Project Title: A robust PNW sweet cherry breeding and genetics program, 2022-2024

Report Type: Final Project Report

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

Project Duration: 3 Year

Total Project Request for Year 1 Funding: \$ 183,524 Total Project Request for Year 2 Funding: \$ 182,948 Total Project Request for Year 3 Funding: \$ 201,863

Other related/associated funding sources:

Awarded

Amount: \$458,022

Funding Duration: 2020-2024 (No-cost extensions in 2022, 2023)

Agency Name: WTFRC/OSCC

Notes: "Understanding little cherry disease pathogenicity". PI. Scott Harper. Co-PIs: Alice Wright,

Per McCord

Awarded

Amount: \$599,807

Funding Duration: 2022-2025 Agency Name: USDA NIFA—AFRI

Notes: "Improving grading methods to infer eating quality in sweet cherries under different cold

chain scenarios". PI: Carolina Torres. Co-PI's: Rene Mogollon, Per McCord

WTFRC Collaborative Costs: None

Budget 1

Primary PI: Per McCord

Organization Name: Washington State University

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Station Manager/Supervisor: Naidu Rayapati

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Item	2022	2023	2024
Salaries	\$52,744.00	\$54,854.00	\$57,048.00
Benefits	\$17,375.00	\$18,070.00	\$18,793.00
Wages	\$39,426.00	\$41,003.00	\$42,643.00
Benefits	\$9,514.00	\$9,894.00	\$10,290.00
RCA Room Rental			
Shipping			
Supplies	\$29,561.00	\$31,605.00	\$33,181.00
Travel	\$6,100.00	\$6,100.00	\$6,100.00
Plot Fees	\$8,700.00	\$10,656.00	\$12,080.00
Miscellaneous	\$2,500.00	\$2,500.00	\$2,500.00
Total	\$165,920.00	\$174,682.00	\$182,635.00

Footnotes: Salaries includes 1.0 FTE research technician. Wages includes temporary labor for crossing, harvesting, seed extraction/transplanting, plus farm crew wages. Supplies includes costs for fruit evaluation, DNA extraction/genotyping, embryo rescue, propagation supplies/services, orchard maintenance, and equipment maintenance. Travel includes fuel, insurance, vehicle maintenance, and lodging/per diem costs (the latter during pollination season).

If project duration is only 1 year, delete Year 2 and Year 3 columns.

(Complete the following budget tables if funding is split between organizations, otherwise delete extra tables.)

Budget 2

Co PI 2: Kelsey Galimba

Organization Name: Oregon State University **Contract Administrator:** Charlene Wilkinson

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Station Manager/Supervisor: Brian Pearson

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Item	2022	2023	2024
Salaries	\$3,655.00	\$2,718.00	\$5,198.00
Benefits	\$2,637.00	\$1,946.00	\$3,723.00
Wages	\$3,439.00	\$865.00	\$4,034.00
Benefits	\$625.00	\$87.00	\$690.00
RCA Room Rental			
Shipping			
Supplies	\$4,599.00	\$1,000.00	\$3,057.00
Travel			
Plot Fees	\$2,649.00	\$1,650.00	\$2,526.00
Miscellaneous			
Total	\$17,604.00	\$8,266.00	\$19,228.00

Footnotes:

- 1. Salary: for one FRA to perform PGR applications, training, thinning, netting and data collection.
- 2. Wages: for hourly employees and students to assist with orchard activities and quality tests.
- 3. Supplies: include irrigation, trellising, block maintenance, and training supplies.
- 4. Research plot fees (\$3,500/acre).

Original Objectives

- 1) Continue to generate seedlings, and rigorously evaluate seedlings and selections at all phases of the breeding program, including those now in Phase 3.
- a) Develop protocols for fruit evaluation via a small-scale commercial grade optical sorter (externally funded).
- 2) Test the effects of plant growth regulators on selections that have been advanced to Phase 3.
- 3) Increase the number of targeted cross made, seeds germinated, and seedlings transplanted
- a) Continue to utilize DNA information for superior and complementary parent selection and seedling screening.
 - b) Deploy newly developed DNA tests for cracking susceptibility and fruit firmness.
- 4) Continue to implement timely and proper practices for orchard management (training/pruning, pest and disease monitoring and control, nutrient management).

Significant Findings

- An average of 285 Phase 1 seedlings evaluated in the fruit lab each year (2022-2024). **Three Phase 1 seedlings were advanced** to Phase 2 trials.
- Phase 2 trials (planted 2021) harvested for two seasons (will be one more in 2025)
 - o Most promising are selections R47, CR01T078, and R35B
 - A new Phase 2 location added near Naches (Valley View) to focus on late-season selections
- Phase 3 selections **R19** and **R3** harvested for two seasons (will be one more in 2025)
 - o **R19** ('Chelan' timing) performed well in 2024, **expected to be released** as new cultivar as early as 2025
 - o **R3** ('Black Pearl' timing) performed well in 2024, **expected to be released** as new cultivar (pending sufficient performance in 2025) as early as 2025
- Optical sorter (single-lane Tomra InVision2) installed in 2023, used for grading fruit from Phases 2 and 3 for 2023 and 2024 seasons
 - Sorter maps developed for both harvest and postharvest grading
 - Labor savings \rightarrow 2 fewer summer employees in future years
- ~3100 seedlings planted in Phase 1 field2022-2024. DNA tests for self-fertility, powdery mildew resistance, low cracking, and high firmness were used to eliminate >1200 seedlings prior to planting, with expected saving of ~\$47,000 during Phase 1 compared to planting all seedlings
- Seed production (2022-2024) totaled 27,325, with 99% from targeted bi-parental crosses
- PCR testing for Little Cherry Disease (183 samples in 2024) identified 6 weakly-infected trees, which will be removed during fall/winter
- PCR pathogen testing of breeding parents in 2024 identified higher incidence of Prune dwarf virus and Prunus necrotic ringspot virus than in prior years

Results and Discussion

Breeding Pipeline (Objective 1)

In 2021 (the year prior to this project), 3 selections had been advanced and planted to Phase 3 (larger scale pre-commercial trials), and 6 selections had been advanced and planted to Phase 2 (small replicated trials) (Table 1). These selections began to fruit in 2023 and have now been evaluated for two of the three seasons slated for Phase 2 and 3 trials.

Table 1. List of cherry selections planted in 2021 Phase 3 and Phase 2 trials.

Phase	Selection	Market Class	Status
3	R19	Early Mahogany	Under evaluation
"	R3	Early Mahogany	Under evaluation

66	R29	Midseason Mahogany	No data yet (delays in tree establishment); first harvest 2025
2	R46	Early Mahogany	Dropped in 2024 (soft fruit)
"	R47	Early Mahogany	Under evaluation
"	CR01T078	Midseason Mahogany	Under evaluation
	R50	Midseason Mahogany	Dropped prior to 2023 (small fruit)
"	R45	Mid-late Mahogany	Under evaluation
"	R35B	Mid-late Blush	Under evaluation

R19 is early ripening ('Chelan' timing), but self-fertile with larger fruit, greater firmness, and higher SSC (°Brix) than 'Chelan'. Although color and sizing was poor in 2023 (likely due to the shortened development time caused by a late bloom followed by warm temperatures), it performed much better in 2024 (Table 2). Small amounts of certified budwood of R19 were sent to local nurseries in 2022 and again in 2024.

R3 ripens 4-7 days later than 'Chelan' but is considerably larger and sweeter, with comparable firmness. Budwood of R3 was sent to interested nurseries in Fall 2024. While R3 mother trees tested positive for cherry virus A (which has no known negative impacts), they tested negative for X-disease, Little Cherry virus 2, Prune dwarf virus, and Prunus necrotic ringspot virus. The breeding program will continue its efforts to obtain certified wood of R3.

Pending a favorable harvest in 2025, Dr. McCord will recommend to the WSU Variety Release Committee that R3and R19 be released as new cultivars. Upon release, Plant Variety Protection or a Plant Patent will be sought. R19 could be available for commercial pre-orders as early as spring 2026. R3 will likely be 1-2 years behind this due to budwood availability.

Table 2. Characteristics of R19 and R3 (Phase 3), with 'Chelan' as a standard. Performance results are from 2024 season. Estimated yield and packout data are from the entire trial, which was harvested and run over a commercial packing line; all other results are based on fruit pooled from 5 trees and evaluated in the breeding program fruit lab.

Selection	S-	Harvest	Weight	Firmness	°Brix/TA	Estimated	Location
	alleles	Date	(g)/ Row	(g/mm)		Yield/Packout	
			size				

R19	S4'1S9	6/6	8.3/10.0	335	23.1/0.28	7.3 tons/89%	Cherry
							Barn
R3	S1	6/10	11.4/9.0	285	25.3/	(small trees)	66
'Chelan'	S3S9	6/6	5.8/Under	319	17.3/0.44	/93%	"
R3		6/10	11.4/9.5	224	/	3.76 tons/78%	Area 51
'Chelan'		6/3	7.0/11.5	212	17.8/0.31		66

Footnotes: ¹Self-fertile allele.

Of the Phase 2 selections (Table 3), R47 is the earliest, ripening more than a week before 'Bing'. It is the firmest cherry in our current Phase 2 trials, with excellent size (especially for the timing) and strong flavor. CR01T078 ripens generally a few days after 'Bing' and has very large fruit with good flavor. R45 is the latest of the group, ripening with or slightly later than 'Skeena'. It is self-fertile, with very large fruit. R35B is the lone blush selection in this group. It ripens 3-4 days after 'Rainier'. Crops are lighter, but fruit size is very large with excellent firmness, and the tree is self-fertile. The coloring of R35B was light this year; we will observe this carefully in 2025 and postpone harvest as needed to allow for color development. Pending a favorable harvest in 2025, we expect to advance several of these selections (resources permitting) to Phase 3 trials.

Table 3. Characteristics of R47, CR01T078, R45, and R35B (Phase 2), with 'Benton', 'Bing', 'Rainier', and 'Skeena' as standards. performance results are from the 2024 season. Results are averaged (where possible) across 3 locations [IAREC (Prosser), Sagemoor (Pasco), and MCAREC (Hood River)].

Selection	S- alleles	Timing (vs. 'Bing')	Weight (g)/ Row size	Firmness (g/mm) ¹	°Brix/TA	Notes
R47	S1S9	-8	12.5/8.4	303	22.6/0.73	Firm, strong flavor
'Benton'	S4'2S9	-5	9.3/9.8	248	25.4/0.92	Standard
'Bing'	S3S4	0	9.2/9.9	206	22.0/0.59	Standard
CR01T078		+3	12.6/9	252	22.2/0.60	Good size, flavor
'Skeena'	S1S4'	+9	11.2/9.6	243	22.7/0.45	Standard
R45	S4'S9	+8	13.4/8.7	258	23.6/0.49	Consistent yield across sites

'Rainier'	S1S4	-1	9.7/10	208	21.4/0.41	Standard
R35B	S4'	+2	13.9/8.6	292	22.7/0.42	Lighter color (need to pick later), lighter crops vs. 'Rainier'

Footnotes: ¹Firmness measured on fruit at room temperature. ²Self-fertile allele.

In addition to the Phase 2 selections described above, we have planted or are awaiting nursery trees for an additional 9 selections (Table 4).

Table 4. List of additional Phase 2 selections in the breeding program pipeline.

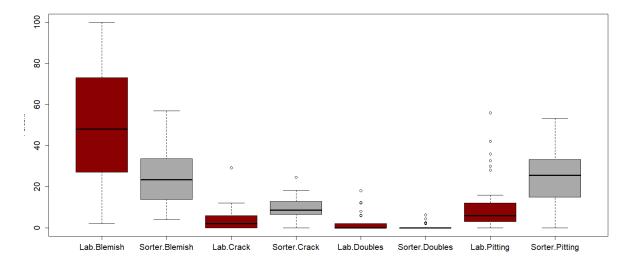
Selection	Market Class	Planting Year
FR09T084	Early Mahogany	2022
CR11T019	Late Mahogany	2022
FR31T011	Mid-season Mahogany	2023 (2024 at MCAREC)
R37B	Late Blush	2023
CR21T043	Late Mahogany	2023
R25	Early Mahogany	2024
CR20T046	Late Mahogany	2025
PSC2019003-WPM	Early Mahogany	2026
PSC2020019-120	Early Mahogany	2026

Optical Sorter (Objective 1a)

The purchase and installation of the optical sorter was made possible by generous financial support from AgWest Farm Credit and the WSU Tree Fruit Endowment Advisory Committee, and industry members also participated in reviewing bids. A two-lane (one active) Tomra InVision2 sorter was selected. The former pilot winery in the West Building on the Prosser campus (IAREC) was remodeled, and the sorter was installed in time for the 2023 harvest season. A cross-feed conveyor belt was donated by Monson Fruit and installed for the 2024 season. Breeding program personnel have received extensive training from Tomra and local Van Doren Sales trainers. We have developed harvest maps (sorting algorithms) for dark red and blush varieties, and a postharvest map for dark red

cherries. In 2023 and 2024 we gained valuable experience in operating the sorter, and compared the results of the sorter with our current fruit lab protocols in which breeding personnel record systematic observations. An example of the comparison for the 2024 season is shown in **Figure 1.** The sorter detected fewer blemishes, but this is likely due to the fact that the fruit went over the sorter first and were damaged before going to the fruit lab. It is also likely that some samples scored by lab personnel as having blemishes were scored by the sorter as having pitting. The sorter is more consistent, and can routinely be run with only two people (one to operate the sorter, one to load and move fruit). In addition, entire plots of hundreds to potentially thousands of fruit can quickly be graded, compared to only 50 fruit per plot currently tested in the fruit lab. We will continue to use the fruit lab for firmness testing, juice quality, and some postharvest traits (stem color and skin luster), but moving forward we expect that using the sorter will remove the need for 2 temporary worker positions devoted to these evaluations, saving the breeding program > \$9000 per year or redirecting such resources to other critical breeding operations.

Figure 1. Comparison of defect detection (percentage of total fruit) between fruit lab personnel and the new Tomra Invision2 optical sorter.



Effects of PGRs on Phase 3 Selections (Objective 2)

We utilized the older Phase 2 planting at MCAREC to test the effects of PGRs on R19 (Retain[®], Parka[®]), R3 (ProGibb[®]) and R29 (ProGibb[®]). These experiments suffered in 2022 and 2023 from combinations of poor weather, animal predation, and high *Pseudomonas* pressure in the old Phase 2 block. In 2022, applications of ProGibb[®] (GA₃) did not have significant effects on color, diameter, individual fruit weight, firmness, stem retention force, soluble solids concentration, or titratable acidity for R29 or R3. Parka[®] did not have significant effects on cracking, color, diameter, individual fruit weight, or firmness for R19. Retain[®] did not have significant effects on fruit set, color, diameter, individual fruit weight, firmness, stem retention force, soluble solids concentration, or titratable acidity for R19. We suspect that the small amounts of fruit that survived to be harvested and quality tested limited the robustness of the data and as a result, we elected not to continue the PGR studies in 2024. The old Phase 2 block at MCAREC will be removed in the near future.

Crossing, Seedlings, and DNA Testing (Objective 3)

In 2024, the breeding program made 74 crosses that produced an estimated 7188 seed. Favorable pollination weather enabled a greater number of crosses compared to 2022 and 2023. We continued the successful use of bumblebees in the crossing greenhouse, and increased the number of hives from one to two. We also purchased custom-built insect-proof cages for use in the crossing greenhouse. The bumblebees were then moved to the field (which blooms later) to supplement the mason bees we traditionally use for controlled crossing in cages. No open-pollinated seed was collected in 2024, because we focused all crosses on those where both parents were carefully chosen. Priority target traits for crossing included early/late ripening, powdery mildew resistance (especially combined with late ripening), and fruit size, firmness, and cracking resistance. Additional traits targeted included self-fertility, resistance to X-disease, late blooming, and genetic diversity. We embryo-rescued approximately 2170 seeds in 2024, including 1500 from crosses targeting early ripening. Across the project period of 2022-2024, the breeding program made a total of 191 crosses, producing an estimated 27,325 seed. Also during this period, approximately 3100 seedlings enriched for target attributes were transplanted to the field. Prior to transplanting, we utilized DNA tests for selffertility, powdery mildew resistance, cracking, and firmness to eliminate more than 1200 seedlings. These less-desirable seedlings would otherwise have taken up more than 1.3 acres of land and cost approximately \$47,000 to maintain over the life of the seedling orchard (~ 5-6 years).

Orchard Management (Objective 4)

As in prior years, the orchards at Prosser (Roza and IAREC blocks) were sprayed regularly for insect and disease control. A delayed dormant spray was applied on 30 March. Aside from the delayed dormant and postharvest sprays for leafhopper control (which ended on 21 September), spraying occurred at 2-week intervals. In addition to leafhoppers, target insects included aphids, cherry fruit fly, cherry maggot, spider mites, and spotted wing *Drosophila* (SWD). Fungicide sprays were applied to control powdery mildew, except in the Phase 1 plantings where we encourage disease pressure to enable selection for resistance. Natural powdery mildew incidence was relatively mild in 2024.

Trees in the Roza orchard were winter-pruned. The younger trees at IAREC were managed by a combination of winter and summer pruning, including summer hedging of the most vigorous seedling blocks. Fertilizer recommendations were provided by Jeff Sample based on soil test results, and feeding occurred via fertigation (IAREC) or broadcast (Roza).

We routinely test parental trees for the presence of Prune dwarf virus (PDV) and Prunus necrotic ringspot virus (PNRSV). In prior years, we have detected one or both viruses at a relatively low rate (24% in 2022, 10% in 2023). However, in 2024, the rate of detection was much higher (93% for PDV, 42% for PNRSV). The most likely reason for the higher rate of detection is cleaner extractions in 2024, resulting in higher sensitivity. For samples with mild infections (high Cp values in the PCR test), it is also possible that these trees only recently became infected (perhaps by the use of infected pollen). The majority of trees do not display obvious symptoms. We are removing the most highly infected trees, and are exploring the possibility of using cryotherapy (a new tissue culture technique) to clean up our parental collection. Preliminary results of cryotherapy from the Clean Plant Center Northwest (CPCNW) are promising, but the technique needs to be confirmed as both effective and cost-conscious before we proceed.

For Little Cherry Disease (LCD), the breeding program tested 101 samples. These samples included parental trees in the B53 and C53 blocks at the Roza, symptomatic trees at IAREC, mother trees of

budwood sent for propagation (IAREC), newly planted nursery-grown trees (IAREC), and a sample of R3 and R19 trees in the Phase 3 plantings at Cherry Barn. All samples tested negative for X-disease and Little cherry virus 2. An additional 82 trees in the B53 block were tested by Scott Harper's lab, and six were found to have low-level X-disease infection. These trees will be removed this fall/winter.

Executive Summary

Project Title: "A robust PNW sweet cherry breeding and genetics program, 2022-2024"

Key words: breeding, DNA testing, optical sorting

Abstract

The PNW cherry breeding program continues its progress since the 2018 relaunch. Two promising Phase 3 selections, the early-ripening R19 and R3, are nearing the end of their 2023-2025 trial period. Pending favorable results in 2025, these two are expected to be released as new cultivars later that year. Newer selections in Phase 2 are also performing well, and one or more of these are expected to advance to Phase 3 after the 2025 season. The deployment of a new optical sorter, tested in 2023 and 2024, will enable cost-saving labor reductions as well as producing more consistent results. More than 3000 new Phase 1 seedlings have been planted in the last three years, derived almost exclusively from bi-parental crosses targeting industry priority traits. The breeding program has also successfully deployed newly developed DNA tests for fruit firmness and cracking along with existing tests for self-fertility and powdery mildew, eliminating > 1200 inferior seedlings at the greenhouse stage, with projected savings of thousands of dollars in planting and management costs.