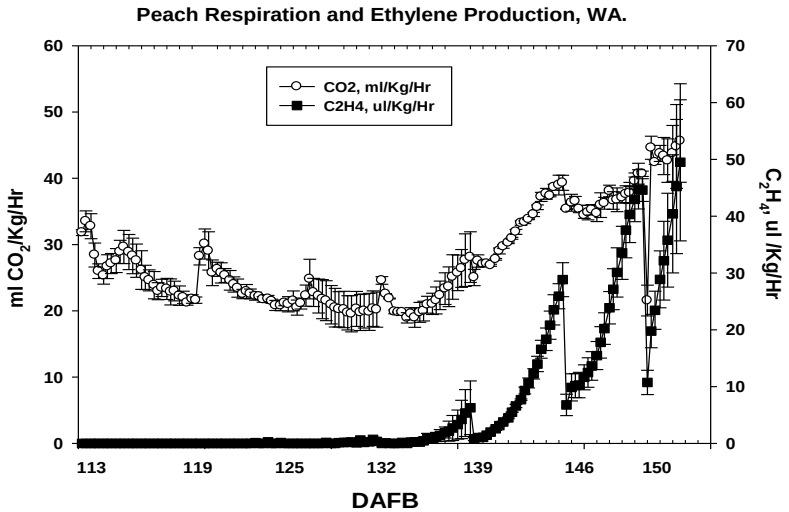


Figure 2. 'Golden Elberta' peach respiration (o) and ethylene production (■).

Ethylene enzymes and products:

ACO enzyme was measured by standard methods based on the sensitive detection of ethylene by gas chromatography. ACO activity was measured as the ethylene produced in a sealed vial when tissue (or a tissue extract) is incubated in the presence of the substrate ACC.

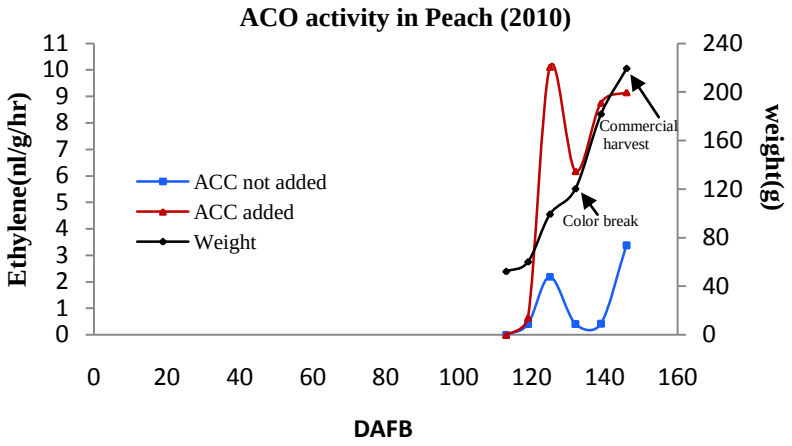
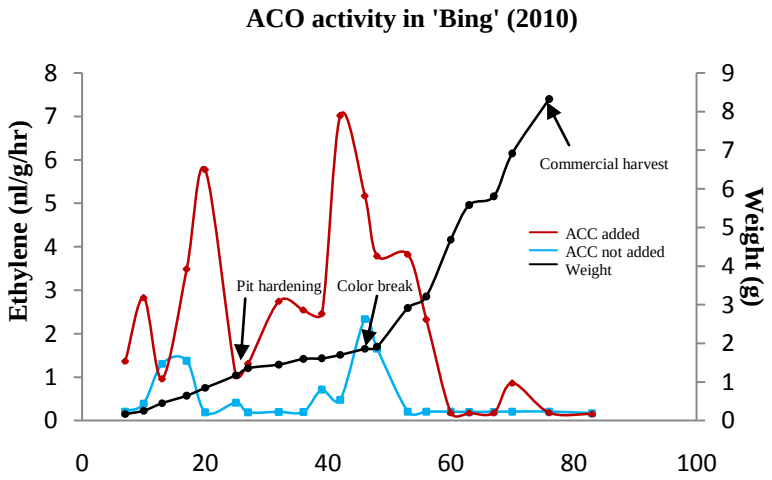


Figure 3. ACO enzyme assay on peach, with (red) and without (blue) addition of ACC and formation of ethylene was measured by Gas Chromatography.



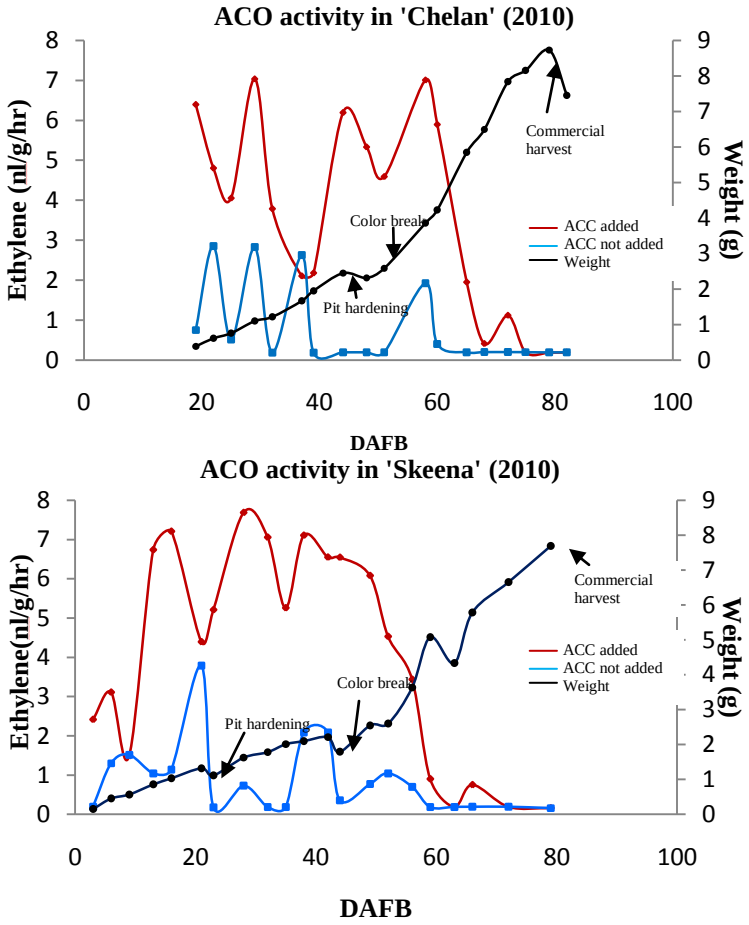


Figure 4. ACO enzyme assay done on 'Bing', 'Chelan' and 'Skeena', with (red) and without (blue) addition of ACC and formation of ethylene was measured by gas chromatography.

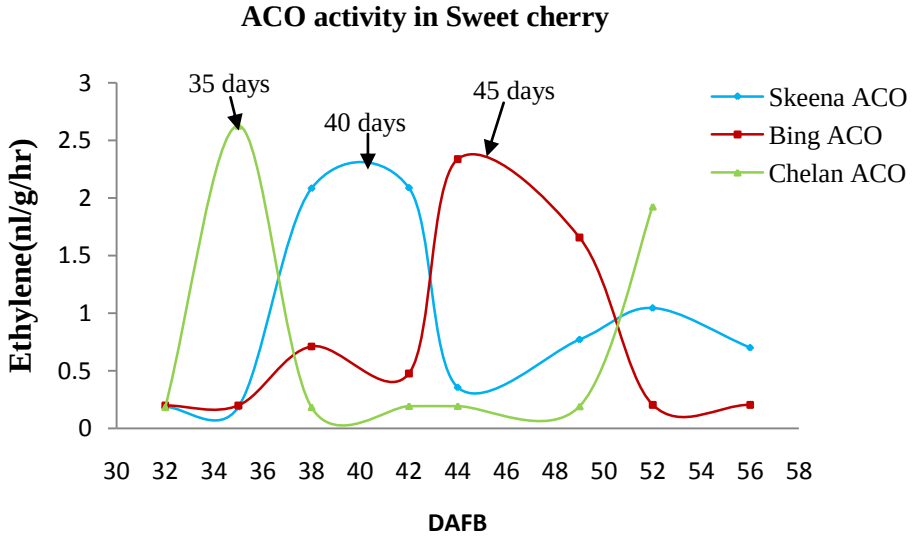


Figure 5. ACO activity peak in 'Bing', 'Chelan' and 'Skeena'. This figure is an inset of the ACO enzyme graphs.

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

Project Title: Improving sweet cherry yield security and fruit quality

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Cooperators:

Total Project Request: Year 1: 74,344 **Year 2: 77,778**

Other funding sources

Agency Name: Horticulture Australia Limited (HAL)
Amt. requested/awarded: $231,000
Notes: This funding will support counter-seasonal research with similar objectives in Australia

WTFRC Collaborative expenses: None

Budget 1
Organization Name: WSU **Contract Administrator:** Mary Lou Bricker/Lisa Bruce
Telephone: 509 335-7667 **Email address:** mdesros@wsu.edu/lisa-bruce@wsu.edu

Item	2010	**2011**	
Salaries	28,914	**35,071**	
Benefits	4,365	**4,539**	
Wages	24,264	**25,235**	
Benefits	3,301	**3,433**	
Equipment	6,500		
Supplies	2,000	**2,000**	
Travel	5,000	**7,500**	
Miscellaneous			
Total	74,344	**77,778**	

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

OBJECTIVES

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

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

SIGNIFICANT FINDINGS

- Daily, fruit set varies significantly
- Pollen germination rate and growth rate don't appear to limit fruit set in cultivars with low productivity
- Short period of ovule longevity appears to limit productivity (fruit set) in Regina, Benton
- Fruit set in Regina, Tieton, Benton can be improved with PGRs applied during bloom
- Fruit quality potential is related to timing of flowering at high crop load
- Fruit quality potential is unrelated to timing of flowering at low crop load

- The earlier the thinning, the better the fruit quality response
- Trials with BA, ethephon, and NAA showed no efficacy as post-bloom thinners
- PCa + ABA shows potential as a post-bloom thinner

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

METHODS

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

NATURAL VARIABILITY/BLOOM ONTOGENY (WA & TAS) Within marked sections of flowering 2 and 3 year old wood we will flag *individual* flowers (minimum 100 flowers per replicate limb) with numbered tape on the day that each opens (i.e., is accessible to a bee). The number of flowers that open each day will be recorded. There will be two "sets" of limbs in this experiment – one will be labeled and left to be pollinated naturally, the other will be labeled and manually pollinated. This will eliminate any differences in pollinator activity on fruit set. 'Lapins' pollen will be used for this purpose. Environmental data will be collected from weather stations within 100 feet of the orchard. Natural fruit set will be assessed at harvest by counting fruit numbers for each flowering day. Fruit set data will then be compared with environmental conditions recorded during the flowering period. Over two years these experiments will be conducted on our four model cultivars, 'Bing', 'Regina', 'Benton', and 'Sweetheart' in Washington, and on 'Regina', 'Kordia', and 'Sweetheart in Tasmania. In each location: 4 cultivars x 4 replicate limbs x 100 flowers/limb = 1600 flowers tagged.
Outputs: documentation of variability in fruit set under natural field conditions; role of timing of flowering on fruit quality potential; bloom ontogeny models based on natural conditions and growth chambers.

GROWTH CHAMBER STUDIES (WA ONLY) We propose to study further the role of temperature on bloom ontogeny and components of fruit set using controlled environmental chambers and temperature manipulations in the field. Flowering limbs from our four model cultivars ('Bing', 'Regina', 'Benton', and 'Sweetheart') will be collected from the field and placed in buckets with water into one of three growth chambers. Limbs will be harvested at early stages of bloom. Temperature programs will be designed to mimic diurnal variation of 1) 20-year mean temperatures during bloom, 2) 10^{th} percentile of temperatures, and 3) 90^{th} percentile of temperatures. This ensures relevance to PNW growers. As flowers open, they will be manually pollinated and labeled. At regular timings post-pollination, populations of 10 flowers will be collected and stored in fixative immediately for subsequent assessments of pollen germination, pollen tube growth, and ovule viability. To assess stigma receptivity we will manually pollinate flowers on the day of anthesis and at 24 hr intervals post-anthesis, up to 5 days (self-sterile cultivar flowers will be unpollinated in growth chamber until we apply pollen; self-fertile cultivars will be emasculated to prevent self-pollination). Flowers will be collected 48 hr following manual pollination and stored in fixative before analyses for pollen germination and growth. We will also record the progression of flowering in the different environments by counting open flowers daily until full bloom (to complement field studies of bloom ontogeny).
Outputs: defining effective pollination period and the influence of temperature; bloom ontogeny models; understanding of factors limiting fruit set; identify flowers/buds that have the greatest quality potential.

2. Identify the best time to thin – To better understand the potential for post-bloom thinning, and the optimum timing for potential chemical bloom and post-bloom thinning programs, entire trees will be subjected to manual removal of either blossoms or developing fruit at approximately 2-week intervals from bloom until harvest. Thinning treatments will be imposed at phenologically distinct stages of flowering/fruiting (e.g., full bloom, shuck fall, early pit hardening, straw, etc.). The earliest thinning treatments will thin buds or flowers to 2/spur and 4/spur, respectively. These treatments will be subsequently thinned to 2 fruit per spur at early pit hardening. Fruit yield and quality will be evaluated. This experiment will be conducted in year 1 in Washington on 'Bing' and 'Sweetheart' and in Tasmania on 'Van' and 'Sweetheart'.
Outputs: relationship between timing of thinning and yield/quality response

3. Investigate potential post-bloom thinners – a post-bloom thinning program would offer the obvious advantage of allowing an assessment of fruit set and the need for thinning. Bloom thinning needs to be more predictable and/or shown to be important for final fruit quality to be pursued further. If there is no advantage to thinning during bloom, post-bloom thinning programs must be developed. Again, we will build upon previous, preliminary investigations of potential post-bloom thinners, studying efficacy of various thinners and their effect on fruit yield and quality. Among others, potential chemical post-bloom thinning agents Ethephon, PCa + ABA, will be applied to entire trees at approximately 2 weeks after full bloom (generally considered the earliest opportunity to assess fruit set). Programs will be tested in Washington and Tasmania in cultivars prone to oversetting. Fruit set/drop will be assessed along with fruit yield and quality.

In addition, we will further develop a protocol for targeted application of hormones to pedicel-spur abscission zones. This technique uses thread drawn through the spur tissue to wick solutions of hormones directly through the vascular tissue. With this approach we will be able to screen multiple hormones and at many rates readily.
Outputs: identify optimum timing for thinning; screening potential post-bloom thinners

4. Understand timing of mesocarp cell division & expansion cycles and their relative role in fruit quality (WA/OR & TAS). – We will combine lab studies of fruit cell expansion and division with field studies of PGRs to influence both stages.

First, we will study cell size and cell division cycles seasonally, beginning in the fall, during flower bud differentiation, continuing until fruit harvest. Using a new technique that creates single cell suspensions from tissue we will assess fruit mesocarp cell size at approximately 14-day intervals post anthesis until harvest. Treatments will be applied to the same cultivars in both WA and OR, allowing comparisons to be made between sites, and offering protection from crop-destroying events at a single site. Two experiments will be established to compare genetic and environmental effects on cell division and expansion cycles:

A) Role of genetics - we will sample mesocarp tissue from both genetically large cultivars (e.g., 'Tieton', 'Regina') as well as genetically small cultivars (e.g., NY54 and 'Emperor Francis');

B) Role of environment - we will collect fruit samples from trees thinned during the dormant season to 1 fruit bud/spur during the dormant period and trees with heavily-cropped spurs to compare fruit development patterns when fruit are source limited and source unlimited (i.e., over- and under-cropped).

Briefly, mesocarp sections will be digested enzymatically and osmotically balanced to yield single cell suspension. Cell size is measured using image analysis software to assess images collected from light microscopy. PD Whiting will test this protocol in the current season in Tasmania. To determine cell division periods, we will collect samples at 3 to 4 day intervals post anthesis and store in fixative before embedding and sectioning for analysis by confocal microscopy at the Francheschi EM Center in Pullman. Further, we will investigate the possibility of assessing cell division cycles and cell size by flow cytometry, a technique that determines the percent of actively dividing cells within single cell suspensions. This resource is available in Pullman and University of Tasmania. This cell size/cell division work will be in collaboration with Dr. Einhorn at Oregon State University – we have a post-doctoral research associate budgeted for this research. The post-doc will be responsible for setting up thinning treatments in Oregon and Washington, collecting samples, analyzing samples, analyzing results, summarizing findings, and outreach and manuscript preparation. This individual will be based out of MCAREC.

5. Develop counter-seasonal collaboration with University of Tasmania and leverage WTFRC/OSCC funding via Horticulture Australia Limited – to hasten research discovery in areas of common concern to the cherry industries in the PNW and Australia (identified above), we propose to develop collaboration with the University of Tasmania using funding from Horticulture Australia Limited (HAL). Monies funded from WTFRC and OSCC can be leveraged through HAL to add capacity (i.e., personnel) and an additional growing season within a calendar year, the advantages of which are significant. We have submitted to HAL a proposal complementary and contingent upon/to the current proposal. With funding from HAL we will be in position to hire a partial post-doctoral associate, an additional Ph.D. student, and a technician at 50%. It is proposed that the post-doc and student spend significant time in the PNW conducting research, 8 months and 4 months/yr, respectively. Having personnel conducting the experiments in both locations will ensure effective collaboration and a consistent approach. Work will be conducted on genotypes that are relevant to both industries. We have met with industry representatives in Tasmania to outline the collaboration. This proposed collaboration was supported fully. Based upon the budget of the current proposal we expect to add another 41% from HAL funding, funding from Australian grower levies, and an assistantship from the University of Tasmania.

RESULTS & DISCUSSION

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

From tagging individual flowers on the day of opening we were able to study fruit set on a daily basis, throughout the flowering period. We documented tremendous variability in fruit set

under field conditions in Lapins, Kordia, and Sweetheart throughout the bloom period. For example, fruit set varied from a low of 10% to 100% in Lapins, across the 18-day bloom period (Fig. 1). At this stage we are analyzing variability in fruit set with daily weather conditions to identify patterns and key environmental factors. Interestingly, fruit set from hand pollinatinons was similar to that of open pollinated flowers on most days. This suggests there weren't many days when pollinator activity was limiting to fruit set.

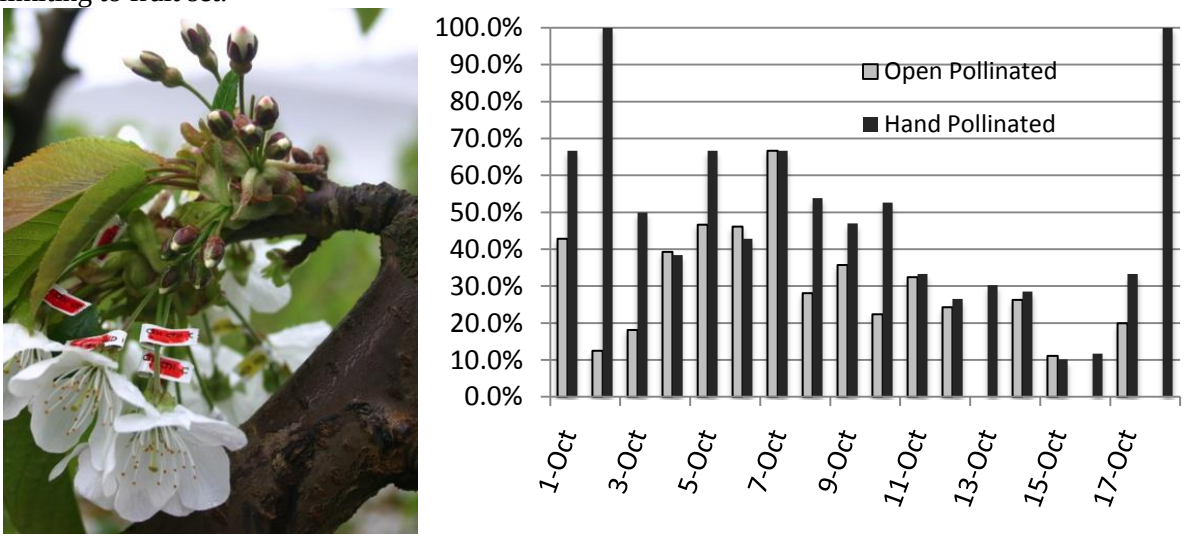


Figure 1. Variability in fruit set (% available flowers) throughout the bloom period in 'Sweetheart' sweet cherry in a commercial orchard in the Huon valley, Tasmania. Individual pedicels were labeled on the day of opening (photo).

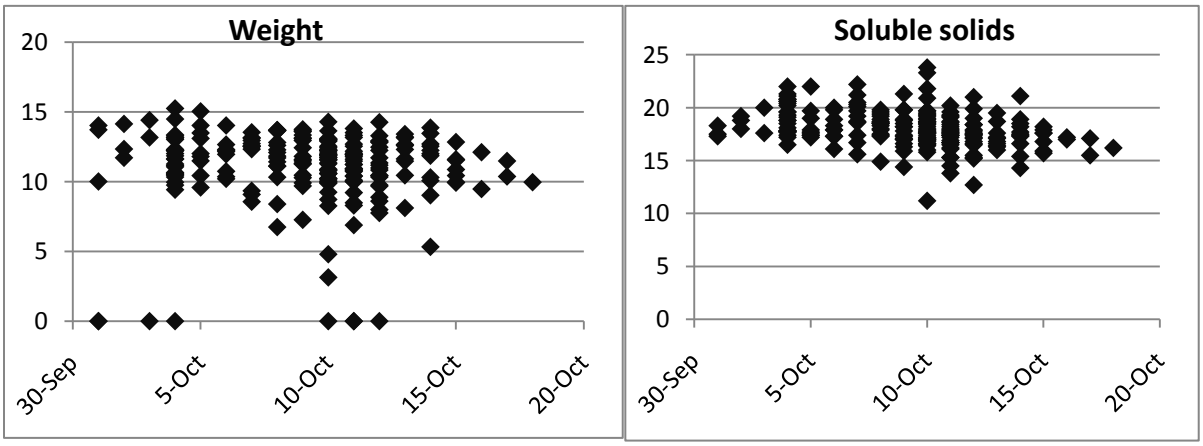


Figure 2. Relationship between fruit weight (g; left) and soluble solids (%; right) at commercial harvest and date of flowering in 'Sweetheart'.

At commercial harvest there was no apparent relationship between fruit quality (any attribute evaluated) and the date that the flower opened during bloom (Fig. 2). This was true for each cultivar evaluated and contradicts data collected in Prosser that showed that quality potential was highest in the earliest-opening flowers. We believe this is due to differences in crop load, which was heavy in Prosser and light in Tasmania. Trials are established in Tasmania now to evaluate the role of crop load on the effect of timing of flowering on fruit quality potential. However, these data suggest fruit quality

potential is determined, in part, at the time of flowering (a possibility we are investigating).

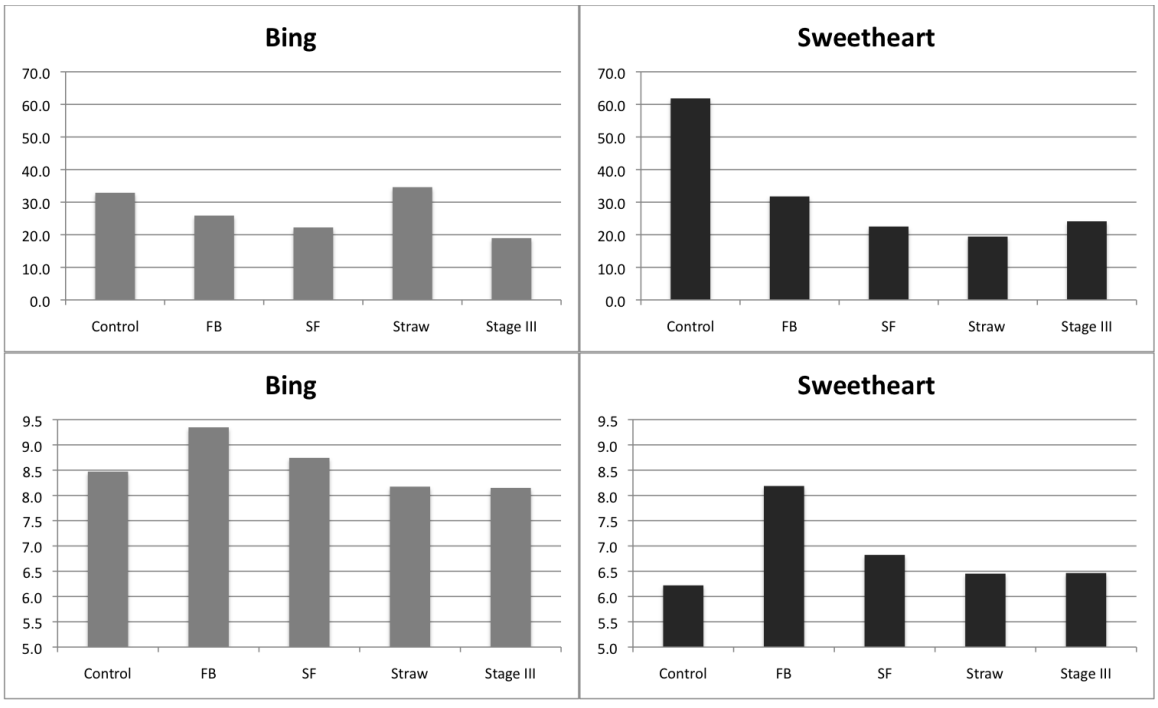
Our investigations into practical strategies for improving fruit set have been based upon our discovery that ovule longevity appears to limit fruit set. In 2010 we treated 'Tieton' at about 75% full bloom with 4-CPA, GA_3+GA_{4+7}, and AVG. Each treatment improved final fruit set significantly (Table 1). Field trials in Tasmania have also confirmed the efficacy of AVG for improving fruit set of 'Regina'. We have anecdotal evidence from two orchards that two applications of AVG, made at about 20% and 50% of full bloom are effective for improving fruit set.

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

Table 1. Effect of PGRs applied to whole trees at about 75% full bloom on fruit set of 'Tieton' sweet cherry. Data with different letters are significantly different at $P < 0.010$)

2. Timing of thinning - we investigated the effects of the timing of thinning at key phenological stages of fruit development on fruit yield and quality relationships for Bing and Sweetheart.

For both cultivars, earlier thinning was beneficial compared with thinning later in the season (Fig. 3). Thinning reduced crop yield in all cases except the thinning at straw in Bing. It isn't clear why this thinning timing did not reduce yield – it was not due to increases in fruit weight. Individual fruit weight was improved by thinning in Sweetheart and Bing. Again, earlier thinning was more effective than later thinning for both cultivars. Thinning at bloom increased fruit weight in Bing by ca. 12% whereas later thinning was. Thinning post straw was largely ineffective for improving fruit weight. This suggests that thinning programs should be imposed as early as possible in the fruiting timeline.



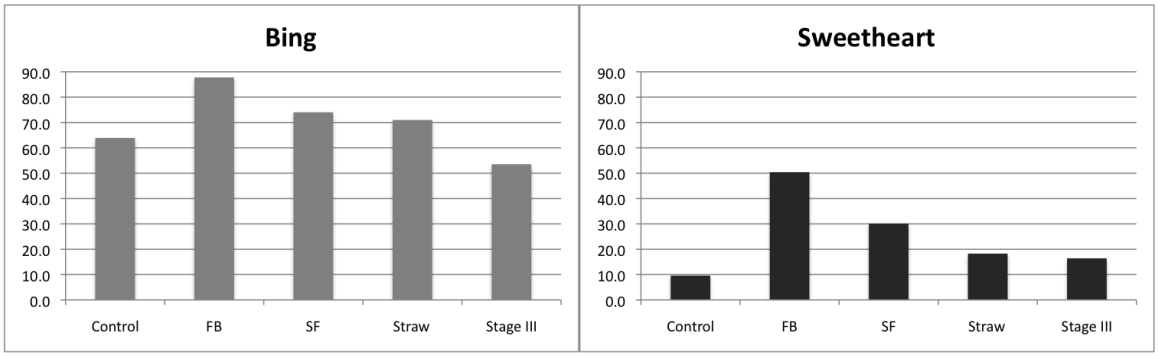


Figure 3. Effect of timing of thinning on yield (lb/tree; top), fruit weight (g; middle), and the percent of fruit 10-row and larger (bottom) of Bing and Sweetheart on 'Gisela®'5. FB = full bloom, SF = shuck fall.

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

5. Develop counter-seasonal collaboration with University of Tasmania and leverage WTFRC/OSCC funding via Horticulture Australia Limited – considerable effort was made this year to work through contract negotiations for the collaboration. The general process is outlined in Fig. 4. A project that complements the current project was submitted to HAL with PIs Whiting and Close. The full amount of funding from the WTFRC awarded to the current project was sent to HAL as a 'voluntary contribution' to the HAL project. These funds will be matched with HAL funds at about 41% (i.e., the $74,344 funded in year 1 will be leveraged to about $104,000). These matched funds will then be returned to the WTFRC to be issued to WSU. The HAL proposal has been funded fully for 2 years and puts in place technical support in Tasmania to work on issues of concern common to Tasmania, Washington, and Oregon. Currently the contract is being negotiated and it appears that this will be settled shortly. Currently, none of the $74,344 has been awarded to WSU.

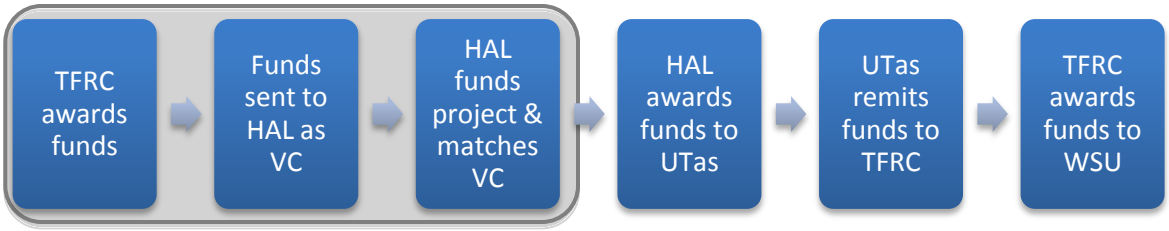


Figure 4. Process for leveraging WTFRC funds with HAL funding and establishing a counter-seasonal collaboration in Australia. Shaded components are completed.