FINAL PROJECT REPORT

Project Title: Fire blight resistance and fruit quality in new Washington cultivars

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Notes: **Title** 'RosBREED: Combining disease resistance with horticultural quality in new rosaceous cultivars'; Norelli: Co-PI and Team Leader for Pathology; Evans: Co-PI; Peace: Co-Project Director

Total Project Funding: $106,110

Budget History:

Item	Year 1:	Year 2:	Year 3:
Salaries[1]	6,132	6,255	6,380
Benefits	491	500	510
Wages[2]	4,000	9,152	9,518
Benefits	392	897	933
Supplies[3]	6,050	7,050	6,150
Travel[4]	560	1,120	1,120
Plot Fees[5]	4,500	4,000	3,100
Miscellaneous[6]	10,300	3,000	14,000
Total	32,425	31,974	41,711

1: summer student for trait measurement, Kearneysville, **2**: wages for time-slip labor for orchard management and trait measurement, Wenatchee, **3**: combined total orchard, greenhouse and laboratory supplies for both Kearneysville and Wenatchee, **4**: travel to field plots in Wenatchee, **5**: Kearneysville and Wenatchee, **6**: genotyping, Kearneysville and Pullman.

ORIGINAL OBJECTIVES

1. Determine the best sources of fire blight resistance in *Malus sieversii* to incorporate in the WSU apple breeding program.

2. Determine fire blight resistance levels in RosBREED reference germplasm for current use in the WSU apple breeding program.

3. Develop DNA tests to enable the fire blight resistance of 'Enterprise' and 'Splendour' to be efficiently evaluated in seedlings and to evaluate genetic resistance in current elite selections.

The overall goal of this project was to enable selection of new Washington apple varieties that are fire blight resistant and have superior fruit quality, as soon as possible. The specific goal of the first objective was to select among the highly resistant *M. sieversii* accessions the best parents to use in the WSU apple breeding program (**WASB**) for strong fire blight resistance based upon the accession's fruit quality. The goal of the 2nd objective was to determine the fire blight resistance-influencing loci (**FBL**) among the current parents and seedling of the WABP. The goal of the third objective was to develop and evaluate DNA markers for known FBL among select parents that have been used in the WABP.

This research addressed the 2015 Apple Crop Protection High Priority Research topic "Fire Blight" and the Apple Horticulture High Priority Research topic "Improved scion and rootstock genetics".

SIGNIFICANT FINDINGS

- Nine *M. sieversii* accessions were identified as potential sources of high level fire blight resistance for use in the Washington apple breeding program (**WABP**) that were resistant to fire blight shoot and blossom infection (Obj. 1)
- Crosses were made with four fire blight resistant *Malus sieversii* accessions to incorporate higher levels of fire blight resistance into the WABP (Obj. 1)
- 1650 trees of 556 elite cultivars and their seedlings were challenged with fire blight in 2016 and 2017 to determine their resistance and facilitate the discovery of fire blight tolerance genes among the existing seedlings and selections of the WABP (Obj. 2)
- Two DNA markers were developed for the fire blight resistance locus on chromosome 7 of 'Enterprise'
- Two DNA markers were developed for the fire blight resistance locus on chromosome 5 of 'Splendour'

RESULTS & DISCUSSION

Obj. 1: Determine the best sources of fire blight resistance in *Malus sieversii* (wild progenitor of the domestic apple) to incorporate in the WSU apple breeding program

A previous WTFRC project identified *M. sieversii* accessions that are highly resistant to fire blight shoot infection. This project evaluated 21 shoot blight resistant accessions to for their resistance to blossom blight and fruit quality to determine the best accessions to incorporate into the WSU apple breeding program.

Evaluation of blossom blight susceptibility: Twenty accessions were evaluated for their resistance to fire blight blossom infection. Of these, 6 accessions (PI657054, PI657085, GMAL3552.v, GMAL3608.h, GMAL4211.a, GMAL4211.d) were found to have unacceptably high levels of blossom blight susceptibility in either the West Virginia or Washington trails.

Evaluation of fruit quality: Fruit of the 21 accessions were harvested from the research plot at USDA-ARS Kearneysville, WV at Cornell starch stage 3 and shipped to the TFREC, Wenatchee for evaluation. Fruit quality was evaluated using the full range of instrumental and sensory traits by the WSU apple breeding program (WABP) on arrival and after two months of refrigerated air storage.

None of the *M. sieversii* accessions in this trial have commercially acceptable fruit quality. However, fruit quality characteristics were used to select among the accessions those most suitable for use in the WABP. In 2016 crosses were made with 3 accessions, including GMAL4002.k. In 2017 an additional cross was made with GMAL3688.c. Some of the seedlings from the 2016 and 2017crosses were evaluated for their resistance to fire blight. Selected seedlings are currently being grown to maturity.

Obj. 2: Determine fire blight resistance levels in RosBREED reference germplasm

While Objective 1 focused on identifying the best sources of fire blight resistance for future use as parents in the WABP, the purpose of Objective 2 was to leverage resources previously developed as part of RosBREED 1 to identify genetic factors associated with fire blight resistance/susceptibility. Although complete immunity or resistance may not be available in the current WABP or RosBREED material, we know that there is a gradient of susceptibility among this material that ranges from highly susceptible to "tolerant". An example of a tolerance is 'Delicious', which at times can become infected with fire blight but rarely are the losses due to this disease devastating in nature. On the other hand, individuals like 'Gala' or 'Jonathan' are highly susceptible and losses can be very severe. Although "tolerance" is potentially a useful type of resistance, the genetic factors controlling "tolerance" are not understood/known. Our goal in this project was to identify fire blight resistance-influencing loci by determining levels of resistance to fire blight in the RosBREED apple germplasm, which is a collection of pedigree connected elite cultivars and their seedlings.

Because fire blight is a sporadic disease from year to year and in its distribution within the orchard, reliable evaluation of fire blight resistance requires artificial challenge of test plants with the fire blight bacteria. Trees of the RosBREED reference germplasm have been grafted onto M.111 rootstock and planted at WSU's Columbia View Orchard. The reference germplasm included several cultivars known to be either susceptible or resistant to fire blight that served as controls to ensure that minimum disease pressure thresholds were achieved in the tests. Vigorously growing shoots will be challenged by dipping a pair of scissors in a suspension of the bacteria and then cutting the youngest leaves of the shoot tip. Resistance will be determined by measuring the percent of the current seasons shoot length that

becomes infected. Because economic losses from fire blight are the result of the death of young trees and woody tissue, rating cultivar resistance based upon progression of disease in shoot tissue has proven a reliable method of accessing fire blight resistance.

The number of shoots inoculated per tree ranged from 3-10 with an average of about 6 shoots per tree. The resistance response to fire blight inoculation was variable ranging from highly susceptible to resistant. The average proportion of healthy tissue ranged from 0.0 (all inoculated shoots were killed) to 1.0 (all inoculated shoots did not develop symptoms of fire blight infection). Of the 556 individuals evaluated, about 16% had an average proportion of healthy tissue of ≤ 0.25, which is indicative of high susceptibility. In contrast, about 55% of individuals had average proportions of healthy tissue ≥0.25 but <0.75 and should be considered susceptible. Approximately, 29% of individuals had average proportions of healthy tissue ≥0.75 and should be considered tolerant to fire blight. Average age of wood infected ranged from 0.0 to 2.5 with average ratings of >1.0 indicating that on average fire blight moved into the previous season's growth. 63.8% individuals had a maximum rating ≥ 2.0, indicating that in at least one challenged shoot the pathogen moved into 2 year or older tissue. Higher maximum ratings (>1.0) indicate more severe infections.

The fire blight resistance / susceptible results for many of the apple varieties grown in Washington State are given in Table 2. Among the cultivars listed in Table 2, Enterprise and Tsugaru were the most resistant to fire blight. Aurora Golden Gala, Cosmic Crisp®, Delicious, Empire, Golden Delicious, and Fuji were classified as moderately resistance in both years, with Fuji performing better than anticipated. GingerGold and Winter Banana were the most susceptible cultivars to fire blight. Gala and Granny Smith were also consistently rated either highly susceptible or moderately susceptible. The most variable cultivars were Piñata® and Spartan which were evaluated as highly susceptible one year and moderately resistant the other. The complete data will be available soon through the WSU Tree Fruit Extension website and the WSU Decision Aid System once the peer-reviewed manuscript* describing the research is accepted for publication.

Although information on the fire blight resistance of apple cultivars is available on the worldwide-web, information on new cultivars has been lacking and much of the available information is anecdotal, based on personal accounts rather than research. This study was the first systematic fire blight evaluation of many new cultivars with controlled pathogen challenge in replicated field trials over multiple years. The results of this study have been written up and submitted for peer review publication in the *Plant Pathology* journal. The information will also be made available to cooperative extension and commercial farm advisors.

Furthermore, the results are currently being analyzed by Sarah Kostick, a graduate student with Kate Evans, using FlexQTL software to identify and predict fire blight resistance-influencing loci. This software was developed and used in the "RosBREED" project utilizing high-resolution genome scans that were previously completed by the project and our analysis will draw upon expertise from the project. The fire blight resistance-influencing loci identified will allow for the future development of additional DNA tests for fire blight resistance.

Table 2. Fire blight resistance and susceptibility of many of the apple varieties grown in Washington State. Resistance was evaluated by the proportion of the current season's shoot length blighted following challenge with the fire blight pathogen (*Erwinia amylovora*). The "control" cultivars used to determine resistance class are highlighted in bold (see sidebar).

Variety	Resistance/Susceptibility Class (2016 / 2017)
'Aurora Golden Gala'	MR / MR
'Cripps Pink' (Pink Lady®)	I / MS
'Cameo'	MS / MR
'Delicious'	**MR/MR**
'Empire'	MR / MR
'Enterprise'	HR / MR
'Fuji'	MR / MR
'Gala'	MS / HS
'GingerGold'	HS / HS
'Golden Delicious'	MR / MR
'Granny Smith	MS / HS
'Honeycrisp'	MS / MR
'Jonagold'	MR / MS
'Jonathan'	**HS/HS**
'McIntosh'	**MS/MS**
'Minnewashta' (Zestar!®)	MS / I
Pinova' (Piñata®)	MR / HS
'Rome Beauty'	MS / MR
Russian seedling #12740-7A	**HR/MR**
'Spartan'	HS / MR
'Splendour'	MR / I
'Tsugaru'	HR / MR
'WA2' (Sunrise Magic®)	MS / MR
'WA38' (Cosmic Crisp®)	MR / MR
'Winter Banana'	HS / HS

Key to resistance / susceptibility class codes:

HR: highly resistance (significantly different from both MS control cultivar (McIntosh) and HS control (Jonathan)).

MR: moderately resistant (significantly different from HS control, but not MS control)

I: intermediate (not significantly different from any of the control cultivars)

MS: moderately susceptible (significantly different from HR control cultivar (Russian seedling #12740-7A), but not the MR control cultivar (Delicious))

HS: highly susceptible (significantly different from both MR and HR control cultivars)

Obj. 3: Develop DNA tests to enable the fire blight resistance of 'Enterprise' and 'Splendour' to be efficiently evaluated in seedlings and to evaluate genetic resistance in current elite selections

Although most of the genetic factors controlling "tolerance" to fire blight in the WABP are currently not known, we do know that some of the parents previously used in the program, such as 'Enterprise', and 'Splendour' are tolerant to fire blight. The goal of Objective 3 was to develop DNA tests for the genetic factors controlling fire blight resistance in these parents to enable efficient evaluation of their progeny seedlings and evaluate the genetic resistance of current elite selections within the WABP.

Before a DNA test could be developed the loci needed to be genetically characterized, and identifying the loci controlling the fire blight resistance of 'Enterprise' and 'Splendour' was beyond the scope of this project. However, this was part of both the USDA-NIFA RosBREED (which targeted 'Splendour') and the European FruitBreedomics (which targeted 'Enterprise') projects. We originally anticipated results from these projects to be available to us to develop DNA tests within the third year of this project. Unfortunately, the loci took longer than anticipated to identify due to unforeseen complexities in the inheritance of fire blight resistance and we therefore requested a no cost extension to complete the development of the DNA in 2018.

A genetic locus controlling fire blight resistance in 'Enterprise' was identified on chromosome 4 (van de Weg *et al.* 2018). Fei Xiong Luo, a graduate student in Cameron Peace's program, developed and evaluated several DNA markers for this locus by comparing DNA test results of seedlings with results from direct challenge of seedlings with the fire blight pathogen. In the end, two distinct seedling populations were used to validate the 'Enterprise' DNA test.

Using new genomics methods candidate loci for fire blight resistance in 'Splendour' were identified in the RosBREED project. Stijn Vanderzande, a post-doctoral RosBREED scientist working with Cameron Peace, similarly developed and evaluated DNA markers for a fire blight resistance locus found on chromosome 5.

The DNA tests for the fire blight resistance loci from 'Enterprise' and 'Splendour' were both used successful in RosBREED experimental crosses this past month and will be available for use in the WABP in 2019.

LITERATURE CITED

Eric van de Weg, Mario Di Guardo, Melanie Jänsch, Didier Socquet-Juglard, Fabrizio Costa, Isabelle Baumgartner, Giovanni A.L. Broggini, Markus Kellerhals, Michela Troggio, François Laurens, Charles-Eric Durel, and Andrea Patocchi. 2018. Epistatic fire blight resistance QTL alleles in the apple cultivar 'Enterprise' and selection X-6398 discovered and characterized through pedigree-informed analysis. Molecular Breeding 38:5. https/doi.org/10.1007/s11032-017-0

EXECUTIVE SUMMARY

Fire blight is a devastating disease affecting the Washington apple industry. The disease can kill young trees outright or result in permanent structural damage to mature trees. Spray application of antibiotics is currently the most effective control for fire blight however its effectiveness is limited by the development of streptomycin resistance in the pathogen and its lack of efficacy in controlling the shoot blight phase of the disease. Breeding for resistance to fire blight offers one of the most effective and sustainable options for managing this disease. The goal of this project was to develop the resources and tools necessary to effectively incorporate fire blight resistance into the WSU apple breeding program (**WABP**).

Four significant accomplishments of this project have made this goal attainable:

1) A DNA marker was developed for the major fire blight resistance locus present in 'Enterprise' which will facilitate the efficient screening of 'Enterprise' progeny within the WABP.
2) A DNA marker was developed for a fire blight resistance locus present in 'Splendour' which will also facilitate the efficient screening of 'Splendour' progeny within the WABP.
3) Four crosses were made to incorporate a high-level fire blight resistance from wild *Malus sieversii* into the WABP. Although several additional crosses will be required before the progeny of these crosses are expected to have superior fruit quality, the process has begun.
4) The fire blight resistance of over 500 elite cultivars and their seedlings was determined under Washington State growing conditions. This information will not only be used for extension purposes and selecting future WABP parents but is also being analyzed to identify fire blight influencing loci within current seedlings of the WABP.

The development of fire blight resistant scion cultivars is a long-term goal. The recent success of fire blight resistant apple rootstocks demonstrates that fire blight resistant cultivars can be developed and that it is an important trait for the apple industry. However, the acceptance barrier for a fire blight resistance scion cultivar will be much higher because unlike rootstocks which are selected based upon horticultural performance, scion cultivars are selected based upon market demand. None the less, the increased efficiency of DNA informed breeding and the growing trend toward new cultivars in the apple industry indicate that the scion cultivars of the future could, and should, be more resistant to fire blight. Incorporating fire blight resistance into the WABP apple breeding program will lead to the release of new cultivars with fire blight resistance similar to or greater than that of 'Delicious' and thereby greatly reduce the occurrence of fire blight and reduce the need for high-priced applications of antibiotics.