RESEARCH RESULTS FOR THE YEAR 2015 'FASTRACK' - A REVOLUTIONARY APPROACH TO LONG-GENERATION CYCLE SPECIALTY CROP BREEDING

Ralph Scorza, Chris Dardick, Doug Raines, Ann Callahan - USDA-ARS Appalachian Fruit Research Station Kearneysville, WV

Sarah Castro, Ted DeJong - University of California, Davis

ABSTRACT

Plum pox virus (PPV), the most serious disease of stone fruits, and plum in particular, continues to spread world-wide. It was eradicated in Pennsylvania at a cost of over $60. M and a loss of 1600 acres of stone fruits, it was also reported in Michigan and New York in 2006 and was soon eradicated in those states. In the fall of 2015 a new area of infection was reported in NY. Canada has discontinued its eradication program and is "managing" the virus in growing areas close to the US border. It is not beyond reason that Canada will be a continual potential source of virus spread in the U.S. The 2015 infection in New York may be a case in point.

There are few sources of high level resistance to PPV. We have developed a breeding method using genetic engineering to rapidly develop resistant germplasm and new varieties highly resistant to PPV. The resistance and the breeding system are approved by the US regulatory agencies and new resistant varieties could be deployed relatively rapidly in the event of the spread of PPV to California. We have made significant progress in the past year of the project in improving the efficiency of the breeding system and developing advanced PPV resistant generations of plums using breeding lines from California as parents. In the past 6 years we have advanced breeding populations to a level that would normally have required almost 20 years of conventional breeding.

OBJECTIVES

The U.S. stone-fruit industry is under threat from *Plum pox virus* (PPV) (aka "Sharka" disease). This exotic invasive pathogen, spread by aphids, compromises tree health, causes premature fruit drop and reduces fruit quality. Infected fruit of most stone fruit is neither suitable for fresh or for most processed products. First identified in Europe in the early 20th century, PPV has rapidly spread world-wide since the late 1980s, likely due to the globalization of trade and travel. PPV has now been reported from all major fruit growing countries except the U.S., Australia, New Zealand, and South Africa. The central valleys of California are particularly vulnerable to this threat because of its high concentration of *Prunus* crops and a lack of geographical barriers to the spread of the disease by insects. Breeding PPV resistant varieties of plum can provide a long-term solution to what is an ongoing threat. Plum breeding is a decades-long process since each generation of crosses requires nearly 4 year to fruit. Additionally there are few effective sources of natural resistance to PPV in domestica plum. The specific objective of the project is to rapidly develop Plum pox virus (PPV) resistant plum germplasm and varieties using FasTrack breeding

to incorporate the high level PPV resistance in the genetically engineering plum variety 'HoneySweet' into California adapted breeding lines.

PROCEDURE

To address the long-term nature of plum breeding we have developed a system to shorten the plum breeding cycle by incorporating a flowering-related gene from poplar (FT) to induce trees to flower early and continually and to produce viable fruits and seeds within one year.
To address the need for high level stable resistance to PPV we have developed a genetically-engineered (GE) plum cultivar 'HoneySweet' that has been tested in Europe for over 18 years under natural conditions of high infection pressure from virus-carrying aphids without a single tree having becoming infected. 'HoneySweet' has received complete regulatory approval in the U.S. and regulatory approval is being pursued in Europe. By using the FT gene in a greenhouse breeding program we Aim to rapidly incorporate the PPV resistance from 'HoneySweet' into the California-adapted prune germplasm.

RESULTS

The following accomplishments are reported for 2015:

After 6 years of work on this project we are at a breeding stage that would have taken 20 years through conventional breeding if we consider a four-year generation cycle for plums.

Specific accomplishment 1: Null segregant PPV resistant lines were analyzed and prepared for shipment to UC Davis for evaluation. The first crop of 12 null segregants that resulted from crosses of California-adapted genotypes with PPV resistant 'HoneySweet' were analyzed for the absence of the early flowering FT gene and prepared for shipment to the UC Davis dried plum breeding program. Null segregants are defined as those progeny that do not contain the early flowering gene or DNA from the early flowering construct. As such, these segregants are not considered by USDA-APHIS to be regulated articles and may be freely planted without APHIS permitting. These 12 null segregants will have the typical vegetative phenotype and generation cycle to flowering and fruiting as conventional plums (**Figure 1**). California Department of Food and Agriculture plant quarantine permitting for shipment and planting of these trees was obtained through the efforts of Sarah Castro. The ancestry of the null segregant lines is shown on **Table 1**. Upon flowering and fruiting they may provide new useful PPV resistant varieties for the California industry and/or may be used as parents in continued hybridizations with California-adapted material to produce further generations of PPV resistant phenotypes.

Specific accomplishment 2: Early flowering plum trees were field-planted to be used for hybridizations in the spring of 2016 to provide an increased number of hybrid seedlings. The FasTrack breeding system offers a great advantage in utilizing plants year-round for breeding and provides in the greenhouse an environment where plants may be hybridized in an environment free from biotic hazards such as damaging winter temperatures, spring frosts, and insect and disease depredations. The disadvantage of breeding with potted trees in the greenhouse is the rather low number of hybrids that can be produced from small greenhouse-grown potted plants. Early flowering plum trees in the field are much larger than the same clones in the greenhouse and produce more flowers and fruit. Field-grown trees continue to produce flowers and fruit throughout spring and early summer with flowering resuming in the

fall. While crosses cannot be made in the fall in the field due to low winter temperatures during the winter in West Virginia, it may be possible in locations in areas of mild winter temperatures such as southern California to pollinate trees in the fall to obtain a second crop of hybrids each year. The prolonged flowering period in spring/summer can allow for the escape from spring frost events. For example, in the spring of 2015 while an early spring freeze event destroyed the flowers and developing stone fruits in our orchard, the early flowering lines were able to recover to produce flowers and fruit as the season progresses. In order to take advantage of the more robust tree growth in the field, and the larger crop of flowers available for pollination, in June of 2015 a selection of 10 early flowering plum trees were field-planted and will be used for hybridizations in the spring of 2016 to provide an increased number of hybrid seedlings for the 2015-2016 project hybridization cycle.

OUTREACH ACTIVITIES

Presentations at scientific meetings have led to increased interest in 'FasTrack' breeding and the wider use of the early flowering plum trees that we have developed. In 2015 under an agreement with NASA early flowering plum lines are being evaluated for future manned Mars missions. Plums may be the first fruit to travel on interplanetary space flights and early flowering plum trees may form the first "orchard" on Mars to provide fresh fruit to Mars colonists. NASA scientists have provided valuable information that can be applied to improving the FasTrack breeding for the current "earth" project. For example, NASA scientists developed a rooting protocol for propagation of elite early-flowering clones. This will allow us to multiply plants and increase the production of fruit from crosses of specific elite parents.

BUDGET NARRATIVE

All funds have been used to support salary and benefits of a full-time technical position for the project. Funds were also used, with prior approval, to support the travel of the PI to present a project report at the December 2015 Prune Workgroup Research Conference, Sacramento.

Plant #	Parent or Seedling ID	Gus Stain	FT-PCR	DNA blot confirmation
45	FT91 x **French** x Honeysweet	+	-	yes
46	FT91 x **French** x Honeysweet	+	-	yes
48	FT91 x Honeysweet x **French**	+	-	yes
35	FT3 x **F9N-21** x Honeysweet	+	-	yes
2	FT157S **x 5-3-4** x **D10S-8** x Honeysweet	+	-	yes
14	FT157 x **5-3-4** x **D10S-18** x Honeysweet	+	-	yes
33	FT157 x **5-3-4** x **D10S-8** x Honeysweet	+	-	yes
34	FT157 x **534-5** x Honeysweet	+	-	yes
29	FT34 x Honeysweet x **G39N-57**	+	-	yes
30	FT34 x Honeysweet x **G39N-57**	+	-	yes
31	FT34 x Honeysweet x **French**	+	-	yes
32	FT34 x Honeysweet x **French**	+	-	yes

Table 1. Null segregant [no early flowering (FT) gene] seedlings that combine the *Plum pox virus* (PPV) resistance gene with California prune genetics and the PCR and DNA blot tests that indicate that the FT gene is not present and the GUS test indicating that the PPV resistance gene from 'HoneySweet' is present. These plants are approved by APHIS for unregulated field planting and by the Calif. Dept of Agriculture plant quarantine to enter California. These plants will be sent to the UC Davis prune breeding program in January 2016 for evaluation of fruit quality and for continued future PPV resistance breeding. (California genetics indicated in bold in the table)

Figure 1. Null segregant plants, not containing the FT early flowering gene (R), from crosses of early flowering, HoneySweet, and UC Davis breeding program selections and early flowering parental clones (L) showing the typical differences in phenotype between early flowering and null segregants plum plants.