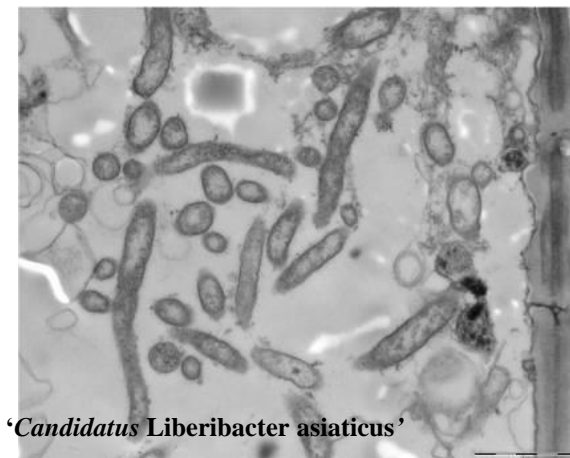
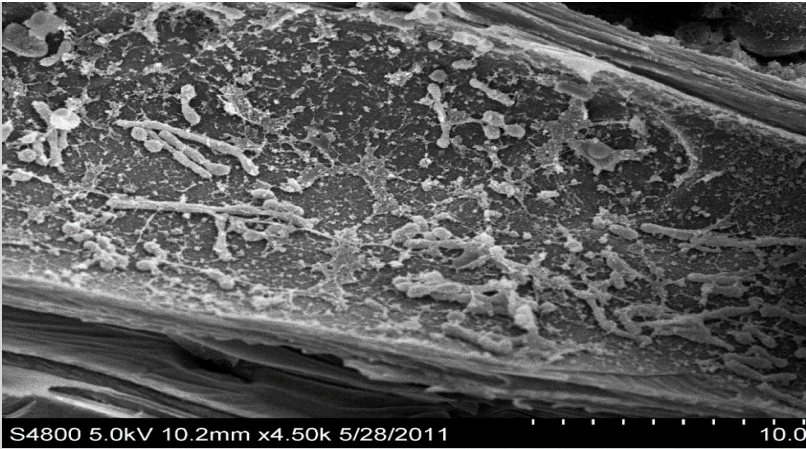


Rapid Selection and Evaluation of Citrus Bud Sports for HLB Resistance/Tolerance

Yong-Ping Duan
USDA-ARS-USHRL, Fort Pierce, Florida
04-12-2022, Citrus Show, Fort Pierce, FL





Two different Las strains on one citrus host genotype

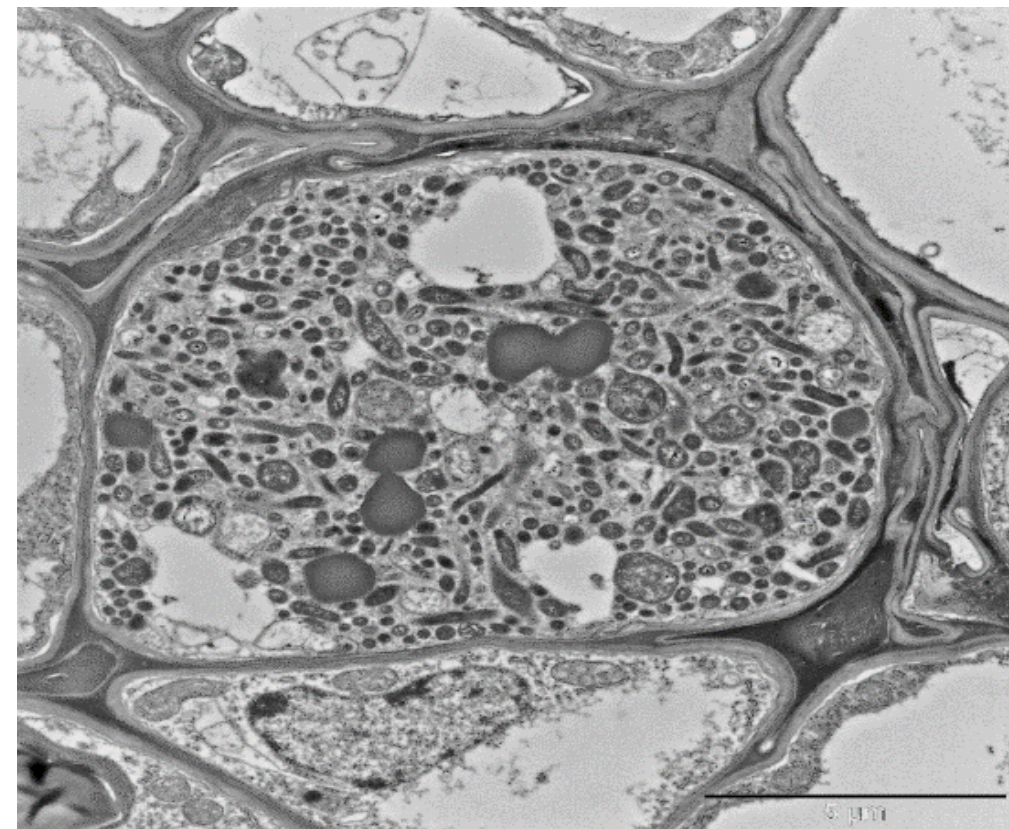


R5-T4 on Duncan grapefruit
Ct=23;



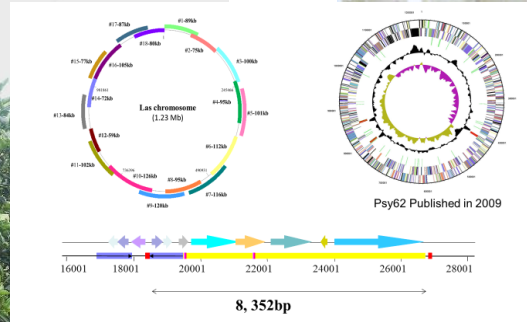
R4-T21 on Duncan
Ct=28;

A



Mc isolate contained only one prophage (FP1), and was associated with more severe symptoms and high bacterial titer.

Las infection over 13 years



Variety	Tree	Average Ct with Li primer	W & M	Deletion mutants
Sour Orange	R7T6, R7T11	25.4	90	10.0%
Grapefruit	R1T11, R1T19	22.7	47	41.25%
Pumelo	R8T4, R8T6	22.0	63	21.25%
Tangelo	R9T8	21.7	6	85.0%
Lemon	R11T5, R11T27	24.5	79	1.25 %
Sweet Orange (Lima)	R5T4	20.9	6	88 %
(Valencia)	R4T3 , R4T9	22.40	78	2.5%

16 years after HLB infection



HLB-affected plants have been grown well for over 16 years in insect-proof greenhouses.

low titer infection (Ct=36)
from *in vitro* cultured Las
via psyllid inoculation



Plants do not grow.

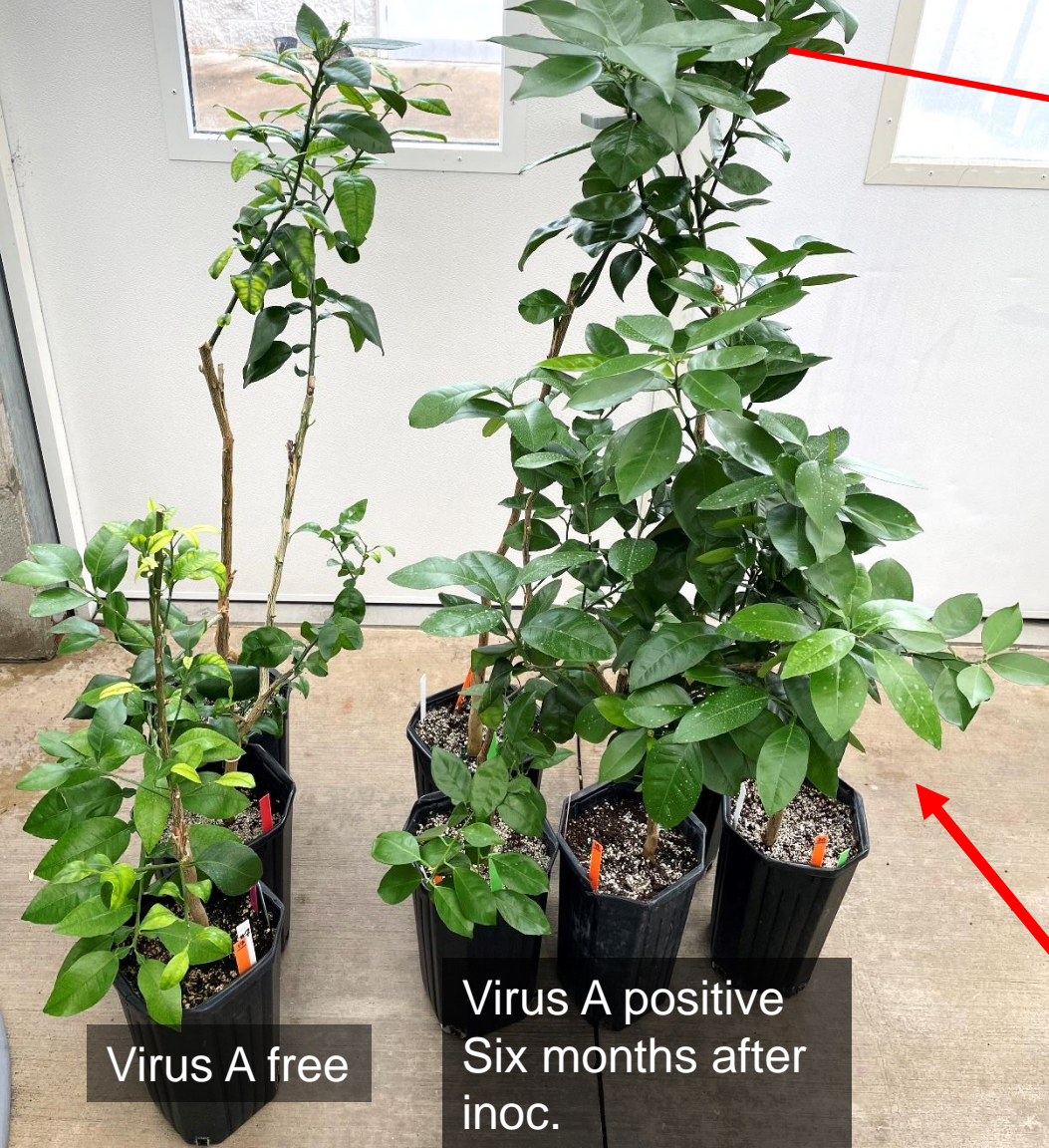


3 years old

One years old



Las titers Ct=19-23



Virus A free

Virus A positive
Six months after
inoc.



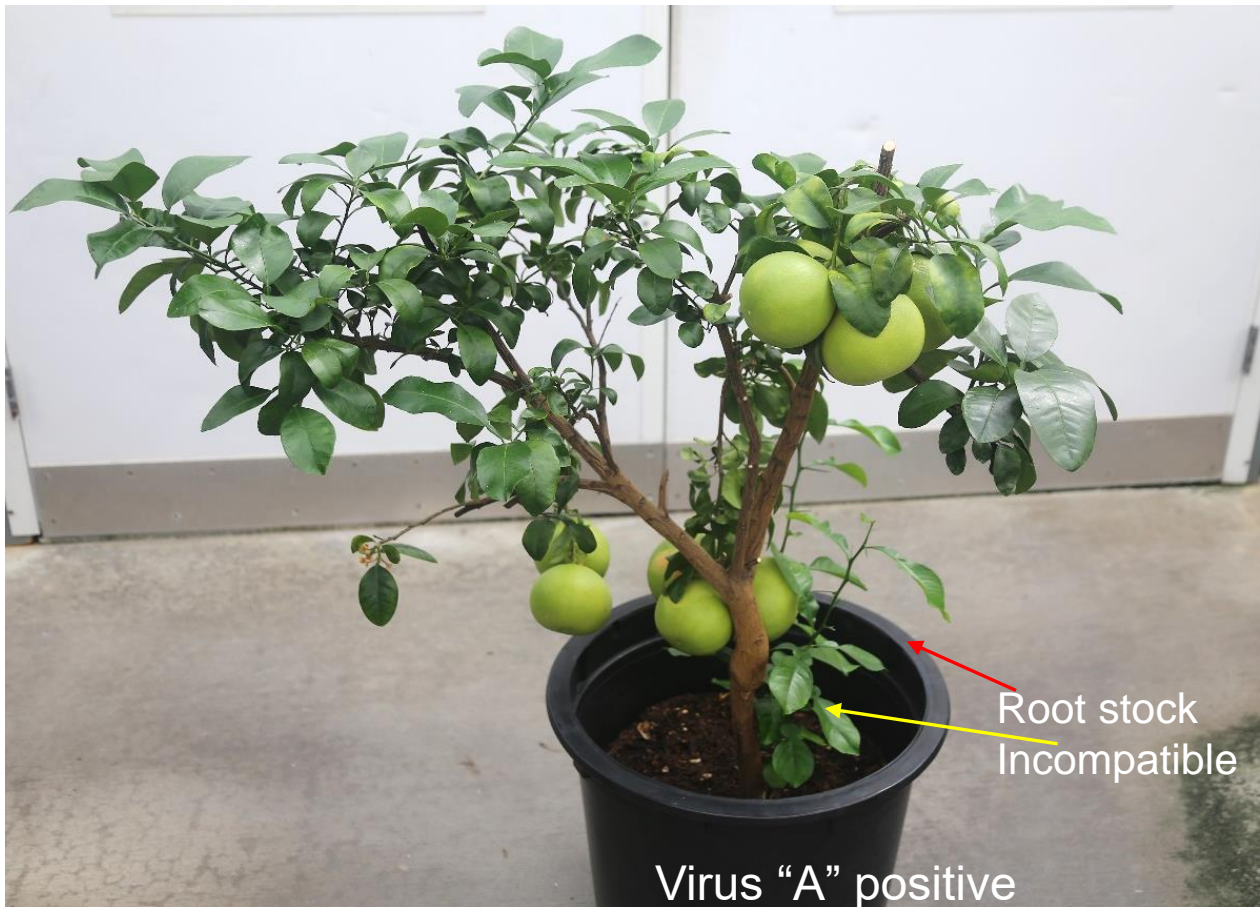
Las+virus A co-infection

Healthy Valencia

HLB-affected plants became asymptomatic after inoculated with a virus, and Valencia propagates **grow normal** as healthy control though they have **high titers of Las.**

HLB-free for **over 4.0**
years at Picos' Farm

Much **higher brix** compared to its siblings and
control varieties in field and greenhouse
settings.

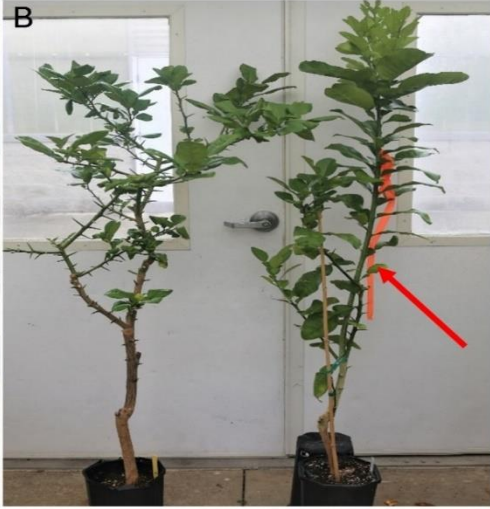


How to rapidly select **variant** citrus plants with **stable** and **broad spectrum** HLB resistance/tolerance

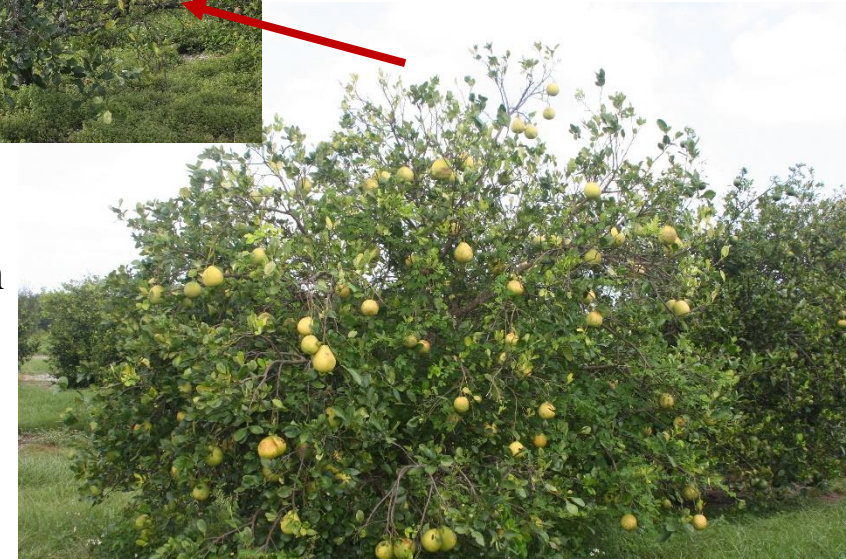
Hypothesis:

- 1). **Mutation(s)** or segregation that results in **lost of functions** of susceptibility gene(s) or gaining of functions of resistance genes
- 2). Mutation(s) that result in **nutrient deficiency** for Las bacterial growth

- A. bud sport** selection and evaluation from natural or radiation-induced mutations: both in greenhouse and field
- B. Selection of variant plants from seedlings:** both in greenhouse and groves



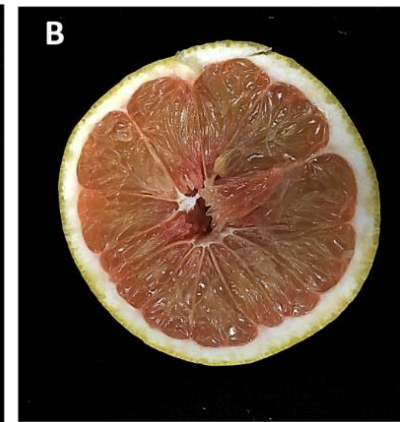
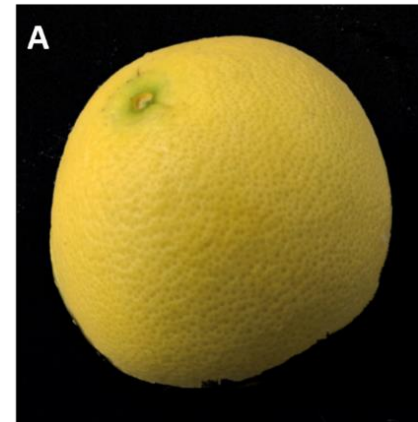
HLB-affected tree in 2010



Mato Buntan
Pumelo



Ct=24-25



Both **brix** and **acid** rich in the new fruit

Wu et al., 2021 *Frontier in Plant Science*
<https://doi.org/10.3389/fpls.2021.739108>

Flame grapefruit



A

Red Ruby grapefruit



C

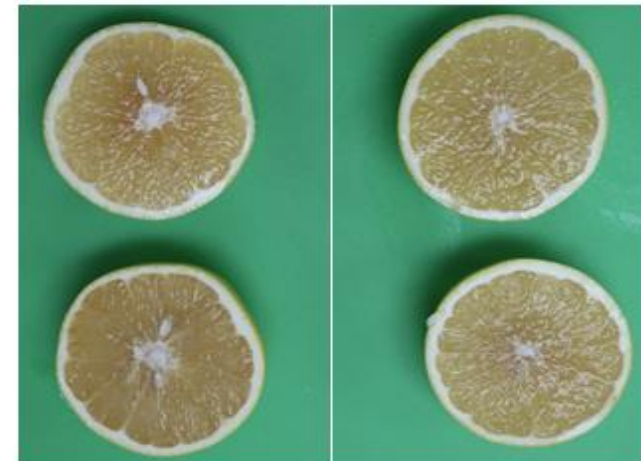
White grapefruit ?



B



D



Selection and evaluation of volunteer grapefruit seedlings

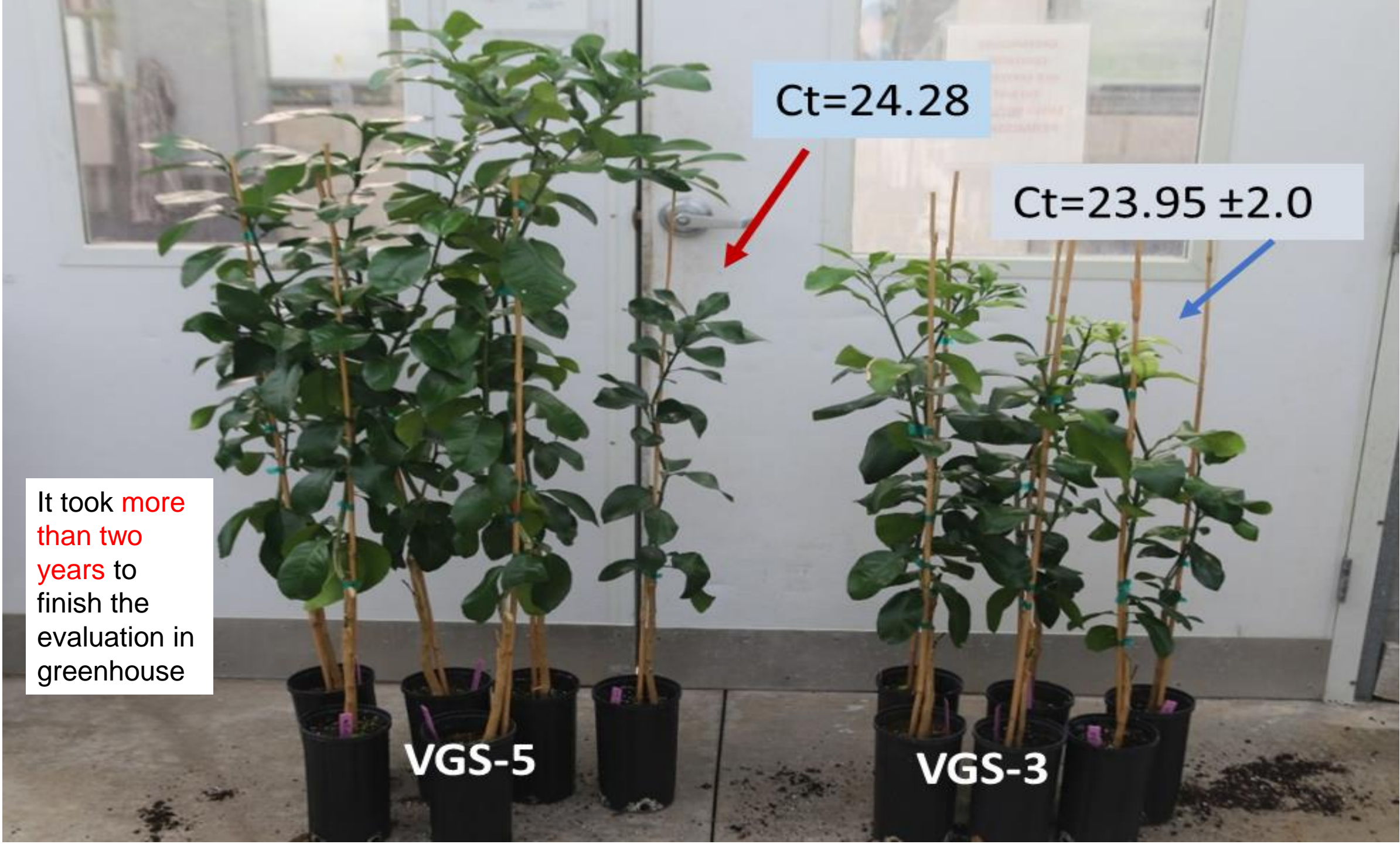
It took **more than two years** to finish the evaluation in greenhouse

Ct=24.28

Ct=23.95 ±2.0

VGS-5

VGS-3



It took **more than five years** to have convincing data from a field trial.



Five years after planting of selected grapefruit bud-sports at USHRL Picos' Research Farm Photographed on June 25, 2022



Five years after planting of selected pumelo bud-sports at USHRL Picos' Research Farm Photographed on June 25, 2022

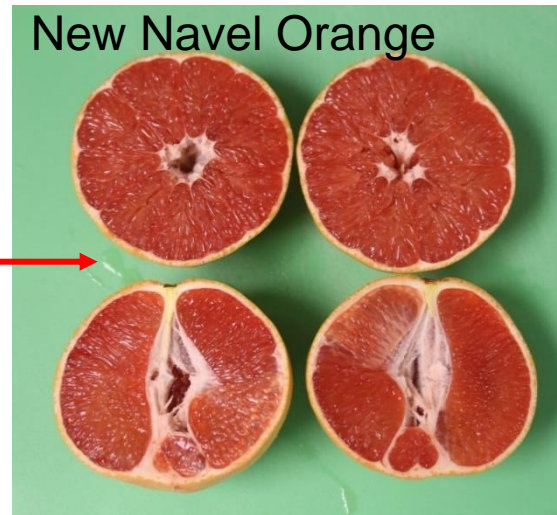
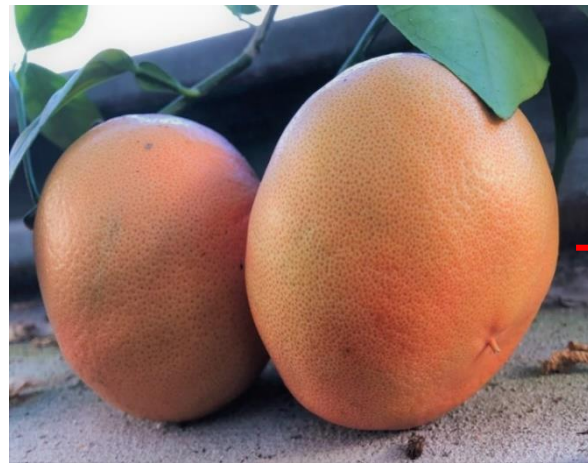
In the past 7 years, we have obtained more than 10 citrus selections with improved HLB resistance/tolerance, which have been cleared up by DPI:

4 Grapefruits—Red Ruby and Flame

4 Valencia and one Ridge Pineapple sweet orange

2 Pumelo and one sour orange

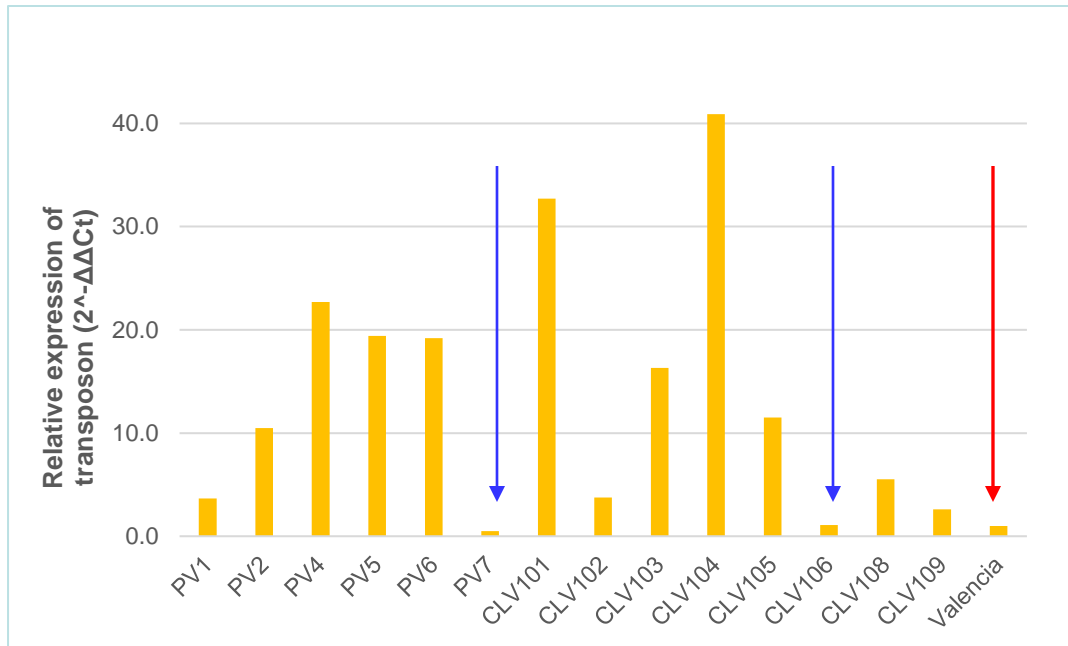
In addition, we have obtained nearly thornless Carrizo mutant, and a flame grapefruit mutant with Mandarin-like leaves.



- 1) Select **aggressive** Las inoculum—"hot" psyllids or HLB-affected plants.
- 2) Inoculate target explants onto HLB-affected plants with severe symptoms.
- 3) Graft-inoculate **multi strains** of Las simultaneously.



- 1) Keep **lateral flushes** to increase the chance to re-select a bud mutant.
- 2) Use RT-qPCR to measure **transposon** activities, and **predict** the variation and stability of bud sport progenies.



Relative expression of transposon-II ($2^{-\Delta\Delta Ct}$)



Summary

- The **bud-sports** and **seedling variants** are important resources for quickly obtaining **stable and broad spectrum** HLB resistant/tolerant commercial varieties.
- HLB bacterial strain **variations** should be considered during evaluation of the bud sports and seedlings variants.
- **Segregation** of resistant/tolerant progenies is also a common phenomenon among the citrus variants; and transposon activities can be used to predict their segregation and **stabilization**.

Acknowledgement

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- **Bo Wu**
- **Rachel Patterson**
- **Christina Latza**



• **Collaborators:**

- **Feng Lou, University of Clemson**
- **Zhanao Deng, UF**
- **Fred Gmitter, UF**
- **Chuck Powell, UF**



We are looking forward to having more collaborators and growers to conduct more field trials.