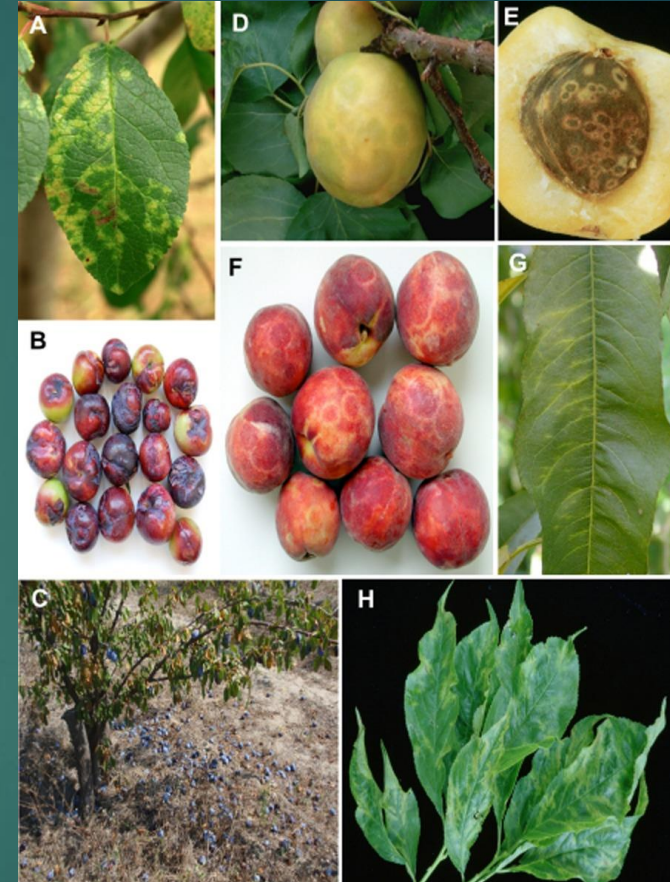


Plum Pox Virus or PPV

- ▶ Called sharka after the Bulgarian word for pox
- ▶ Vecteded by aphids
- ▶ Worldwide, one of the most important diseases of stonefruit
- ▶ Wide-spread in Europe and Middle East; also present in Canada, Asia, South America
- ▶ Eradicated from Pennsylvania



Typical symptoms induced by *Plum pox virus* on a domestic plum leaf (A), domestic plum fruits (B), premature domestic plum fruit drop (C), an apricot fruit (D), an apricot stone (E), peach fruits (F), a peach leaf (G) and Japanese plum leaves (H). From Garcia et al, MPP 15:226 2014

Can almond serve as a reservoir host for Plum Pox Virus?

Chris Dardick¹ and Elizabeth Rogers²

¹USDA ARS, Appalachian Fruit Research Station, WV.

²USDA ARS, Fort Detrick, MD.



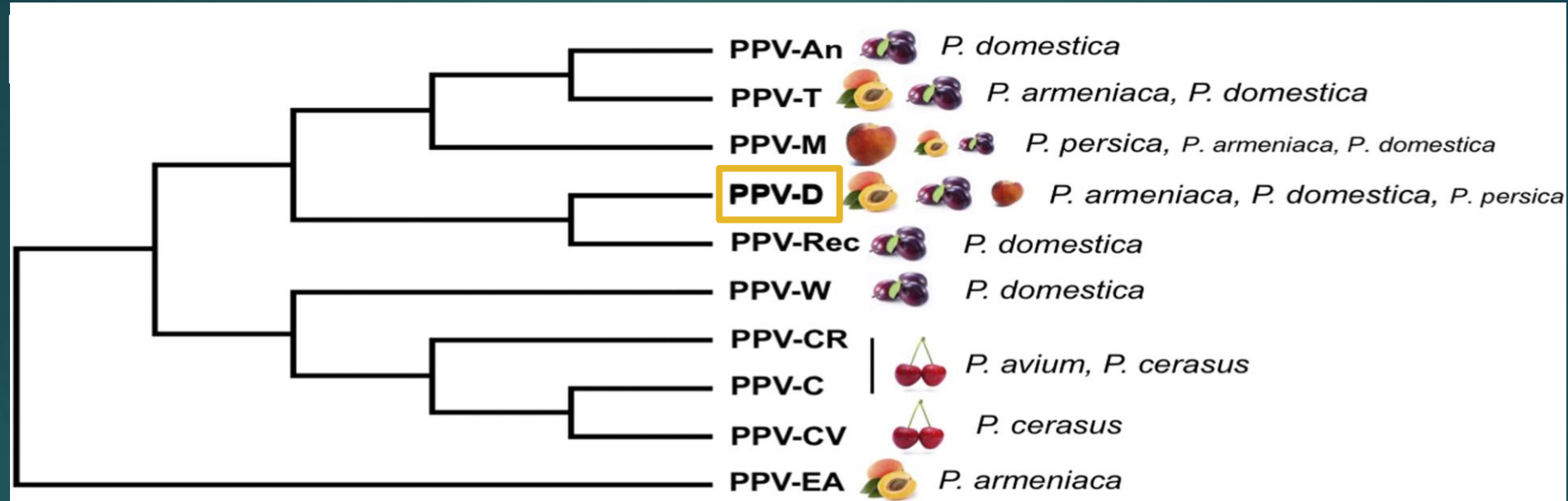
Almonds in California

- ▶ Valued at approximately \$5 billion per year; produces more than 95% of world crop
- ▶ Grown in close proximity to other stone fruits (peaches, plums, apricots, etc)
- ▶ Almond Board of California is funding work to test the susceptibility of CA almond cultivars to PPV-D

Literature on susceptibility of Almond to PPV is mixed

- Šafářová, D., Neoralová, V., James, D. and Navrátil, M. (2017). Almond (*Prunus dulcis* L.) - not a natural host of Plum pox virus in the Czech Republic. *Acta Hortic.* 1163, 123-128
- İlbağrı, H., Çıtır, A. Detection and partial molecular characterization of Plum pox virus on almond trees in Turkey. *Phytoparasitica* 42, 485–491 (2014).
- Rubio M., Martínez-Gómez P., García J.A., Dicenta F. (2013) Interspecific transfer of resistance to Plum pox virus from almond to peach by grafting. *Ann. Appl. Biol.* 163:466–474.
- Martínez-Gómez, P., Rubio, M., Dicenta, F., & Gradziel, T.M. (2004). Resistance to Plum Pox Virus (Dideron Isolate RB3.30) in a Group of California Almonds and Transfer of Resistance to Peach. *Journal of the American Society for Horticultural Science* 129(4), 544-548.
- Rubio M., Martínez-Gómez P., Dicenta F. Resistance of almond cultivars to Plum pox virus (sharka) *Plant Breed.* 2003;122:462–464.
- Dallot, S., Bousalem, M., Boeglin, M., Renaud, L.Y. And QUIOT, J.B. (1997), Potential role of almond in sharka epidemics: susceptibility under controlled conditions to the main types of plum pox potyvirus and survey for natural infections in France . *EPPO Bulletin*, 27: 539-546.

PPV: at least 10 recognized strains



- ▶ PPV-D is the only strain known to be present in North America
- ▶ PPV-D produces some of the mildest disease symptoms

Foreign Disease/Weed Science Research Unit (USDA-ARS; Ft. Detrick, MD)

- ▶ 7500 sq ft BSL-3 plant pathogen greenhouse
- ▶ Attached 1000 sq ft laboratory space
- ▶ Separate building with lab, office, propagation and insectary greenhouse space



Early PPV-D host range studies

***Prunus* Host Range of *Plum pox virus* (PPV) in the United States by Aphid and Graft Inoculation**

V. D. Damsteegt, USDA, ARS, Foreign Disease-Weed Science Research Unit, Ft. Detrick, MD 21702; R. Scorza, USDA, ARS, Appalachian Fruit Research Station, Kearneysville, WV 25430; A. L. Stone and W. L. Schneider, USDA, ARS, FDWSRU, Ft. Detrick, MD 21702; K. Webb and M. Demuth, USDA, ARS, AFRS, Kearneysville, WV 25430; and F. E. Gildow, Department of Plant Pathology, Penn State University, University Park, PA 16802

ABSTRACT

Damsteegt, V. D., Scorza, R., Stone, A. L., Schneider, W. L., Webb, K., Demuth, M., and Gildow, F. E. 2007. *Prunus* host range of *Plum pox virus* (PPV) in the United States by aphid and graft inoculation. *Plant Dis.* 91:18-23.

hosts of PPV in Poland in 2001, and walnut (*Juglans regia*) was reported as a natural host by Baumgartnerova in 1997 (3), although this has not been confirmed. Nemeth (26) lists eight *Prunus* species as natural hosts and an additional 33 *Prunus*

- ▶ Butte and Texas Mission almonds were aphid inoculated
 - ▶ 17/30 positive by ELISA
 - ▶ 3/30 had visual symptoms

Canine PPV Patrol

- ▶ 2015-2018: training site for detector dogs (Tim Gottwald and Gavin Poole, USDA-ARS Ft. Pierce Florida)
- ▶ Detect PPV with high accuracy as soon as two weeks after inoculation and in infected budwood



Almonds infected with PPV-D Penn4 for Canine Patrol

- ▶ Butte, Nonpareil and Texas Mission scions; Nemaguard rootstock (peach); no side shoots
- ▶ Subset of samples tested by qRT-PCR; positive
- ▶ No PPV symptoms

Questions:

- ▶ Can we confirm if almonds are susceptible to US isolates of PPV-D?
- ▶ Can almonds be a source of inoculum for aphid transmission of PPV to more susceptible Prunus species?

Experimental Design



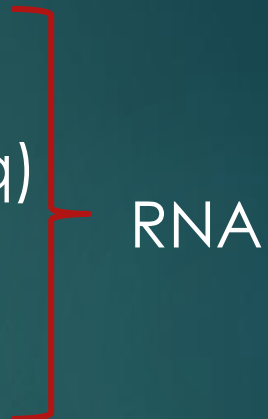
- ▶ Scions: Tuono or Texas Mission almond
- ▶ Rootstock: GF305 (peach) or Nemaguard (peach)
- ▶ Some almonds on their own rootstock as well

- ▶ Viral isolate: PPV-D Penn4
- ▶ Inoculated using *Myzus persicae* green peach aphid

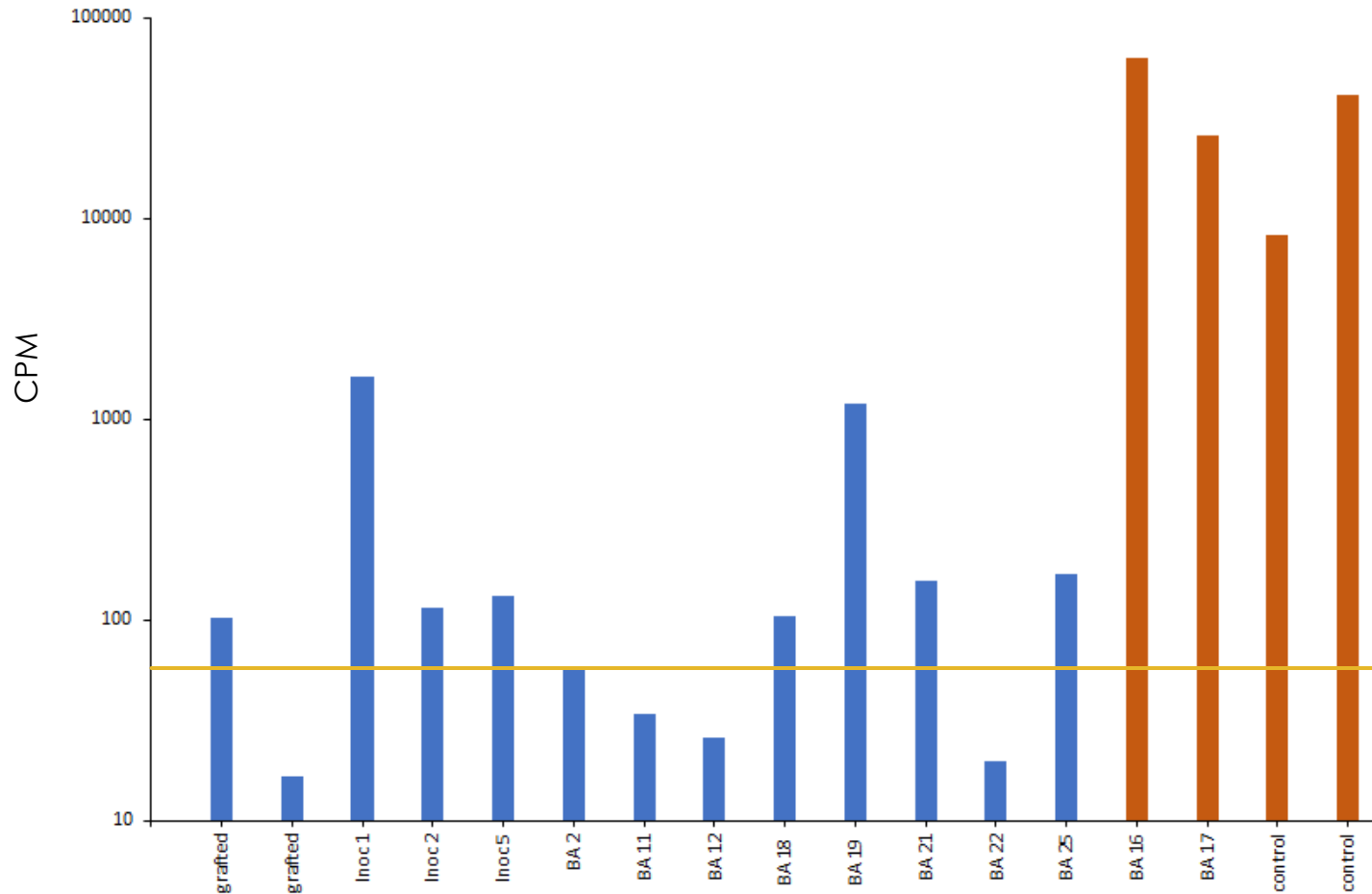
Pandemic Experimental Timeline

- ▶ Almond trees grafted and inoculated (also 2 peach GF305 inoculated as positive controls)
 - ▶ Vernalized once (artificial winter-like conditions) **Post vernalization #1 and pre-back assay**
 - ▶ Sampled and subset of trees used as source for back assays to healthy peach GF305 seedlings
 - ▶ Vernalized again **Vernalization #2 and post-BA**
 - ▶ Sampled again
-
- ▶ Back assay recipients vernalized, sampled, most discarded
 - ▶ A few kept, vernalized again (**V2**) and sampled again

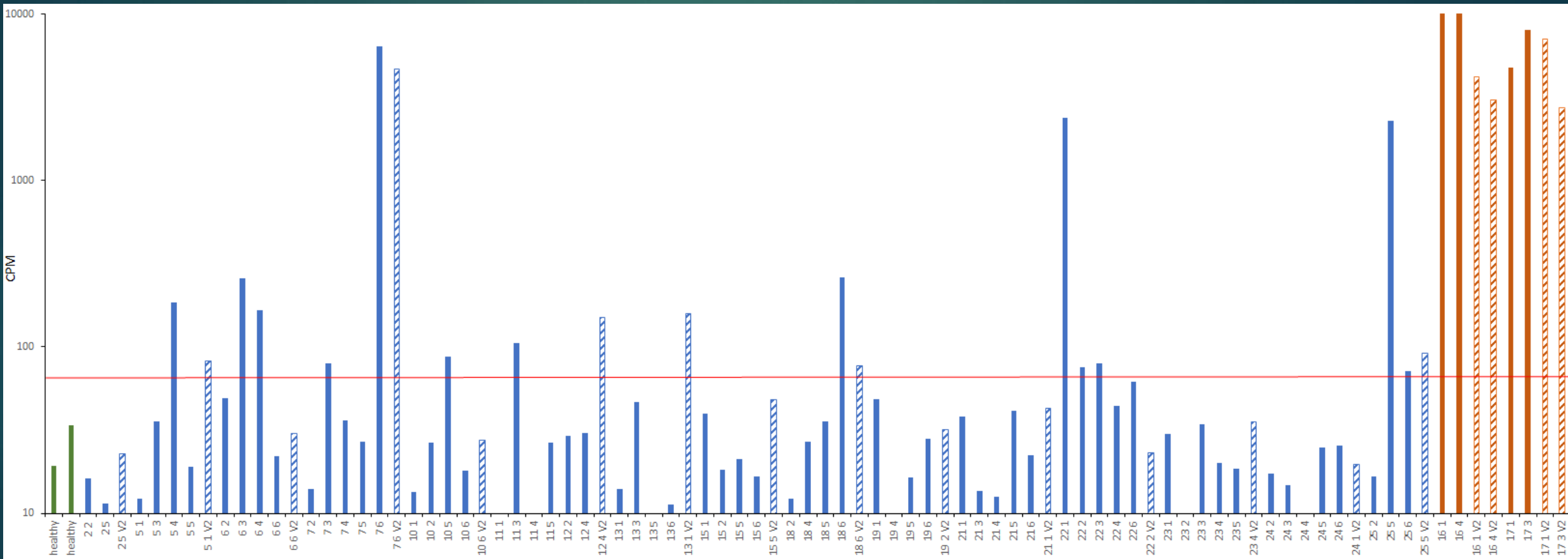
PPV Detection methods

- ▶ NGS – Illumina platform
 - ▶ Libraries made by Erik Burchard (Illumina/brbSeq)
 - ▶ Sequenced by Azenta/GeneWiz
 - ▶ qRT-PCR (assay adapted by Tami Collum)
 - ▶ ELISA (detects protein, not RNA)
 - ▶ Problems:
 - ▶ many samples missing after -80 freezer failure
 - ▶ hard to get good quality RNA from almond
- 

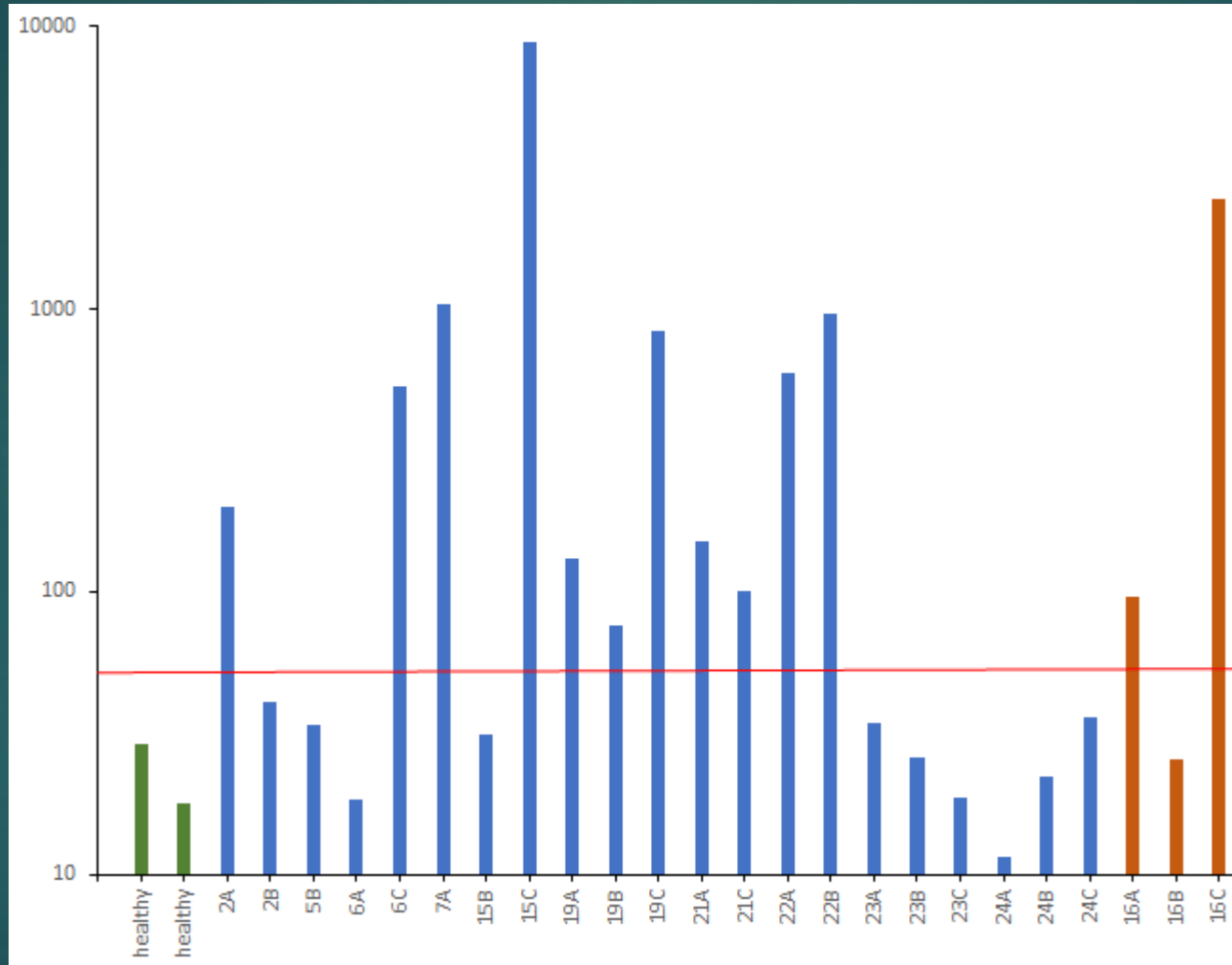
PPV detected by NGS in almonds post V1 and at time of back assay



PPV detected by NGS in back assay recipients:



PPV detected by NGS in source almonds, post V2:



Source Almonds

Aphid transmission to Peaches

BA	status	Source pre-BA NGS	Source post-V2 NGS	Source post-V2 qRT-PCR	Source post-V2 ELISA	Recipients post-V1 NGS	Recipients post-V1 qRT-PCR	Recipients post-V2 NGS	Recipients post-V2 qRT-PCR
2	infected	positive	positive	not positive	not positive	not positive	positive	not positive	positive
5	infected	not tested	not positive	not positive	not positive	positive	positive	positive	positive
6	infected	not tested	positive	positive	not positive	positive	positive	not positive	positive
7	infected	not tested	positive	not tested	not positive	positive	positive	positive	positive
10	infected	not positive	not tested	not tested	not tested	positive	positive	not positive	positive
11	infected	positive	not tested	not tested	not tested	positive	positive	not tested	not tested
12	infected	positive	not tested	not tested	not tested	not positive	positive	positive	positive
13	infected	not tested	not tested	not tested	not tested	not positive	positive	positive	positive
15	infected	not tested	positive	positive	positive	not positive	positive	not positive	positive
18	infected	positive	not tested	not tested	not positive	positive	positive	positive	positive
19	infected	positive	positive	not positive	not positive	not positive	positive	not positive	positive
21	infected	positive	positive	positive	not positive	not positive	positive	not positive	positive
22	infected	positive	positive	not positive	not positive	positive	positive	not positive	positive
23	not infected	not tested	not positive	not positive	not positive	not positive	not positive	not positive	positive
24	not infected	not positive	not positive	not positive	not positive	not positive	not positive	not positive	positive
25	infected	positive	not tested	not tested	not tested	positive	positive	positive	positive
16	positive control	positive	positive	positive	positive	positive	positive	positive	positive
17	positive control	positive	not tested	not tested	not tested	positive	positive	positive	positive

Conclusions:

- ▶ NGS was more sensitive than RT-PCR or ELISA in almond
- ▶ PPV-D Penn4 can infect almond and almond is an aphid transmission-competent host
- ▶ PPV titers in almond are significantly lower in almond than peach (100 fold)
- ▶ RT-PCR and ELISA may not be good assays to detect PPV in almond

Elizabeth E. Rogers^{1*}, Andrew L. Stone¹, Erik Burchard², Diana J. Sherman¹, and Christopher Dardick²
(2023) Almond can be infected by Plum Pox Virus-D isolate Penn4 and is a transmission-competent host.
Plant Disease. *In review.*

Further research:

- ▶ Does rootstock influence almond susceptibility to PPV?
- ▶ Are CA Almond varieties susceptible to PPV? Can they serve as a host for infection of plum or peach?
- ▶ Need improved assays for detection of PPV in almond.

Acknowledgments PPV project:

- ▶ Ft. Detrick:
 - ▶ Elizabeth Rogers
 - ▶ Andrew Stone
 - ▶ Diana Sherman
- ▶ Kearneysville:
 - ▶ Chris Dardick
 - ▶ Erik Burchard
 - ▶ Mark Demuth
- ▶ Former ARS scientists:
 - ▶ Ralph Scorza
 - ▶ Vern Damsteegt
 - ▶ Bill Schneider
- ▶ Funding: Department of Homeland Security - Science and Technology Directorate

Can ReTain application minimize crop loss during heat at bloom?

- ▶ In the last 17 years, prune crop failures in California (statewide or regionally) occurred in 2004, 2005, 2007, 2013, and 2020. Unusually cool or hot weather during bloom in those years coincided with low production (a state average of less than 1.

Posted on March 6 2023 by Franz Niederholzer



AVG – ethylene inhibitor



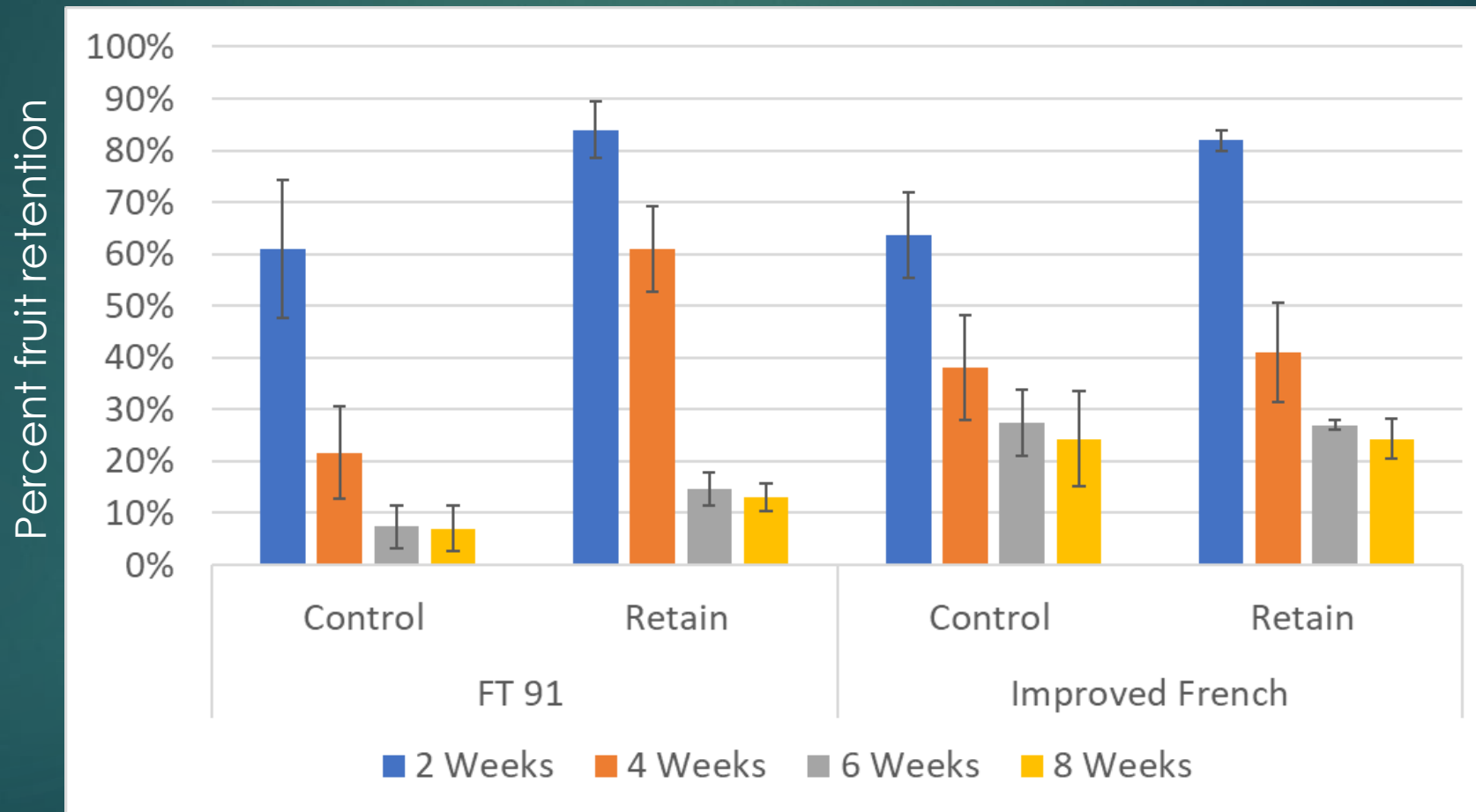
Flowering Locus T 1 (FT1) Over-expressing Plums

- Small tree size
- Continual flowering
- No dormancy required
- Set fruit year-round
- Flowering controlled by temperature.



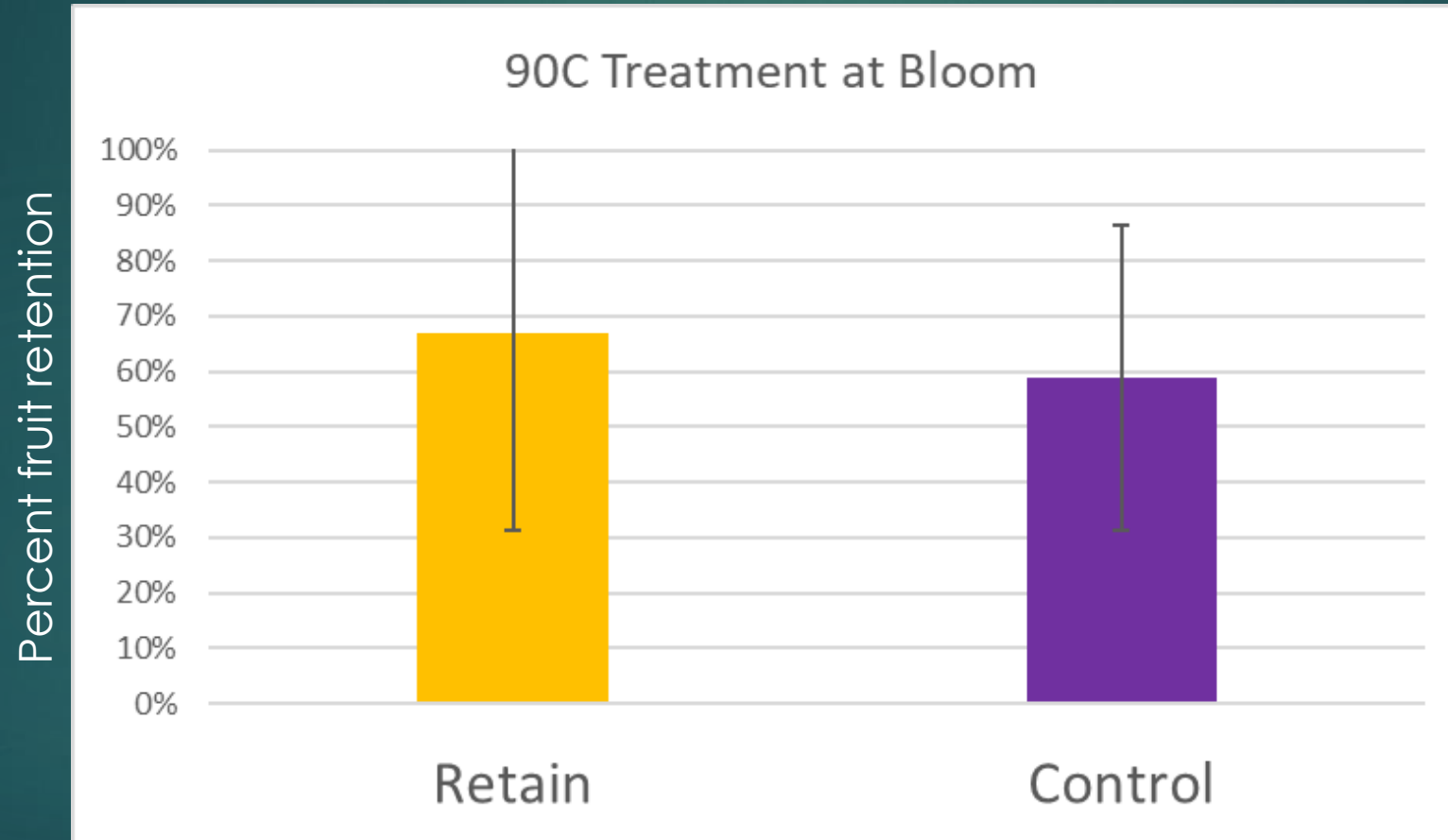
Srinivasan C, et al. Plum (*Prunus domestica*) trees transformed with poplar FT1 result in altered architecture, dormancy requirement, and continuous flowering. *PLoS One*. 2012;7(7):e40715.

Control Retain Treatment (field)



Retain Treatment Upon Heat at Bloom

(Greenhouse study)



*Trees kept at 90C for 3 days and flowers were pollinated daily.