Project/Proposal Title: Supporting a robust PNW sweet cherry breeding and genetics program

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Cooperators: Allan Bros. Fruit, Cherry River Farms, Custom Orchards, Inc. Orchardview Farms, Stemilt Growers, Breeding Program Advisory Committee (BPAC) members

Report Type: Final Project Report

Project Duration: 3 Year

Total Project Request for Year 1 Funding: $48,623
Total Project Request for Year 2 Funding: $174,559
Total Project Request for Year 3 Funding: $183,584

Other related/associated funding sources:

Awarded
Funding Duration: 2014-2019
Amount: $10 million
Agency Name: USDA NIFA—SCRI
Notes: "RosBREED: Combining disease resistance with horticultural quality in rosaceous cultivars". PI: Amy Iezzoni. Multiple Co-PI's including Cameron Peace and Per McCord

Awarded
Funding Duration: 2019-2022
Amount: $188,165
Agency Name: WSDA Specialty Crop Block Grant
Notes: "Reducing Cold Damage in Tree Fruit". Co-PI: Matt Whiting

Awarded
Funding Duration: 2019
Amount: $79,000
Agency Name: WTFRC/OSCC
Notes: "Equipping the re-launched PNW cherry breeding program"

Awarded
Funding Duration: 2019-2020
Amount: $88,000
Agency Name: WTFRC/OSCC
Notes: "Durable genetic solutions to powdery mildew infection in sweet cherry". PI: Cameron Peace. Co-PIs: Per McCord, Prashant Swamy.

Awarded
Funding Duration: 2020-2022
Amount: $458,022
Agency Name: WTFRC/OSCC
Notes: "Understanding little cherry disease pathogenicity". PI: Scott Harper. Co-PIs: Alice Wright, Per McCord.

WTFRC Collaborative Costs: None

Budget 1
Primary PI: Per McCord
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Item	(2019)	(2020)	(2021)
Salaries[1]	$45,760	$37,440	$38,938
Benefits	$19,493	$16,230	$17,327

Wages[2]	$31,200	$32,450	$33,750
Benefits[3]	$10,564	$5,390	$5,606
Equipment			
Supplies[4]	$9,760	$33,325	$52,363
Travel	$4,000	$5,500	$6,100
Miscellaneous[5]	$40,000	$19,259	$2,500
Plot Fees	$4,275	$7,630	$8,800
Carryover from 2018 request	-$132,665		
Total	$32,387	$157,224	$165,384

Footnotes: [1]Includes Horticultural Support in 2019 (only), plus 1.0 FTE research technician. [2]Includes temporary labor for crossing, harvesting, seed extraction/transplanting, plus farm crew wages. [3]Reduction of benefit costs for 2020-21 reflects a more accurate estimate based on actual 2019 expenses. [4]Supplies for fruit evaluation, DNA extraction/genotyping, embryo rescue, propagation supplies/services, orchard maintenance. Amount is increased from original request as a result of more detailed expense information. [5]Irregular expenses. Amount is reduced from original request as a result of more detailed expense information.

Budget 2
Co PI 2:
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Item	Type year of project start date here	(Type year start date of year 2 here if relevant)	(Type year start date of year 3 here if relevant)
Salaries[1]	$5,405	$6,005	$6,305
Benefits	$4,486	$4,985	$5,234
Wages[2]	$3,840	$3,840	$4,032
Benefits	$384	$384	$403
Equipment			
Supplies			
Travel			
Miscellaneous[3]	$2,121	$2,121	$2,226
Plot Fees			
Total	$16,236	$17,335	$18,200

Footnotes: [1] Estimated salary for technician to complete pruning, thinning and data collection. [2] Wages for one part-time employee ($16/hr) to assist with orchard activities. [3] Fees include per-acre research plot fees ($3104/acre), 2 months cold storage room fee ($1.24/square foot) and miscellaneous lab supplies.

Original Objectives & Significant Findings

1. *Build a well-trained support team to maintain and improve horticultural practices in the breeding orchard and maximize breeding efforts*
    - WTFRC/OSCC and WSU startup-funded technicians hired in January 2019
        - New WSU-funded technician hired October 2020
        - WTFRC/OSCC funded technician voluntarily separated July 2021
        - WSU-funded technician voluntarily separated September 2021
        - Moving forward, will request support for one WTFRC/OSCC funded technician, second technician (approximately 0.3 FTE) supported via breeding program royalties
    - Solid training in horticultural practices received from Bernardita Sallato, Matt Whiting, Mark Hanrahan, Tom Auvil, and other industry partners.
    - Breeding program transitioning from the Roza to IAREC headquarters (all plantings since 2019 have been here)
    - Orchard blocks fertilized according to results of soil and foliar analyses. Fertigation for new P1 plantings
    - Parental trees and P2 selections screened for presence of *Prune dwarf virus* (PDV), *Prunus necrotic ringspot virus* (PNRSV), *little cherry virus 1,2* (LChv-1,2), and X-disease phytoplasma (XDP)
        - PDV is common in breeding blocks, PNRSV and XDP rare (but not absent)
        - No known infections with LChv-1,2
        - All trees known to be infected with XDP removed (including seedlings)

2. *Continue to rigorously evaluate existing selections in Phase 2 (P2) and seedlings in Phase 1 (P1). Advance selections as warranted to Phase 3 (P3)*

    - Incorporated modified atmosphere packaging for postharvest samples
    - Digital data collection at Prosser (no more paper forms)
    - Evaluated 17 P2 selections (10 multi-location, 7 only at Prosser)
    - Three selections advanced to Phase 3 trials: 'R3', 'R19', 'R29'
    - P1 seedlings advanced to P2: 2 (2019), 2 (2020), 3 (2021)
    - Removed oldest seedlings from C52 (361 trees)
    - New P2 plantings made at Prosser, Pasco (Sagemoor), and Hood River (MCAREC)
        - Replicated, randomized trials (12-15 trees per selection per site)
3. *Increase the number of targeted crosses made, seeds germinated, and seedlings transplanted*
    - 230 crosses made since program restart (2018) resulting in more than 22,400 seed
        - More than 68% of seed from bi-parental crosses
        - Over 4,170 new seedlings transplanted to the field, guided by DNA tests for self-fertility and powdery mildew resistance
    - Successfully employed embryo rescue for crosses targeting early ripening. Recovery rate increased via seed coat removal.
4. Enhance precocity and reduce external variation in the seedling blocks (*delayed 1 year*)
    - Low success rate (<10%) with initial attempts in 2020
    - In 2021, test budding of greenhouse-grown, cold-acclimated seedlings onto Gisela-12 rootstock (in the field) had success rate of 69% (single budding)
    - Applied limb bending and girdling in 2019 seedling planting (own roots); will evaluate for presence of floral buds after leaf drop in 2021 and during bloom in 2022.

Results & Discussion

1. *Support team and horticultural practices*

Michael Stein (WSU-funded) and Corina Serban (WTFRC/OSCC-funded) both began working for the CBP as full-time technicians in January 2019. Dr. Stein left in June 2020, and was replaced in October 2020 by Juhi Chaudhary. Ms. Serban left in July 2021 to work for WSU Extension, and Dr. Chaudhary left in September 2021 to work for Syngenta. Moving forward, the CBP will be requesting funds for a new 3-year project to support one technician at 1.0 FTE. A second technician will be hired on a part-time basis (approximately April-August), supported by royalty income. The breeding program has built a good working relationship with the IAREC farm manager and farm crew, allowing them to accomplish the majority of orchard maintenance under Dr. McCord's overall direction. This relationship should allow the program to continue to function at an adequate level without two full-time technicians.

Throughout the project, training in orchard management and horticultural practices has been provided by Bernardita Sallato, Dr. Matt Whiting, Mark Hanrahan, Tom Auvil, and other BPAC and industry members. Through the efforts of Dr. McCord, Ms. Serban, and close cooperation with our crop consultant (Jeff Sample) and the IAREC farm manager and crew, we have been able to maintain good orchard management practices. Control of insect pests and powdery mildew was acceptable, and irrigation has been accomplished in a timely manner. In 2021, postharvest sprays to control leafhoppers have been applied at 2-3 week intervals, and an October application of urea and zinc sulfate should speed defoliation, further discouraging leafhopper feeding.

As in prior years, the Hood River (MCAREC) and Pasco (Sagemoor) blocks were pruned during the winter, as well as the main parental block at the Roza (B53), and the 2019 P1 planting at IAREC headquarters. The RosBREED block (C53) and younger P1s at the Roza were pruned during the summer, as well as the 2020 P1 planting at IAREC.

Throughout the project, trees were evaluated for disease via bioassays (on 'Shirofugen' indicator rootstock), ELISA, or PCR. The main diseases of interest were the ilarviruses (Prune dwarf virus (PDV) and *Prunus* necrotic ringspot virus (PNRSV), little cherry viruses 1 and 2 (Lchv-1,2), and X-disease phytoplasma (XDP). Presence of PDV is high in the breeding program. We have removed the majority of known PDV-infected trees, but since there are rarely if ever symptoms of PDV (aside from lower bud take), we are focusing our efforts more on avoiding the use of infected material in making crosses. All trees known to be infected with PNRSV and XDP have been or will be removed by the end of October 2021 (no trees have been found to be infected with Lchv-1 or 2). PCR inhibitors in samples taken by CBP personnel for XDP/Lchv1,2 screening have limited our in-house ability to screen for these pathogens. We will continue to work with the Clean Plant Center NW (CPCNW) to refine our protocols, including cleanup and retesting of archived samples.

2. *P1 and P2 evaluations*

As in prior years, BPAC members were invited to inspect P1 seedlings during the fruiting season. Walkthroughs were conducted 1-2 times per week, with BPAC members visiting once per week. Selection criteria in the field was based on fruit size, firmness, and flavor. Fruit from selected P1 seedlings, all current P2 selections, and standard cultivars were evaluated in the laboratory for defects (harvest and post-harvest), weight, diameter, firmness, stem pull force (P2 only), color, Brix, and titratable acidity.

In order to maximize efficiency, P1 selections that did not meet the thresholds of weight (minimum 9 grams) or firmness (minimum 270 g/mm) generally were not evaluated for downstream traits. An 'induced pitting' protocol for post-harvest analysis was implemented by putting fruit in a bucket on an orbital shaker platform for 3 minutes at 200 rpm. When sufficient fruit was available, we also performed an induced cracking test based on a 4-hour soak in deionized water. Prior to storage, P2 samples were treated with a fungicide soak (Shield-Brite® FDL-230SC). Fruit sampled for post-harvest analysis was

placed in modified-atmosphere packaging and stored in a walk-in cooler for 4 weeks at approximately 35°F.

In 2021, a total of 106 P1/P1.5 selections passed field criteria and were evaluated in the lab. This was a good deal lower than in 2020 (Table 1), likely due to heat stress. Three selections showed good performance over multiple years, and were advanced to P2 for planting in 2023. Summary data can be seen in Table 2, which also includes data from selections advanced to P2 in 2019 and 2020. Over the course of this project, we have advanced seven P1/P1.5's to P2.

From 2019-2021, 14 P2 selections were evaluated, including 7 only present at Prosser. From this group, three were advanced to P3 (Table 3). R3 and R19 are early ripening cherries with superior fruit quality vs. 'Chelan', the early standard variety.

Of the 7 P2's those only at Prosser, four were advanced to a full (multi-location) P2 trial. The new P2 trial (including the first P1/P1.5 selections in Table 2) was planted in 2021 at Prosser (IAREC headquarters), Pasco (Sagemoor), and Hood River (MCAREC). Each location was planted in a completely randomized design with 4-5 replicates per selection, and 3 trees per replicate. Standard varieties ('Bing', 'Rainier', and 'Skeena') were planted with 3 replicates per variety, and 2 trees per replicate. The improved replication and randomization in the new P2 trials should yield higher quality data to guide advancement decisions, and provide sufficient budwood for propagation.

Table 1. Numbers of P1/P1.5 selections passing field criteria (size, firmness, flavor) and evaluated in the laboratory.

Year	Laboratory Evaluation	Rate of Field Selection
2019	143	7.5%
2020	169	11%
2021	106	7%

Table 2. Characteristics of P1/P1.5 selections advanced to Phase 2.

ID	Color	Timing	Fruit Weight (g)	Row Size/Diameter (mm)	Firmness (g/mm)	Brix/ TA	Advancement Year
CR01T078	Mahogany	Bing +8	11.5	~9/29.5	354		2019
R35	Blush	Bing +15	12.7	9 /30.1	324		2019
FR09T084	Mahogany	Bing -10	9.7	9.5/27.6	352	18.7/ 0.58	2020
CR11T019	Mahogany	Bing +26	9.6	9.5/27.6	335	23.1/ 0.72	2020
FR31T011	Light Mahogany	Bing +1	14	8.5/31.4	294	20.0/ 0.5	2021
R37	Blush	Bing + 12	12.6	8.5/32.0	280	23.4/ 0.89	2021
CR21T043	Mahogany	Bing +26	10.1	9.5/28.7	272	26.4/ 0.60	2021

Table 3. Characteristics of selections advanced to Phase 3.

ID	Color	Timing	Fruit weight (g)	Row size/Diameter (mm)	Firmness (g/mm)	Brix/TA	Notes
R3	Mahogany	Chelan +3-4	10.0	9.6/28.1	326	20.5/0.51	Meaty texture, ripen fully for best flavor
R19	Mahogany	Chelan + 0	9.0	9.8/27.6	335	23.3/0.59	Early, sweet and firm, nose cracking, self-fertile but light crops, storage challenges
R29	Mahogany	Bing + 3	12.1	8.9/30.3	302	21.1/0.40	Very large, self-fertile, mild flavor

3. *Crossing and seedling production*

In 2021, the CBP made 65 crosses, producing an estimated 5,854 seed. Seventeen of these crosses (producing 854 seed) were made using potted trees in the new hoop house, which protects trees from frost. Since the CBP began making crosses again in 2018, 230 crosses have been made, resulting in more than 24,000 seed. We have increased the proportion of seed resulting from bi-parental crosses, where identity of both parents is known (Figure 1). Crosses have been made targeting industry-relevant traits, including maturity (early/late, with a focus on early ripening), size, firmness, self-fertility, powdery mildew resistance, and resistance to X-disease phytoplasma. Cross combinations were guided by a combination of DNA information and field performance. Pollen viability testing, begun in 2019, was augmented in 2021 with testing for PDV and PNRSV in an effort to eliminate infected pollen donors from the breeding program. We built upon our previous research using ReTain to increase fruit set, by testing additional plant growth regulators (PGRs). Although late frosts limited the number of hand pollinations, we did see a beneficial effect on fruit set using a combination of ReTain and Harvista (a formulation of 1-MCP). DNA testing was used each year of the project to focus on seedlings with desired traits. Self-fertility (S4') and powdery mildew resistance (*pmr*-1) were the most-used tests. Additional tests were developed in 2021 for cracking and firmness, and will also be employed for seedling selection.

Embryo rescue for seeds from early-ripening crosses was begun in 2019, with approximately 780 embryos cultured under sterile conditions. Although we cultured essentially the same number of embryos in 2020, recovery of viable seedlings was higher (and germination more rapid) via removal of the seed coat. (Table 4). In 2021, the procedure was greatly expanded, and we began testing new growth media and the addition of PGRs. Data collection from 2021 is ongoing.

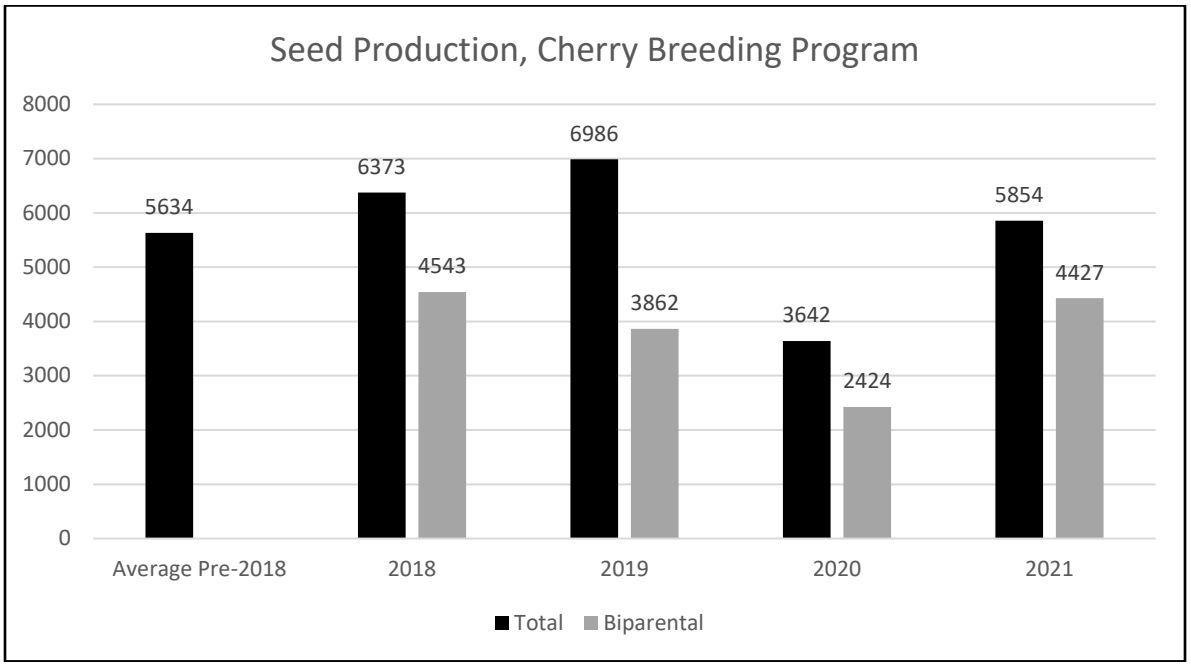
During the course of the project, the CPB transplanted more than 4,170 seedlings to the field at IAREC headquarters. The number transplanted each year varied depending on seed production and germination (752 in 2019, 2,488 in 2020, 933 in 2021). Moving forward, we have established an upper limit of 2,000-2,500 transplanted seedlings per year to fit with available land and personnel resources. The use of microsprinklers and drip tape for prompt irrigation and fertigation has allowed for strong establishment and healthy growth of seedlings.

Table 4. Progress in use of the embryo rescue technique by the CBP.

Year	Embryos rescued	Percent recovery	Notes
2019	780	33	
2020	800	50	Seed coat removed
2021	1550	33 (in-process)[1]	New media, PGRs

Footnotes: [1]The largest family in the group, with more than 500 seed, contained a large number of albino seedlings which germinated but will not survive.

Figure 1. Seed production in the CBP.



4. *Enhancing precocity and reducing variation in seedling blocks*

Our initial efforts to bud cherry seedlings onto precocious rootstocks were unsuccessful, most likely due to inexperience. We attempted to bud Gisela-12 rootstocks in the greenhouse during the winter of 2019-2020, using greenhouse-grown seedlings (2019 crosses) as scions. Despite using the more mature wood at the base of the seedling, none of the buds took. In the spring of 2020, our attempt to spring bud Gisela-6 rootstocks in the field using winter-collected wood from 2018 crosses had a bud take of only 9%. In spring of 2021, we single-budded a small number (13) of greenhouse-grown seedlings on Gisela-12 rootstocks that had been planted in the field the previous year. Prior to budding, the seedlings were given an 8-week cold treatment after reaching a height of 2-3 feet. Bud take was 69%, which is very encouraging. In addition, we applied girdling and limb bending to the cohort of seedlings transplanted in 2019 to test these effects on seedling precocity. As these trees begin to flower in 2022, we will be able to evaluate the results of these training methods compared to the traditionally pruned control seedlings.

In conclusion, the CBP has devoted significant efforts to develop superior sweet cherry cultivars for the Pacific Northwest. We have engaged university and industry experts to establish sound orchard management practices. We have re-started the crossing and P1 portion of the breeding pipeline, which is critical for long-term success, and are employing techniques such as embryo rescue and DNA-informed breeding to target important traits and make the process more efficient. We have also identified and advanced promising selections in the program, including three selections now in P3, two of which are targeting the critical early-ripening sector.

Executive Summary

Project Title: "Supporting a robust PNW sweet cherry breeding and genetics program"

Key words: breeding, embryo rescue

Abstract: 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. Since the re-launch of the program in 2018, significant improvements have been made. The breeding pipeline has been re-started in earnest. The majority of seed produced is from bi-parental crosses targeting industry-relevant traits, and embryo rescue has been successfully implemented for early ripening and interspecific crosses. More than 4,000 new seedlings (Phase 1/P1) have been planted over the past three years. Six new selections have been planted in replicated and randomized Phase 2 (P2) trials at Prosser, Pasco, and Hood River, and three selections have been advanced to on-farm Phase 3 (P3) trials for pre-commercial evaluation, including two early-ripening selections (R3 and R19), which have the potential to increase the profitability of the Pacific Northwest cherry industry. Efforts are also being made to shorten the breeding cycle by budding seedlings onto precocious rootstocks, and training own-rooted seedlings to enhance fruiting.

ADDENDUM

Final report for 1-year project, "Equipping the Relaunched PNW cherry breeding program"

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Total Project Request: **Year 1:** $79,000

Budget 1
Organization Name: Washington State University **Contract Administrator:** Katy Roberts
Telephone: (509)-335-2885 **Email address:** arcgrants@wsu.edu

Item	2019		
Salaries			
Benefits			
Wages			
Benefits			
Equipment	$79,000		
Supplies			
Travel			
Miscellaneous			
Plot Fees			
Total			

Original Objectives

The objectives of this proposal were to purchase/build critical equipment and infrastructure needed for a successful cherry breeding program. Specifically, funds were requested for the following:

- Laminar flow hood to generate sterile conditions for embryo rescue of seeds from crosses targeting early ripening, a critical trait for the industry
- Growth chamber for initial culturing of rescued embryos
- Tissue grinder to prepare leaf samples for DNA extraction prior to DNA marker testing
- A greenhouse (approximately 90 X 30 feet) for making crosses using potted trees

Results and Discussion

After determining that a combination of a walk-in cooler (for stratification) and a simple light cart was sufficient for initial growth of embryo-rescued seedlings, the CBP has procured two laminar flow hoods and LED grow lights instead of a single hood and a single growth chamber. In 2021 alone, 1550 embryos were rescued, resulting in more than 500 viable seedlings targeting early ripening.

A 'Beadbeater-96' tissue homogenizer has been used to extract DNA from more than 4,000 seedlings, as well as processing samples for pathogen detection and DNA marker discovery and test development.

After delays in design/procurement and the COVID-19 pandemic, a 96 X 30 foot hoop house was constructed in early 2021. It is equipped with double layer polyethylene sheeting and an inflation fan, as well as exhaust fans and a cooling wall. In addition to protecting potted trees from frost, the double sheeting and cooling wall provided adequate cooling even during the summer. A total of 854 seed were produced during the first season of using the hoop house, and its use in crossing will continue to expand.

These critical investments in the cherry breeding program are enabling the techniques and throughput required to more rapidly produce superior sweet cherry varieties for the Pacific Northwest.