Apple CGC Minutes
August 20, 2020; 1:00-2:30 EDT
Teleconference hosted by Plant Genetic Resources Unit (PGRU)
Geneva, NY

Attendees: Gayle Volk (chair), Herb Aldwinckle, Jackson Bartell, Peter Bretting, Susan Brown, Dawn Dellefave, Molly Dexter, Laura Dougherty, Gennaro Fazio, Ben Gutierrez, Alejandra Jimenez, Awais Khan, John Keeton, Gary Kinard, Jim Luby, Diane Miller, Greg Peck, Reece Perrin, Tim Rhinehart, Tim Widmer, David Zakalik, Gan-Yuan Zhong

Minutes provided by: Gayle Volk

Announcement: Ben Gutierrez is now the permanent apple and tart cherry curator for the Plant Genetic Resources Unit Geneva, NY collections.

Curator Report (Gutierrez, see attached for PDF version)
Highlights: Fire blight has been an ongoing concern in the PGRU apple collection. The collection was originally grafted onto EMLA 9 rootstock and there were multiple fireblight outbreaks in the 1990s. The collection was switched to EMLA 7 in 2000. Fire blight has continued to be an issue, likely in part because the collection has a very long bloom time, making it difficult to spray at all the timepoints during the bloom season. In addition, some cultivars are particularly susceptible to the disease.

In 2002, streptomycin resistant fire blight was reported in the Finger Lakes Region. In 2020, the specific strain of fireblight was determined for 47 trees in the collection. All trees harbored the streptomycin resistant strain. Field assessments were performed in the collection in 2020 to determine the extent of the outbreak: Percentage of first year shoots with fireblight & 1 to 7 rating of the extent of pruning performed in response to the fire blight. Overall, the accessions grafted onto B9 (dwarfing rootstock in the core collection planting) fared better than those grafted on EMLA 7 (semi dwarf). Malus domestica and Malus sargentii were the most affected by the outbreak, but many other species (Malus sieversii, Malus ioensis, etc.) experienced symptoms. In one example, a tree (PI 307518) progressed from 35% damage to 80% damage in three weeks (June to July).

As of August 2020, 326 trees have been targeted for repropagation in response to the fire blight damage. Of these, 160 were severely diseased and 303 are currently available in a cryopreserved state at the National Laboratory for Genetic Resources Preservation.

New control measures were proposed for 2021 and beyond including:

1) Alternative chemical applications such as Blossom Protect, Kasugamycin, and Copper
2) Expand spraying program to include early and late blooming trees
3) Lighter pruning for fire blight to leave scaffolds for regrowth
4) Enhanced scouting and removal of fire blight in the orchards
5) Evaluation of compatibility of alternative rootstocks (Geneva series or possibly B9) for future repropagation efforts.

There were about 7000 PGRU apple distributions in 2020. The apple curation team proposed sending a letter in response to budwood requests, explaining that dormant budwood will not be distributed in the winter of 2020.

CGC Committee Discussion:
The committee agreed that budwood should not be distributed for propagation purposes in the winter of 2020. There may be research exceptions (pathology projects etc) granted after communication with the curator. Distributions may resume in future years, when the spread of fire blight-contaminated budwood is no longer a concern.

A letter, approved by National Program Staff, will be provided to customers who ask for dormant budwood in Winter 2020-2021.

Geneva 890 rootstock has been used for orchard tree repropagation efforts. This will serve as an assessment as to how compatible this rootstock is with other *Malus* species.

Apple cryopreservation efforts will intensify in winter 2020-2021 to back-up at-risk infections before the 2021 season. There was a question about trueness-to-type after cryopreservation. There are now a number of trees in the Geneva collection that have been recovered from the cryopreserved state. An ongoing experiment with Gennaro Fazio may also address this question.

The committee supports the proposed control methods for 2021 and beyond.

There was a suggestion that the apple curation team collaborate with others at Cornell University to better understand the safety of the proposed pesticide control regime.

The CGC continued to express concern that there is not adequate duplication of trees in the collection for research purposes. Perhaps, one solution could be the planting of 2 trees per accession on Geneva 890 rootstock or 4 trees per accession on Geneva 41 rootstock. These are both dwarfing rootstocks, so the overall space use would be similar. Before this is pursued, the extra cost of trellising must be evaluated and the curation team would have to ensure that adequate quantities of budwood would be available for distribution.

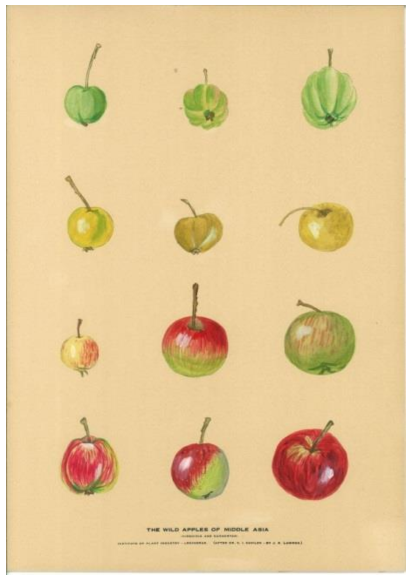
Vulnerability Statement and Quad Chart updates. The current apple vulnerability statement has been published: Volk et al. 2015. The vulnerability of U.S. apple (Malus) genetic resources. Genetic Resources and Crop Evolution 62: 765–794. Final revisions for the Quad Chart and Vulnerability Statement update will be completed and forwarded to the committee for feedback prior to the September 30 deadline.

Subcommittees. The CGC agreed that these subcommittees are important and the planned efforts should continue. Ben Gutierrez has agreed to coordinate the New Core Subcommittee and Land Use Efficiency Subcommittee activities.

Other news:
Genomes of Gala, *Malus sieversii*, and *Malus sylvestris* will soon be published as a collaboration between PGRU and Cornell University/Boyce Thompson Institute.

PGRU Update to Apple CGC

Ben Gutierrez
Laura Dougherty
Molly Dexter
John Keeton
Gan-Yuan Zhong



Kerik Cox
Anna Wallis
Awais Khan

Operations

Welcome!

- Laura Dougherty, PGRU Post-Doc
- Molly Dexter (Hobart and William Smith Colleges), PGRU intern

Vacancies

- Clonal Curator position (Grape)
- Molecular Biologist (vice Schwaninger)

PGRU Apple Collection and Fire Blight

Natural Occurrence of Fire Blight in USDA Apple Germplasm Collection After 10 Years of Observation

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Geneva, New York 14456-0462, USA

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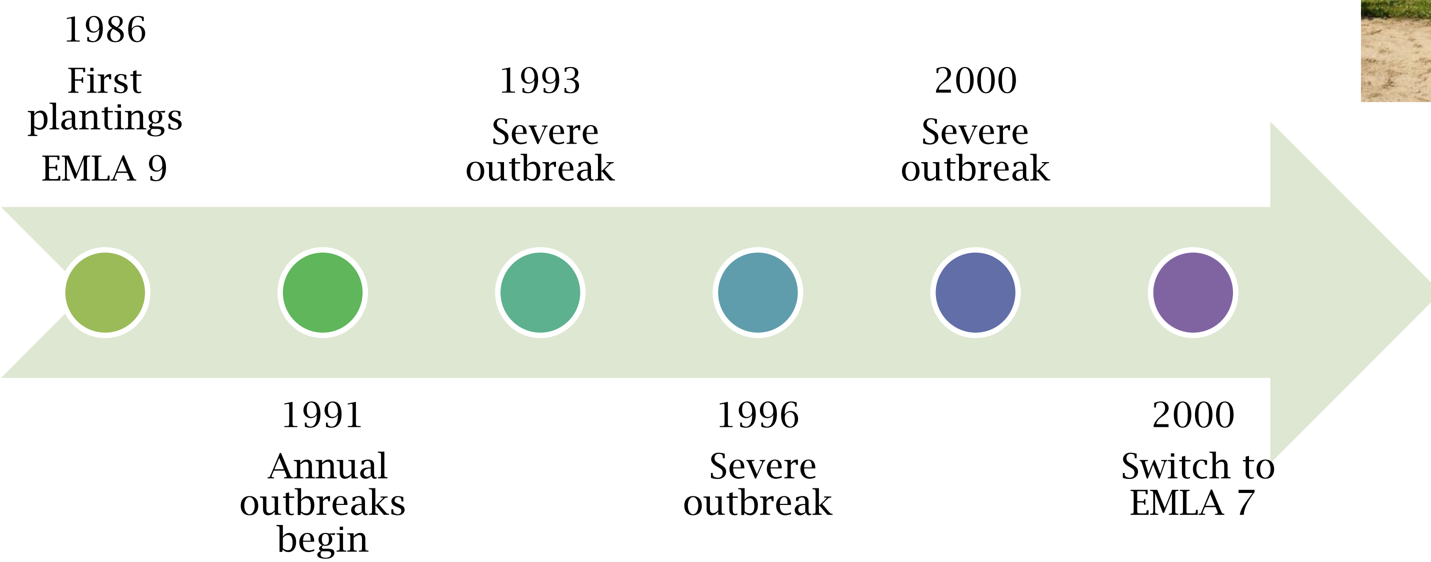


Streptomycin-Resistant *Erwinia amylovora*

Isolation of Streptomycin-Resistant Isolates of *Erwinia amylovora* in New York

Nicole L. Russo and **Thomas J. Burr,** Department of Plant Pathology, Cornell University, Geneva, NY 14456; **Deborah I. Breth,** Cornell Cooperative Extension, Albion, NY 14411; and **Herb S. Aldwinckle,** Department of Plant Pathology, Cornell University

ABSTRACT

Russo, N. L., Burr, T. J., Breth, D. I., and Aldwinckle, H. S. 2008. Isolation of streptomycin-resistant isolates of *Erwinia amylovora* in New York. Plant Dis. 92:714-718.

Streptomycin is currently the only antibiotic registered for the control of fire blight, a devastating disease of apple (*Malus*), pear (*Pyrus*), and other rosaceous plants caused by the bacterium *Erwinia amylovora*. Resistance of *E. amylovora* to streptomycin was first identified in California pear orchards in 1971 and is currently endemic in many parts of the United States. The Northeast remains the only major U.S. apple-growing region without streptomycin-resistant isolates of *E. amylovora*. In 2002, during a routine survey for streptomycin resistance, isolates from two neighboring orchards in Wayne County, NY were found to be highly resistant to streptomycin at a concentration of 100 µg/ml. This constitutes the first authenticated report of streptomycin resistance in New York State. Infected trees were shipped at the same time from a single nursery in Michigan. Resistance was caused by the acquisition of the *strA-strB* gene pair, inserted into the ubiquitous nontransmissible *E. amylovora* plasmid pEA29. Previously, streptomycin-resistant *E. amylovora* populations from Michigan were described with a similar mechanism of resistance, although the *strA-strB* genes are not unique to Michigan. These findings illustrate how unintentional movement of nursery material could undermine efforts to prevent the spread of antibiotic-resistant *E. amylovora*.

Additional keywords: antibiotic resistance

originated in *Pantoea agglomerans* and was acquired by *E. amylovora* through conjugal transfer (7,22). The tandem *strA-strB* genes code for aminoglycoside-3-phosphotransferase and aminoglycoside-6-phosphotransferase, respectively (9). These aminoglycoside-modifying enzymes modify streptomycin, and previously have been described in resistant populations of *Pseudomonas syringae* pv. *papulans*, *P. syringae* pv. *syringae*, and *Xanthomonas campestris* pv. *campestris* (17,23,38), as well as some bacteria of human and animal origin (22). Although the *strA* and *strB* genes function independently, both must be present to confer high levels of antibiotic resistance (9). The *strA-strB* genes have been found on broad host range plasmids such as pRSF1010 (pEa8.7) (30) or located within the transposable element Tn*5393* inserted into strain-specific plasmids like pEa34 (8). Tn*5393*, with *strA-strB*, also has been discovered inserted into

First reported in Western New York in 2002

Same strain identified in PGRU collection in 2020 (Anna Wallis)

- Isolated *E. amylovora* from 47 randomly distributed trees
- All isolates were streptomycin resistant
- Determined strain identity for 11
    - All had space array patterns 41:23:38
- Distribution suggests outbreak likely caused by single strain

Fire Blight Scoring

GRIN fire blight descriptor: 1-5 rating

Distribution of Values for Fire Blight Shoot (Natural) (FBSHNAT)

Code	Definition	Number of Accessions
1	Very resistant - no occurrence	598
2	Moderately resistant - only light rating	129
3	Intermediate - light to medium rating	173
4	Moderately susceptible - medium to heavy rating	361
5	Very susceptible - very heavy rating	1093

2020 Fire Blight Evaluations (two approaches)

1. **Fire blight incidence**

$$\frac{Number\ of\ 1st\ year\ shoots\ with\ fire\ blight}{Total\ number\ of\ 1st\ year\ shoots} \times 100 = \text{Incidence of fire blight (\%)}$$

2. **Fire Blight Pruning rating (M7 and E7)**
   - M7 collection pruned before collecting incidence data
   - 7-point rating for pruning due to fire blight from zero visible cuts to severe pruning of scaffolds and trunk.

Fire Blight Incidence (%)

2 %

50 %

75%

86%

- Two rootstocks: Bud-9 (dwarf) and EMLA 7 (semi-dwarf)
  - B9: 236 accessions (*Malus* Core)
  - E7 and partial M7 orchards: 1,187 accessions (1,417 trees)
    - 1,417 trees in June
    - 585 trees in July (E7)
- 50 species & hybrids

Cut Severity Rating

0 No visible cuts | **0.5** | **1** Light cuts | **1.5** | **2** Medium cuts | **2.5** | **3** Heavy cuts

Cut Score	Criteria (fire blight resistance)
0	No visible cuts (highly resistant)
0.5	Very light cuts, not noticeable (resistant)
1	Light cuts, small branches removed, no effect on tree shape (resistant)
1.5	Light cuts, small/medium branches removed, little effect on tree shape (mildly resistant)
2	Medium cuts, large branches removed (susceptible)
2.5	Medium/Heavy, large cuts, >50% tree removed (susceptible)
3	Heavy cuts, >75% tree removed (highly susceptible)

- M7 and E7 scored (2,665 trees)
- B9 scored (236 trees)

Fire Blight Evaluation

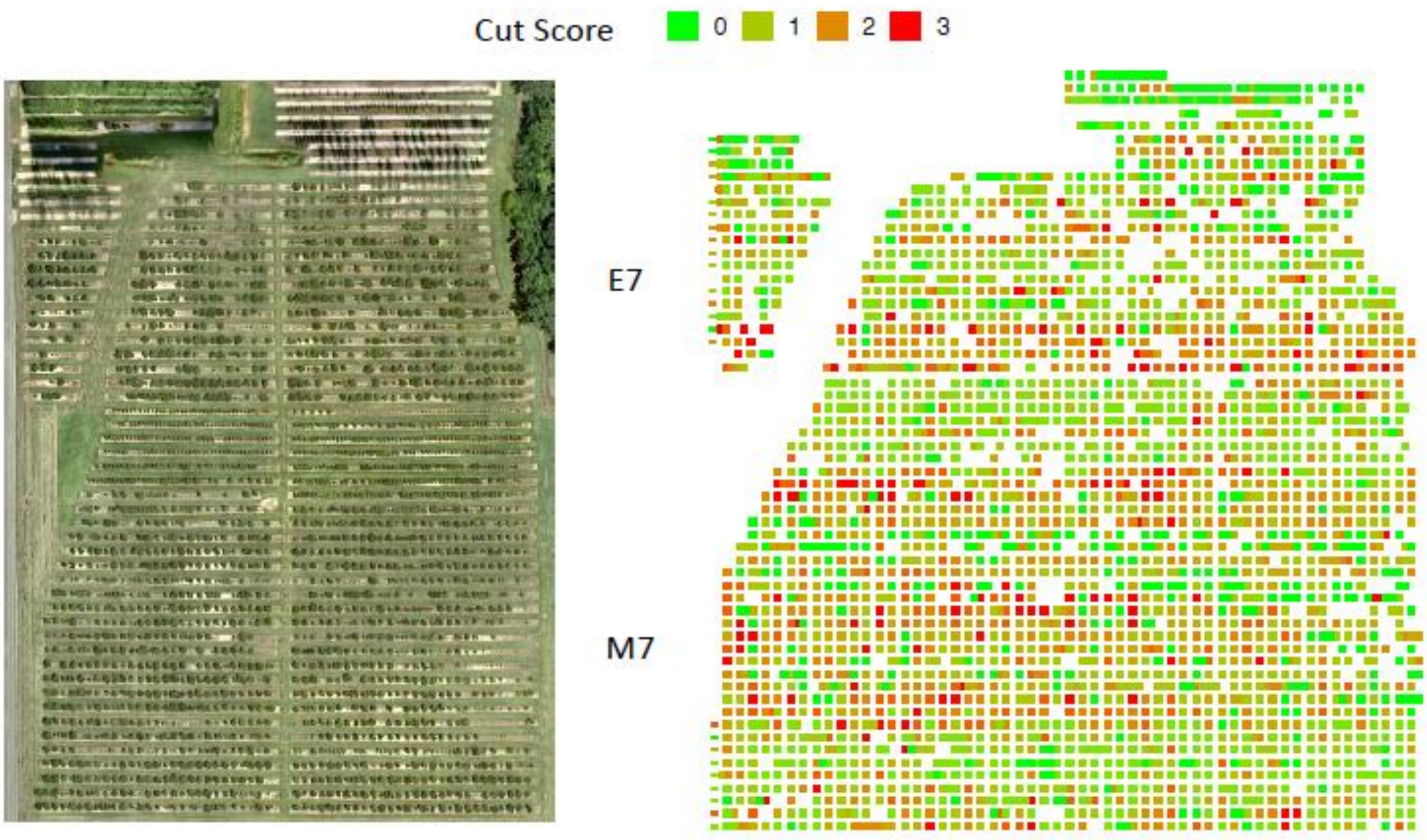
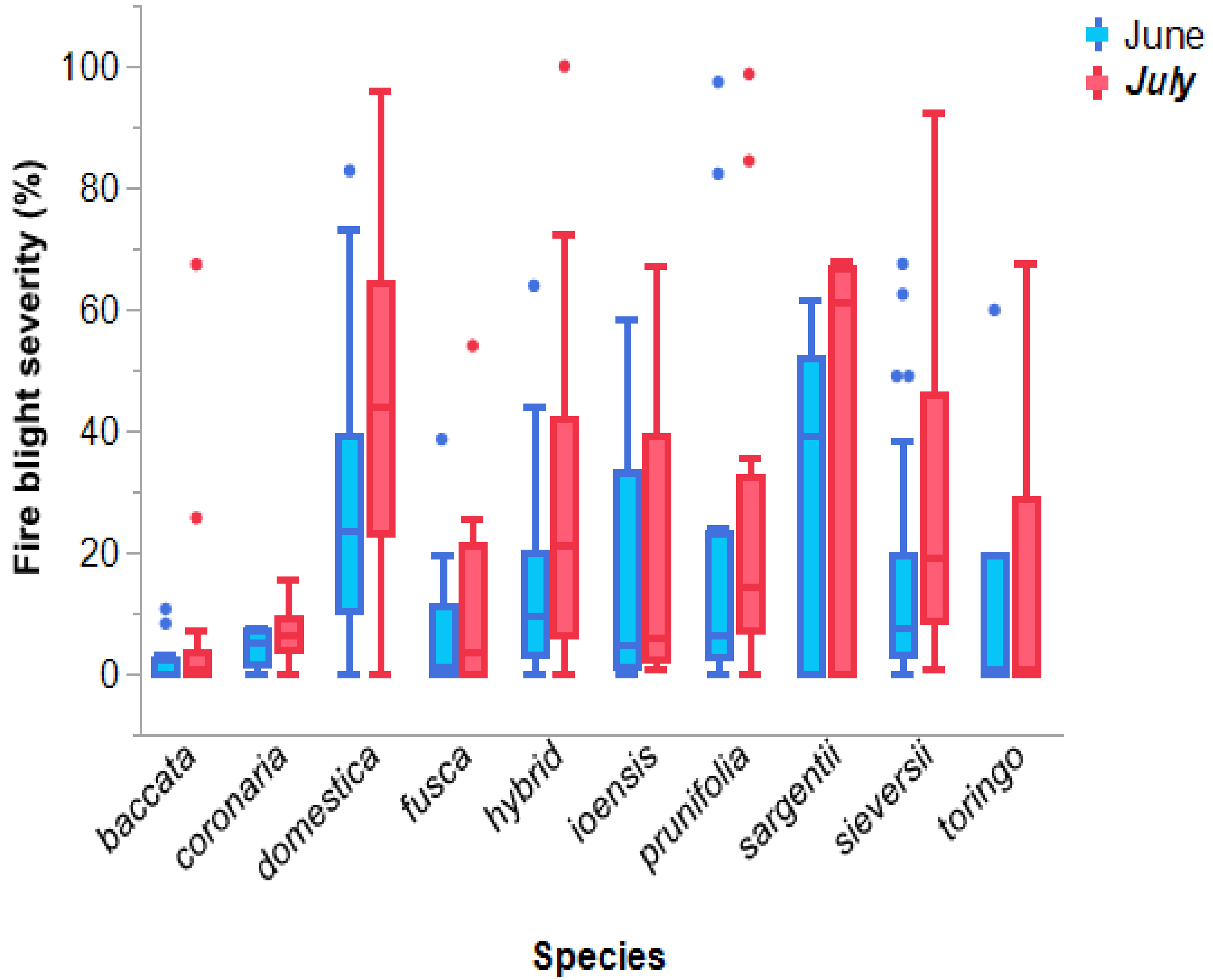


Figure 4. (A) Aerial image of orchards E7 and M7.[4-5] (B) Heat map of the intensity of cutting in E7 and M7. A gradient from green to red is used, with green indicating no visible cuts and red indicating heavy cuts.

Fire blight severity on EMLA7 rootstock



Disease Progression

35% Incidence

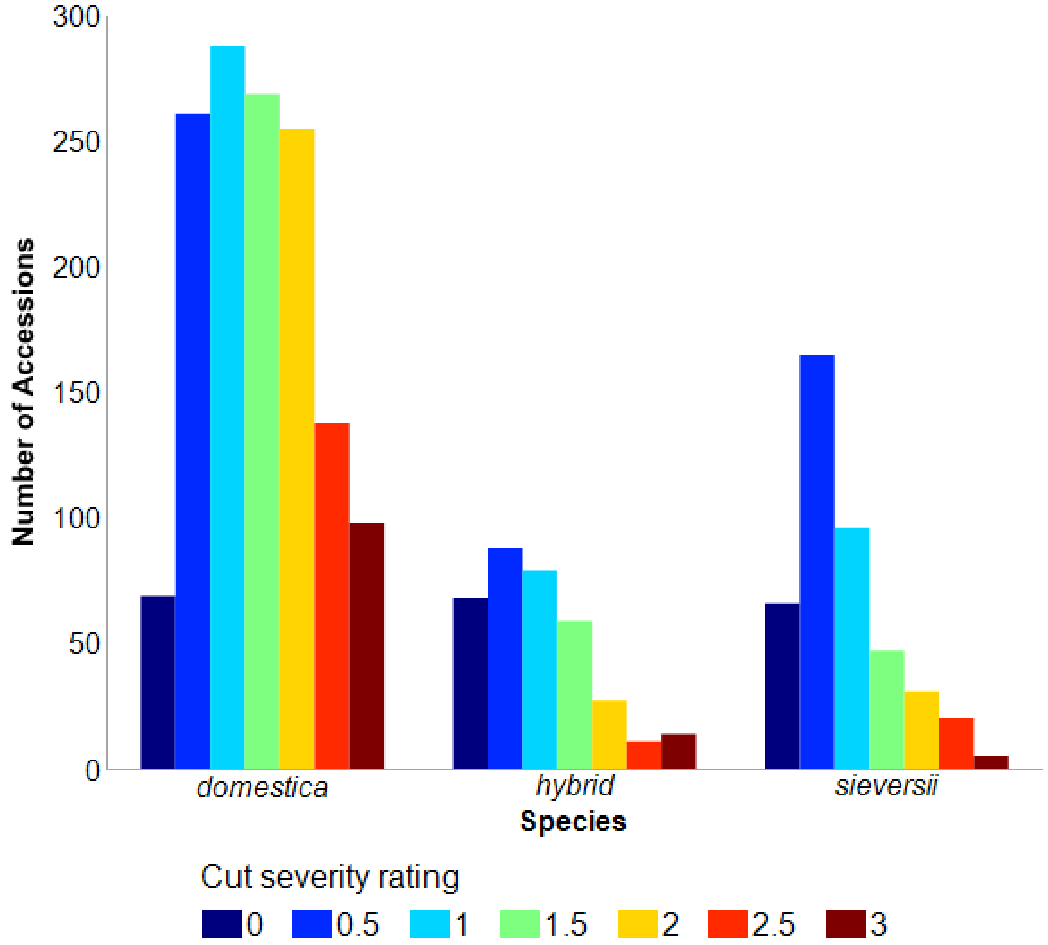
6/23/2020

80% Incidence

7/14/2020

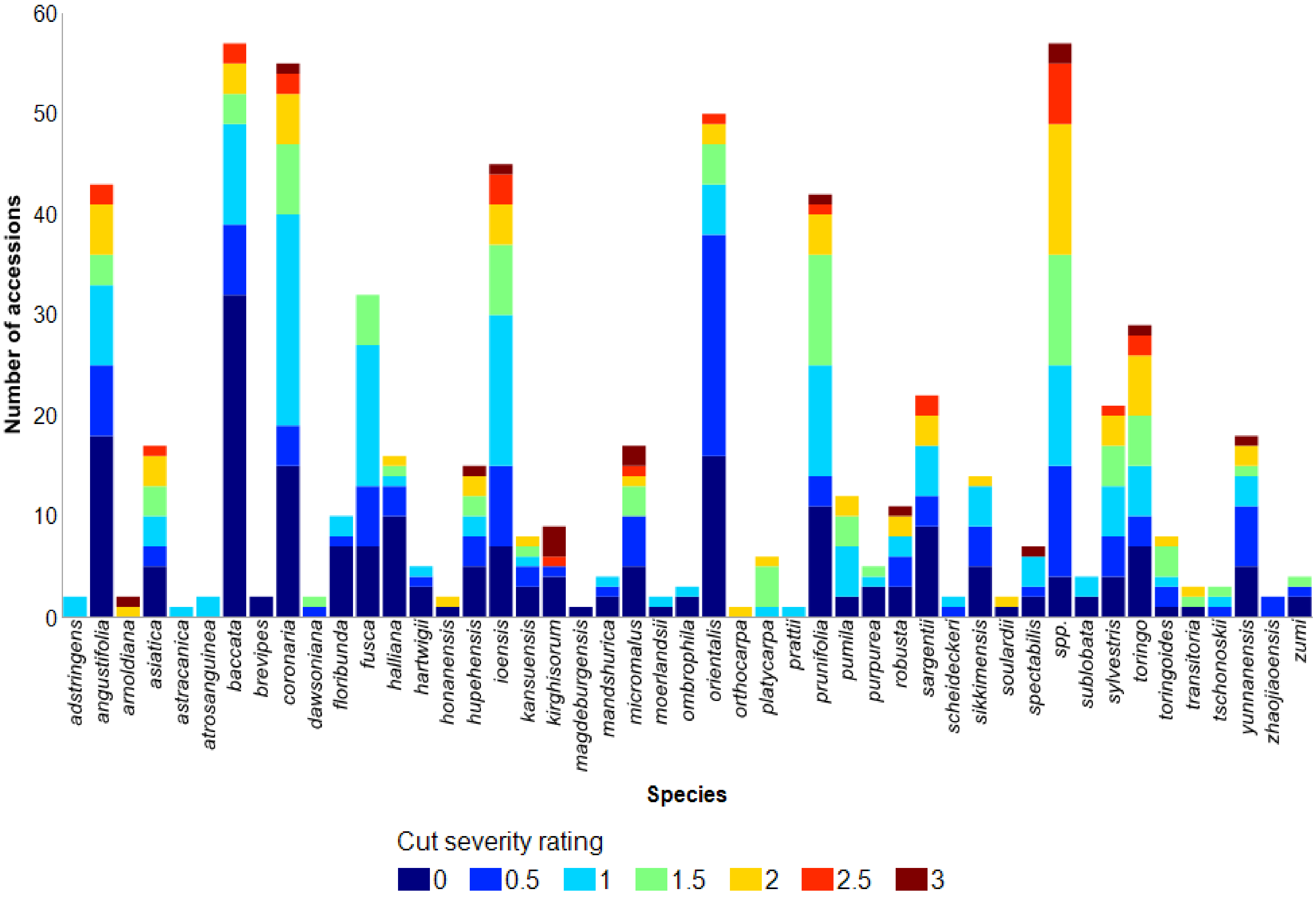
PI 307518
3 weeks apart

Cut severity data on EMLA 7 rootstock



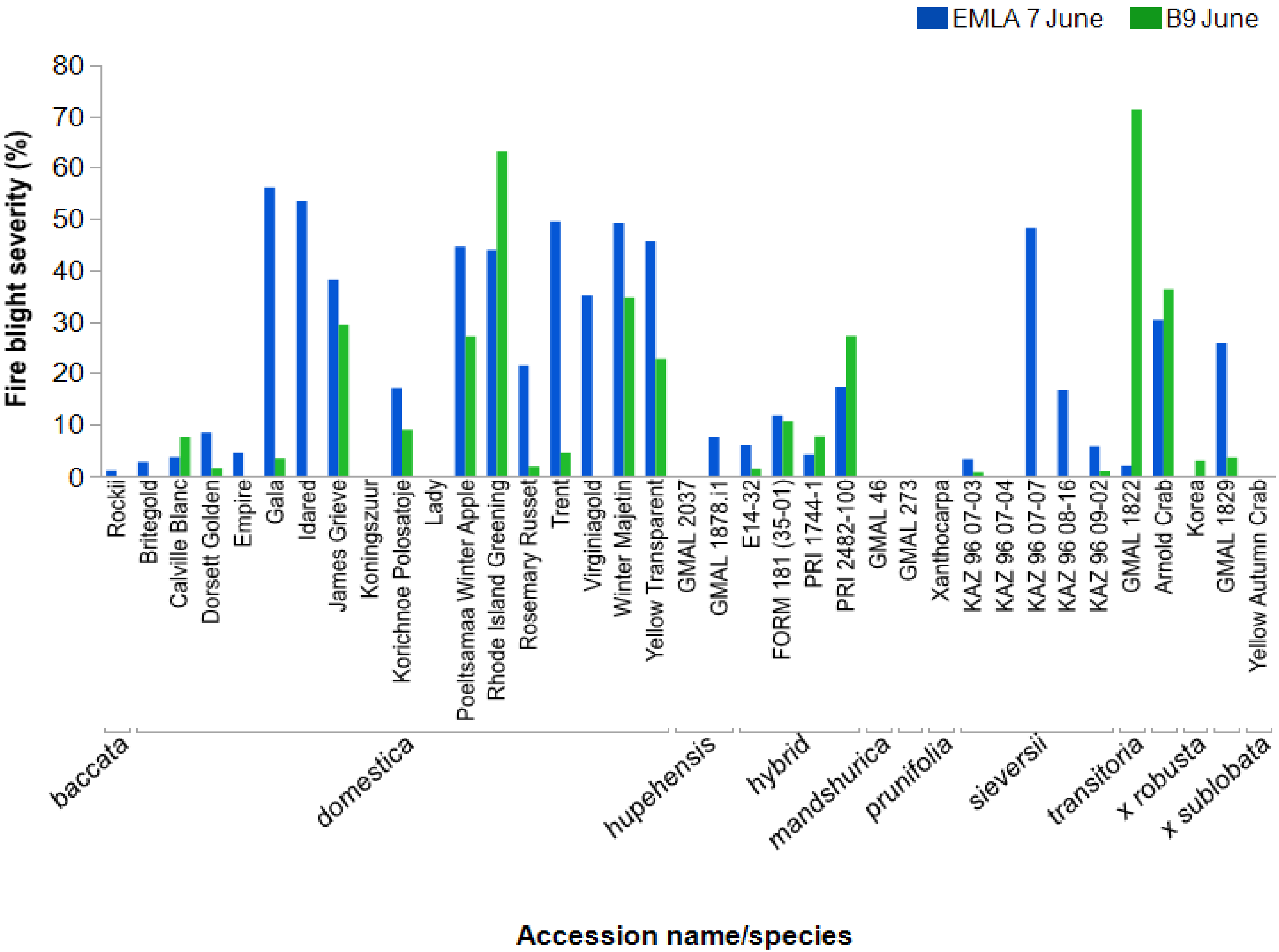
domestica: 1378
hybrid: 346
sieversii: 430

Cut severity data on EMLA 7 rootstock

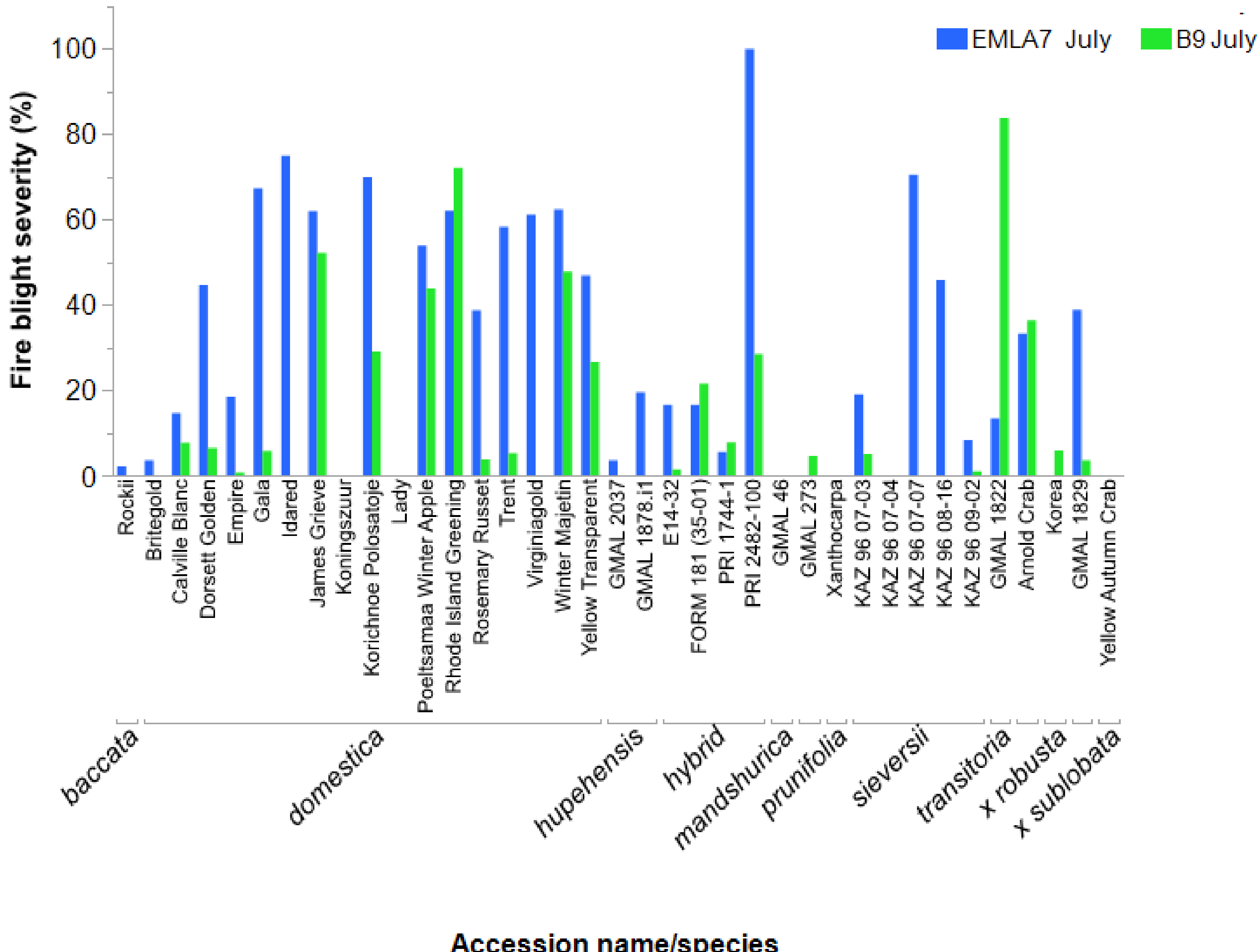


*All species have under 100 accessions

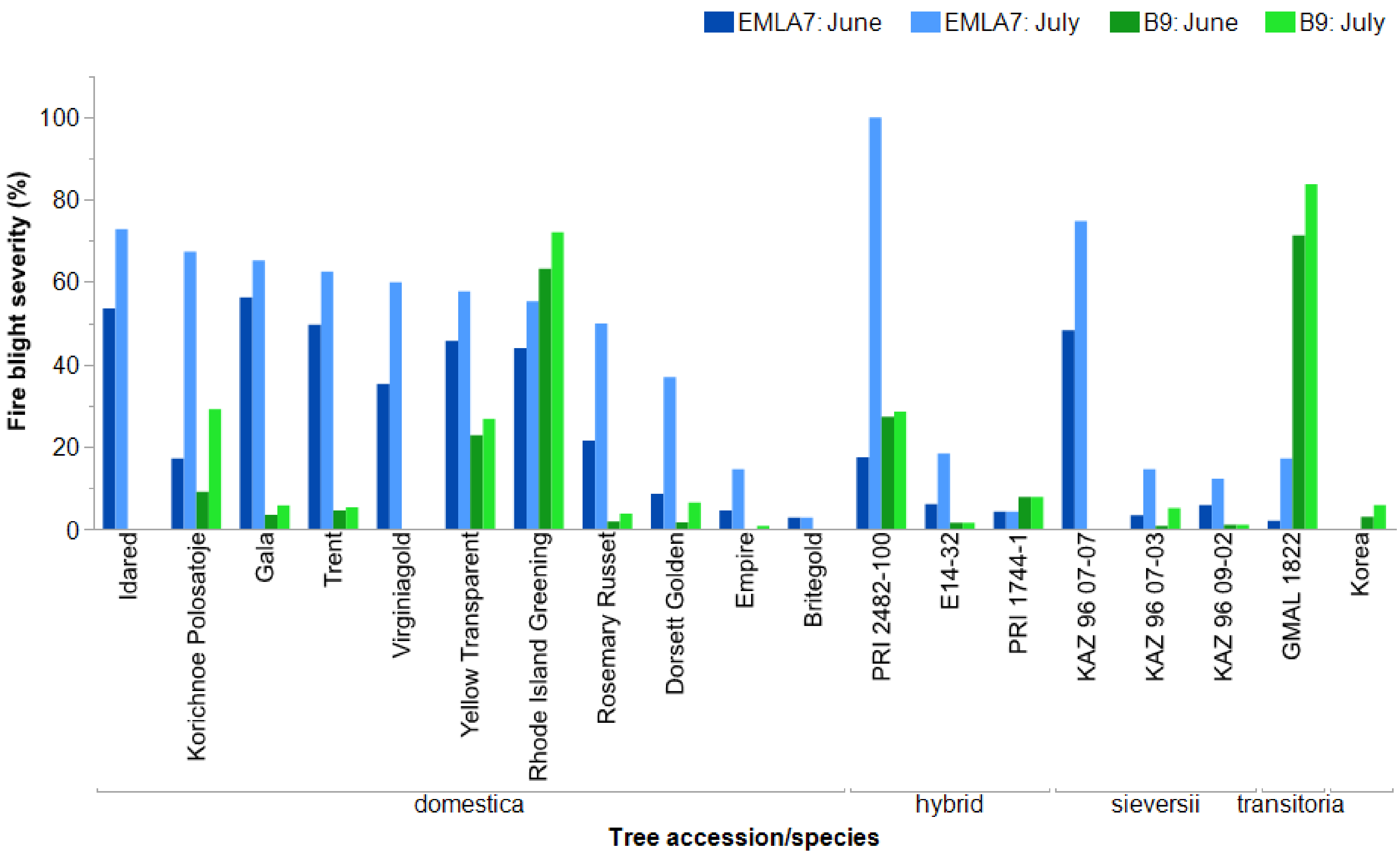
Rootstock comparisons in June



Rootstock comparisons in July



Rootstock comparisons over 2 months

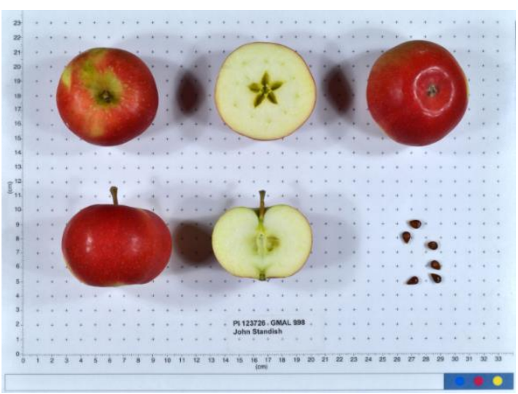


GRIN-Global Fire Blight Data

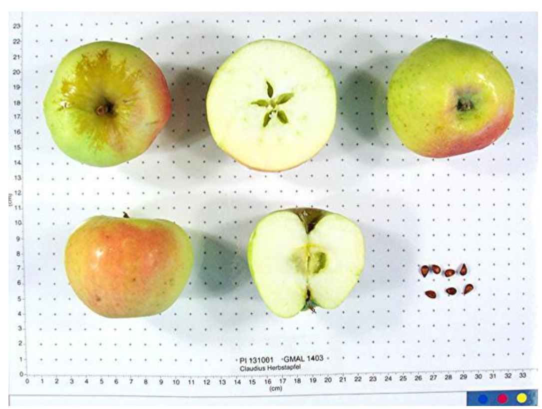
Low correlation between GRIN data and 2020 Incidence and Cut severity datasets

- r=0.21 for Cut severity
- r=0.28 for Incidence
- Different Rootstock (EMLA 9 VS EMLA 7)
- Strain specificity and disease resistance
- Random distribution of pathogen (escapes)

‘John Standish’ PI 123726
GRIN: Very resistant (1)
2020: 55% fire blight incidence



‘Claudius Herbstapfel’ PI 131001
GRIN: Very Susceptible (5)
2020: 5% fire blight incidence



‘Doux-AMR’ PI 122616
GRIN: Very Susceptible (5)
2020: 80% fire blight incidence

Malus Repropagation 2020

Species	Sum
M. angustifolia	2
M. arnoldiana	1
M. asiatica	1
M. baccata	2
M. coronaria	5
M. domestica	228
M. hartwigii	1
M. honanensis	3
M. hupehensis	1
M. hybr.	26
M. ioensis	5
M. micromalus	3
M. orientalis	1
M. prunifolia	2
M. robusta	1
M. sargentii	1
M. sieversii	25
M. spectabilis	1
M. spp.	5
M. sylvestris	1
M. toringo	4
M. toringoides	1
M. transitoria	2
M. yunnanensis	3
M. zhaojiaoensis	1
Total	**326**

326 accessions targeted for repropagation

- 160 were severely diseased
- 16 had no viable budwood, but are cryopreserved
- 303/326 are cryopreserved

Summer budding completed by Bob Martens

Malus Field Maintenance 2021

Working with Cornell University pathologists to develop approach to manage disease.

- New chemical applications to target streptomycin resistance
  - Blossom Protect, kasugamycin, and copper
    ***May observe increased fruit russeting
- Expand spraying program to include phenological extremes
  - We miss high risk periods for extreme early or late blooming accessions
- Lighter touch with fire blight pruning (leave scaffolds for regrowth)
- Enhanced scouting and removal of fire blight
- More focused evaluations of fire blight resistant rootstocks (B9 or Geneva series)
  - Rootstock compatibility with diverse *Malus* is essential

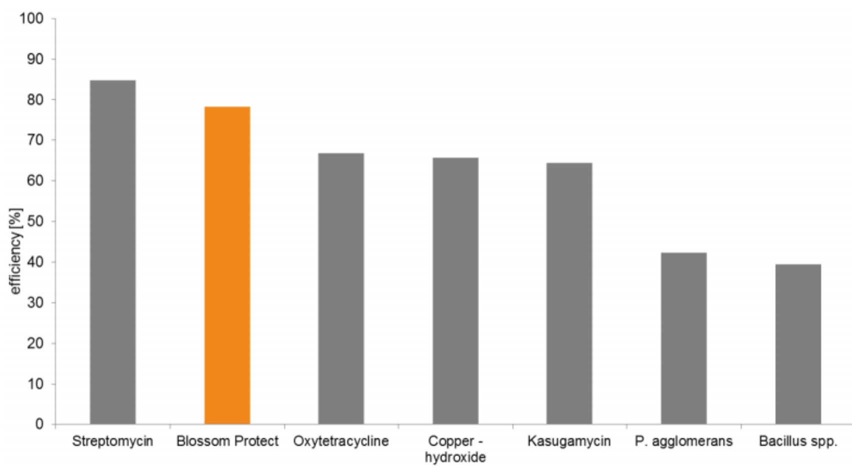


Fig. 7 Efficiency of Blossom Protect™ +Buffer Protect in field trials in the USA between 2008 and 2014 after artificial inoculation of the blossoms of apple or pear. In courtesy of Westbridge Agricultural Products

Impact on PGRU Distribution

Plant Disease • 2017 • 101:186-191 • http://dx.doi.org/10.1094/PDIS-06-16-0892-RE

Fire Blight Symptomatic Shoots and the Presence of *Erwinia amylovora* in Asymptomatic Apple Budwood

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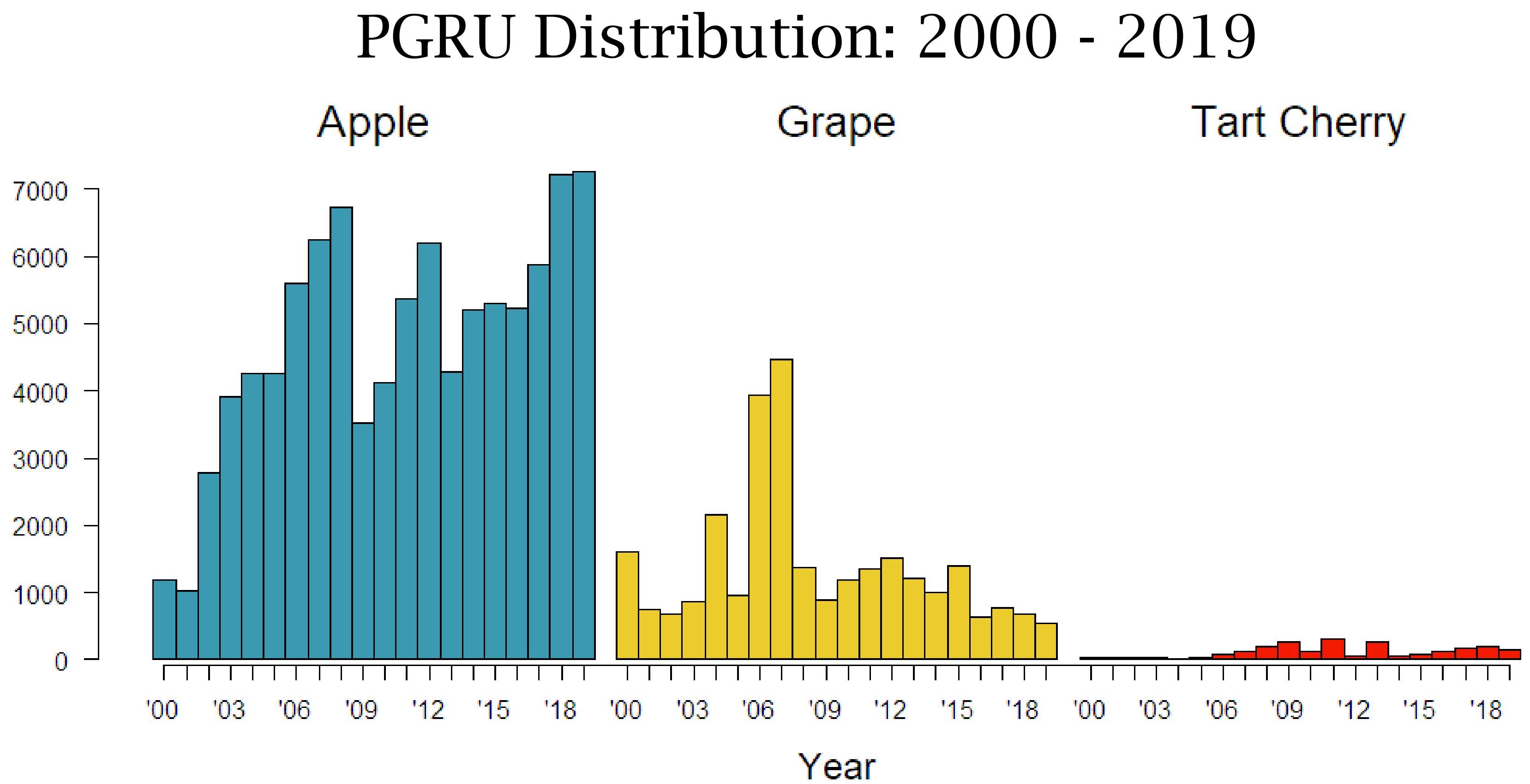
Abstract

Erwinia amylovora, the causal agent of fire blight, causes considerable economic losses in young apple plantings in New York on a yearly basis. Nurseries make efforts to only use clean budwood for propagation, which is essential, but *E. amylovora* may be present in trees that appear to have no apparent fire blight symptoms at the time of collection. We hypothesized that the use of infected budwood, especially by commercial nursery operations, could be the cause, in part, of fire blight outbreaks that often occur in young apple plantings in New York. Our goal was to investigate the presence of *E. amylovora* in asymptomatic budwood from nursery source plantings as it relates to trees with fire blight symptoms. From 2012 to 2015, apple budwood was collected from two commercial budwood source plantings of 'Gala' and 'Topaz' at increasing distances from visually symptomatic trees. From these collections, internal contents of apple buds were analyzed for the presence of *E. amylovora*. *E. amylovora* was detected in asymptomatic budwood in trees more than 20 m from trees with fire blight symptoms. In some seasons, there were significant ($P \leq 0.05$) differences in the incidence of *E. amylovora* in asymptomatic budwood collected from symptomatic trees and those up to 20 m from them. In 2014 and 2015, the mean *E. amylovora* CFU per gram recovered from budwood in both the Gala and Topaz plantings were significantly lower in budwood collected 20 m from symptomatic trees. Further investigation of individual bud dissections revealed that *E. amylovora* was within the tissue beneath the bud scales containing the meristem. Results from the study highlight the shortcomings of current budwood collection practices and the need to better understand the factors that lead to the presence of *E. amylovora* in bud tissues to ensure the production of pathogen-free apple trees.

Impact on PGRU Distribution



867 GRIN Requests in 2020
About 30 were research (legitimate) requests

Malus Distribution Letter

Dear Requestor,

Thank you for your interest and support of our apple conservation program. During the 2020 season we had an outbreak of streptomycin resistant fire blight throughout our apple orchards. As a result, ***we are not distributing apple summer budwood or dormant scion wood this year***. Although we are managing this disease and are confident our collection can recover, spread of this pathogen through distribution would negatively impact growers and research and breeding programs. Our apologies if this hinders your projects.

For this season, we recommend an internet search for scions or contact your local nurseries for suitable alternatives. Plant Information Online (https://plantinfo.umn.edu/) is a resource to discover nurseries in North America with specific cultivars.

We are honored to be stewards of one of the world's largest and diverse apple collections and look forward to serving you in the future.

Sincerely,

Ben Gutierrez, Ph.D.
Plant Genetic Resources Unit
USDA-ARS
630 W. North St.
Geneva, NY 14456
315-787-2439

PGRU will review each request and either send our "rejection letter" for non-research requests or the "fire blight letter." We will record and report the impact on apple distribution.

Discussion and Questions

Thoughts on:
- Fire blight management
- Distribution and communication to stakeholders
- Potential impact to research and breeding
- Evaluation methods