The Development of Improved Citrus Rootstocks to Facilitate Advanced Production Systems and Sustainable Citriculture in Florida

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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.

ROOTSTOCK IMPROVEMENT

An Exercise in "Creative Packaging"

WHAT DO WE NEED?

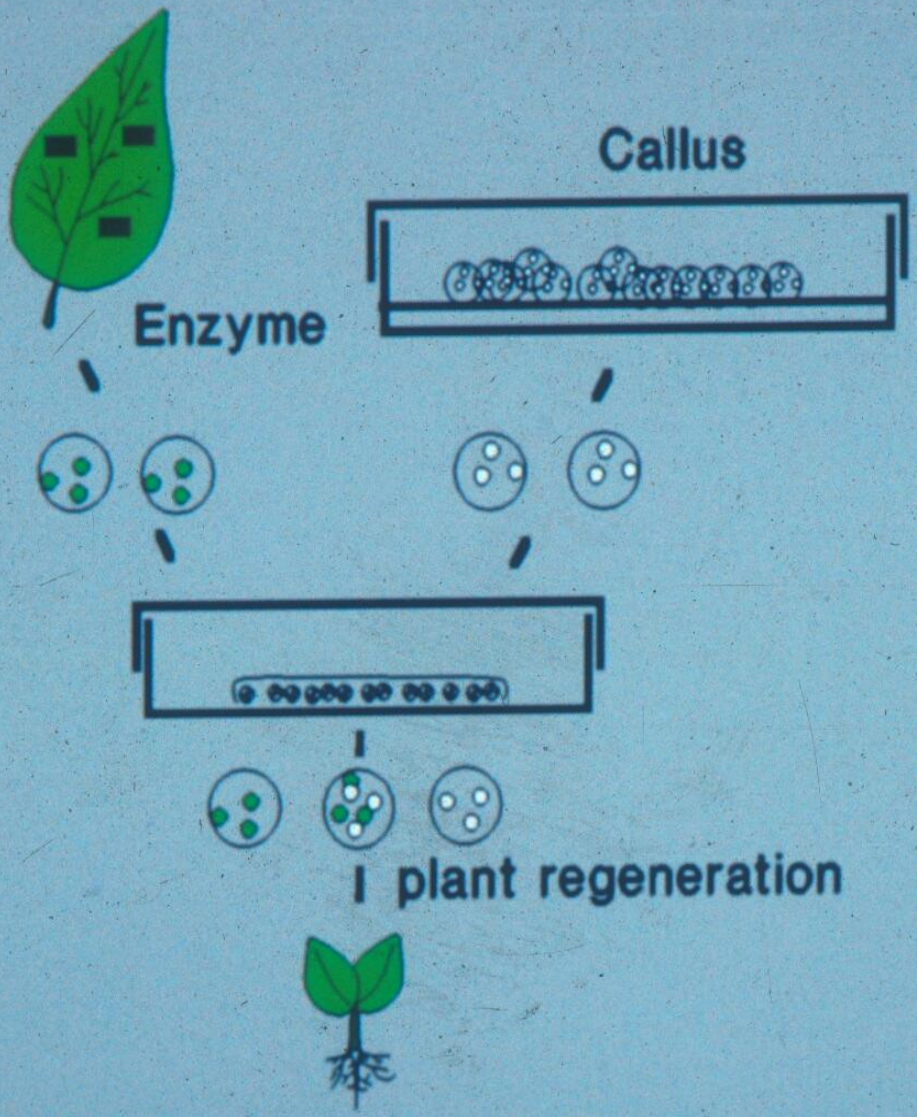
- Wide adaptability, tolerance of high pH, calcareous and heavy soils
- Resistance to CTV-induced quick decline
- Resistance to Citrus Blight
- High yields of good fruit quality
- Optimum tree size for new production systems
- New rootstocks must be able to tolerate mechanical damage inflicted by ***Diaprepes*** larvae, and resist secondary infections of ***Phytophthora nicotianae*** and ***P. palmivora***, and other invading fungi
- Must be capable of vigorous root growth following ***Diaprepes*** damage.

-tolerance of salinity in some areas

--NOW – Tolerance to HLB (huanglongbing)!

Our primary strategy for rootstock improvement has been to produce allotetraploid somatic hybrids by combining complementary diploid rootstocks.

Tetraploid rootstocks usually (but not always) have a built in tree-size control mechanism due to some unknown physiological reaction with the diploid scion.


Callus
Enzyme
plant regeneration

Effect of Polyploidy on Tree Size (based on % of Carrizo average canopy volume) – Sweet orange scion, McTeer Trial, Dundee, Somatic hybrids indicated by plus sign. Trees 4-7 years old.

Rough lemon 8166	141
CARRIZO	100
Cleo	100
Kinkoji	95
C-35	90
Sour orange + Carrizo	84
Swingle	78
Sour orange + rangpur	65
WGFT+50-7	63*
Cleo + Carrizo	61
Cleo + Argentine trifoliate orange	58
Nova + Hirado Buntan pummelo zyg.	56
Cleo + rangpur	54
SO+50-7	51*
Nova + Palestine sweet lime	49
Milam + Kinkoji	42
Changsha + Benton	38
Cleo + Swingle	35
Cleo + Flying Dragon	35
Hamlin + Flying Dragon	34
Flying Dragon	33
Sour orange + Benton	29

Average Diameter/Estimate of Optimum Planting Density and Projected Yield
With a **20 foot**-middle (Based on 2000 tree size and 2000/2001 yields)
US Sugar/CREC Somatic Hybrid Rootstock Trial – Typical Flatwoods Site
Rohde Red Valencia Planted 1992, 1993*

Rootstock	Average Diameter	Trees/acre 20 ft.middle	Boxes/acre 2000	Boxes/acre 2001	Cummulative Total .
Succari + Argentine trifoliate orange*	9.4 (ft)	232	309	320	629
Cleo + Swingle*	9.8	222	369	346	715
Cleo + Flying Dragon	10.0	218	307	233	540
Sour + Rangpur*	10.1	216	330	389	719
Cleo + Argentine trifoliate orange*	10.4	209	355	299	654
Sour + Palestine sweet lime	10.9	200	**488**	**464**	**952**
Cleo + Sour orange	11.5	189	354	276	630
Hamlin + Rough lemon	11.5	189	382	361	743
Valencia + Femminello	12.0	182	412	366	778
Hamlin + Rangpur	12.1	*180*	*428*	*454*	*882*
Cleo + Rough lemon	12.4	*176*	*422*	*509*	*931*
Cleo + Volk	12.6	*173*	*394*	*495*	*889*
Sour orange	12.6	*173*	*457*	*391*	*848*
Cleo + Rangpur	12.8	*170*	*430*	*491*	*921*
Swingle	12.8	*170*	*410*	*427*	*837*
SFS	13.0	*168*	*382*	*428*	*810*
Palestine sweet lime	14.5	*150*	*496*	*591*	*1087*
Cleo*	14.6	*149*	*328*	*408*	*736*
Volk	14.7	*148*	*546*	*599*	*1145*
Carrizo	15.0	*115*	*334*	*367*	*801*
Rough lemon	15.0	*1 15*	*455*	*472*	*927*

Red italics = middle too close for equipment

Projected Valencia fruit yield with trees planted at optimum spacing in rows with 20 foot middles (based on 2011 actual yield data).

Somatic Hybrid	In-row spacing	Trees/Acre	Boxes/Acre
SO+50-7	8.2 (ft)	265	488
WGFT + 50-7	9.0	242	522
Changsha + 50-7	**8.7**	**250**	**563**
SO+Carrizo	8.4	259	541
Changsha + Benton	10.0	218	512

10-year old Valencia/Changsha + 50-7

CITRUS BLIGHT

-still a major killer of trees in Florida

-if you solve the sour orange problem, you solve blight!

-trifoliate orange hybrids susceptible – strategy is to dilute trifoliate contribution while retaining disease resistance and fruit quality attributes

-field screening of new hybrids by resetting in blight holes– Primary cooperator is Mr. Orie Lee, Secondary cooperator David and Sharon Garrett.

-build in tree size control along with the CTV resistance

NEW STRATEGY FOR BUILDING A BETTER SOUR ORANGE

-Somatic Hybridization of Superior Pummelo + Mandarin parents.

- All pummelos are not created equal. We have developed an effective greenhouse screen for selecting zygotic pummelo seedlings adapted to high pH, calcareous soils, and that are resistant to both ***Phytophthora nicotianae*** and ***P. palmivora.*** A trememdous amount of genetic diversity can be quickly screened. Superior individuals will serve as fusion partners with selected mandarins including widely adapted Shekwasha and ***C. amblycarpa***. Resulting somatic hybrids should have tremendous rootstock potential.

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

Screening Zygotic Pummelo Seedlings from a superior pummelo seed parent

Screening of Open-Pollinated Pummelo Seedlings for Tolerance of Phytophthora and High pH, Calcareous Soil

Tree I.D.	Pummelo Variety	% Seedling Survival
DPI 8-1	Liang Ping Yau sdlg. (China)	69%
DPI 7-2	Large Pink Pummelo (SE Asia)	67%
J.L.C.	Hirado Buntan sdlg. (Japan)	56%
DPI 5-4	Red Shaddock Pummelo (SE Asia)	47%
DPI 4-3	Sha Ten Yau sdlg. (China)	30%
DPI 7-3	Siamese Sweet (Thailand)	27%
DPI 4-4	Siamese Pummelo (Thailand)	18%
DPI 5-1	Hirado Buntan sdlg. (Japan)	17%
DP”I 8-2	Pummelo NW (Florida)	12%
DPI 7-1	Chinese Pummelo (China)	4%
DPI 6-1	Kao Phuang (Thailand)	0%

>200 seedlings selected for fusion experiments

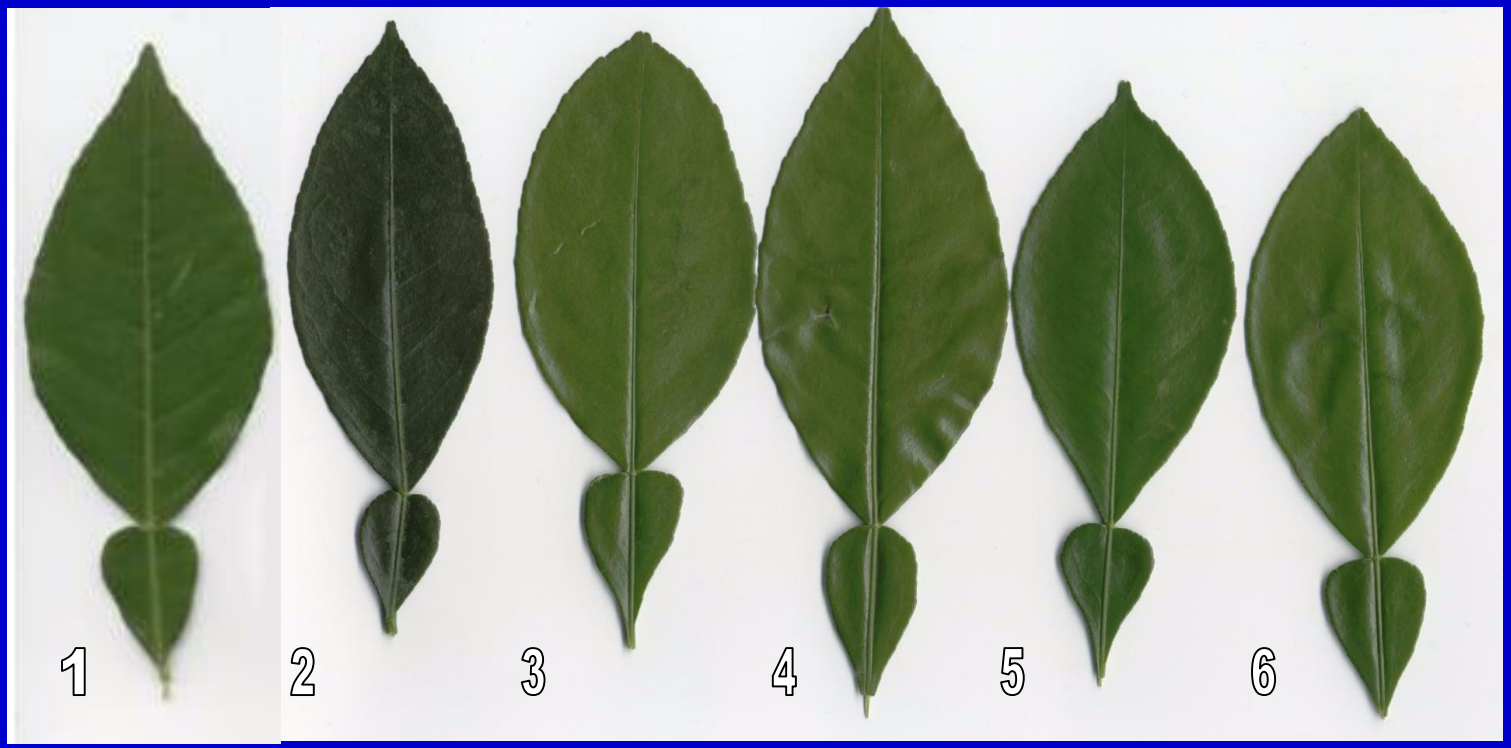


Figure.1. Leaf morphology of sour orange and some new somatic hybrids of mandarin + pummelo ('mandelos'). 1- Sour orange; 2 - Amblycarpa + C. grandis 'Chandler'; 3 - Amblycarpa + HBP (Hirado Buntan Pummelo); 4 - Amblycarpa + HBP sdl-JL-2B; 5 - Amblycarpa + HBP sdl-5-1-99-1B; 6 - Amblycarpa + 'LingPing Yau'-sdl-8-1-99-4A.

Field Assay for Tristeza Virus Resistance – Sour orange not growing, Mandarin + Pummelo somatic hybrid not affected by virus!
(Azza Mohamed)

Sour orange

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.

SOMATIC HYBRID BREEDING PARENTS BEING USED FOR ROOTSTOCKS IMPOVEMENT:

Females (produce high % of zygotic seed):

1. **Nova mandarin + Hirado Buntan sdl. pummelo**
2. **Sour orange + rangpur**
3. **Succari sweet orange + Hirado Buntan sdl. pummelo**

Pollen Parents:

1. **Cleopatra + Argentine trifoliate orange**
2. **Cleopatra + Swingle citrumelo**
3. **Sour orange + Carrizo**
4. **Red Marsh grapefruit + Argentine trifoliate orange**
5. **Cleopatra + Sour orange**
6. **Sour orange + Palestine sweet lime**
7. **Sour orange + Flying dragon trifoliate orange**
8. **Cleopatra + rangpur (for salinity tolerance)**
9. **Succari sweet orange + Argentine trifoliate orange**
10. **Nova mandarin + *C. ichangensis***
11. **Hamlin sweet orange + *Microcitrus papuana***

Vigorous trifoliate hybrid growing in high pH-calcareous soil infested with P. nicotianae and P. palmivora

SALINITY TOLERANCE

Selection of progeny from crosses designed for salinity tolerance: healthy seedlings from Nova + HBpummelo with Cleo + rangpur

The latest PROBLEM before HLB:

-New rootstocks must be able to tolerate mechanical damage inflicted by ***Diaprepes*** larvae (the sugar cane root weevil), and resistant to secondary infections of ***Phytophthora nicotianae*** and ***P. palmivora***, and other invading fungi

- Must be capable of vigorous root growth following ***Diaprepes*** damage.

"Tetrazyg" selection with severe Diaprepes damage.

"Tetrazyg" selection with minimal Diaprepes damage.

Fully recovered "tetrazyg" cuttings.

(Nova + HBPummelo) x (Cleo + Arg. Trifoliate orange)
“TETRAZYGS”

Amenability of Tetrazyg Rootstock Candidates to Traditional Seed Propagation

Hybrid	Seeds per Fruit	Seed Type (based on SSR**)
Orange 1 (Nova+HBPxCleo+APT)	15	Nucellar
Orange 2	10	Nucellar
Orange 3	4	Nucellar*
Orange 4	23	Nucellar
Orange 6	3	Zygotic
Orange 8	18	Zygotic
Orange 12	13	Zygotic
Orange 13	14	Nucellar
Orange 14	18	Nucellar
Orange 15	6	Zygotic
Orange 19	20	Nucellar*
White 4 (Nova+HBP x Succ+APT)	20	Nucellar*
Green 2 (Nova+HBP x SO + CZO)	23	Zygotic
Green 6	20	Zygotic
Green 7	21	Nucellar
Purple 2 (Nova+HBP x Cleo+SO	20	Zygotic
Purple 4	15	Zygotic
Blue 1 (Nova+HBP x SO+PSL)	15	Nucellar
Blue 2	12	Zygotic
Blue 3	7	Nucellar
Blue 4	26	Zygotic
Blue 9	7	Zygotic
6058x2060-00-S1 (salt-tolerant)	8	Nucellar*

•hybrid performed well in Diaprepes screen, **SSR = Simple sequence repeats (microsatellite)

•analyses performed by J. Gmitter and C. Chen.HBP = 'Hirado Buntan' pummelo seedling;

•APT = Argentine trifoliate orange; SO = sour orange; Succ = 'Succari' sweet orange;

•PSL = Palestine sweet lime

Rootstock Selections Showing Promise vs. Diaprepes/Phytophthora Complex – From the Top 50 of 7 Screening Experiments (>500 hybrids screened – with assistance from Angel Hoyte)

Tetrazygs:

- 2247x2071-00-10 (Orange 10)
- 2247x2071-00-21 (Orange 21)
- 2247x1571-00-04 (White 4)
- 2247x2072-00-18 (Orange 18)
- 2247x2071-00-19 (Orange 19)
- 2247x2071-00-03 (Orange 3)
- 2247-6056-00-05 (Blue 5)
- 2247x2060-00-03 (Purple 3)
- 2247-2075-01-12
- 2247x2075-01-1
- 2247-OP-A2 'The Buster Pratt Special'
- 2247-OP-A5
- 2257x6056-00-6 (Blue 6)
- S0+PSL-OP-04-11
- 2247x1523-02-19
- 2247x2055-02-6
- 2247x2055-02-11
- 2247x2075-02-9
- N+HBP-OP-04-S15
- Suc+HBPxCl+Czo-04-17
- N+HBP-OP-04-S1
- N+HBPxCl+Czo-04-18
- N+HBP-OP-04-S20
- N+HBP-OP-04-S12
- S+HBPxCl+Czo-04-10
- R-02-52
- N+HBP-OP-04-20
- R-4x-10
- 2247x2071-01-A4

Diploid Sour orange-like Hybrids:

46x32-02-20
46x32-02-S6
46x32-02-3
46x32-02-5
46x32-02-R51
46x31-02-11
46x31-02-13
46x31-02-14
46x32-02-3

Sour orange-like Somatic Hybrids:

Amb+HBJL-2B
Murc+SN3
Amb+Chandler
Murc+HBP
Amb+5-1-99-1B
Amb+8-1-99-4a

Other Somatic Hybrids:

Amb+Carrizo
Milam + HBP

Tetrazyg nucellar liners prior to budding at commercial nursery

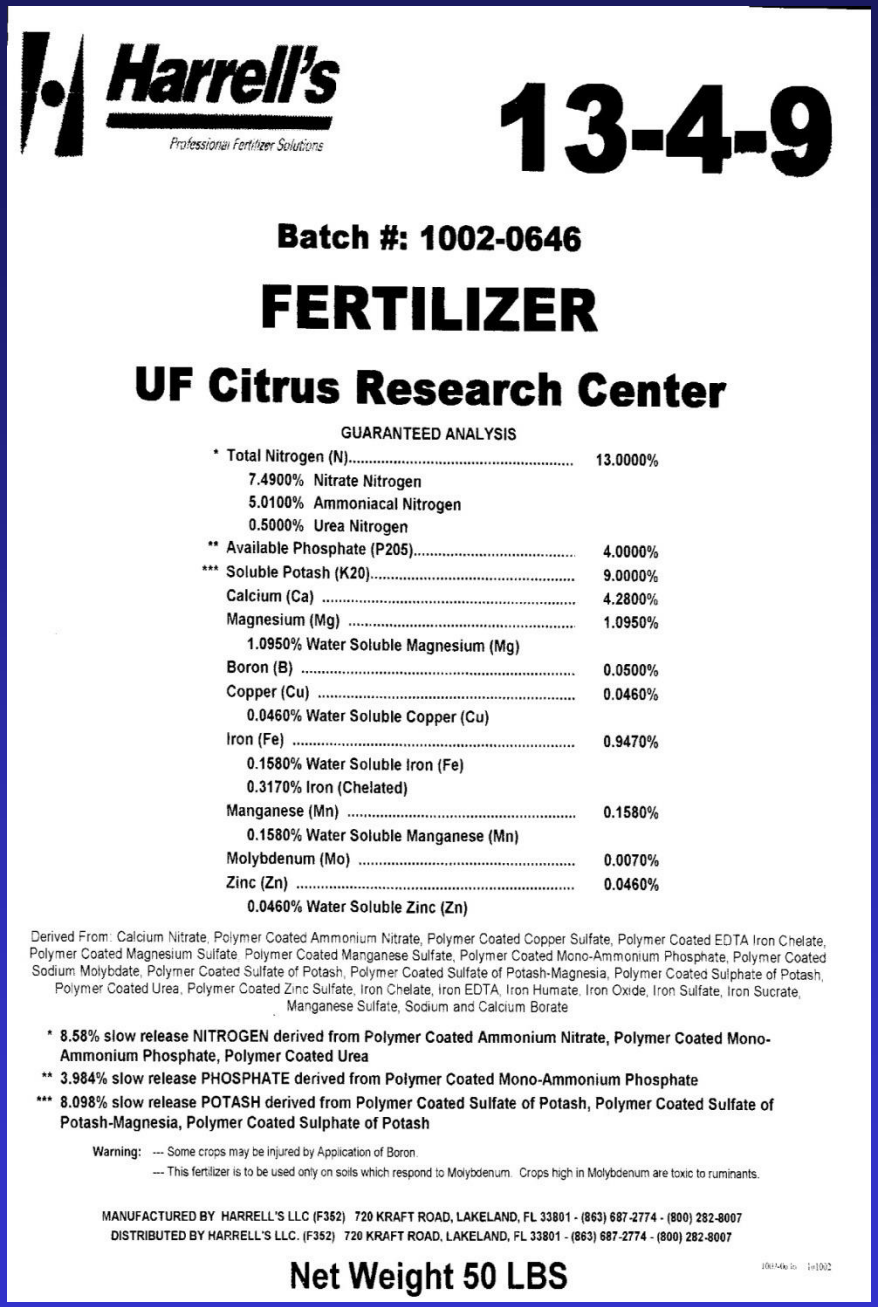
The Old Gauntlet

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. Propagation of seed source trees
6. Propagation via rooted cuttings to produce replicate plantlets for Diaprepes/Phytophthora screen and blight field screen

5 year old Valencia on Orange#14, between two rough lemon trees planted at the same time – blight resets in Alligator grove (Mr. Lee)

Juice Quality Data from New Tetrazyg Rootstocks – 5-Year Old Valencias – 2010 – Alligator Grove

Rootstock	Acid	Brix	Ratio	LB Solids/Bx
Orange #2	0.95	12.23	12.87	5.9
Orange#3	0.71	11.80	16.16	5.5
Orange#4	0.84	11.30	13.45	5.0
Orange#14	0.91	11.09	12.19	5.6
Orange#19	0.87	11.83	13.60	5.7
Green#2	0.87	12.66	14.55	5.6
Green#7	1.01	11.92	11.80	5.9
White#1	0.79	11.96	15.14	5.6
White#3	0.79	11.88	15.04	5.3
White#4	0.86	12.95	15.06	6.0
Rough Lemon	0.70	11.57	14.65	5.0



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
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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!

St. Helena Project – Top Thirty by Yield, 2012 (47 months)

Scion	Rootstock	Lbs. Solids Per Box 2012	Yield (per tree) 2012	# 4-tree reps
VALQUARIUS	WHITE 1805	5.1698	1.188	2
VERNIA	VOLK	3.3425	1.125	1
VERNIA	VOLK	3.8556	1.125	1
VALQUARIUS	ORANGE 14	5.4063	1.083	1
VALQUARIUS	ORANGE 1804	4.2035	1.017	2
VALQUARIUS	ROUGH LEMON	3.6614	1.000	2
VALQUARIUS	AQUA 1803	4.5813	0.981	2
VALQUARIUS	68-1G-26-F6-P20	5.8357	0.875	1
VERNIA	SWINGLE	5.1100	0.850	1
VALQUARIUS	ORANGE 13	4.8762	0.844	2
VERNIA	BLUE 1	5.7146	0.844	2
VALQUARIUS	FG 1793	5.5322	0.833	1
VERNIA	ORANGE 14	5.5414	0.833	1
VALQUARIUS	ORANGE 15	4.8405	0.813	2
VALQUARIUS	ORANGE 16	5.0880	0.813	2
VERNIA	AQUA 1803	5.4920	0.806	2
VALQUARIUS	CH+50-7	5.6397	0.781	2
VERNIA	ROUGH LEMON	3.6700	0.775	1
VALQUARIUS	PINK 1802	3.9876	0.763	2
VALQUARIUS	MG-11	4.9770	0.750	1
VALQUARIUS	ORANGE 4	4.5651	0.750	2
VALQUARIUS	WHITE 1801	5.3474	0.750	1
VERNIA	CLEO+CZO	5.8767	0.750	2
VERNIA	KCZ	4.3354	0.750	1
VALQUARIUS	ORANGE 2	5.2627	0.738	2
VALQUARIUS	ORANGE 3	5.5036	0.719	2
VERNIA	CH+BENTON	5.6640	0.719	2
VERNIA	ORANGE 19	5.7920	0.713	2

St. Helena Project – 2012 Top 30 lbs. solids (47 months)

Scion	Rootstock	Lbs. Solids Per Box	Yield (per tree) 2012	n
VERNIA	BLUE 2	6.1629	0.413	2
VERNIA	ORANGE 1804	6.0989	0.250	2
VERNIA	SO+CZO	6.0217	0.250	2
VERNIA	WHITE 1801	5.9323	0.325	1
VERNIA	SO+50-7	5.9173	0.475	1
VERNIA	WHITE4	5.8923	0.250	2
VERNIA	CLEO+CZO	5.8767	0.750	2
VERNIA	ORANGE 13	5.8750	0.481	2
VERNIA	ORANGE 18	5.8381	0.650	1
VALQUARIUS	68-1G-26-F6-P20	5.8357	0.875	1
VALQUARIUS	FG 1731	5.8273	0.675	1
VERNIA	MG-11	5.8218	0.544	2
VALQUARIUS	68-1G-26-F2-P12	5.7976	0.625	1
VERNIA	ORANGE 19	5.7920	0.713	2
VALQUARIUS	CH+BENTON	5.7850	0.425	1
VERNIA	BLUE 4	5.7698	0.613	2
VERNIA	BLUE 9	5.7684	0.250	2
VALQUARIUS	WHITE 4	5.7582	0.563	2
VERNIA	BLUE 1	5.7146	0.844	2
VERNIA	WHITE 1805	5.7044	0.250	1
VALQUARIUS	FG 1707	5.6997	0.325	1
VALQUARIUS	SO+CZO	5.6946	0.656	2
VERNIA	CH+50-7	5.6749	0.625	2
VERNIA	CH+BENTON	5.6640	0.719	2
VERNIA	ORANGE 1	5.6566	0.250	2
VALQUARIUS	CH+50-7	5.6397	0.781	2
VERNIA	ORANGE 3	5.6112	0.667	3
VERNIA	PURPLE 2	5.5624	0.450	1
VERNIA	ORANGE 14	5.5414	0.833	1

Candidate for ACPS

Valquarius on Orange #15 tetrazyg rootstock – just < 5 years at St. Helena, Dundee FL

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 1712	1	1	1	100%
FG 1714	1	1	1	100%
FG 1715	1	0	0	0%
FG 1722	1	0	1	100%
FG 1731	5	0	1	20%
FG 1733	5	1	1	20%
FG 1792	2	0	0	0%
FG 1793	5	1	2	40%
FG 1794	2	0	0	0%
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	Trees 4.5 years old	# HLB infected on or	Total # infected	% trees
Rootstock (4X)	# of trees	before Aug 2012	as of Oct 2012	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 3	1	0	0	0%
Green 6	6	0	1	17%
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

Considerations for Fast Track Rootstock Release

1. Large scale trialing at multiple locations (i.e. one acre per rootstock selection per site, combined with new robust sweet orange clones). Propagation at commercial nurseries in progress. Cooperators have been lined up to accomplish this. CRDF now engaged!

2. Propagation and protection of seed trees – in progress.

3. Alternative mass propagation: in vitro propagation and rooted cuttings. The CREC Citrus Improvement Team is establishing a collaboration with the highly regarded international woody plant propagation company Agromillora, which has demonstrated expertise in Citrus (Spain, Brazil, and Oregon/CA). We have also initiated collaboration on citrus micropropagation with AgriStarts (Apopka), and Phillip Ruck's Nursery. A new CRDF-funded grant with Barrett Gruber (IRREC) will propagate (via rooted cuttings) and field test promising selections (gauntlet survivors) in the IR.

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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)

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; 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).

Valencia on a Sour orange-like somatic hybrid of Amblycarpa (Nasnaran) + Hirado Buntan pummelo – blight tolerant monoembryonic female? (9-year old tree, Batton Block blight screen, Mr. Orie Lee). Many hybrids in the gauntlet!

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 – after 10 months in the field – Valencia/tetrazyg [Nova+HBP] x [(sour orange+rangpur) x Cleo+Arg. trifoliate orange)]

HLB Status of the First Round of 'Gauntlet Survivors' after 10 months in the field at Picos Farm, Fort Pierce

Local Sample Id	Block	Cal_ct	Diagnosis	Scion	Rootstock
1	19	36.92	No HLB Found	VALENCIA	A+HBP X 6058 + 2071-08-2-S6
2	19	40.00	No HLB Found	VALENCIA	A+HBP X 6058 + 2071-08-2-26
3	19	40.00	No HLB Found	VALENCIA	A+HBP X 6058 + 2071-08-2-S15
5	19	40.00	No HLB Found	VALENCIA	A+HBP X 6058 + 2071-08-2-S12
6	19	40.00	No HLB Found	VALENCIA	GREEN6 X ORANGE 14 -09-24
7	19	40.00	No HLB Found	VALENCIA	GREEN6 X ORANGE 14 -09-23
8	19	35.62	No HLB Found	VALENCIA	A+HBJL-2B X ORANGE 14 -09-13
9	19	40.00	No HLB Found	VALENCIA	GREEN 6 X ORANGE 14-09-21
10	19	40.00	No HLB Found	VALENCIA	MILAM+HBP X ORANGE 14-09-15
11	19	40.00	No HLB Found	VALENCIA	A+HBJL-1-09-4
12	19	40.00	No HLB Found	VALENCIA	A+HBJL-2B X ORANGE 14 -09-7
13	19	40.00	No HLB Found	VALENCIA	N+HBP X 6058 + 2071-08-2-15
14	19	37.08	No HLB Found	VALENCIA	A+HBJL-1-09-14
15	19	40.00	No HLB Found	VALENCIA	A+HBJL-1-09-18
16	19	40.00	No HLB Found	VALENCIA	A+HBJL-1-09-34
17	19	40.00	No HLB Found	VALENCIA	GREEN 6 X ORANGE 14-09-14
18	19	30.09	**Questionable**	VALENCIA	A+HBP X 6058 + 2071-08-2-21
19	19	40.00	No HLB Found	VALENCIA	A+HBJL-1-09-8
20	19	31.81	**Questionable**	VALENCIA	A+HBJL-2B X ORANGE 19 -09-31
21	19	40.00	No HLB Found	VALENCIA	A+HBJL-2B X ORANGE 19 -09-53
22	19	40.00	No HLB Found	VALENCIA	A+HBJL-2B X ORANGE 19 -09-16
23	19	37.48	No HLB Found	VALENCIA	A+HBJL-2B X ORANGE 19 -09-13
34	19	40.00	No HLB Found	VALENCIA	AMB X FD-09-1
35	19	20.50	**HLB Positive**	VALENCIA	A+HBJL-2B X ORANGE 14 -09-3

HLB Status of the First Round of 'Gauntlet Survivors' after 10 months in the field at Picos Farm, Fort Pierce

Local Sample Id	Block	Cal_ct	Diagnosis	Scion	Rootstock
77	19	35.65	No HLB Found	VALENCIA	A+HBJL-2B X ORANGE 19 -09-29
78	19	40.00	No HLB Found	VALENCIA	A+HBP X 6058 + 2071-08-2-33
80	19	36.69	No HLB Found	VALENCIA	A+HBJL-2B X ORANGE 19 -09-28
81	19	40.00	No HLB Found	VALENCIA	A+HBJL-2B X ORANGE 19 -09-23
84	19	38.01	No HLB Found	VALENCIA	A+HBJL-2B X ORANGE 19 -09-18
85	19	35.24	No HLB Found	VALENCIA	A+HBJL-2B X ORANGE 19 -09-11
87	19	40.00	No HLB Found	VALENCIA	MILAM+HBP X ORANGE 14-09-2
88	19	22.26	HLB Positive	VALENCIA	A+HBP X 6058 + 2071-08-2-29
89	19	40.00	No HLB Found	VALENCIA	MILAM+HBP X ORANGE 14-09-3
90	19	23.58	**HLB Positive**	VALENCIA	BLUE 2 X ORANGE 14-09-3
91	19	36.30	No HLB Found	VALENCIA	MILAM+HBP X ORANGE 14-09-19
92	19	40.00	No HLB Found	VALENCIA	N+HBP X 6058 + 2071-08-2-1
93	19	40.00	No HLB Found	VALENCIA	A+HBP X 6058 + 2071-08-2-16
94	19	23.11	**HLB Positive**	VALENCIA	A+HBJL-1-09-22
95	19	21.01	**HLB Positive**	VALENCIA	A+HBP X 6058 + 2071-08-2-31
96	19	23.27	**HLB Positive**	VALENCIA	MILAM+HBP X ORANGE 14-09-20
97	19	22.54	**HLB Positive**	VALENCIA	N+HBP X 6058 + 2071-08-2-10
98	19	40.00	No HLB Found	VALENCIA	MILAM+HBP X ORANGE 14-09-12
99	19	37.11	No HLB Found	VALENCIA	BLUE 2 X ORANGE 14-09-1
100	19	40.00	No HLB Found	VALENCIA	MILAM+HBP X ORANGE 14-09-9
101	19	40.00	No HLB Found	VALENCIA	MILAM+HBP X ORANGE 14-09-4

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.

Concluding Remarks:

1. Tolerance/resistance to canker and HLB is being discovered in commercial quality CREC scion and rootstock germplasm – making these epidemics a mixed blessing for our breeding program.

2. Successful rootstocks should be tolerant/resistant to all known vascular diseases of citrus including CTV-quick decline and blight (is there a blight/HLB synergy, the double whammy??). Commercial rootstocks are lacking in this regard – especially Swingle and Carrizo!

3. Management programs should minimize any stress on the trees, including nutritional or water stresses; and provide constant nutrition. Don't forget about ground fertilizer applications!

4. New rootstock candidates are showing a lower frequency of HLB infection, and reduced severity of disease symptoms once trees become infected. Tolerant rootstock types being identified include complex tetrazygs, citranges, citrandarins, and sour orange types.

5. Complex new rootstocks in combination with the right tree care and good psyllid control have potential to prevent and/or mitigate HLB. Imagine the potential of ACPS when combined with the use of HLB-tolerant, tree-size controlling precocious rootstocks!

6. Differences observed in HLB responses among unselected new rootstock candidates suggest great potential for the selection of rootstock genotypes that can completely protect trees – a solution for all commercial scions. Stay tuned for the second wave of new rootstocks!

THERE IS HOPE!

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