Wild apple species as a source of fire blight resistance for sustainable productivity of apple orchards

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This research was partially supported by the New York Apple Research and Development Program

The susceptibility of commercial apple scion varieties and rootstocks to pathogens and insects greatly reduces orchard productivity and fruit quality. Since its first report almost 200 years ago in a pear orchard in the Hudson Valley, NY, fire blight has been a major threat to apple and pear production in New York State (Norelli et al. 2003). The causative bacteria (Erwinia amylovora) can enter via natural stomatal and blossom openings, physical injuries/wounds caused by hail storms, farm equipment, and rain splashes, making their entry difficult to stop. Insects and bees can spread the bacteria during bloom time from infected trees to healthy trees, as can contaminated tools; for example, pruners and shears. Rain after an infection event washes bacteria down and can spread it to rootstocks, which also can ultimately lead to the death of the tree. Fire blight causes economic losses exceeding $100 million each year in the US through blossom, shoot, or rootstock blight alone. A major epidemic of fire blight in 2000 in Michigan led to the loss of more than 600 acres of orchards, or over $42 million loss for the growers (Norelli et al. 2003). Chemicals can be used to prevent fire blight infection, but once the pathogen is inside the plant tissue, there is no effective chemical treatment. The use of chemical control methods with antibiotics has also led to the emergence of antibiotic-resistant bacteria strains that could cause a sudden fire blight epidemic (Cox et al. 2012). In addition, both management of fire blight and use of preventive chemical sprays contribute significantly to production costs. The actual cost incurred by disease damage and management is difficult to estimate, because of the recurring cost of sprays, pruning of infected parts, waste of fruit due to decreased quality, and the multi-year impact due to loss of productive trees.

Fire blight control via chemicals and pruning of infected parts is difficult and costly; therefore, developing resistant apple cultivars is considered to be the best option for fire blight management (Luby et al. 2002). Apple cultivars with improved disease resistance are needed to meet market demands and for optimal performance in the face of not only the challenge of existing pathogens, but also for newly emerging diseases, the changing population of the pathogen strains, as well as abiotic stresses. Strains of Erwinia amylovora have shown high genetic variability and rapid changes in the genome in response to the use of antibiotics. Mutated strains of fire blight could escape sprays and infect previously resistant apple cultivars (Smits et al. 2014). However, existing resistance genes in the natural apple (Malus) germplasm will allow breeding of new cultivars with enhanced disease resistance, as long as the genes are clearly identified.

Approximately 28 regions in the apple genome have been identified as sources of major or minor resistance to fire blight (Khan et al. 2012). The five major regions were identified on chromosome 3 in Malus robusta 5, chromosome 7 in cv. Fiesta, chromosome 10 in ‘Evereste’, and chromosome 12 in Malus fusca and Malus x arnoldiana (Khan et al. 2007; Parravicini et al. 2011 Fahrentropp et al. 2013; Emeriewen et al. 2014, 2017). All of these major resistance genomic regions originate from wild apple species except in cv. Fiesta. A DNA-based marker test of the cv. Fiesta pedigree showed that it inherited this resistance from apple cv. Cox’s Orange Pippin and that other cultivars that share a pedigree with the cv. Fiesta also carry this resistance region (Khan et al. 2007). Most of these regions have a major gene underlying the resistance and they are fire blight bacterial strain-specific. The major fire blight resistance gene on chromosome 3 from Malus robusta 5 has been transformed by a European group into the fire blight-susceptible cv. Gala, which then showed significantly lower infection from artificial inoculations with E. amylovora (Broggini et al. 2014). There is great potential for tapping into natural resistance in wild apple species and incorporating it into susceptible but commercially important and consumer favorite cultivars using conventional or genome-assisted breeding or transgenic approaches, where appropriate and accepted. In an apple breeding program, DNA markers linked to the resistance genes are used to select apple seedlings from a cross between susceptible and resistant parents.

High genetic variability in E. amylovora and its capacity to rapidly mutate in response to high selection pressure can allow it to overcome cultivar resistance. For example, a highly aggressive E. amylovora strain can overcome major resistance from Malus robusta 5 (Peil et al. 2011). Additionally, even a minor variation in the genome of a host plant can cause a major change in gene function, either through complete loss of resistance or altering the resistance spectrum. Disease severity of the host depends on...
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on the interaction of its resistance genes and aggressiveness of the corresponding pathogen strains. At any one time, several *E. amylovora* strains exist in an apple orchard with varying aggressiveness, resulting in different levels of fire blight infection (Norelli et al. 1986). Therefore, it is important to test the response of host plants to the multiple bacteria strains commonly found in local orchards. Exposing *Malus* germplasm to multiple bacteria strains will not only test the stability and effectiveness of resistance genes to a large number of bacterial strains, but can lead to the identification of novel resistance genes.

The USDA-ARS National Plant Germplasm System (NPGS) *Malus* collection at the Plant Genetic Resources Unit (PGRU), Geneva, NY represents great diversity for horticultural traits, fruit quality, and disease resistance. This is the world’s most diverse *Malus* collection, holding 6,778 *Malus* accessions from 52 species, of which 5,196 accessions are trees in the field and 1,582 accessions are seed lots from Asia, Europe, and North America. These accessions were obtained from research institutions, breeding programs, and genebanks around the world, or collected through expeditions in Central Asia between 1986 and 1996 by Phil Forsline (PGRU, Geneva, NY) and Herb Aldwinckle (Cornell University, Geneva, NY). A large component of this collection is old and new cultivars from *M. domestica* and hybrids with different wild *Malus* species. A significant part of the collection is maintained in the field at Geneva, including a *Malus* core collection maintained on B.9 rootstock, a permanent collection on EMLA 7 rootstock, *M. sieversii* seedlings from Kazakhstan, and wild *Malus* species seedlings from Armenia, China, Georgia, Russia, and Turkey. This germplasm is routinely shared with the research and breeding community in the US and internationally to assess disease resistance, fruit quality, and horticultural traits and as a potential source of genes for use in genetic improvement of scion cultivars and rootstocks of apple for resistance to economically important diseases. This collection has primarily been evaluated for preliminary descriptors and to assess genetic diversity in general. Part of this collection, at different times, has also been evaluated for fire blight and apple scab resistance by plant pathologists at Cornell University, the PGRU staff at Geneva, and other research groups in the US, Canada, and Europe (Volk et al. 2015; Harshman et al. 2017). Although the collection contains great naturally occurring and functionally diverse disease resistance, the lack of detailed characterization and availability of this data limits their utilization. With the recent advances in genome sequencing and disease phenotyping, the collection could be comprehensively characterized at DNA levels, and for specific diseases.

**Materials and Methods**

Field observation data for shoot fire blight for 2,362 *Malus* accessions from 52 species and blossom blight of 694 *Malus* accessions from 41 species, characterized by PGRU, were downloaded from the Germplasm Resources Information Network (GRIN-Global database). We also accessed fire blight inoculation data from a two-year (2011 and 2012) greenhouse experiment using a subset of the *Malus* collection. Artificial inoculation was done in the greenhouse on young *Malus* plants grafted onto B.9 using *E. amylovora* strain Ea273 (Khan et al. 2013). Field observation data was collected as qualitative scores; 1 (very resistant) and 5 (very susceptible) as described by Postman et al. (2010, Table 4) and [http://www.ars-grin.gov/ngps/descriptors/apple](http://www.ars-grin.gov/ngps/descriptors/apple). Data in the greenhouse was collected as average percentage of fire blight necrosis length (PLL). In order to compare datasets, we used data for accessions that were either resistant (below 10 PLL) or susceptible (above 35 PLL) in the greenhouse experiment, and that were also present in the field study.

**Results and discussion**

The blossom fire blight data downloaded from USDA GRIN-Global was for 694 accessions from 41 species. Only 638 accessions from 14 species had more than 5 accessions/species; i.e., 27 species had less than four accessions per species. *M. prunifolia, M. baccata, M. hybrid, and M. domestica* each had blossom blight data for more than 11 accessions. Our analysis showed highly resistant scores (1-2) for more than 65% of accessions from *M. angustifolia, M. ioensis, M. spp*, *M. sieversii, M. toringo*, and *M. coronaria*. *Malus sieversii* from Central Asia, the main progenitor of modern day apples, had 33% of accessions highly susceptible to blossom blight. European crab apple (*M. sylvestris*), which has also contributed significantly to the genome of domesticated apples, had 80% of accessions that were highly susceptible to blossom fire blight. Domesticated apples had 21% of the accessions rated as resistant and approximately 60% as highly susceptible (4-5).

Shoot fire blight data was available for 2,362 apple accessions from 52 species. There were 2,318 accessions from 33 species with at least 5 accessions per species. *M. prunifolia, M. ioensis, M. fusca, M. baccata, M. coronaria, M. sieversii, M. hybrid, and M. domestica* each had more than 35 accessions with shoot fire blight data. More than 65% of accessions from *M. ombrophila, M. prattii, M. fusca, M. spp.*, *M. sieversii, M. haliiana, M. coronaria, M. x hartwigii, and M. x asiatica* had highly resistant scores (1-2) for shoot blight. From cultivated apples (*M. domestica*), 18% of accessions were resistant (1-2) and almost 75% accessions were highly susceptible (4-5) to shoot blight. *Malus sieversii* had 71% and 25% of accessions in resistant and susceptible categories, respectively, while European crab apple had 33% and 66% accessions rated as resistant and susceptible, respectively.

There are 26 species with shoot fire blight data for at least 10 accessions. Of these, we found approximately 31% and 11% potential shoot blight and blossom blight-resistant accessions (score 1-2), respectively, which could be a valuable resource for identifying new resistance genes with potential use in apple breeding and biotechnology (Figure 1). Field observation data can have false positives and negatives due to the sporadic nature of fire blight and random distribution of inoculum and strains in the field. Comparison with greenhouse fire blight inoculation results using a subset of this collection showed a consistent ranking for several overlapping accessions between the two data sets (Figure 2), although there are inconsistencies as well. These discrepancies could be from environmental factors that can influence the interaction between the host and pathogen in the field, escape of the host plant from infection, especially for accessions that were identified as highly resistant (score of 1), or strain and host specificity. For example, accessions that are scored 5 (very susceptible) in the field, but show very low infection to Ea273 strain in the greenhouse experiment, could be susceptible to other, highly virulent strains, present in the field. Also, the ranking of wild *Malus* species based on average
rating of accessions per species in field observation data is consistent with other controlled studies (Figure 1). Therefore, this data can provide a basis for confirmation of resistance through detailed and systematic characterization, selection of Malus accessions for identifying novel resistance genes, and as disease resistance donors for future apple breeding and research. Accessions of promising species such as M. sieversii, M. fusca, and M. toringo should be further evaluated for shoot and blossom fire blight, especially for bacterial strains prevalent in New York.

In addition to fresh fruit, cider, and juices, an increase in apple value-added products, pick-your-own orchards, increased concerns about human health, and rising incomes, demand development of new varieties with market-specific attributes. However, improved disease resistance of scion cultivars and rootstocks is the foundation for all these market-specific uses, including organic orchard production. Emergence of new diseases and new pathogen strains is a common problem across the entire cropping system, but is more pronounced in high-density apple orchards. The majority of the top ten commercial scion cultivars and rootstocks produced and sold in the US – e.g., Gala, Red Delicious, Granny Smith, Fuji, Golden Delicious, Honeycrisp, McIntosh, Rome, Cripps Pink/Pink Lady®, Empire, and M.9 – are all susceptible to bacterial, fungal, soil-borne, and post-harvest diseases. Application of antibiotics and fungicides significantly increases production costs, with environmental and health concerns, and leads to emergence of new resistant strains. Therefore, a well-characterized resistant Malus collection can act as a donor of new resistance genes to efficiently deploy into elite apple cultivars and develop new disease-resistant and consumer-preferred cultivars via breeding or biotechnology (Khan et al. 2013; Volk et al. 2015; Harshman et al. 2017). New resistant varieties will protect the US apple industry from the losses associated with fire blight, increase orchard profitability, as well as limit the impact and cost of antibiotic use, to have a positive economic impact on apple production in the US and its competitiveness in the international market.

Acknowledgements
This work was funded in part by USDA-ARS grant 58-8080-7-006, developing apple germplasm donor stock for novel strain-specific and broad-spectrum alleles and genes for fire blight genetic research and resistance breeding. This work was also supported in part by the New York Apple Research and Development Program (ARDP). Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the United States Department of Agriculture (USDA).

References


Figure 2. Fire blight inoculation results from natural shoot blight in the field (observational data) and average percentage of fire blight necrosis length (PLL) from two-year (2011 and 2012) artificial inoculation data in the greenhouse with E. amylovora strain Ea273 (Khan et al. 2013). Data is shown for accessions that were either resistant (below 10 PLL) or susceptible (above 35 PLL) in the greenhouse experiment, and that were also present in the field study. Natural blight scores are 1 (very resistant) and 5 (very susceptible) as described Postman et al. (2010, Table 4) and http://www.ars-grin.gov/npgs/descriptors/apple.

Figure 3. Shoots of young apple accessions from a subset of the apple collection at PGRU grafted onto B.9 rootstock and inoculated (using a hypodermic needle) in the greenhouse with a bacterial culture of Erwinia amylovora strain Ea273 at University of Illinois, Urbana-Champaign. Note bacterial oozing, wilting, and typical shepherd’s crook symptoms observed on susceptible shoots and regeneration of new tissue on resistant shoots. a) Resistance response: point of inoculation but no progress in disease, b) highly susceptible with bacterial ooze and necrosis of leaves and shoot, c) susceptible with shepherd’s hook and dead stem tissue, d) moderately susceptible, with slow progress, e) susceptible with shepherd’s hook and dead stem tissue, f) highly resistant.


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