USDA APHIS PPQ FIELD OPERATIONS
Plant Germplasm Quarantine Program (PGQP)
USDA APHIS Quarantine facility located in the old Airport site
Building 580 hosts S&T and FO (PGQP)
PGQP current workflow - Overview

- Poaceae Program
- Vegetable Program
- Pomes Program
- Stones Program
- Woody & Ornamentals Program

Foreign plant material

Greenhouse tests
- Molecular test
- NGS test*

Tissue Culture Therapy

Post-Therapy

Release pathogen-free germplasm

Pomes Field Testing 3-4 years
Quarantine period per crop

- Rice: 1 year
- Grasses: 2 years
- Sugarcane: 3 years
- Sorghum: 4 years
- Bamboo: 5 years
- Potatoes: 1 year
- Sweet potatoes: 2 years
- Cassava: 3 years
- Ribes: 4 years
- Prunus: 5 years
- Apples: 1 year
- Pears: 2 years

The longest program is for Bamboo, Sugarcane, Sorghum, Ribes, Prunus, Apples, and Pears, each requiring 5 years in quarantine.
Summary of Pathogens detected on imports
Period 2016-2019

Bioassays (Greenhouse)

PCR-Lab
Treatments conducted (Period 2016-2019)

Majority of importations required treatment (>60%)
Ramping up Bioassays for 2020 (Russian, Spy, M. micromalus)

960 bioassays <> ~4000 grafts
Nucleic Acid extraction calendar for PCR-based diagnostics

- **Virus**
  - RNA extraction
  - April-May

- **Viroids**
  - RNA extraction
  - June-July

- **Phytoplasmas**
  - DNA extraction
  - August-September
**PCR-based testing panel-Pomes (July 2019)**

<table>
<thead>
<tr>
<th>Family</th>
<th>Genus</th>
<th>Species</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Betaflexiviridae</td>
<td>Foveavirus</td>
<td>Apple stem pitting virus</td>
<td>ASPV</td>
</tr>
<tr>
<td>Betaflexiviridae</td>
<td>Capillovirus</td>
<td>Apple stem grooving virus</td>
<td>ASGV</td>
</tr>
<tr>
<td>Betaflexiviridae</td>
<td>Trichovirus</td>
<td>Apple chlorotic leaf spot virus</td>
<td>ACLSV</td>
</tr>
<tr>
<td>Bromoviridae</td>
<td>I larvirus</td>
<td>Apple mosaic virus</td>
<td>ApMV</td>
</tr>
<tr>
<td>Phenuiviridae</td>
<td>New</td>
<td>Rubbery wood virus</td>
<td>ARW-1, 2</td>
</tr>
<tr>
<td>Bunya</td>
<td>Coguvirus</td>
<td>Citrus concave gum associated virus</td>
<td>CCGaV</td>
</tr>
<tr>
<td>Bunya</td>
<td>Coguvirus</td>
<td>Citrus Virus A</td>
<td>CiVA</td>
</tr>
<tr>
<td>Luteoviridae</td>
<td>Luteovirus</td>
<td>Apple Luteovirus 1</td>
<td>ALV-1</td>
</tr>
<tr>
<td>Pospiviroidae</td>
<td>Apscaviroid</td>
<td>Apple dimple fruit viroid</td>
<td>ADFVd</td>
</tr>
<tr>
<td>Pospiviroidae</td>
<td>Apscaviroid</td>
<td>Apple scar skin viroid</td>
<td>ASSVd</td>
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<tr>
<td>Pospiviroidae</td>
<td>Apscaviroid</td>
<td>Apple fruit crinkle viroid</td>
<td>AFCVd</td>
</tr>
<tr>
<td>Pospiviroidae</td>
<td>Apscaviroid</td>
<td>Pear blister canker viroid</td>
<td>PBCVd</td>
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<td>Avsunviroidae</td>
<td>Pelamoviroid</td>
<td>Apple hammerhead viroid</td>
<td>AHVd</td>
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<tr>
<td>Pospiviroidae</td>
<td>Hostuviroid</td>
<td>Hop stunt viroid</td>
<td>HSVd</td>
</tr>
</tbody>
</table>

AND Phytoplasma PCR detection
## Molecular Lab issues PCR reports

### RT-PCR: Apple chlorotic leaf spot virus (ACLSV) in Pomes

**October 2018**

**Requestor – R. Jones**

#### Results

<table>
<thead>
<tr>
<th>Sample</th>
<th>RT-PCR Reaction</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>Positive</td>
<td>Yes</td>
</tr>
<tr>
<td>Sample 2</td>
<td>Negative</td>
<td>No</td>
</tr>
<tr>
<td>Sample 3</td>
<td>Positive</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**Overall Conclusions**

Of the 157 samples tested, the following samples tested positive for Apple chlorotic leaf spot virus (ACLSV) by RT-PCR using the primer set ACLSV-F/ACLSV-R.

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**Digitally signed by CODY KEPNER**

Date: 2018.10.05 09:32:28 -04'00"
### 2019 Pomes Processed samples

#### Molecular Diagnostic Lab

<table>
<thead>
<tr>
<th>CROP</th>
<th>Unique Imports</th>
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</thead>
<tbody>
<tr>
<td>Apples</td>
<td>146</td>
</tr>
<tr>
<td>Pears</td>
<td>112</td>
</tr>
<tr>
<td>Quinces</td>
<td>16</td>
</tr>
</tbody>
</table>

#### Over 10,000 RT-PCRs

<table>
<thead>
<tr>
<th>Source</th>
<th># Imports</th>
</tr>
</thead>
<tbody>
<tr>
<td>Public</td>
<td>156</td>
</tr>
<tr>
<td>Private</td>
<td>118</td>
</tr>
</tbody>
</table>

2019: Provisional Release 20; Final Released: 15
Field indexing

1 apple importation = 9 field indicators (3 varieties in triplicates)
1 pear importation = 15 field indicators (5 varieties in triplicates)

17 Apples, 13 Pears, 1 Quince (2019-2021)
50 Apples, 45 Pears, 6 Quince (2020-2022)
USDA APHIS PGQP field block (6 acres)
Implementing HTS: PGQP Sequencing Lab

RNA extraction
- Qiagen QIAcube

RNA & Library QA/QC
- Agilent 4200 Tapestation

Sequencing
- Illumina NextSeq 550

Server
- 24 core processor
- 256 GB memory
- 40 TB
PGQP committed to implement HTS

Target 2022
IMPORT CLEAN
Import from Certified Sources
Life will be much easier

Acknowledgments:
PGQPers
Clint McFarland (Executive Director-District 4)
FPS-Maher Al Rwahnih & Kristian Stevens
Cornell-AgriTech-Marc Fuchs & Khan Awais
NCPN-Prosser-Scott Harper
CFIA-Allison Gratz & Mike Rott