FINAL PROJECT REPORT

Project Title: Incorporating fire blight resistance into Washington apple cultivars

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Cooperators: Cameron Nursery, LLC, Eltopia, WA donated 4,000 MM.111 EMLA rootstocks to project for tree propagation.

Other funding sources

Agency Name: USDA-ARS-National Plant Germplasm System
Amt. awarded: $10,000
Notes: *Title:* 'Genotyping By Sequencing (GBS) *Malus sieversii* accessions to identify and characterize new sources of resistance to *Erwinia amylovora'.* Funds were provided to identify genetic markers in the accessions of *M. sieversii* used in this WTFRC project.

Total Project Funding: 84,479

Budget History:

Item	2012	2013	2014
Salaries		$11,990[1]	$13,760[1]
Benefits		$1,079	$1,216
Wages			
Benefits			
Equipment			
Supplies		$1,760	$1,800
Travel	$500	$2,650	$3,070
Plot Fees	$2,200	$2,200	$14,600[2]
Miscellaneous	$500		$27,154[3]
Total	$2,200	$19,679	$62,600

Footnotes: 1: 2 summer students to assist with fire blight inoculation, recording data and plant maintenance; **2:** plot fees higher in year 3 due to planting of orchard associated with obj. 2, **3:** propagation of trees for obj. 2.

OBJECTIVES

1. Identify the best *M. sieversii* (wild apple) accessions to be used as sources of fire blight resistance in the WSU apple breeding program.

2. Establish planting of RosBREED apple Crop Reference Set and Washington State Breeding Pedigree Set in Wenatchee, WA for future fire blight evaluation.

The goal of this project was to develop the genetic resources necessary to incorporate the selection of fire blight resistance into the Washington State University (WSU) apple breeding program.

The goal of Objective 1 was to identify the best sources of fire blight resistance within *Malus sieversii*, the wild large-fruited progenitor of domesticated apple, for use in scion breeding.

The goal of Objective 2 was to establish a planting of the RosBREED reference germplasm for future fire blight evaluation. This will allow us leverage advances made by the RosBREED project to enable marker-assisted breeding of fire blight resistance in existing seedlings and selections of the WSU apple breeding program.

SIGNIFICANT FINDINGS

- Field plantings of 194 wild apple accessions and 8 control cultivars were established at WSU's Columbia View Orchard and USDA-ARS, Kearneysville, WV in 2012 for the purpose of identifying the best wild apple accessions to be used as sources of fire blight resistance in the WSU apple breeding program.

- Based upon controlled challenge with the fire blight pathogen in 2013 and 2014 at both the Wenatchee, WA and Kearneysville, WV plantings, 12 wild *Malus sieversii* accessions were identified as highly resistant to fire blight and will serve as good sources for introducing strong fire blight resistance into the WSU apple breeding program.

- The RosBREED reference germplasm (approximately 600 cultivars) was budded onto MM.111 rootstock at Willow Drive Nursery and 3 replicate trees of each cultivar will be planted at WSU's Columbia View Orchard in spring 2015.

RESULTS & DISCUSSION

Objective 1: Identify the best *M. sieversii* accessions to be used as sources of fire blight resistance in the WSU apple breeding program.

The approach we used in evaluating resistance to fire blight in these wild apple (*Malus sieversii*) accessions was to use a severe inoculation procedure with a high dose of the fire blight pathogen (*Erwinia amylovora*) to ensure that a fire blight infection will be initiated, if possible, and then evaluate resistance based upon how far fire blight disease progresses in the infected shoot. An alternative approach would be to evaluate the trees based on their resistance to the incidence of infection, such as determining the number of blossom infection that occurred after flowers are sprayed with a relatively low dose of the fire blight pathogen. These types of evaluation methods are appropriate when evaluating chemical or biological control treatments, but they are not appropriate when selecting sources of resistance for breeding. Although resistance to disease progression, or severity of infection, is usually correlated with resistance to the incidence of infection, there is not a one to one association between these two types of resistance. We believe that growers could tolerate a relatively high level of fire blight blossom infection if those infections do not progress systemically through the tree. Because economic losses from fire blight are the result of the death of young trees and woody tissue, we believe rating cultivar resistance based upon progression of disease in shoot tissue is the most useful and appropriate method of accessing fire blight resistance.

As expected, after challenging vigorously growing shoot tips with the fire blight pathogen we observed diverse responses among the wild apple accessions ranging from highly susceptible to highly resistant. After challenge with the fire blight pathogen, typical fire blight infections developed on many of the wild apple accessions. In some cases the infections progressed through the current season's shoot growth into the previous season's wood or into 2 or 3year-old wood (highly susceptible response) and in a few cases into 4 year-old wood causing the death of trees. In other cases the infections progressed through much of the current season's shoot growth but did not penetrate into the previous season's growth (intermediate response). In several cases only minor evidence of disease was observed in the challenged leaves and infections did not progress from the leaf into the shoot (highly resistant response).

The amount of disease observed after challenge with a pathogen is the result of an interaction between the pathogen, the host plant and the environment. Fire blight severity is strongly influenced by environment and when evaluating plant material for its resistance it is important to separate the effect of the host's genetic resistance from the effect of environment. By evaluating the *M. sieversii* accessions in 2 very different environments, Wenatchee, WA and Kearneysville, WV, over 2 different years, we in-effect evaluated the material under 4 different environmental conditions. In many cases, an accession may have appears highly resistant in some of the tests, but appeared susceptible in others. This is the result of resistance that is strongly influenced by environment and not useful resistance to use in the breeding program. Although approximately 10-30% of the accessions may have appeared highly resistant in any individual test, only 6%, or 12 accessions, were consistently, highly resistant in multiple tests.

Table 1. The percent of the current season's shoot growth that developed fire blight symptoms following controlled challenge with the fire blight pathogen. Twelve *M. sieversii* accessions were consistently rated as highly resistant when evaluate in Wenatchee, WA (WA) or Kearneysville, WV (WV) in 2013 and 2014. "PI#" indicated *M. sieversii* accessions that are Plant Introductions into the permanent US National Plant Germplasm System. "GMAL#" indicated *M. sieversii* accessions that have not yet been assigned to the permanent collection. "N" is the number of shoots challenged in the test.

Genotype	WA 2013	N	WA2014	N	WV2013	N	WV2014	N
Robusta 5					0.00%	20	0.00%	27
GMAL4002.k			0.0%	4	0.00%	20	0.00%	40
PI657115			0.0%	4	0.00%	20		
PI657116			0.1%	4	0.00%	15	0.00%	15
GMAL3616.o			0.0%	4	0.00%	15	0.20%	31
GMAL4002.m					0.00%	20	0.30%	38
GMAL4211.d			0.0%	5	0.70%	20	0.00%	39
PI657054					0.00%	20	0.40%	40
PI657085					0.00%	19	0.50%	37
GMAL4211.a					0.00%	20	0.90%	28
GMAL3975.c			0.0%	4	0.30%	20	2.00%	38
GMAL3688.c	0.0%	5			0.00%	14	2.80%	27
GMAL3989.c			0.0%	4	0.60%	19	3.30%	38
Delicious					1.10%	18	3.80%	38
Golden Delicious			5.6%	17				
Gala							24.50%	31
Jonathan					74%	19		

Robusta 5 is a wild apple with small, bitter and astringent fruit that has been used as a source of fire blight resistance in the Geneva rootstock breeding program. The fire blight resistance of GMAL4002.k, PI657115, and PI657116 appeared equivalent to Robusata 5 in more than one test. GMAL3616.o, GMAL4002.m, GMAL4211.d, PI657054, PI657085 and GMAL4211.a had some slight fire blight development in one of the tests, but also appear comparable with Robusta 5 in resistance. GMAL3975.c, GMAL3688.c and GMAL3989.c did develop fire blight comparable with 'Delicious', which is a resistant cultivar, in the WV 2014 test. However, these accessions were also judged as useful sources of resistance since they performed well in other tests and cultivars with fire blight resistance comparable with Delicious would be desirable.

Not all accessions were evaluated in every test. The *M. sieversii* (wild apple) planting at WSU Columbia View Orchard sustained heavy deer damage in the spring and early summer of 2013. This resulted in a limited number of usable shoots for fire blight challenge in 2013 and only 32 of the 194 wild apple accession had a sufficient number of shoots for reliable fire blight evaluation. The orchard has since been protected by deer fencing and 2014 tests at Columbia View Orchard were more extensive. However, the number of replicate shoots evaluated at the Columbia View Orchard was lower.

Initially, the wild apple accessions were quantitatively ranked based upon the average distance the fire blight infection progressed in the shoot and then assigned into categories of highly resistant,

resistant, intermediate, susceptible or highly susceptible based upon comparison to known control cultivars in the trial. Those that responded similarly to Robusta 5 were rated as highly resistant; those similar to 'Empire' or 'Golden Delicious' were rating intermediate and those similar to 'Gala' or 'Jonathan', which was severely damaged by the fire blight challenge, were rated highly susceptible.

In addition to the average distance the fire blight infection progressed in the shoot, several other measures of resistance were evaluated and the ratings of the wild apple accessions were adjusted in a conservative manner. Other measures of resistance included the average age of the oldest tissue infected and the percent of the current season's shoot length infected. The analysis of these other measures sometimes led to the "conservative" adjustment of the resistance based on an accession's worst performance, not its best performance or average performance. For example, if an accession was rated as resistant based upon distance of disease progression but intermediate based upon age of wood infected, its rating would be adjusted down from resistant to intermediate; however if the cultivar ranked higher based upon the analysis of another measure of resistance, its rating would not increase from resistant to highly resistant. Similarly, if an accession was rated differently in the WA and WV trials, or in 2013 and 2014, the accession would be given the lower of the two ratings. Although this conservative adjustment of resistance rating may be considered somewhat "unscientific" or unfair, it should help to ensure that the accessions selected as sources of resistance for the breeding program are in fact resistant.

Other observations of fire blight development were also considered in adjusting the resistance rating of the wild apple accessions. Because we were looking at a genetically diverse collection of wild apples we looked for the unexpected. Atypical from observations in most domesticated apple cultivars, we observed several wild accessions that appeared quite resistant to the initiation of fire blight infection, but when an infection did occur it progressed rapidly into older wood. In the case of GMAL4028.h, only one of 35 shoots challenged with the fire blight pathogen in 2013 and 2014 developed into a shoot infection but that infection progressed through 2 year-old wood into the central leader, destroying the young tree (Fig. 1). Because only 1 of 34 challenged shoots were infected, the accession's average distance of progression and average age of wood infected was still quite low, however this is obviously not a useful type of resistance to incorporate into the breeding program. To eliminate this type of resistance from consideration, any accession with a single infection that progressed into 2 year-old wood was rated as highly susceptible and an accession with an infection that progressed into the previous season's growth was ranked as susceptible.

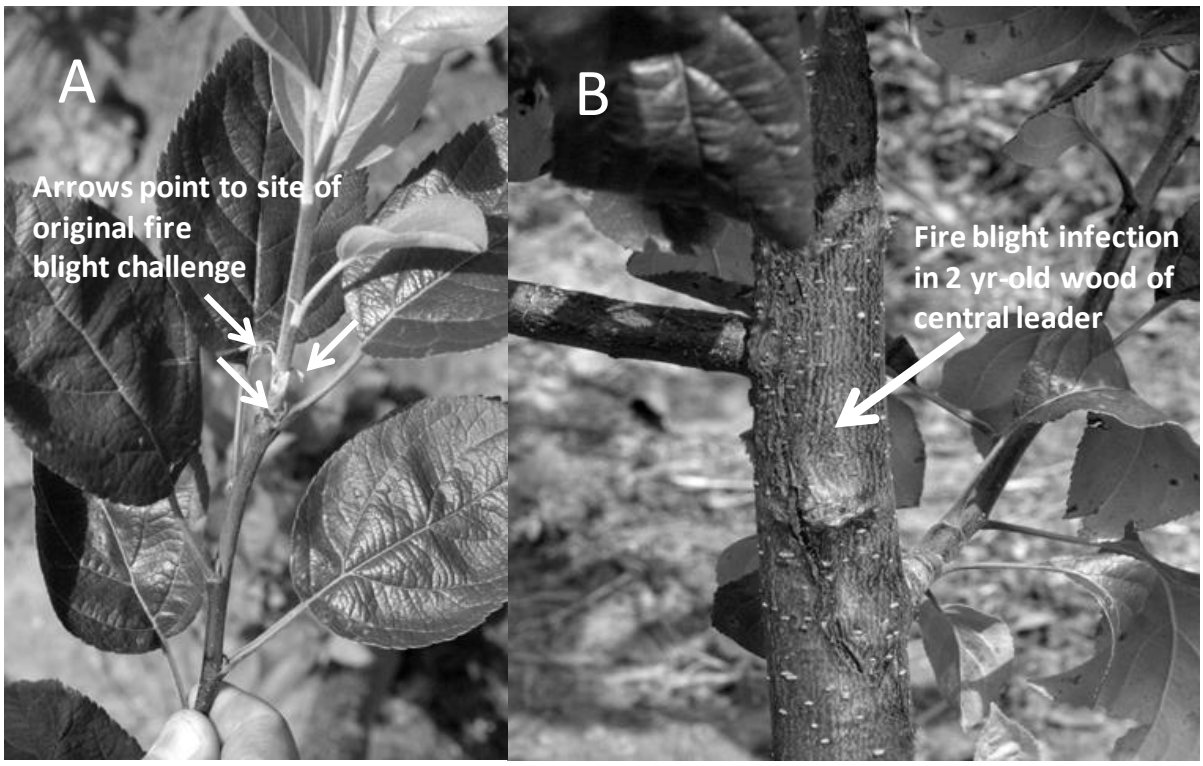

Figure 2: Evaluation of fire blight resistance of *M. sieversii* GMAL4028.h. **A**: In 34 of 35 fire blight shoots challenged in 2013 and 2014 no evidence of infection could be observed 6 weeks after inoculation. **B**: In 1 of 35 fire blight challenged shoots, fire blight progressed into 2 year-old wood of central leader.

In addition, naturally occurring blossom and shoot infections (infection that were not the result of our controlled fire blight challenge) were recorded and monitored, and resulted in downward adjustment to an accession's resistance rating if infection resulted in significant fire blight damage to the tree. In 2013, after adjustment 26 wild apple accessions were rated as highly resistant, 38 were rated resistant and 128 were rated intermediate, susceptible or highly susceptible (Fig. 2). Similar results were obtained in 2014.

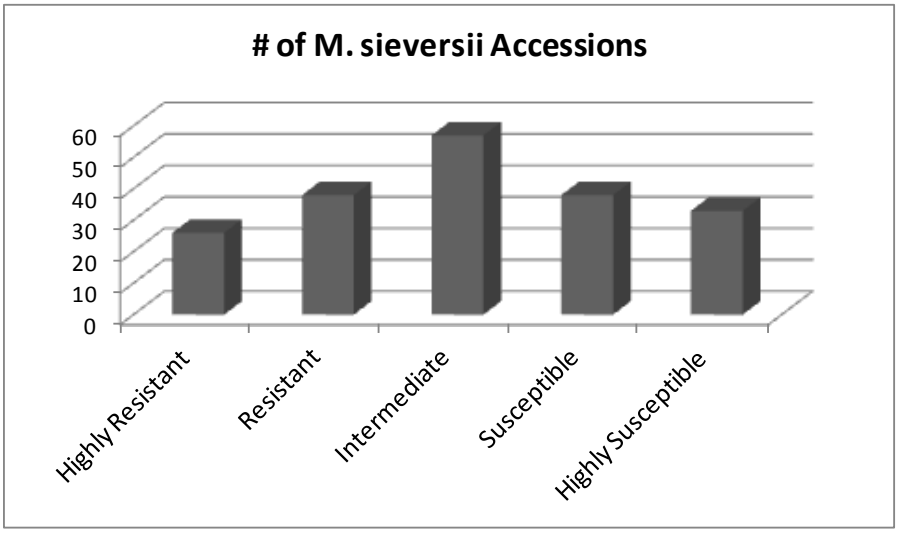

Figure 2. Number of wild apple accessions rated in different classes of resistance to fire blight.

This project has allowed us to identify excellent sources of fire blight resistance for use in the WSU apple breeding program. Several years ago the USDA-ARS Plant Genetic Resources Unit established a large collection of *M. sieversii*, the main progenitor of the domestic apple, collected from Central Asia (Kazakhstan). The 194 *M. sieversii* accessions used in the project were selected from over 1,000 *M. sieversii* seedling based upon their fire blight resistance and performance in an orchard grown at the USDA-ARS facility in Geneva, NY. The 12 accessions we have now identified as highly resistant to fire blight by replicated controlled pathogen challenge at multiple locations probably represent the best available sources of strong fire blight resistance available for apple scion breeding. Although many wild apples have been identified that are highly resistant to fire blight, most have extremely poor fruit quality. *M. sieversii* is the only wild species with large, edible fruit. Kate Evans thinks the most effective way to select the 1-3 accessions to start using in the WSU apple breeding program will be to evaluate the fruit of the 12 accesions in the standardized fruit quality evaluation protocols of the WSU apple breeding program. These trials are planned for 2015.

Objective 2: Establish planting of RosBREED apple Crop Reference Set and Washington State Breeding Pedigree Set for future fire blight evaluation**.**

The goal of this objective is to determine the fire blight resistance of the RosBREED reference germplasm so that we can utilize RosBREED resources to identify markers for fire blight resistance. Although we identify excellent sources of fire blight resistance in Objective 1 to be used in future crosses, it will not facilitate selection of fire blight resistance among the existing seedlings and selections of the WSU apple breeding program. Evaluating the RosBREED apple reference germplasm for its resistance/susceptibility to fire blight will allow us to leverage the significant financial investment of RosBREED in marker and software development to enable marker-assisted breeding of fire blight resistance in existing seedlings and selections in the WSU apple breeding program. Because fire blight disease can result in major structural damage of trees, and in some cases tree death of susceptible cultivars, existing plantings of the RosBREED apple Crop Reference Set and WSU Breeding Pedigree Set established to evaluate fruit quality traits cannot be used to evaluate fire blight resistance. In order to keep the cost of this project as low as possible, a single planting located at WSU Columbia View Orchard was established.

Trees for this planting have been propagated at Willow Drive Nursery in Ephrata, Washington. Budwood of the RosBREED apple Crop Reference Set and WSU Breeding Pedigree Set was collected at WSU-TFREC Wenatchee, WA or obtained from the other RosBREED core breeding programs at the University of Minnesota and Cornell University, or the USDA-ARS-Plant Genetic Resources Unit in Geneva and budded onto M.111 rootstock during the 2013 growing season. MM.111 rootstock was selected because of its tolerance to fire blight to prevent tree loss due to rootstock infection. Trees will be planted this spring within the current grant cycle at the WSU Columbia View orchard with protective deer fencing.

Because fire blight resistance will be determined on shoots, the tests for resistance can be conducted on young trees and the planting is expected to be of short term duration (3 to 4 years), allowing for planting at high density. Because fire blight challenge of the planting will be required for future evaluation of fire blight resistance, the planting will be situated on the Columbia View Orchard just north of Wenatchee.

Executive Summary

Project Title: **Incorporating fire blight resistance into Washington apple cultivars**

This project had two goals: 1) identify the best sources of fire blight resistance within *Malus sieversii*, the wild large-fruited progenitor of domesticated apple, for use in scion breeding; and 2) establish a planting of the RosBREED reference germplasm for future fire blight evaluation. Both of the goals were successfully completed.

Twelve *M. sieversii* accessions were determined to be highly resistant to fire blight shoot infection in multiple tests conducted in 2013 and 2014 at WSU's Columbia View Orchard in Wenatchee, WA and the USDA-ARS Appalachian Fruit Research Station Farm in Kearneysville, WV. A total of 194 *M. sieversii* accessions were evaluated in the trial that had been selected from over 1,000 seedling accessions collected in Kazakhstan. Many of the accessions appeared as resistant as *Malus x robusta* Robusta 5 which has been successfully used as a source of fire blight resistance in the Geneva rootstock breeding program. However, unlike Robusta 5 which has small, inedible fruit, the *M. sieversii* accessions have more typical apple fruit that are larger and edible. This will make the *M. sieversii* accessions a much more suitable source of resistance for scion breeding. Although the fruit are edible, they are not of commercial quality and their resistance will need to be bred with elite selections to improve fruit quality while maintaining fire blight resistance. The resistance of the 12 *M. sieversii* accessions is far stronger than the type of resistance normally observed in fire blight resistant cultivars, such as 'Delicious' or 'Enterprise'. To choose the best of these 12 accessions to start incorporating into the WSU apple breeding program we plan to evaluate the accessions for their fruit quality. Although some fruit quality data exists in the USDA database of these accessions (which was used as a factor in selecting them for the trial), Kate Evans thinks that seeing the performance of the fruit in the WSU breeding program's standardized fruit quality evaluation protocols will give her a much better knowledge base for selecting the accession(s) to work with. We plan on using the current *M. sieversii* plantings in Wenatchee and Kearneysville to evaluate the fruit of the 12 highly resistant accessions in 2015 and 2016.

The project also established a planting of the RosBREED apple reference germplasm set (elite cultivars and their seedlings, 3 replicate trees, total n=3,500) at WSU's Columbia View Orchard for the purpose of evaluating their fire blight resistance. A vast dataset was developed for this germplasm in the previous RosBREED project, including comprehensive fruit quality evaluations, high-resolution genome scans, and predictive genotypes at fruit quality-influencing loci. Determining the fire blight resistance of this reference germplasm will allow us to leverage the significant financial investment of RosBREED to enable marker assisted breeding of fire blight resistance of existing seedlings and selections in the WSU apple breeding program. The Columbia View Orchard will be planted spring 2015 and evaluated for resistance to fire blight shoot infection in 2016-2017 (Obj. 2). This should allow us to identify predictive genotypes for fire blight resistance loci 2017-2018. Markers for these loci would then be developed and evaluated. Once validated they would be used in breeding.