Highlights from the UF/IFAS/CREC Citrus Improvement Program

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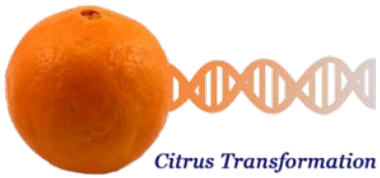

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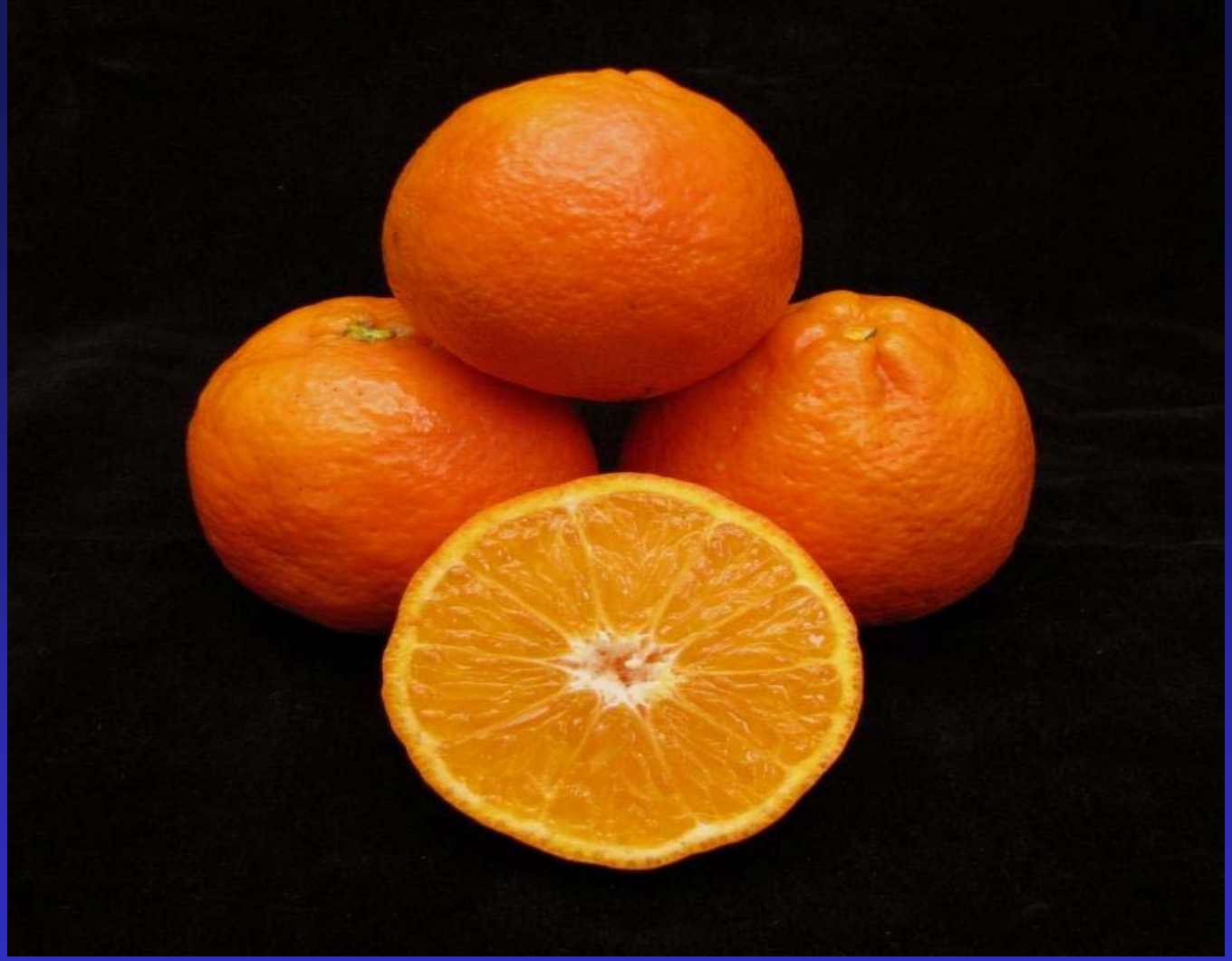
Nova + Osceola
harvested December 6, 2005
brix= 14.8, acid=1.15, ratio= 12.9



Triploid mandarin hybrid – fathered by somatic hybrid Nova + Succari – We now have more than 16,000 triploid hybrids, most are in the ground!

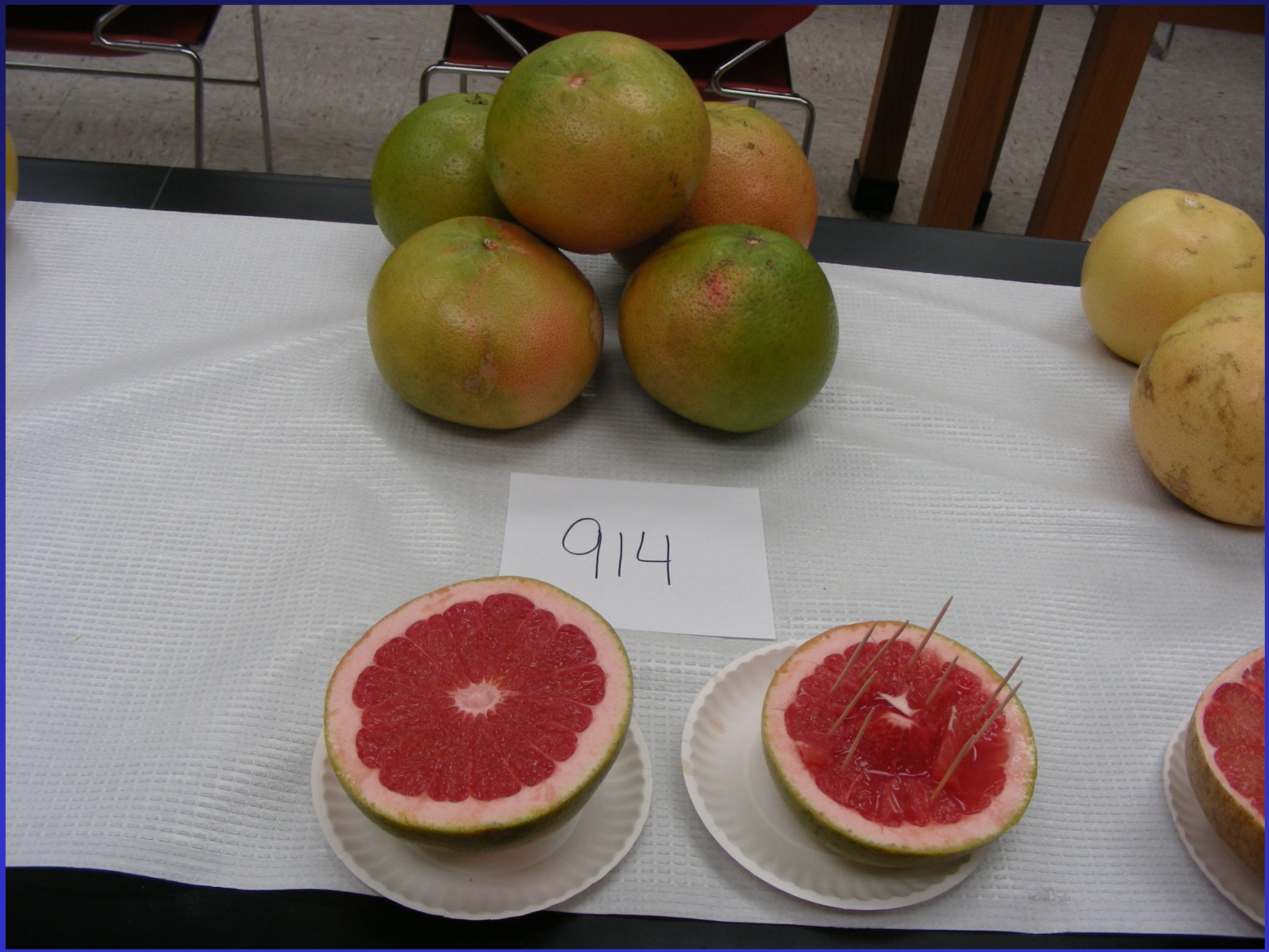
Breeding Canker Tolerant Triploid Seedless Grapefruit-like Hybrids

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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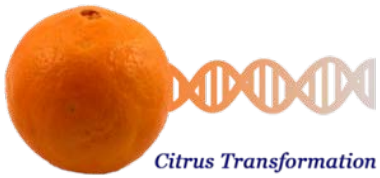
Three 2010 Releases,
Our First Three!



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

Valenfresh ™ sweet orange, a seedless selection of Valencia for fresh market or processing, late maturing and holds quality into June!

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

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Eight Probable Releases in 2011, all approved by the IFAS Citrus Advisory Committee

Red Pummelo 5-1-99-5
Proposed name: ‘ShirleyRed’ Pummelo
-thin rind, good color and flavor with a hint of grapefruit bitterness.

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Red Pummelo 5-1-99-2
Proposed name: ‘Pummelette’
-grapefruit sized, delicious, high-ratio fruit

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B9-65 Valencia for processing
Proposed name: ‘ValAries’ sweet orange
- A high yield, high solids selection with typical Valencia maturity, best of 30 selections in trial at Conserve II.

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Seedless Valencia T2-21 – for fresh or processing.
Proposed name: “The Uncle Tony Sweet Orange”
Delicious, seedless Valencia selection, fruit holds well on the tree

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Citrus Selection ‘900’
-A beautiful, delicious, easy to peel mandarin that ripens before ‘FallGlo’; excellent shelf life.

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‘Seedless Snack’, delicious unique flavored early-maturing tangor, not a zipper-skin but can be peeled, good in November.

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Citrus Selection ‘950’, a delicious, easy-to-peel tangerine, good for the Christmas market;
- Referred to as the ‘Florida Clementine’ by some.

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Citrus Selection ‘411’, a delicious, large fruited, easy-to-peel mandarin, eating good NOW!

-affectionately known as ‘Heather’

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HLB and Canker – similar transgenic strategies for control?

- Bacterial diseases of Citrus and caused by Gram negative bacterium.
- No natural resistance to HLB in any commercially cultivated sweet orange, grapefruit or tangerine while most cultivars are susceptible to canker.
- A broad spectrum antimicrobial gene can theoretically be used to combat both diseases.

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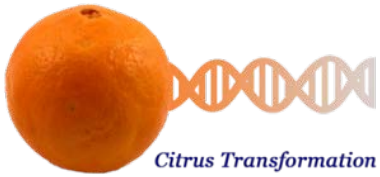
Antimicrobial gene(s) currently under evaluation

- AttacinE - Lytic peptide gene from *Hyalophora cecropia*.
- CEAD - Codon optimized cecropin A-cecropin D lytic peptide gene.
- CEMA - Codon optimized cecropin A-melittin lytic peptide gene.
- CEME - Codon optimized cecropin A-melittin lytic peptide gene (differs at the C terminus from CEMA).
- LIMA-A and LIMA-B – Synthetic hybrid lytic peptide genes obtained from Dr. Dennis Gray, MREC, UF/IFAS.
- PTA - Codon optimized N terminally modified Temporin A gene.

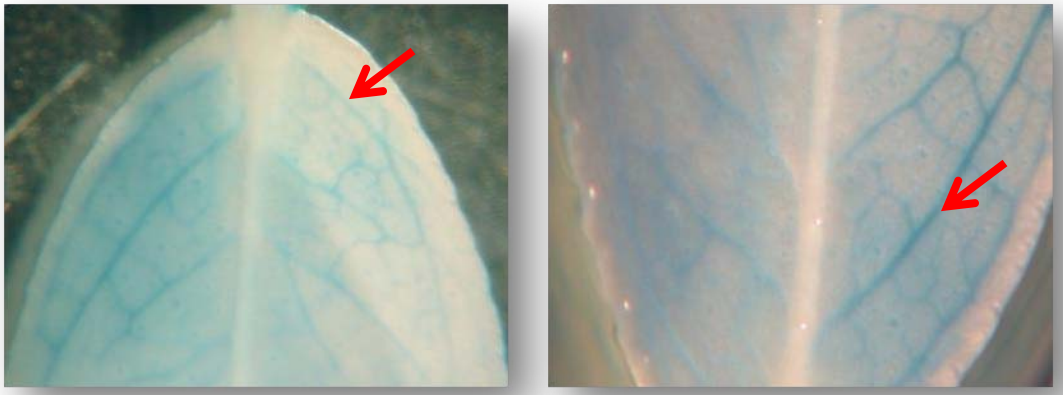


SAR genes currently under evaluation

- **SABP2** (Salicylic Acid-Binding Protein 2 gene from tobacco)
  - High Affinity for SA.
  - It may be required to convert Methyl Salicylate to SA as part of the signal transduction pathways that activate systemic acquired resistance.

- **NPR1** (Nonexpresser of PR Genes1 gene from *Arabidopsis*)
  - NPR1 is a key regulator in the signal transduction pathway that leads to SAR.
  - Mediates the salicylic acid induced expression of pathogenesis-related (PR) genes and systemic acquired resistance.

Use of phloem specific promoters to restrict trans-protein in phloem tissues



GUS expression in citrus leaf phloem tissue using the Rice Sucrose Synthase promoter

- HLB resides in the phloem.
- Targeting the trans-protein in the phloem resolves issues of the presence of the protein in the fruit and juice.
- Two phloem specific promoters are currently under evaluation
  1) *Arabidopsis* Sucrose synthase promoter and
  2) Rice Sucrose synthase promoter.

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Rapid propagation of transgenics

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Transgenic trees ready for testing

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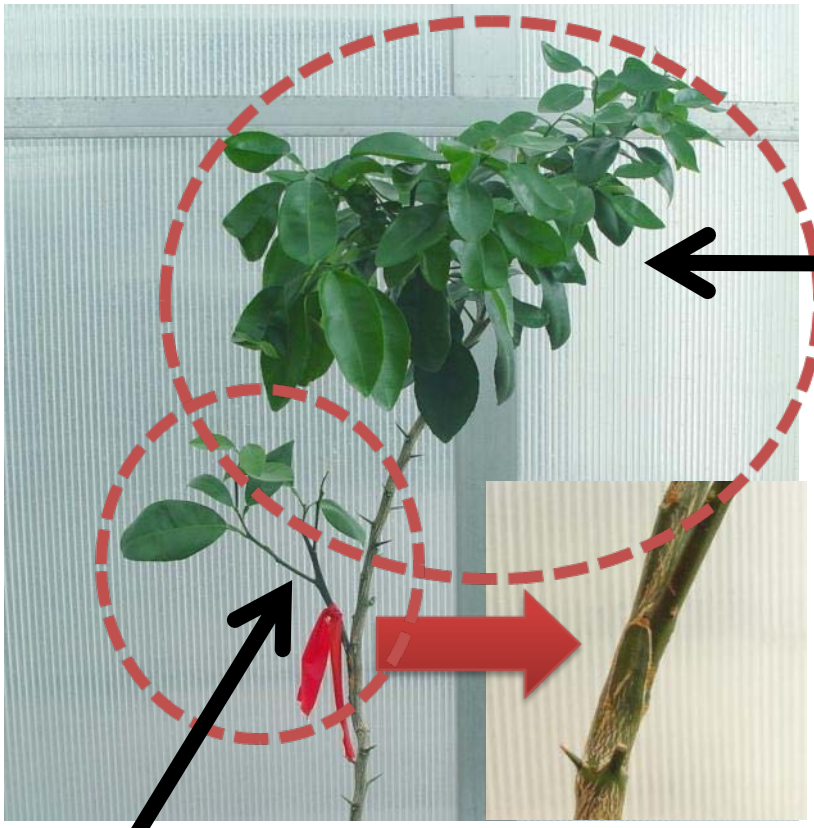
Transgenic plant challenge via grafting with HLB infected budwood



HLB infected budstick

Transgenic citrus plant after 15 months of **inoculation** with HLB + sweet orange bud stick

Wide range of symptoms observed after inoculation with HLB + budwood

Plants with general mottle on top of leaf to plants with no visual symptoms

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Field trials of transgenics in HLB/canker ‘hotspots’

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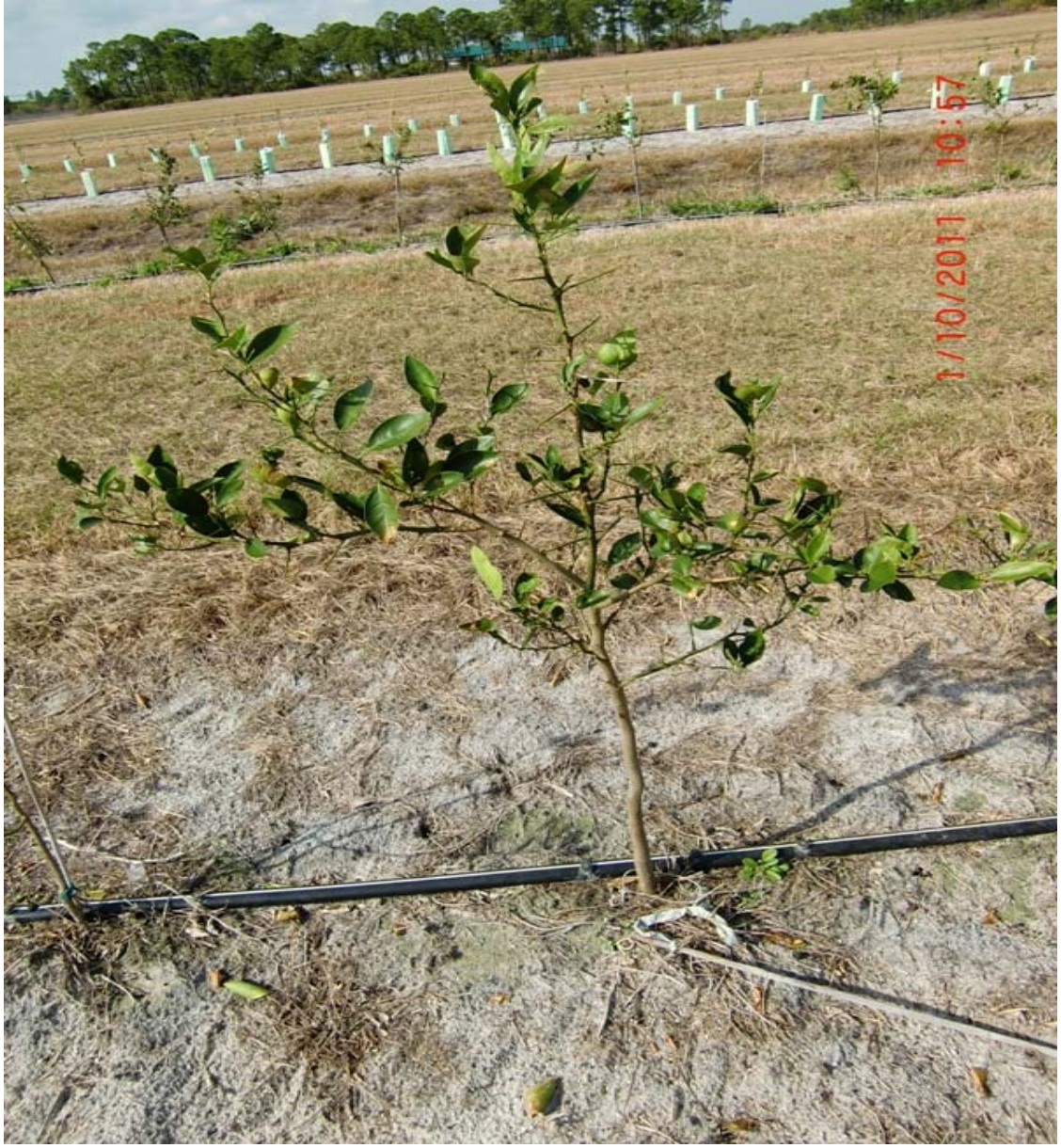

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One of 900 transgenic trees after one year in the field, enduring three freezes.

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Approaches for genetic resistance studies

- Screen a large number of transgenic trees to select individuals with the highest level of resistance.
- Challenge putative tolerant plants with "hot" psyllids – in collaboration with Mike Irey @ SGC. 350 plants ready to go!
- Field testing in a high disease pressure environment. APHIS permit issued, 900 trees planted in field 12/09 'hotspots' ! 300 more to be planted in March, 2011.

Transgenic plants for Field Testing

- **Plants in ground (planted December, 2009).**
  - Site 1: 600
  - Site 2: 300
- **New plants ready for planting**
  - 250
- **Additional plants to be planted by Fall 2011**
  - 300

Issue of Juvenile vs. Mature Explant Transformation

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The RES – Rapid Evaluation System
*** Flowering in 11 months! ($-NVDMC)**

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Can complex new rootstocks in combination with the right tree care prevent or mitigate HLB?

***Exploring the rootstock/nutrition interaction – looking for clues!**

•Rootstocks have different efficiencies for specific nutrient uptake and transport to the scion. Successful rootstocks must more efficiently provide nutrients shown to be deficient in HLB infected scions (i.e. calcium and zinc)

•Successful rootstocks should prevent or suppress HLB replication, so what comes down does not go back up to infect additional parts of the scion.

•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.

•Management programs should minimize any stress on the trees, including nutritional or water stresses.

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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)

5 year old Valencia on rootstock Green#7, between two small rough lemon trees planted at the same time – blight resets in Alligator grove (Mr. Orie Lee), fruit quality and size are excellent!

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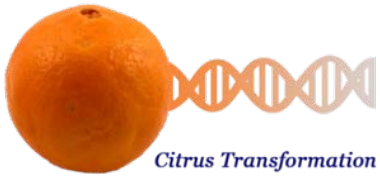
Tolerant?
Rootstock
Green #7

Screening complex rootstock hybrids by growing Valencia scion from HLB-infected budwood. Greenhouse tree grown from highly symptomatic PCR+ budwood of Valencia on rootstock Green #7 (Nova+HBPummelo x Sour orange+Carrizo)

THANKS!

- FCPRAC (now CRDF)
- NVDMC/FDOC
- Industry Collaborators, especially Orie Lee, Lee Jones and Gardinier Citrus, Barney Greene and Greene Citrus, Tom Hammond, Becker Citrus, Conserve II, etc.
- USDA-HRS Fort Pierce w/ Ed Stover
- USDA-CSREES
- UF/CREC Core Citrus Transformation Facility
- CREC Faculty and Staff



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