FINAL PROJECT REPORT

Project Title: Screening for fruit powdery mildew resistance in the breeding program

PI:	Cameron Peace	**Co-PI (2):**	Claudia Probst → Prashant Swamy
Organization:	WSU-Horticulture	**Organization**:	WSU-Plant Pathology
Telephone:	509-335-6899	**Telephone**:	509-786-2226
Email:	cpeace@wsu.edu	**Email**:	prashant.swamy@wsu.edu
Address:	Johnson Hall 39	**Address**:	WSU – IAREC
Address 2:	PO Box 646414	**Address 2**:	24106 N Bunn Rd
City:	Pullman	**City**:	Prosser
State/Zip:	WA 99164	**State/Zip**:	WA 99350

Co-PI (4):	Bernardita Sallato	**Co-PI (5):**	Daniel Edge-Garza
Organization:	WSU-Horticulture	**Organization:**	WSU-Horticulture
Telephone:	509-439-8542	**Telephone:**	509-335-0544
Email:	b.sallatocarmona@wsu.edu	**Email**:	daniel.edgegarza@wsu.edu
Address:	WSU – IAREC	**Address:**	Johnson Hall 149
Address 2:	24106 N Bunn Rd	**Address 2**:	PO Box 646414
City:	Prosser	**City**:	Pullman
State/Zip:	WA 99350	**State/Zip**:	WA 99164

Cooperators: Gary Grove, Neusa Guerra, and Per McCord (WSU IAREC), Alexandra Johnson (graduate student, WSU Pullman)

Other funding sources

Agency Name: WTFRC/OSCC
Amount awarded: $150,000 (2017)
Notes: "Streamlining the Pacific Northwest Sweet Cherry Breeding Program." PI: Peace. Co-PI: Sallato.

Agency Name: WTFRC/OSCC
Amount awarded: $150,000 (2018)
Notes: "Sweet cherry breeding: identifying genetically superior selections." PI: Peace → McCord. Co-PIs: Sallato, Peace.

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" PIs: Iezzoni & Peace. Co-PIs include McCord.

Total Project Funding: $57,823

Budget History:

Item	Year 1: 2017	Year 2: 2018
WTFRC expenses		
Salaries [a]	9,001	12,454
Benefits	3,872	5,323
Wages [b]		8,000
Benefits		800
Equipment		
Supplies [c]		7,096
Travel [d]		2,327
Plot Fees [e]	4,475	4,475
Miscellaneous		
Total	17,348	40,475

[a] 0.25 FTE for an associate in research Prosser (2017 & 2018); 1 month salary and benefits for genetic screening technician in the WSU Tree Fruit Genotyping Lab (2018)

[b] Time slip field workers (2018)

[c] Bags for artificial inoculation of fruit; DNA test development consumables

[d] Prosser-Pullman travel for meetings among PIs

[e] Plot fees and maintenance of block C53

RECAP ORIGINAL OBJECTIVES

Overall goal: Develop a reliable, efficient assay for revealing genetic potential for fruit powdery mildew (PM) resistance that can be routinely used in the PNW sweet cherry breeding program.

Specific objectives:

1. Determine genetic potential for fruit powdery mildew resistance/tolerance in the PNW sweet cherry breeding program by evaluating a large, representative germplasm set using natural and artificial inoculation
2. Convert large-effect genetic factors discovered for fruit powdery mildew resistance/susceptibility into a diagnostic DNA test for routine breeding use

SIGNIFICANT FINDINGS

- Fruit PM resistance = Foliar PM resistance, genetically
- One major factor of genetic resistance ("Pmr1") was determined to be widespread in our breeding program, inherited from PMR-1, 'Moreau', and Mildew-Immune Mazzards
- Complete absence of fruit and foliar PM infection was associated with a dominant allele inherited from any of the above-mentioned sources
- The Pmr1 status (lacking or carrying one or two copies of this genetic factor) was established for selections and parents in the breeding program
- Two effective screening methods are now available for identifying PM resistance vs. susceptibility in the breeding program:
  - Detached leaf disk assay
  - DNA test
- Other genetic factors for resistance and/or suppression of infection severity appear to exist in the breeding program

RESULTS & DISCUSSION

Over two seasons, resistant and susceptible breeding individuals were successfully discriminated for both fruit and foliar PM infection. In the orchard in 2017 and 2018, a high degree of disease spread on fruit and leaves was achieved throughout the experimental block. In the pathology lab in 2017, the detached leaf disk assay was found to be as effective as reliable quantitative PCR for pathogen presence/absence (indicating susceptibility/resistance). The clear discrimination across genetically variable germplasm with known pedigree structure allowed strong conclusions to be made about the genetic control of fruit PM infection:

(1) The main genetic factor giving fruit resistance is the same as that giving foliar resistance (Figure 1).
(2) The foliar resistance genetic factor "Pmr1" from the Toyama selection PMR-1 (that it inherited from 'Moreau', as did 'Chelan') is the same factor as that from the small-fruited Mildew-Immune Mazzards.

(3) This resistance allele is dominant, such that plants only need to inherit one copy to be resistant to both fruit and foliar PM.
(4) Seedlings with genetic resistance to fruit-and-foliar PM infection are common throughout the PNW sweet cherry breeding program because of extensive use of parents with the resistance allele for the past decade.

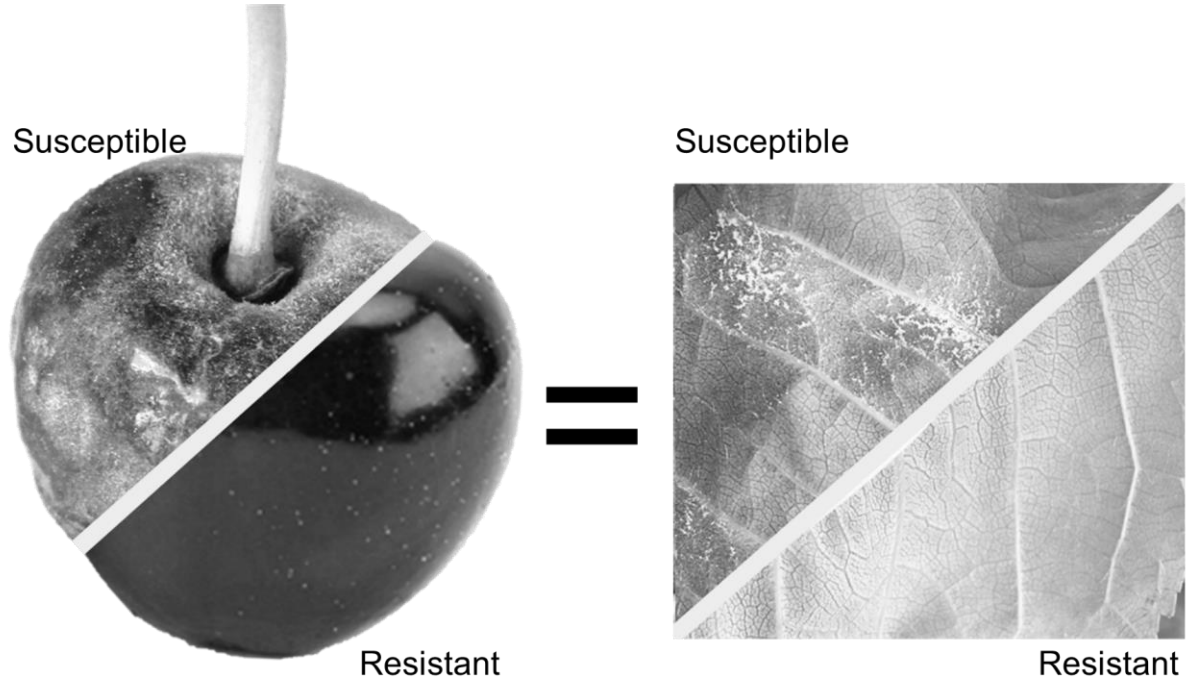

Figure 1: The main discovery of the project. Genetically, resistance vs. susceptibility to powdery mildew of fruit is the same as for leaves. The genetic factor (allele) responsible for resistance is called "Pmr1", which several breeding parents have. Trees having Pmr1 in their genetic makeup are resistant to both fruit and foliar PM. Trees without Pmr1, which includes most cultivars of the PNW industry, are susceptible to PM.

From 2018's field evaluation of the breeding program's parent block (B53), across which the pathogen was allowed to spread, all parents having Pmr1 in their genetic makeup had no PM infection symptoms – zero mildew for all replicate trees examined. Of the ~40 parents without Pmr1, only four had zero mildew for all of their replicate trees – which included 'Regina' and 'Hedelfinger'.

Other sources of resistance might exist in the material tested. In the genetically variable families of the evaluated RosBREED block (C53), some cases of zero-mildew (both fruit and foliar) were observed in trees of offspring not carrying a Pmr1 resistance allele. Mirroring observations in the B53 parent block, 'Regina' was a parent of several of these offspring, as was 'Venus' (whose mother is 'Hedelfinger'). Some families had offspring trees with no fruit incidence and low foliar incidence, for which 'Regina' and 'Venus' were also commonly the parents. However, the 2018 season's orchard evaluations of fruit infection did not include multiple days of assessment through the season and had several discrepancies between tree replicates of the same genotype and with the 2017 season's orchard observations. These issues undermined our ability to effectively dissect the genetics of quantitative resistance beyond Pmr1 that the 2017 results had pointed to.

From whole-genome DNA profiling (results of the RosBREED project), ‘Regina’, ‘Venus’, several other parent cultivars*, and at least a third of selections of the breeding program were determined to carry an allele at the *Pmr1* chromosomal region that looks similar to that of Mildew-Immune Mazzards – so we call this the “Pmr1-like” allele. Two seasons of observations found that most offspring with the Pmr1-like allele became PM-infected. However, in 2018 about one in every four ‘Regina’ offspring with the Pmr1-like allele were not infected (6 of 22) whereas all ‘Regina’ offspring (26) without that allele became infected. So, the Pmr1-like allele might be a “leaky” version of normal Pmr1 that sometimes provides the possibility for zero or low mildew infection but depends on environmental conditions or depends on alleles of other genomic regions present in some offspring. Or, the resistance (or lower susceptibility) of ‘Regina’ and offspring might be entirely due to genetic factors independent of Pmr1.

* Other cultivars we determined from DNA profiling to have a Pmr1-like allele include: ‘Venus’, ‘Schneiders’ (the mother of ‘Regina’), ‘Hedelfinger’ (the mother of ‘Venus’), ‘Sato Nishiki’, ‘Early Burlat’, and ‘Cristobalina’.

The clear, “single-gene” resistance provided by Pmr1 leads to the question of whether it can be overcome by the pathogen. If not, a powerful breeding strategy would be to eventually have all new cultivars carry this allele. If it can be overcome, it would still be useful to continue to enrich the breeding genepool with this allele. Another question revolves around the genetic control of the alternative PM resistance (or reduced susceptibility, tolerance, or leaky resistance) that a few cultivars such as ‘Regina’ have. If those further genetic factors could be identified, having a DNA test for them and knowing which parents, seedlings, and selections carry them would facilitate additional breeding strategies to combat this high-priority disease problem for the PNW cherry industry.

Two assays are now available for routinely discriminating resistance vs. susceptibility for both fruit and foliar PM resistance in cherry breeding germplasm: a DNA test and the detached leaf assay. These two efficient assays were confirmed to be effective for both fruit and foliar PM resistance, and both assays can be used on seedlings through to mature trees without exposing the whole plants to the pathogen. The DNA test can be integrated into routine operations of greenhouse-stage DNA testing of seedlings. The DNA test can also be used to confidently plan crosses that result in a high proportion of seedlings resistant to fruit and foliar PM, and to ascertain which selections have genetic resistance.

EXECUTIVE SUMMARY

This two-year project aimed to develop a reliable, efficient assay for revealing genetic potential for fruit powdery mildew (PM) resistance for routine used in the PNW sweet cherry breeding program. The aim was achieved by evaluating a large, representative germplasm set containing genetic variability for PM resistance. Two assays are now available for routinely discriminating resistance vs. susceptibility for both fruit and foliar PM resistance in cherry breeding germplasm: a DNA test and the detached leaf assay.

Over two seasons, resistant and susceptible breeding individuals were successfully discriminated for both fruit and foliar PM infection. In the orchard, a high degree of disease spread on fruit and leaves was achieved throughout the experimental block. In the pathology lab, the detached leaf disk assay was as effective as reliable quantitative PCR for pathogen presence/absence. The clear discrimination across genetically variable germplasm with known pedigree structure allowed strong conclusions to be made about the genetic control of fruit PM infection:

(1) The main genetic factor giving fruit resistance is the same as that giving foliar resistance.
(2) The foliar resistance genetic factor "Pmr1" from the Toyama selection PMR-1 (that it inherited from 'Moreau', as did 'Chelan') is the same factor as that from the small-fruited Mildew-Immune Mazzards.
(3) This resistance allele is dominant, such that plants only need to inherit one copy to be resistant to both fruit and foliar PM.
(4) Seedlings and selections with genetic resistance to fruit-and-foliar PM infection are common throughout the PNW sweet cherry breeding program because of extensive use of parents with the resistance allele in the past decade.

Field observations of the 2018 season confirmed those of 2017 for the strong effect of Pmr1. However, the second season's orchard PM evaluations of the genetically variable RosBREED germplasm set were not fine-scaled enough to dissect degrees of resistance beyond Pmr1, i.e., that of 'Regina', 'Venus', and some other cultivars.

The clear, "single-gene" resistance provided by Pmr1 leads to the question of whether it can be overcome by the pathogen. If not, a powerful breeding strategy would be to eventually have all new cultivars carry this allele. If it can be overcome, it would still be useful to continue to enrich the breeding genepool with this allele. Another question revolves around the genetic control of the alternative PM resistance (or reduced susceptibility) that a few cultivars such as 'Regina' have. If those further genetic factors could be identified, having a DNA test for them and knowing which parents, seedlings, and parents carry them would facilitate additional breeding strategies to combat this high-priority problem for the PNW cherry industry.