Progress on Developing Scions and Rootstocks for an HLB-Endemic Florida

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Citrus Research and Education Center

Jude Grosser, Fred Gmitter, and Bill Castle

UF-CREC Citrus Genetic Improvement Team
2016

Scion Breeding

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UF-CREC Citrus Genetic Improvement Team
2014

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!

Sugar Belle® is HLB tolerant! Trees near Fort Pierce, HLB+ for more than six years – normal production!

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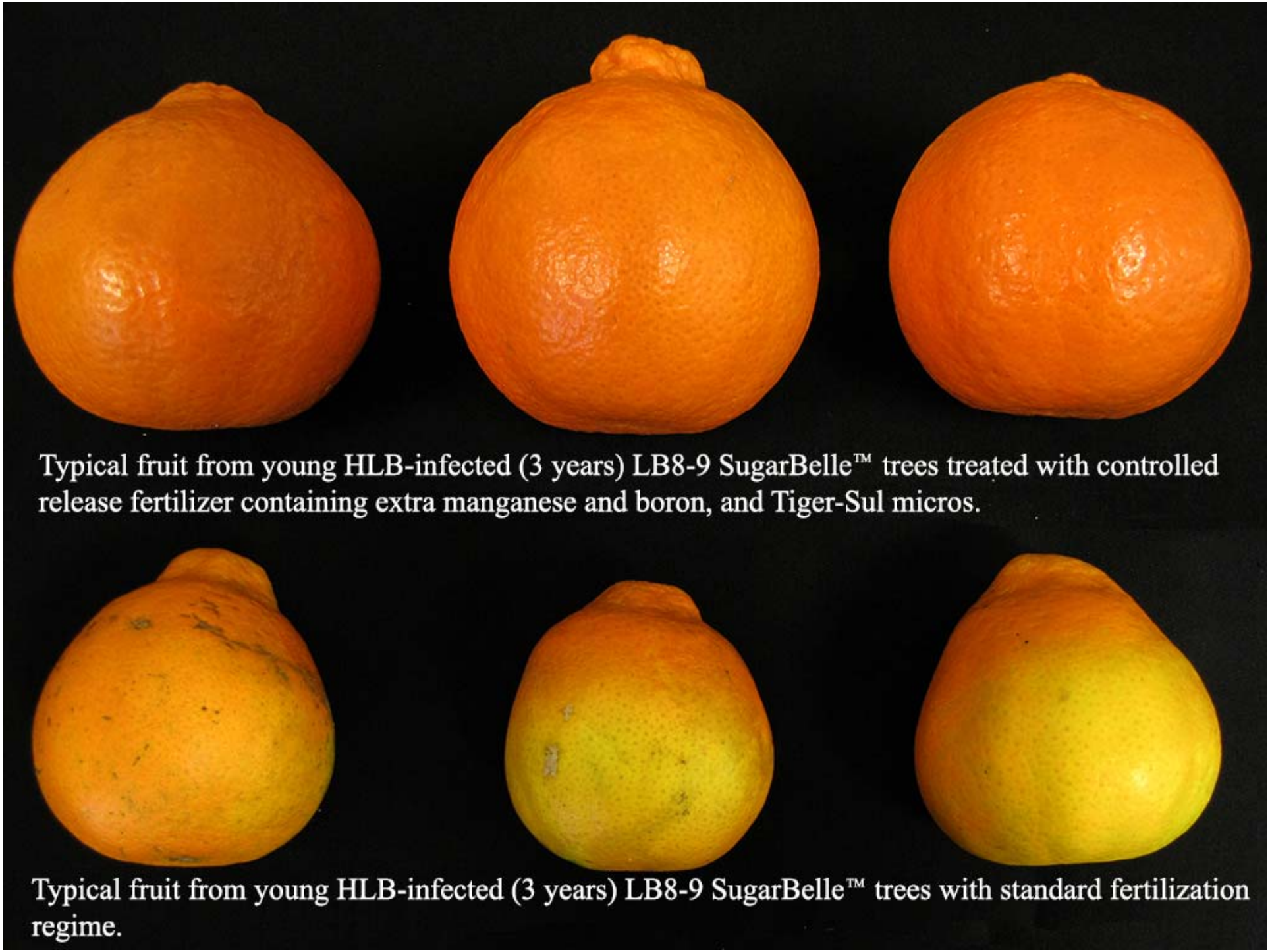

Typical fruit from young HLB-infected (3 years) LB8-9 SugarBelle™ trees treated with controlled release fertilizer containing extra manganese and boron, and Tiger-Sul micros.

Typical fruit from young HLB-infected (3 years) LB8-9 SugarBelle™ trees with standard fertilization regime.

HLB-tolerant SugarBelle can transmit it's HLB tolerance to seedless triploid progeny: above is a triploid hybrid from a cross of SugarBelle with the Nova+Osceola somatic hybrid.

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Low seeded cybrid Dancy on UFR-5. 2 trees on left treated with CRF + extra Mn and boron – good fruit. Two trees on right just standard CREC program – no edible fruit. NUTRITION!

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HLB-tolerant Mandarin Breeding Parent Cybrid 304: 2x and 4x versions. Large easy to peel fruit, sweet orange-like juice, amenable to processing; being used in interploid crosses.

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HLB-tolerant Somatic Hybrid Tetraploid Mandarin Breeding Parent W.Murcott+UF-03B. Large easy to peel fruit, good color and flavor; being used in interploid crosses.

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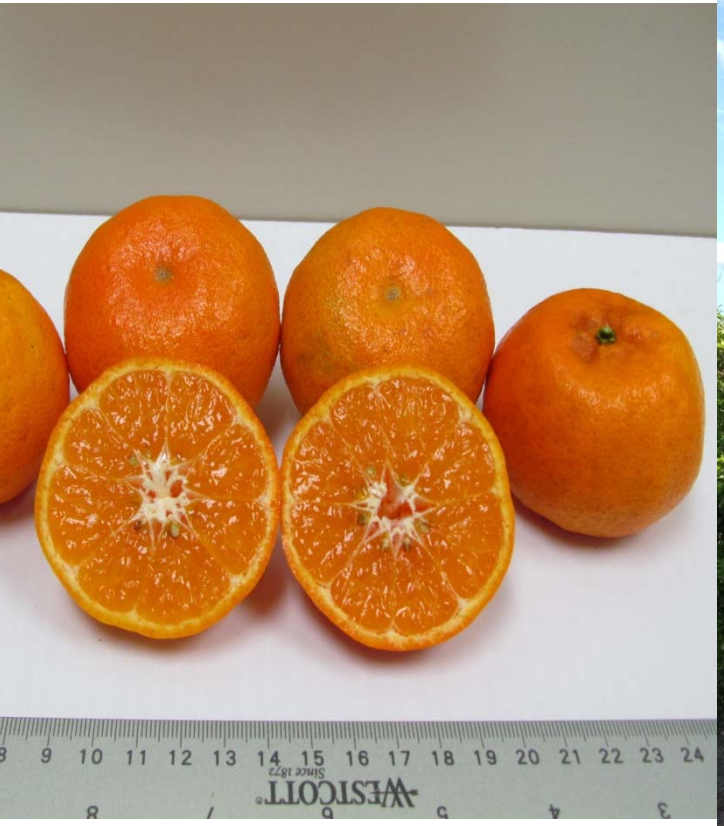

6-27 ‘Bingo’ – easy-peel, seedless mandarin approved

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

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Differential response of sweet orange clones to HLB

UF-CREC Citrus Genetic Improvement Team
2013

ORANGES

‘OLL-8’

ttributes: Excellent color and quality, extends
st window of ‘Valencia’ quality juice
ces round oranges with internal and external
similar to ‘Rhode Red Valencia’
s on the tree exceptionally well, and maintains
y into the summer
appear to yield better than standard ‘Valencia’
juice content and good pounds solids
easier than a standard ‘Valencia’
its added color, could also be a valuable addition
Florida fresh market portfolio
precocious bearing clone among the OLL
clones

‘OLL-8’

For more information on ‘OLL-8’ or ‘OLL-4’, please contact Florida Foundation Seed Producers, Inc. www.ffsp.net

‘OLL-4’

‘OLL-4’

- Key attributes: excellent color and quality, extends harvest window of ‘Valencia’ quality juice; also believed to be higher yielding than ‘Valencia’
- Produces fruit with excellent internal and external quality with exceptional juice color scores, juice content and soluble solids
- Holds on the tree exceptionally well
- Maintains quality into the summer; however, it matured earlier, and with better ratios than ‘Valencia’ in 2014
- Has been the highest yielding tree among the OLL somaclones

Juice Quality Data from OLL-Series Somaclones

Data 3-2-2012

iety	Lbs. Juice Per Box	Acid	Total Brix	Ratio	Lbs. Solids Per Box	Color
ontrol	54.41	1.00	12.03	12.03	6.55	38.60
	53.23	0.84	12.10	14.40	6.44	40.50
	54.99	0.84	12.34	14.69	6.79	39.80
	57.58	0.79	12.81	16.22	7.38	40.20
	56.30	0.94	12.97	13.80	7.30	39.90
	49.48	0.99	14.56	14.71	7.20	39.50
	51.55	0.77	12.77	16.58	6.58	41.00
	54.52	0.98	12.92	13.18	7.04	40.30
	55.52	0.92	12.60	13.70	7.00	40.70
	57.51	0.94	13.20	14.04	7.59	41.20
ontrol/SW	48.46	0.79	12.91	16.34	6.26	39.10
	58.20	0.77	12.38	16.08	7.20	40.30
	55.55	0.82	12.42	15.15	6.90	40.60
	53.92	0.93	13.13	14.12	7.08	40.10
	57.21	0.81	12.12	14.96	6.93	39.90
	54.02	0.84	12.32	14.67	6.65	40.90
	55.10	0.90	12.90	14.33	7.11	41.50
	58.19	0.96	13.85	13.39	7.48	40.80

t orange OLL #7 topworked onto severely symptomatic

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Scion/Rootstock interaction – Synergy against HLB

ear old OLL-8 trees on rough lemon (Lee Alligator Grove, St.

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Juice Quality Data from Two New Early-Maturing Valencia Somaclones. Data from fruit harvested first week of December, 2014, 6-year old trees on rough lemon rootstock, Alligator Grove, St. Cloud, Florida.

Variety	Lbs. Juice Per Box	Acid	Total Brix	Ratio	Fruit Ct	Lbs. Solids Per Box	Lbs. Solids Per Box	juice color
Vernia	53.906	0.87	11.04	12.69	78	5.9512	5.95	35.3
B7-70	50.913	0.71	11.30	15.92	61	5.7532	5.75	36
Hamlin	52.914	0.94	11.17	11.88	64	5.9105	5.91	34.5
Valuarius	50.728	0.84	9.87	11.75	55	5.0069	5.01	35.7
SF14W-65	50.299	0.67	11.06	16.51	63	5.5631	5.56	36
Valencia	53.443	0.98	9.53	9.72	58	5.0931	5.09	35.4

: Early-maturing Valencia somaclone B7-70; photo Dec. 1, 2014. EV-1 and EV-2

Breeding Rootstocks for the HLB World

Jude Grosser, Fred Gmitter & Bill Castle

UF-CREC Citrus Genetic Improvement Team

2014

Differential Response to HLB from rootstock candidates not pre-selected for HLB tolerance

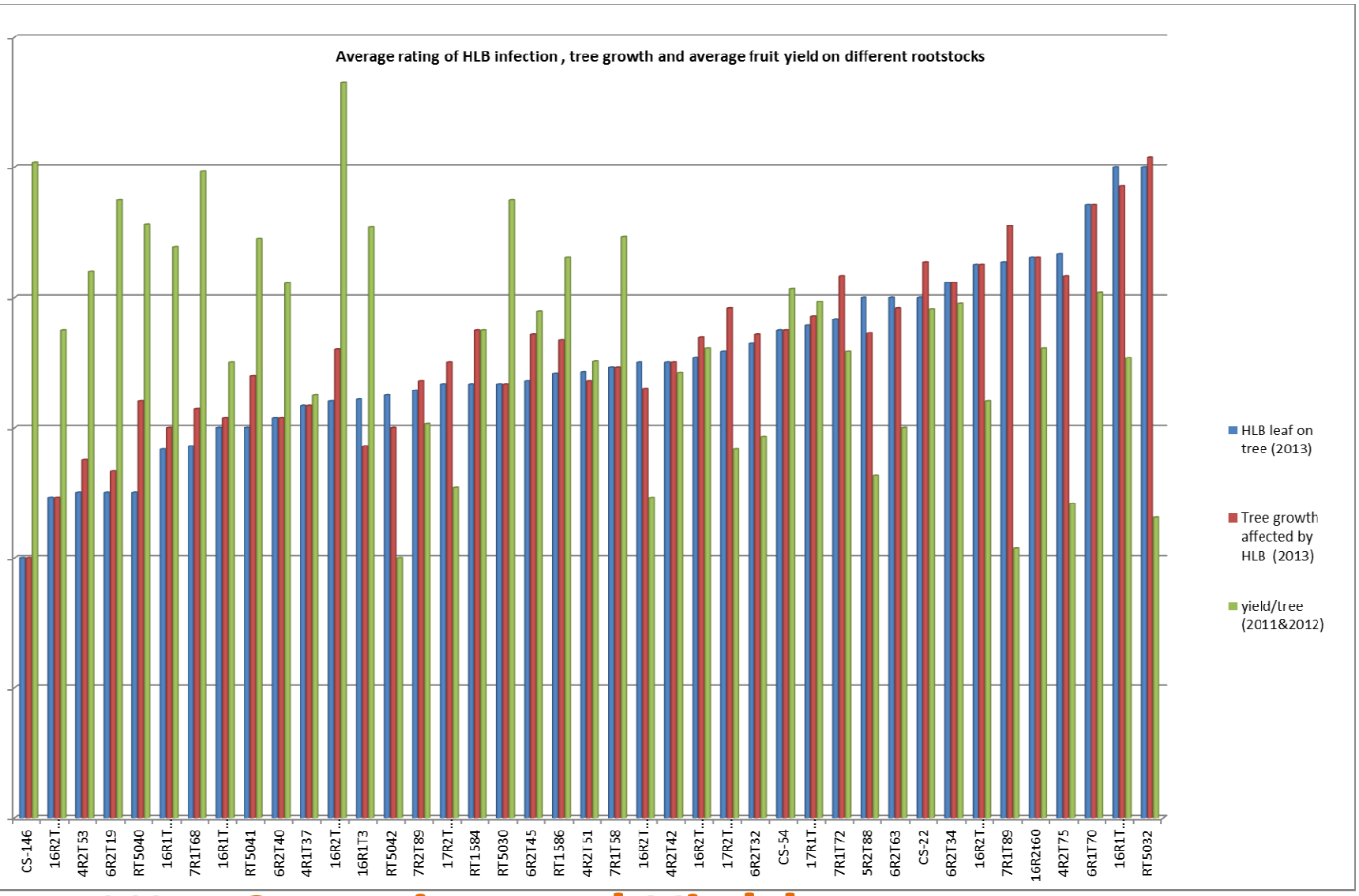
Citranges

Jude Grosser, Fred Gmitter, and Bill Castle

UF-CREC Citrus Genetic Improvement Team

2015

Premier Grapefruit Rootstock Trial – Fort Pierce



HLB Severity and Yield

2,7,8,9,10,11&13 best citranges

Differential Response to HLB from rootstock candidates not pre-selected for HLB tolerance

Sour Orange-like genetics
(half pummelo/half mandarin)

*** Potential for wide soil adaptation and tolerance to blight!**

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UF-CREC Citrus Genetic Improvement Team

2015

2013

2014

Valencia/HBPxCleopatra 46x20-04-48

4.5 year old resets in high HLB/blight pressure area

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elena Trial 2012 4 years

2014 6.5 years

t c/o Mr. Orie Lee – Dundee Florida
nore than 80 rootstocks under evaluation.
eason Vernia and Valquarius scions
grown with 100% CRF, evolving formulas

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

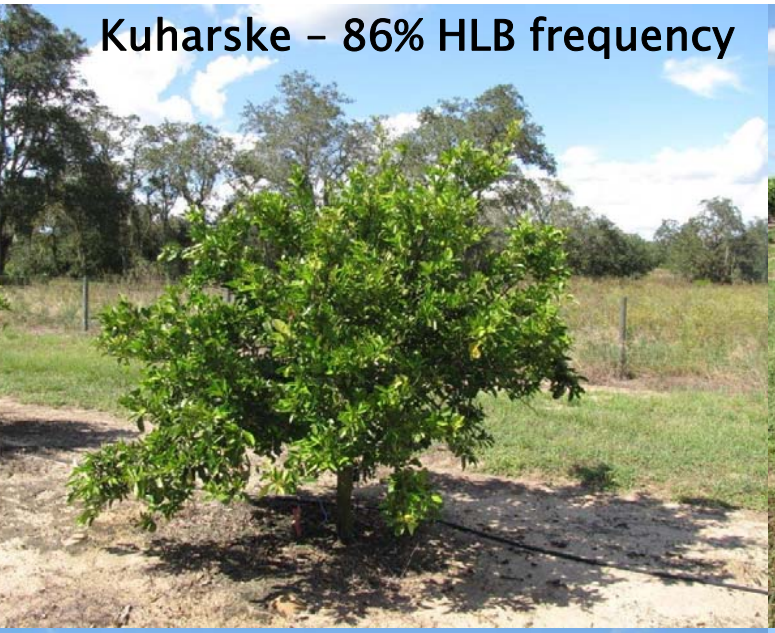
-Use of allotetraploid somatic hybrid breeding parents allows the mixing of genes from 3-4 diploid rootstocks at once.

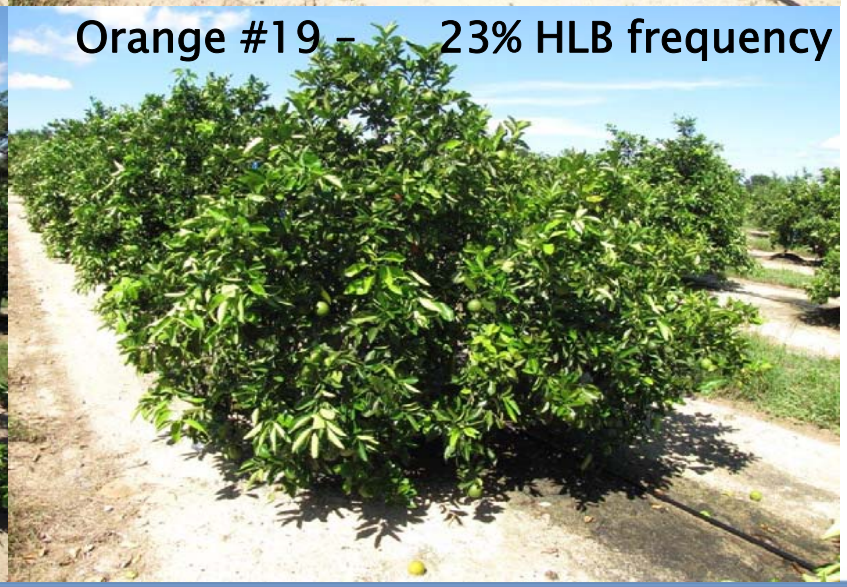
- Progeny can be screened at the seed/seedling level for wide soil adaptability and Phytophthora resistance.

- Products can have direct rootstock potential including adequate polylembryony, ability to control tree size due to polyploidy, and improved disease resistance.

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

Candidate
for ACPS





HLB-infected trees in the St. Helena Project

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elena Project, Dundee (C/) Orie Lee– Projected Cummulative PS/Acre – 2011-2015
5 combinations. Trees 7-years old in April, 2015; now 95% infected with HLB.

Rootstock	Tree Width	OptimalTrees/ acre	Boxes/ acre 2014	Boxes/ acre 2015	PS / Acre 2014	PS / Acre 2015	Cumulative PS / Acre	% change in yield 2014-2015
UFR-13	8.3	264.0	528.0	628.3	3373.9	3795.1	12170.8	19.0
Blue1	7.6	285.6	462.7	517.0	2461.8	2946.9	10311.2	11.7
Cleo+CZO	8.4	258.1	467.2	709.9	2280.0	4223.7	10107.9	51.9
Chang+Bent	6.9	316.8	456.2	377.0	3015.4	2062.1	10072.2	-17.4
Org14	7.8	281.0	373.8	539.6	1767.9	3264.5	9804.3	44.4
Cleo+CZO	7.8	281.0	368.2	359.7	2260.5	2305.8	9343.6	-2.3
UFR-1	8.9	243.7	441.1	750.6	2064.3	3662.8	9338.6	70.2
Org13	8.4	260.1	390.1	455.1	2305.4	2676.0	9296.4	16.7
Aqua1803	10.3	211.2	329.5	528.0	2032.8	3231.4	9086.2	60.3
Aqua1803	9.1	238.7	496.5	386.7	2675.9	2026.1	8943.2	-22.1
UFR-14	10.0	217.8	326.7	435.6	1793.6	2940.3	8877.6	33.3
White1805	11.0	198.0	469.3	445.5	2266.5	2619.5	8854.0	-5.1
SO+50-7	5.8	378.8	424.2	473.5	2511.5	2528.4	8850.8	11.6
AMB+HBJL1	7.3	300.4	225.3	375.5	1198.7	1971.5	8637.7	66.7
Purple4	7.0	311.1	448.0	348.5	2822.7	2317.4	8562.7	-22.2
Blue2	7.4	292.8	348.5	421.7	2188.5	2715.7	8470.6	21.0
Amb+HBJL-2B	7.9	274.4	332.0	354.0	2058.5	2297.3	8357.5	6.6
WGFT+50-7	8.2	266.0	348.5	415.0	2272.1	2444.3	8231.5	19.1
FG1709	7.9	276.7	368.1	462.2	2248.9	2689.8	8189.9	25.6
Blue9	8.3	264.0	264.0	594.0	1380.7	3326.4	8171.5	125.0
UFR-5	8.5	256.0	350.7	384.0	1971.0	2185.0	8104.1	9.5
UFR-6	8.4	258.1	291.7	338.2	1636.4	1829.4	8048.2	15.9
Blue4	7.5	292.0	274.5	341.7	1669.0	1875.7	8016.8	24.5
Org2	7.2	303.0	303.0	369.7	1875.7	2170.1	8013.2	22.0

ena Project – Dundee, FL (C/O Orie Lee) – Comparison of UFR-Fast Track released ocks versus Commercial Control Rootstocks after 7 years.

Rootstock	Tree Width	OptimalTrees/ acre	Boxes/ acre 2014	Boxes/ acre 2015	PS / Acre 2014	PS / Acre 2015	Cumulative PS / Acre	% change in yield 2014-2015
UFR-13	8.3	264.0	528.0	628.3	3373.9	3795.1	12170.8	19.0
UFR-1	8.9	243.7	441.1	750.6	2064.3	3662.8	9338.6	70.2
UFR-14	10.0	217.8	326.7	435.6	1793.6	2940.3	8877.6	33.3
UFR-5	8.5	256.0	350.7	384.0	1971.0	2185.0	8104.1	9.5
UFR-6	8.4	258.1	291.7	338.2	1636.4	1829.4	8048.2	15.9
UFR-2	9.7	225.3	338.0	500.2	1973.7	2881.1	7720.2	48.0
UFR-4	9.8	222.0	290.8	319.6	1986.0	1796.3	7719.5	9.9
UFR-6	8.0	274.0	274.0	298.7	1934.4	1717.3	7573.8	9.0
UFR-5	9.4	230.8	302.3	403.9	1865.3	2366.7	7521.2	33.6
UFR-3	9.3	235.5	367.3	485.0	1961.5	2740.5	7503.7	32.1
UFR-1	9.4	232.7	283.9	388.6	1876.5	2222.8	7322.1	36.9
UFR-3	9.6	227.8	214.1	398.6	1393.8	2443.3	7182.4	86.2
Kuharske	11.4	191.6	216.5	478.9	1175.4	3237.3	6835.9	121.2
Volk	11.6	187.4	374.9	539.8	1237.1	2661.3	5890.7	44.0
Swingle	9.5	229.3	201.8	458.5	1246.8	2714.5	5890.6	127.3
Rough Lemon	10.2	213.8	239.4	395.5	1142.2	1965.7	5613.5	65.2
UFR-2	8.8	247.1	215.0	232.3	1378.3	1226.6	5438.6	8.0
UFR-4	8.9	243.7	212.0	243.7	1257.2	1357.4	5315.6	14.9
Cleo	10.3	212.5	159.4	452.6	924.3	2507.4	5313.7	184.0
Swingle	9.8	223.4	167.5	279.2	854.4	1549.7	5148.0	66.7
Volk	12.3	177.8	200.9	467.6	721.3	2305.3	4703.9	132.7
KCZ	9.8	223.4	111.7	252.4	577.4	1322.7	4178.5	126.0

The New Gauntlet in the HLB world

Crosses of superior parents made at diploid and tetraploid levels
Seed harvested from crosses planted in bins of calcareous soil (pH=8), inoculated with *P. nicotianae* and *P. palmivora* (JH Graham)
Selection of robust seedlings based on growth rate, health and color (most don't make it!)
Transfer to 4x4 pots in commercial potting soil
Top of new tree goes for seed source tree production; remaining liner to the HLB screen
Hybrid liner is grafted with HLB-infected budstick of Valencia sweet orange; remaining rootstock top removed, forced flushing from HLB-infected sweet orange budstick
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.

nitially Susceptible (including dead trees) – The Duds

B screening of complex new rootstock candidates by grafting 'hot' PCR+ HLB-infected encia budsticks into each hybrid (after propagation of seed trees). Valencia trees wing out from the infected tissue with little or no symptoms after the 2nd flush, are

od First Flush

Good Second Flush

didates on right are passed through the 'hot psyllid' house until feeding

6 Field Planting will include trees on left; featuring 3 superior crosses:
5-12 pummelo x papeda; A+HBP x White 1 and A+HBP x sour orange+rangpur.

Gauntlet Survivor at Picos Farm

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Gauntlet trees showing promise – 2016

A+HBJL1—OP-09-23
Green 6 x Orange 14-09-21
Green 6 x Orange 14-09-6
Green 6 x Orange 14-09-32
Green 6 x Orange 14-09-31
N+HBP x 6058x2071-08-2-15
A+HBJL-2B x Orange 14-09-7
A+HBJL-2B x Orange 19-09-16
A+HBJL-2B x Orange 19-09-1
A+HBJL-2B x Orange 19-09-11
A+HBJL-2B x Orange 19-09-9
A+HBJL-2B x Orange 19-09-5
A+HBP x Green 7-12-40
A+HBJL1-OP-09-36
Pink-Black-09-1
Orange 5xH+RL-11-14
Blue 2 x Orange 14-09-2

Gauntlet trees showing promise – 2016

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
A+HBP x Green 7-12-40
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

Gauntlet trees showing promise – 2016

Diploid Flying Dragon Hybrids (candidates for ACPS):

N-40-R2-T14-11-7
B11-5-60-11-12
B11-R5-T25-11-2
B21-R1-T25-11-6
B21-R1-T25-11-9
B21-R1-T2-11-1
B21-R1-T2-11-2
B11-R5-T2-11-4

Gauntlet Survivor at Picos Farm

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1-year old Gauntlet trees showing promise - 2016

46x31-00-S10 x US812-11-2
S10xS11-11-S20 (salt tolerant Shekwasha/pummelo)
A+8-1-99-4A x Orange 15-12-19
A+8-1-99-4A x Orange 15-12-57
A+8-1-99-4A x Orange 15-12-65
8-1-99-2B x C22-12-15
8-1-99-2B x C22-12-6
8-1-99-2B x C22-12-27
S10xS15-12-25 (salt tolerant Shekwasha/pummelo)
S10xS15-12-48 (salt tolerant Shekwasha/pummelo)
S10xS15-12-35 (salt tolerant Shekwasha/pummelo)
S10xS15-12-32 (salt tolerant Shekwasha/pummelo)
S10 x x639-12-16

1-year old Gauntlet trees showing promise - 2016

A+HBP x Orange 3-12-5
A+HBP x Orange3-12-10
N+HBP x Orange 4-12-17
N+HBP x Orange 4-12-6
N+HBP x Orange 19-12-15
N+HBP x Orange 19-12-3
A+HBP x Orange 19-12-4
A+8-1-99-4A x Orange 15-12-17
A+HBP x Changsha+50-7-12-70
A+HBP x Changsha+50-7-12-18
A+HBP x Changsha+50-7-12-31
A+HBP x Changsha+50-7-12-24
A+HBP x Changsha+50-7-12-47
A+HBP x Changsha+50-7-12-40
A+HBP x Changsha+50-7-12-42
A+HBP x Changsha+50-7-12-26
A+Chandler x Orange 4 -12-6
N+HBP x 6058x2071-02-08-17

Gauntlet Survivors at Picos Farm

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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, and can be mass-propagated by tissue culture (Ruck's Nursery, Agristarts, Agromillora, Citrific, etc.).

Many of the most promising hybrids are being entered into expanded field trials via a MAC grant (w/ Kim Bowman) that will test 48 new rootstocks (24 from UF and 24 from USDA); along with new trials with other industry cooperators.

To HALL OF FAME CITRUS GROWER-RESEARCHER
and Outstanding Industry Collaborators: Mr. Orie Lee

Funding: Mr. Orie Lee, Citrus Variety Improvement Grants from the Citrus Research and Development Foundation (CRDF) , USDA/CSREES; and the Citrus Research and Education Foundation (CREF).

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Thanks!

UF-CREC Citrus Genetic Improvement Team
2015