Update on Breeding Rootstocks for an HLB-Endemic Florida

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IFAS
Citrus Research and Education Center

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Rootstock Genetics/Nutrition and HLB

Optimized Nutrition (Year-round) Required to Maximize HLB Tolerance of Any Rootstock

https://citrusagents.ifas.ufl.edu/events/GrowersInstitute2020/grosser.shtml

Link to 2020 Citrus Institute Talk:
"Improved Genetics and Nutrition: The Way Forward"

UF/CREC-Germplasm into MAC Trials

1. Rogers MAC Project – CREC Lake Alfred (planted)

- 70 sweet orange/rootstock combinations (19 early orange; 51 late orange); SugarBelle on 4 rootstocks; all new rootstock candidates from the UF/CREC citrus improvement program.
- Plot size: 50 trees/plot; >12,000 trees, four plots for most combinations; 70+ acres

2. Grosser/Gmitter/Bowman MAC Rootstock Project (planted)

- Vernia/Valencia B9-65 and OLL-8 on 48 rootstocks (24 from UF)
- 12 trials, 12 4-tree replications, nearly 16,000 trees all planted

3. Vidalakis/McCollum MAC Project – CA/FL- Experiment 1 – HLB Tolerant Rootstock Effects on Fruit Quality (first planting underway)

- 5 standard scions on 12 improved rootstocks (6 from UF);3 Sites in Florida, 7 replications per site, 3780 trees

4. Vidalakis/McCollum MAC Project – CA/FL - Experiment 2 – Evaluation of Putatively HLB Tolerant Scions

- Four Control Scions: Valencia, Hamlin, Tango & SugarBelle; 6 UF and 7 USDA mandarins (13 total selections);7 UF and 5 USDA sweet orange/sweet orange-like (12 total selections); includes several true sweet oranges, 3 sites, 18,900 trees on US-942

Lykes/UF Rootstock Trials: Camp Mack and Basinger

Two major sister 40-acre rootstock trials replicated at two sites: Camp Mack (Ridge) and Basinger (Flatwoods). 11/12 replications per rootstock (for most rootstocks).

Hamlin on 57 rootstocks
Valencia on 52 rootstocks

Rootstocks included from UF, USDA, CA and Spain

We have collected 4 years of young tree data, with our colleague Dr. Ute Albrecht (PI on the Trial Evaluation Project).

Improving delivery of trial information to growers

- Citrus Improvement Team Website Now Online – one stop shopping for growers/processors, packers. Data from 26 trials at present, ongoing trials updated annually (**Dr. Filomena Valim**):

- https://crec.ifas.ufl.edu/citrus-research/rootstock-trials/

Table 2. St. Helena Rootstock Survey Trial – 2018/19 vs. 2019/20 vs. 2020/21 **Vernia** - Yield and Estimated* PS/acre (PS=pounds solids). **Trees planted in 2008.**

Rootstock	2018/19 Yield [boxes/tree]	2019/20 Yield [boxes/tree]	2020/21 Yield [boxes/tree]	2018/19 [PS/box]	2019/20 [PS/box]	2020/21 [PS/box]	% Yield Variation from 2018/19 to 2019/20	% Yield Variation from 2019/20 to 2020/21	Optimum trees/acre*	Estimated PS/acre [increase 2017/18 to 2018/19, %]	Estimated PS/acre [increase 2018/19 to 2019/20, %]	Estimated PS/acre [increase 2019/20 to 2020/21, %]
Purple 2	2.0	1.6	2.6	6.1	6.0	4.6	-21%	65%	250	3026 [29]	2357 (-22)	3013 [28]
MG 11	2.3	2.1	2.6	6.8	6.5	5.6	-7%	20%	206	3209 [91]	2848 (-11)	2977 [5]
Aqua 1803	2.2	2.1	2.1	6.6	6.4	5.9	-5%	3%	226	3363 [130]	3019 (-10)	2872 [-5]
Orange 1804	2.9	2.8	2.3	6.5	6.4	6.0	-4%	-18%	208	3906 [147]	3854 (-6)	2836 [-26]
White 1805	1.4	1.4	1.5	6.9	6.4	6.5	-4%	12%	282	2776 [95]	2442 (-12)	2772 [14]
Blue 1	2.5	2.0	2.1	6.5	6.1	6.4	-20%	4%	209	3346 [201]	2534 (-24)	2767 [9]
Yellow 1800	2.4	1.8	2.0	6.5	6.5	6.1	-25%	9%	230	3580 [134]	2669 (-25)	2741 [3]
UFR 2: Orange 4	1.1	0.9	1.6	6.7	6.4	6.6	-15%	65%	254	1901 [105]	1537 (-19)	2580 [68]
UFR 1: Orange 3	1.6	1.4	1.8	6.8	6.1	6.1	-14%	28%	227	2434 [117]	1897 (-22)	2456 [29]
Cleo+CZO	1.8	1.8	1.4	6.9	6.7	6.8	1%	-25%	256	3136 [65]	3093 (-1)	2370 [-23]
Blue 4	1.0	1.0	1.3	6.5	6.4	6.5	-3%	29%	289	1855 [69]	1783 (-4)	2335 [31]
Wgft+50-7	1.9	1.2	1.5	6.7	6.6	6.4	-39%	25%	250	3079 [115]	1906 (-38)	2311 [21]
Blue 3	0.9	1.0	1.1	6.7	6.6	6.7	9%	9%	320	2020 [168]	2048 (2)	2275 [11]
Volk	3.5	2.6	2.5	5.6	5.6	5.1	-27%	-4%	180	3548 [115]	2601 (-27)	2266 [-13]
Orange 14	1.1	0.9	1.3	6.8	6.4	6.3	-17%	42%	279	2090 [99]	1624 (-22)	2265 [39]
Orange 1	1.5	1.1	1.4	7.0	6.4	6.3	-27%	27%	256	2607 [178]	1775 (-32)	2239 [26]
UFR 3: Orange 15	1.2	1.0	1.5	6.7	6.7	6.4	-14%	47%	233	1830 [86]	1567 [-14]	2230 [42]
Orange 21	1.9	1.5	1.5	6.6	6.1	6.0	-19%	-2%	246	3099 [101]	2240 (-27)	2226 [-1]
UFR 4: Orange 19	1.9	1.6	1.7	6.4	6.1	5.9	-16%	5%	226	2773 [150]	2173 (-21)	2224 [2]
UFR 6: Changsha+TF 50-7	1.4	1.0	1.1	7.3	6.8	7.0	-26%	8%	277	2841 [221]	1932 (-32)	2135 [11]

(*) Calculated using measured average width and hypothetical 20 ft. between all rows.

CREC Citrus Plant Improvement

Plant species have thrived for thousands of years in the presence of evolving, hostile pathogens – HOW? They have created their own genetic diversity, and through the process of natural selection, tolerant or resistant genotypes overcome the threat and allow the species to evolve.

In Citrus, this process has been largely interrupted by man, with Citriculture now approaching monoculture – leading to the problem that has brought us all together.

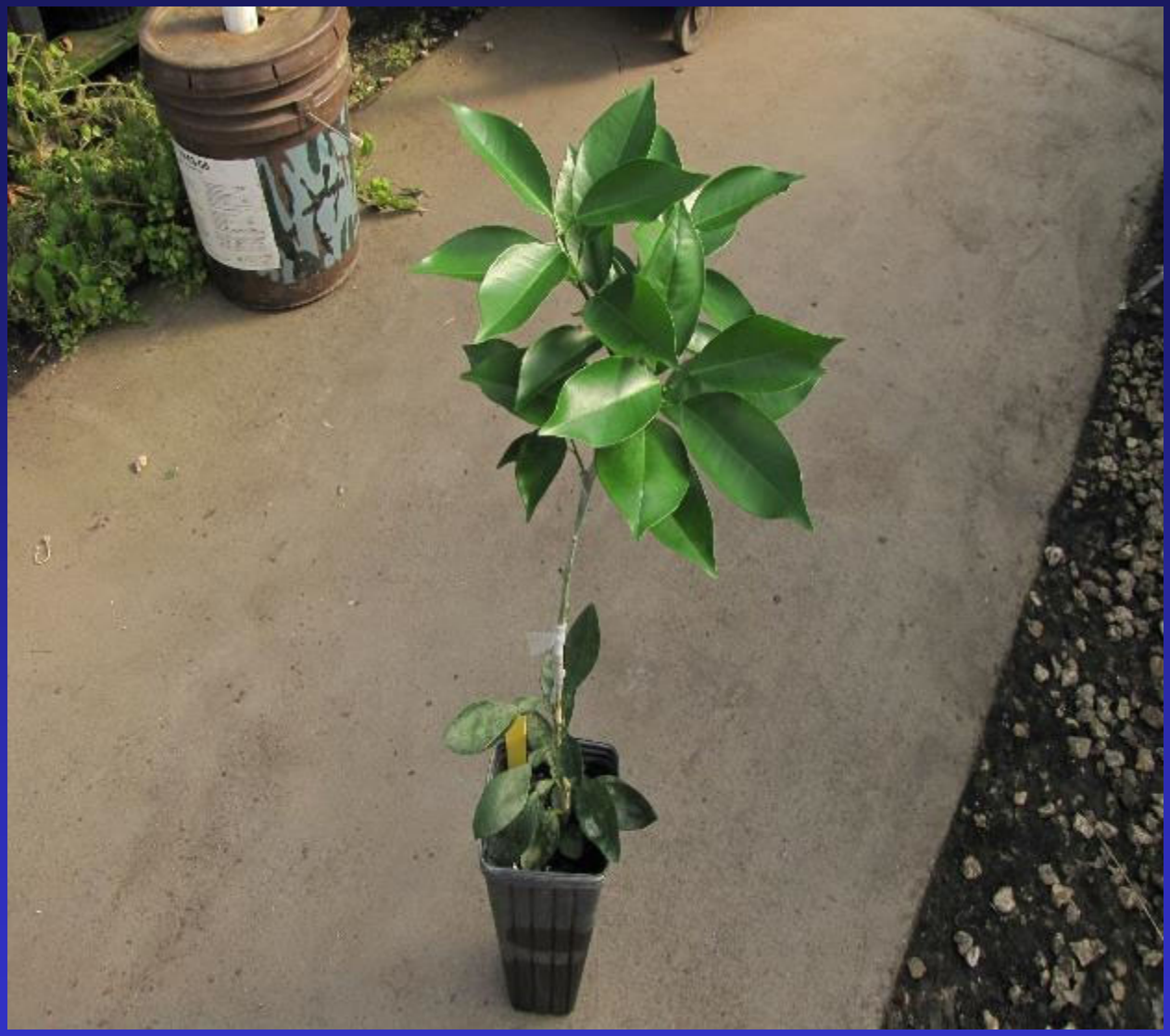
Facilitated by biotechnology, citrus breeders have the opportunity to artificially reinstate this process by creating broad and unique genetic diversity from elite parents, followed by robust screening. Maybe this is the answer for solving the HLB and other disease problems!

The New Gauntlet in the HLB world
High Throughput Screening Method
>12,000 hybrids screened to date

1. Crosses of superior parents made at diploid and tetraploid levels
2. Seed harvested from crosses planted in bins of calcareous soil (pH=8), inoculated with *P. nicotianae* and *P. palmivora* (JH Graham)
3. Selection of robust seedlings based on growth rate, health and color (most don't make it!)
4. Transfer to 4x4 pots in commercial potting soil
5. Top of new tree goes for seed source tree production; remaining liner to the HLB screen
6. Hybrid liner is grafted with HLB-infected budstick of Valencia sweet orange; remaining rootstock top removed, forced flushing from HLB-infected sweet orange budstick
7. Trees monitored for HLB symptoms – healthy appearing trees entered into 'hot psyllid' house for 4 weeks, followed by field planting at Picos Farm (under DPI permit).

Rootstock cross with good Phytophthora resistance.

Gauntlet trees are produced by 'stick' grafts. HLB-infected Valencia budsticks wrapped in parafilm are grafted into selected rootstock candidates. Rootstock tops are used to produce rooted cuttings for seed trees on their own roots.



Quite often the first flush is symptom free, selection is based on the 2nd flush, which usually shows symptoms.

2016 Field Planting will include trees on left; featuring 3 superior crosses: C2-5-12 pummelo x papeda; A+HBP x White 1 and A+HBP x sour orange+rangpur. Candidates on left already passed through the 'hot psyllid' house.

Gauntlet Survivor at Picos Farm
-Valencia on Milam+HBP x Orange #14-09-14

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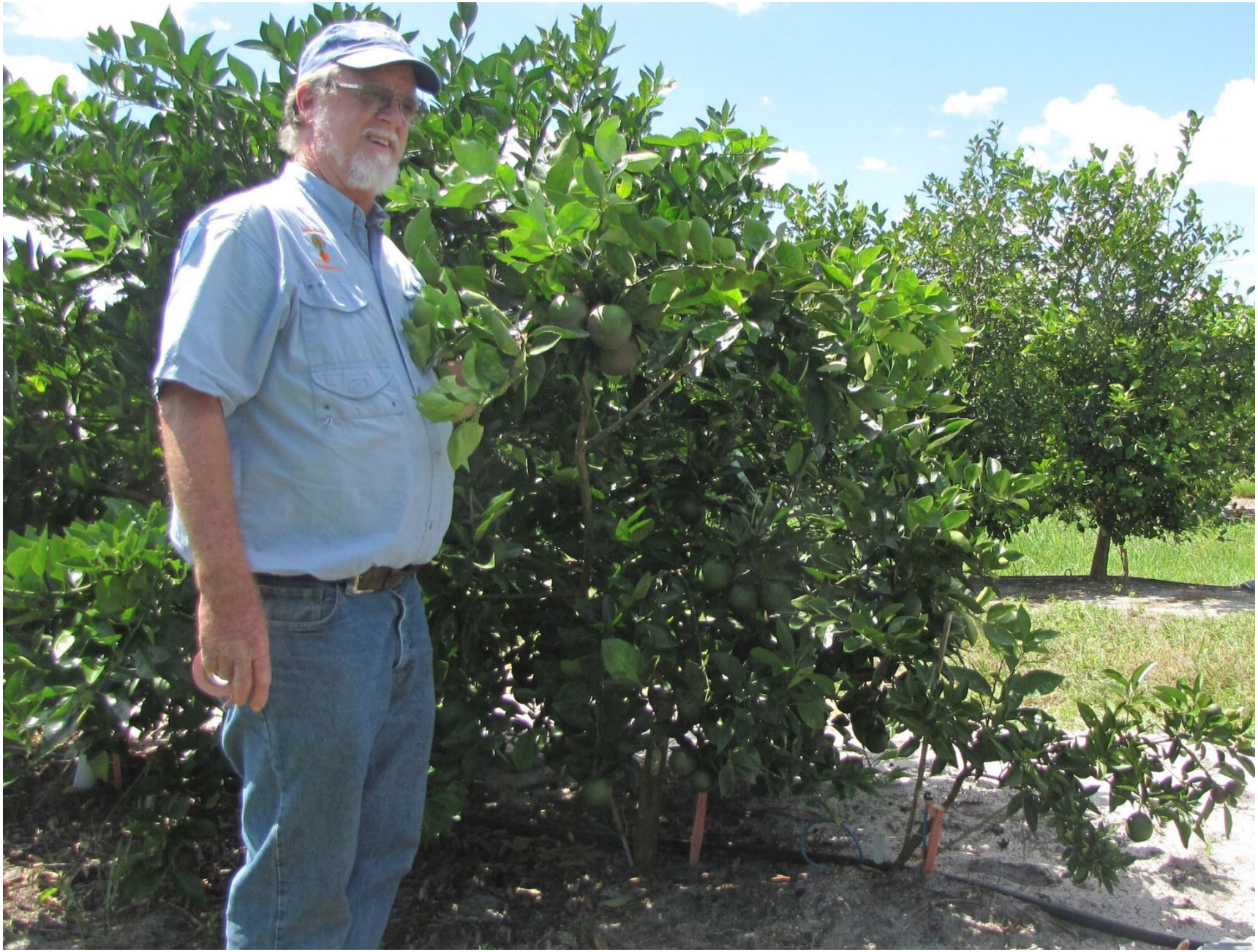

3-year old Valencia on gauntlet rootstock A+HBPxSORP-13-60 at USDA Picos Farm, planted HLB+, tested negative 3 times.

GAUNLET SCREENING to date

*Approximately 16,000 hybrid seed planted from diploid and tetraploid crosses covering a wide range of genetic diversity

- >800 rootstock candidates made it to Picos Farm
  - 625 merited qPCR testing in 2021
  - 54% tested questionable or negative
- Most promising rootstock candidates being cleaned up and propagated for advanced trials

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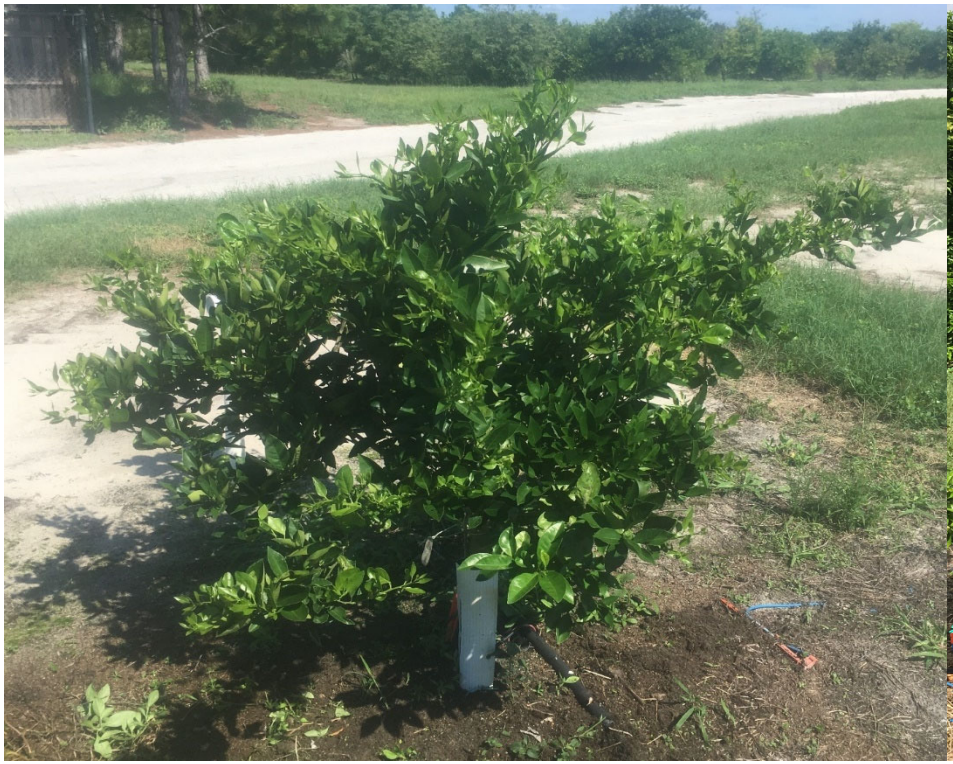
Possible tolerance from SugarBelle in rootstock hybrids?



2-year old ‘gauntlet’ tree of Valencia/Sugar Belle x S13-15-16, infected with *CLas* before planting – note massive healthy new flush, typical of pre-HLB tree. Psyllids have also been observed on every new flush since planting. This is one of several rootstocks with SugarBelle as a parent doing well in the ‘gauntlet’ screen.

1-year old ‘gauntlet’ tree of Valencia/SugarBelle x S10-15-9, infected with *Clas* before planting. Most vigorous of 150 ‘gauntlet’ trees planted at the same time, set lots of fruit!

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The **Ultimate Solution to HLB** is an HLB-resistant rootstock that can transfer it's resistance to any grafted scion!

Such a rootstock would bring the Early Valencias, Hamlin, all grapefruit, the 'Honey' Murcott (now nearly seedless selections available), etc, back into play HLB-risk free!

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Exploiting **Genetic Diversity** is the answer to multiple severe problems confronting mankind.

Climate change-induce heating and acidification of the oceans is causing massive destruction of our coral reefs. Coral reefs are also chimeric, but instead of scion and rootstock, you have the coral cells containing a symbiotic algae that performs photosynthesis

https://www.pbs.org/wgbh/nova/video/reef-rescue/

Solutions underway (with multiple parallels to our approaches to solving the HLB crisis) include:

*Identification of resistant corals (aerial and underwater surveillance)

*Assisted Breeding combining resistant corals

*Identification of heat tolerant symbiotic algae

*Swapping heat tolerant algae into adapted corals

*Testing hybrid corals and new coral/algae combinations

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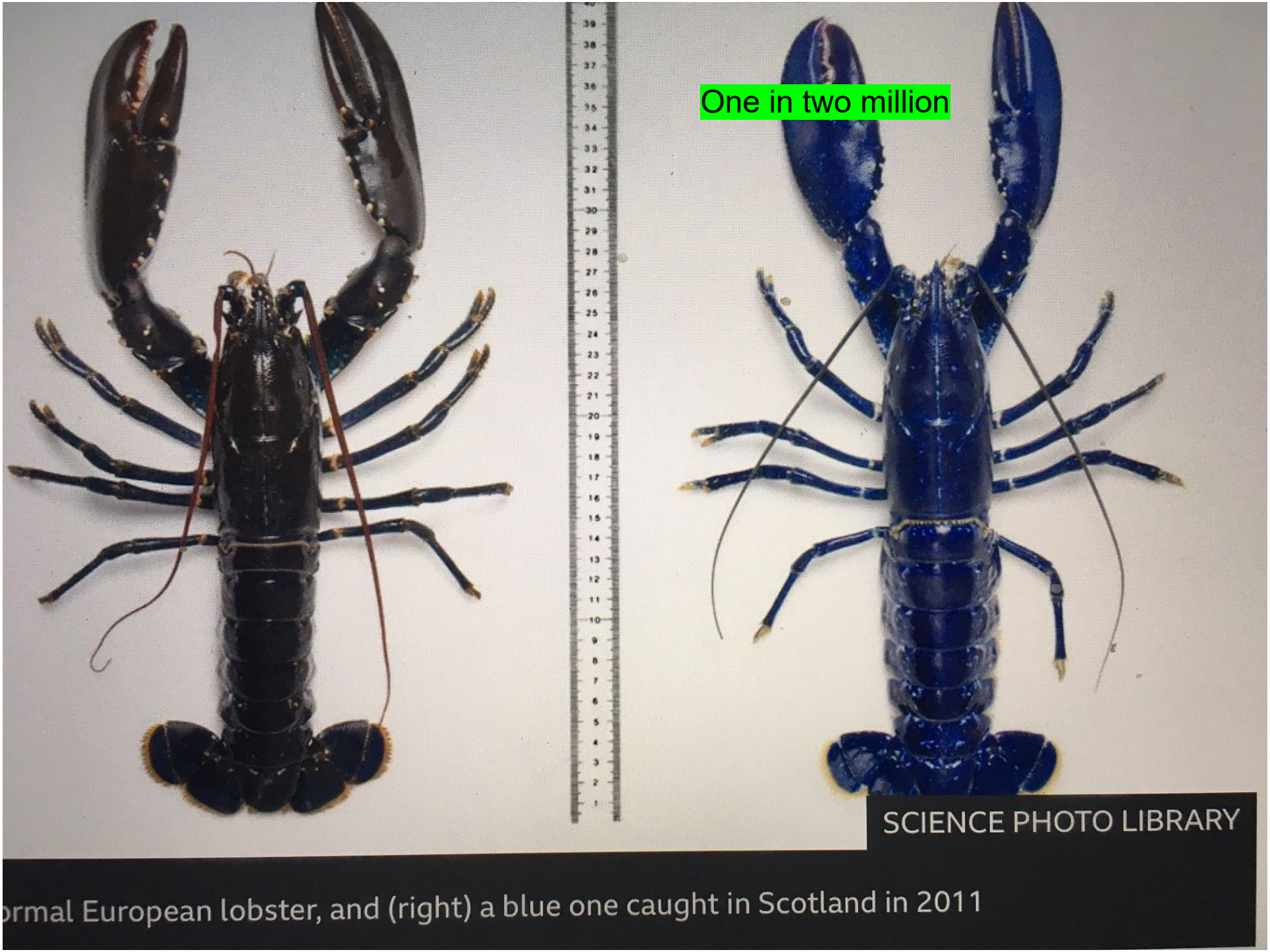

ormal European lobster, and (right) a blue one caught in Scotland in 2011

Can new rootstocks in the citrus breeding pipeline confer **HLB-resistance** to susceptible scions?

Is it one in a hundred, one in a thousand, one in a million, or possible at all????

How do you find the needle in the haystack?

What should the 'haystack' be?

What is the fastest and most efficient way to screen the haystack?

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qPCR testing of roots and leaves

ct values are inversely proportional to Clas titers. The lower the score, the higher the bacterial population. Most commercial trees have leaf ct values ranging from 24-28.

strong infection 24-28
tolerable infection 28-30
questionable infection 30-32
non-active infection 32-39
no bacteria detected 40

All samples collected by Lili Cano Lab, IRREC

Root sample PCR run by Lili Cano Lab,

Leaf sample PCR run by Southern Gardens Diagnostic Lab, via Mike Irey

qPCR testing – industry averages

Average of Cal_ct	Column Labels			
Row Labels	Hamlin	Valencia	Vernia	Grand Total
2006	30.40	28.37		28.71
2007	27.34	26.88	36.14	27.01
2008	23.60	23.83	20.20	23.78
2009	24.04	23.99	28.74	24.00
2010	25.44	25.70	26.84	25.66
2011	24.76	24.44	24.11	24.52
2012	25.09	24.91	22.42	24.96
2013	27.83	29.14		28.05
2014	25.36	25.37		25.36
2015	24.85	24.54		24.69
2016	26.21	26.33		26.27
2017	27.91	26.81		27.15
2018	27.60	26.67		27.28
2019	28.61	28.05	28.68	28.27
2020	30.79	28.14	36.21	29.06
2021		28.81		28.81
Grand Total	25.08	24.81	27.47	24.90

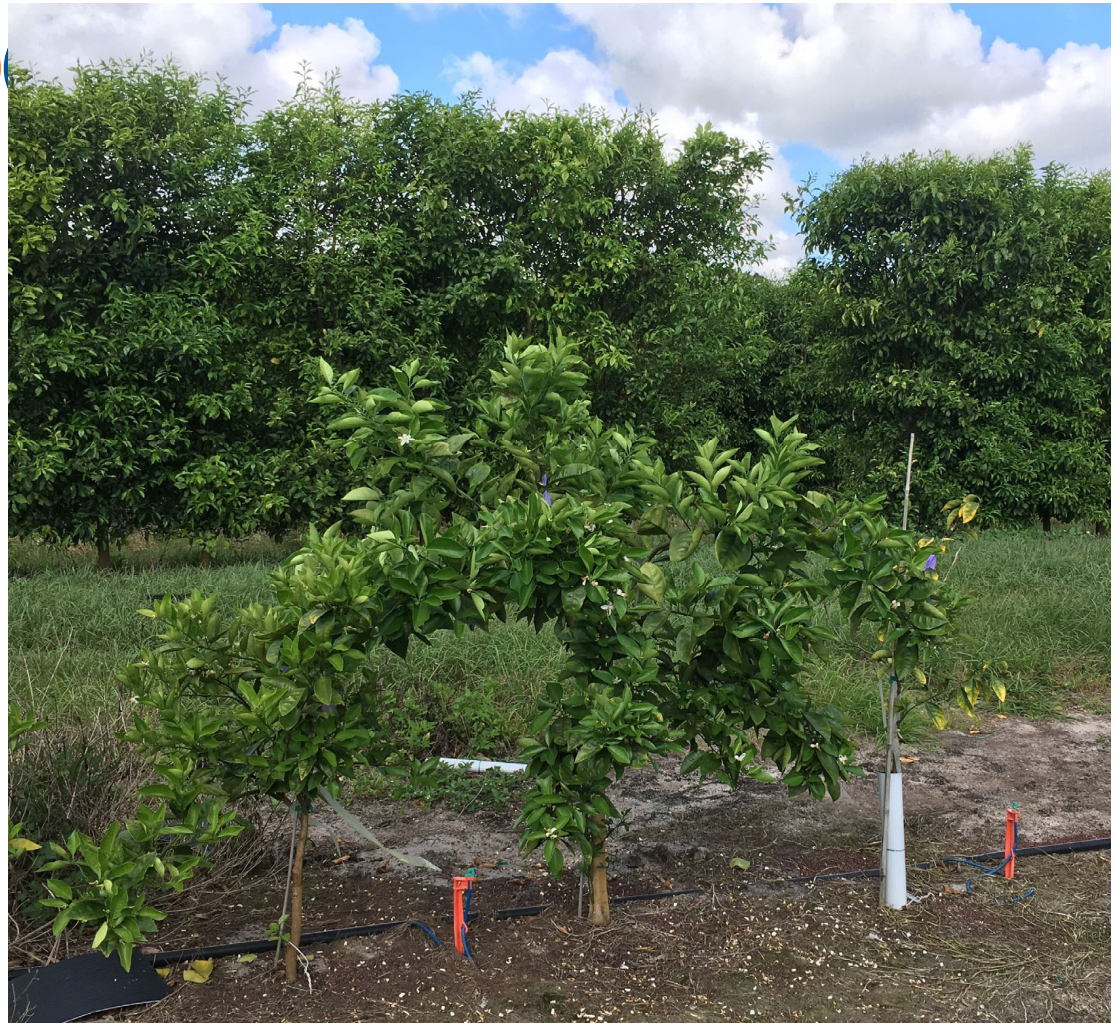
Data kindly provided by Mike Irey/Southern Gardens Diagnostic Laboratory

Selected Gauntlet Trees (all with Valencia scion, planted HLB+) – From best trees

Row/Tree	Rootstock	Root ct	Leaf ct	Ploidy
24-27	C2-4-1x3246x2071-05-16-40	40	**34.7**	2x
24-59	S11x50-7-6-12	40	**37.3**	2x
19-175	N+HBPxOrange19-12-3*	40	30.1	4x
19-6	Green 6xOrange 14-09-24	40	29.8	4x
19-9	Green 6xOrange 14-09-21	40	29.6	4x
19-17	S10xS11-11-S16	40	28	2x
19-127	A+VolkxOrange19-11-5	40	30.9	4x
19-135	B21-R1-T25-11-2	40	30.5	2x
19-137	A+VolkxOrange19-11-1	40	28.4	4x
19-173	8-1-99-2xC-22-12-15	40	29.2	2x
19-187	A+HBPxCH+50-7-12-4	40	31.1	4x
19-92	A+HBJL2BxOrange19-9-7	40	29.5	4x
19-113	A+VolkxOrange19-11-21	40	29.5	4x

* high brix fruit

Possible Resistance via the rootstock?



One-year old ‘gauntlet’ tree of HLB+ Valencia/S11x50-7 [(salt tolerant HBPummelo X Shekwasha) x trifoliate orange 50-7)]. No bacteria detected in roots, and almost none in the scion.

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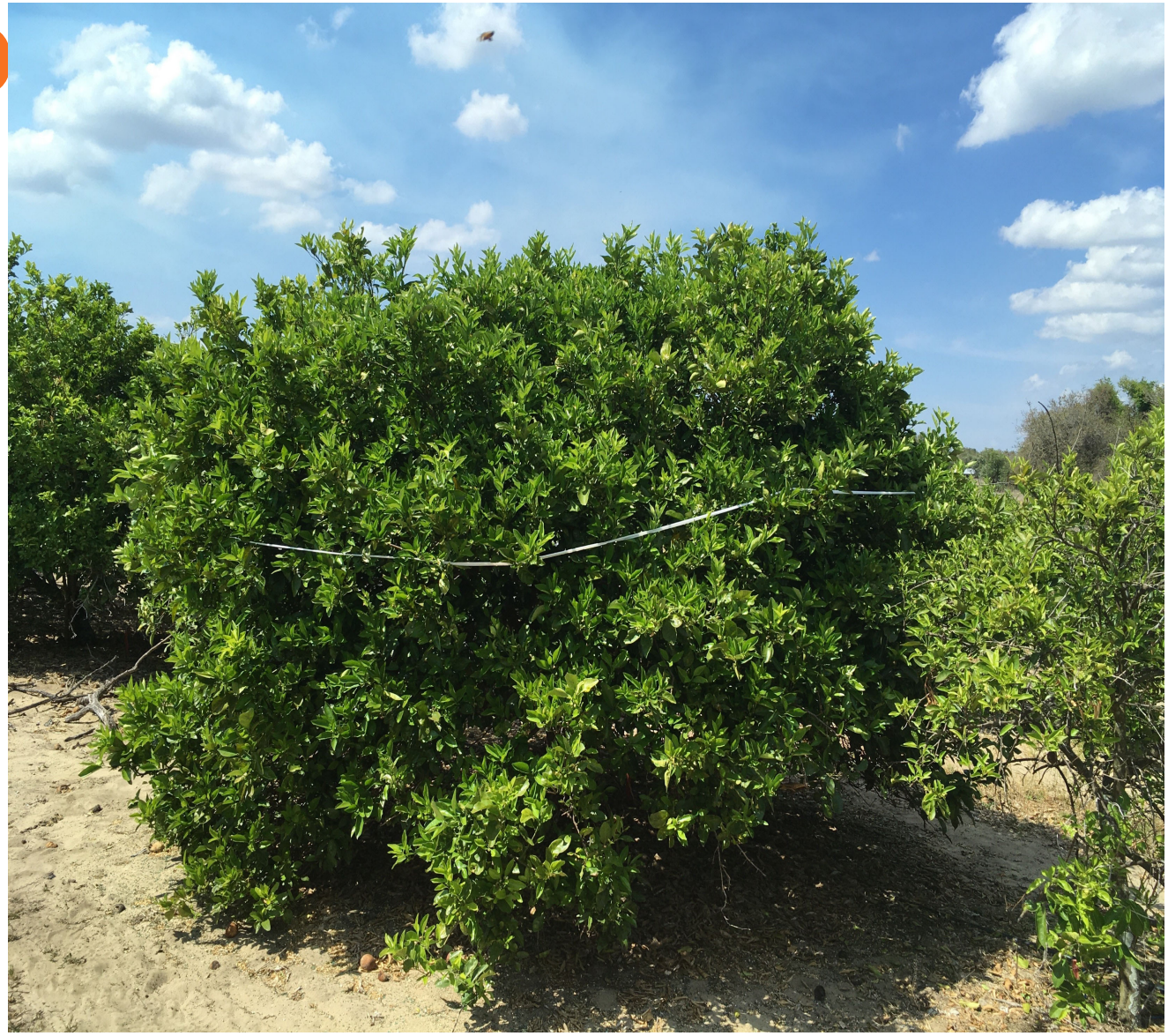
Selected Gauntlet Trees (all with Valencia scion, planted HLB+) – From best trees

24-1	43x20-04-12x50-7-16-9	33.1	**32.9**	2x
24-4	LB8-9xS10-15-26	33.9	**34.9**	2x
24-29	N+PxWGFT+50-7-16-14	36.9	**34.4**	4x
24-54	S11x50-7-16-6	34.9	30.1	2x
13-20	SR+SH-99-18xWGFT+50-7-14-9	34.4	28.7	4x
13-23	A+5-1-99-3xSO+50-7-7-1	32.9	28.4	4x
19-18	A+HBJL2BxOrange19-9-31	35.8	28.9	4x
19-31	A+HBPxGreen7-12-40	32.4	29.9	4x
19-32	A+HBPxGreen7-12-13	32.9	28.4	4x
19-81	Milam+HBPxOrange14-09-19	33.1	30	4x
19-109	Milam+HBPxOrange14-09-14	33	30.1	4x
19-104	A+VolkxOrange19-11-9	35.2	30.6	4x
19-128	B11-R5-T25-11-3*	34.7	**34.4**	2x
19-139	B21-R1-T2-11-1	33	28	2x
19-181	A+HBPxCH+50-7-12-11*	34.5	31.5	4x
19-184	S10xS15-12-25	37	30.3	2x
29-128	S10xX639-12-32	34.5	31	2x
29-138	S10xS15-12-51	34	31.9	2x
29-183	A+HBPxOrange3-12-10	34.9	30.5	4x

* high brix fruit

Another exciting source of **Genetic Diversity** being investigated is probably zygotic rootstocks coming from seed sources that produce zygotic progeny at a high rate.



Vernia on zygotic HBPummelo x Shekwasha at St. Helena; 3.5 boxes fruit, 12.5 brix. One of 5 trees at St. Helena on probable zygotic rootstocks selected for propagation and further testing.

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WHAT HAVE WE LEARNED ?

- Rootstocks can impart HLB-tolerance to grafted scions
- Rootstocks may be able to impart HLB-resistance to grafted scions
- Mechanisms may include enhanced micronutrient mining and translocation; enhanced feeder root growth and preservation; enhanced phloem regeneration capacity; and suppression of Clas replication
- Rootstocks with abiotic stress tolerance performing well against HLB
- Focus also on tolerant/resistant rootstocks that induce early bearing of high soluble solids fruit, as necessary to battle industry-wide juice quality issues

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Combination of good scion genetics, good rootstock genetics and evolving nutrition (McKenna nitrate program): OLL-8 sweet orange/UFR-4 rootstock, 4 year old trees - Working!

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The Ticket For the Immediate Future

Improved Scion Genetics for HLB Tolerance

Plus

Improved Rootstock Genetics for HLB Tolerance

Plus

Improved Affordable Production Systems w/ Enhanced Root Nutrition

$$ Success $$

Thanks! The UF/CREC Citrus Improvement Team Appreciates Supporting Grants from CRDF and NIFA/SCRI!

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Thanks also to Steve Mayo, Ed Stover and the USDA Picos Farm Crew, Troy Gainey and the CREC Farm Crew, Orie Lee Family Groves, Agromillora and Philip Rucks Nursery TC Labs (Beth Lamb) and many others.

UF-CREC Citrus Genetic Improvement Team
2021