Solving Citrus Greening with Genetics and Nutrition

UF UNIVERSITY of FLORIDA
IFAS
Citrus Research and Education Center

Jude Grosser
Fred Gmitter and Bill Castle

UF-CREC Citrus Genetic Improvement Team
2020

What is Citrus greening?

- Citrus greening or Huanglongbing (HLB) a bacterial plant disease that is **fatal** for citrus trees.

- Diseased trees produce bitter, hard, misshapen fruit and die within a few years after infection.

- None of the commercial citrus Sweet Orange or Grapefruit cultivars grown in Florida have resistance to this disease.

http://californiacitrusthreat.org/huanglongbing-citrus-greening.php

What causes Citrus greening/HLB

- Citrus Greening is caused by the gram negative bacterium *Candidatus* Liberibacter sp.

- In the United States and Asia, HLB is caused by the species asiaticus (CLas).

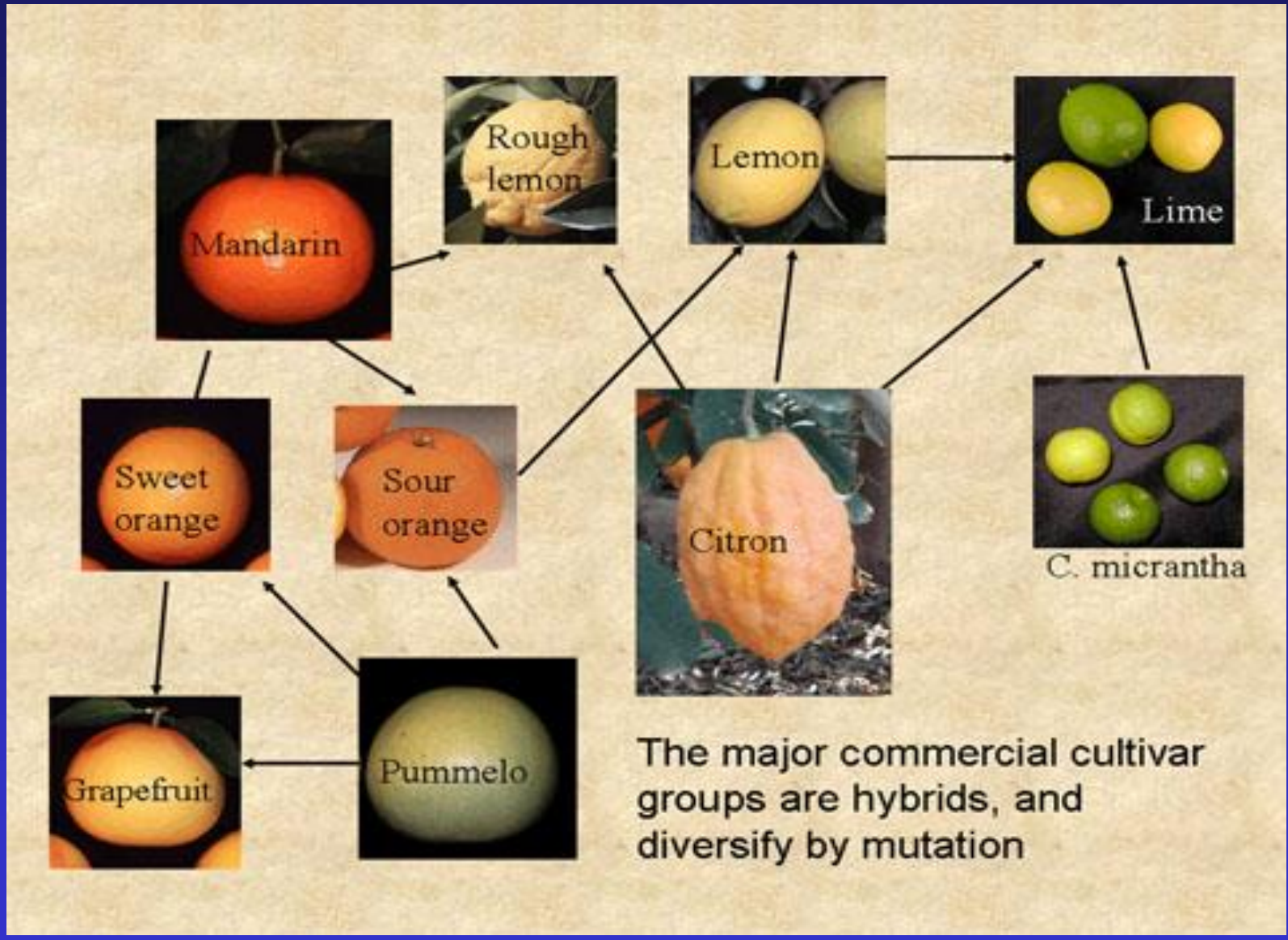
http://www.goletamonarchpress.com/wp-content/uploads/2014/07/ACP-adults-005-Rogers.jpeg

- Other species include:
  - Candidatus Liberibacter africanus (HLB in Africa)
  - Candidatus Liberibacter americanus (HLB in Brazil)

- Transmission of the bacteria into the citrus tree is a two-step process.
  - First, psyllids acquire it from infected trees.
  - Second, infected psyllids pass on the bacteria to other trees.

Four Progenitor Species

1. Pummelo (*Citrus grandis* L. Osbeck)
2. Mandarin (*C. reticulata* Blanco)
3. Citron (*C. medica* L.)
4. Papeda (*C. micrantha* Wester)



Provided by Mikeal Roose, UC Riverside

1. Biotechnology applications in scion breeding

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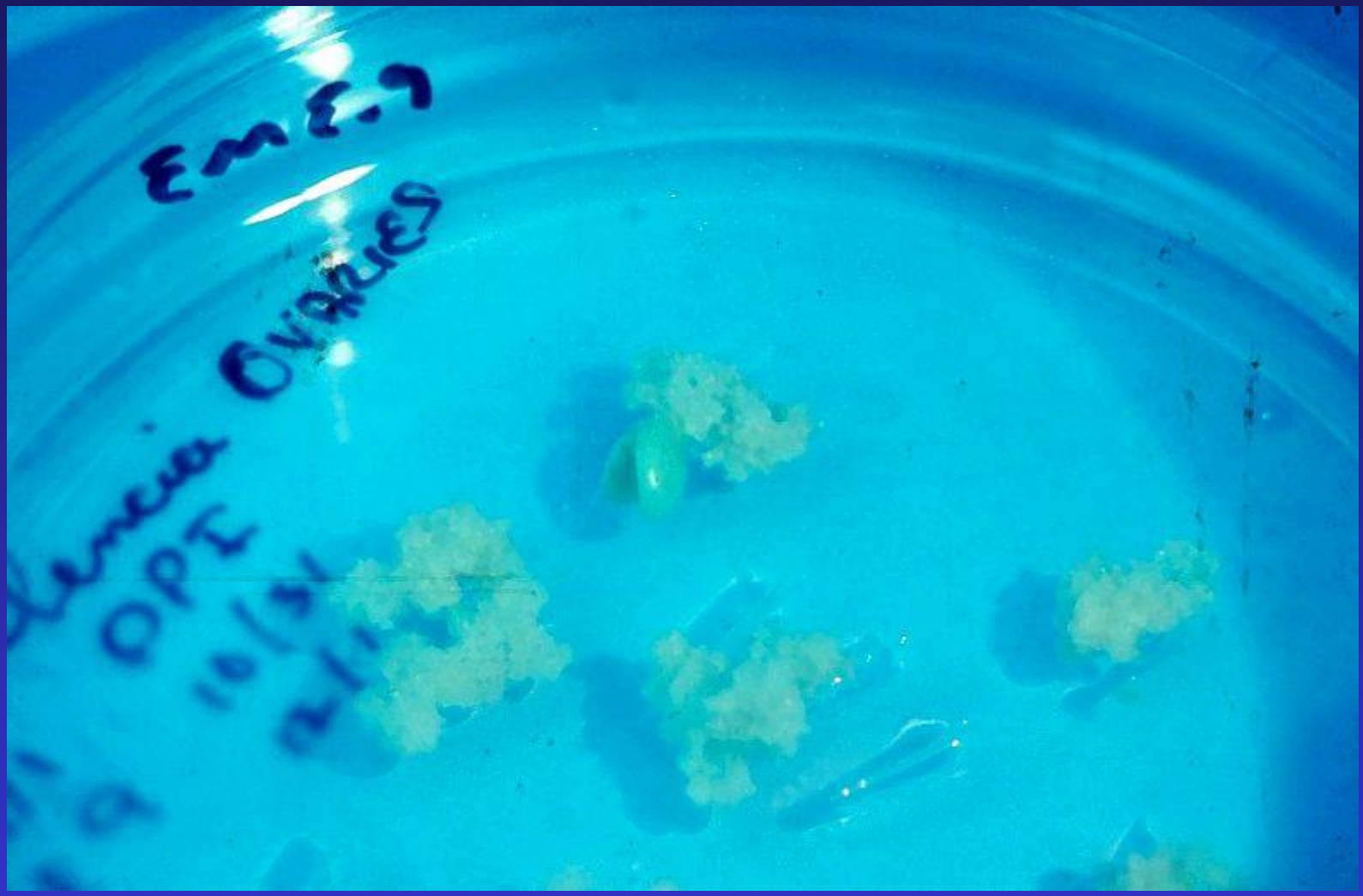
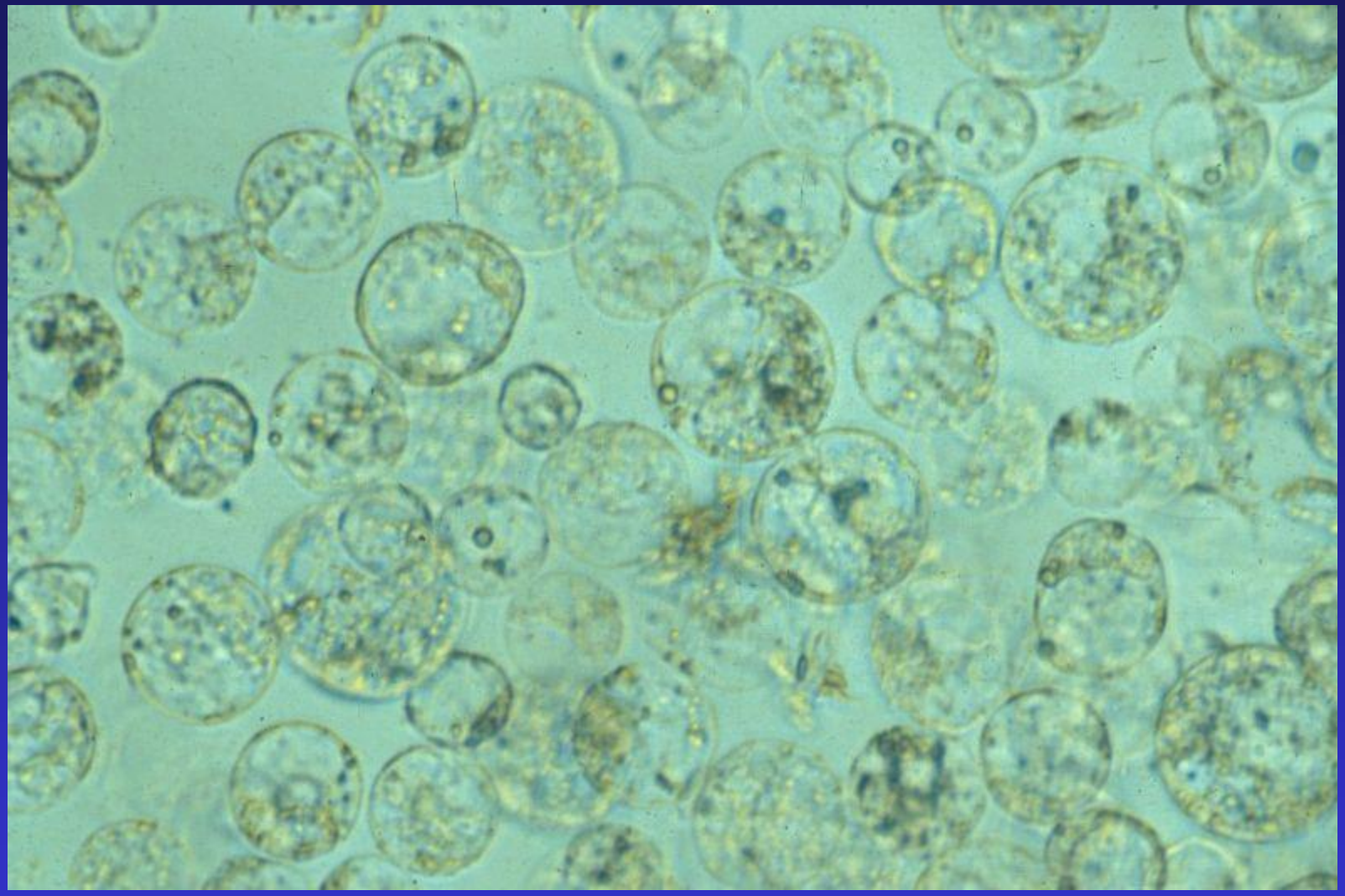
2016



SWEET ORANGE EMBRYOGENIC CALLUS

SWEET ORANGE ORGANOGENESIS
ADVENTITIOUS SHOOT BUD INDUCTION



SWEET ORANGE SUSPENSION CULTURE PROTOPLASTS

Somaclonal Variation: variability in plants regenerated from tissue culture that is either induced or uncovered by a tissue culture process. Most somaclonal variation is negative, but if enough plants are examined, positive changes can usually be recovered.

Sources of somaclones in citrus:
organogenesis, somatic embryogenesis, protoplasts

Valquarius ™ processing sweet orange, an early-maturing selection of Valencia that can be harvested mid-January through February, with typical Valencia quality.

Citrus Transformation

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B9-65 Valencia (PP27,144) for processing

- A high yield, high solids selection with typical Valencia maturity, best of 30 selections for yield and soluble solids in trial at Conserve II.

Table1. Yield Boxes / tree of Late Season sweet orange selections (somaclones, seedling introductions and controls) on Carrizo citrange rootstock planted 15' x25' at Water Conserv II, Winter Garden, FL, planted March, 2000.

Late season selections	2005-06	2006-07	2007-08	2008-09	2009-10	Cumulative Yield	Cumulative Rank
B-9-65	2.21	1.28	5.38	1.4	4.8	15.06	1
Appleby	2.46	0.98	4.61	1.82	4.91	14.78	2
T-4-43	1.8	1.02	4.4	0.73	5.81	13.76	3
T-2-25	2.08	1.27	4.15	1.08	4.68	13.26	4
S441-54-3 (Juv. 10-12-7)	2.27	1.24	4.4	0.83	4.38	13.13	5
T-3-62	1.59	1.58	3.8	1.21	4.52	12.7	6
S822-111-5 (Mat.10-12-7)	1.94	1.53	3.13	2.31	3.72	12.62	7
B-8-66	2.03	1.18	3.88	1.23	4.25	12.58	8
B-10-81	1.89	1.29	4.25	1.18	3.94	12.56	9
B-6-68	2.13	1.11	4.21	1	4.1	12.53	10
B-12-71	2.25	0.75	4.19	1.13	4.2	12.52	11
Jenner	2.08	1.18	4.52	1.18	3.48	12.44	12
Natal	1.48	1.46	4.07	1.04	4.27	12.32	13
Smith	2.11	0.99	3.93	1.01	4.26	12.3	14
T-1-13	2.35	0.84	4.28	0.91	3.8	12.18	15
T-1-23	1.82	0.7	4.07	1.42	4.18	12.18	16
T-2-62	1.8	1.06	3.82	1.22	4.18	12.08	17
Frost	2.14	0.79	4.52	0.84	3.77	12.06	18
B-8-76	1.84	0.89	4.43	0.66	4.13	11.96	19
T-1-25	1.61	0.69	4.61	0.43	4.38	11.72	20
T-1-26	1.66	0.72	4.29	0.81	4.22	11.7	21
T-1-33	1.62	0.67	3.75	0.77	4.47	11.27	22
Rohde Red Valencia	1.51	1.49	3.47	1.57	3.19	11.22	23
Valencia SF8-2-35	1.72	1.19	3.11	1.88	3.31	11.21	24
B-10-68	1.82	0.96	4.12	1.07	3.2	11.16	25
Vernia	1.35	1.34	3.18	1.29	3.12	10.28	26
Valencia SF11-1-69	1.54	1.17	2.06	1.47	3.4	9.64	27
B-10-62	0.99	0.74	2.58	1.25	2.27	7.82	28
Valencia SF9-1-86	0.93	0.73	2.04	1.05	2.83	7.58	29
B-6-66 (seedless, dwarfing)	0.82	1.06	1.48	1.03	2.38	6.77	30

ORANGES

‘OLL-8’

- Key attributes: Excellent color and quality, extends harvest window of ‘Valencia’ quality juice
- Produces round oranges with internal and external color similar to ‘Rhode Red Valencia’
- Holds on the tree exceptionally well, and maintains quality into the summer
- Trees appear to yield better than standard ‘Valencia’
- High juice content and good pounds solids
- Peels easier than a standard ‘Valencia’
- With its added color, could also be a valuable addition to the Florida fresh market portfolio
- Most precocious bearing clone among the OLL somaclones

‘OLL-8’

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

‘OLL-4’

Juice Quality Data from OLL-Series Somaclones

Data 3-2-2012

Variety	Lbs. Juice Per Box	Acid	Total Brix	Ratio	Lbs. Solids Per Box	Color
Valencia Control	54.41	1.00	12.03	12.03	6.55	38.60
OLL 20	53.23	0.84	12.10	14.40	6.44	40.50
OLL 27	54.99	0.84	12.34	14.69	6.79	39.80
OLL 25	57.58	0.79	12.81	16.22	7.38	40.20
OLL 9	56.30	0.94	12.97	13.80	7.30	39.90
OLL 15	49.48	0.99	14.56	14.71	7.20	39.50
OLL 2	51.55	0.77	12.77	16.58	6.58	41.00
OLL 3	54.52	0.98	12.92	13.18	7.04	40.30
OLL 4	55.52	0.92	12.60	13.70	7.00	40.70
OLL 7	57.51	0.94	13.20	14.04	7.59	41.20
Valencia control/SW	48.46	0.79	12.91	16.34	6.26	39.10
OLL 21	58.20	0.77	12.38	16.08	7.20	40.30
OLL 10	55.55	0.82	12.42	15.15	6.90	40.60
OLL 5	53.92	0.93	13.13	14.12	7.08	40.10
OLL 23	57.21	0.81	12.12	14.96	6.93	39.90
OLL 19	54.02	0.84	12.32	14.67	6.65	40.90
OLL 1	55.10	0.90	12.90	14.33	7.11	41.50
OLL 6	58.19	0.96	12.85	13.39	7.48	40.80
OLL 3-10 dwarf	53.80	0.80	12.51	15.64	6.73	41.20
OLL 8	56.43	0.77	11.99	15.57	6.77	40.70

‘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

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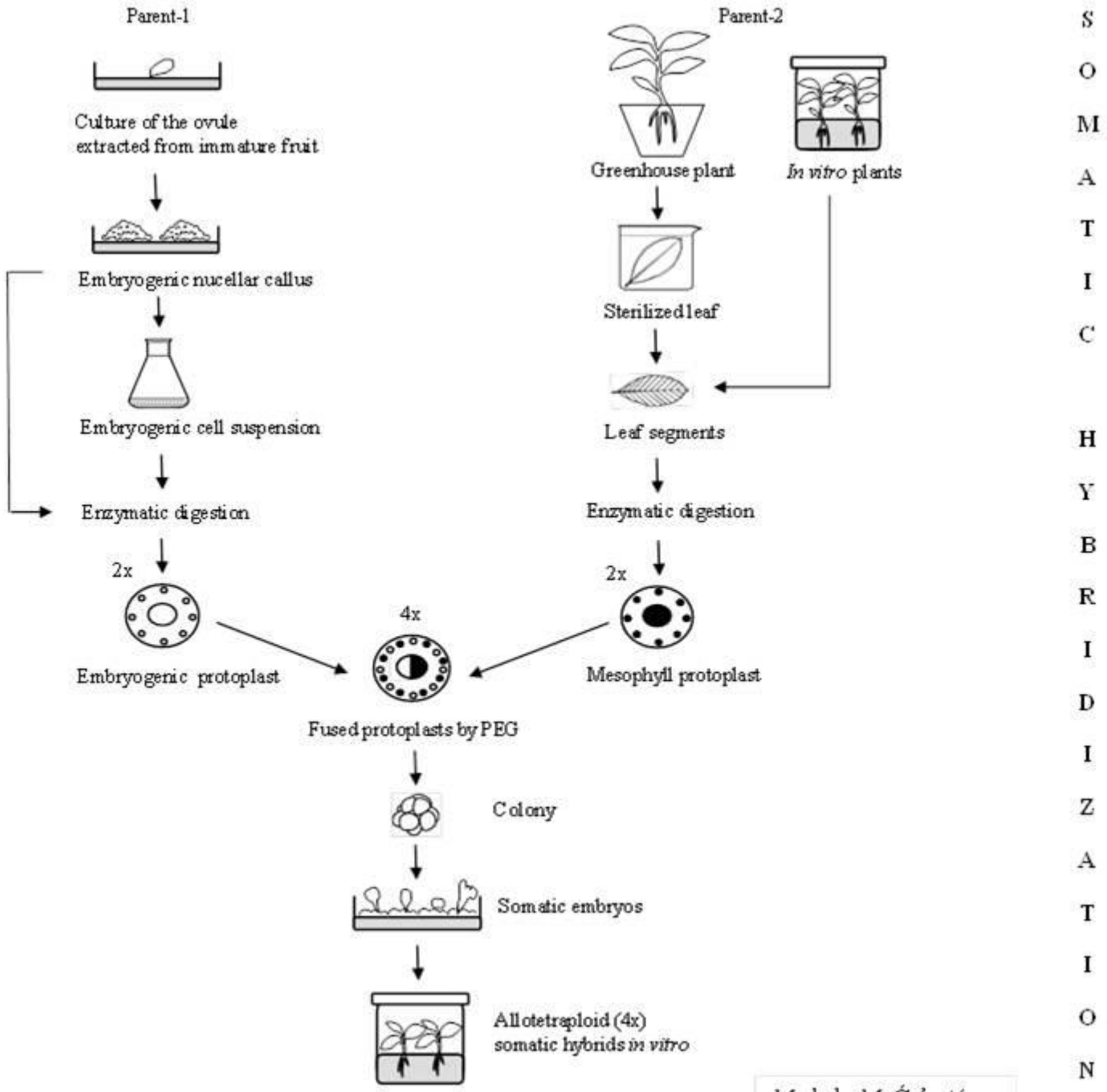
UNIVERSITY of FLORIDA

Our Primary Approach for Developing Novel Seedless Fresh Citrus Fruit – Triploidy! (just like banana and new watermelons)

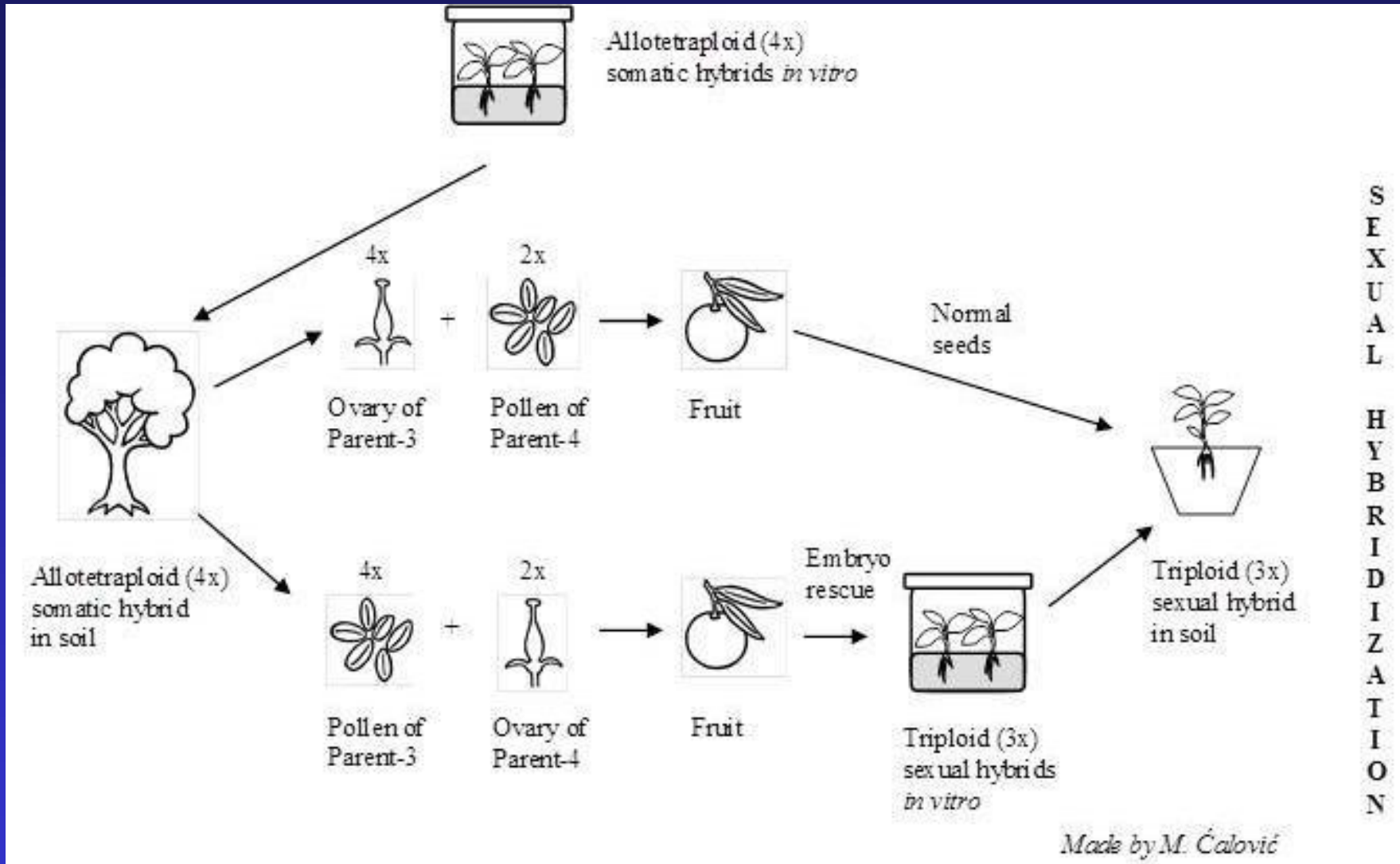
1. Somatic Hybridization Via Protoplast Fusion to Generate Elite Allotetraploid Breeding Parents (Mostly Interspecific Combinations)

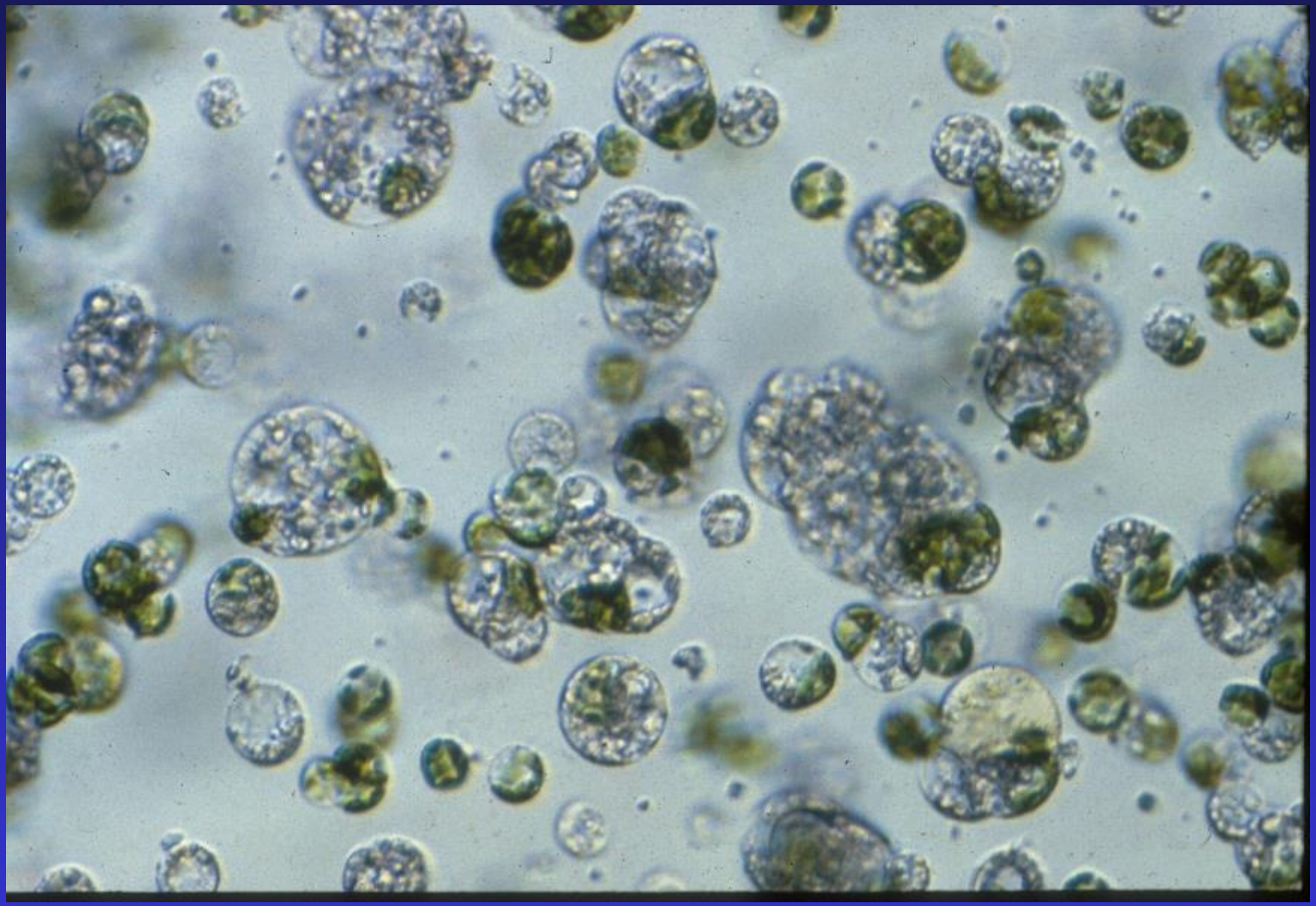
2. Interploid Hybridization (Mostly Interspecific)– Crosses of Diploid Monoembryonic Citrus with Selected Tetraploids as Pollen Parents (Reciprocal Crosses when Possible)

3. Embryo Rescue and Micro-grafting to Expedite Seedless Triploid Recovery (Crosses with Tetraploids as the Female do not Require Embryo Rescue)

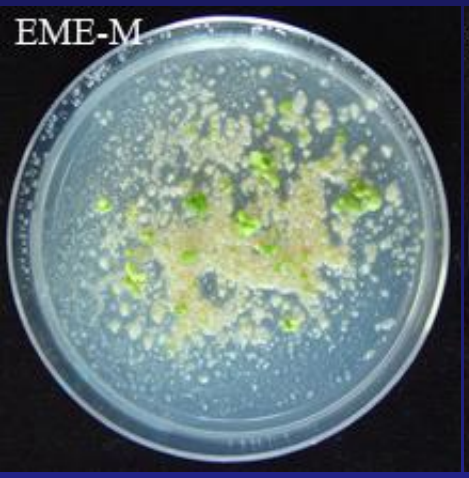


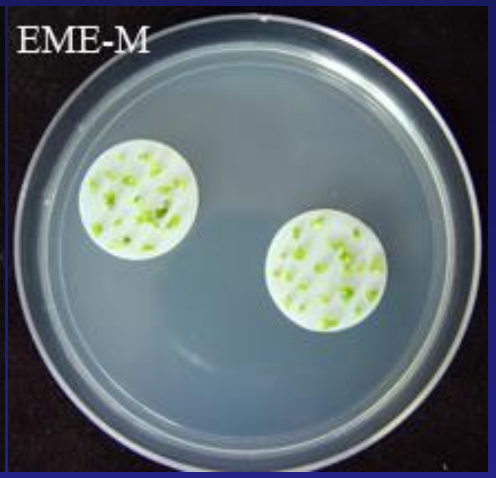
SOMATIC HYBRIDIZATION

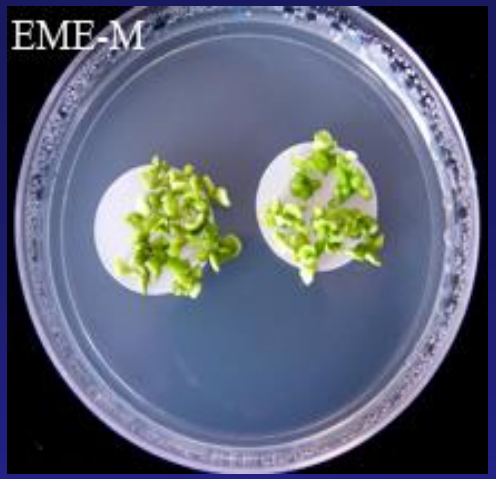

Allotetraploid (4x)
somatic hybrids *in vitro*
4x
2x
Ovary of
Parent-3
Pollen of
Parent-4
Fruit
Normal
seeds
Allotetraploid (4x)
somatic hybrid
in soil
4x
2x
Pollen of
Parent-3
Ovary of
Parent-4
Fruit
Embryo
rescue
Triploid (3x)
sexual hybrids
in vitro
Triploid (3x)
sexual hybrid
in soil
SEXUAL HYBRIDIZATION
Made by M. Ćalović

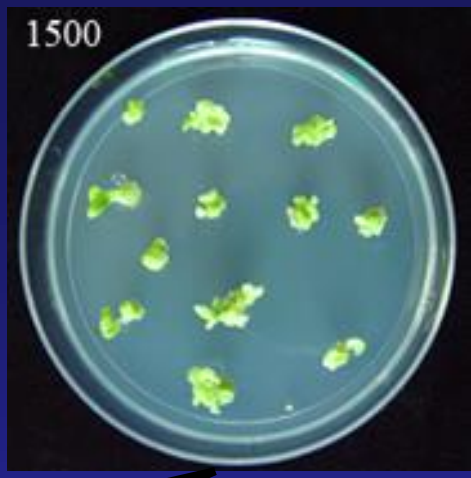


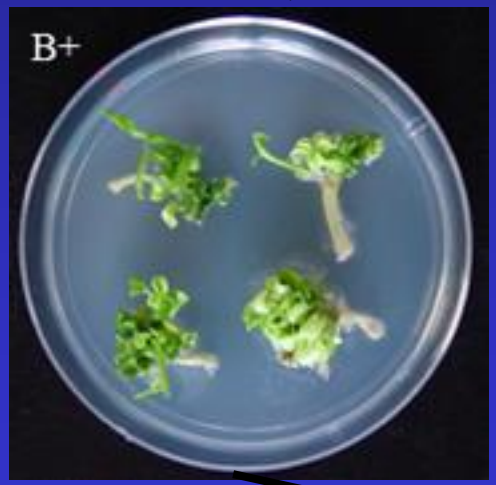
TYPICAL SUSPENSION PROTOPLAST + LEAF PROTOPLAST PEG-INDUCED FUSION

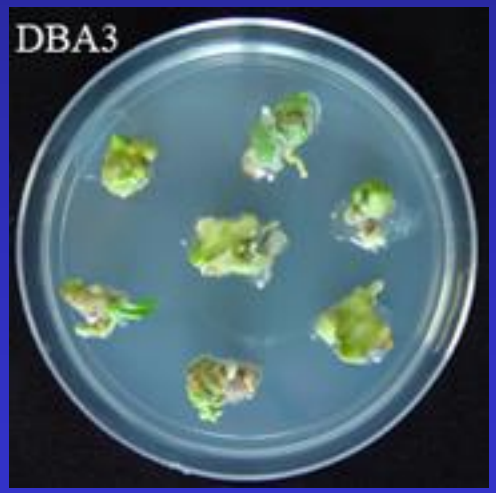


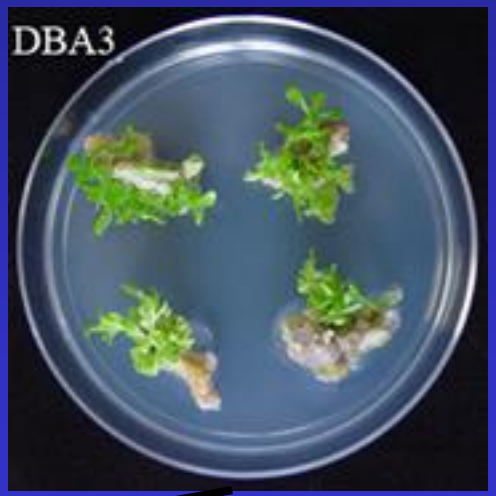




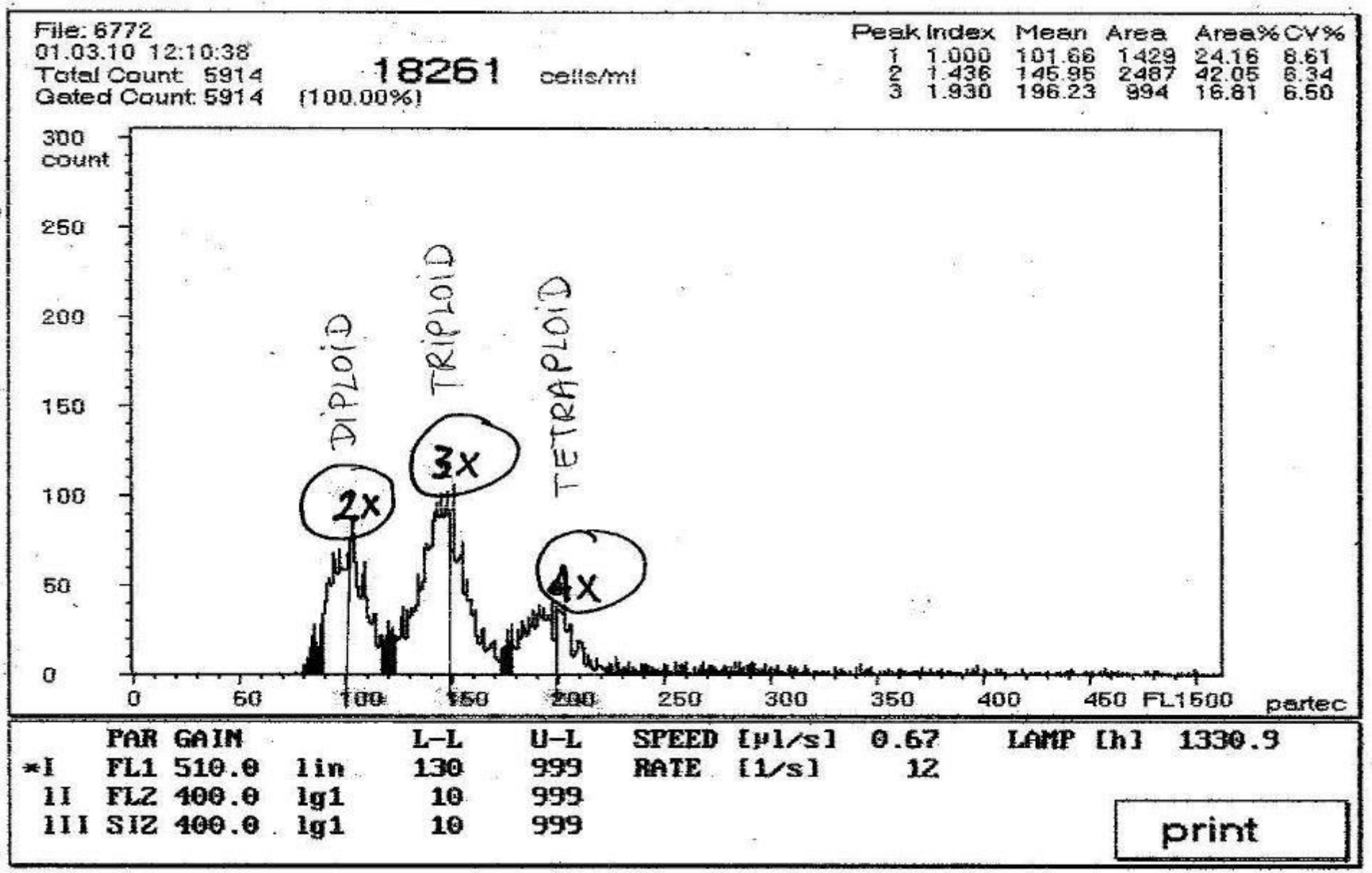








RMAN



Ploidy Analysis Showing Diploid, Triploid and Tetraploid Peaks , Using a Partec Tabletop Flow Cytometer

NOVA + SUCCARI SOMATIC HYBRID TREE

Interploid hybridization using tetraploid somatic hybrids as pollen parents to produce seedless triploids for mandarin improvement:

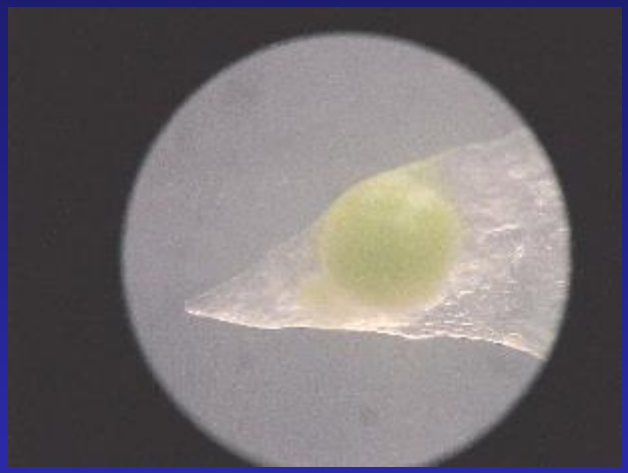
- **more than 16000 triploids produced to date,many fathered by somatic hybrids (under direction of FG Gmitter,CREC)**

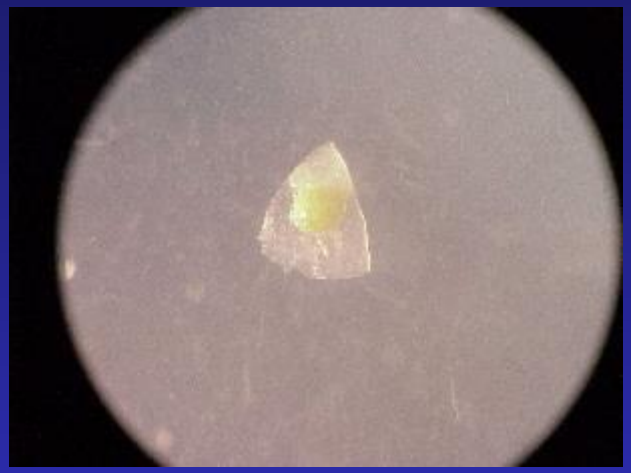
- **oldest hybrids are now fruiting and most are seedless!!!!!**

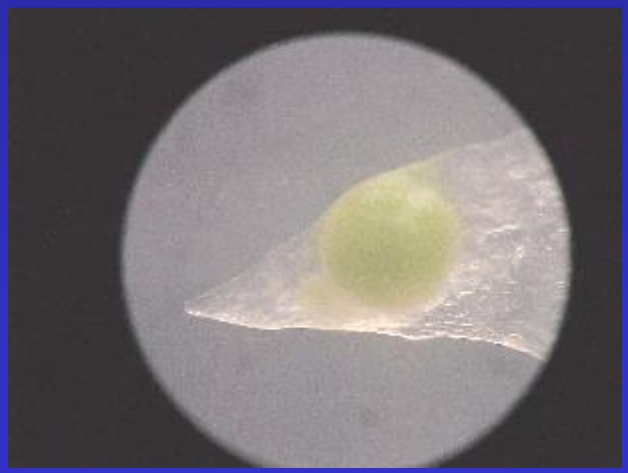
Rohde Red Valencia + Dancy
harvested January 28,2004
brix=11.4, acid=1.57, ratio= 7.26
3 seeds/fruit

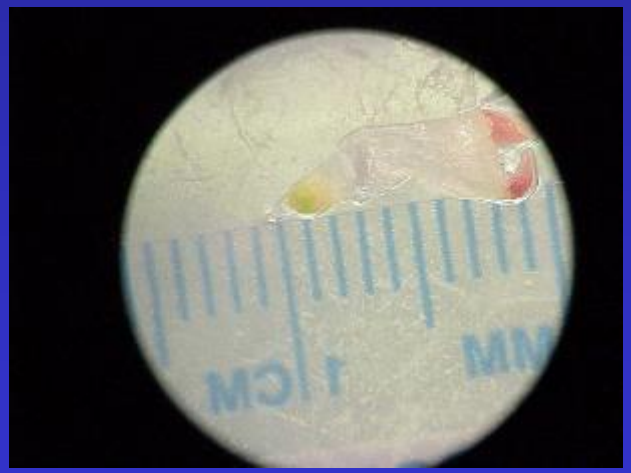
Page + (Clementine x Satsuma)
First zipperskin tetraploid with large fruit size and rich flavor!
First triploids in the ground!

Embryos at Rescue









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

Sugar Belle® near Vero Beach, HLB+ >8 years !

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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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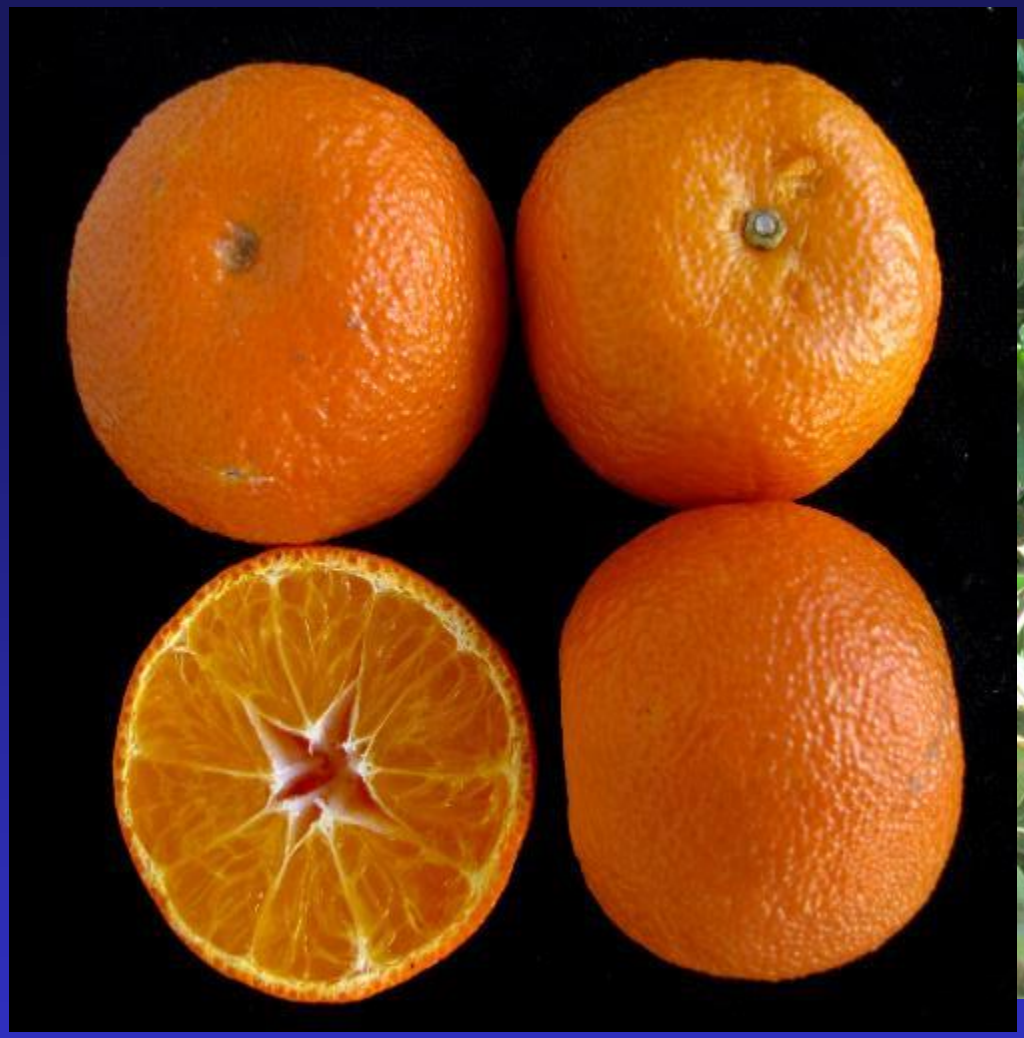
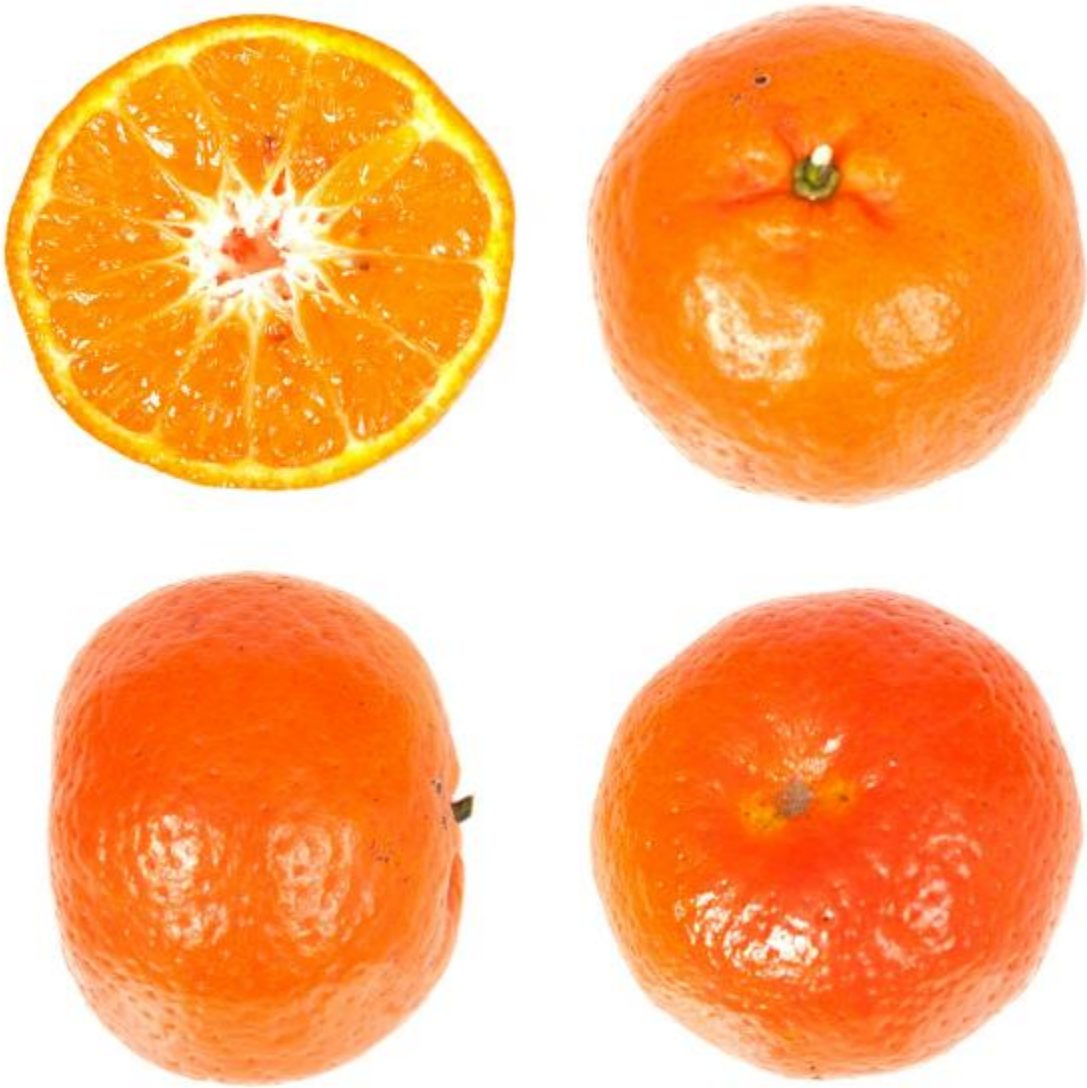


Triploid mandarin hybrid – C4-15-19: Sugarbelle x Nova mandarin+ Succari sweet orange somatic hybrid. First triploid hybrid ever released fathered by a somatic hybrid! Nick-named “Kid’s Favorite”

UF 950

- EZ to peel, seedless
- Clementine size
- Convenient to eat
- Crisp texture
- Better color and flavor than Clementine
- December maturity
- Alternaria resistant
- HLB tolerant
- Moved into commercial plantings



‘BINGO’

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‘Bingo’ on 4/4/16

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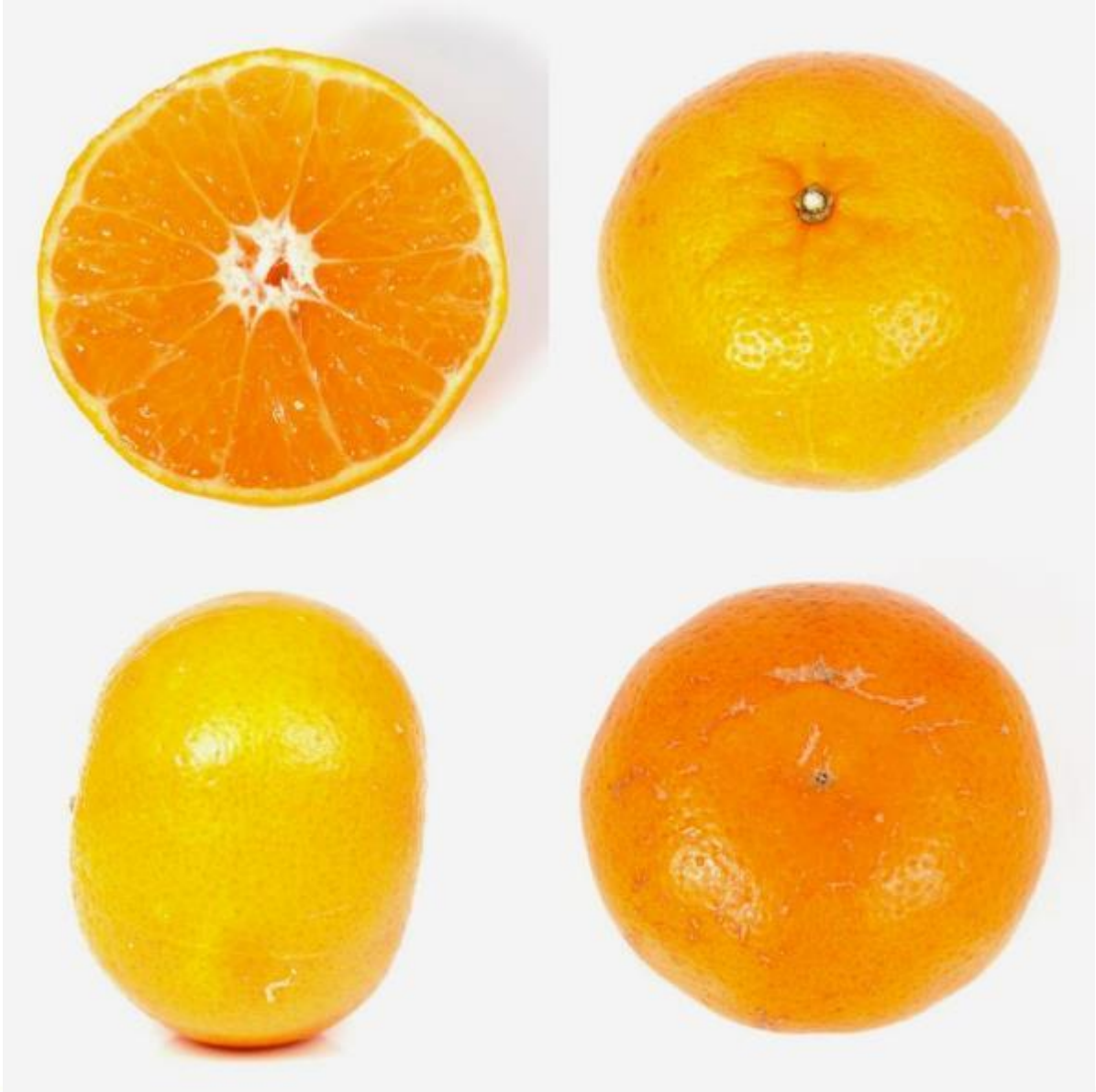

*

‘Marathon’

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- **Brix: 12.5 Acid: 0.89 Ratio: 14.04 19 August 2015**
- **Brix: 13.0 Acid: 0.59 Ratio: 22.03 25 September 2015**
- **Brix: 14.0 Acid: 0.69 Ratio: 20.28 29 October 2015**
- **Brix: 17.4 Acid: 0.62 Ratio: 28.06 17 December 2015**
- **Fruit remained in sound condition until early January**
- **No clipping required**

1420: Long Maturity Window

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Mexican lime (*C. aurantifolia*) x [Valencia sweet orange (*C.sinensis*) + Femminello lemon (*C. limon*)]. EARLY

Development of New Grapefruit and Grapefruit-like Varieties for Florida

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Citrus Research and Education Center

Jude Grosser, Fred Gmitter
Ahmad Omar, Mayara Murata, Christine Chase, Qibin Yu, Aditi Satpute, Melinda Grosser, Jim Graham and Ethan Nielsen

Wheat Breeder's Conference
- 2018

Breeding Canker Tolerant Triploid Seedless Grapefruit-like Hybrids

Tetraploid Somatic Hybrid

X

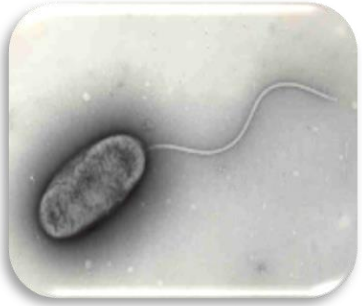
Canker Tolerant Pummelo

-Several good breeding parents now flowering
-Hundreds of triploid hybrids already produced
-Embryo rescue not required when tetraploid parent is used as the female

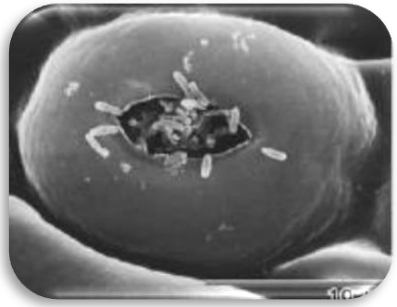
Triploid hybrid fruit – 2/3 pummelo, 1/3 sweet orange 15 ratio October 1st!

CITRUS CANKER

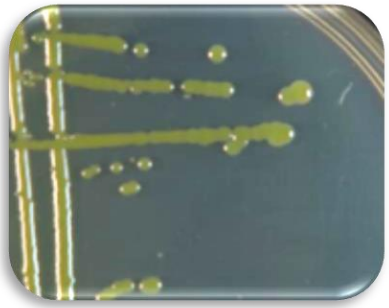
- *Xanthomonas citri* subsp. *citri* (Xcc)



Jaciani, 2008



Graham et al., 2004



Mayara Murata

- Symptoms

Gottward et al., 2012

Fundecitrus, 2012

Mayara Murata

Red pummelo 8-1-99-5B

Murcott+Chandler #81
somatic hybrid (4x)

neighbor

Breeding for canker & HLB resistance: canker epidemic causing a naturalscreen of CREC germplasm – leading to the identification of superior canker &HLB tolerant diploid and tetraploid breeding parents for use ininterploid crosses to generate seedless triploids – crosses underway! Hundreds of triploids have been produced.

CITRUS CANKER RESISTANCE SCREENING

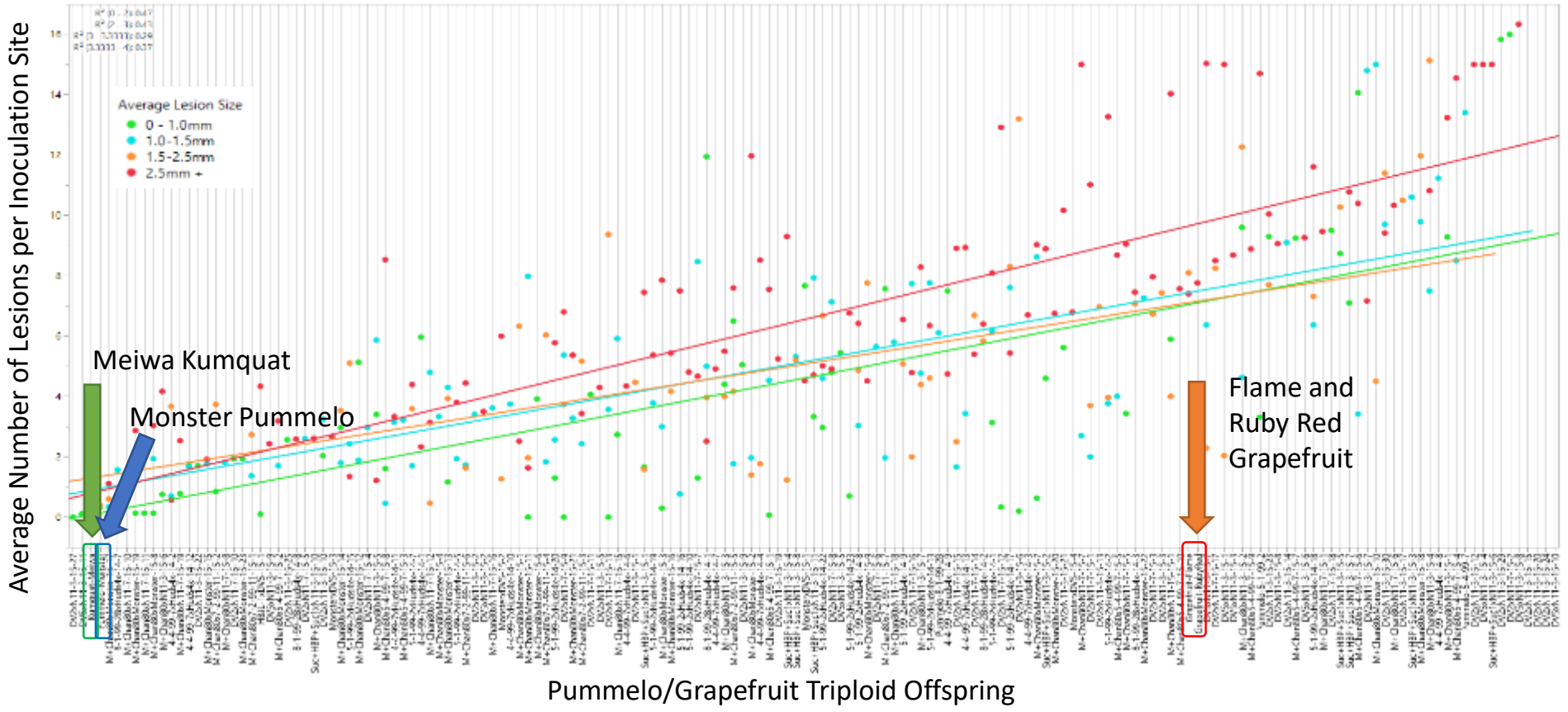
Infiltration inoculation assay (attached leaf assay)

- 10 cybrids and positive (Flame and Marsh grapefruit) and negative controls (Meiwa kumquat)
- Bacterial suspension (Xcc): concentration 10^4 CFU/ml
- 6 inoculation point per leaf: ~ 2 µl (water soaked area of 5-6 mm)
- 6 to 8 leaves per plant
- 5 plants per cybrid
- Experiment done two times

Testing of Triploid Pummelo/Grapefruit Hybrids for Citrus Canker Resistance

Citrus leaves 4 weeks post inoculation with *Xanthomonas citri* var. *citri*, Meiwa kumquat on left

Wide Range of Response of Inoculations with Citrus Canker Among Offspring



Love grapefruit but can't eat it because you're taking prescription drugs?

We may have the answer!!!

UF 914

- Red flesh color
- Attractive peel blush
- Grapefruit size (+)
- Grapefruit flavor and aroma
- Tender and juicy flesh
- Very low in FC’s (GJE)
- Seedless
- Good brix, lower acid
- Slightly thicker peel than grapefruit

SOMATIC CYBRIDIZATION

w/ Chris Chase for genotyping

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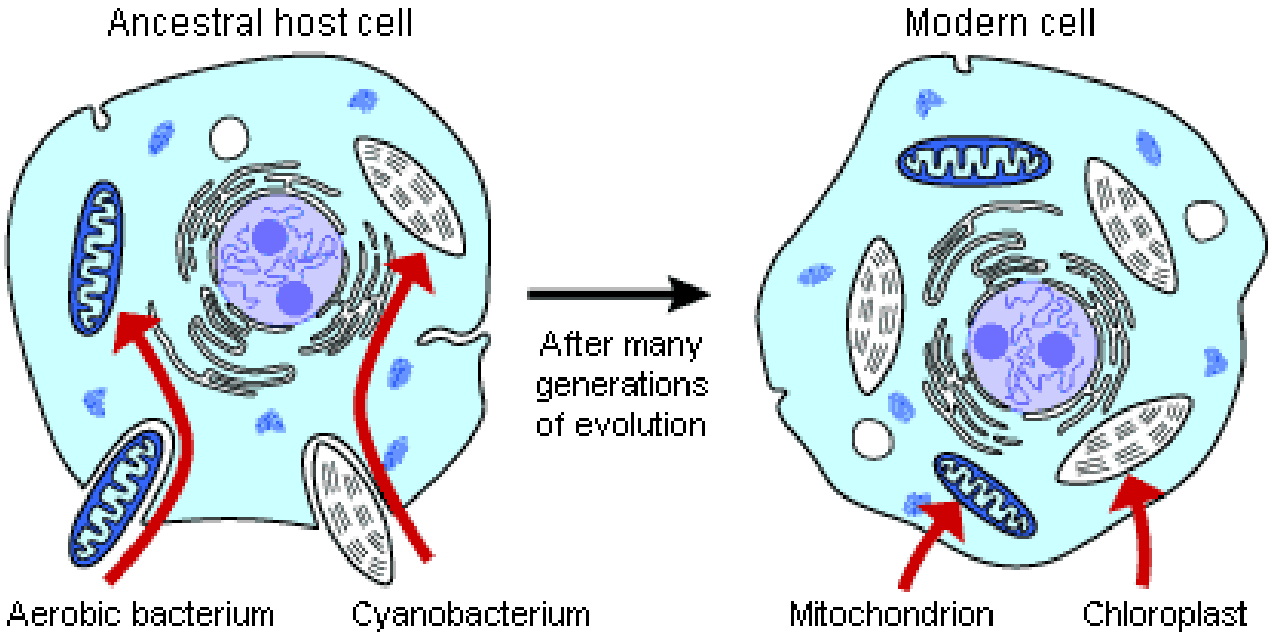
Lynn Margulis



From: http://evolution.berkeley.edu/evolibrary/article/history_24

EndoSymbiosis Theory – Lynn Margulis (1970) published her argument in: *The Origin of Eukaryotic Cells*.

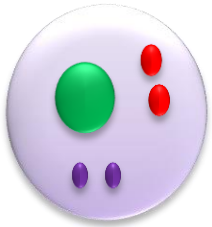
CYBRIDIZATION PROCESS

Ahmad Omar

Highly Susceptible
Fresh fruit market

Ahmad Omar

Highly Resistant



Grapefruit
Citrus paradisi

Kumquat
Fortunella crassifolia

- All genes are not housed in the nucleus. Both mitochondria and chloroplasts have small genomes.
- Citrus cybridization often occurs in citrus somatic hybridization experiments (via protoplast fusion).
- Citrus cybrids from embryogenic culture + leaf cell fusions always have the mitochondrial genome from the culture cell parent, the chloroplast genome is generally randomly inherited. Allows for study of nucleo-cytoplasmic interaction.

Accidental Cybridization – creation of the N2-28 'Summer Gold' grapefruit

Efforts to generate triploids directly by fusing protoplasts isolated from mandarin embryogenic callus with protoplasts isolated from grapefruit pollen tetrads (haploid) resulted in the regeneration of plants with grapefruit morphology. These plants produce delicious grapefruit with an exceptionally long harvest window. We hypothesize that they regenerated from fusions of mandarin suspension cells with contaminate grapefruit pollen wall cells.

UF-CREC Citrus Genetic Improvement Team

2013

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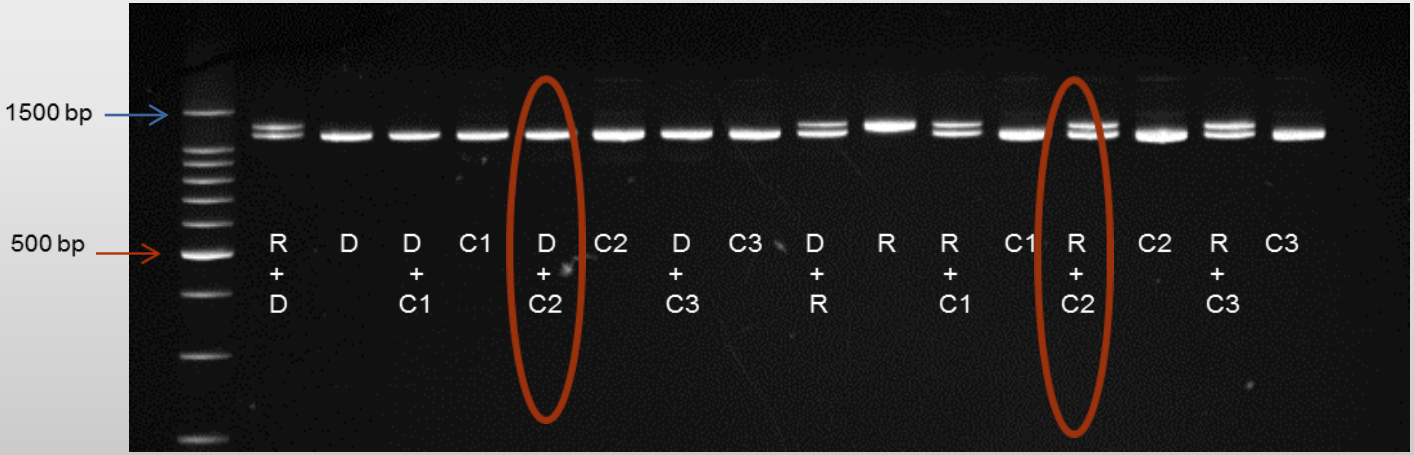
Mitochondrial Genotyping

PCR amplified Cybrid DNA (C1, C2 and C3) in mixture with Dancy (D) and Ruby Red (R) DNA to reveal polymorphism using intron based marker *nad7i1*.



The *nad*7i1 primer set developed by Grosser et al. (in preparation) amplifies a mitochondrial genome target

N2-28 Summer Gold Grapefruit

Table 1. Comparison between summer N2-28 'Summer Gold Grapefruit' and controls 'Ruby Red' and 'Pink Marsh" for the Brix, color and titrable acidity value (average of 20 fruit per selection, test conducted July, 2013).

	Brix°	Color	Titrable Acidity (ml)
Summer Gold N2-28	11.6	34	0.98
'Ruby Red' grapefruit	9.4	34.5	0.85
'Pink Marsh' grapefruit	8.2	34.3	1.2

- Cybrid with 'Dancy' cytoplasm
- Sweeter than Ruby Grapefruit
- Harvest from December to August!
- No granulation or seed germination

TARGETED SOMATIC CYBRIDIZATION

w/ Jim Graham and Mayara Murata

Citrus Transformation

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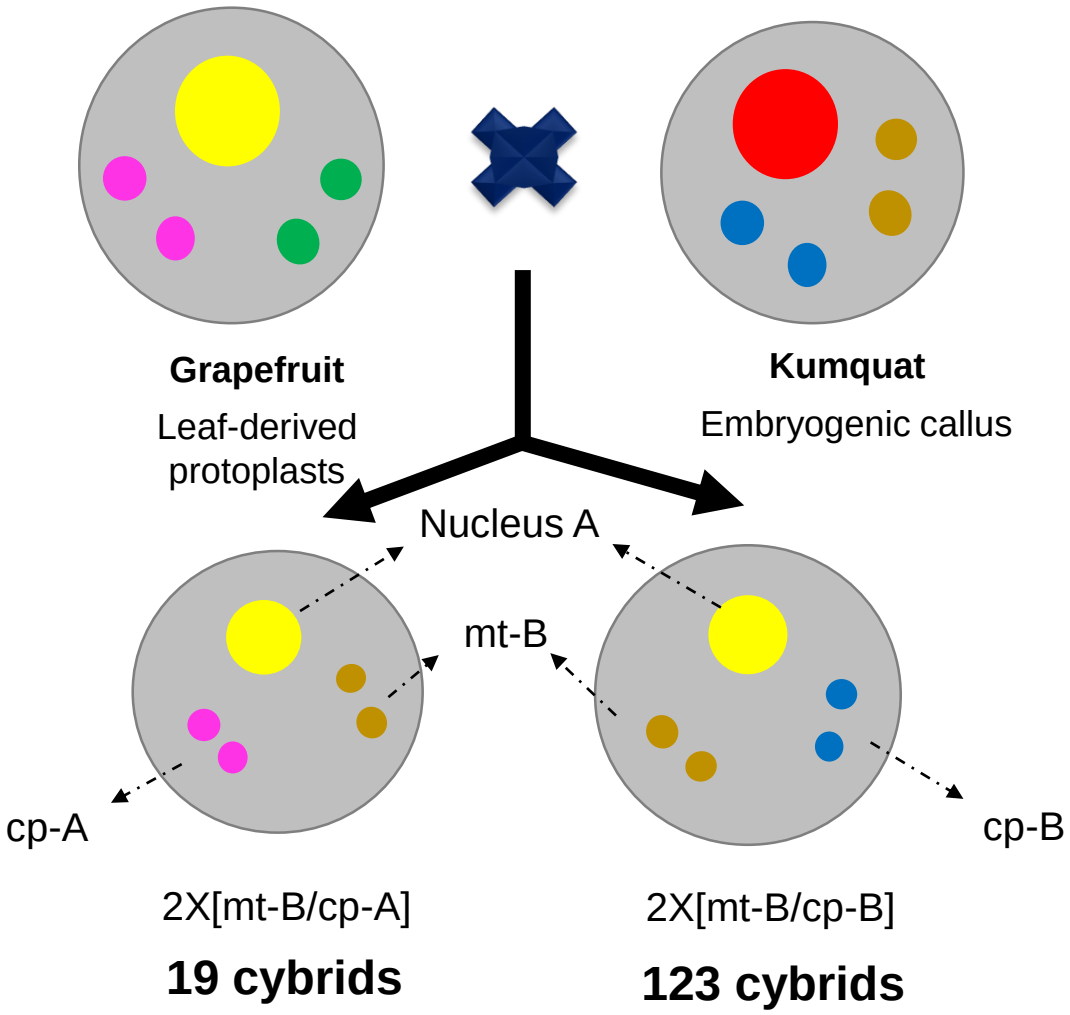
Example of grapefruit cybridization with kumquat cytoplasm



Somatic cybrid plants of Grapefruit containing Mewia ‘Kumquat’ cytoplasm (mitochondrial genome).

Somatic cybrid plants of White Marsh, Flame and red somaclone N11-11 grapefruit containing Meiwa Kumquat cytoplasm (mitochondrial genome)

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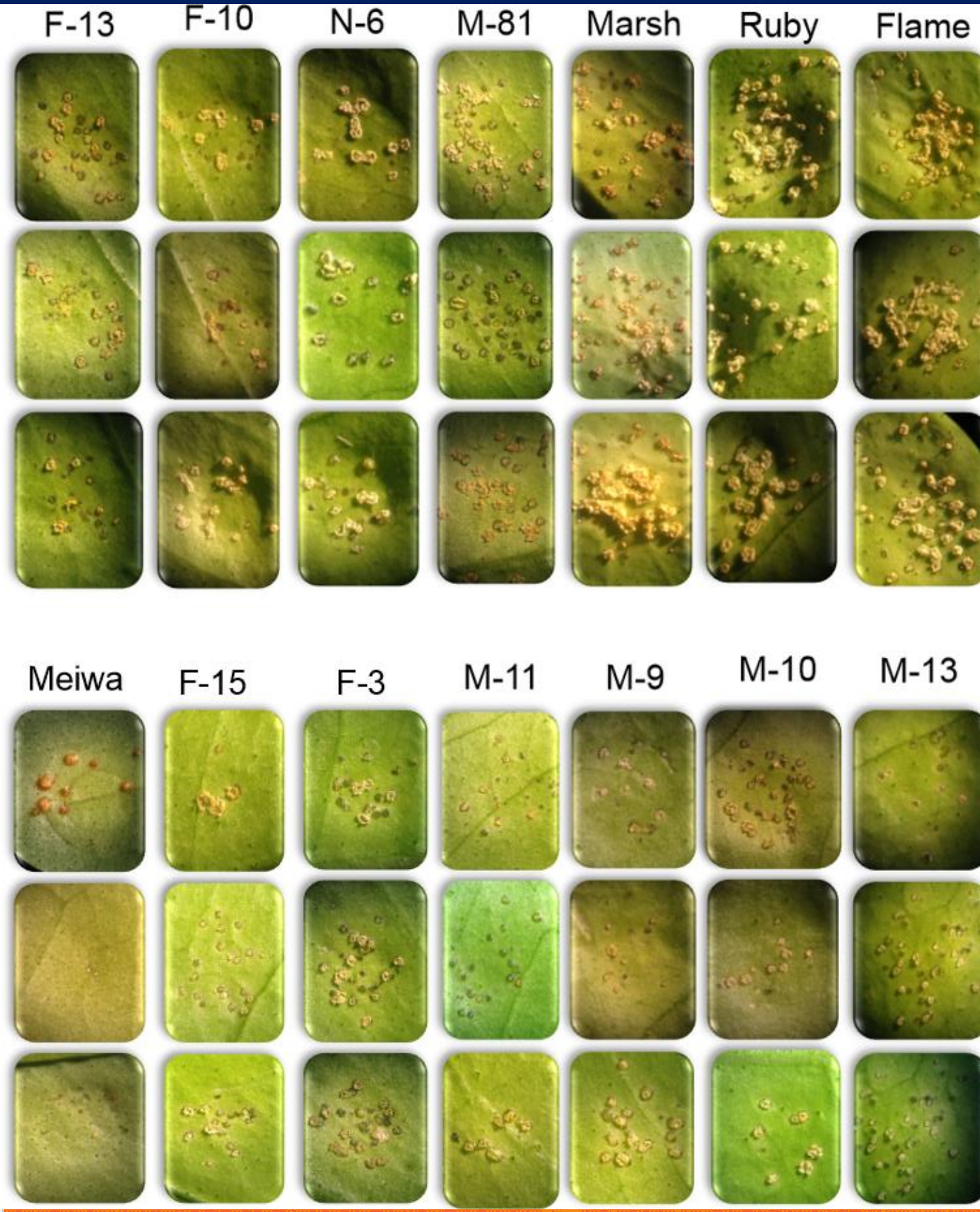

OBJECTIVE 2

Citrus Rootstock Breeding for an HLB-Endemic Florida: The Way Forward

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Citrus Research and Education Center

Jude Grosser
Fred Gmitter and Bill Castle
w/ Ahmad Omar, Lili Cano and Anas Fadli

January, 2020

Citrus Rootstock Breeding:

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 to HLB for the future, regardless of the scion.

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 (from somatic hybridization and 4x breeding)
Citranges: UFR 7-12
Ichangensis hybrid: UFR 13
Sour orange types: UFR 14-16

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.

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/Box [2019]	Boxes/Tree [2019]	PS/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 (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)

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

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

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

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

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

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

Rootstock cross with good Phytophthora resistance.

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

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

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

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

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

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Top ‘Gauntlet’ rootstock trees in Row 13 at USDA Picos Farm; planted with HLB+ Valencia scion – January 2020.

Sample code	Row	Tree	Rootstock	Height	Health	Fruit	Cal_ct	Diagnosis
1	13	57	MILAM-HBP x Orange 4	6	4	3	30.61	Questionable
2	13	58	C-2-5-12 x C. Latipes-13-75	5.5	4	2	27.71	HLB Positive
3	13	62	A-HBP x SORP-13- 72	5	4	2	31.68	Questionable
4	13	64	C-2-5-12 x C. Latipes-13-54	7	4	2	26.51	HLB Positive
5	13	68	C-2-5-12 x C. Latipes-13- 44	6	4	2	30.10	Questionable
6	13	71	A-HBP x White 1-13- 25	6	4	2	27.20	HLB Positive
7	13	72	A-HBP x White 1-13- 13	6.5	4	3	33.17	No HLB Found
8	13	76	C-2-5-12 x C. Latipes-13- 5	7	4	2	30.22	Questionable
9	13	91	A-HBP x White 1-13- 37	6	3	3	28.16	HLB Positive
10	13	102	A-HBP x White 1-13- 1	5.5	3	3	26.26	HLB Positive
11	13	109	C-2-5-12 x C. Latipes-13-1	5.5	3	3	27.52	HLB Positive
12	13	116	A-HBP x SORP-13- 79	6	4	2	37.28	No HLB Found
13	13	129	A-HBP x White 1-13- 20	6	4	1	33.67	No HLB Found
14	13	138	A-HBP x SORP-13- 12	5	3	3	25.08	HLB Positive
15	13	141	Orange 15 mutation 16-2X	5	4	1	25.64	HLB Positive
16	13	146	A-HBP x SORP-13- 50	5.5	4	3	27.18	HLB Positive
17	13	156	C-2-5-12 x C. Latipes-13- 53	6	3	3	26.48	HLB Positive
18	13	160	Orange 15 mutation 25-4X	5.5	3	3	34.00	No HLB Found
19	13	167	Orange 15 mutation 34-4X	7	4	3	40.00	No HLB Found
20	13	172	C-2-5-12 x C. Latipes-13- 74	3.5	4	2	30.95	Questionable
21	13	173	A-HBP x SORP-13- 48	4.5	3	3	33.30	No HLB Found
22	13	180	C-2-5-12 x C. Latipes-13- 11	7	4	2	31.85	Questionable
23	13	183	A-HBP x SORP-13- 10	6.5	4	3	40.00	No HLB Found
24	13	184	A-HBP x SORP-13- 20	4	3	3	40.00	No HLB Found
25	13	192	C-2-5-12 x C. Latipes-13- 77	6.5	4	3	40.00	No HLB Found
26	13	193	A-HBP x SORP-13- 60	4	3	3	40.00	No HLB Found

PCR performed at Southern Gardens diagnostic lab, c/o Mike Irey

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!

3-year old Valencia on gauntlet rootstock A+HBPxSORP-13-60 at USDA Picos Farm, planted HLB+, now HLB-negative (photo from October, 2019).

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						
	144			**111**			**147**

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

Improving delivery of trial information to growers

- Citrus Improvement Team Website Now Online – one stop shopping for growers/processors, packers. Data from 16 trials at present:
- https://citrusresearch.ifas.ufl.edu/rootstock-field-data/
- Website will eventually have tabs to General Information, CRDF supported trials, MAC trials, and NIFA grant progress.
- Website will also provide links to FFSP, NVDMC, etc. for information on scion and rootstock access/licensing, etc.

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 2nd '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.

Clues Emerging Regarding the Relationship of Nutrition and Root Health in HLB-infected trees

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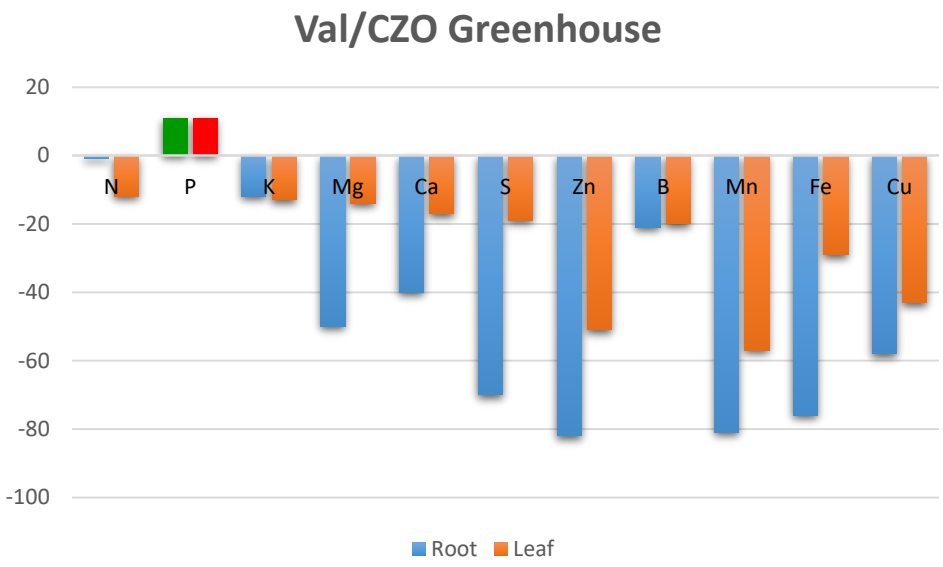
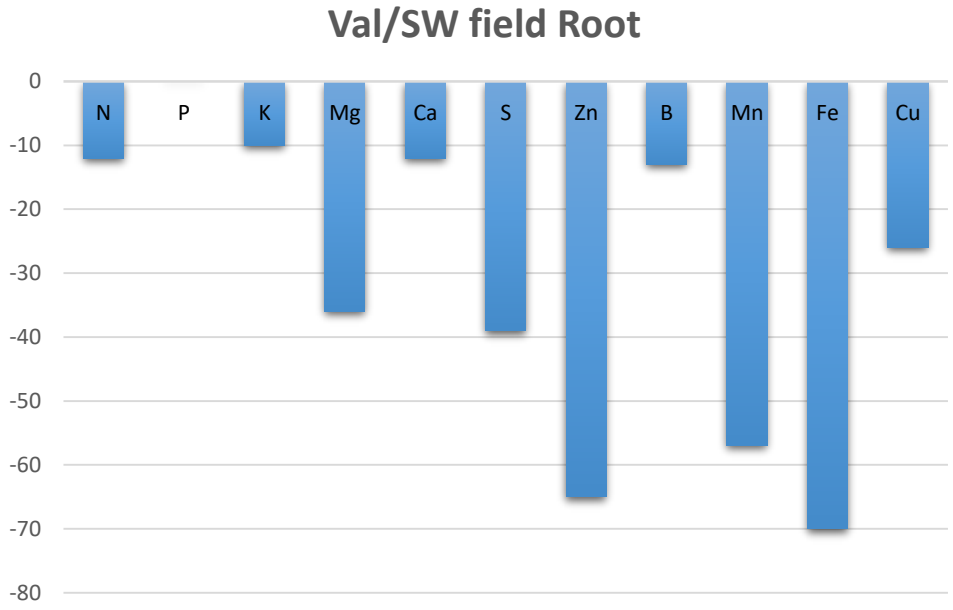
Orie Lee, Frank Rogers, Ed English, Alton Green,
Jobie Sherrod, Jim Graham, Bryan Belcher, Matt Shook, Trey Whitehurst, Brian Patterson, Jim Chason, Ward Gunter, Jack Zorn, &

Lake County – CREC 2018

HLB Impacts Root Micro-nutrient Metabolism



- Comparison of Healthy/Symptomatic trees (average of 10 trees) - % change compared to healthy GH trees
- Root deficiencies are much higher than leaf deficiencies



- Comparison of Healthy/Symptomatic trees - % change compared to healthy field trees
- Same patterns as greenhouse trees
- Soil pH and micro-nutrient content not responsible!
- Foliar sprays do not address this!

Supplemental Nutrients in Controlled Release Forms

<u>Micronutrients – applied at 3x concentration</u>
Tiger-Sul Micronutrients Zinc 18% (18% Zn, 65% S)
Tiger-Sul Micronutrients Iron 22% (22% Fe, 55%S)
Tiger-Sul Micronutrients Manganese 15% (15%Mn, 65% S)
Tiger-Sul 'Arnolds mix' (3.85% Fe, 7.50% Mn, 5.85% Zn, 63% S)
Florikote Polymer Coated Sodium Borate (8.82% B)
Florikote Polymer Coated Magnesium Sulfate (13.9%)
Florikote Polymer Coated Triple Super Phosphate (40% P_2O_5)
Florikote $FeSO_4$ Polymer Coated Ferrous Sulfate (28% Fe, 17% S)
BioChar from Southern Yellow Pine (97%)

<u>Macaronutrients—applied at 2x concentration</u>
Florikote Polymer Coated Mini Ammonium Sulfate (19% N)
Florikote Polymer Coated Sulfate of Potash (47% K_2O)
Florikote Polymer Coated Urea (42% N)

Polycoated Florikote products kindly provided by Brian Patterson (Florikan Corp.)

Stick-graft method – Valencia budstick taken from heavily HLB-impacted field tree. Graft wrapped with budding tape, Budstick wrapped with parafilm. 10 trees per treatment.

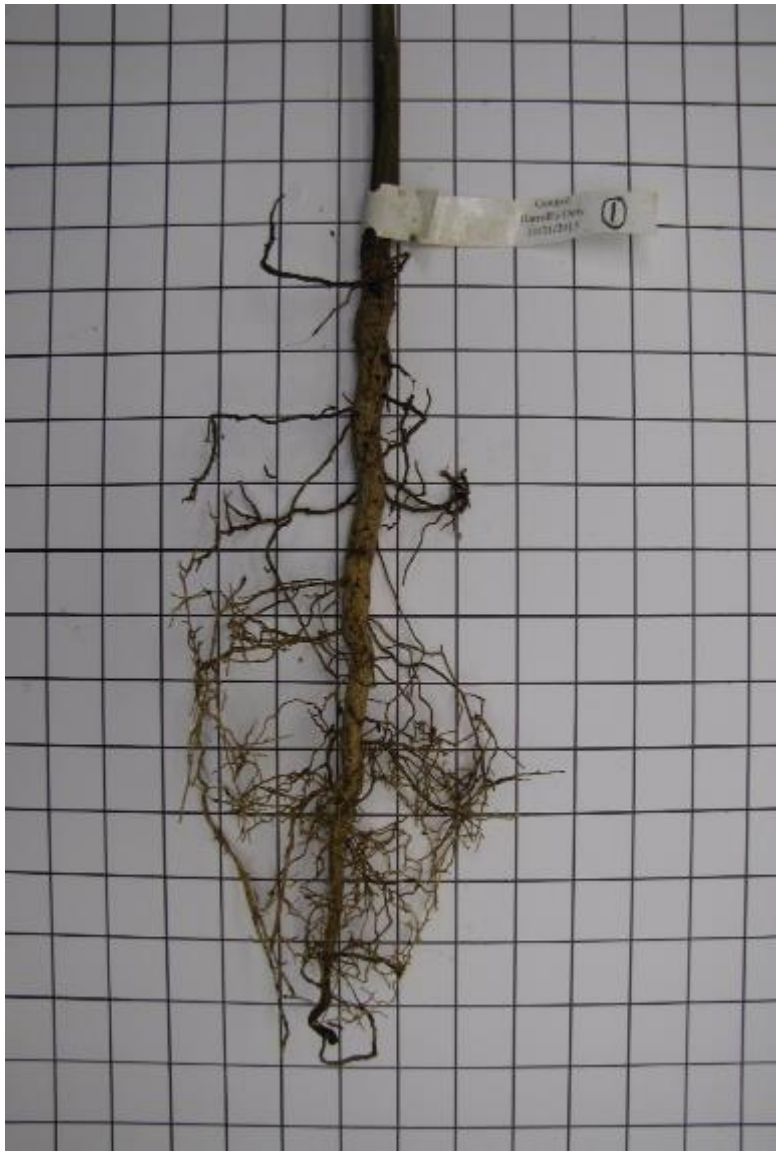
Greenhouse Study – Effects of nutrient overdoses on HLB-infected Valencia on UFR-3 (Orange #15) tetrazyg rootstock after 1 year. Total Root length (cm) , determined by winRhizo washed root image analysis.

Treatment	N	Mean*	Standard Deviations	Tukey Grouping
Harrell's + 3x TigerSul Mn	10	2361	848	A
Harrell's + 3x Tiger-Arnold's Mix (Mn, Fe, Zn)	9	2270	933	A
Harrell's + 3x TigerSul-Arnold's + Biochar	9	1955	1237	AB
Harrell's + 3x Tigersul Zinc Sulfur	10	1672	1039	AB
Harrell's - Control	8	1670	900	AB
Harrell's + 3x Florikan Sodium Borate	10	1554	1466	AB
Harrell's + 3x Tigersul Fe	7	1419	704	AB
Liquid Fertilizer Only - Control	6	1349	1273	AB
Harrell's + 3x Florikan Magnesium Sulfate	8	1315	1025	AB
Harrell's + 2x Florikan Ammonium Sulfate	8	1276	805	AB
Harrell's + 2x Florikan Urea	8	1173	766	AB
Harrell's + 3x Florikan Iron Sulfate	7	1032	544	AB
Harrell's + 3x Florikan Super triple Phosph	6	910	642	AB
Harrell's + 2x Florikan potash	4	902	226	AB
Harrell's + Biochar	9	559	403	B

* Means with the same letter are not significantly different at 95% confidence

Control liquid fertilizer Harrell's CRF+TigerSul Mn

HLB-infected greenhouse trees after one year; Valencia/UFR-3.



Harrell’s CRF Control #1

Harrell’s+TigerSul Mn #10

Hughes Post Office Block Yields – New 'Hybrid' nutrition program with micronutrient treatments

13 year old Valencia/Vernia trees on mostly Swingle and some C-35, 100% HLB

Overall yield for 2015 harvest bottomed out at **1.25** boxes per tree, severe drop

2015: change from traditional program to 50-50 traditional/Basacote CRF (200 lbs. N per acre), 2 applications plus the per row treatments below.

Data per 2 rows (approx. 95 trees per row):

Treatment	Boxes/tree 2016	Boxes/tree 2017
Arnolds TigerS mix#	1.50	1.70
3x polycoated boron*	1.72	1.71
3x TigerS manganese	1.69	1.74
3x Tiger mn + 3x pc boron	1.79	1.68
Arnolds Mix + 3x mn + 3x boron	1.89	**2.00**

#Arnolds Mix: TigerSul Fe + TigerSul Mn + TigerSul Zn
*Florikan product
One box = 90 lbs. or 40.8 kg.

Hughes Post Office Block (Haines City, 10 acres) - Overall Yield and Lbs. Solids

13 year old Valencia/Vernia trees on mostly Swingle and some C-35, 100% HLB

Year	Total Boxes	Lbs. Solids/box	Total Lbs. Solids	% change lbs. solids
2015	1567	5.77	9,041.6	___
2016	2282	5.80	13,228.5	+46.3
2017	2233	6.06	13,524.4	+02.2

One box = 90 lbs. or 40.8 kg.

2017 crop reduced slightly by PFD

13-year old Valencia/Swingle, 100% HLB-infected; after 2 years on 50/50 CRF/dry soluble fertilizer program (2 applications/year);Trees have good crop, fruit sizing well – 2nd consecutive yield increase expected.

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.

What are ‘HYBRID’ Nutrition Programs?

Any program that combines multiple sources of nutrient delivery with a goal of providing a constant supply of all required nutrients year round (including winter!) at an affordable cost. Can be tailored to address micronutrient deficiencies in HLB-impacted roots. Continued fine-tuning will improve results and lower costs!

Examples:

1. Fertigation supplemented with CRF (Controlled Release Fertilizer) during the rainy season (Tropicana program).

2. Traditional dry soluble N & K, monthly liquid micronutrient nitrates; separate liquid phosphoric acid (E. English program).

3. Traditional NPK supplemented with CRF: (Duda program) – 30/70% CRF/WS January; 50/50% CRF/WS April; 30/70% CRF/WS September.

NO SILVER BULLET, BUT THESE PROGRAMS WORK!!!

Ed English (Alton Green) Program:

Citra-Guard Nitrate Soil Ammendment 7-0-0; monthly treatment. Material is being applied through airblast sprayer, bottom two nozzle ports open on each side. It is applied at 50 GPA.

7% Nitrate nitrogen
0.75% copper
4.60% iron
3.80% manganese
3.80% zinc
all nitrate derived

Phosmax (Phosphorous Acid) @ 1 quart per acre. The goal is to get 60 lb of phosphorus per acre per year.

Dry and Foliar: 11-37-0

Revived 100% HLB-infected Valencia/Swingle trees in Alva, Ed English (Alton Green) monthly liquid nitrate program. Concept of ‘tree momentum’

Inside fruit on Ed English Valencia/Swingle trees; 7.4 lbs. solids!

Evolution to the McKenna Liquid Program:

- they are applying the 5-0-7 'strawberry mix' at 10gal/acre (calcium, potassium and magnesium nitrates) mixed with 1 quart/acre of the CitraGuard (micro-nitrates – see below). This is alternated weekly with a 9-4-9. They do not apply during weeks when they are spraying and during periods of heavy rain.

Citra-Guard Nitrate Soil Amendment 7-0-0; It is applied at 50 GPA.

7% Nitrate nitrogen
0.75% copper
4.60% iron
3.80% manganese
3.80% zinc
all nitrate derived

Results: increased yield and fruit quality, reduced fruit drop in first year across several groves, scion/rootstock combinations and trees of different ages! Take care of your roots!

14 year-old Valencia/rough lemon +CRF + extra Mn and boron

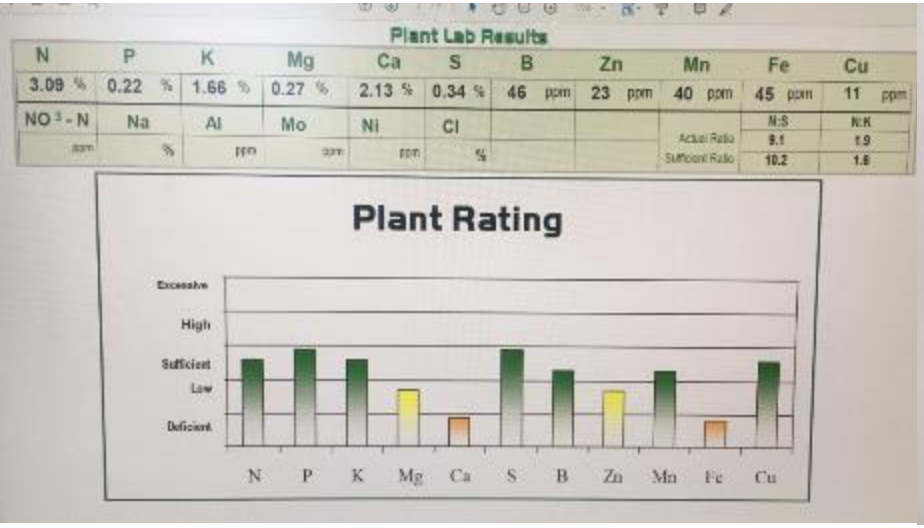
Alligator Matthew Block Nutrition Study – 2017 December PCR and yield results

Vernia/rough lemon (10-years old; treatments started fall of 2015, 12 trees per treatment (2-six tree reps); 2017 harvest heavily impacted by PFD. Last column is boxes per treatment (12 trees) since trial began (2-years of production). Products: Harrells CRF St. Helena mix, TigerSul mn, Florikan polycoated boron. CT value 32 or above considered negative for Liberibacter.

Treatment	CT Value mean	SD	# trees 32+ ct	Yield (B/T): 2016	2017	2018	Cumm
1 standard	23.19	4.8	0	1.67	0.56	1.71	27.2
2 + Harrells	27.81	5.3	5	1.50	1.02	1.75	33.2
3 + Harr/2x mn	27.57	5.3	3	1.50	0.83	1.54	28.4
4 +Harr/2x bn	29.48	5.4	5	1.92	0.83	1.71	30.5
5 +Harr/2x mn&bn	30.32*	5.5	5	1.50	0.94	1.71	31.8
6 +4x mn	**32.75***	**5.7**	**7**	**1.75**	**0.92**	**2.21**	**37.6**
7 + 4x bn	28.07	5.3	5	1.58	0.44	1.63	24.8
8 +4x mn & bn	23.81	4.8	0	1.50	0.90	1.79	32.3

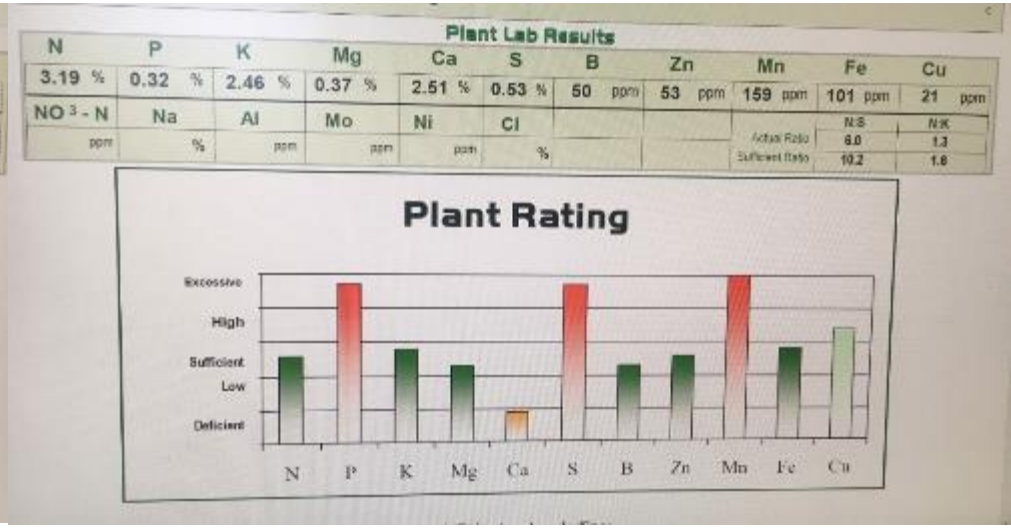
*significantly different than standard at 95% CI

Evidence: Overdoses of manganese can be therapeutic against HLB!!!!!!!



Red grapefruit /Sw on standard CREC soluble dry nutrition

qPCR Ct value 25.4



Recovering Cybrid Flame/Sw on CREC + Harrells 14-3-11 + Florikan Mn

qPCR Ct value 32.9

14-3-11

Harrell's
Growing a Better World®

FERTILIZER

Batch #: 1901-0679

GUARANTEED ANALYSIS

Total Nitrogen (N)*	14.0000%
5.1250% Nitrate Nitrogen	
4.5050% Ammoniacal Nitrogen	
4.3700% Urea Nitrogen	
Available Phosphate (P_2O_5)*	3.0000%
Soluble Potash (K_2O)*	11.0000%
Calcium (Ca)	2.8000%
Magnesium (Mg)	1.3400%
1.3400% Water Soluble Magnesium (Mg)	
Sulfur (S)	10.3430%
6.2130% Free Sulfur (S)	
4.1300% Combined Sulfur (S)	
Boron (B)	0.0360%
Copper (Cu)	0.0360%
0.0360% Water Soluble Copper (Cu)	
Iron (Fe)	0.5000%
0.1600% Chelated Iron (Fe)	
Manganese (Mn)	1.0550%
0.0550% Water Soluble Manganese (Mn)	
Molybdenum (Mo)	0.0050%
Zinc (Zn)	0.5230%
0.0360% Water Soluble Zinc (Zn)	

Derived From: Polymer Coated Ammonium Nitrate, Polymer Coated Ammonium Phosphate, Polymer Coated Calcium Nitrate, Polymer Coated Muriate of Potash, Polymer Coated Sulfate of Potash, Polymer Coated Urea, Elemental Sulfur, Ferric Oxide, Kieserite, Manganese Oxide, Polymer Coated Copper Sulfate, Polymer Coated Iron EDTA, Polymer Coated Magnesium Sulfate, Polymer Coated Manganese Sulfate, Polymer Coated Sodium Molybdate, Polymer Coated Zinc Sulfate, Sodium Borate, Zinc Oxide

* 14% coated slow release Nitrogen(N) from Polymer Coated Ammonium Nitrate, Polymer Coated Ammonium Phosphate, Polymer Coated Calcium Nitrate, Polymer Coated Sulfate of Potash, Urea, 3% coated slow release Available Phosphate(P_2O_5), and 11% coated slow release Soluble Potash (K_2O)

NOTICE: The application of fertilizing materials containing Molybdenum(Mo) may result in forage crops containing levels of Molybdenum(Mo) which are toxic to ruminant animals. Product contains Boron(B). Do not apply to Boron sensitive crops.

Chlorine (Cl), Not more than..... 4.2500%

F302 Density - 65 lb./cu. ft.

Directions for Use

Information regarding the contents and levels of metals in this product is available on the internet at: http://www.aapfco.org/metals.html

Net Wt. 50 LBS. (22.7 KG)

1901-0679 211901

Warning

Dust may cause Serious Eye Damage
Skin, Eye and Respiratory Tract irritant. Harmful if swallowed.

Precautionary Statements: Wear safety glasses with side shields or goggles when handling product. Avoid breathing dust. Wash face, hands and exposed skin thoroughly after handling. Do not eat, drink or use tobacco products when using this product.

First Aid
If in Eyes: Flush with water for 15 minutes. Call a doctor for treatment advice if irritation persists.
If on Skin: Wash thoroughly with soap and water. Launder clothing before reuse.
If Inhaled: Remove affected person from source of exposure. Call 911 if breathing is difficult.
If Ingested: Do not induce vomiting. Get medical attention.
Have the product label with you when calling a doctor or going for treatment.

MANUFACTURED AND GUARANTEED BY HARRELL'S, LLC PO BOX 897 LAKELAND, FL 33802 (863) 687-2774 (800) 282-8007

florikan CRF

with GAL-Xs ONE

12-4-8 Citrus Ag Max

WITH FLORIKAN HUMIC

GUARANTEED ANALYSIS:

Total Nitrogen (N)*	**12%**
5.70% Nitrate Nitrogen	
6.40% Ammoniacal Nitrogen	
Available Phosphate (P_2O_5)*	**4%**
Soluble Potash (K_2O)*	**8%**
Calcium (Ca)	0.50%
Magnesium (Mg)*	2.00%
1.04% Water Soluble Magnesium (Mg)	
Sulfur (S)*	6.10%
6.10% Combined Sulfur	
Boron (B)*	0.26%
Copper (Cu)*	0.05%
0.02% Water Soluble Copper (Cu)	
Iron (Fe)*	2.33%
0.68% Chelated Iron (Fe)	
0.55% Water Soluble Iron (Fe)	
Manganese (Mn)*	1.45%
1.01% Water Soluble Manganese (Mn)	
Molybdenum (Mo)	0.001%
Zinc (Zn)*	0.62%
0.18% Water Soluble Zinc (Zn)	

DERIVED FROM: Polymer Coated Ammonium Nitrate, Polymer Coated Ammonium Phosphate, Polymer Coated Calcium Phosphate, Polymer Coated Ammonium Polyphosphate, Polymer Coated Potassium Sulfate, Polymer Coated Iron EDTA, -Ammonium Sulfate, Calcium Sulfate, Sodium Borate, Manganese Sucrate, Manganese Sulfate, Zinc Sucrate, Zinc Sulfate, - Polymer Coated Magnesium Sulfate, Polymer Coated Iron EDTA, Polymer Coated Iron Citrate, Polymer Coated Manganese Sulfate, Polymer Coated Copper Sulfate, Polymer Coated Zinc Sulfate, -Polymer Coated Magnesium Sulfate, -Polymer Coated Sodium Borate, -Ferrous Sulfate, Iron Sucrate, Iron EDTA, Iron Citrate, Magnesium Sulfate, Magnesium Sucrate, Manganese Sucrate, Zinc Sucrate, Copper Sucrate, Sodium Borate, Sodium Molybdate, | *The Following Nutrients have been Polymer Coated to Provide 12% Slow Release Nitrogen (N), 4% Slow Release Available Phosphate (P_2O_5), 8% Slow Release Soluble Potash (K_2O), 0.6% Slow Release Magnesium (Mg), 4.5% Slow Release Sulfur (S), 0.132 Slow Release Boron (B), 0.02% Slow Release Copper (Cu), 0.55% Slow Release Iron, 0.88% Slow Release Manganese (Mn), and 0.02% Slow Release Zinc (Zn)

The below rates are provided as a guide only. Specific rates will vary by region, soil type, and performance requirements. Before deciding which formulation, release type, and rate to use, contact your Florikan Technical Representative. They will advise you in choosing the right product for your crop and conditions.

DIRECTIONS FOR USE - Florikan CRF™ should be applied according to soil or growing media conditions and the specific nutrient requirements of the crop being grown. It is recommended that a soil analysis is done prior to incorporation of Florikan CRF™. If the soil or growing media is sandy and loose with good drainage (low Cation Exchange Capacity), a higher rate of the product should be used. For heavier, clay-type soils (high Cation Exchange Capacity) a lower rate of Florikan CRF™ should be used.

NOTICE - Warranty of this product, either expressed or implied, is limited to a guarantee of the composition as shown on the label in as much as uses are beyond the seller's control. For the same reason, seller is not liable for any injury to living things, crops, soils or materials which may result from the use of this product.

PER TREE APPLICATION RATES

Type	UP TO 360 DAYS
Item #	12-4-8-360
YEARS IN GROVE	Lbs.
1 YEAR	1.25
2 YEARS	1.75
3 YEARS	2.5
4 YEARS	3-3.25
5 YEARS	3.5-4

This product was developed in conjunction; and with assistance from NASA using cutting edge research, quality control and scientific methodology.

SKU: 12-4-8-360-CIT

U.S. STANDARD - NET WEIGHT 50 LB. (22.68 KG.)

Signal Word: NON-REGULATED MATERIAL

Hazard Statement: Harmful to aquatic life with long lasting effects

Precautionary Statement: Avoid release to the environment - dispose per US laws

UP TO: 360 DAYS

AVG MEDIAN TEMPS

60°		70°		80°	
420	14	390	13	360	12
Days	Mnths	Days	Mnths	Days	Mnths

GUARANTEED BY: F1062

QUALITY CONTROL BATCH NUMBER:

WARNING TO PURCHASER, THIS IS A DISCLAIMER. The performance warranty and/or release of this product, whether stated or implied, is null and void if improperly stored.

Heavy Metal Statement: Information regarding the contents and levels of metals in this product is available on the internet at http://www.aapfco.org/metals.html

CAUTION: KEEP OUT OF THE REACH OF CHILDREN HARMFUL IF SWALLOWED. This product must be stored under cool and dry conditions.

Environmental Caution: As a precaution, fertilizer products should not be applied within 10 feet of any water body.

Caution: The application of fertilizer containing Molybdenum (Mo) may result in forage crops containing levels of Molybdenum which may be toxic to ruminant animals.

WARNING: This product contains Boron (B) and should be used according to directions only, especially on crops sensitive to Boron (B) uptake.

NOTE: Fertilizer products can be hydroscopic and may be affected by humidity and/or heat during improper storage. Please keep this fertilizer product in a dry condition and away from heat and moisture which may start release in the bag.

gv-8213-2

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!

UF UNIVERSITY of FLORIDA
IFAS
Citrus Research and Education Center

To HALL OF FAME CITRUS GROWER-RESEARCHER
And Outstanding Industry Collaborator Mr. Orie Lee

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Thanks!

UF-CREC Citrus Genetic Improvement Team
2019