Breeding Scions and Rootstocks for the HLB World

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Jude Grosser & Fred Gmitter

UF-CREC Citrus Genetic Improvement Team
2014

Scion Breeding

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Can we breed seedless (or low-seeded) sweet orange hybrids tolerant of HLB? Maybe! Above tree is a 3x hybrid containing 8% trifoliate orange, with juice indistinguishable from commercial sweet oranges! Midseason fruit with 37 color score and high brix.

Wide hybridization capturing HLB resistance genes from trifoliate orange

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2012

2013

2014

A Grand Experiment in Natural Selection: HLB-tolerant tetraploid breeding parent Hirado Buntan Pink pummelo x 4x Succari sweet orange; PCR+ for HLB for 4 years, shows blotchy mottle in fall, but no effect on fruit!

Interploid Cross: HLB-tolerant tetraploid breeding parent Hirado Buntan Pink pummelo x 4x Succari sweet orange allotetraploid x Page orange (tangelo): recovered 2x, 3x and 4x hybrids from same cross!

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A Grand Experiment in Natural Selection – New Breeding Parents Being Identified

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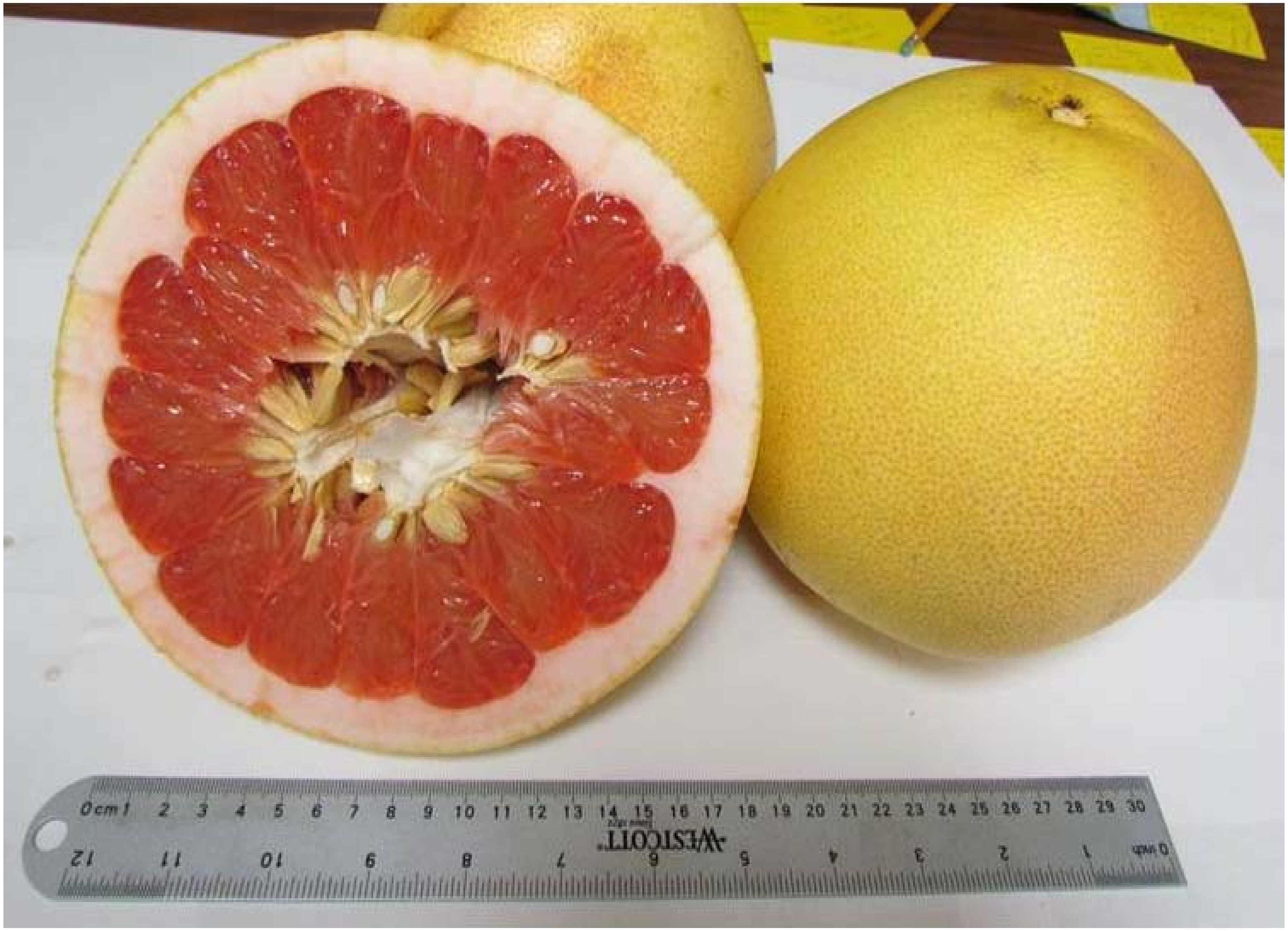

C2-5-12 Red Pummelo – delicious, showing best tolerance to HLB so far!

Breeding for canker resistance: canker epidemic causing a natural screen of CREC germplasm – leading to the identification of superior canker tolerant diploid and tetraploid breeding parents for use in interploid crosses to generate seedless triploids – >100 triploid hybrids recovered from 2012 crosses.

When it comes to HLB, all processing sweet orange clones are not the same!

Differential response of sweet orange clones to HLB

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Topworked OLL#7 onto a severely HLB-impacted Valencia/Swingle tree – Alligator.
OLL #4 looks to be the best!

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Rootstock Breeding

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WHAT IS SOUR ORANGE?

RAPD Markers Identified in Various Citrus Genotypes
For Analysis of Origin (from Nicolosi et al. 2000).

Genotype	Markers	Markers shared with Pumm	Mand	Citron	Sweet	Sour	Extra
sweet or.	71	35	36				
sour or.	84	42	36				6
grapefr.	72	45			27		
lemon	78			45		31	2
Volk	56		6	27		22	1
rough lem	79		32	46			5
Palestine	73			48		21	4
Rangpur	85		32	46			7

How do we exploit what we know about sour orange genetics?

A. Use sour orange as a breeding parent:

- -diploid traditional crosses
- -somatic fusions to produce allotetraploids
- -traditional crosses at the tetraploid level
- -add genenetic resources beyond pummelo/mandarin

B. Resynthesize pummelo/mandarin hybrids

- -diploid traditional crosses with superior parents
- -somatic fusions of superior parents
- -traditional crosses at the tetraploid level
- -add gene resources beyond pummelo/mandarin

Traditional Diploid Breeding:

A. Pummelo x Mandarin Crosses Conducted

-Hirado Buntan pummelo x Shekwasha mandarin
-Hirado Buntan pummelo x Amblycarpa mandarin
-Hirado Buntan pummelo x Cleopatra mandarin

Problem: few polyembryonic hybrids recovered

Fast Track Releases (polyembryonic, vigorous trees, good fruit quality, reduced HLB frequency of infection in limited trials):

-UFR-15 (Hirado Buntan pummelo x Cleopatra)
-UFR-16 (Hirado Buntan pummelo x Shekwasha)

Valencia/HBPxCleopatra 46x20-04-48
3-year old resets in high HLB pressure area

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Ruby Red/HBPxCleopatra 46x20-04-S13
3-year old resets in high HLB pressure area (Vero)

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Traditional Diploid Breeding:

B. Using sour orange-like parents as females:

For example,

Female: 46 x 31-02-S10 (salinity tolerant, monoembryonic)

Pollen Parents: US-812, x639, MG-11, 46x31-02-S15

Several progeny from these crosses performing well in our HLB screening gauntlet

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 polylembryony, ability to control tree size due to polyploidy, and improved disease resistance.

Harrell's

Professional Fertilizer Solutions

13-4-9

Batch #: 1002-0646

FERTILIZER

UF Citrus Research Center

GUARANTEED ANALYSIS

* Total Nitrogen (N)	13.0000%
7.4900% Nitrate Nitrogen	
5.0100% Ammoniacal Nitrogen	
0.5000% Urea Nitrogen	
** Available Phosphate (P205)	4.0000%
*** Soluble Potash (K20)	9.0000%
Calcium (Ca)	4.2800%
Magnesium (Mg)	1.0950%
1.0950% Water Soluble Magnesium (Mg)	
Boron (B)	0.0500%
Copper (Cu)	0.0460%
0.0460% Water Soluble Copper (Cu)	
Iron (Fe)	0.9470%
0.1580% Water Soluble Iron (Fe)	
0.3170% Iron (Chelated)	
Manganese (Mn)	0.1580%
0.1580% Water Soluble Manganese (Mn)	
Molybdenum (Mo)	0.0070%
Zinc (Zn)	0.0460%
0.0460% Water Soluble Zinc (Zn)	

Derived From: Calcium Nitrate, Polymer Coated Ammonium Nitrate, Polymer Coated Copper Sulfate, Polymer Coated EDTA Iron Chelate, Polymer Coated Magnesium Sulfate, Polymer Coated Manganese Sulfate, Polymer Coated Mono-Ammonium Phosphate, Polymer Coated Sodium Molybdate, Polymer Coated Sulfate of Potash, Polymer Coated Sulfate of Potash-Magnesia, Polymer Coated Sulphate of Potash, Polymer Coated Urea, Polymer Coated Zinc Sulfate, Iron Chelate, Iron EDTA, Iron Humate, Iron Oxide, Iron Sulfate, Iron Sucrate, Manganese Sulfate, Sodium and Calcium Borate

* 8.58% slow release NITROGEN derived from Polymer Coated Ammonium Nitrate, Polymer Coated Mono-Ammonium Phosphate, Polymer Coated Urea

** 3.984% slow release PHOSPHATE derived from Polymer Coated Mono-Ammonium Phosphate

*** 8.098% slow release POTASH derived from Polymer Coated Sulfate of Potash, Polymer Coated Sulfate of Potash-Magnesia, Polymer Coated Sulphate of Potash

Warning: --- Some crops may be injured by Application of Boron.
--- This fertilizer is to be used only on soils which respond to Molybdenum. Crops high in Molybdenum are toxic to ruminants.

MANUFACTURED BY HARRELL'S LLC (F352) 720 KRAFT ROAD, LAKELAND, FL 33801 - (863) 687-2774 - (800) 282-8007
DISTRIBUTED BY HARRELL'S LLC. (F352) 720 KRAFT ROAD, LAKELAND, FL 33801 - (863) 687-2774 - (800) 282-8007

Net Weight 50 LBS

St. Helena Project – c/o Mr. Orie Lee

-mimic principles of OHS to minimize tree stress

-two applications per year of Harrell's/UF mix slow-release fertilizer (January, July); thanks to Arnold Schumann for assistance in developing the formula

-daily irrigation unless adequate rainfall

-evaluation of >75 rootstocks, 19.9 acres

-bad neighbor issue – unsprayed grove on one side, organic grove on other side

St. Helena Project c/o Mr. Orie Lee

8/9/12 photo, trees 4.5 years old

Bad neighbor effect - Rootstock effects on HLB emerging!

Candidate for ACPS

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

St. Helena Project	4.5 year old trees	# HLB infected	Total # infected	% trees
Rootstock (2X)	# of trees	as of Aug 2011	as of Oct 2012	infected
68-1G-26-F2-P12	10	0	2	20%
68-1G-26-F4-P2	12	1	2	17%
68-1G-26-F4-P6	13	0	0	0%
68-1G-26-F6-P20	17	0	3	18%
69-LTX-AM-F14 P37	4	0	2	50%
Aqua 1803	19	3	5	26%
CLEO	16	0	4	25%
FG 1702	2	0	0	0%
FG 1707	3	0	1	33%
FG 1709	4	0	0	0%
FG 1731	5	0	1	20%
FG 1733	5	1	1	20%
FG 1793	5	1	2	40%
Kuharske	63	24	58	92%
MG11	40	1	5	13%
Orange 1804	18	3	7	39%
Pink 1802	18	3	4	22%
Rough Lemon	18	3	10	56%
Swingle	20	6	14	70%
Volk	20	7	17	85%
White 1801	11	1	5	45%
White 1805	19	1	4	21%
Yellow 1800	11	1	2	18%
Total	305	58	152	50%

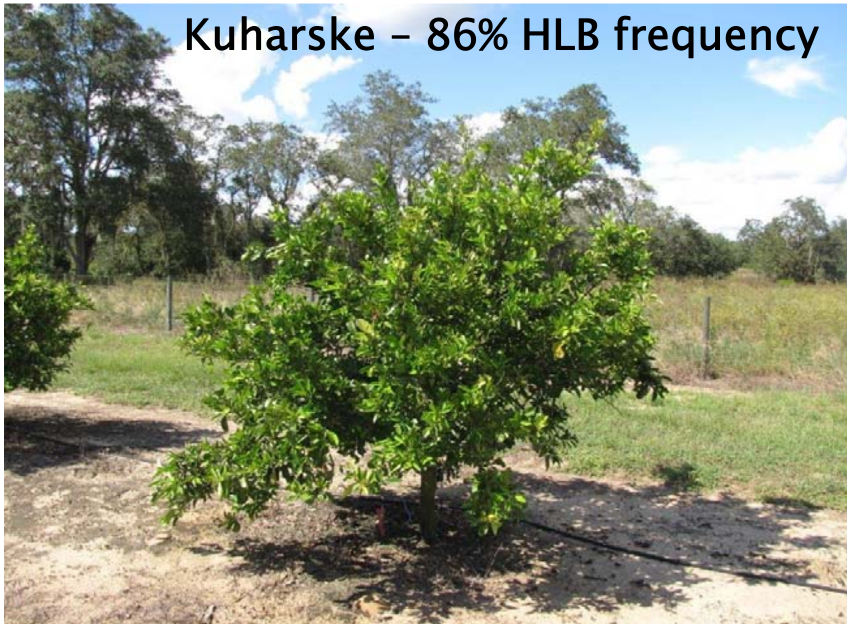
St. Helena Project Rootstock (4X)	Trees 4.5 years old # of trees	# HLB infected on or before Aug 2012	Total # infected as of Oct 2012	% trees infected
AMB+HBJL1	12	1	3	25%
Blue 1	69	7	13	19%
Blue 2	24	1	4	17%
Blue 3	44	1	10	23%
Blue 4	37	4	10	27%
Blue 9	30	1	7	23%
Chang+50-7	60	11	21	35%
Chang+Bent	34	1	7	21%
Cleo+CZO	160	22	59	37%
Green 2	**16**	**2**	**2**	**13%**
Green 7	69	9	18	26%
Milam+Kinkoji	8	0	0	0%
Orange 1	24	3	6	25%
Orange 10	20	2	4	20%
Orange 12	33	2	4	12%
Orange 13	50	5	16	32%
Orange 14	62	2	15	24%
Orange 15	**43**	**1**	**3**	**7%**
Orange 16	**27**	**3**	**6**	**22%**
Orange 18	45	5	13	29%
Orange 19	**128**	**8**	**19**	**15%**
Orange 2	74	3	9	12%
Orange 21	46	1	8	17%
Orange 3	60	5	11	18%
Orange 4	70	8	22	31%
Orange 8	46	1	9	20%
Purple 2	20	2	6	30%
Purple 3	5	0	1	20%
Purple 4	64	6	15	23%
SO+50-7	45	4	6	13%
SO+CZO	265	17	53	20%
WGFT+50-7	86	15	32	37%
White 1	24	0	8	33%
White 4	72	8	15	21%
Total	1735	161	436	25%

HLB tolerance from new rootstocks?

Vernia/Orange #4

Vernia/Orange #19

New photos of trees PCR+ since last September – St. Helena



HLB-infected trees in the St. Helena Project
-differences in infection frequency & disease severity

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Rootstock Data from 5-year old trees in the St. Helena trial - Dundee.

White = diploid; Orange = somatic hybrid; Blue = tetrazyg

NS = not significant fruit na = data not available * = control commercial rootstock Commercial control rootstocks in red

Scion	Rootstock	Lbs Solids/Box		Yield Boxes/Tree			Cumulative Yield (Boxes)	Tress With Symptoms as of March 2013	Number of Trees in Trial	Percentage with HLB as of March, 2013 (5 years)
		2012	2013	2011 (35 mo.)	2012 (47 mo.)	2013 (59 mo.)				
VALQUARIUS	**UFR-6** CH+50-7	5.64	5.43	0.5	0.78	1.94	3.22	25	60	42%
VERNIA	**UFR-6** CH+50-7	5.67	6.01	0.4	0.63	1.41	2.44			
VALQUARIUS	**UFR-1** ORANGE 3	5.5	4.87	NS	0.72	2.23	2.95	15	60	25%
VERNIA	**UFR-1** ORANGE 3	5.61	6.28	0.31	0.67	1.33	2.31			
VERNIA	**UFR-2** ORANGE 4	5.47	5.93	0.35	0.25	1.38	1.98	22	73	30%
VALQUARIUS	**UFR-2** ORANGE 4	4.57	5.37	NS	0.75	1.73	2.48			
VALQUARIUS	**UFR-3** ORANGE 15	4.84	5.05	NS	0.81	1.97	2.78	6	43	14%
VERNIA	**UFR-3** ORANGE 15	5.46	5.82	0.37	0.38	1.82	2.57			
VERNIA	**UFR-4** ORANGE 19	5.79	6.07	0.54	0.71	1.73	2.98	30	129	23%
VALQUARIUS	**UFR-4** ORANGE 19	4.65	5.07	NS	0.65	1.59	2.64			
VALQUARIUS	**UFR-5** WHITE 4	5.76	5.72	0.33	0.56	1.80	2.69	20	72	28%
VERNIA	**UFR-5** WHITE 4	5.89	5.34	0.42	0.25	1.93	2.60			
VALQUARIUS	**UFR-13** FG 1731	5.83	6.81	NS	0.68	2.20	2.88	1	5	20%
VALQUARIUS	**UFR-14** FG 1733	5.12	5.63	NS	0.67	2.77	3.44			
VERNIA	SWINGLE*	5.11	5.79	0.33	0.85	1.08	2.26	14	20	70%
VALQUARIUS	SWINGLE*	NS	5.61	NS	NS	1.50	1.50			
VERNIA	CLEO*	4.79	5.51	NS	0.50	0.83	1.33	6	16	38%
VALQUARIUS	CLEO*	NS	5.21	NS	NS	1.7	1.7			
VERNIA	R. LEMON*	3.67	na	NS	0.78	na	0.78	12	18	67%
VALQUARIUS	VOLK*	NS	4.12	NS	NS	2.58	2.58	18	20	90%
VERNIA	VOLK*	3.6	4.73	0.4	1.13	0.83	2.36			
VALQUARIUS	KUHARSKE*	NS	5.75	NS	NS	2.2	2.2	56	65	86%
VERNIA	KUHARSKE*	4.34	5.83	0.15	0.75	1.08	1.98			

LB8-9, Sugar Belle™: a New Diploid Interspecific Mandarin Hybrid

CREC/McTeer Rootstock Trial with SugarBelle – Haines City
-almost all HLB+ summer of 2011, treated with Harrell's UF mix and biochar in January, 3-year old trees.

SugarBelle/Orange #4
2 trees on left

SugarBelle/Orange #19
5 trees on left

New photos of trees HLB+ since last September – McTeer

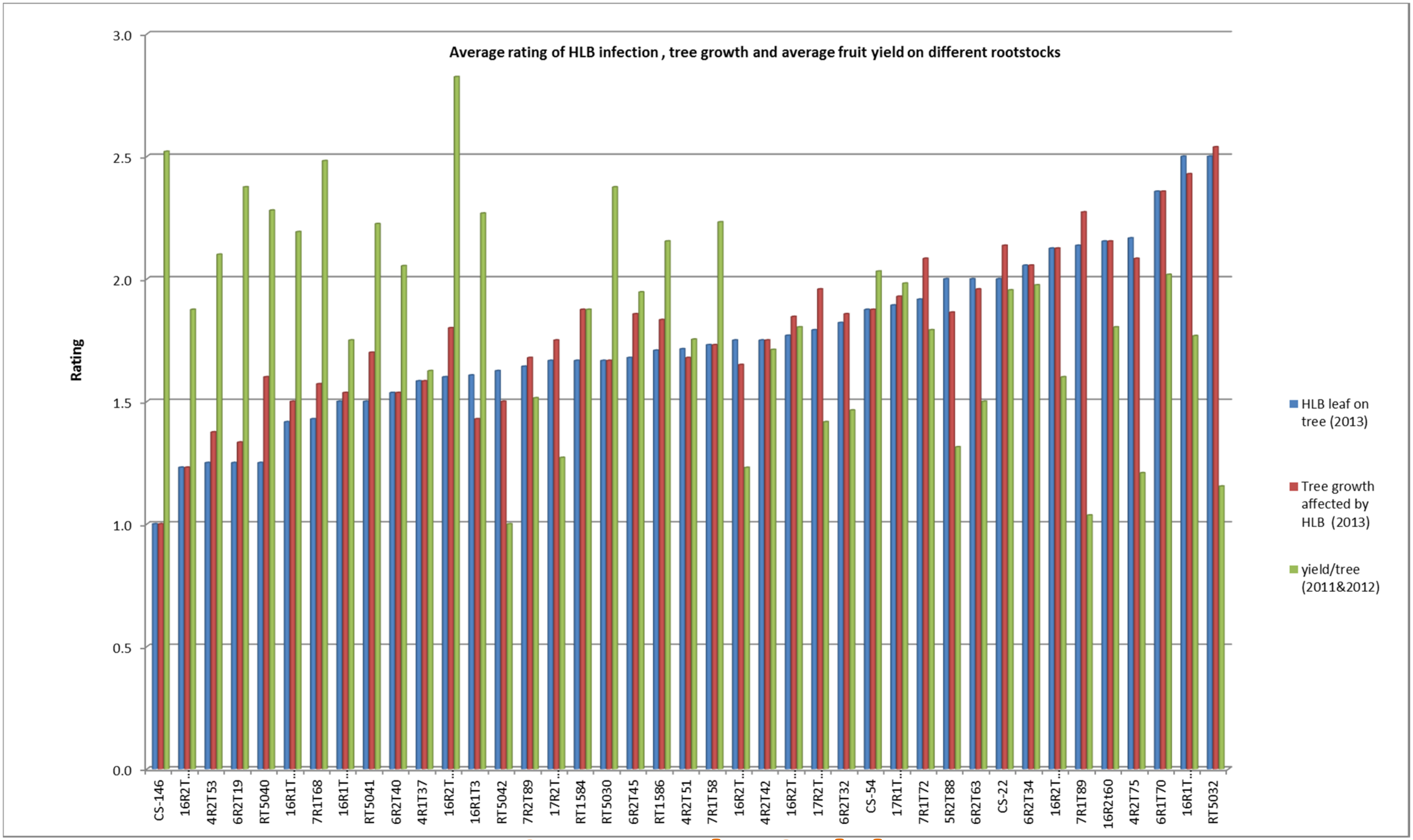
McTeer SugarBelle Rootstock Trial – Haines City, 4 years old

Rootstock	Average tree score (0-dead, 5-healthy)	% needing replacement
White 4 (UFR-5)	3.92	0
Orange 19 (UFR-4)	3.86	0
Orange 1	3.81	4.8
Trifoliate 50-7	3.50	12.5
Orange 4 (UFR-3)	3.53	5.0
SO+50-7	3.33	10.5
Changsha+50-7 (UFR-1)	3.18	11.8
C-35	3.13	10.2
Swingle	3.13	13.6
w/biochar	3.42	8.3
no biochar	2.8	20
WGFT+50-7	3.26	20
SO+Carrizo	3.0	25
Purple 4	2.86	28.6
Flying Dragon	2.77	32
Rich Trifoliate	2.6	29.6
Purple 3	1.5	66.7

Grapefruit Rootstock Trial

Grapefruit Rootstock Trial

Grapefruit Rootstock Trial



HLB Severity and Yield

UFR-7,8,9,10,11&12 -best citranges

3-year OLL#8 resets at Alligator – symptoms last winter disappeared

4-year old Vernia trees at St. Helena

Tetrazyg Green#2 – another new candidate for the CRDF rootstock matrix

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Susceptible rootstock Orange #1

Tolerant? Rootstock Green #7

Screening complex rootstock hybrids by growing Valencia scion from HLB-infected budwood. Left 3 trees: rootstock Orange # 1 (Nova+HBP x Cleo+trifoliate orange); Right 3 trees: rootstock Green #7 (Nova+HBPummelo x Sour orange+Carrizo)

Vernia/Green #7 at St. Helena, HLB+ for >3 years
-recovering from severe symptoms
-shows potential for rootstock breeding

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Scion/Rootstock Interaction-Synergy against HLB
Valencia BHG2-68/Orange #19 (UFR-4)
-inoculated twice, 2.5 years @ Picos Farm!

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Scion/Rootstock interaction – Synergy against HLB
– 3 trees Valencia N7-3/White grapefruit + 50-7
– only orange trees in block free of HLB symptoms

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Research article: **Rootstock-regulated gene expression patterns associated with fire blight resistance in apple**

By Philip J Jensen[1], **Noemi Halbrendt**[1,2], **Gennaro Fazio**[3], **Izabela Makalowska**[4], **Naomi Altman**[5], **Craig Praul**[6], **Siela N Maximova**[7], **Henry K Ngugi**[1,2], **Robert M Crassweller**[7], **James W Travis**[1,2] and **Timothy W McNellis**[1*]

BMC Genomics 2012, 13:9

In apple, rootstock genetics effect scion gene expression – in this case affecting fire blight resistance (also caused by a gram-negative bacterium). Thus, something being produced by the rootstock is being translocated to the scion that affects disease resistance – why wouldn't this happen in citrus as well, especially with complex tetraploid rootstocks?

Rootstocks differentially translocate nutrients, phytohormones (plant growth regulators), micro-RNAs, small proteins (pathogenesis related?), and other metabolites to the scion. This could have both direct and indirect, quantitative and quantitative affects on scion gene expression, and possibly Lilberibacter pathogenesis in citrus – especially with unique complex allotetraploid rootstocks.

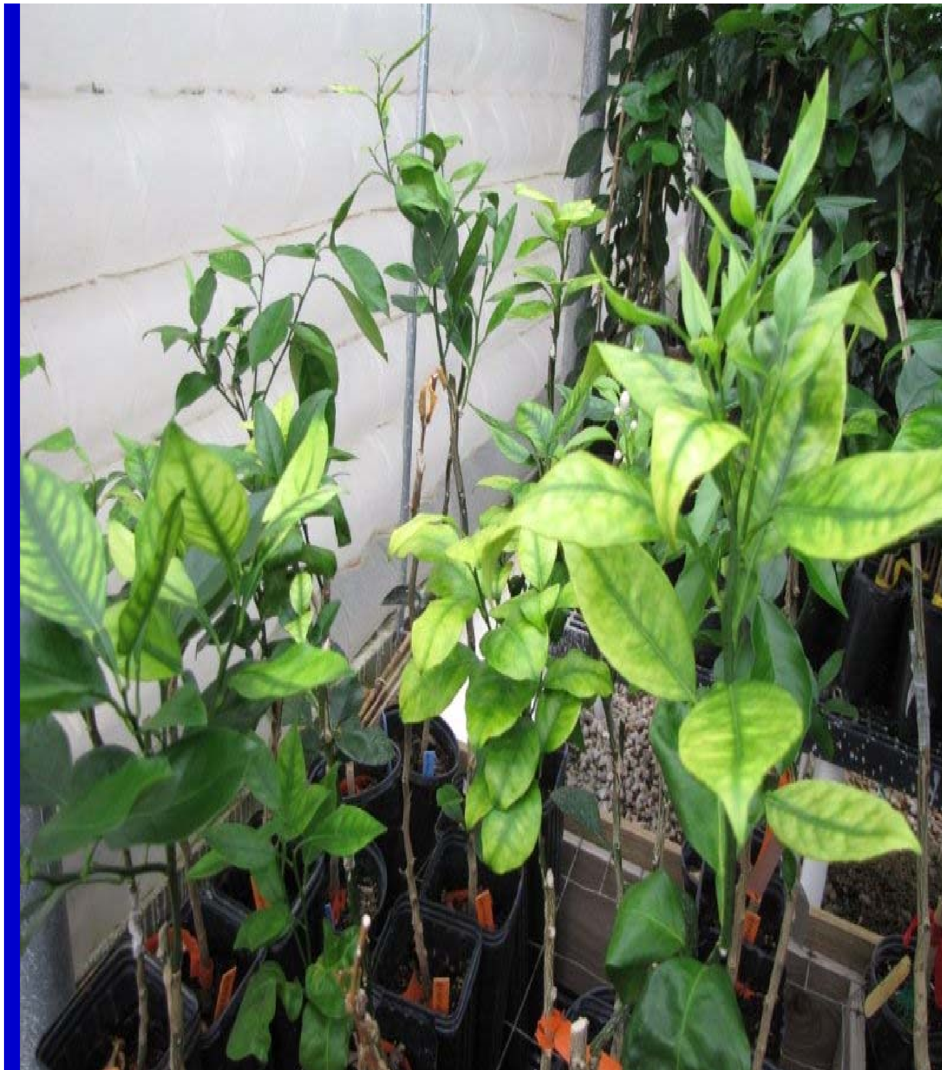
Plant species have thrived for thousands of years in the presence of evolving, hostile pathogens – HOW? They have created their own genetic diversity, and through the process of natural selection, tolerant or resistant genotypes overcome the threat and allow the species to evolve.

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

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 (now via rooted cuttings); 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).
8. Superior hybrids and superior crosses being identified!



Initially Susceptible (2 dead)

Initially Tolerant

HLB screening of complex new rootstock candidates by grafting 'hot' PCR+ HLB infected Valencia budsticks into each hybrid (after propagation of seed trees). Valencia trees growing out from the infected tissue with no symptoms are passed through a 'hot' psyllid house, then planted in the field at a high-HLB pressure location.

Gauntlet Survivor at Picos Farm
-Valencia on A+Volk x Orange #19-11-31

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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 – these will still require efficient psyllid control and optimized production systems.

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.

Integrated Tree Management (ITM)

1. Best scion genetics (clone selection)
2. Best rootstock genetics
3. Best production system for your situation

    a. soil amendments; **biochar**, sludge, compost, etc.

    b. optimum psyllid control/foliar nutrition

    b. first 2-3 years: use either CRF + TigerSul (Schumann mix Fe, Zn, Mn) or fertigation supplemented with CRF+TigerSul (constant nutrition and efficient nutrient uptake)

    d. Year 3-4: hybrid nutrition program (traditional dry + CRF + TigerSul, 3 applications?) or fertigation supplemented with CRF+TigerSul

To HALL OF FAME CITRUS GROWER-RESEARCHERS
And Outstanding Industry Collaborators:
Mr. Orie Lee and the late Mr. Harold McTeer

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Thanks!

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