

# **Citrus Scion & Rootstock Development for an HLB- Endemic Florida: The Way Forward**



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September 2019, CRDF Select Committee



## A relevant quote from a world renowned biologist:

“Scientific research is thereby seldom straightforward. It rarely takes major leaps straight to the top. It moves obliquely, pressing forward at angles, reformulating, twisting, filling the subject out, waiting, looking around, describing parts more exactly, describing causal lineages more firmly, then, like a crack in a cave wall, a guiding beam of light comes through”. .....Edward O. Wilson

- **Development of rootstocks that can impart HLB tolerance or resistance to grafted scions.**
- **Breeding of HLB tolerant or resistant processing sweet oranges and orange-like hybrids.**
- **Screening of the UF-CREC germplasm collection to identify and validate HLB tolerant or resistant selections**
- **Advanced field trials, release and commercialization of promising HLB tolerant/resistant scion and rootstock cultivars.**

## **Project 15-010 Objectives**

- **Overcome the devastation of HLB**
- **Develop new, or recapture old, markets**
  - **Sweet oranges for the juice business**
  - **Mandarin hybrids**
  - **Grapefruit and grapefruit like hybrids**
  - **Acid fruit, e.g. lemons**

**Citrus breeding is a continuum, and requires a delicate and common sense balance between short/medium-term and long-term objectives**

# **Our Priorities**

- **First somatic hybrids in citrus**
- **First to exploit somaclonal variation for sweet orange**
- **First HLB-tolerant citrus cultivar ‘LB8-9’ Sugar Belle®**
- **First cybrid citrus cultivar: N2-28 Summer Gold grapefruit**
- **First triploid scion cultivar fathered by a somatic hybrid: C4-15-19**
- **OLL series, UF 914, EV1&2, Bingo, Marathon, Vernia, Valquarius, Valencia B9-65, 36+ Hamlin**
- **UFR Rootstock Series**
- **UF-914 grapefruit hybrid – low furanocoumarins, no drug interactions**

# **Our Track Record**

- **First to develop genetic engineering techniques for citrus**
- **First to publish on phloem-specific promoters in citrus**
- **First to publish on long-term transgenic field tolerance in citrus**
- **First to demonstrate successful graft transmission of early flowering (FT) from transgenic rootstock to juvenile scions**
- **First molecular marker systems for genetic mapping**
- **First publicly available citrus genome sequences**

# **Our Track Record**





**GREC-BALM Rescue:** The UF/CREC Citrus Improvement Team recently lost 50 acres of program citrus germplasm due to the water crisis at the GREC. The entire 50 acres was indexed for HLB tolerance, and 2200 tolerant selections were Identified. We have successfully rescued about 1600 of these, to be planted soon.



## Now to Rootstocks:

The UF/CREC Citrus Improvement Team has worked very hard to establish a common sense delicate BALANCE between providing viable/profitable rootstock options for the short-medium term, and developing the HOMERUN rootstock(s) that will be the ANSWER for the future, regardless of the scion.



# Citrus Budwood Annual Report 2017-2018, Rootstocks

2018		# budded	2017	2016
1	Swingle	683,378	Swingle	Kuharske
2	US-942	661,003	US-942	X-639
3	Kuharske	504,639	X-639	S/O
4	Sour Orange	418,407	Kuharske	US-897
5	X-639	349,046	Sour Orange	SWG
6	US-897	243,781	US-802	US-942
7	US-802	169,465	US-897	US-802
8	Own Root	163,966	UFR-04	US-812
9	US-812	146,410	US-812	Cleopatra
10	Volkamer	119,276	C-35	UFR-04
11	C-35	86,181	Cleopatra	Volkamer
12	UFR-04	71,231	Volkamer	Kinkoji
13	P Trifoliata	53,935	UFR-03	UFR-03
14	Rough Lemon	33,909	C-22	Carrizo
15	UFR-03	32,853	Carizzo	Rough Lemon
16	UFR-06	28,617	Rough Lemon	Research
17	Cleopatra	21,760	UFR-17	UFR-02
18	C-22	19,361	Kinkoji	UFR-17
19	US-896	18,205	UFR-01	UFR-16
20	UFR-05	16,735	Flying Dragon	Sun Chu Sha



# University of Florida Rootstocks (UFR's)

In 2011, we were asked to assess our new rootstock germplasm and release a group of rootstocks that we thought had the best potential against HLB, not waiting for the usual traditional data sets.

We chose to divide this up into appropriate categories, and we released the following 17 following UFR rootstocks, based on limited but positive data:

Tetraploid Rootstocks: UFR 1-6; 17 (sour orange hybrid)

Citranges: UFR 7-12

Ichangensis hybrid: UFR 13

Sour orange types: UFR 14-16

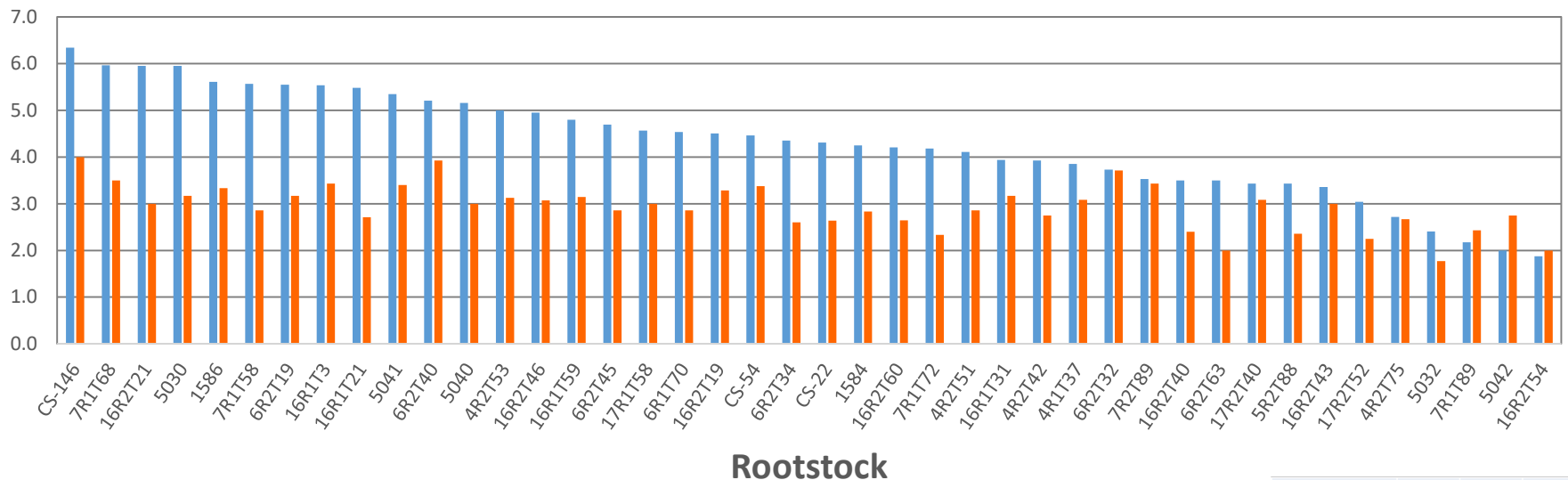


**Premier Grapefruit Rootstock Trial – Fort Pierce**  
**Several new citranges performing well!**



# Premier Citrus Trial

Premier Marsh Rootstock Trial - Cum 3-year yield 2010-11, 2011-12, 2015-16  
[box/tree] and HLB rating 2017



HLB ratings

■ cum 3-year yield ■ HLB Rating [2017]

HLB rating scores			
0-0.5	=	no HLB symptom or few leave symptoms	
1-1.5	=	10%-20% HLB symptomatic canopy	
2-2.5	=	25%-50% HLB symptomatic canopy	
3-3.5	=	more than 50% HLB symptomatic canopy	
4	=	decline to death	

# NEW STRATEGY: BREEDING SOMATIC HYBRID ROOTSTOCKS AT THE TETRAPLOID LEVEL – CREATION OF **‘TETRAZYGS’**

- Use of allotetraploid somatic hybrid breeding parents allows the mixing of genes from 3-4 diploid rootstocks at once.
- Progeny can be screened at the seed/seedling level for wide soil adaptability and Phytophthora resistance.
- Products can have direct rootstock potential including adequate polyembryony, ability to control tree size due to polyploidy, and improved disease resistance.



Candidate  
for ACPS

Valquarius on Orange #15 tetrazyg rootstock - just < 5 years at  
St. Helena, Dundee FL - released as UFR-3



St. Helena Project 2019 Top 30 Data – Rootstock Recovery Contest under Improved Nutrition

Scion/Rootstock Combination 10-year old trees						PS/Acre	Boxes/Acre	cum PS/acre
	PS/Box [2019]	Boxes/Tree [2019]	PS/tree [2019]	Optimum Trees/Acre	Optimum Sq ft/tree [2019]	Projected [2019] (% increase 2019/2018)	Projected [2019] (% increase 2019/2018)	[projected] 2011-19 (9 years of data)
Vernia:Orange1804	6.5	3.0	19.1	207	210	3951 (150)	611 (114)	16509 (9)
Vernia:KCZ	6.9	2.6	17.7	212	205	3756 (119)	543 (87)	13466 (9)
Valquarius:Orange1804	5.9	3.3	19.6	189	230	3713 (121)	629 (95)	13479 (8)
Vernia:Yel1800	6.5	2.4	15.4	235	185	3619 (136)	559 (122)	13516 (9)
Vernia:Blue1	6.5	2.5	16.0	223	195	3573 (NA)	546 (173)	18278 (9)
Vernia:MG-11	6.8	2.3	15.6	229	190	3571 (113)	527 (72)	16081 (9)
Vernia:Orange21	6.6	1.9	12.5	281	155	3518 (129)	534 (83)	14550 (9)
Vernia:Purple2	6.1	2.0	12.1	281	155	3411 (46)	556 (21)	17951 (8)
Vernia:Volk	5.6	3.5	19.7	171	255	3362 (104)	604 (84)	14339 (9)
Valquarius:FG1793	6.1	3.0	18.1	185	235	3341 (150)	551 (139)	13651 (8)
Valquarius:Orange13	6.6	2.0	12.9	256	170	3312 (127)	502 (93)	16746 (9)
Vernia:Aqua1803	6.6	2.3	15.4	203	215	3131 (114)	473 (73)	16612 (9)
Vernia:Cleo+CZO	6.9	1.8	12.4	249	175	3097 (NA)	448 (39)	18089 (9)
Vernia:Chang+50-7 [UFR 6]	7.3	1.4	10.3	300	145	3079 (249)	420 (156)	13902 (9)
Valquarius:Amb+HBJL-2B	6.4	2.3	14.8	207	210	3067 (223)	482 (177)	15128 (8)
Valquarius:681G26F4P6	6.2	2.5	15.1	198	220	2993 (70)	485 (53)	15716 (8)
Valquarius:Aqua1803	6.2	2.3	14.4	207	210	2971 (145)	482 (112)	14538 (8)
Valquarius:White1805	6.1	2.8	17.2	171	255	2942 (138)	484 (112)	15380 (8)
Vernia:Wgft+50-7	6.7	1.9	12.3	235	185	2891 (NA)	435 (104)	16168 (9)
Valquarius:Pink1802	6.4	1.7	10.5	272	160	2868 (73)	449 (53)	12977 (9)
Vernia:White1805	6.9	1.4	9.9	290	150	2866 (101)	415 (69)	13868 (9)
Valquarius:MG-11	6.0	2.1	12.4	229	190	2834 (109)	476 (69)	13885 (8)
Valquarius:FG1707	6.8	1.9	13.2	212	205	2790 (72)	409 (45)	14208 (8)
Vernia:Orange19 [UFR 4]	6.4	1.9	12.2	223	195	2730 (147)	426 (102)	14720 (9)
Valquarius:FG1733	6.3	1.8	11.4	229	190	2609 (172)	412 (110)	15233 (8)
Vernia:Orange18	6.7	1.5	10.1	256	170	2573 (NA)	384 (49)	13824 (9)
Vernia:Orange3 [UFR 1]	6.8	1.6	10.8	235	185	2542 (127)	376 (86)	12726 (9)
Vernia:SWC	6.7	1.8	12.1	207	210	2513 (114)	377 (88)	12500 (9)
Vernia:Purple4	7.5	1.1	7.9	311	140	2466 (NA)	330 (42)	15659 (9)
Valquarius:Orange3 [UFR 1]	6.5	1.8	11.5	212	205	2445 (93)	377 (82)	16295 (8)

## St. Helena Project -Top 15 Among 8-year old trees – 2019 Data Rootstock Recovery Contest under Improved CRF Nutrition

Scion/Rootstock Combination 8-year old trees	PS/Box [2019]	Boxes/Tree [2019]	Optimum Trees/Acre	Optimum Sq ft/tree [2019]	PS/Acre Projected [2019] (% increase 2019/2018)	Boxes/Acre Projected [2019] (% increase 2019/2018)	cum PS/acre [projected] 2011-19 (Years of data)
Vernia:46x31-02-13 (UFR-16)	6.4	2.1	280	155	3751 (109)	582 (89)	9823 (5)
Valquarius:White1	6.2	3.1	189	230	3615 (238)	586 (212)	9682 (6)
Valquarius:6058-2071-01-02	5.9	1.7	311	140	3048 (41)	513 (32)	7467 (5)
Vernia:46x31-02-S3	6.3	1.8	264	165	3046 (129)	486 (104)	9613 (6)
Vernia:Amb+Volk	6.5	1.4	293	180	2666 (136)	410 (75)	9909 (6)
Vernia:Nova+7-2-99-2	6.6	1.4	272	160	2548 (139)	389 (108)	8203 (6)
Valquarius:SO+RPxSH99-5	6.9	1.1	323	135	2414 (70)	349 (50)	10139 (6)
Vernia:46x31-02-S9	6.9	1.1	317	137	2366 (13)	342 (-2)	8783 (6)
Vernia:Green2 (UFR-17)	6.3	1.8	203	215	2314 (173)	367 (125)	8341 (5)
Vernia:6058x6056-002	5.9	1.7	218	200	2104 (92)	360 (67)	6070 (5)
Vernia:Amb51992	6.5	1.0	300	145	1855 (69)	285 (48)	8372 (6)
Vernia:6058-2071-01-02	6.4	1.1	264	165	1771 (99)	277 (86)	7271 (6)
Vernia:A-Mac	6.2	1.1	242	180	1674 (-10)	269 (-23)	7135 (6)
Vernia:SO+RPxSH99-4	6.1	1.0	281	155	1665 (88)	273 (91)	3989 (5)
Vernia:Wmur+HBjL-7	6.7	1.0	235	185	1500 (13)	223 (10)	6837 (6)

## UFR-17 Emerging as good HLB-tolerant rootstock for higher-density plantings.



Valencia on UFR-17, February 2018



July 2018



February 8, 2019

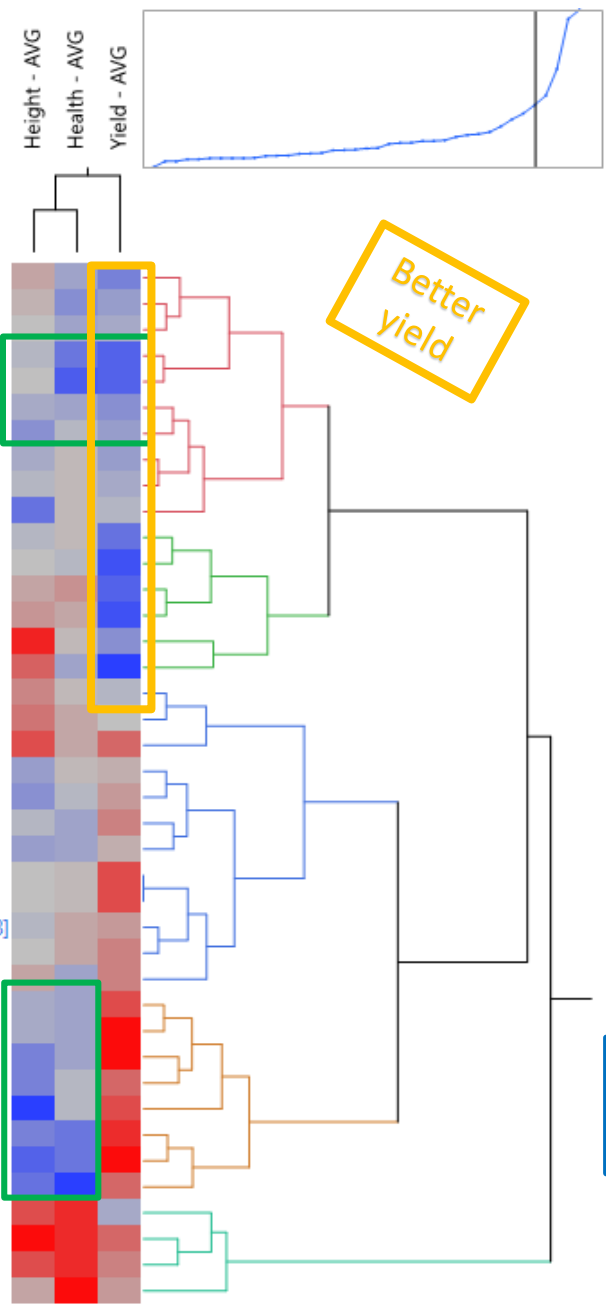
6-year old Valencia/UFR-17 resets at Orie Lee Alligator Grove; HLB+ over 4 years, grown with no psyllid control; picked 2.13 boxes/tree in 2018 season; 2.5 in 2019. UFR-17 is [Nova+HBPummelo x sour orange+Carrizo].

Combined Heat Map: Sour orange-type rootstocks in southwest FL, planted in 2014, English Bros., Jackson Citrus, and Wayne Simmons trials. Approximately 5000 trees.

Better ones



- B9-65/43x20-04-12[131]
- B9-65/46x20-04-47[117]
- B9-65/46x20-04-64[137]
- B9-65/UFR17[178]
- OLL20/UFR17[133]
- B9-65/UFR15(46x20-04-37)[118]
- OLL20/White1[17]
- OLL20/UFR2[53]
- OLL20/UFR4[98]
- B9-65/UFR2[59]
- B9-65/46x20-04-09[165]
- B9-65/46x20-04-42[78]
- B9-65/46x20-04-48[79]
- B9-65/UFR4[90]
- B9-65/46x20-04-12[52]
- B9-65/White1[39]
- B9-65/46x20-04-2[119]
- OLL20/43x20-04-12[122]
- OLL8/43x20-04-12[142]
- OLL20/46x20-04-09[168]
- OLL20/46x20-04-42[82]
- OLL20/46x20-04-47[110]
- OLL8/UFR15(46x20-04-37)[143]
- OLL20/46x20-04-2[25]
- OLL8/46x20-04-47[154]
- OLL20/UFR15(46x20-04-37)[148]
- OLL8/46x20-04-48[113]
- OLL8/46x20-04-2[133]
- OLL20/46x20-04-48[70]
- OLL8/46x20-04-64[116]
- OLL20/46x20-04-64[151]
- OLL8/46x20-04-09[229]
- OLL8/46x20-04-42[132]
- OLL8/UFR17[90]
- OLL8/UFR4[343]
- OLL8/UFR2[73]
- OLL20/46x20-04-12[19]
- OLL8/43x20-04-2[129]
- OLL8/46x20-04-12[10]
- OLL8/White1[23]



Better yield

Scions:  
Valencia B9-65  
OLL-20  
OLL-8

Blue: High  
Red: Low



Best of 125 hybrid rootstock selections  
originally being tested against blight.



9-year old Valencia on 46x20-04-6 (HB Pummelo x Cleo) grown at Lee Alligator Grove (St. Cloud) with only 2 psyllid sprays per year and no special nutrition.





# Time is the Enemy



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 2<sup>nd</sup> 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**





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





Two complex 'tetrazyg' rootstocks performing well in the 'gauntlet' HLB screen are now making polyembryonic seed, allowing for rapid large scale testing – now underway (above: Milam+HBPxOrange 14-09-10 and A+VolkxOrange 19-11-8). Trees on right were budded with our new early Valencia release EV-1. These and other selections have been entered into the DPI Parent Tree Program and are being provided to rootstock tissue culture micropropagation companies.



**1. Genetic Patterns Emerging! Gauntlet trees showing promise – complex tetraploids, combining phloem regeneration capacity from the lemon group with the feeder root preservation trait of Orange 19 (and sibling Orange 14).**

A+Volk x Orange 19-11-23

A+Volk x Orange 19-11-9

A+Volk x Orange 19-11-26

A+Volk x Orange 19-11-8

A+Volk x Orange 19-11-31

A+Volk x Orange 19-11-1

A+Volk x Orange 19-11-5

A+Volk x Orange 19-11-13

Milam+HBP x Orange 14-09-3

Milam+HBP x Orange 14-09-19

Milam+HBP x Orange 14-09-12

Milam+HBP x Orange 14-09-9

Milam+HBP x Orange 14-09-4

Milam+HBP x Orange 14-09-6

Milam+HBP x Orange 14-09-14

Milam+HBP x Orange 14-09-10

Milam+HBP x Orange 14-09-11

**2. Genetic Patterns Emerging! Gauntlet trees showing promise from rootstock hybrids stacked with abiotic stress tolerance genes (HLB is a stress reactive disease).**

Sour orange types:

S10xS11-11-S20 (salt tolerant Shekwasha/pummelo)

S10xS15-12-25 (salt tolerant Shekwasha & Cleo/pummelo)

S10xS15-12-48 (salt tolerant Shekwasha & Cleo/pummelo)

S10xS15-12-35 (salt tolerant Shekwasha & Cleo/pummelo)

S10xS15-12-32 (salt tolerant Shekwasha & Cleo/pummelo)

S10 x x639-12-16 (salt tolerant Shekwasha/Cleo/trifoliolate orange)

S10 x x639-12-31 (salt tolerant Shekwasha/Cleo/trifoliolate orange)

46x31-00-S10 x US812-11-2 (salt tolerant Shekwasha, Sunki,  
trifoliolate orange)



46x31-02-S10x46x20-04-S15-12-25

46x31-02-S10x46x20-04-S15-12-48

Hybrids selected for abiotic stress tolerance performing exceptionally well in 'gauntlet' rootstock screen. Abiotic stress tolerance and HLB-biotic stress tolerance genetics may overlap! Rootstocks of above trees (grown with HLB+ Valencia) are hybrids of parents both previously selected for tolerance to 3500 ppm NaCl; [Hirado buntan pummelo x Shekwasha mandarin] x [Hirado buntan pummelo x Cleopatra mandarin]. Tree on right was HLB+ in 2016, HLB- in 2017!





S10xS15-12-25 (Shekwasha/Cleo/pummelo)



Several hybrids of 8-1-99-2B x C22 (pummelo x citrandarin)

**GAUNTLET rootstock screening (Final stage at USDA-Picos Farm, Fort Pierce, FL); HLB+ Valencia trees grown from the get-go with Clas-infected budsticks; also passed through a hot psyllid house. More than 10,000 hybrids screened to date. Some now in large-scale trials!**

# Is HLB-Resistance in the Scion Possible from a Rootstock?

Clas	Non-Detected	NR
1.	S10xX639-12-4	4
2.	S10xS15-12-25	4
3.	S10xS15-12-33	3
4.	8-1-99-2BxC-22-12-32	4
5.	A+VolkxOrange19-11-24	3
6.	B11-R5-T25-11-6 (FD hybrid)	3
7.	Milam+HBPxOrange14-09-19	4
8.	B11-R5-T4-11-2 (FD hybrid)	4
9.	S10xS15-12-29	4
10.	A+HBPxCH+50-7-12-57	4
11.	S10xX639-12-7	3

**Table 3.** Grosser/Gmitter: 2019 qPCR (Cano) older 'gauntlet' trees showing non-active CLas infection – potential resistance being transmitted to the Valencia scion? Trees 4-6 years old at USDA-Picos Farm.





Sour+Rangpur Seed Tree



HLB+Valencia/ A+HBPxSORP-13-29

Genetic Pattern 3. Gauntlet HLB+ Valencia on a complex rootstock hybrid of Amblycarpa+HBPummelo x Sour orange+Rangpur. The Sour orange+Rangpur parent seed trees are planted at 3 locations, and all are exceptionally tolerant of HLB. However, it does not make a good rootstock itself due to slow growth and excessive zygotic seed production. Hybridizing it with the tolerant A+HBP parent has corrected the vigor problem!



Top Performing Gauntlet Rootstock Status					
Rootstock	Seed Tree	Producing Seed	PTP Status	TC	MAC
A+VolkxOrange19-11-8	Yes	Yes	Available	Yes	Yes
Milam+HBPxOrange14-09-10	Yes*	Yes*	In process	Yes	Yes
A+VolkxOrange19-11-31	Yes	No	Available	Initiated	No
A+HBJL1-OP-09-36 (sour type)	No	No	Soon	No	No
Milam+HBPxOrange14-09-9	No	No	Soon	No	No
Milam+HBPxOrange14-09-14	No	No	Available	Initiated	No
A+HBPxOrange19-08-2	Yes	Zygotic	Available	Initiated	No
Flying Dragon Hybrids (for ACPS)					
B21-R1-T2-11-2	No	No	Available	No	No
B21-R1-T25-11-10	No	No	Available	No	No
B21-R1-T25-11-6	No	No	Available	No	No
A+FDxOrange19-11-10	Yes	Yes	Soon	No	No
R&D Tissue Culture Collaborators: Phillip Rucks Nursery TC Lab and Agromillora FL					

# Sugar Belle (LB8-9) Rootstock Hybrid Cuttings Inventory

Hybrid*	Number	Hybrid*	Number	Hybrid*	Number
LB8-9 X S10-15-5	5	LB8-9 X S13-15-1	8	Sugar Belle LB-Zyg x 50-7-16-25	11
LB8-9 X S10-15-7	10	LB8-9 X S13-15-2	5	Sugar Belle LB-Zyg x 50-7-16-26	12
LB8-9 X S10-15-9	6	LB8-9 X S13-15-4	6	Sugar Belle LB-Zyg x 50-7-16-23	13
LB8-9 X S10-15-10	6	LB8-9 X S13-15-6	12	Sugar Belle LB-Zyg x 50-7-16-6	22
LB8-9 X S10-15-11	8	LB8-9 X S13-15-8	9	Sugar Belle LB-Zyg x 50-7-16-12	15
LB8-9 X S10-15-12	7	LB8-9 X S13-15-9	5	Sugar Belle LB-Zyg x 50-7-16-7	21
LB8-9 X S10-15-14	5	LB8-9 X S13-15-10	4	Sugar Belle LB-Zyg x 50-7-16-5	8
LB8-9 X S10-15-17	5	LB8-9 X S13-15-11	8	Sugar Belle LB-Zyg x 50-7-16-4	33
LB8-9 X S10-15-18	13	LB8-9 X S13-15-12	5	LB8-9 x 50-7-16-4	4
LB8-9 X S10-15-19	10	LB8-9 X S13-15-13	8	LB8-9 x 50-7-16-2	8
LB8-9 X S10-15-20	9	LB8-9 X S13-15-14	6		
LB8-9 X S10-15-21	4	LB8-9 X S13-15-15	8		
LB8-9 X S10-15-25	11	LB8-9 X S13-15-16	6		
LB8-9 X S10-15-28	5	LB8-9 X S13-15-18	4		
LB8-9 X S10-15-29	7	LB8-9 X S13-15-22	6		
LB8-9 X S10-15-30	11	LB8-9 X S13-15-23	4		
LB8-9 X S10-15-41	11	LB8-9 X S13-15-24	7		
LB8-9 X S10-15-47	4				
LB8-9 X S10-15-15	5				
LB8-9 X S10-15-16	2				
	<b>144</b>		<b>111</b>		<b>147</b>

\*Name: Sugar Belle X OP Pummelo + Trifoliolate 50-7-XX-X (The Last Number is designates the sibling #. LB-ZYG = Lime Block Zygotic Or LB8-9 X OP Pumelo + Trifoliolate 50-7-XX-X

## SugarBelle Rootstock Hybrids – Moving Through the ‘Gauntlet’



No stone unturned! Left: 1-year old HLB+Valencia on SugarBelle x 46x20-04-S15-15-16, under heavy psyllid pressure; Right: HLB+Valencia on various SugarBelle rootstock hybrids prepared for ‘Gauntlet’ field screen at USDA-Picos Farm in Fort Pierce.





# 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



# New Lykes Trials: Camp Mack and Basinger

Working with John Gose and the Lykes Nursery, two major sister 40-acre rootstock trials replicated at two sites: Camp Mack and Basinger. 11/12 replications per rootstock.

Hamlin on 57 rootstocks

Valencia on 52 rootstocks

Rootstocks included from UF, USDA, CA and Spain

We have collected 2 years of young tree data, and will work with colleague Dr. Ute Albrecht (PI on the Trial Evaluation Project) to continue robust data collection and analysis.



## OTHER STUFF

- Development and screening of HLB-tolerant dwarfing rootstocks for high density plantings, by combining Flying Dragon with widely adapted complementary parents, both at diploid and tetraploid level; some performing well in 'gauntlet' screen.
- Testing 'Super-root' rootstock mutants (and zygotics) identified and provided by Beth Lamb at Ruck's Nursery. Fast-28 (from UFR-1) looks quite promising.
- Testing HLB-tolerant pummelo and pummelo/mandarin **interstocks** with Valencia/Swingle, small field trial underway with Tamiami in Avon Park. After 3 years, most of the trees look good, but there are differences in cropping – data to be collected this season.
- Still experimenting with ultra-susceptible HLB+Murcott as a more robust rootstock screening tool (albeit on a small scale).





## Improving delivery of trial information to growers

- Citrus Improvement Team Website Now Online – one stop shopping for growers/processors, packers. Data from 10 trials at present
- Website will eventually have tabs to General Information, CRDF supported trials, MAC trials, and NIFA grant progress.
- Our team hired Dr. Filomena Valim to facilitate trial data statistical analyses and easily understood presentations.
- Website will also provide links to FFSP, NVDMC, etc. for information on scion and rootstock access/licensing, etc.

Following All Leads!



3 trees from middle of 10 tree plot of OLL-7/Orange 14 at Tropicana/CREC trial; exceptional middle tree appears to be on a zygotic Orange 14 (all other trees in the plot look like the two outside trees). Using the wedge-cut technique, rootstock shoots were induced, harvested, and are being propagated. SSR marker analysis is underway to determine if the rootstock is a zygotic.

## **Rootstock improvements regarding HLB are like likely to come in stages:**

First stage: Rootstocks that reduce the frequency of HLB infection, and reduce the severity of the disease once infected – already proven to work with mid- and late-season oranges when grown with optimized nutrition programs focusing on root health.

Second stage: Potential rootstock mitigation of the disease – research is underway to possibly identify rootstocks that can protect the entire tree – regardless of the scion. Psyllid control may not be necessary. No horticultural performance data would be available on such selections initially, but the hybrids would have good rootstock pedigree, and can be mass-propagated by tissue culture (Ruck's Nursery, Agristarts, Agromillora, Citrific, etc.).

Many of the most promising hybrids have been entered into expanded field trials via MAC grants. The first MAC grant (w/ Kim Bowman @ USDA) is testing 48 new rootstocks (24 from UF and 24 from USDA) with industry cooperators at 9 locations. The 2<sup>nd</sup> 'Rogers' MAC grant is testing 79 scion/rootstock combinations in LA. A third MAC grant will test putatively tolerant scions and rootstocks in Florida and California (w/ Vidalakis and McCollum, USDA). Numerous other trials are also planned and underway.



- **Renewed field team personnel**
- **More grove visits in the last 2 years than ever before**
- **More data collected in the past year than ever before**
- **New focus on summary reports from field trials**
- **New web presence now online and being expanded**

**A few more things...**

# CONCLUSIONS

- Enhanced ground nutrition featuring ‘hybrid’ programs that include CRF and/or liquid applications can help restore and sustain production from HLB-infected trees – but it takes time!

## 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 \$\$**



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





**THANK YOU CRDF!**

**2019 Yield data (boxes/tree) from Lee Groves 'Karen's Block OLL somaclone/rootstock trial; planted June, 2013. Two-trees per plot, trees all produced from field budwood under DPI permit; trial grown with no psyllid control, past two years trees received supplemental Harrells 12-3-9 or 14-3-11 and Florikan Mn. Trial propagated from field budwood under DPI permit.**

	1	3	7	2	4	5	6	8	
RS	OLL 7	OLL 4	OLL 20	OLL 8	OLL 5	OLL 10	OLL 6	OLL 9	X Box/8Tree
Swingle	1.0	1.0	0.5	1.0	0.5	0	0.5	0	4.5
x639	1.75	1.5	0.7	1.25	1.5	1.0	0.65	0.13	8.48
Carrizo	1.83	1.25	0.63	1.25	0.63	0.63	0.25	0.25	6.72
White 1	1.5	2.0	1.13	1.75	1.5	1.0	1.13	0.38	10.39
White 3	1.13	1.0	0.75	0.75	0.88	0.63	0.63	0.38	6.15
UFR 6	1.75	1.25	0.85	1.25	1.25	1.0	0.63	0.88	8.61
UFR 4	2.0	1.88	1.9	1.25	1.25	0.63	0.88	0.13	9.92
UFR 1	1.83	1.75	0.5	2.0	1.0	1.5	1.0	0.5	10.08
UFR 2	1.5	0.5	1.8	1.75	1.0	1.25	1.63	0.38	9.81
Orange 14	2.0	1.75	0.85	2.15	1.25	1.75	1.75	1.0	12.5
Orange 15	0.85	0.88	0.63	0.9	0.5	1.38	0.75	0.88	6.77
Orange 2	0.87	0.75	0.75	1.25	1.38	0.13	0.63	0.75	6.51
Orange 13	1.0	0.75	0.65	1.0	0.75	1.5	0.63	0.75	7.03
Orange 16	1.4	2.25	0.6	2.0	1.63	1.5	1.75	0.5	11.63
UFR 5	1.5	1.5	0.63	2.5	1.75	2.13	1.25	0.75	12.01
Green 7	1.5	1.25	0.38	0.5	1.13	1.5	0.63	0.5	7.39
AMB+CZO	0.85	1.0	0.35	0.5	1.5	1.0	0.5	0.38	6.08
Green 2	2.85	1.25	0.6	1.0	1.75	0.75	0.63	0.13	6.96
	25.11	23.51	14.20	24.05	21.15	19.28	15.82	8.67	
	1.4	1.31	0.78	1.34	1.18	1.07	0.88	0.48	

2019 Juice quality data – Karen’s Block 13W – Lee Groves planted 2013, no psyllid control, enhanced nutrition.

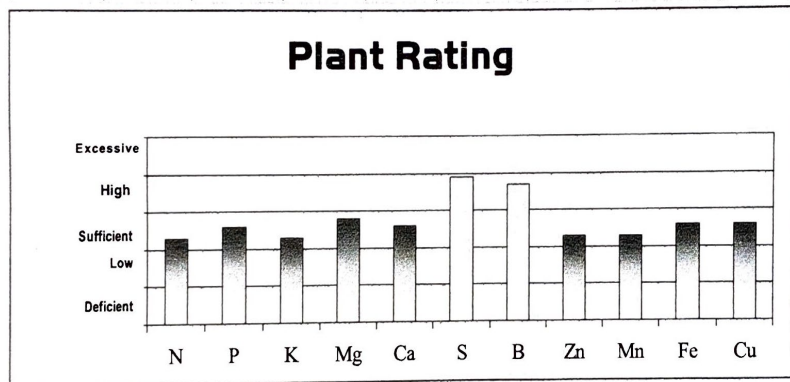
	Sample ID		Wt. Sample	Wt. Juice	Lbs. Juice Per Box	Acid	Total Brix	Ratio	Lbs. Solids Per Box	Juice Color
1	Carrizo	OLL4	27.99	16.65	53.537	0.94	12.92	13.74	6.92	38.2
2	Carrizo	OLL8	27.32	16.21	53.400	0.98	13.93	14.21	7.44	37.5
3	White 1	OLL4	26.44	15.97	54.361	0.91	12.66	13.91	6.88	38.2
4	White 1	OLL8	27.00	16.68	55.600	0.90	11.89	13.21	6.61	38.6
5	White3	OLL4	25.13	15.27	54.688	0.96	12.34	12.85	6.75	38.4
6	White3	OLL8	24.92	15.35	55.437	0.89	12.31	13.83	6.82	38.5
7	UFR-6 (Chang+50-7)	OLL4	24.65	15.00	54.767	0.92	12.40	13.48	6.79	38.4
8	UFR-6 (Chang+50-7)	OLL8	24.73	15.57	56.664	0.96	13.51	14.07	7.66	38.7
9	UFR-4 (Orange 19)	OLL4	30.74	18.44	53.988	0.85	11.65	13.71	6.29	37.8
10	UFR-4 (Orange 19)	OLL8	23.46	14.37	55.128	0.86	12.46	14.49	6.87	38.3
11	UFR-1 (Orange 3)	OLL4	28.26	17.18	54.713	0.95	12.40	13.05	6.78	38.2
12	UFR-1 (Orange 3)	OLL8	24.00	14.90	55.875	0.92	11.85	12.88	6.62	38.2
13	UFR-2 (Orange 4)	OLL4	30.82	18.44	53.848	0.80	10.47	13.09	5.64	38.1
14	UFR-2 (Orange 4)	OLL8	23.22	14.24	55.194	0.81	11.85	14.63	6.54	39.1
15	Orange 14	OLL4	27.48	16.82	55.087	0.80	11.07	13.84	6.10	37.8
16	Orange 14	OLL8	24.17	14.52	54.067	0.94	11.23	11.95	6.07	37.8
17	UFR-3 (Orange 15)	OLL4	29.98	18.27	54.847	0.82	10.69	13.04	5.86	38.1
18	UFR-3 (Orange 15)	OLL8	24.26	15.36	56.983	0.80	11.44	14.30	6.52	38.4
19	Orange 2	OLL4	29.87	18.35	55.290	0.93	13.18	14.17	7.29	38.6
20	Orange 2	OLL8	25.36	15.46	54.866	0.79	12.33	15.61	6.76	37.8
21	Orange 13	OLL4	27.48	16.57	54.269	0.86	11.82	13.74	6.42	37.7
22	Orange 13	OLL8	26.46	16.02	54.490	0.85	12.21	14.36	6.65	38.5
23	Orange 16	OLL4	26.75	15.80	53.159	0.90	11.70	13.00	6.22	38.1
24	Orange 16	OLL8	25.54	15.89	55.995	0.88	11.91	13.53	6.67	38.0
25	UFR-5 (White 4)	OLL7	29.45	18.16	55.497	0.90	11.86	13.18	6.58	38.3
26	UFR-5 (White 4)	OLL4	29.34	17.66	54.172	1.02	13.03	12.77	7.06	37.8
27	UFR-5 (White 4)	OLL20	24.65	15.23	55.606	0.86	11.86	13.79	6.59	38.6
28	UFR-5 (White 4)	OLL8	24.24	14.84	55.099	0.96	12.43	12.95	6.85	38.6
29	UFR-5 (White 4)	OLL5	26.90	16.52	55.271	0.95	12.00	12.63	6.63	38.6
30	UFR-5 (White 4)	OLL10	26.29	16.40	56.143	0.93	12.47	13.41	7.00	38.1
31	UFR-5 (White 4)	OLL6	22.79	14.24	56.235	1.04	12.88	12.38	7.24	38.1
32	UFR-5 (White 4)	OLL9	28.25	16.90	53.841	1.08	12.49	11.56	6.72	36.0
33	Green 7	OLL4	29.11	17.29	53.456	0.98	12.40	12.65	6.63	37.8
34	Green 7	OLL8	23.06	13.12	51.206	0.94	13.55	14.41	6.94	37.6
35	AMB+Carrizo	OLL4	25.25	15.26	54.392	0.87	12.65	14.54	6.88	38.1
36	AMB+Carrizo	OLL8	24.15	14.45	53.851	0.88	11.96	13.59	6.44	38.6
37	Green 2	OLL4	28.51	17.23	54.391	0.97	11.61	11.97	6.32	38.2
38	Green 2	OLL8	26.71	16.39	55.227	0.93	11.71	12.59	6.47	38.2
41	639	OLL4	24.57	15.21	55.714	1.07	11.00	10.28	6.13	38.6
42	639	OLL8	23.28	14.06	54.356	0.89	12.81	14.39	6.96	38.0
43	Swingle	OLL4	24.31	14.90	55.162	1.06	12.82	12.09	7.07	38.5
44	Swingle	OLL8	24.90	15.24	55.084	0.99	12.56	12.69	6.92	38.2



**Average ct values (+ s.d.) per rootstock in the Lee Groves Karen's Block 13W after 5 years with no psyllid control; OLL scions all grafted from field budwood under DPI permit.**

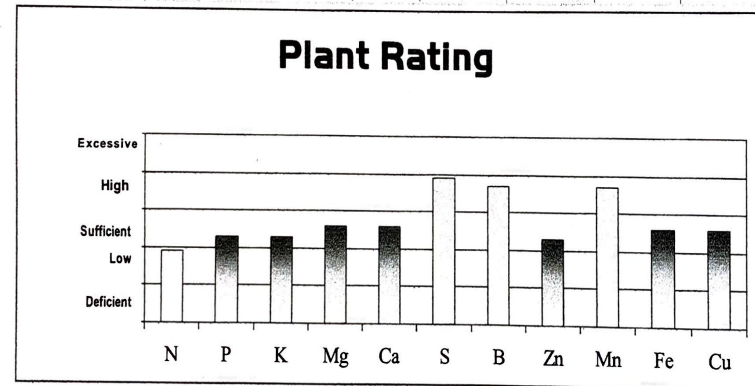
<b>Rootstock</b>	<b>Scion</b>	<b>Average ct value</b>	<b>standard deviation</b>	<b>Trees 32 or above</b>
Swingle	OLL	38.4	3.14	7/8
X639	OLL	38.4	2.23	8/8
Amb+Czo	OLL	28.6	4.42	3/16
UFR-6	OLL	32.9	4.40	8/16
Carrizo	OLL	30.2	4.71	4/16
Green 2	OLL	27.8	3.33	2/16
Green 7	OLL	36.4	4.92	14/16
Orange 13	OLL	31.3	5.65	7/16
Orange 14	OLL	27.5	3.92	1/16
UFR-3	OLL	29.41	4.64	3/16
Orange 16	OLL	31.8	4.55	7/16
UFR-4	OLL	36.5	3.98	14/16
UFR-1	OLL	29.9	4.28	6/16
Orange 2	OLL	27.3	3.46	1/16
UFR-2	OLL	34.4	7.03	11/16
White 1	OLL	30.0	4.73	6/16
White 3	OLL	27.7	3.34	2/16
UFR-5	OLL	27.5	3.44	1/16

Plant Lab Results											
N	P	K	Mg	Ca	S	B	Zn	Mn	Fe	Cu	
2.7 %	0.2 %	1.41 %	0.51 %	3.76 %	0.43 %	118 ppm	27 ppm	45 ppm	95 ppm	10 ppm	
NO <sup>3</sup> -N	Na	Al	Mo	Ni	Cl			Actual Ratio		N:S	N:K
ppm	%	ppm	ppm	ppm	%			6.3	1.9		
								Sufficient Ratio	10.2	1.6	



Sweet Orange on standard Lee Groves soluble dry

Plant Lab Results											
N	P	K	Mg	Ca	S	B	Zn	Mn	Fe	Cu	
2.47 %	0.15 %	1.21 %	0.42 %	4.12 %	0.43 %	121 ppm	38 ppm	113 ppm	92 ppm	10 ppm	
NO <sup>3</sup> -N	Na	Al	Mo	Ni	Cl			Actual Ratio		N:S	N:K
ppm	%	ppm	ppm	ppm	%			5.7	2.0		
								Sufficient Ratio	10.2	1.6	



Karen's OLL trial block on standard + Harrells + FI-Mn

