Resistance and Tolerance to Citrus Greening Disease AKA Huanglongbing or HLB

Ed Stover- USDA/ARS Ft. Pierce, FL

Huanglongbing, AKA Citrus Greening

- Caused by a bacterium, Liberibacter asiaticus (Las), which in trees lives only in the plumbing which distributes sugar throughout the plant (called the phloem)

- Transmitted in the US only by an insect that feeds on citrus leaves (and specifically the phloem) called the Asian Citrus Psyllid (ACP), much as mosquitoes carry the malaria pathogen
- Most distinctive symptom is blotchy mottle of the leaves in which the yellow areas are not symmetrical on either side of the leaf midrib (see below). In severely infected trees, many fruit are lopsided with aborted seeds and poor color development (also below)
- Within a few years of infection, many citrus trees become weak, have poor quality fruit, with lots of fruit drop, and trees may die or become useless

Photos Bové, 2006

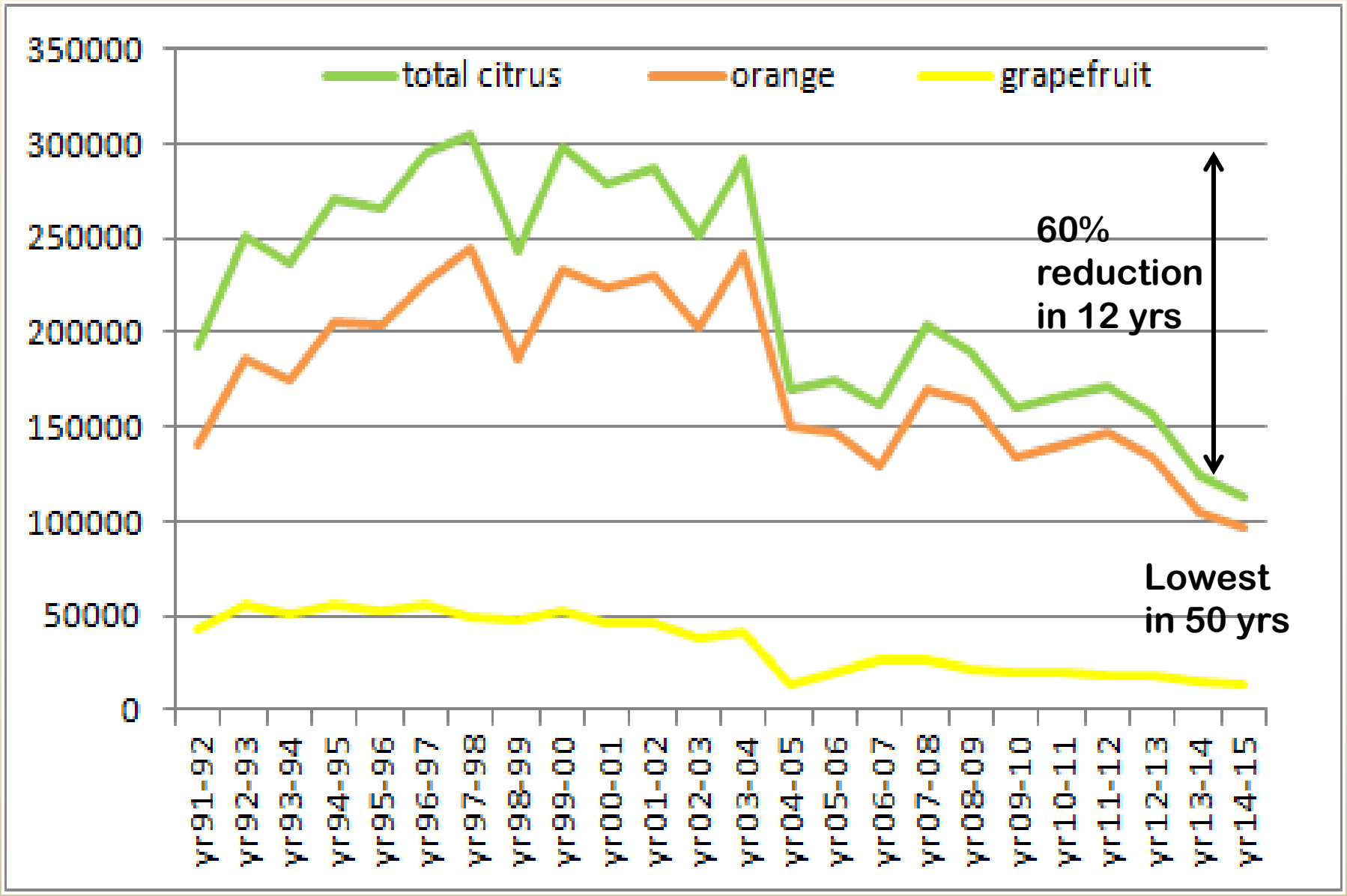
World Experience (Bové, 2006)

- “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

Should I be concerned as a grower?

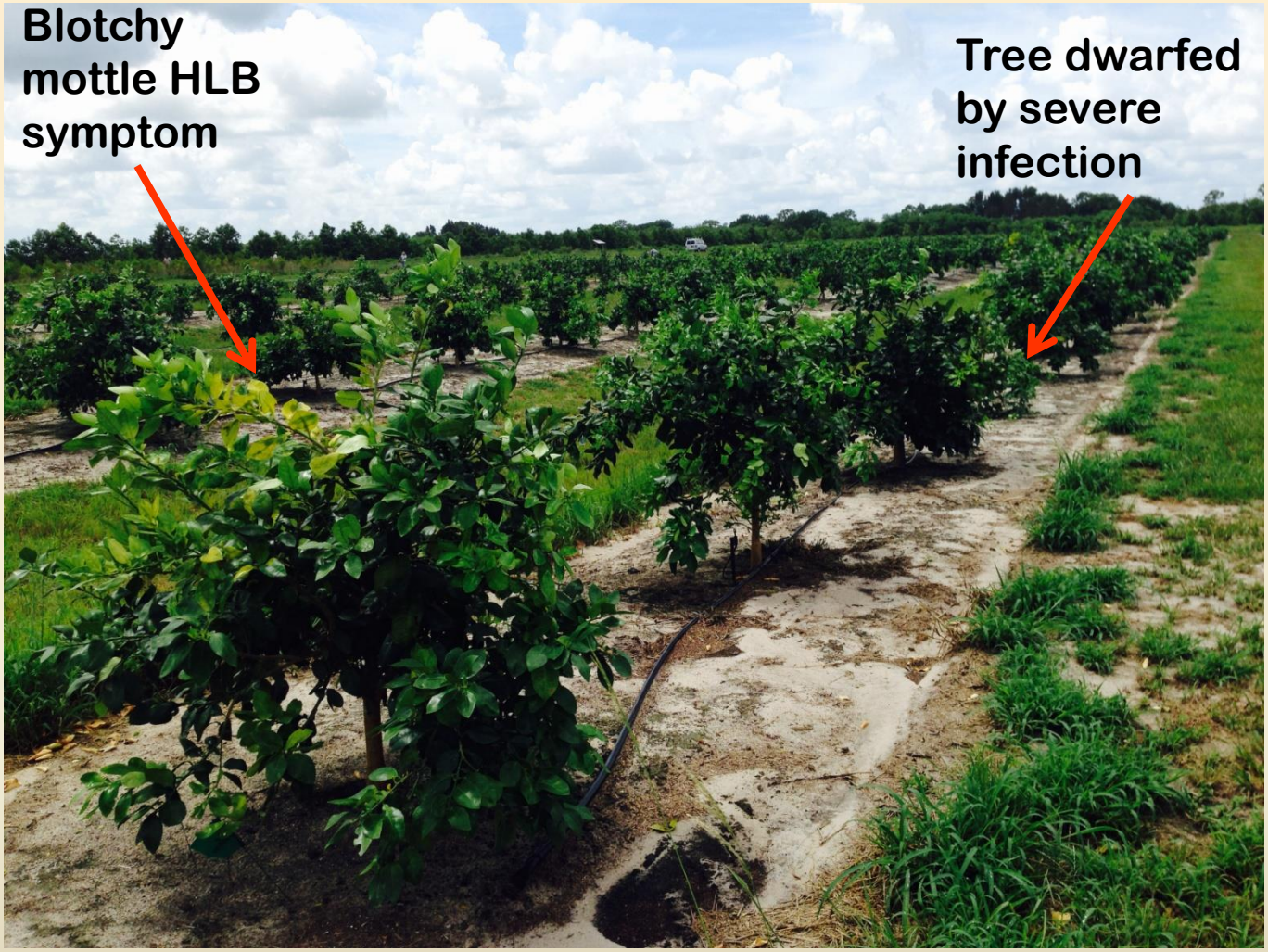
- Where HLB is widespread (like Florida now), it is the most limiting factor to economic citrus production.
  - In FL, it is estimated that infected trees have a crop reduction of 40% compared to healthy trees (Singerman, 2015)- around 70% of all FL trees are infected and many folks are already out of business
  - FL citrus production dropped a great deal in 2004-5 as a result of three back to back hurricanes, but due to HLB it has never recovered and declined further.
  - FL has produced the smallest citrus crop in over 50 years
  - HLB was first identified in Florida in August 2005 and rapidly spread throughout the state

Trend in Florida Citrus Production



Four years of HLB progression in same Hamlin Sweet Orange tree. Note thinner canopy, lighter leaf and fruit color and presence of many green fruit on tree in 2015

This represents fairly successful management using extensive foliar nutrition and aggressive psyllid control, and likely is around the 40% cropping reduction of typical HLB-infected sweet orange in Florida. Photo courtesy of Dr. Steve Futch-
http://citrusagents.ifas.ufl.edu/hlb_photos/index.shtml



Infection in a young grove means it is unlikely trees will ever be productive

Advanced infection in mature groves greatly reduces productivity. This grove won’t repay production costs.

Abandoned unproductive groves from HLB infection are widespread (>130,000 acres in Florida 2015)

Fruit quality, flavor and preharvest drop:

- Fruit quality affects of HLB indicate highly symptomatic are worst, with slightly metallic or bitter flavor and lower sugar; non-symptomatic fruit are slightly different from healthy but acceptable (ARS-FL)
- Preharvest fruit drop is much greater in HLB-infected trees, even with nutrient enhancement.
- Some loads of fruit have been refused at the juice plant because HLB reduced quality

S. Rogers
http://ircitrusleague.org/interactive-virtual-citrus-groves/

HLB- tree in decline

HLB-infected but cropping well but lots of preharvest drop

Can we cure infected trees?

- Once a citrus tree is infected with HLB there is no evidence that it can be cured with existing technologies
- Thermotherapy (heating trees above 100ºF for more than 48 hours) has been shown to greatly reduce the disease-causing bacterium and increase tree health for many months, but so far no rapid method or procedure suitable for treating lots of trees has been proven
- Antimicrobial materials have shown some promise for temporarily improving tree health, but no legal treatments are available
- A section 18 application is in place for Oxytetracycline and Streptomycin- looks like they will help but won't return trees to full health

How can I prepare for HLB?

- There is no current substitute for keeping the disease causing bacterium and its insect carrier away from your trees!
- Maintain awareness of how close the disease and the carrier insect are to your area
- Monitor your trees for HLB symptoms and the presence of ACP, and coordinate with your Dept. of Agriculture and other growers- looks like major error to not control ACP
- Follow disease progress in Florida and learn from industry experience
- Wide-spread agreement that HLB resistant trees will be the long-term sustainable solution

Resistance and Tolerance to HLB

Support Scientists and Postdocs

Randy Driggers
Guixia Hao
Sharon Inch
Malu Oliveira
Ric Stange
Godfrey Miles

Principle Collaborators:

David Hall

Greg McCollum

Bob Shatters

YongPing Duan

Goutam Gupta

Gloria Moore

Richard Lee

Manjunath Keramane

Chandrika Ramadugu

Bill Belknap

Jim Thomson

Fred Gmitter

Jude Grosser

Mikeal Roose

Malcolm Smith

HLB tolerance/resistance solutions in short-, medium-, and long-term

- Existing cultivars and most advanced selections
- New selections with conventional citrus cultivar genetics
- New hybrids entering testing
- Hybrids with more-resistant citrus relatives
- Transgenics- best chance of total immunity

All these solutions require replanting!

Categories of resistance:

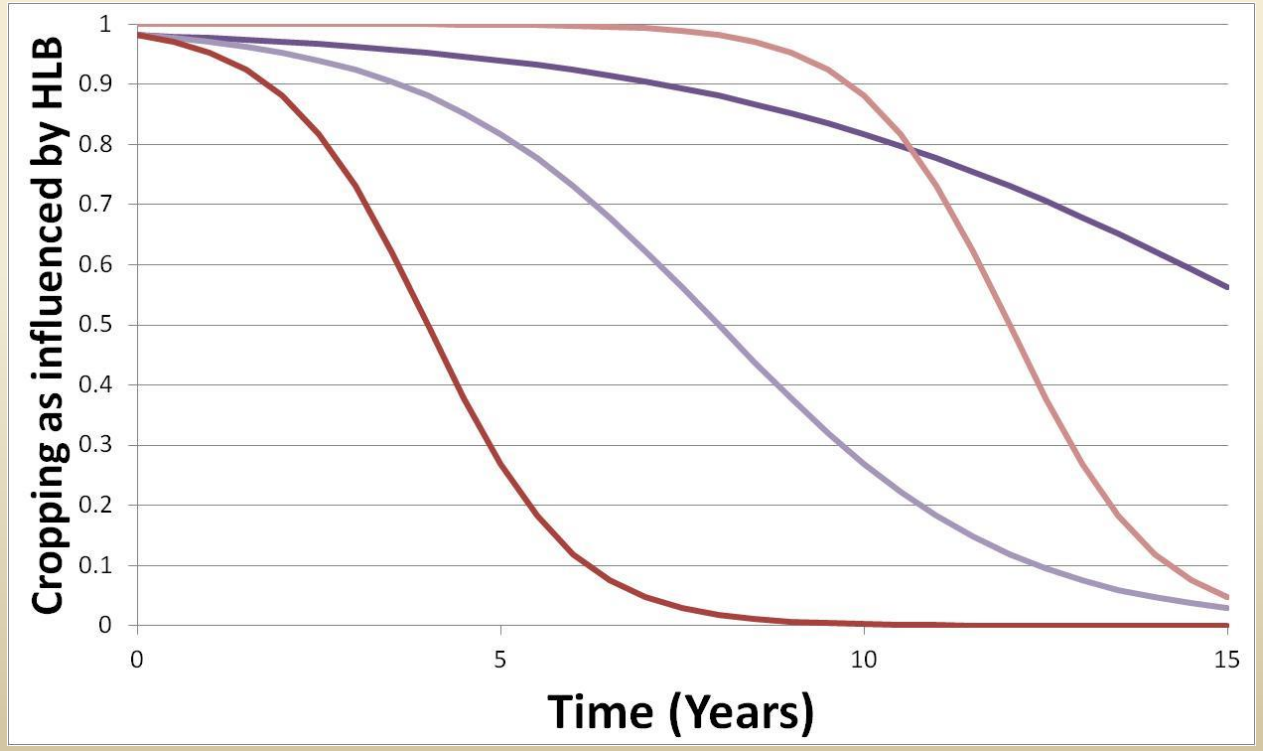
Immune- is the best! No disease whatsoever. Example: humans (and most plants) are immune to HLB! No good evidence of HLB-immune citrus

Resistant- slower to develop infection and/or slower to develop symptoms and/or lower levels of the pathogen result; also lower levels of symptoms

Tolerant- some symptoms and even the pathogen may develop at levels similar to those in susceptible individuals/genotypes. However, they continue to grow (and produce) fairly normally.

>Example: sweet orange is tolerant to sour orange-decline CTV on appropriate stock

- In time truly immune trees will be found… in the meantime
- How much of a delay in symptom development / compromise of cropping is needed to be useful?



Overview of US Citrus

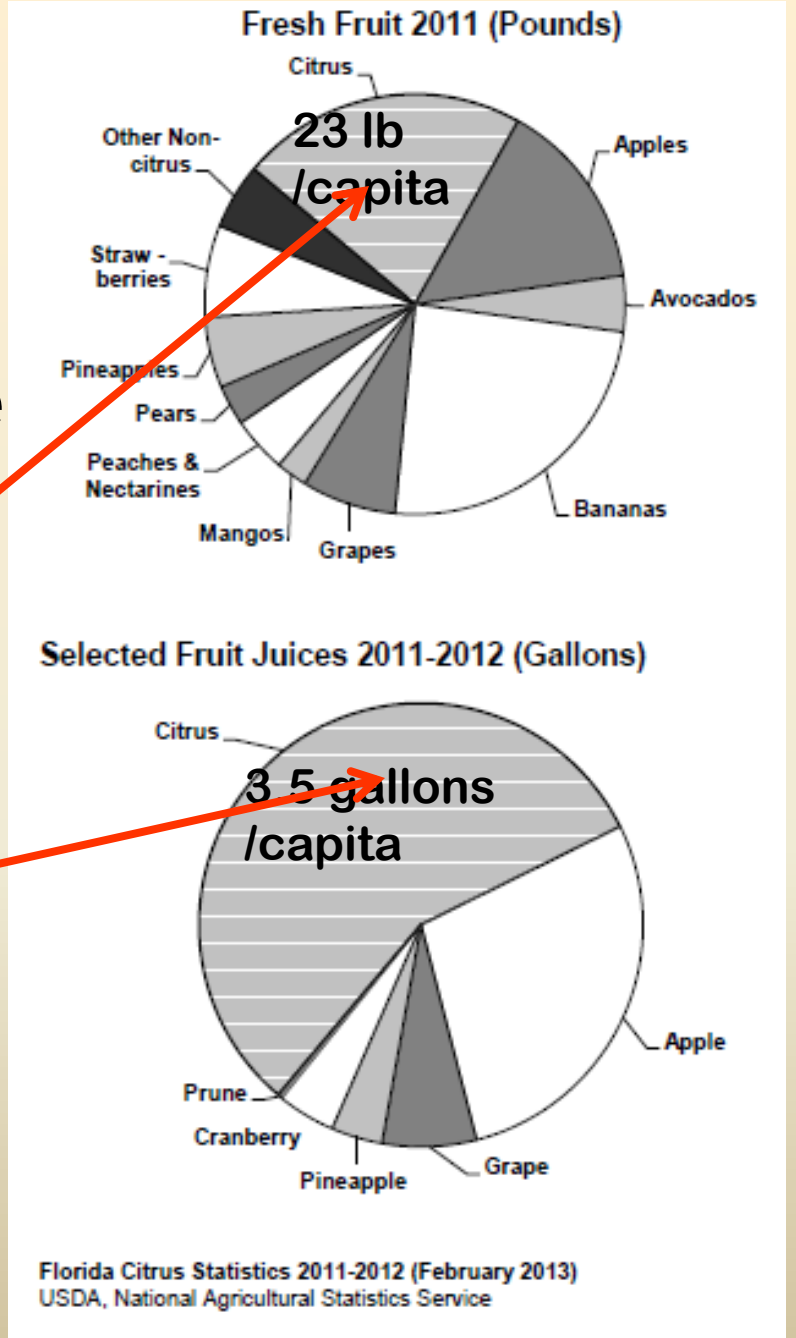
In US: total value of citrus industry is ~$9 billion
-75% production is sweet orange
-11% grapefruit

FL: 66% of US Citrus total
-85% oranges (96% juiced)
-12% grapefruit (58% juiced)

Citrus #1 fruit consumed in US

50x10^6/ yr cartons fresh exports

Fruit by % US Consumption



US Citrus is almost a monoculture

- All sweet oranges are mutants of a hybrid that arose in China a thousand or more years ago, and so are almost genetically identical
- Commercial grapefruit are similarly mutants of a hybrid (between sweet orange and pummelo) that arose in the Caribbean a few hundred years ago
- The genetic homogeneity of US citrus provides great vulnerability to introduced pathogens or pests
- As it happens, sweet orange and grapefruit display among the most severe decline from HLB of any citrus tested!

Significant tolerance to HLB in existing cultivars? Survey in groves with multiple types in 2010 -disease introduced into trees at maturity

Liberibacter per sample by cultivar

Stover & McCollum

	Pathogen titer: mean # CLas /100 mg sample by PCR	% trees "HLB+"
Minneola	304	43%
Murcott	168	44%
Sweet orange	236	31%
Grapefruit	40	20%
Temple	9	15%
Fallglo	13	18%
Sunburst	107	13%

Planting in Jan 2010

Planting in Jan 2014

Grapefruit vs. Near Grapefruit

	3 yr Cumulative				2011-2012			
Cultivar	Fruit per tree		Fruit drop		Disease rating		TSS/TA ratio	
Flame	129.4	bc	50%	b	4.2	b	7.0	b
Marsh	66.5	c	53%	b	4.4	b	5.7	c
Jackson	219.9	ab	14%	a	2.5	a	10.6	a
Triumph	255.1	a	15%	a	2.4	a	9.6	a
F&M vs. T&J	0.0002		<0.0001		<0.0001		0.0001	

- Fruit quality assessments were made each growing season with 'Triumph'/'Jackson' showing generally acceptable commercial fruit quality
- 'Flame'/'Marsh' had too low Brix/acid.
- In 2011/2012 many 'Flame'/'Marsh' were small and/or misshapen while 'Triumph'/ 'Jackson' displayed normal size and shape.
- Similar levels of HLB bacterium

More evidence of tolerance

Ongoing Evidence that some Mandarins have substantial HLB

Clementine

Fairchild (Clem x Orl)

Fortune (Clem x Dancy)

Bower (Clem x Orl)

Dancy

Kunembo (*C. nobilis*)

New planting looking at wide range of Clem x Orlando to map tolerance genes- Stover & Roose

5-51-2
Clementine x Orlando cross by J. Hearn

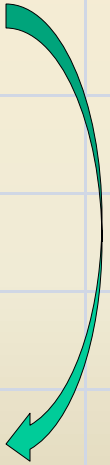
Several USDA C x O are displaying potentially useful HLB tolerance

What if trees are exposed to CLas at planting?

> 6 yr replicated trial at Picos Farm

>CLas titers not significantly different

Scion/Rootstock	Mortality (%)		Fruit/tree Oct 2015 (no.)		Health Oct 2015 (3 pt)		Change in diam. (mm)	
Fallglo/Kinkoji	20	a	28.4	b	1.9	cd	23.8	b
Hamlin/Cleopatra	20	a	18.6	bc	2.2	bc	20.4	b-d
Hamlin/Kinkoji	10	a	12.9	cd	1.9	cd	14.5	d
Ruby/Kinkoji	10	a	4.6	e	1.6	d	20.7	bc
SugarBelle/Sour	0	a	81.3	a	2.9	a	46.1	a
Tango/Kuharske	0	a	88.1	a	2.9	a	32.2	a
Temple/Cleopatra	18	a	35.6	a	2.3	ab	23.8	b



- Some scion/rootstock combinations continued to develop even with high titers of CLas and and strong mottle symptoms
- Not “tolerant” rootstocks used so likely a scion effect

SugarBelle/SourOr and Tango/Kuharske look particularly good and are producing more fruit

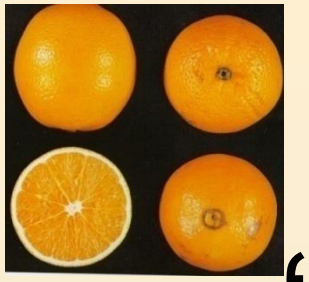
Hamlin/Kinkoji

SugarBelle/Sour Orange

Tango/Kuharske

We are most importantly a breeding program, and make >2000 new hybrids each year. All new hybrids from crosses intended to achieve HLB tolerance/ resistance are planted at the Picos farm in Ft. Pierce

It’s important to know how our new selections hold up to HLB! Replicated plants of 50 USDA selections and standards planted in the field after no-choice hot-ACP for a week and 4 months in the hot-ACP house



New “Sweet Orange” Hybrids

-different genetic makeup than all other sweet oranges- MAY have greater resistance/tolerance and currently being tested

-Have been propagated and planted in commercial field tests (McCollum lead)

Finally making progress on truly sweet-orange-like hybrids!

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**

Volatile profile comparison of USDA sweet-orange-like hybrids vs. ‘Hamlin’ and ‘Ambersweet’

Jinhe Bai, Elizabeth Baldwin

Randall Driggers, Jack Hearn and Ed Stover

Further afield-Considerable HLB resistance in citrus gene pool! Ramadugu et al. 2016

- Field experiment with genebank at Riverside CA of 85 citrus relative genotypes - showed *Poncirus* among most-resistant to HLB and also psyllid colonization (ARS CA and FL)

- *Eremocitrus* and *Microcitrus*, also showed strong Las and psyllid resistance and we have new collaboration with Queensland citrus breeder Malcolm Smith

Trifoliate genes for HLB resistance

- U of Florida (Fred Gmitter), UC Riverside (Roose) and USHRL (Stover) collaborating on trial to identify genes associated with HLB-resistance in citranges
- When mapped and identified, can use gene markers in conventional breeding and in intragenics
- Includes near commercial quality, advanced Poncirus hybrids

Poncirus is deciduous, cold-hardy, Citrus cross-compatible, but tastes terrible

USDA started using *Poncirus* as parent 110 yrs ago for cold-hardiness: looks like may pay off for HLB-tolerance

Sweet Orange like fruit-Navel in alligator-hide

Apparent tolerance to HLB

1/16 *Poncirus*

No off-flavor

Being used in many crosses

100+ Poncirus hybrid genotypes all replicated and exposed to HLB/ACP for 36 months

Gnarlyglo trees are the largest (7 ft vs 3 ft in sweet orange) healthiest trees in the entire planting, even though produced from field budwood

Grosser, Das, Gmitter

Promising observations on rootstocks inducing HLB-tolerance

-almost all HLB+ summer of 2011, 3-year old trees.

SugarBelle/Rootstock #4
2 trees on left

SugarBelle/Rootstock #19
5 trees on left

Would be ideal if could protect susceptible scions with a rootstock!
Industry leading extensive field tests as part of their development program

Transgenics for HLB- Resistant Citrus

- Tolerance and resistance is great….. IF you have decided to live with HLB
- Transgenics appear to be the most promising solution for strong HLB resistance and perhaps <u>immunity</u>
- Another major advantage is ability to improve an existing cultivar with essentially no other changes: HLB-resistant Washington Navel,Tango etc.

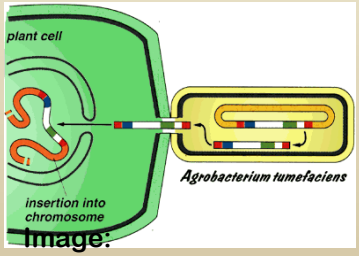


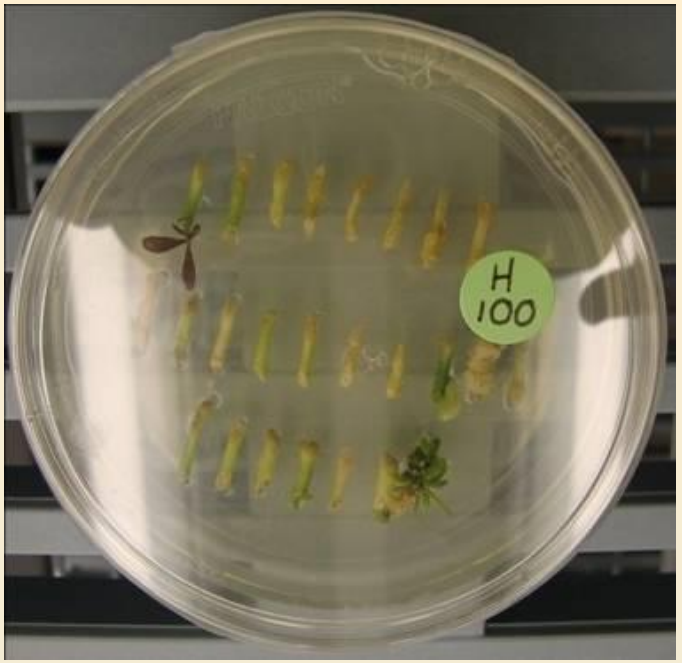
Image: www.plantsci.cam.ac.uk/.../GFP/plantrans.html

Future of transgenic Citrus

- Genetically engineered (GE) cultivars deregulated for commercial use in ~25 different agricultural crops
- GE crops are grown on ~12% of global arable land, mainly four field crops: soybean, maize, cotton and canola
- Several GE horticultural crops are being produced commercially since they provide solutions to otherwise intractable threats, much as HLB seems for citrus
- Commercial GE citrus is likely inevitable and GE crop concerns will likely decline with time
- NO released transgenic in any crop for bacterial resistance

Categories: Transgenic Strategies

- Direct attack on the pathogen
  - >Antimicrobials
  - >”Antibodies” for exposed pathogen proteins
- Host pathogen interactions
  - >Basal defense genes
  - >CLas gene products that target host (nuclear localization protein, flagellin etc.)
- Citrus physiology
  - >Possibly overactive defense response
- Psyllid targets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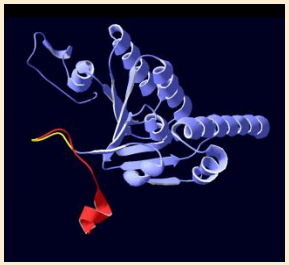
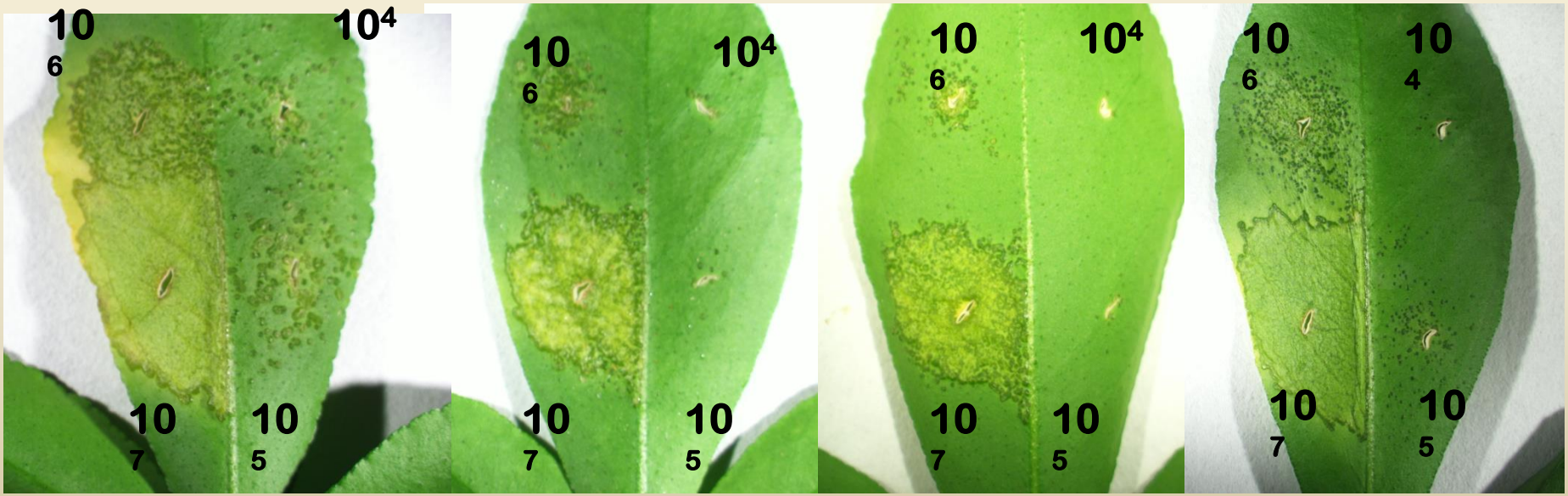
Thionin and chimeral antimicrobial peptides, designed by Goutam Gupta (Los Alamos National Laboratory)



Chimera of a citrus serine protease (cyan) joined to the lytic D4E1 peptide (red) by a GSTA linker (yellow)

Xcc Infiltration results with transgenic plants containing thionin, D4E1 and chimera



Non transformed control **Thionin-C12** **Chimera-C9** **D4E1-C20**

Transgenic Carrizo grafted with HLB+Rough lemon- in progress

Chimera **Control** **Thionin**

<u>After 9 months, significant @ 1% level</u>

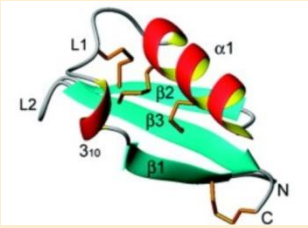
Control 3347 CLas/100 mg root

Thionin 16 CLas/100mg root

Aggressive challenge begins with no-choice exposure to CLas infected psyllids- led by D. Hall

Trees in greenhouse with free-flying CLas infected psyllids plus source plants-led by D. Hall

Spinach Defensins for HLB resistance: Erik Mirkov Texas A&M University and Southern Gardens Citrus



- Furthest along in deregulation- data package well along
- Trees being tested in field -Red Grapefruits and Sweet Orange
- Indicate have stronger HLB-resistance with *two different* spinach defensins expressed in same trees, in greenhouse

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

By AMY HARMON JULY 27, 2013 776 COMMENTS

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

In Which I Actually Endorse One Use of GMOs

—By ***Tom Philpott*** | Wed Aug. 7, 2013 5:00 AM PDT

Carl Kiilsgaard/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:

The FUTURE for Citrus production looks bright!

- Still many dark days to get there, at least in Florida

- Massive research investment is generating options for future plantings with HLB-resistant/tolerant trees
- Research is poised to identify game-changing technologies to protect uninfected susceptible trees such as ability to quickly identify and remove newly infected trees or prevent ACP from carrying Clas
- **Acceptance of "replacement" citrus varieties or transgenics may be essential to an HLB solution**
- An important missing piece in FL is therapy to maintain production on existing trees, needed to provide cash for implementation of new solutions

Thanks!

- Florida Citrus Research & Development Foundation
- NIFA- Specialty Crops Research Initiative
- New Varieties Development and Management Corp
- Florida Citrus Research Foundation (Whitmore)
- California Citrus Research Board
- DPI Budwood Office (especially Peggy Sieburth)
- USDA/ARS Funding and USDA/APHIS

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