The Clemson Clean Plant Center: Updates and Progress

PLANT VIROLOGY LAB
College of Agriculture, Forestry and Life Sciences

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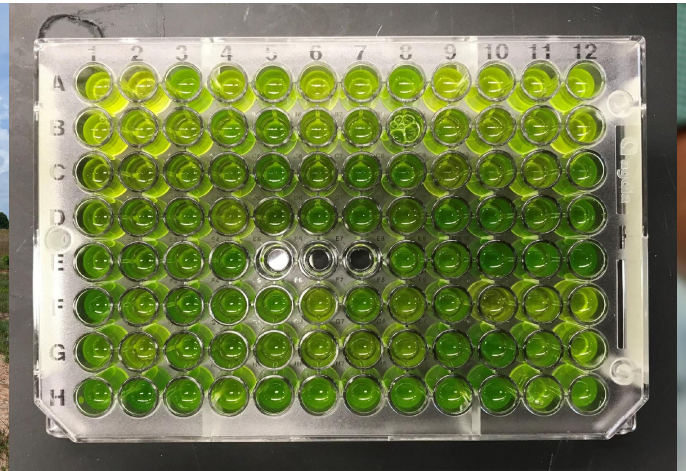


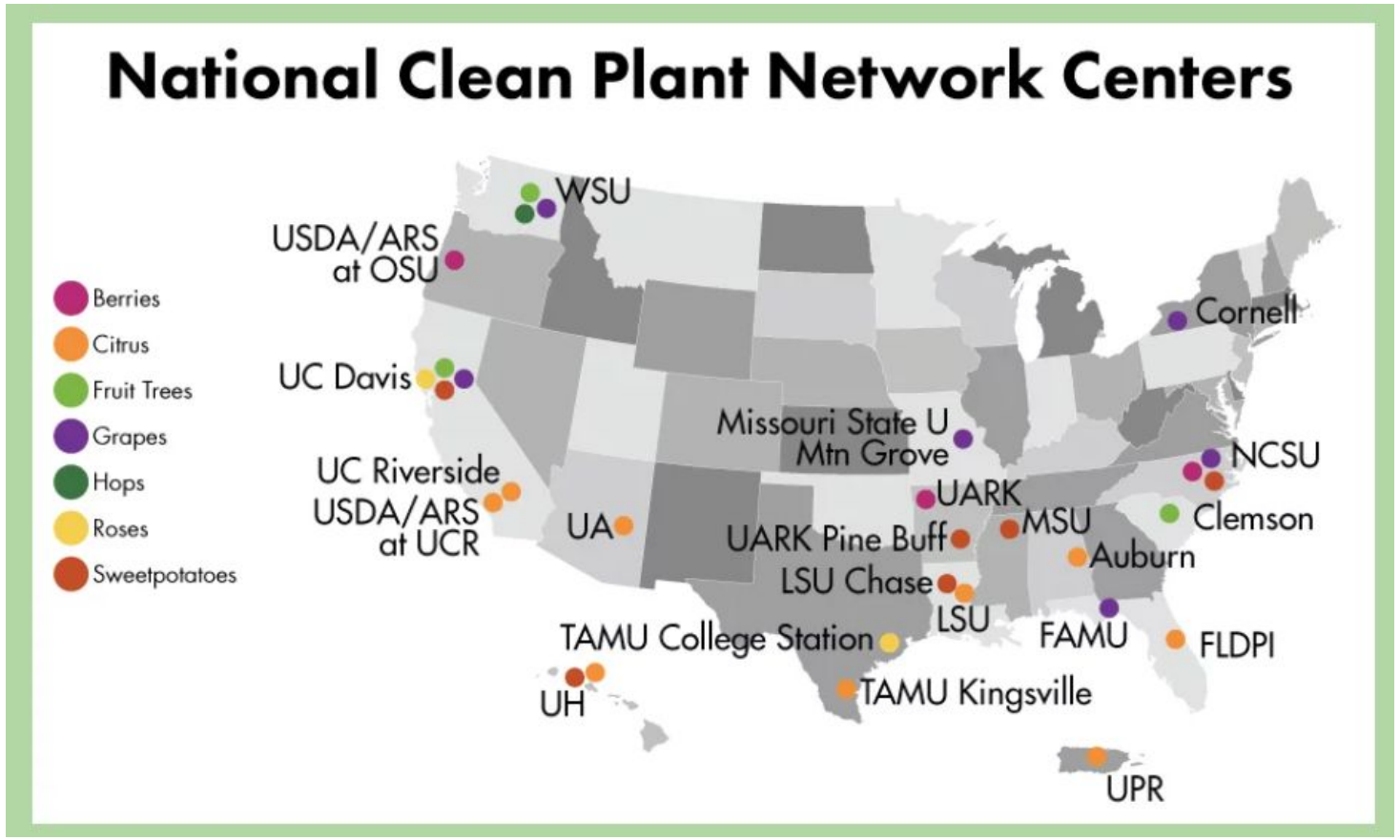
National Clean Plant Network (NCPN)

MISSION

The Network produces and distributes asexually propagated plant material free of targeted graft-transmissible plant pathogens to ensure the global competitiveness of specialty crop producers and to protect the environment.

National Clean Plant Network (NCPN)

- Implemented in 2009
- APHIS, ARS, and NIFA
- Supports clean plant centers throughout the U.S.
- Fruit trees, grapes, citrus, berries, hops, roses, and sweet potato
- Fruit tree centers at Prosser WA, Davis CA, and Clemson SC
- Members: researchers, regulators (fed and state), growers, nurseries



Why is virus prevention important?

Damage to Orchards

- Reduced yield
- Reduced fruit quality
- Tree weakening
- Interaction with other pathogens

Economic Losses

- Cost of tree removal and replacement
- Time until new orchard becomes productive
- Weakened trees lost revenue

Once infected with a virus, a tree remains infected for life.

Management options are limited once viruses are present in the orchard.

Which viruses* are important?

**And other graft-transmissible pathogens*

Major Graft-transmissible Diseases of Peach in the Southeast

Peach stunt disease

- Synergy between prune dwarf virus and Prunus necrotic ringspot virus
- Disease severity varies by cultivar

Photos by Simon Scott

Phony peach disease

- *Xylella fastidiosa*
- Re-surging in some areas
- Sharpshooter-vectored
- Graft-transmissible (?)

Photos by Kendall Johnson

Prune dwarf virus (PDV) is graft and pollen-transmitted

- Widespread globally in stone fruits
- Symptoms: stunting, shortened internodes, gumming, bark splitting
- Preliminary evidence of increased susceptibility to bacterial spot in PDV-infected trees

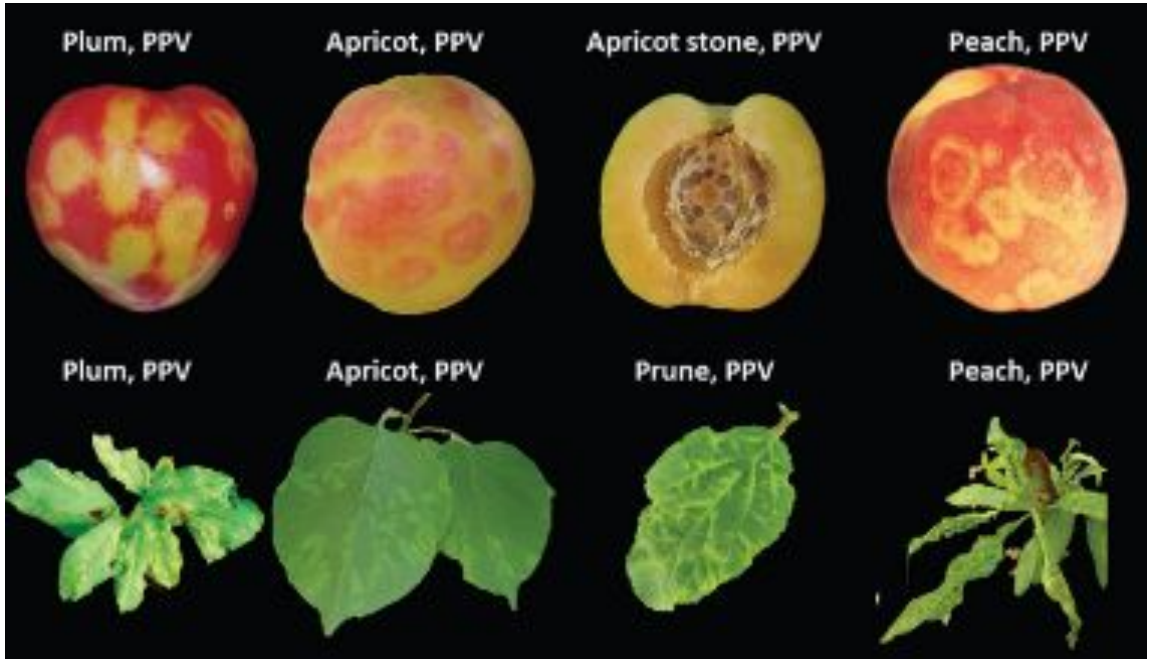
Prunus necrotic ringspot virus (PNRSV) is graft and pollen-transmitted

- Widespread
- Symptoms: initial shock symptoms are severe, chronic symptoms usually less conspicuous

Shot-holing usually only observed in early shock phase, easily mistaken for copper damage

Plum pox virus (PPV) has not been detected in the Southeast, but we must remain vigilant

- Potyvirus (aphid-vectored)
- Detected in Pennsylvania in 1999, then in Michigan and New York
- Now “eradicated” from U.S.
- Extremely devastating, harsh eradication procedures
- Can kill the tree



Tomato ringspot virus (ToRSV)

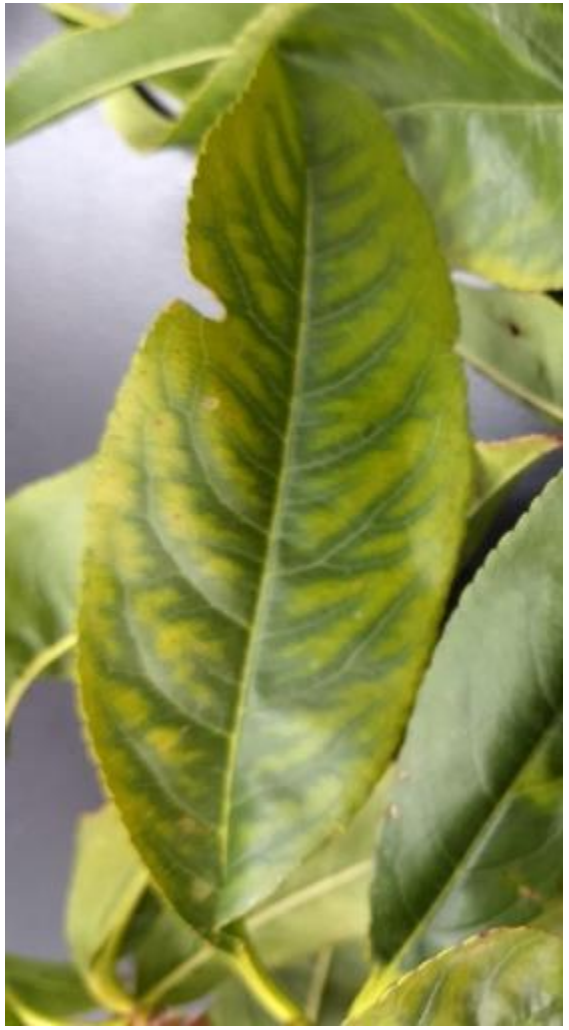
- Nematode-vectored
- Can persist in weeds and nematode populations
- Thickened, necrotic bark, stem pitting, decline of orchard
- Rare in southeastern stone fruits?

Photo: https://www.canr.msu.edu/news/fruit_trees_in_a_sea_of_viruses

X-Disease Phytoplasma in Peach in the PNW

Healthy

Early Stage XDP



Late Stage XDP

Photos: Dr. Scott Harper, WSU

Viruses should be easily prevented, but can quickly spread if inoculum is high

Heavily PDV-infected peach block

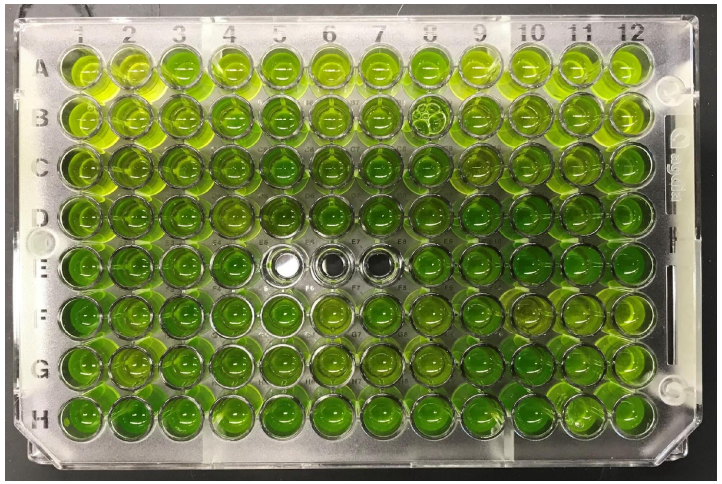
6251	6250	6231	6230	6211	6210
6252	6249	6232	6229	6212	6209
6253	6248	6233	6228	6213	6208
6254	6247	6234	6227	6214	6207
6255	6246	6235	6226	6215	6206
6256	6245	6236	6225	6216	6205
6257	6244	6237	6224	6217	6204
6258	6243	6238	6223	6218	6203
6259	6242	6239	6222	6219	6202
6260	6241	6240	6221	6220	6201

Sporadic virus infections

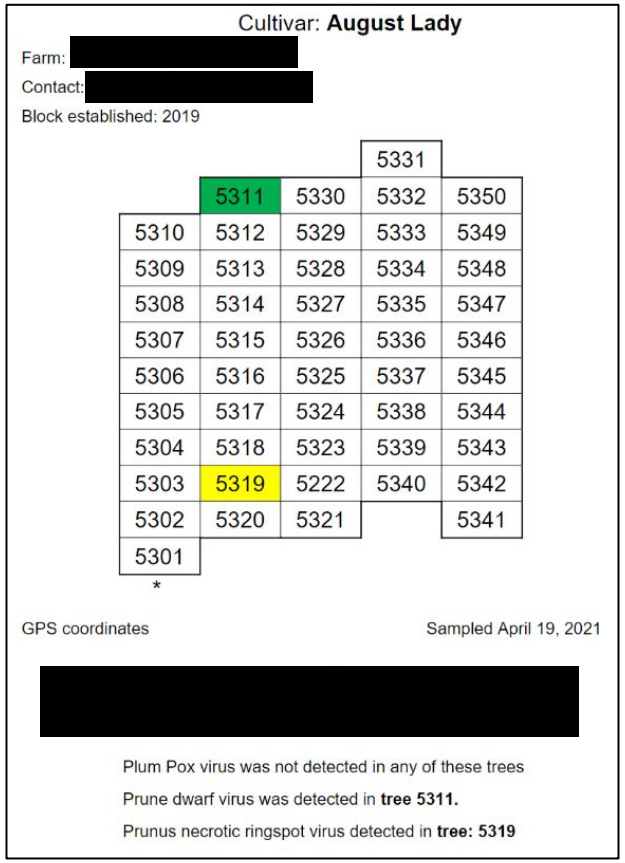
	6176	6175	6146	6145	6116	6115
	6177	6174	6147	6144	6117	6114
	6178	6173	6148	6143	6118	6113
	6179	6172	6149	6142	6119	6112
	6180	6171	6150	6141	6120	6111
6200	6181	6170	6151	6140	6121	6110
6199	6182	6169	6152	6139	6122	6109
6198	6183	6168	6153	6138	6123	6108
6197	6184	6167	6154	6137	6124	6107
6196	6185	6166	6155	6136	6125	6106
6195	6186	6165	6156		6126	6105
6194	6187	6164	6157	6134	6127	6104
6193	6188	6163	6158	6133	6128	6103
6192	6189	6162	6159	6132	6129	6102
6191	6190	6161	6160	6131	6130	6101

Clemson Clean Plant Center –
1. Southeastern Budwood Program virus diagnostics

Blocks of 50-100 trees/cultivar are established on farms in South Carolina and Georgia

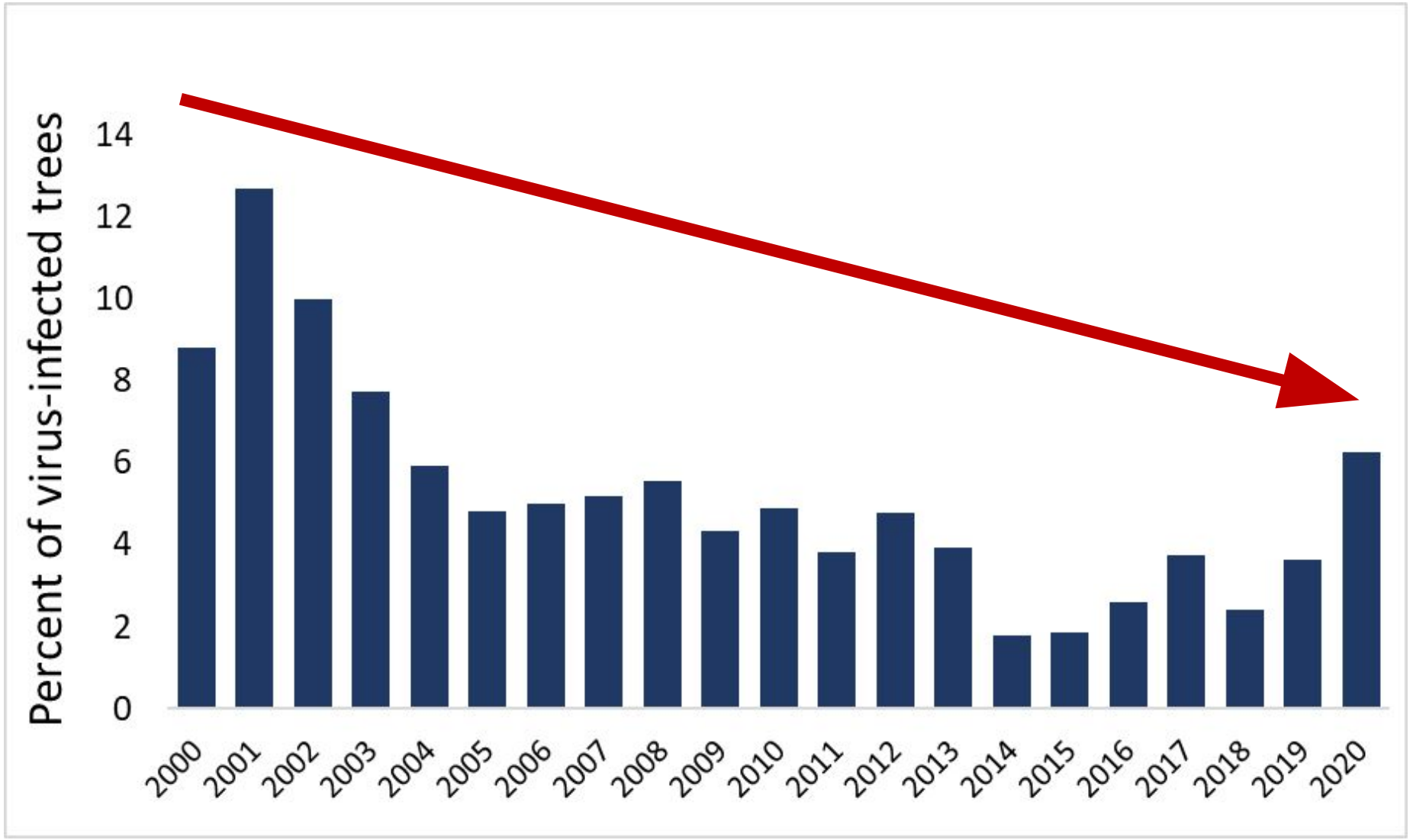


Leaf samples are collected in spring and tested for PDV, PNRSV and PPV by ELISA



Maps of the blocks with virus infection status are produced and provided to participating nurseries

The Budwood Program has resulted in lower virus incidence in SC and GA over the last two decades



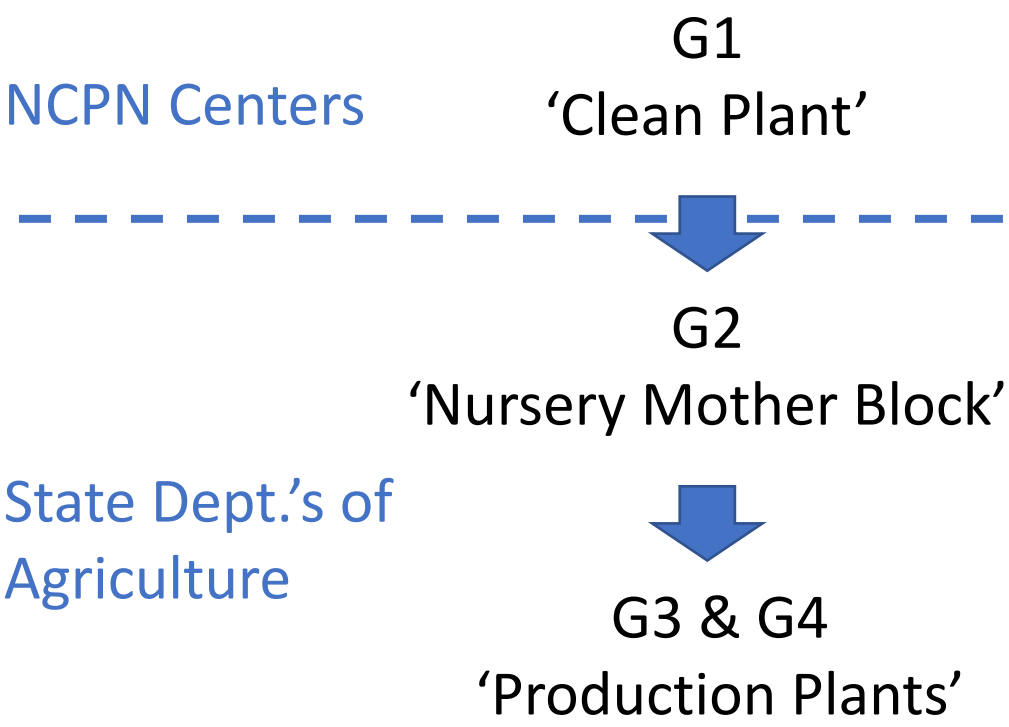
Southeastern Budwood Program in 2021

- **38** peach cultivars tested for PDV, PNRSV, and PPV
- **2,700** trees tested

Results

- **3.6%** infected with PNRSV
- **0.6%** infected with PDV
- No PPV

What is a clean plant?



- Not every state has a certification program
- SE Budwood Program trees are negative for 3 viruses

'Clean' plants are negative for all viruses known to cause damage

G1-G4 Certification Model

Regulations (required testing, visual inspections, etc.)

G1: Foundation

Trees maintained at clean plant centers, rigorous testing for pathogens

G2-G3 level can be blurred

G2: Mother blocks

Stock produced from foundation, conditions to minimize infection, frequently maintained by nurseries

G3: Increase blocks

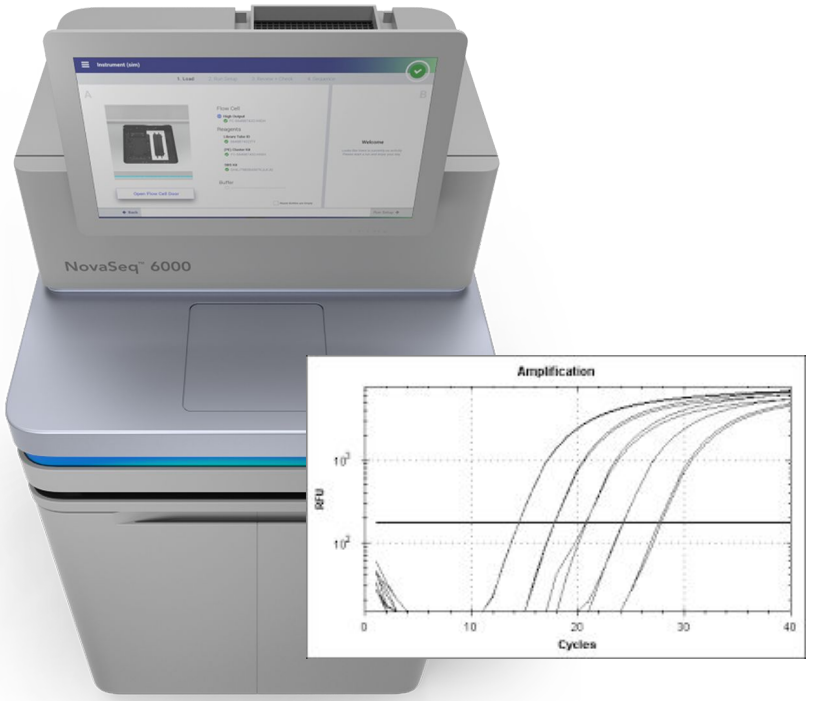
From G2, usually maintained in secondary increase blocks/certified nursery blocks

G4: Production trees

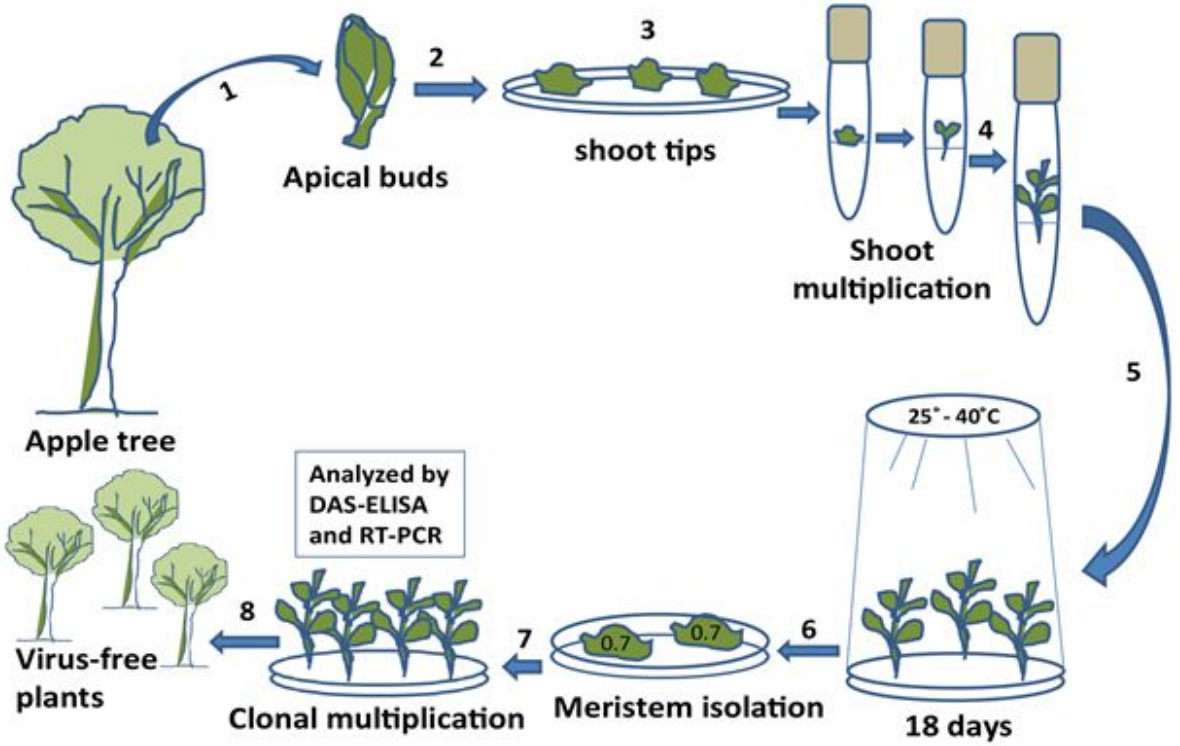
Propagated from G3, certified plants destined for delivery to the grower

ALL certified material originates at foundation (tested for all viruses, highest sanitary standards, true to type)

Clemson Clean Plant Center –
2. *Prunus* panel diagnostics and virus elimination therapy

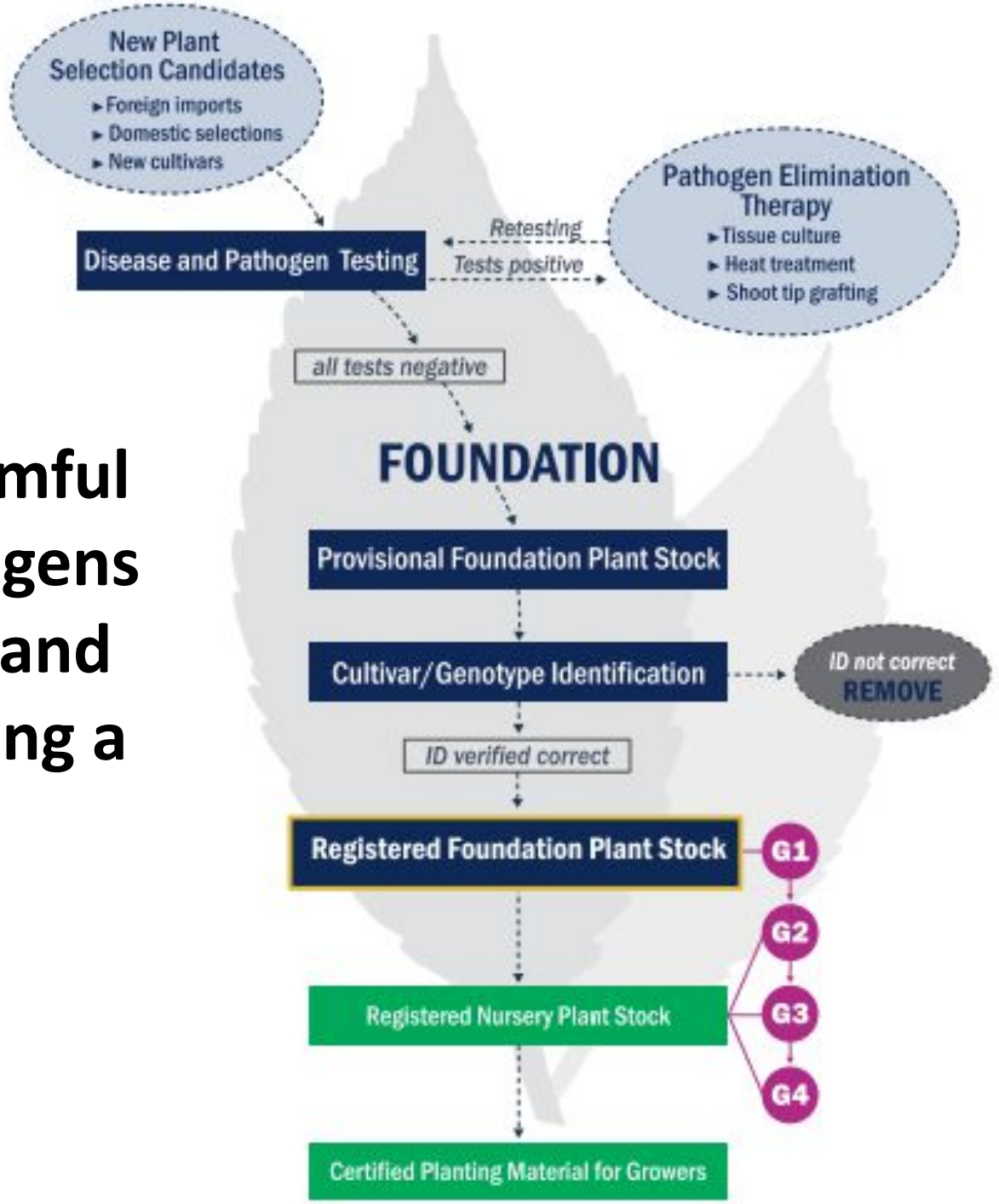


Advanced diagnostics for 26 viruses, viroids, and graft-transmissible bacteria using high throughput sequencing and PCR



Virus elimination therapy using thermotherapy and tissue culture

The diagnostic process targets harmful and economically important pathogens at the optimal times of detection and tests multiple times before declaring a plant clean.



Diagnostics for *Prunus* viruses and graft-transmissible pathogens

Shifting to entirely molecular methods

- High throughput sequencing
- qRT-PCR
- qPCR

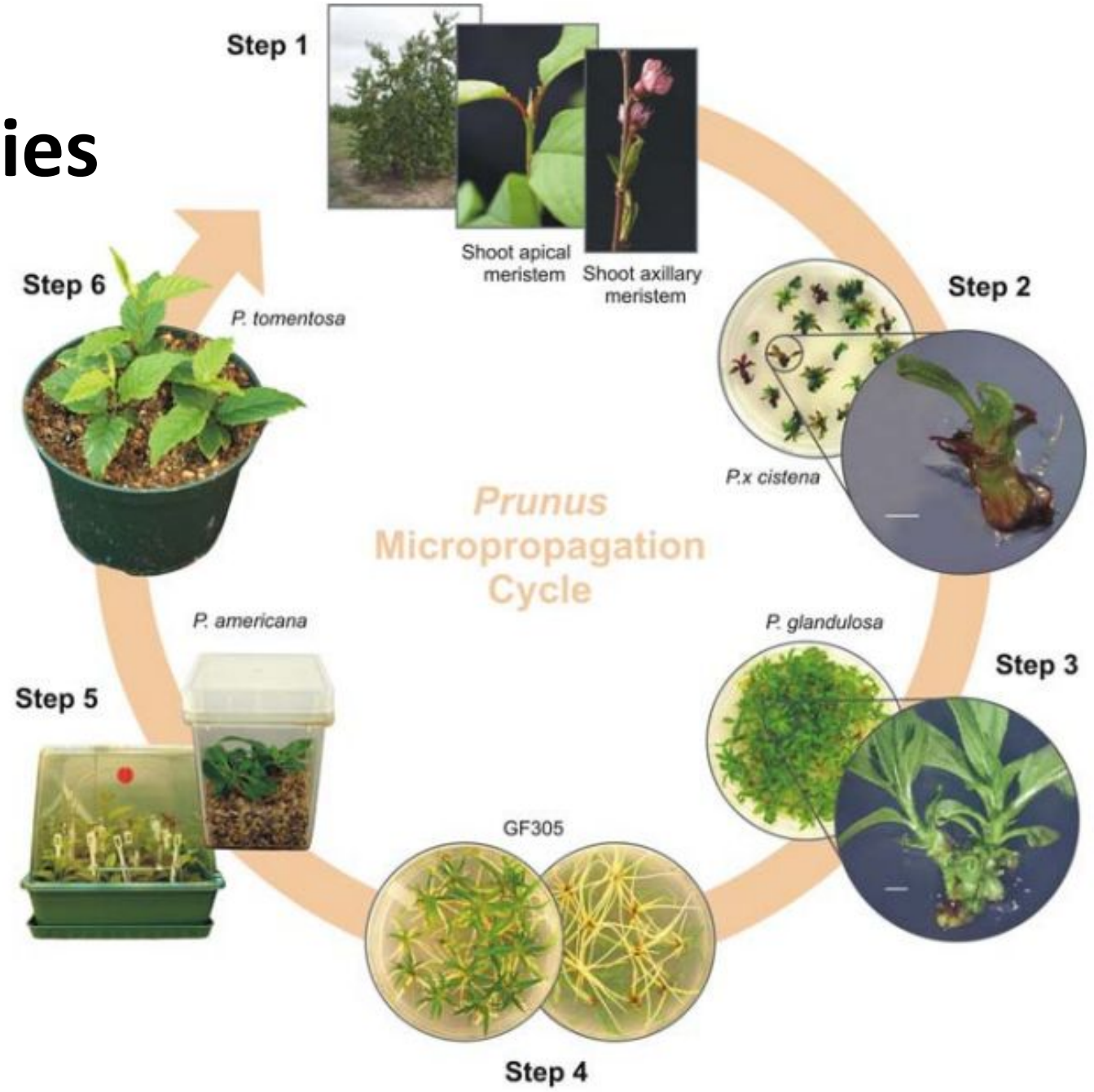
✔ Higher sensitivity

✔ Better specificity

✔ Can test for multiple pathogens at once

Virus elimination therapies can be conducted on infected selections

- Thermotherapy
- Meristem tip tissue culture



Clemson Clean Plant Center – 3. *Prunus* Foundation

Virus-negative, clean Prunus cultivars important to the Southeast are maintained in indoor and outdoor Foundation collections under conditions that minimize the risk of re-infection.

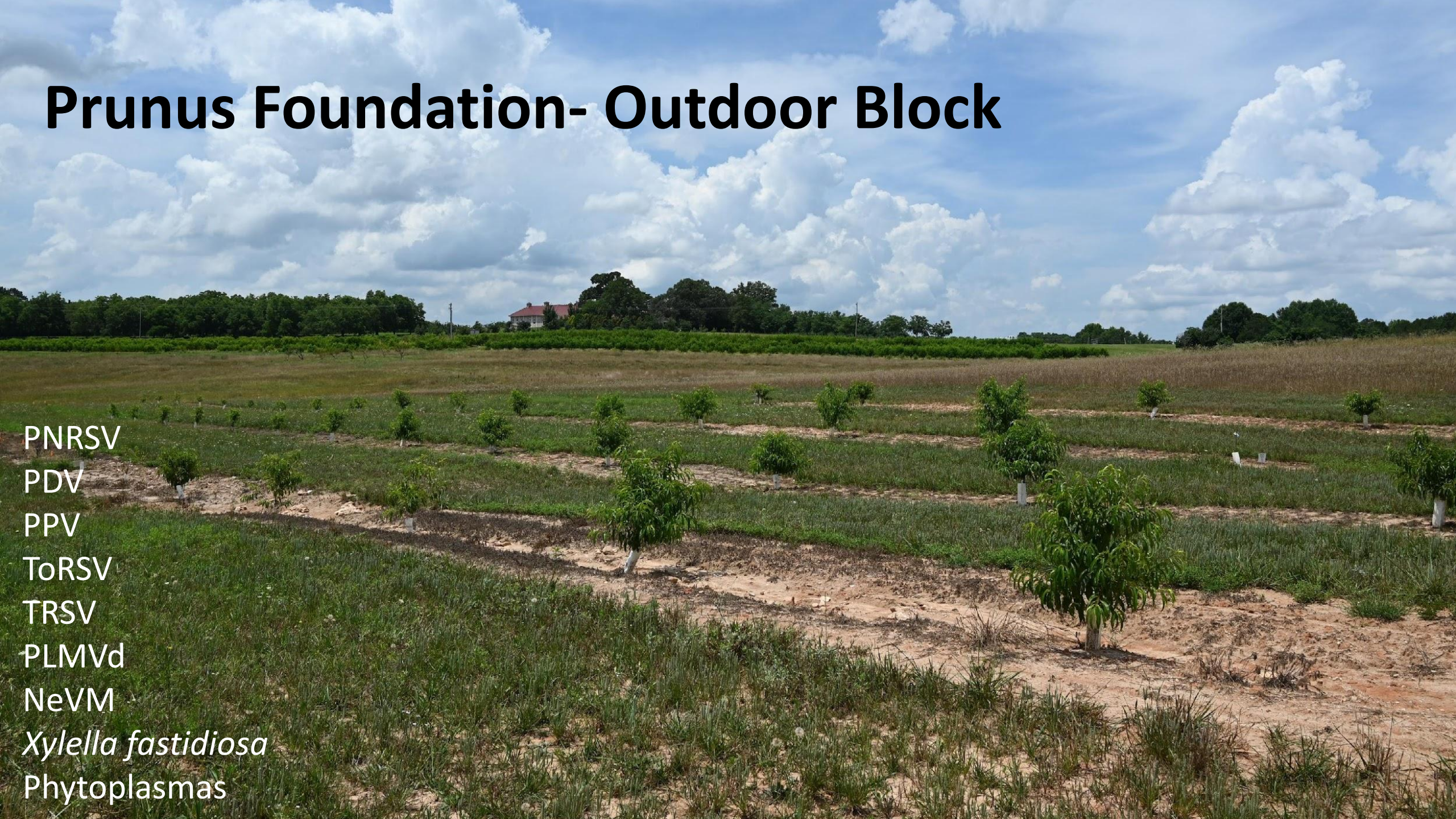
Foundations are tested annually for a subset of the Prunus panel pathogens.

This Foundation collection serves as a source of clean, nuclear stock available for propagation.

Prunus Foundation- Indoor Potted

PNRSV
PDV
PPV
PLMVd
Xylella fastidiosa
Phytoplasmas
1/5 by HTS each year

Prunus Foundation- Outdoor Block



PNRSV
PDV
PPV
ToRSV
TRSV
PLMVd
NeVM
Xylella fastidiosa
Phytoplasmas

Current Prunus Foundation at Musser Farm

Screenhouse

Field Block

PEACH CULTIVARS

- **Big Red**
- Carymac
- Cresthaven
- E. Augustprince
- **Flameprince**
- **Juneprince**
- **O'Henry**
- Redglobe
- Rubyprince
- **Summergold**
- Winblo
- Belle of Georgia
- **Flavorich**
- **Flavorcrest**
- **Harvester**
- **Carored**

ROOTSTOCKS

- **Nemaguard**
- **Nemared**
- **Flordaguard**
- **Lovell**
- **Guardian**

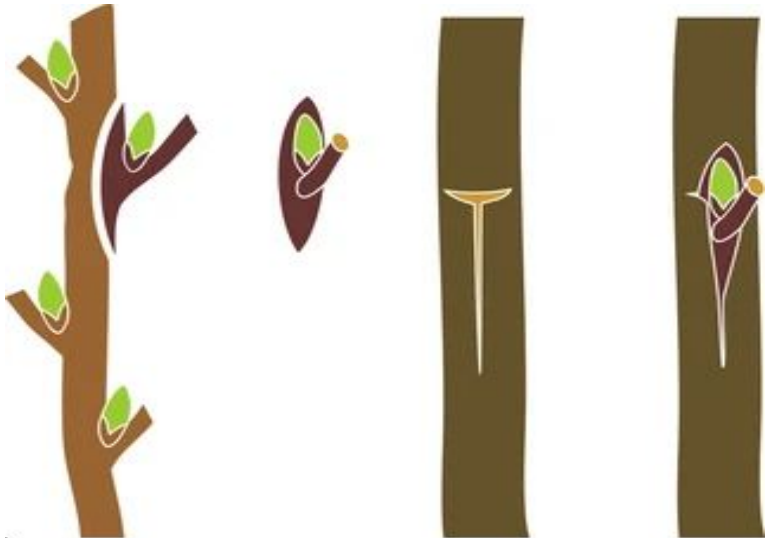
PLUMS

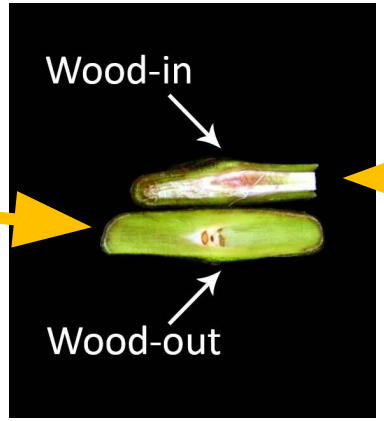
- Ruby Queen
- Spring Satin

Budwood Program	Foundation (G1)
4 participating nurseries and several participating growers each year	Public program, can be used by anyone
Mother trees tested for PPV, PNRSV, and PDV	Mother trees tested for 26 viruses/viroids/bacteria
Budwood source trees (50-100/block) are full-size, providing maximum amount of budwood	Foundation plants (2-3 indoor) are in pots and provide small amounts of budwood
Time to establish a budwood block is a couple weeks	Making a clean plant can take several years

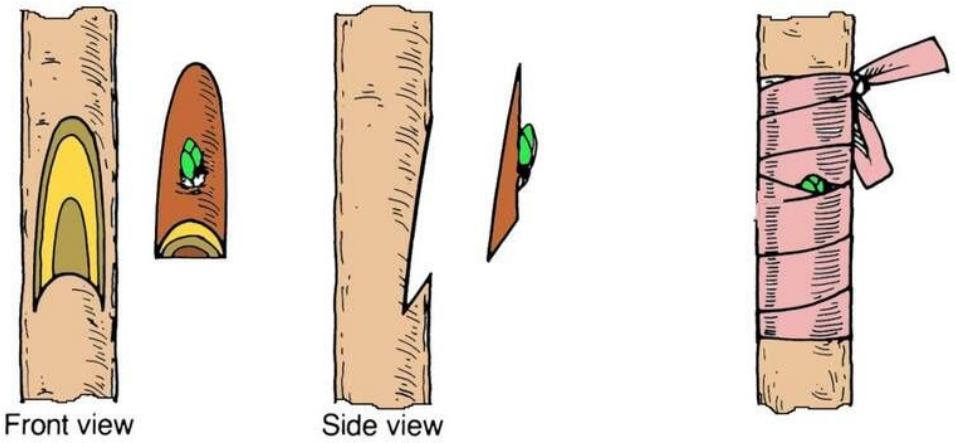
Is *Xylella fastidiosa* transmissible through grafting in peach?

T-Budding





Chip Budding



T-budding completed in summer 2021.

Chip budding will occur in winter 2021-2022.

Plant Disease • 2021 • 105:14-26 • https://doi.org/10.1094/PDIS-05-20-1061-FE

M. Fuchs†, C. V. Almeyda, M. Al Rwahnih, S. S. Atallah, E. J. Cieniewicz, K. Farrar, W. R. Foote, D. A. Golino, M. I. Gómez, S. J. Harper, M. K. Kelly, R. R. Martin, T. Martinson, F. M. Osman, K. Park, V. Scharlau, R. Smith, I. E. Tzanetakis, G. Vidalakis, and R. Welliver

Economic studies highlighted the critical nature of NCPN centers for the sustainability of viticulture and fruit tree production with documented returns on public investments ranging from 10:1 to 150:1

(Fuchs et al. 2021)

Acknowledgements

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- Brodie Cox, Garner Powell, and the rest of the Cieniewicz Lab
- Dr. Scott Harper (Clean Plant Center Northwest, WSU)
- Dr. Maher Al Rwahnih (Foundation Plant Services, UC-Davis)
- Clemson Peach Team
- Musser Farm crew
- South Carolina Peach Council
- Georgia Peach Council
- National Clean Plant Network

www.cleanplant.Clemson.edu