FINAL PROJECT REPORT

Project Title: New genomic regions controlling production and fruit disorder traits

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Other funding sources

Agency Name: WTFRC/OSCC
Amount awarded: $442,847 (2012–2014)
Notes: "PNW sweet cherry breeding and genetics program." PI: Oraguzie. Co-PI: Peace.

Agency Name: WTFRC/OSCC
Amount awarded: $141,000 for 3 years from 04/01/2014 to 03/31/2016
Notes: After RosBREED: Developing and deploying new sweet cherry DNA tests led by Dr Peace with Oraguzie as Co-PI

Agency Name: WTFRC/OSCC
Amount awarded: $52,092 for two years from 04/01/2014 to 03/31/2015
Notes: Sweet cherry toolbox project led by Dr Main with Oraguzie as Co-PI

Agency Name: USDA-NIFA Specialty Crop Research Initiative
Amount awarded: $10.0 M (Sep 2014 – Aug 2019)
Notes: "RosBREED: Combining disease resistance with horticultural quality in new rosaceous cultivars." PI: Iezzoni. Co-PIs include Peace, Oraguzie, and Main.

Agency Name: USDA-NIFA Specialty Crop Research Initiative
Amount awarded: $2.74 M (Sep 2014 – Aug 2019)
Notes: "Genome Database for Rosaceae: Empowering specialty crop research through big-data driven discovery and application in breeding." PI: Main. Co-PIs include Peace, Oraguzie, and Main

Total Project Funding: $52, 844

Budget History:

Item	2014	2015
Salaries	**14,585**	**15,168**
Benefits	**6,417**	**6,674**
Wages	**2,800**	**2,800**
Benefits	**272**	**272**
Equipment		
Supplies	**972**	**972**
Travel	**1,000**	**1,000**
Plot Fees		
Miscellaneous		
Total	**26,002**	**26,842**

Footnotes: Salaries and benefits are for 0.42 FTE Research Associate. Wages and benefits are for a temporary assistant working 20 hours/week for 14 weeks at the rate of $10/hr. Supplies include reagents, lab supplies and other consumables.

Objectives: The specific objectives of this study were to:

1. Refine the marker-locus-trait (MLT) associations already determined for PFRF, bacterial canker and powdery mildew incidence.

2. Obtain phenotypic data on cracking incidence and pitting susceptibility and establish MLT associations using genotypic data developed in both RosBREED and the Stem-less cherry projects. Utilize the 'Selah' x 'Cowiche' population for specific trait phenotyping (pitting, PFRF and cracking susceptibility).

Significant findings and achievements

1. We have identified a genomic region on chromosome 2, *PFRF_G2b*, explaining 27.5% of the phenotypic differences in PFRF. This genomic region is common to both 'Selah' x 'Cowiche' population and the 600 sweet cherry accessions used in the RosBREED project. A genetic test is being developed for this locus in the project, CH-14-102 (led by Peace and Oraguzie). In addition, we have also identified genomic regions on chromosomes 3, 5 and 8 specific to 'Selah' x 'Cowiche' population.

2. A genomic region on chromosome 5, *PM_G5*, observed in multiple years associated with foliar powdery mildew (PM) incidence has been identified. This locus explains 40.5% of the differences among individuals in PM incidence and has been targeted for DNA test development in CH-14-102. A study to understand the genetic mechanisms underlying PM fruit infection has been initiated in the current RosBREED project.

3. A genomic region on chromosome 5, *BC_G5*, explaining 15.5% of the phenotypic variation in bacterial canker disease response in the green houses has been identified.

4. The cracking index of ~46 named cultivars in the PNW has been characterized. In addition, the genomic regions associated with cracking index have been identified on chromosomes 1, 2, and 3, across years in the 'Selah' x 'Cowiche' population. The high LOD score for the genomic regions on Chromosome 1 in 2014 (5.4) and chromosome 2 in 2015 (4.6), as well as, the occurrence of both regions over two years suggest that both would be candidates for DNA test development.

5. Approximately 46 PNW cultivars have been characterized for pitting index. We have identified several genomic regions associated with pitting index, however, the genomic region on chromosome 1 which is common across years appears to be a suitable candidate for DNA test development for MAB.

Results and Discussion

Two sets of germplasm were used in these studies. One set consisted of 600 accessions including old cultivars, current commercial cultivars, advanced selections and selected breeding progenies developed in the RosBREED SCRI project, whereas, the other set comprised 110 individuals belonging to the 'Selah' x 'Cowiche' mapping population developed in the Stem-free cherry SCRI project. The genotypic data sets each consisted of a 6K Infinium SNP array.

The objectives of this project as presented in this report focused around phenotypic data collection and integration of both phenotypic and genotypic datasets to identify genomic regions underlying pedicel-fruit retention force (PFRF), bacterial canker (BC) infection, foliar powdery mildew (PM) incidence, cracking index (CI) and pitting index (PI). A pedigree-based analysis in the FlexQTL™ software and interval mapping in MapQTL were used to identify genomic regions in the 600 accessions and in the 'Selah' x 'Cowiche' mapping population, respectively.

Objective 1: Refine the marker-locus-trait (MLT) associations already determined for pedicel-fruit retention force (PFRF), bacterial canker and powdery mildew incidence

a. Pedicel-fruit retention force (PFRF)

Fruit samples from each of 600 accessions were harvested randomly from single tree plots with pedicels attached at physiological maturity determined by color, taste and firmness. Five largest fruits from each individual were selected for PFRF measurements (in grams) in the laboratory using a mechanical force gauge (Imada DPS-11, Northbrook, IL, USA) with a custom fitted polyvinyl chloride attachment for fruit detachment. Data were recorded in 2010, 2011 and 2012. Following data analysis, we identified a genomic region on chromosome 2 (G 2) across years and in the combined data associated with PFRF (Table 1). The percentage of phenotypic variation in PFRF explained by this genomic region in the combined data was 27.5%. Other genomic regions were also observed on G 1 and G 2 in 2011 and one on G 4 in 2012 explaining one third of the phenotypic variation.

Table 1. Marker intervals and additive effect sizes of the PFRF genomic regions detected in the 600 pedigree-linked accessions. Please note that sample size increased with years.

Year	Chr	Locus	Marker interval (cM)	Peak position (cM)	Flanking markers	Posterior Intensity	Additive effect (g)	PVE (%)
2010	2	*PFRF_G2a*	0-16	1	ss490548700 ss490559390	0.04	125.8	3.5
2011	1	*PFRF_G1*	6 – 14	10	ss490545589 ss490546281	0.29	285.5	14.3
	2	*PFRF_G2b*	12 – 23	19	ss490548989 ss490549569	0.16	181.7	11.6
	8	*PFRF_G8a*	1 - 13	11	ss490557717 ss490557851	0.08	218.4	12.7
	8	*PFRF_G8b*	38 - 52	41	ss490551301 ss490558696	0.04	210.0	11.3
2012	2	*PFRF_G2b*	19-31	21	CPSCT038 Ss490547354	0.08	105.3	7.8
	4	*PFRF_G4*	12-15	13	ss490552535 ss490552620	0.76	314.2	33.0
Combined data	2	*PFRF_G2b*	12-14	13	ss490548989 ss490549037	0.59	211.8	27.5

Chr = chromosome; PVE-phenotypic variance

In the ‘Selah’ x ‘Cowiche’ population, 10-15 fruit per individual were screened in 2012, 2103 and 2014, and interval mapping in MapQTL was used to identify genomic regions associated with PFRF (Table 2). The genomic regions on G1, G 2, G 3 and G 5 each were observed across years, while a genomic region on G 8 was observed between years in 2012 and 2013.

Table 2. Genomic regions associated with PFRF in 'Selah' x 'Cowiche' population.

Year	Chromosome	Peak (cM)	LOD
2012	1	85.6	4.23
	1	107.4	4.19
	2	67.9	3.70
	3	15.6	1.72
	5	60.6	1.27
	8	24.5	2.56
2013	1	0.0	2.86
	1	108.3	2.34
	2	62.9	1.15
	3	17.6	1.72
	5	60.6	1.37
	8	20.3	1.76
2014	1	2.6	5.02
	2	67.9	2.85
	5	59.6	3.39
2015	1	2.6	1.48
	2	67.9	1.61
	3	14.6	2.59
	5	59.6	2.23

Note: The gray color indicates that the genomic regions have a LOD score less than 3, but were consistent between years. LOD= Log likelihood ratio.

The genomic region on G 2 was consistent between the 600 accessions and 'Selah' x 'Cowiche' population and this locus has been followed up with genetic test development in the project, CH-14-102. It appears that the genomic regions on G 3, G 5 and G 8 may be specific to 'Selah' x 'Cowiche' population and will be targeted for further DNA test development to select individuals from that population that have low, medium or high PFRF combined with superior fruit quality.

b. Powdery mildew incidence

Powdery mildew infection on sweet cherry leaves was assessed in the field in 2011, 2012, 2013 and 2014 on 600 sweet cherry accessions based on a six point scale where, 0= no infection and 5= severe infection (Chavoshi et al. 2014). Following data analysis in FlexQTL™, we identified a genomic region on G 5 that was consistent across years while another on G1 was observed in 2011 and 2013. A genomic region on G 2 was identified in 2011 and 2014. Genomic regions on G 3 and G 6 were identified in single years (Table 3). Combining all 4 years data, we observed two genomic regions; one on G2, *PM_G2*, explaining 3.7% of the phenotypic variation and another on G5, *PM_G5*, explaining 48.1% of the phenotypic variation. Genetic tests are now being developed for the *PM_G5* region, in the project, CH-14-102, to facilitate marker assisted breeding for foliar PM resistance in sweet cherry.

Although there was no specific funding for PM infection on fruit, we took advantage of the high disease pressure in 2014 to score for PM infection on fruit. However, only 161 accessions which had limited fruit on the tree were assessed in late August, a time when most trees had no fruit or the fruit had dried out. Following analysis, we identified genomic regions on G 4 and G 6 explaining 19.6% and 55.2% of the phenotypic variation, respectively. Due to low disease pressure in 2015, we were unable to take advantage of natural infection in the field to score for fruit PM. With funding from RosBREED, we inoculated a subset of cultivars with the PM pathogen to facilitate developing a standard protocol for fruit infection

for use in screening a larger germplasm set in future for identification of genomic regions for fruit infection.

Table 3. Marker intervals and additive effect sizes of PM genomic regions detected.

Chr	Year	Locus	Marker interval (cM)	Flanking markers	Max Posterior intensity	Additive effect	PVE (%)
1	2011, 2013	*PM_G1*	9.30 – 11.58	ss490545821 - ss490546020	0.19	0.86	30
2	2011, 2014	*PM_G2*	26.04 – 26.98	ss490549912 - ss490550007	0.11	0.85	17-22
3	2014	*PM_G3*	14.02 – 18.10	ss490547825 - ss490551374	0.05	0.53	2
5	2011, 2012, 2013, 2014	*PM_G5*	4.64 – 7.13	ss490553865 - ss490553929	0.34	1.22	37-42
6	2011	*PM_G6*	48.8 – 50.9	ss490555836 - ss490555878	0.10	0.64	4

Chr = chromosome; PVE-phenotypic variance

c. Bacterial canker infection

Bacterial canker screening was conducted in 2013 and 2014 according to Mgbechi-Ezeri (2015). Data analysis based on separate years indicated a positive evidence (BF≥8) for a genomic region on G 5, *BC_G5a*, explaining 17.0% of the phenotypic variation in 2013 while in 2014, there was a decisive evidence for a genomic region on G 5 explaining 15.3% of the variation. Combining both years, there was a decisive evidence for a genomic region on G 5, explaining 15.1% of the variation, and yet another genomic region on G 7, explaining 6.3% of the phenotypic variation.

Please note that these results were obtained from disease response studies following leaf inoculation in the lab with the pathogen, *Pseudomonas syringae* var *syringae*. There was no opportunity to carry out field tests since bacterial canker is considered a quarantined disease in Washington. However, we have identified isolates of other *Pseudomonas* spp from WSU-IAREC Prosser, Roza farm, which we plan to make available for use in field tests in the future.

Table 4. Bacterial canker genomic regions, chromosomes & percentage variance explained by region

Year	Chr	Marker interval	Peak position	Posterior Intensity	Additive effect	PVE (%)	Flanking markers
2013	5	2, 13	9	0.200	0.467	17%	Ss490553738-ss490553963
	7	3, 7	5	0.320	0.387	11%	Ss4905536403-ss490556479
2014	5	(2, 11)	4	0.353	.598	15.3%	Ss490553738-ss490553817
	2	40, 46	43	0.2603	0.407	7.1%	Ss490550529-ss490550691
Combined data	5	2, 13	9	0.282	0.440	15.1%	Ss490553738-ss490553963
	7	4, 7	4	0.274	0.300	6.3%	Ss4905536403-ss490556485

Chr = chromosome; PVE = phenotypic variance

Objective 2: Obtain phenotypic data on cracking incidence and pitting susceptibility and establish MLT associations using genotypic data developed in both RosBREED and the Stem-less cherry projects. Utilize the 'Selah' x 'Cowiche' population for specific trait phenotyping (pitting, and cracking susceptibility)

a. Cracking susceptibility and identification of genomic regions

Fruit were harvested at commercial maturity from ~80 seedlings belonging to 'Selah' x 'Cowiche' mapping population and from ~46 named cultivars in 2014 and 2015. Approximately 10-25 and 60 fruit samples per tree, respectively, at commercial maturity, were selected for phenotyping in the 'Selah' x 'Cowiche' population and from the named cultivars. The 60 fruit sample was subdivided into three groups of 20 fruit representing three replicates in each named cultivar, whereas, only a single replicate of 10-25 fruit per seedling (due to low fruit numbers) was assayed in the 'Selah' x 'Cowiche' population. The diameter of each fruit was recorded as well as the combined weight of fruit in each replicate. The fruit samples were soaked in distilled water and cracking incidence as well as split type was recorded every hour up to 5 hours.

Analysis of variance showed significant differences among genotypes in cracking susceptibility at 1h, 2h, 3h, 4h and 5h following soaking in water ($p<0.0001$)(data not presented). The highest incidence of cracking (CI) was observed at 5h (CI = 0.83 in the named cultivars and 0.97 in 'Selah' x 'Cowiche' population) and the lowest at 1h (CI = 0.35 in the named cultivars and 0.603 in 'Selah' x 'Cowiche' population) ($p<0.05$)(data not presented). There was no significant difference between the incidence at 5h and 4h in both the named cultivars and the 'Selah' x 'Cowiche' population ($p>0.05$). The correlation between years (i.e., 2014 and 2015) was 0.84.

The correlation between fruit size/weight and incidence of cracking was high in the cultivars, with correlation coefficients of 0.45–0.63 ($p<0.01$)(data not presented). However, correlation was generally low and non-significant ($p>0.05$) in 'Selah' x 'Cowiche' population, although the correlation at 1h appeared to be the highest at $r=0.18$, with fruit diameter and $r=0.21$ with fruit weight (data not presented).

Following QTL analysis using interval mapping in MapQTL in 'Selah' x 'Cowiche' population (n=64) in 2014, we identified a genomic region on chromosome 3 (LOD = 5.4) across soaking periods including 1h, 2h, 3h, 4h and 5h, suggesting that only one soaking period may suffice for recording CI (data not presented). Other genomic regions were identified on G 1 (LOD = 2.5), G 2 (LOD = 2.30), G 6 (LOD = 4.2), G 7 (two regions at LOD = 3.7 and 2.6), and G 8 (LOD = 3.3 and 3.40). The FlexQTL™ results combining data sets including 'Selah' x 'Cowiche' population (n=62) and 37 named cultivars on the other hand, identified a major genomic region on G 2 explaining 54% of the phenotypic variation (Table 5). In 2015, the genomic regions identified in the 'Selah' x 'Cowiche' population (n=47) include G 1 (LOD = 3.3), G 2 (LOD = 4.3), G 3 (at LOD = 2.6), G 4 (LOD = 2.3), G 5 (LOD = 2.2, and G 6 (LOD = 2.2). FlexQTL™ analysis combining 'Selah x 'Cowiche' data (n= 45) and data from named cultivars (n= 37) identified a major genomic region on G 4 explaining 34% of the phenotypic variation (Table 5).

A study at INRA, Bordeaux, France (Quero-Garcia et al.2010) conducted in 2006, 2008, 2009, 2010 and 2011 using the 'Regina' x 'Lapins' population, identified genomic regions on G 1, G 3 and G 4 which were weak and variable across years while the genomic region on G 5 was stable across years and explained 15% of the phenotypic variation for CI. The genomic regions on G 1, G 2, and G 3 were common between our study and that of Quero-Garcia et al (2010), although different mapping populations were used. The genomic regions on G 2 and G 3 would appear to be candidates for DNA test development. .

Table 5. Genomic regions for cracking index identified based on MAPQTL and FlexQTL™ analyses.

Year	Chr	LOD	Peak(cM)
S x C family			
2014	1	2.5	0
	2	2.3	40.8
	3	5.4	45.6
	6	4.2	25.9
	7	3.7	27.7
	7	2.6	47.7
	8	3.3	10.3
	8	3.4	33.7
2015			
	1	3.3	92.7
	2	4.3	20.6
	2	4.3	45.6
	3	2.6	15.6
	4	2.3	0
	5	2.2	60
	6	2.2	40
FlexQTL™			
	Chr	Bayes factor	PVE (%)
2014	2	>5	54
2015	4	>5	34

Chr=chromosome; S X C='Selah' x 'Cowiche'

b. Pitting susceptibility and identification of genomic regions

Fruit were harvested at commercial maturity from 'Selah' x 'Cowiche' population (n= 80 and 77 in 2014 and 2015, respectively) and from 46 named cultivars in both years. Approximately 3-25 and 60 fruit samples without blemish per individual, were selected after harvest in the 'Selah' x 'Cowiche' population and in the cultivar subgroup, respectively. The fruit samples from each cultivar were subdivided into three groups of 20 fruit representing three replicates, whereas, only a single replicate of fruit from 'Selah' x 'Cowiche' population was assayed. Each replicate was weighed, transferred into ziplock bags and stored at 4 ºC for 4 hours. Pitting was induced on both sites of the fruit in the 'Selah' x 'Cowiche' population and on one side of the fruit in the named cultivars and fruit were held at 1 °C for 2 weeks. Pitting was rated on a 4 point scale where, 1= no pitting, 2= superficial pitting, 3= medium pitting; pit was deeper and wider and had clearly distinct edges; 4= severe pitting, pit was very deep and edges had sunken into the pulp tissue (Toivonen et al. 2004).

Analysis of variance showed significant differences in pitting index (PI) among cultivars (p<0.0001)(data not presented). Tukey's honest studentized test was used to separate the means at p<0.05. The correlation between years among the named cultivars was r = 0.63, whereas, in the 'Selah' x 'Cowiche' population this was 0.50. 'Heldelfingen' showed the lowest PI (1.70) in 2014, while 'Ulster' was the lowest (1.63) in 2015. By contrast, 'Moreau' recorded the highest PI (3.87 and 3.94 in 2014 and 2015, respectively), while 'Regina' had medium PI (2.1 and 2.06 in 2014 and 2015, respectively) (data not presented). The correlation between PI and fruit weight was low at r= -0.07 in the named cultivars and r= -0.05 in the 'Selah' x 'Cowiche' population (p>0.05). In addition, there was a negative and low correlation (r= -0.21) between firmness and PI in the 'Selah' x 'Cowiche' population (data not presented).

Data analysis based on interval mapping in MapQTL (n=79) using the 2014 data showed genomic regions on G 1 (LOD = 5.7), G 3 (LOD = 4.7), G 4 (LOD =3.5), G 6 (LOD = 2.3) and G 7 (LOD = 2.3)(Table 6). FlexQTL™ analysis with combined data set including named cultivars and 'Selah' x 'Cowiche' population identified genomic regions on G 1 (showing a peak at 34 cM and explaining 5% of the phenotypic variation), G 3 (with a peak at 30 cM explaining 2 % phenotypic variation) and on G 4 (with a peak at 14 cM explaining 4 % phenotypic variation). In 2015, the genomic regions identified in the 'Selah' x 'Cowiche' population (n=77) mapped on G 1 (with a peak at 53.7 cM and a LOD score of 7.7), G 2 (peaking at 51.4 cM with a LOD score of 2.6), and G 8 (LOD = 2.0). The FlexQTL™ analyses (based on n=75 and n=42, in the 'Selah' x 'Cowiche' population and the named cultivar group, respectively identified one genomic region on G 1 (with a peak at 25 cM) explaining 30% of the phenotypic variation.

This is the first study on genetic mechanisms underlying pitting susceptibility in sweet cherry and unfortunately there is no other study to compare and contrast our results with. It appears that the genomic region on G 1 which showed up consistently between years and statistical analyses methods may be a candidate for DNA test development for deployment of MAB strategies for PI. This genomic region was detected in the Selah x Cowiche population in 2014, and in 2015, explained 30% of the phenotypic variation from FlexQTL™ analyses (Table 6).

Table 6. Genomic regions for pitting index identified based on MAPQTL and FlexQTL™ analyses.

Year	Chr	LOD	Peak(cM)
S x C family			
2014	1	5.7	61
	3	4.7	24
	4	3.5	29
	6	2.3	70
	7	2.3	50
2015	1	7.7	54
	2	2.6	51
	8	2.0	25
FlexQTL™			
	Chr	Bayes factor	PVE (%)
2014	1	>5	34
	3	>5	30
	4	>5	14
2015	1	>5	25

Chr=Chromosome; S X C= 'Selah' x 'Cowiche'.

References

Chavoshi M.S., Watkins, C.S., Oraguzie, B.O., Zhao, Y., Iezzoni, A., N. Oraguzie. 2014. Phenotyping protocol for sweet cherry (*Prunus avium* L.) to facilitate an understanding of trait inheritance. J. Amer. Pomol.Soc. 68(3): 125-134

Mgbechi-Ezeri J., K. Johnson, and N. Oraguzie. 2015. Effect of inoculum concentration, isolates and leaf age on bacteria canker disease development in sweet cherry (*Prunus avium* L.) cultivars. Acta Hort. (In press)

Toivoven P.M.A., Kappel, F, Stan, S., Mckenzie, D-L, Hocking R. 2004. Firmness, respiration and weight loss of 'Bing', 'Lapins' and 'Sweetheart' cherries in relation to fruit maturity and susceptibility to surface pitting. HortScience 39 (5): 1066-1069

Executive Summary

Knowledge of genetic systems controlling the inheritance of horticulturally important traits is important for selection and breeding decisions and for future predictions of the performance of individuals. In this study we have used two population types including a bi-parental mapping population ('Selah' x 'Cowiche') and a pedigree based population comprising ~600 accessions to map and identify the genomic regions associated with traits of importance to the sweet cherry industry including pedicel fruit retention force (PFRF), bacterial canker infection (BC), foliar powdery mildew infection (PM), cracking index (CI) and pitting index (PI). To our knowledge, this is the first study on the identification of genomic regions underlying pedicel fruit retention force, powdery mildew incidence, bacterial canker incidence, and pitting index in sweet cherry. Association analyses using both phenotypic and genotypic datasets were conducted in MAPQTL and FlexQTL™ for the bi-parental population and the pedigree-based germplasm, respectively. A genomic region was identified for pedicel fruit retention force (PFRF), on chromosome 2 (G 2), tagged *PFRF_G2*. This locus was consistent between years and studies, and explained more than one-quarter of the phenotypic variation among individuals for PFRF. In the case of foliar powdery mildew infection, a major locus, *PM_G5*, was identified which explained ~40% of the variation. This genomic region appeared to co-locate with the genomic region, *BC_G5*, associated with bacterial canker disease response in the green houses. The *BC_G5* locus explained ~15% of the variation in disease response for BC. For pitting index, we identified genomic regions on chromosomes 1, 3 and 4, however, the locus on chromosome 1 was consistent between years and study, whereas, other genomic regions were variable between years. Genomic regions were identified for cracking index in 'Selah' x 'Cowiche' population on chromosomes 1, 2, 3, and 6, whereas, only two genomic regions were identified including G 2 and G 4 based on FlexQTL™ analyses using a combined data set including 'Selah' x 'Cowiche' population and the named cultivars. The results for cracking index using 'Lapins' x 'Regina' population by a research group at INRA, France, also identified genomic regions on G 1, G 2 and G 3 in single years but only the genomic region on chromosome 5 was consistent across years. In addition, the profile of ~46 PNW cultivars for both cracking and pitting indices has been established.

Going forward, we suggest developing DNA tests for the genomic region on chromosome 2, *PFRF_G2*, for MAB for low, medium and high pedicel fruit retention force, *PM_G5*, for foliar powdery mildew resistance, *BC_G5*, for green house screening for low bacterial canker disease response, G.2 and G.3 for low cracking index, and for G 1, for low pitting index. The DNA tests (being developed in CH-14-102) will facilitate deployment of MAB for multiple traits in sweet cherry to improve selection efficiency and cost-effectiveness.