FINAL PROJECT REPORT

Project Title: Sweet cherry breeding: identifying genetically superior selections

PI: Per McCord
Organization: WSU Dept Horticulture
Telephone: 509-786-9254
Email: phmccord@wsu.edu
Address: WSU IAREC
Address 2: 24106 N. Bunn Rd
City/State/Zip: Prosser, WA 99350

Co-PI (2): Cameron Peace
Organization: WSU Dept. Horticulture
Telephone: 509-335-6899
Email: cpeace@wsu.edu
Address: Johnson Hall 39
Address 2: PO Box 646414
City/State/Zip: Pullman, WA 99164

Co-PI(3): Bernardita Sallato
Organization: WSU Extension
Telephone: 509-786-9201
Email: b.sallato@wsu.edu
Address: WSU IAREC
Address 2: 24106 N. Bunn Rd.
City/State/Zip: Prosser, WA 99350

Cooperators: WSU Cherry Breeding Program Advisory Committee (BPAC), Steve Castagnoli (OSU-MCAREC)

Other funding sources

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 McCord and Peace.

Total Project Funding: $150,000

Budget History:WSU[1]

Item	Year 1:	Year 2:	Year 3:
Salaries	11,100		
Benefits			
Wages	29,466		
Benefits[2]	7,395		
Equipment	793		
Supplies			
Travel	3,579		
Plot Fees			
Miscellaneous[3]	34,396		
Total	86,729		

Footnotes: [1]Expenses recorded cover the period from 1 November 2017- 21 October 2018. [2] Combines benefits from both salaried and hourly employees. [3]Supplies and Miscellaneous combined.

Budget History: OSU-MCAREC[1]

Item	Year 1:	Year 2:	Year 3:
Salaries	6,005		
Benefits	4,985		
Wages	3,840		
Benefits	384		
Equipment			
Supplies	2121		
Travel			
Plot Fees			
Miscellaneous			
Total	17,335		

Footnotes: [1] OSU does differentiate amongst sub-accounts within overall projects, but funds were considered to be used in accordance with the proposed budget.

ORIGINAL OBJECTIVES

The Pacific Northwest Sweet Cherry Breeding Program (CBP) was established to provide superior sweet cherry cultivars for the Oregon and Washington industries. Since the departure of the previous breeder in 2016, the program has sought to improve horticultural practices and provide an improved framework for the eventual new breeder. Dr. McCord was hired as the new breeder in April 2018.

Overall goal: Develop superior new cultivars for the Pacific Northwest sweet cherry industry, using a streamlined breeding framework that is objective and resource-driven and quantitatively targets industry priorities

Specific objectives

1) Maintain a robust horticultural management system that efficiently raises and maintains healthy plant materials at all breeding stages
2) Incorporate new tools to deploy a robust performance evaluation system that effectively identifies superior selections in Phase 1 seedling trials and Phase 2 regional trials

SIGNIFICANT FINDINGS

- Automated irrigation timers were installed for the P1, P2, and RosBREED blocks, allowing for night-time irrigation.
- P1, P2, RosBREED blocks, and P1.5 trees (seedlings budded onto precocious rootstock) were fertilized with MAP and Zinc sulfate according to soil test results.
- Dr. McCord worked closely with WSU orchard staff to implement an appropriate spray regime.
- Virus screening of the orchard continued with approximately 400 trees screened via bioassay on 'Shirofugen' indicator. Fifty-seven trees tested positive, and will be removed this fall.
- Leaf tissue from forty cultivars and advanced selections was submitted to the Clean Plant Center Northwest (CPCNW) for virus screening via RT-PCR. These selections are being targeted primarily for use as parents in a protected crossing block comprised of trees in pots in a greenhouse.
- Evaluations were made of ten P2 selections. R19 (early mahogany) and R29 (mid-season mahogany) are of particular interest.
- The P2 site at the Roza orchard is poor (trees are stressed and have variable health). New P2 plantings will go into a different block.
- Field evaluations of P1 seedlings resulted in 58 selections being evaluated in the laboratory. However, none were judged sufficiently superior to warrant advancement to P2.
- Testing of the Mohr MDT-2 penetrometer indicates that its workflow is not compatible with a high-throughput breeding evaluation laboratory. In addition, preliminary analysis suggests that data captured by the instrument is not well-correlated with firmness as measured by the standard FirmTech device, nor is the data better able to discriminate perceived firmness (based on mouth feel) vs. the FirmTech.
- The crossing program was restarted in April 2018 very soon after Dr. McCord's arrival. Twenty-nine bi-parental crosses and ten open-pollinated crosses resulted in approximately 6,000 seeds produced. DNA information was used to guide the majority of bi-parental crosses made.
- Dr. McCord attended the OSU pre-harvest cherry tour in The Dalles in June 2018. He also visited private breeding programs, a commercial nursery, and the USDA-ARS germplasm collection in California (August), and the Agriculture and Agri Food Canada breeding program near Summerland, British Columbia (October)

RESULTS & DISCUSSION

1a) Irrigation and nutrient management, pesticide application

We installed electronic irrigation timers and associated solenoids on blocks C50-53 and B48, which encompasses the seedlings (P1), P2 selections, and the RosBREED collection of genetic stocks. This allowed irrigation to take place at night when it would not interfere with harvesting. Based on soil analysis, these same trees were fertilized with monoammonium phosphate and zinc sulfate, via banding into a shallow trench in the root zone. Promising seedling trees from the old F block that had been budded onto Gisela-6 (phase '1.5') were also fertilized. Dr. McCord worked closely with the WSU orchard manager to ensure that a spray program was followed that provided acceptable levels of control, while also allowing flexibility in harvesting. As in prior years, the RosBREED and adjacent P1 blocks were not sprayed for powdery mildew (PM), to allow for sufficient disease pressure to evaluate the seedlings for PM resistance, and for continued research on the genetics of resistance to PM infection.

1b) Virus screening

As a continuation of the more rigorous virus testing implemented in recent years, we sampled one tree from approximately 400 2-tree replications in the RosBREED block (C53). In late July 2018, a short section of current year's growth was sampled from four points throughout the tree. Bark from these sections was 'grafted' onto limb sections of *Prunus serrulata* 'Shirofugen'. This species is hypersensitive to ilarviruses, of which *Prune dwarf virus* and *Prunus necrotic ringspot virus* are of particular interest. In October 2018, these limb sections were sliced open with a knife to observe any hypersensitive reaction. A total of 57 trees tested positive, with a further 4 listed as questionable. The positive trees will be cut down to a stump, and glyphosate applied to identify any root-grafted neighboring trees that should also be removed. The questionable trees will be tested in the spring via RT-PCR or ELISA.

1c) New protected parental block

The CBP is in the process of transitioning from making crosses in the orchard, to potted trees in a greenhouse. This move is expected to provide greater flexibility in crossing, as well as protection from frost, birds, and vectors of pollen-borne viruses. As a first step, the first 40 potential parents have been identified. These individuals represent a range of genetic diversity as well as targeting high-priority traits such as fruit size, early/late maturity, and powdery mildew resistance. To ensure clean budwood and/or pollen, leaf tissue from these trees was sampled and sent to the CPCNW in October 2018, for RT-PCR testing of the following viruses: *Cherry leaf roll*, *Prune dwarf, Prunus necrotic ringspot*, *cherry virus A*, *cherry rasp leaf*, *little cherry virus* 2, and Western X (phytoplasma).

2a) Phase 2 evaluations

The P2 selections evaluated in 2018 are listed in Table 1. Fruit from P2 selections were evaluated for fruit size (weight and diameter), firmness, color, soluble solids content, and harvest defects (primarily bruising, pitting, and doubles. After two weeks of refrigeration, the samples were evaluated for the same criteria as at harvest, plus storage defects (loss of luster, shrivel, and stem browning).

Table 1. Evaluation status of current P2 selections.

Original name	Selection	Class[1]	Prosser 2018	Pasco 2018	Hood River 2018
FR14T012	R19	EM	Full	Full	
FR09T049	R3	EM	Full	Full	
FR11T059	R16B[2]	EB	Full		Full
FR51T113	R28B	EB	Just bearing	Full	
FR01T002	R1	MM	Full	Full	
FR44T083	R17	MM	Full		
FR36T035	R21	MM	Just bearing	Full	
FR52T095	R29	MM	Just bearing	Full	
FR01T070	R5B	LB			Full
FR01T074	R6	LM	Full		Full

Footnotes: [1]EM= early mahogany; EB= early blush; MM= mid-season mahogany; LB= late blush; LM = late mahogany. [2]B = blush.

Of the ten selections, two of them show the most promise. 'R19' is an early-season mahogany selection, with similar timing to 'Chelan'. However, it is showing larger fruit size, better firmness, and higher soluble solids content (Table 2). It appears to be more susceptible to birds than 'Chelan', possibly due to the higher SSC. DNA test results indicate that it is self-fertile. 'R29' is a mid-season mahogany selection. Data is only available for the Pasco site, as the trees in Prosser are just starting to bear. This selection is comparable to 'Bing', but has much larger fruit (Table 3), and is self-fertile.

Table 2. 2018 performance data for 'R19'.

ID	Harvest date	Fruit Wt. (g)	Fruit width (mm)	Fruit firmness (g/mm)	Juice SSC (ºBrix)	Year	Site
Chelan	12-Jun	8.0	25	297	15.9	2017	Pasco
R19	16-Jun	10.2	29	437	25.7	2017	Pasco
Chelan	8-Jun	7.3	24.5	272.0	18.7	2018	Pasco
R19	6-Jun	9.3	27.3	391.1	21.9	2018	Pasco
Chelan	7-Jun	7.1	22.7	249.8	18.4	2018	Prosser
R19	7-Jun	9.1	27.2	378.5	24	2018	Prosser

Table 3. 2018 performance data for 'R29'.

ID	Harvest date	Fruit Wt. (g)	Fruit width (mm)	Fruit firmness (g/mm)	Juice SSC (ºBrix)	Year	Site
Bing	26-Jun	12.5	30	336	20.6	2017	Pasco
R29	30-Jun	12.5	30	336	20.6	2017	Pasco
R29	21-Jun	13.9	31.0	293.6	21.5	2018	Pasco

2b) Phase 1 evaluations

The seedlings were evaluated generally twice per week. Field evaluations were based on fruit size, perceived firmness, and flavor. Of the approximately 1400 seedlings in the field, slightly more than half (~720) were potentially old enough to be fruiting. The majority of these were rejected for small fruit size. A total of 124 selections were sampled in the field, of which 58 were of enough interest in the field to be sent to the laboratory for evaluation. Three mahogany and four blush seedling selections showed consistently good performance over at least two seasons. However, based on BPAC input, none were deemed sufficiently superior to warrant advancement into P2. This was primarily due to lackluster fruit size, and generally midseason timing. Dr. McCord is focused on making new crosses that target fruit size and early/late maturity. In the meantime, he is propagating 2 of the most promising seedlings onto Gisela 6 for additional observation.

2c) Mohr Penetrometer

The departure of Dr. Blakey in early 2018 made it more difficult to evaluate the Mohr MDT-2 penetrometer for firmness testing. However, we were able to complete a preliminary assessment of the machine that reveals the following:

- The MDT-2 is slower than the standard FirmTech, as each fruit must be manually presented to the penetrometer. This is currently incompatible with the workflow of the CBP lab evaluation pipeline.
- By measuring the same fruit with the FirmTech and the MDT-2, we determined that none of the MDT-2 parameters are well-correlated with the FirmTech firmness output (Table 4).
- The FirmTech is better able to distinguish 'softer' vs. 'firmer' fruit based on mouth feel as compared to the MDT-2, though additional data is probably needed to definitively answer this question (Table 5).

Table 4. Correlation between Mohr MDT-2 with FirmTech firmness.

Parameter	Firmness (mm/gr)
Firmness (mm/gr)	1
Diameter (mm)	-0.17
Max Hardness R1 gr	0.62
Average Hardness R1 gr	0.66
Max Hardness R2 gr	0.38
Average Hardness R2 gr	0.42
Force at the end of R2 (gr)	0.30
Crispness	0.13

Table 5. Ability of Firmtech and MDT-2 to distinguish between different firmness classes (based on bite experience).

Parameters Bite Experience	Firmness (mm/gr)	Max Hardness R1* (gr)	Max Hardness R2** (gr)	Crispness	Force at end R2** gr
Soft	218.4 a	646.6 a	826.3 a	20.6 a	502.4 a
Middle soft	230.2 b	669.2 a	873.2 ab	21.6 a	595.6 ab
Firm	239.3 b	666.3 a	905.3 b	21.1 a	660.2 b
p value	0.006	0.438	0.033	0.625	0.012

*R1; referred as the skin region of the fruit. **R2; referred to the flesh portion of the fruit. Note that the Mohr MDT-2 performs a destructive analysis, so the bite experience was done on other fruit from the same sample.

2d) Crossing

Dr. McCord purposely arrived on the job early (April 2018) in order to be able to make crosses, which had not been done since 2015. Using DNA information provided by Dr. Peace, he and CBP team members made 29 bi-parental crosses, including five self-pollinations. Crosses were made with emasculated flowers and collected pollen, limb cages (for small self crosses), and whole trees in insect-proof cages, with a small hive of bees and a bucket with branches of the pollen parent enclosed. Approximately 4250 seeds resulted from these crosses. Open-pollinated seed were collected from ten additional mother trees selected for their large fruit. This yielded more than 1700 additional seeds, for a total approximately 6000 seed.

As the new breeder, Dr. McCord also spent time familiarizing himself with the breeding program, and conducting strategic travel to meet with breeders and other industry experts. His attendance at the OSU pre-harvest tour at the Dalles in June was an important opportunity to meet many in the PNW cherry industry. In August, he traveled to California to meet breeders from International Fruit Genetics and Zaiger's Genetics where he gained valuable insights into the basics of cherry breeding. He also visited Sierra Gold Nurseries to learn about rootstock propagation and characteristics, and the USDA-ARS germplasm collection in Davis. In October, he traveled to British Columbia to visit the federal Canadian program near Summerland, and the Summerland Varieties Corporation which handles final variety development and licensing of Canadian varieties.

EXECUTIVE SUMMARY

The Pacific Northwest sweet cherry breeding program (CBP) is devoting significant efforts in its mission to develop superior cherry cultivars for the Oregon and Washington industries. Horticultural practices put in place in recent years are being maintained in order to reduce viruses, and enhance tree health and fruit quality. Promising P2 selections have been identified, and rigorous selection criteria are being applied towards P1 seedlings to ensure that only superior selections are advanced. The crossing phase of the breeding program was successfully relaunched, resulting in large numbers of seed from DNA-informed crosses. In addition, Dr. McCord has reached out to experts in the industry to gain relevant information that can be applied to the CBP.