Breeding Citrus Cultivars for a Changing Citrus Industry

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ARS Citrus Improvement

The oldest citrus breeding program in the world?

W. T. Swingle

H. J. Webber

Sub-Tropical Laboratory
Eustis, Fla.
Swingle and Webber
1893-1897

>75% of US citrus industry has rootstock and/or scion from USDA program…. mainly Carrizo/Troyer and Swingle rootstocks!
USDA Citrus Scion Breeding Program- more than 120 years of effort

- Murcott
- Orlando Tangelo
- Minneola Tangelo
- Nova Tangelo
- Lee
- Osceola
- Robinson
- US Early Pride
- Sunburst Tangerine
- Fallglo Mandarin
- Sunstar Orange
- Midsweet Orange
- Gardner Orange
- Flame Grapefruit
- Page
- US Seedless Pineapple

11% of Florida citrus propagations, >17 million trees
Breeding program continues to evolve to keep it vital and productive…. for another100+ years!!
Huanglongbing (HLB), AKA Citrus Greening
First identified in Florida in August 2005, now found in all FL citrus producing counties: verified TX & CA 2012
Estimated that as many as 40% of FL citrus trees are infected (Spann and Schumann, 2012), but some groves are 100% infected and some no longer productive
Associated with a phloem-limited bacterium, *Candidatus Liberibacter asiaticus*, vectored by the Asian Citrus Psyllid (*Diaphorina citri*, in Florida since 1998 and little controlled)
Within a few years of infection, many citrus trees become weak, have poor quality fruit with off-flavor, with lots of fruit drop, and trees may die or become useless
Greening has cost Florida’s economy ~$3.6 billion in lost revenues since 2006 (Hodges & Spreen, 2012)

Photos Bove, 2006
World Experience (Bové, 2006)

• “Probably the most serious disease of Citrus”
• First unambiguous report in China 1940s
• “Practically all commercial citrus species and cultivars are sensitive, regardless of rootstocks”
• Has caused elimination or contraction of citrus production in several citrus growing regions
• Has latent period with few symptoms for several years
• May completely debilitate trees within two years of first symptoms
• Research supports HLB-management by aggressive ACP spraying, regular scouting and roguing of infected trees, and use of disease-free replants
Florida HLB

• Initial rate of disease development, from first find to as much as 39% of a grove in 10 months (Gottwald, 2007)
• Particularly severe symptoms in FL: some expect all trees to be infected in <10 yrs
• Initially most growers scouted and removed infected trees, but rapid spread caused many to question practice…..
• Now most spray for psyllids but maintain infected trees through enhanced foliar nutrition - many now replacing trees (investing in future), but unknown if will produce.
Seriously Bad News For Florida Citrus

- In addition to immediate effects of falling yields and compromised juice quality, there is concern that production may fall below the critical volume needed to sustain processing and packing industries 2013/2014 estimate (133.4 x10^6 boxes OJ, 18.4 GF)

- FL industry redirected ~$16 million/year from ad $$ to research, + ~$11 million in federal and state funds- extraordinary research effort for short, medium, and long-term solutions

- The most sustainable solution for a plant disease is use of resistant cultivars: Is this possible for HLB?
Earlier Reports of Citrus Resistance to HLB

Reports of tolerance or resistance to HLB from various parts of the world:

- **limes** (e.g. Lange et al., 1985; Schwarz et al., 1973; Shokrollah et al., 2009),
- **lemons** (e.g. Cheema et al., 1982; Nariani, 1982; Schwarz et al., 1973),
- **pummelos** (e.g. Koizumi et al., 1997; Schwarz et al., 1973),
- **some mandarin types** (e.g. Koizumi et al., 1997),
- **and some non-cultivated citrus and relatives** (e.g. Folimonova et al., 2009)

• Inconsistent reports may be due to different Citrus genotypes and/or strains of CLAs
Significant Resistance to HLB in mainstream cultivars? Survey in groves with multiple types

Liberibacter per sample by cultivar
Stover & McCollum: HortScience 2011

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Mean # CLas genome/sample</th>
<th>% trees “HLB+”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minneola</td>
<td>304</td>
<td>43%</td>
</tr>
<tr>
<td>Murcott</td>
<td>168</td>
<td>44%</td>
</tr>
<tr>
<td>Sweet orange</td>
<td>236</td>
<td>31%</td>
</tr>
<tr>
<td>Grapefruit</td>
<td>40</td>
<td>20%</td>
</tr>
<tr>
<td>Temple</td>
<td>9</td>
<td>15%</td>
</tr>
<tr>
<td>Fallglo</td>
<td>13</td>
<td>18%</td>
</tr>
<tr>
<td>Sunburst</td>
<td>107</td>
<td>13%</td>
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</table>
USDA Ft Pierce FL Farm with Endemic HLB
Germplasm collection, diverse hybrids, and experiments exposed to HLB/ACP. Is there sufficient resistance or tolerance in Citrus to be economically useful?
Trial Planting: Grapefruit vs. Near Grapefruit

- Planting established 2003 and 2004 by Jose Chaparro (Stover, McCollum, Ritenour, Chaparro, ProcFSHS 2012)

- Goal was assessment of potential for very early grapefruit for juice and fresh-fruit due to lower bitterness of ‘Triumph’ and ‘Jackson’

- A “discovered experiment” in 2009, as ‘Triumph’ (T) and ‘Jackson’ (J) appeared to be less adversely affected by HLB compared to ‘Marsh’ (M) and ‘Flame’ (F)
Planting in Jan 2010

Jackson Grapefruit-like hybrid

Marsh Grapefruit
HLB, Canker and Health all on a 5 point scale such that 1 is healthy and 5 is severely affected

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Health09</th>
<th>HLB09</th>
<th>canker09</th>
<th>Fruit per tree</th>
<th>Fruit drop</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flame</td>
<td>3.3 b</td>
<td>3.7</td>
<td>3.6 b</td>
<td>62.3 bc</td>
<td>62% b</td>
</tr>
<tr>
<td>Marsh</td>
<td>3.5 b</td>
<td>3.5</td>
<td>3.6 b</td>
<td>42.2 c</td>
<td>69% b</td>
</tr>
<tr>
<td>Jackson</td>
<td>2.1 a</td>
<td>3.6</td>
<td>1.6 a</td>
<td>126.1 a</td>
<td>21% a</td>
</tr>
<tr>
<td>Triumph</td>
<td>2.6 a</td>
<td>3.7</td>
<td>2.0 a</td>
<td>100.2 ab</td>
<td>24% a</td>
</tr>
<tr>
<td>F&amp;M vs. T&amp;J</td>
<td>&lt;0.0001</td>
<td>0.7053</td>
<td>&lt;0.0001</td>
<td>0.0028</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Health10</th>
<th>HLB10</th>
<th>canker10</th>
<th>Fruit per tree</th>
<th>Fruit drop</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flame</td>
<td>4.1 b</td>
<td>3.8</td>
<td>2.9 b</td>
<td>22.7 b</td>
<td>5% a</td>
</tr>
<tr>
<td>Marsh</td>
<td>4.6 b</td>
<td>3.7</td>
<td>3.1 b</td>
<td>8.8 b</td>
<td>21% b</td>
</tr>
<tr>
<td>Jackson</td>
<td>3.2 a</td>
<td>3.1</td>
<td>1.5 a</td>
<td>82.4 a</td>
<td>4% a</td>
</tr>
<tr>
<td>Triumph</td>
<td>2.8 a</td>
<td>4.2</td>
<td>1.7 a</td>
<td>94.5 a</td>
<td>5% a</td>
</tr>
<tr>
<td>F&amp;M vs. T&amp;J</td>
<td>&lt;0.0001</td>
<td>0.6663</td>
<td>0.0005</td>
<td>&lt;0.0001</td>
<td>0.0013</td>
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</tbody>
</table>
‘Flame’ (True Grapefruit) vs ‘Triumph’

Highly symptomatic leaves but thick canopy, in ‘Triumph’/’Jackson’

‘Triumph’/‘Jackson’ met commercial fruit quality standards in each year but “true grapefruit” did not.

Encouraging that there is tolerance in conventional Citrus, and being studied in several populations
Triumph/Jackson a hybrid between GF and Sweet Orange?

Combining genes for resistance or losing genes for susceptibility?
Some hybrid breeding populations show considerable tolerance to HLB
‘Flame’
5/8 Pummelo, 3/8 Mandarin

‘Hirado’ x ‘Ninkat’
1/2 Pummelo, 1/2 Mandarin
5-51-2
Clementine x Orlando cross by J. Hearn
5-51-2 on left- Clementine x Orlando (Duncan x Dancy)
> little evidence of HLB in grove where trees are heavily infected
1-43-21 on right Fortune (Clementine x Dancy) x Encore (King x Willowleaf)
• Good news is that there appears to be substantial resistance/tolerance to HLB in conventional citrus germplasm (at least to strains of CLas tested)
• Questions remaining: 1) Degree of tolerance, 2) Willingness of industry and consumers to shift cultivars, 3) How much of a delay in compromise of cropping is needed to be useful?, 4) Are there CLas strains that are more virulent on these genotypes?

![Graph showing cropping as influenced by HLB over time](image)

• Is this of value where HLB is being excluded, or only where the industry is “living with” HLB?
3-year Grant from CRDF: Exploiting Tolerance/Resistance to HLB in Citrus

• Postdoc began 10/8/12
• Verifying levels of tolerance/resistance in promising material and testing all advanced selections
• Comparing growth (and ultimately cropping) in susceptible and tolerant material at various CLas titers
• Exploring non-GM methods to enhance resistance
How about HLB Resistance in Citrus relatives?

- Folimonova et al. (Dawson group at UF-Lake Alfred) tested 30 genotypes for HLB response.
- Severity of symptoms varied greatly, with sweet orange in the most sensitive, highest titer group.
- *Poncirus trifoliata* and *Citrus latipes* displayed the greatest resistance in symptoms and low/no titer.
- *Poncirus* is deciduous, Citrus cross-compatible (parent of many rootstocks), but tastes absolutely terrible. Some hybrids are fuzzy, if you want citrus with a fur coat........
Numerous Studies Showing Poncirus HLB resistance

- 85 Seed accessions from Riverside, Calif. repository: planted June 2009 (Lee et al.)
- 8 plants each randomized in Ft Pierce, Fla.
- Most resistance in field experiment of 85 genotypes: Bergera koenigii (8/8 seedling trees), Eremocitrus (5/5), Microcitrus (5/8; 4/7; 2/3), Poncirus (6/6; 4/8), also x639 (4/7), Rusk citrange (5/8) which are Poncirus hybrids
- Now challenging mapping population of Poncirus x Sweet Orange to find markers (Gmitter, Roose, Stover) for use in conventional breeding and GE

Also exploring resistance further in other genera (Microcitrus and Eremocitrus)
Replicated trifoliate hybrid trial

Trees have been exposed to ACP/HLB for 20 months
USDA started using *Poncirus* as parent 110 yrs ago for cold-hardiness: looks like may pay off for HLB-resistance

Sweet Orange like fruit-
Navel in alligator-hide

Apparent tolerance to HLB
1/16 *Poncirus*

No off-flavor

Being used in many crosses
Gnarlyglo trees are the largest healthiest trees in the entire trifoliate mapping planting, even though produced from field budwood (likely some level of Clas present)

All trees on same rootstock, budded at the same time, and grown together (but others clean)
What do we do if a distant relative has HLB-resistance?

Carrizo transformed with D35S:: Citrus FT Gloria Moore- UF Horticulture

Tool to permit 100 years of plant breeding in 15 years and final cultivars will not be transgenic
Creation of new scions and rootstocks

Sexual Hybridization - slide from McCollum

- Controlled pollinations to combine parents with desirable traits
- Fruit harvested
- Seed extracted
- Seedlings grown for field planting

Evaluation of seedlings 1st Test

Propagation of promising selections

Replicated field trials 2nd Test
Breeding “Sweet Orange” Hybrids

-all sweet oranges are mutants of an original hybrid (3/4 mandarin and 1/4 pummelo) which arose hundreds or thousands of years ago
-Now breeding similar hybrids with different genetic makeup than all other sweet oranges-MAY have greater resistance/tolerance and currently being tested
-Clean budwood (clean-up by Florida DPI, Peggy Sieburth) is being propagated for second tests
FF-1-75-55 Ambersweet x FF-1-30-52. Ripens about the same time as Hamlin, has orange appearance, taste, and aroma volatiles. Can be peeled by hand. Near Valencia Juice Color. Relatively few seeds. Easy peeling
**FF-1-75-113** Ambersweet x FF-1-30-52. Ripens about the same time as Hamlin, slightly smaller than oranges. Has 38 juice color score and juice tastes nearly exactly like orange juice. Relatively few seeds.
FF-1-76-50  Ambersweet x FF-1-30-52. Ripens about the same time as Hamlin. Has orange appearance, taste and aroma volatiles. Has very rich flavor and excellent Valencia-like juice color. Seedy
FF-1-76-52 Ambersweet x FF-1-30-52. Ripens about the same time as Hamlin. Has Orange appearance, taste and aroma volatiles. Many fruit are near Navel Orange size. Has excellent flavor and juice color is near that of Valencia. Fruit usually have relatively few seeds.
Volatile profile comparison of USDA sweet-orange-like hybrids vs. ‘Hamlin’ and ‘Ambersweet’

Note: Ambersweet is parent of new hybrids and is (Clementine x Orlando) x Sweet Orange
Ftp-6-32-64

large fruited mandarin with good flavor
FF-1-29-105 x FF-1-11-61
Ftp-6-34-55
late ripening mandarin
FF-1-12-70 x FF-1-26-1
Remember that some mandarin types display marked resistance to HLB............

Would the processing industry consider a focus on juice from other citrus types as a “substitute” for OJ?

I understand not a trivial conversion to move from well-tested and proven cultivars with established handling methods for consistent product

Compatible with standard juicers?

Many people actually prefer rich taste of juice from these hybrids.......
Ftp-6-16-172 Red Grapefruit/Pummelo
Ftp-6-42-88
Grapefruit-like with *P. trifoliata* in pedigree, monoembryonic and tolerant of HLB- using as a parent

ALL advanced selections are being tested for HLB resistance/tolerance and expect that some will be markedly better than sweet oranges
Transgenics for HLB- Resistant Citrus

• Tolerance and resistance may be great….. IF you have decided to live with HLB

• Transgenics appear to be the most promising solution for strong HLB resistance and perhaps immunity

• Another major advantage is ability to improve an existing cultivar with essentially no other changes: HLB-resistant Ray Ruby, Valencia, Hamlin, Tango etc.

• We are using as another source of variability in plant improvement
A Race to Save the Orange by Altering Its DNA

Trees that are infected by disease are cut down and burned in Clewiston, Fla., at groves owned by Southern Gardens Citrus.  Richard Perry/The New York Times

CLEWISTON, Fla. — The call Ricke Kress and every other citrus grower in Florida dreaded came while he was driving.

“It’s here” was all his grove manager needed to say to force him over to the side of the road.
In Which I Actually Endorse One Use of GMOs

By Tom Philpott | Wed Aug 7, 2013 3:00 AM PDT

Jump to the comments of this posting.

Carl Kilgoard/ZUMA

In a July 27 feature article that set the interwebs aflame, *New York Times* reporter Amy Harmon told the tale of a bacterial pathogen that's stalking the globe's citrus trees, and a Florida orange juice company's effort to find a solution to the problem through genetic engineering.

An invasive insect called the Asian citrus psyllids carries the bacteria, known as Candidatus Liberibacter, from tree to tree, and it causes oranges and other citrus fruits to turn green and rot. "Citrus greening," as the condition has become known, has emerged as a pest nearly wherever citrus is grown globally. Harmon reported that an "emerging scientific consensus" holds that only genetic engineering can defeat it.

Meanwhile, Michael Pollan, a prominent food industry and agribusiness critic, tweeted this:
Harnessing nature’s genetic engineer: *Agrobacterium tumefaciens*

*Agrobacterium tumefaciens* causes crown gall disease in many plant species.

By removing *At* genes for growth regulators and replacing with:

1) promoter
2) gene of interest etc.
3) gene for selectable marker (antibiotic)

...can express genes when and where you want, without gall formation.

Image: [www.plantsci.cam.ac.uk/.../GFP/plantrans.html](http://www.plantsci.cam.ac.uk/.../GFP/plantrans.html)
Citrus Transformation: Major Focus for HLB and Canker Resistance

1. Transformation
2. Selection
3. Regeneration
4. Evaluation
5. Grow out
6. Micro-grafting

Slide K. Bowman
Transgenes we are studying for HLB resistance—Just another source of variability in breeding

- Antimicrobial peptides, including chimeral peptides designed by Goutam Gupta (Los Alamos National Laboratory)
- Transmembrane protein from Liberibacter and bacteria-product resistance triggering genes identified from the \textit{Liberibacter asiaticus} genome (working with Duan group at USHRL).
- Phloem-specific protein induced during HLB infection (Bowman data USHRL)
- Dormancy-inducing gene from peach (Bielenberg)
- Working with ARS scientists in California to develop constructs so that ALL inserted genes are from Citrus! (Belknap)
- New opportunities as they arise…………..
Full genomic sequences now available for host (several citrus species), pathogen and vector

Providing information to target disease from many fronts: transgenics, therapies, etc.
AMP transgenics being tested with CLas infected psyllids
Thanks!

- Florida Citrus Research & Development Foundation
- New Varieties Development and Management Corp
- Florida Citrus Research Foundation (Whitmore)
- California Citrus Research Board
- USDA/ARS Funding and USDA/APHIS

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