



**Grower Day
International Research Conference
HLB IV**

WELCOME!

March 12, 2015

ACKNOWLEDGEMENTS

- Citrus Research and Development Foundation
- UF, IFAS, Citrus Research and Education Center, Lake Alfred
 - Staff at Ben Hill Griffin Auditorium – Jen Dawson, Sarah White, others
- Florida Citrus Mutual
 - Organizers – Clark Baxley and Kevin Metheny
 - Chef Extraordinaire – Rusty Wiygul
- IRCHLB Organizing Committee and Program Committee
- Grower Day Reporting Teams

Housekeeping

- Please move to front seating
- Phones silent – Take calls outside
- Restrooms off of lobby
- Continuing Education Units (CEU) available – Table in lobby
- Agenda at your seat
- Grower survey – Dr. Ariel Singerman, UF, IFAS CREC
- 30 minute presentations + 5 minute Q & A
- Lunch served following session

Goals for Grower Day

Review IRCHLB presentations before, during and after the conference to capture research findings that have the greatest relevance to Florida Growers

- With this goal in mind, the IRCHLB IV Organizing Committee and Scientific Program Committee prepared for this process.
 - They required authors to include a non-technical summary in their abstracts.
 - They requested that the oral or poster presentation include a slide or box providing an updated non-technical summary.
 - Presentations classified into six categories
 - Corresponding teams recruited to summarize information by category for Grower Day

Categories for Presentation at Grower Day

- 1) Non-bearing (yrs 1-3) and 2) Transition into early bearing (yrs 4-7)
- 3) Mature trees (yrs 7+)
- 4) New scientific discoveries that will lead to progress in delivering solutions, perhaps at a later time
- 5) Research from outside Florida (includes other US research and International Research) that may contribute

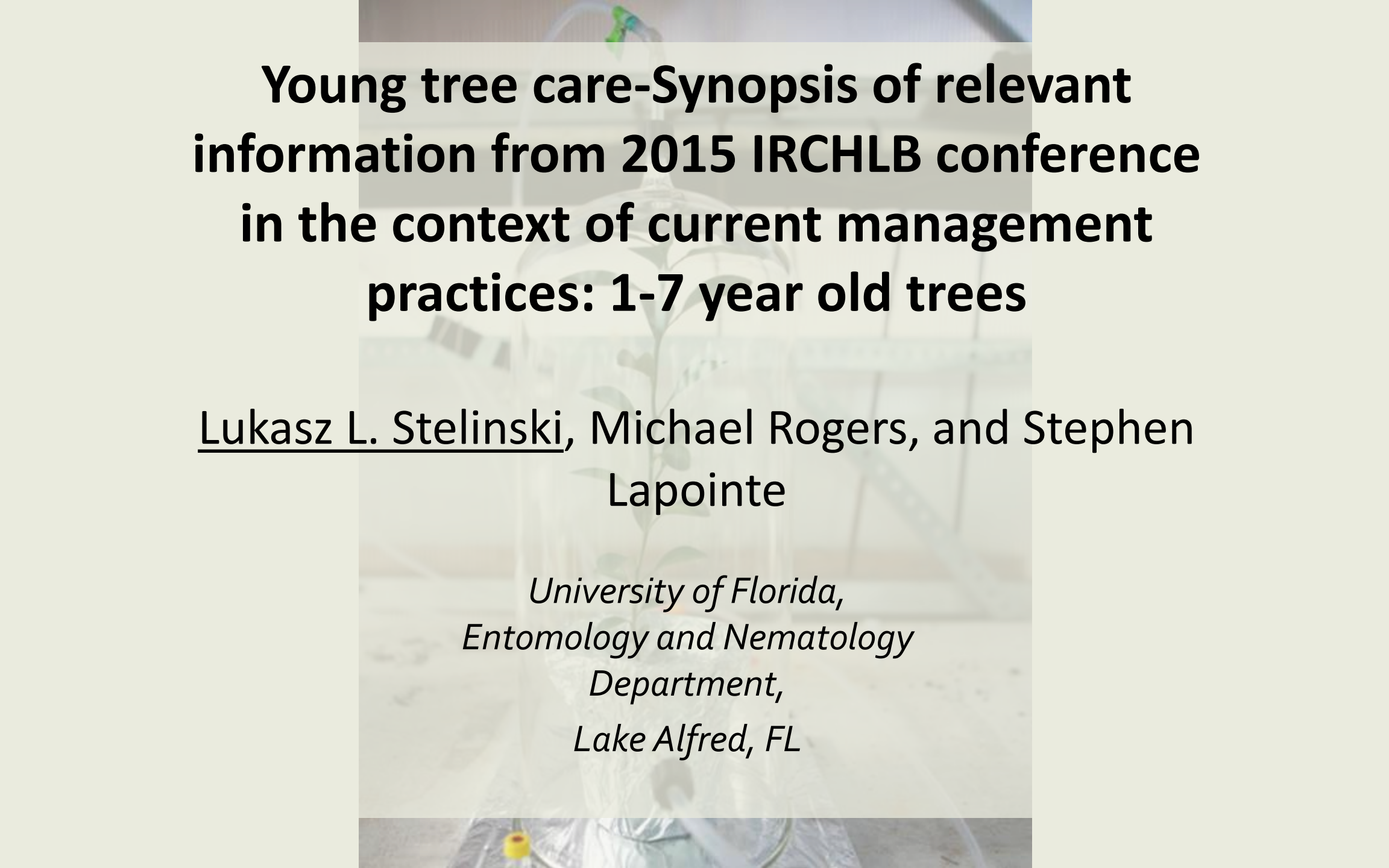
IRCHLB IV Stats

Goals of IRCHLB:

1. Assemble HLB scientists to share HLB progress
 2. Promote cooperation to rapidly develop solutions
- IRCHLB held in 2005, 2009, 2012, 2015
 - > 475 participants – greatest numbers yet
 - 25 countries represented, 20 states
 - ~99 oral presentations organized by topic
 - ~116 posters displayed – discussion sessions
 - Began Monday morning and engaged through Friday noon

Young Tree Care - Synopsis of Relevant Information in the Context of Current Management Practices: 1-7 years old

- Team 1 - Lukasz Stelinski, Michael Rogers, Steve Lapointe
- 17 presentations and posters



Young tree care-Synopsis of relevant information from 2015 IRCHLB conference in the context of current management practices: 1-7 year old trees

Lukasz L. Stelinski, Michael Rogers, and Stephen
Lapointe

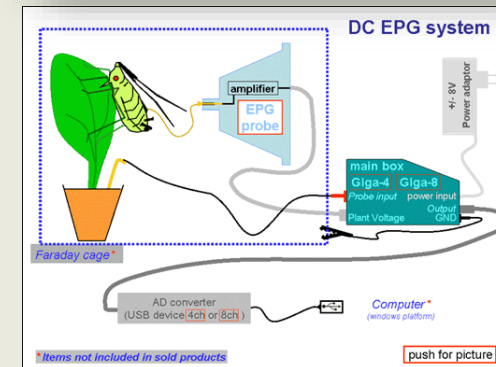
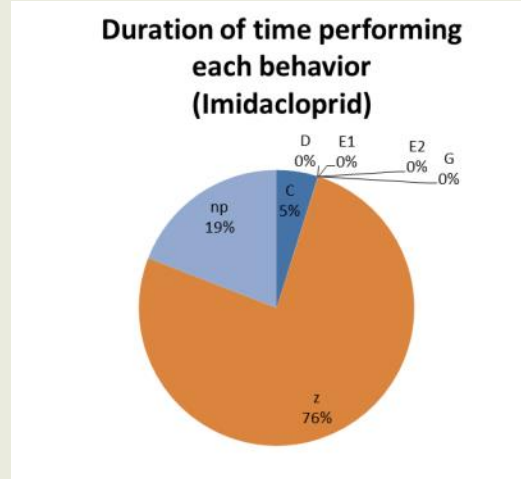
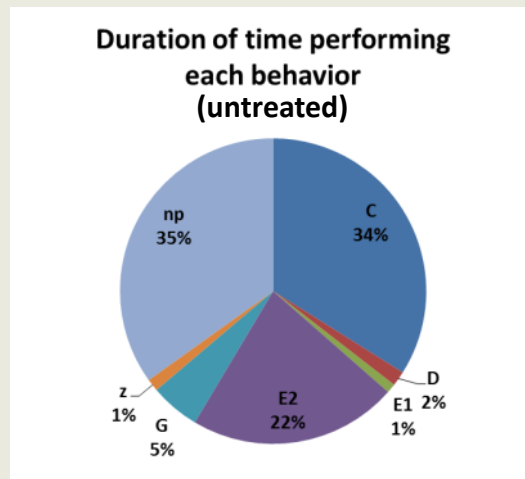
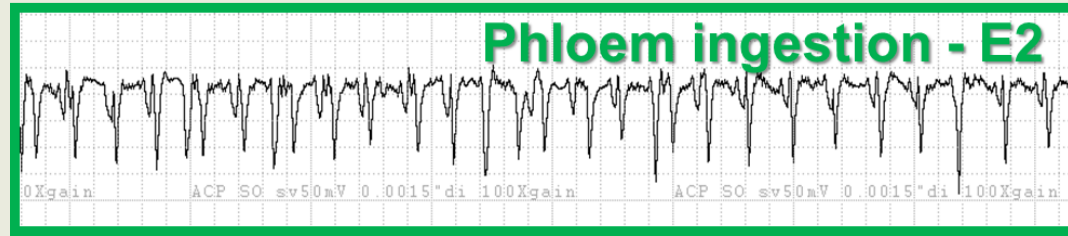
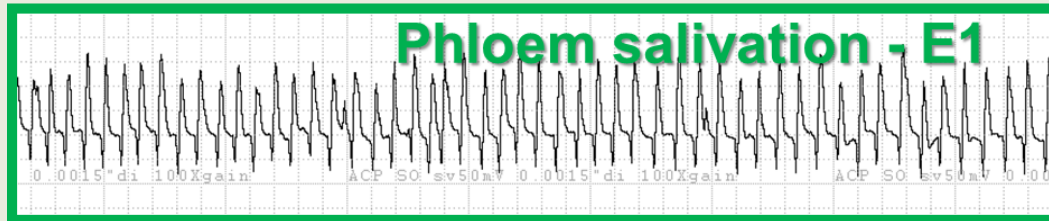
*University of Florida,
Entomology and Nematology
Department,
Lake Alfred, FL*

Soil-applied neonicotinoids

- Foundation of young tree psyllid control programs
 - Admire Pro (imidacloprid)
 - Platinum 75 SG (thiamethoxam)
 - Belay 2.13 SC (clothianidin)



Soil-applied neonics can prevent phloem feeding by psyllids—Rogers et al. previous research



Season-long ACP control

(foliar applications to prevent pesticide resistance to neonics shown in orange)

Tree size	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Reset (<3')	P		A	A	B	B	B	A	A		P	
1-2 yr (3-5')	P		A	B	B	B	B	B	A		P	
3-5 yr (5-9') bearing			B		P	A	B	A				

A= Admire (imidacloprid); B=Belay (clothianidin); P=Platinum (thiamethoxam)

How much protection?

- Soil-applied neonicotinoids will not provide 100% protection from HLB
 - Can expect <1-3% infection rate annually depending on local conditions
 - Distribution of product within plant
 - Affected by accuracy of application
 - Psyllid population pressure (surrounding areas)
 - The lower the psyllid population, the greater the success will be in keeping HLB spread low
 - “numbers game”
 - Foliar sprays will be important
 - CHMAs

HLB infection rates in new plantings



Reports from Growers (2014)

- Varied success in young tree protection
 - Trees 3-4 years of age:
 - Average rate of HLB ~10-12%
 - Best case examples (4 years after planting):
 - As low as 8% HLB where psyllid populations remain under control in surrounding areas
 - Where psyllid populations have not been controlled in surrounding areas up to 80-100% HLB by year 4

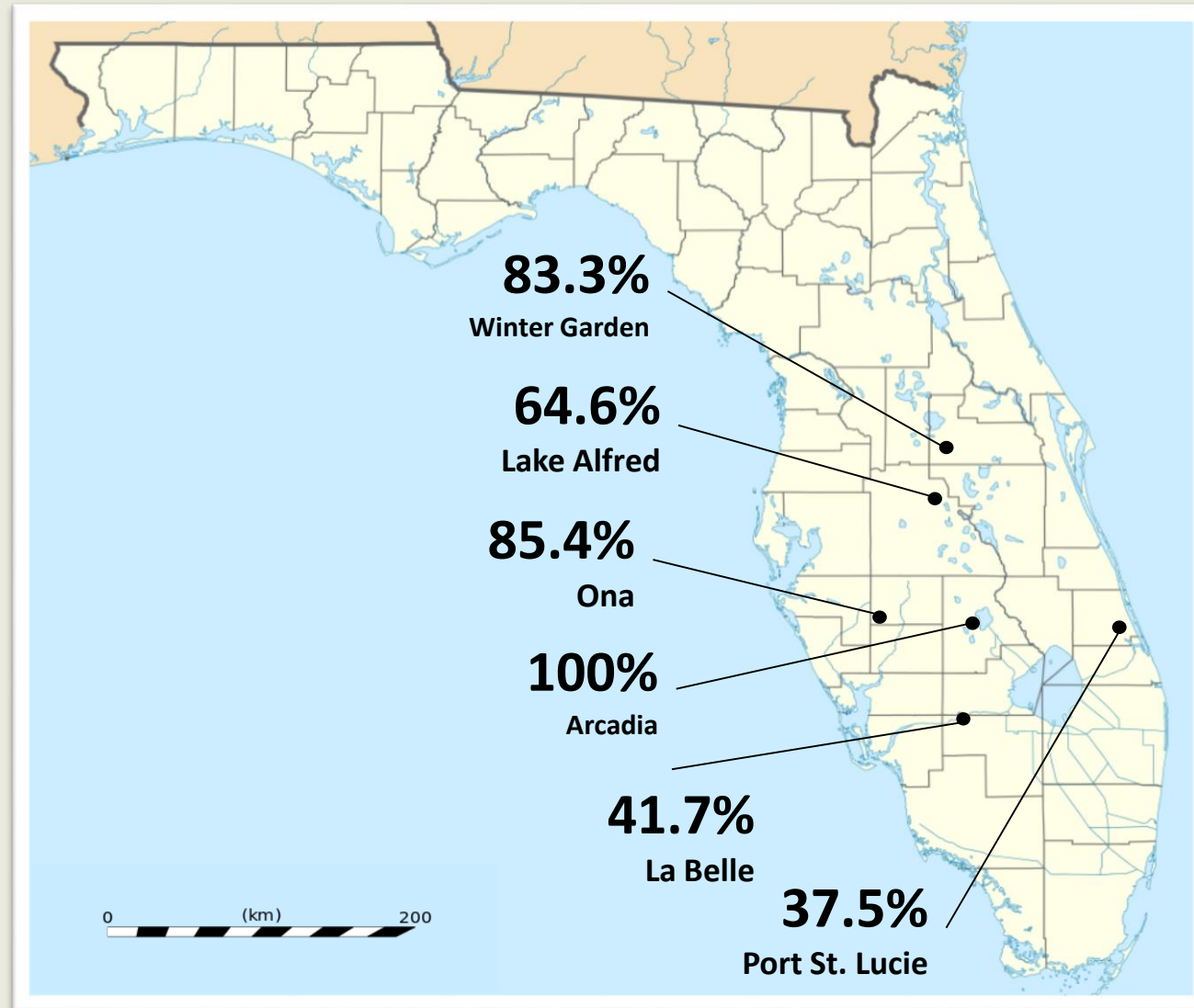
HLB infection rates in new plantings

Groves ~2 years of age (2014)

Psyllid pressure	HLB infection range	Average infection rate	# of groves
1 - low	0 – 4.05%	1.02%	20
2 – medium	1.73 – 65.6%	17.7%	20
3 - high	0 – 36.9%	20.96%	21



Psyllid infection is not 100% in Florida in all locations at all times. Surprising variation. Coy and Stelinski 2015



Uptake Study SWFREC 2010--Stansly et al.

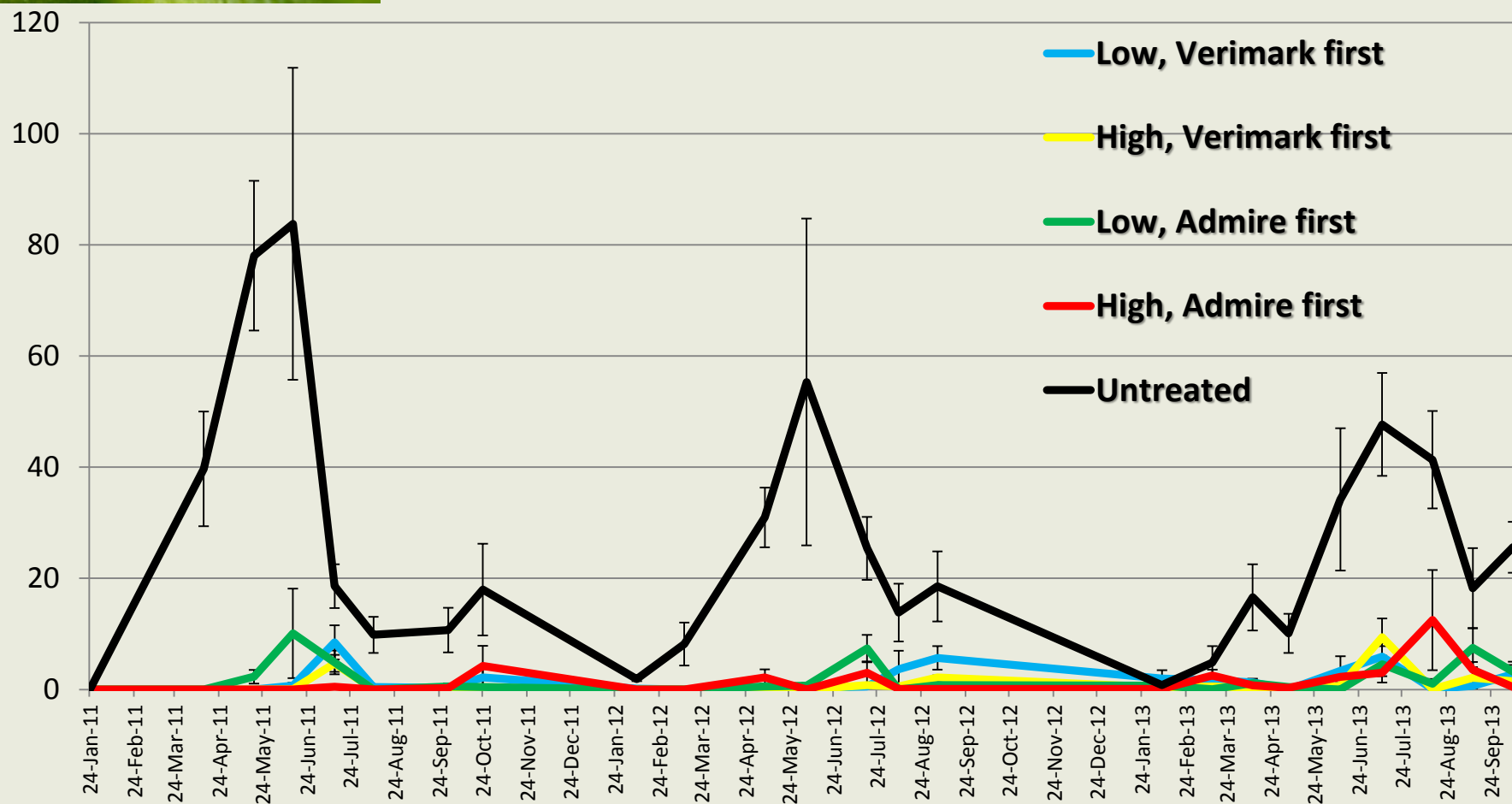
A single 8 oz suspension of each product was applied to bare soil within 9 inches of the trunk of the tree using an EZ-Dose® sprayer operating at a pressure of 45 PSI

Quantity of cyantraniliprole, imidacloprid or thiamethoxam in citrus flush

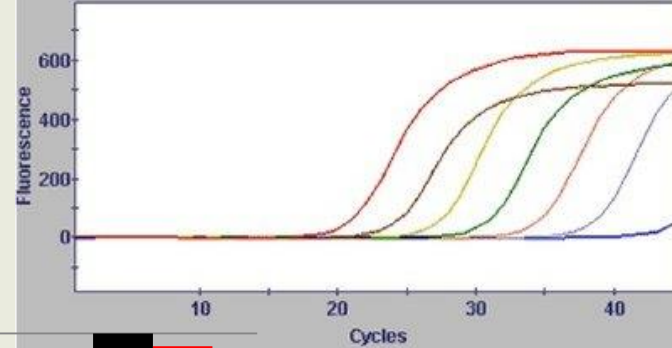
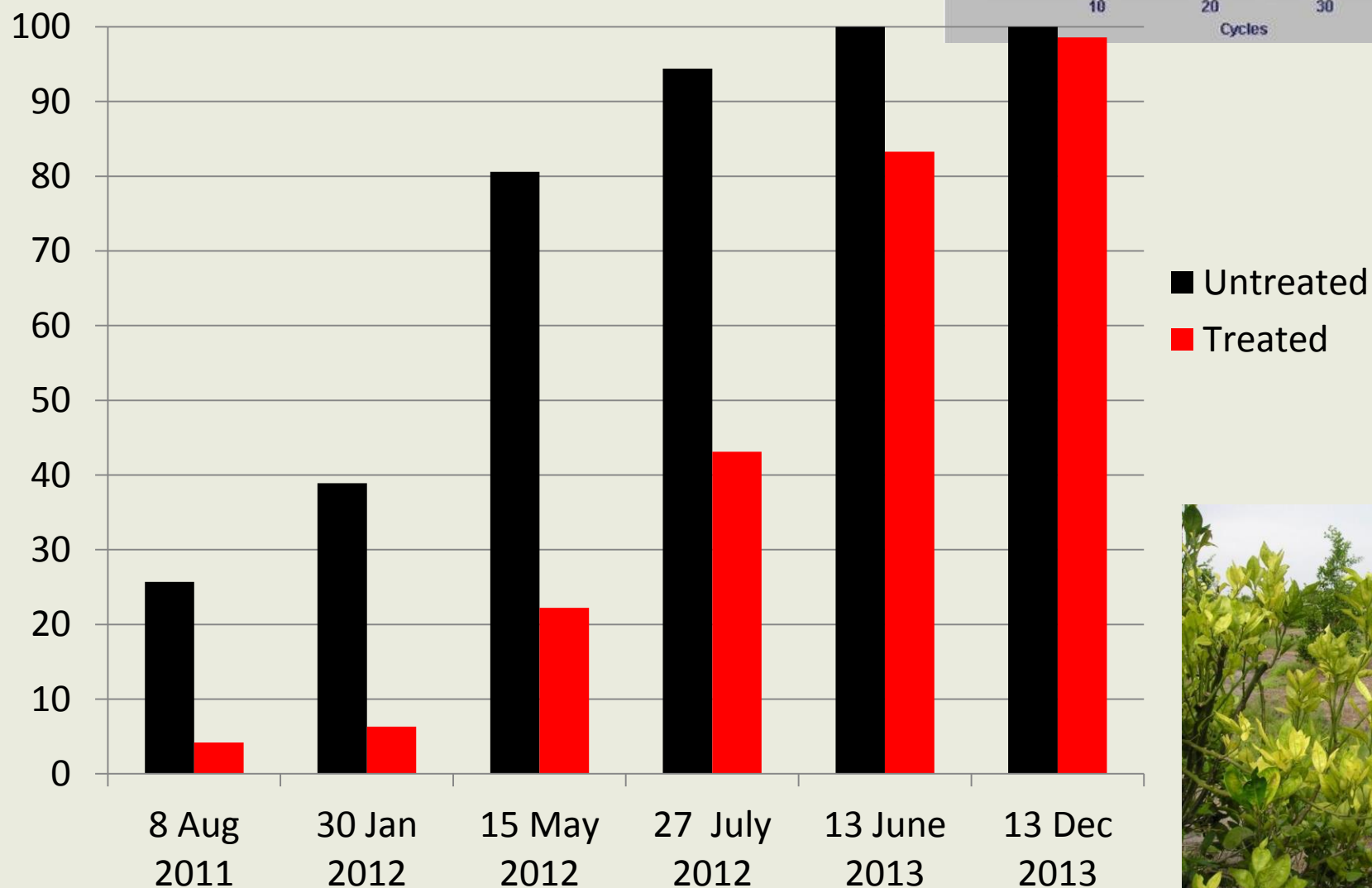
Treatment	Rate	Concentration in leaves (ug ai/g)		
	g ai/ha (oz/A)	8 Sep 2010 (50 DAT)	18 Nov 2010 (118 DAT)	11 May 2011 (295 DAT)
Admire Pro (imidacloprid)	280 (7)	2.86	0.20	0.03
Platinum (thiamethoxam)	140 (2.67)	1.13	0.07	0.00
Verimark (cyantraniliprole)	150 (10.3)	0.57	0.15	0.17
	225 (15.4)	0.46	0.31	0.06
	350 (25)	0.32	0.48	0.16



ACP Nymphs per Shoot

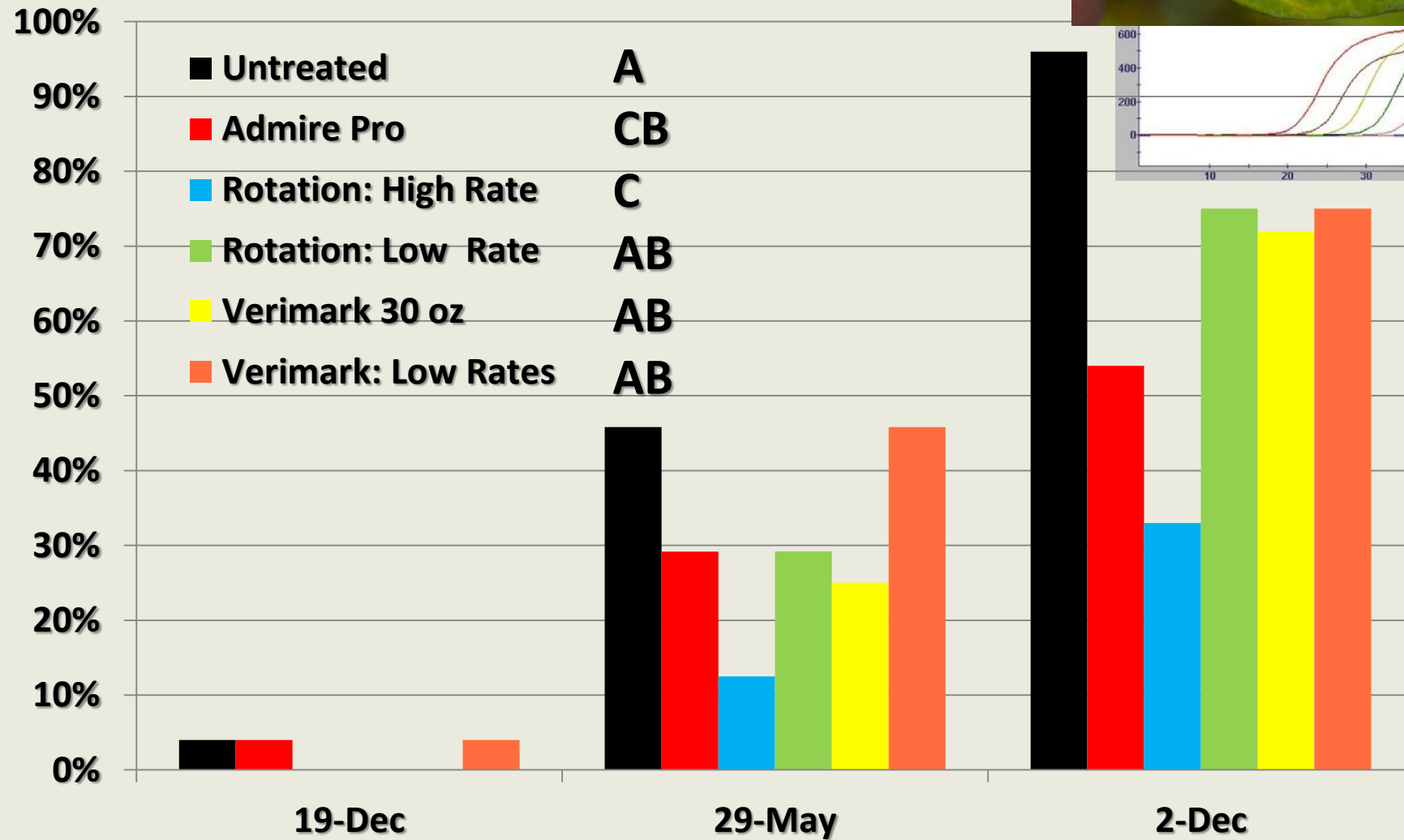


Incidence of HLB (%)



Product/	Rate/	Drench					Drip				
Formulation	Acre	June	Aug	Oct	Dec	Feb	Apr	Jun	Aug	Oct	Dec
Untreated											
Admire Pro 4.6 SC	7.0 oz	x	x	x	x	x					
Admire Pro 4.6 SC	10.5 oz						x	x	x	x	
Admire Pro 4.6 SC	14 oz										x
Admire Pro 4.6 SC	7.0 oz	x				x					
Verimark 20 SC	30 oz		x						x		
Belay 2.13 SC	6.0 oz			x				x			x
Platinum 75 SG	2.67 oz				x						
Platinum 75 SG	3.17 oz						x				
Admire Pro 4.6 SC	10.5 oz									x	
Admire Pro 4.6 SC	7.0 oz	x									
Verimark 20 SC	15 oz		x			x					
Verimark 20 SC	20 oz								x		
Platinum 75 SG	2.67 oz			x							
Belay 2.13 sc	6.0 oz				x					x	
Platinum 75 SG	3.17 oz						x				
Admire Pro 4.6 SC	10.5 oz							x			
Platinum 75 SG	3.67 oz										x
Verimark 20 SC	30 oz	x	x	x	x	x	x	x	x	x	x
Verimark 20 SC	15 oz	x	x	x	x	x					
Verimark 20 SC	20.0 oz						x	x	x	x	x

Incidence of HLB— Stansly et al.



Some conclusions

- Rotations of soil applied systemic insecticides including neonicotinoids and cyantraniliprole were most effective in reducing ACP numbers, HLB incidence and increasing growth and yield compared to untreated trees or trees receiving only one mode of action
- Chemigation with drip after first year most efficient
- Nevertheless, control was not sufficient under conditions of high ACP and HLB incidence

New tools with systemic properties

- Sivanto—New insecticide from Bayer CropScience in the 4D butenolide class
- Effective against both adult and immature ACP
- Reduces ACP feeding
- In our field trials, sometimes less consistent as compared with imidacloprid, but a useful new tool.
- Closer from Dow (sulfoxamine class) is also a useful new tool in the 4C group of insecticides [no or little cross-resistance with neonics reported thus far]

Soil-applied neonics must be applied properly

- Must account for
 - Tree size
 - Planting density
 - Formulation of product being applied
 - Imidacloprid products
 - 4.6F, 4F, 2F...



SEASON-LONG ACP CONTROL ON YOUNG TREES

Tree size	Jan		Feb		Mar		Apr		May		Jun		Jul		Aug		Sep		Oct		Nov		Dec	
Reset (<3')		P			A			A			B			B			A			A			P	
1-2 yr (3-5')		P			A			B			B			B			B			A			P	
3-5 yr (5-9') ¹ bearing								P			A			B			A			B				

A= Admire (imidacloprid); B=Belay (clothianidin); P=Platinum (thiamethoxam); timing of foliar applications with different modes of action to prevent pesticide resistance to neonics depicted by white boxes.

¹EPA SLN No. FL-12008 now permits up to 2 applications of Admire Pro at a rate of 14 fl oz/A per 12 months for trees 5-9' in height; Belay section 18 label permits 2 applications at 12 fl oz/A per 12 months for trees 5-9' in height. Always read and follow label directions.



Resets (< 3')

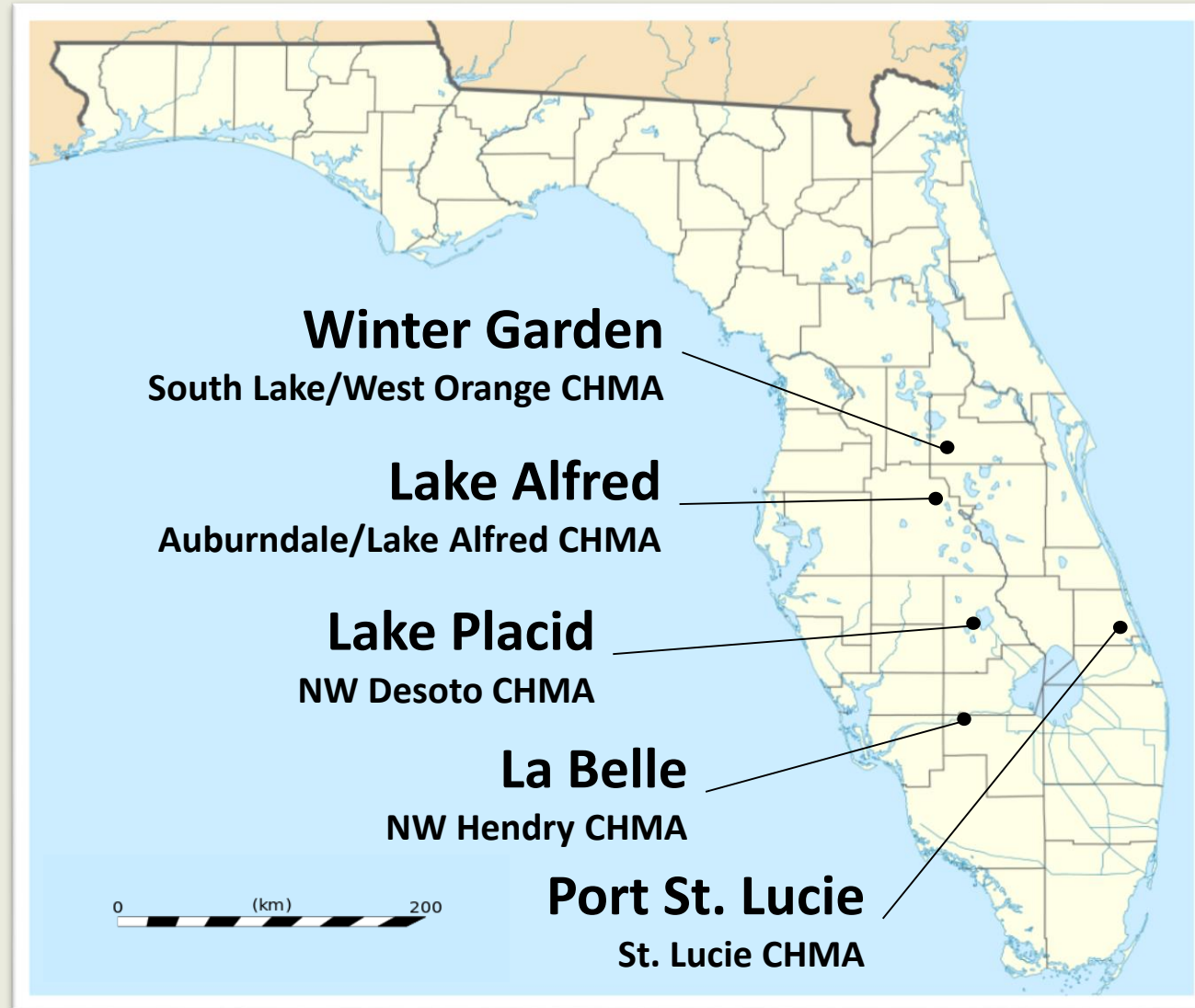


1-2 yr (3-5')

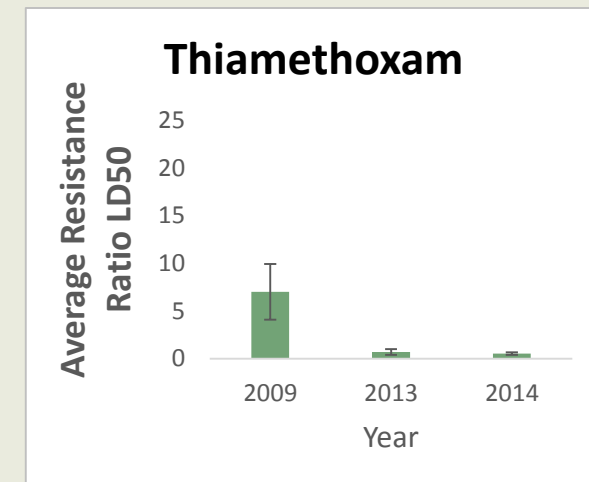
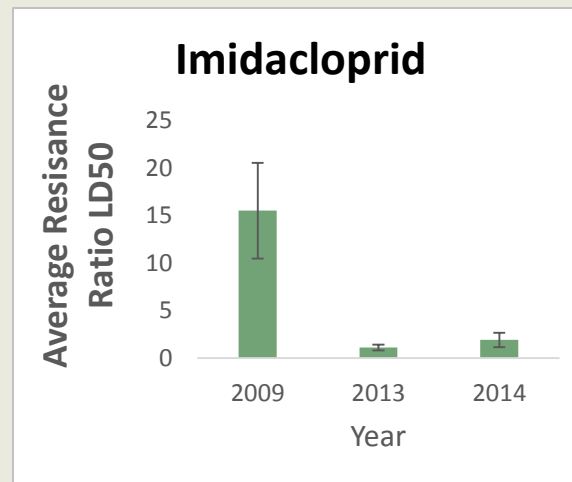
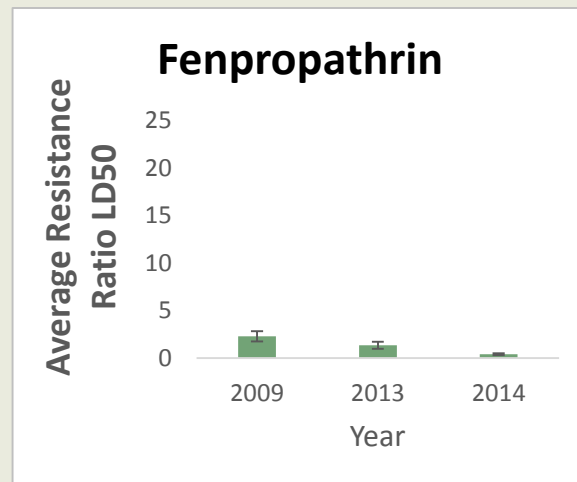
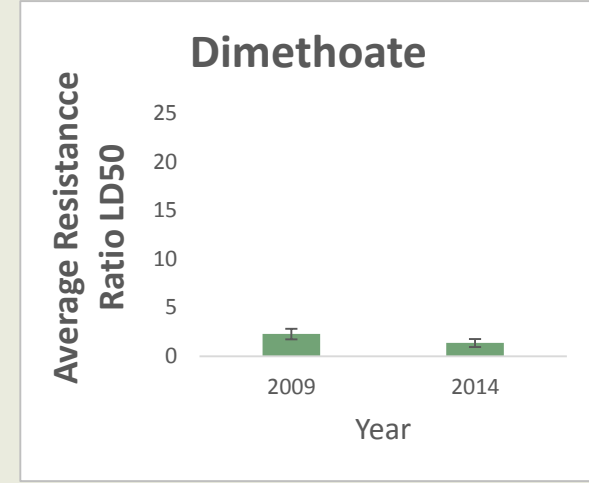
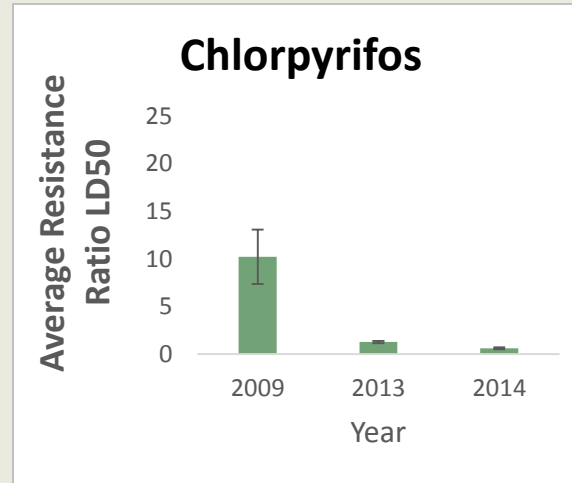
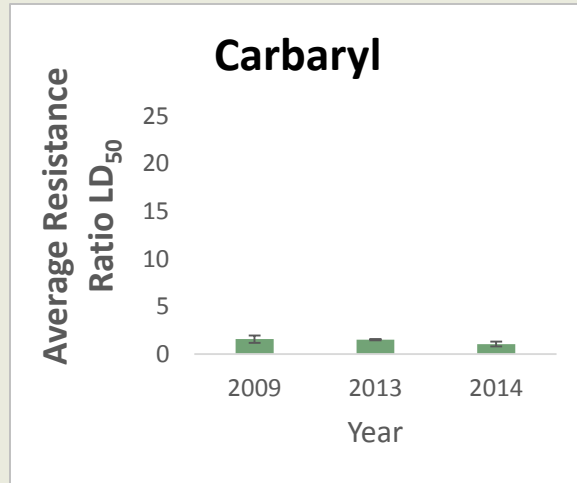


3-5+ yr (5-9')

What's the recent news on resistance?



Things look pretty good currently



Correct use of available tools for ACP control in young trees:

- Don't cut the per tree rate below what is recommended
 - will result in not enough product being taken up by the plant for any true activity
- If short on product at higher planting density...
 - Prioritize soil applications to periods of year when psyllid pressure is greatest
- Don't slack off on psyllid control...its still a numbers game
 - CHMAs are the most cost effective approach

New tools in addition to insecticides?

- Reflective mulches show promise in reducing ACP populations.
- Windbreaks reduce psyllid populations on border rows of orchards. If you already have them, great. If not, consider putting them in.
- New information on potential ACP attractants for monitoring and attract-&-kill tactics.
- Applications of RNAi to affect psyllid biology—more later on that topic.
- Development of a nuPsyllid that does not transmit the pathogen—more on that later.

Epidemiology

- Why does HLB spread if we are doing everything right?
- ACP move widely. Infected psyllids are more likely to move than uninfected.
- HLB was likely here in 1998, but we didn't really start managing ACP in earnest until about 2008—the problem got a **10 year** head start.

Epidemiology

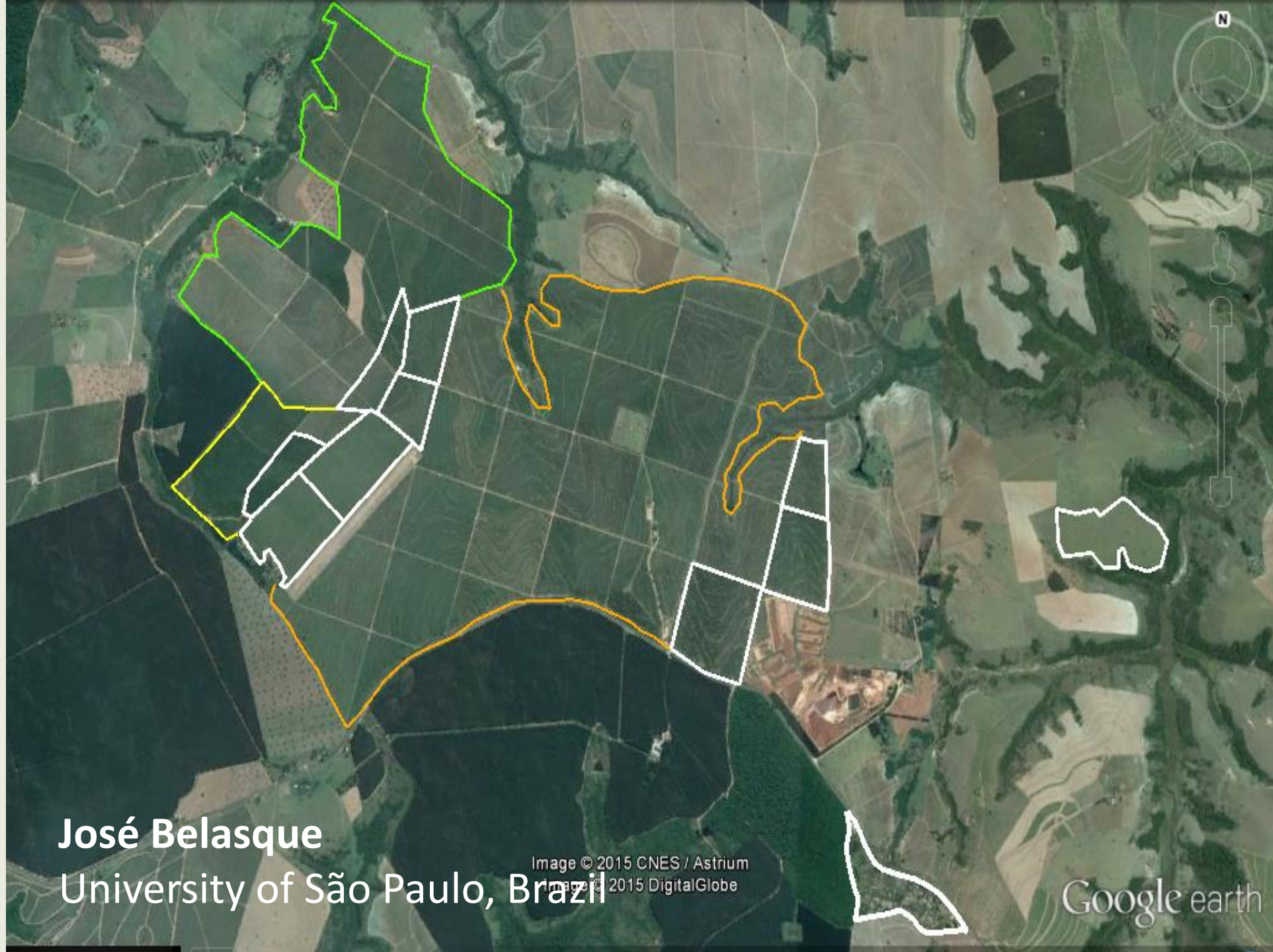
- The epidemiological work continues to show that removal of infected trees combined with vector management is the best way to manage this problem.
- It's too late to take this approach in Florida unless most of the mature inventory were to be replaced.
- Why did it not work in Florida—Lot's of reasons. The 10 year head start; previous canker tree eradication program soured many on tree removal.

An aerial photograph showing a patchwork of agricultural fields. Some fields are lush green, likely citrus groves, while others are reddish-brown, indicating bare soil or a different crop. The fields are separated by narrow roads and paths. The text is overlaid on the top half of the image.

distance and magnitude of influence of HLB-inoculum areas on **well-managed** citrus producing farms

José Belasque

University of São Paulo, Brazil



José Belasque

University of São Paulo, Brazil

Image © 2015 CNES / Astrium
Image © 2015 DigitalGlobe

Google earth

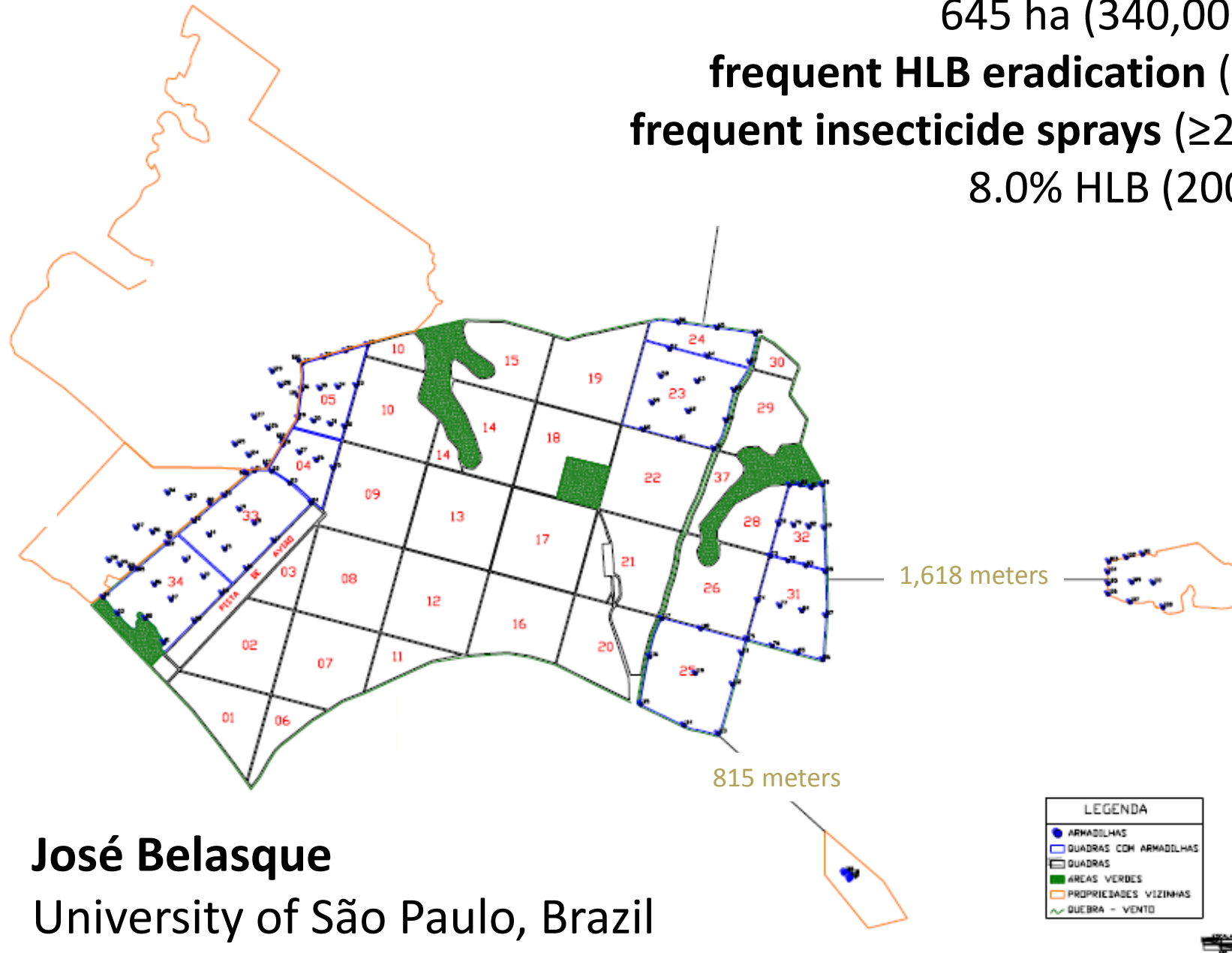
Reference area

645 ha (340,000 plants)

frequent HLB eradication (≥ 4 /year)

frequent insecticide sprays (≥ 2 /month)

8.0% HLB (2007-2014)

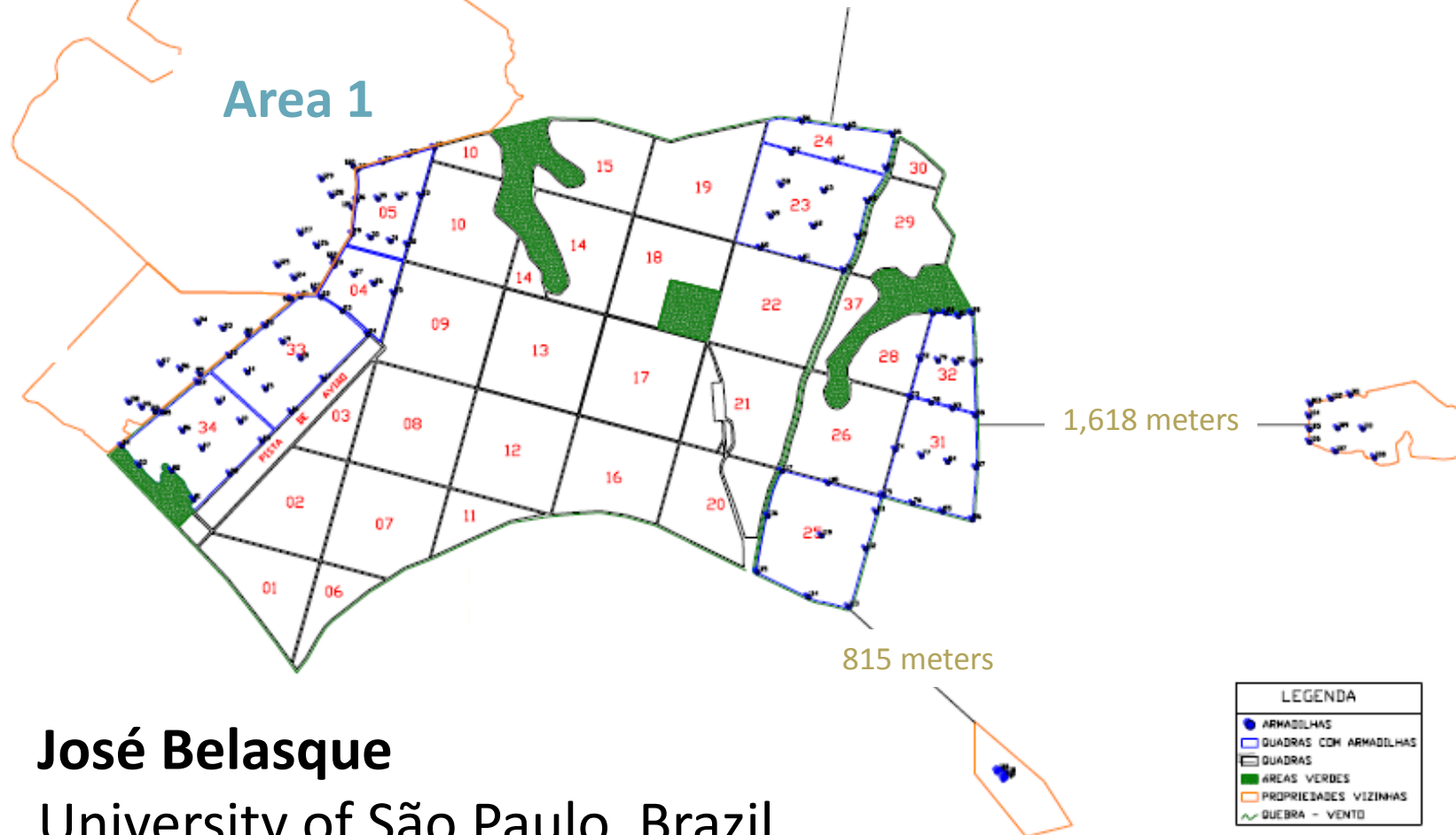


José Belasque

University of São Paulo, Brazil

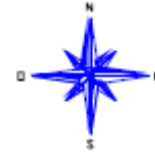
320 ha (115,000 plants)
some **HLB eradication**
insecticide sprays ($\geq$ monthly)
 $\geq 5\%$ HLB

Reference area
645 ha (340,000 plants)
frequent HLB eradication (≥ 4 /year)
frequent insecticide sprays (≥ 2 /month)
8.0% HLB (2007-2014)



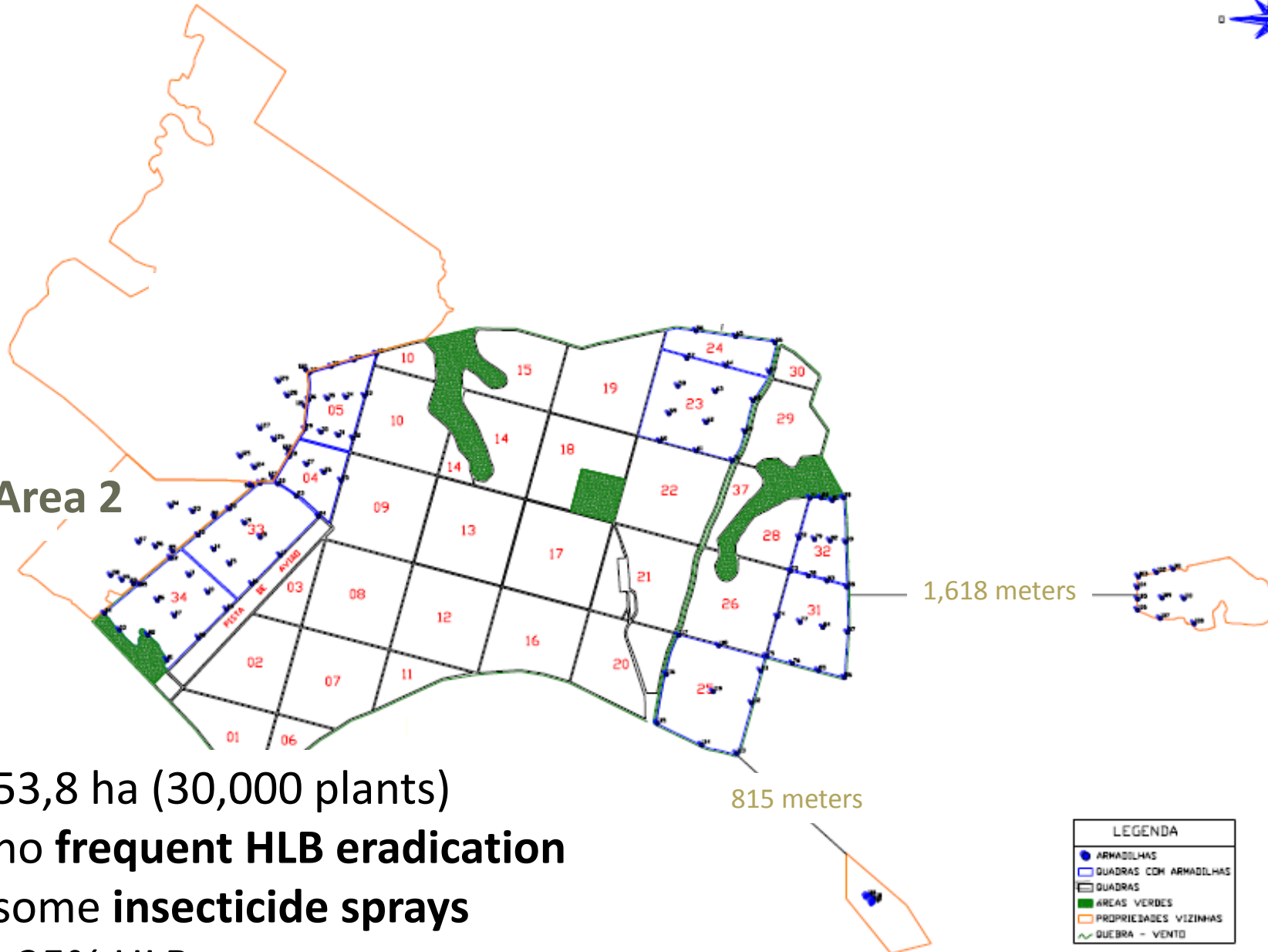
LEGENDA	
	ARMADILHAS
	QUADRAS COM ARMADILHAS
	QUADRAS
	AREAS VERDES
	PROPRIEDADES VIZINHAS
	QUEBRA - VENTO

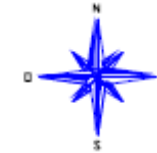




Area 2

53,8 ha (30,000 plants)
no frequent HLB eradication
some insecticide sprays
>25% HLB





Area 3

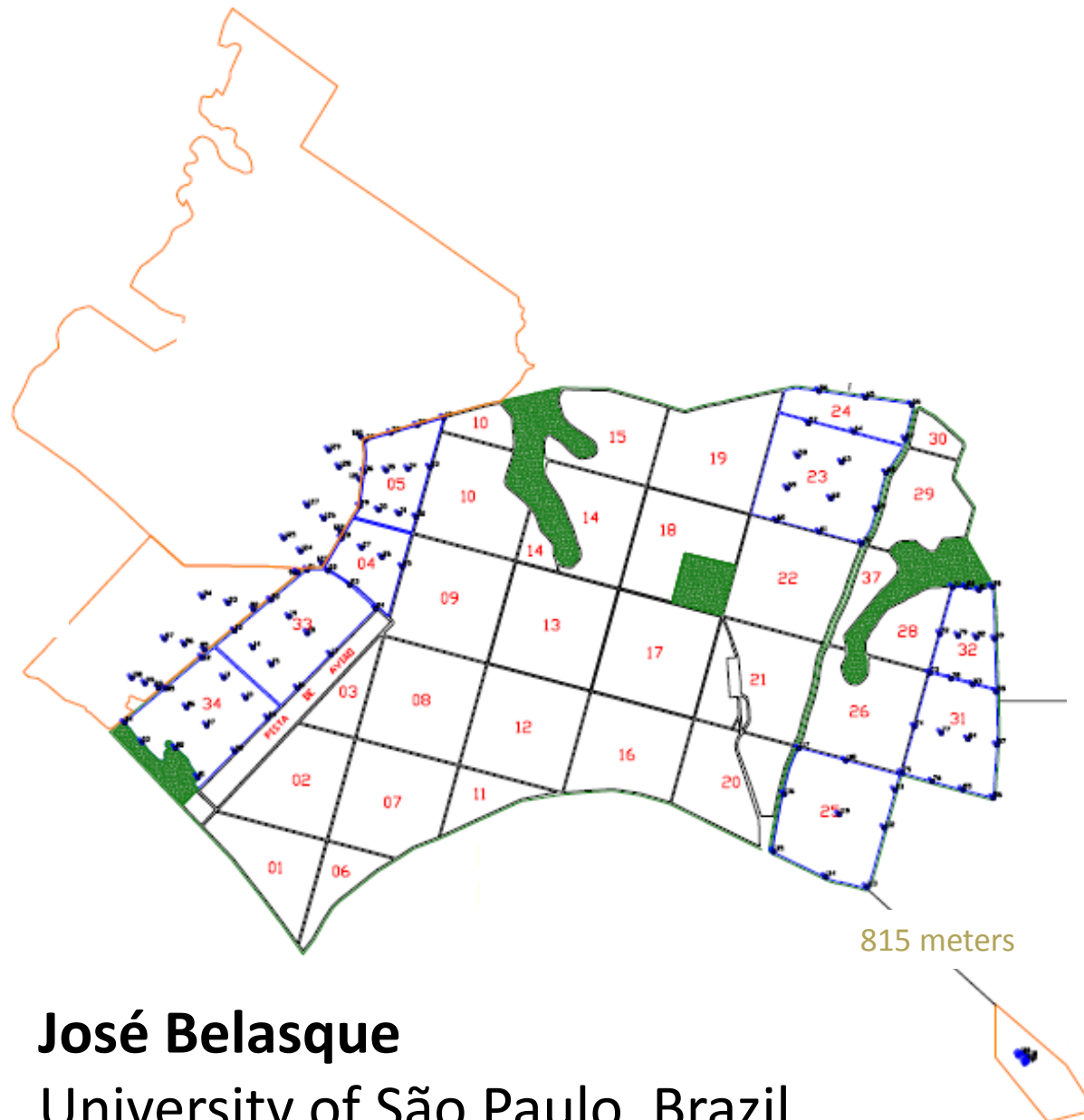
22,4 ha (5,000 plants)

no **HLB** eradication

no **insecticide sprays**

100% HLB

eradicated on 08/2013



1,618 meters

José Belasque

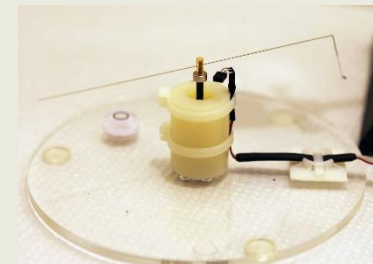
University of São Paulo, Brazil

LEGENDA	
	ARMADILHAS
	QUADRAS COM ARMADILHAS
	QUADRAS
	ÁREAS VERDES
	PROPRIEDADES VIZINHAS
	QUEBRA - VENTO

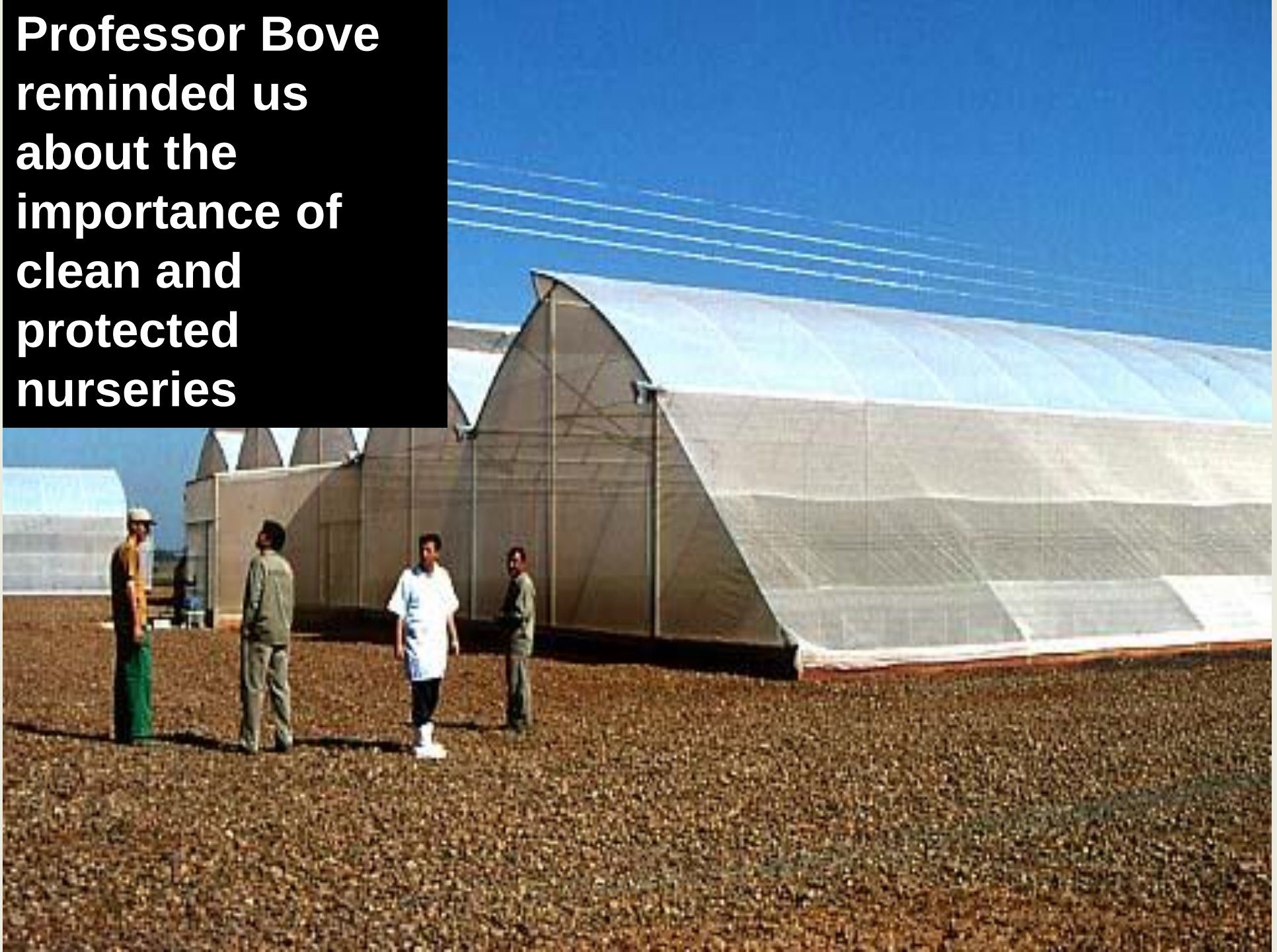


The epidemiology of disease spread and biology of the psyllid vector match

- Psyllids are capable of 3 hours of continuous flight in the lab.
- This translates to approximately four miles of continuous flight.
- We have measured up to 1.25 miles of flight in the field.
- About 1.25 miles is the “risk distance” that epidemiologists seem to calculate.



**Professor Bove
reminded us
about the
importance of
clean and
protected
nurseries**



Need to protect young trees out of the gate (nurseries)

- **Results from California-**

- Imidacloprid remains at target thresholds for psyllid control for 12 weeks.
- Thiamethoxam was as effective as imidacloprid.
- Dinotefuran was least effective out of the three tested.

- **Results from Texas-**

- Recent findings of HLB and perhaps not surprisingly in unenclosed facilities. Need to remain vigilant regarding protecting nursery stock.

Epidemiology and the need for area-wide CHMA-type management

- Old news now-Area-wide or CHMA-type management is the way to go for success.
- It works. The science shows it; the modeling shows it; the proof is there in areas where it is practiced (especially when combined with inoculum removal).
- Not much new here, except for more and more corroborating data.

Grosser, Gmitter, and Castle

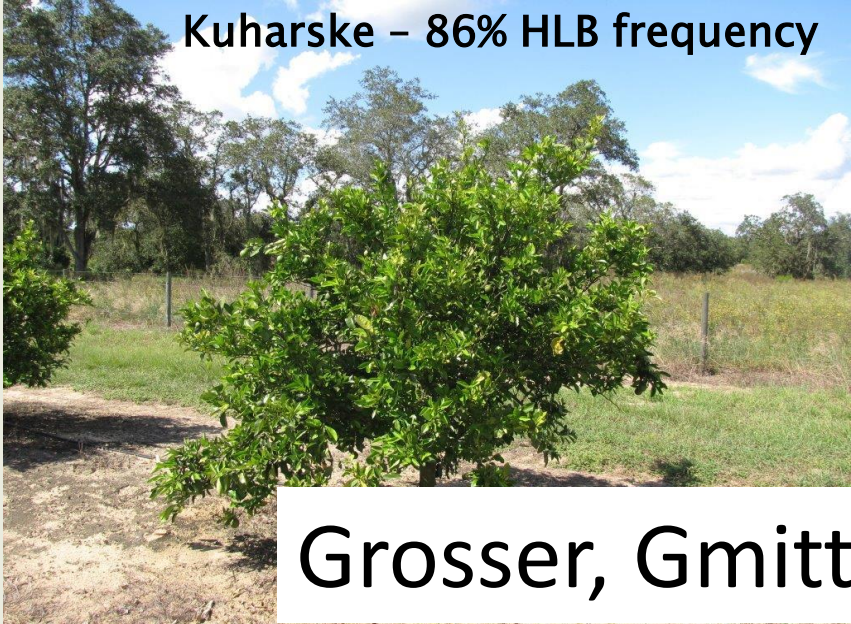
Susceptible
rootstock
Orange #1



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)

Kuharske – 86% HLB frequency

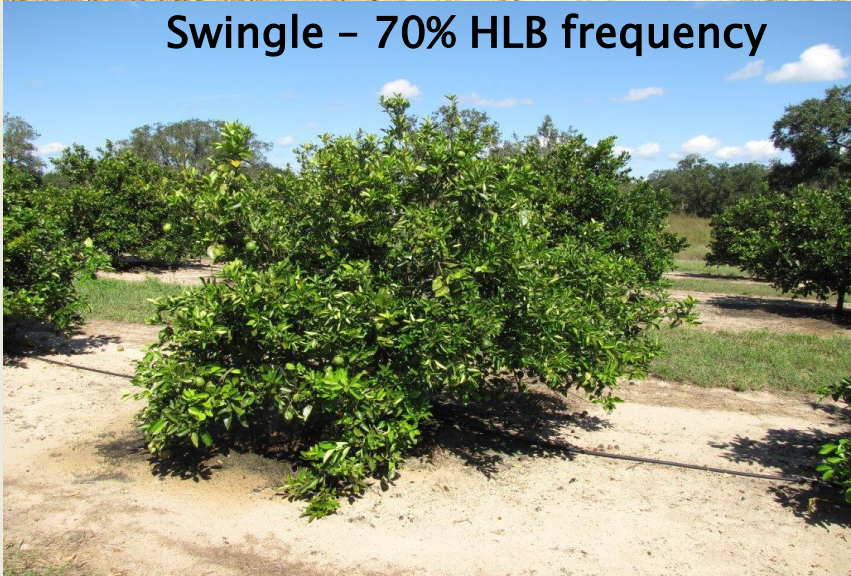


Orange #15 – 14% HLB frequency



Grosser, Gmitter, and Castle

Swingle – 70% HLB frequency



Orange #19 – 23% HLB frequency



**HLB-infected trees in the St. Helena Project
–differences in infection frequency & disease severity**

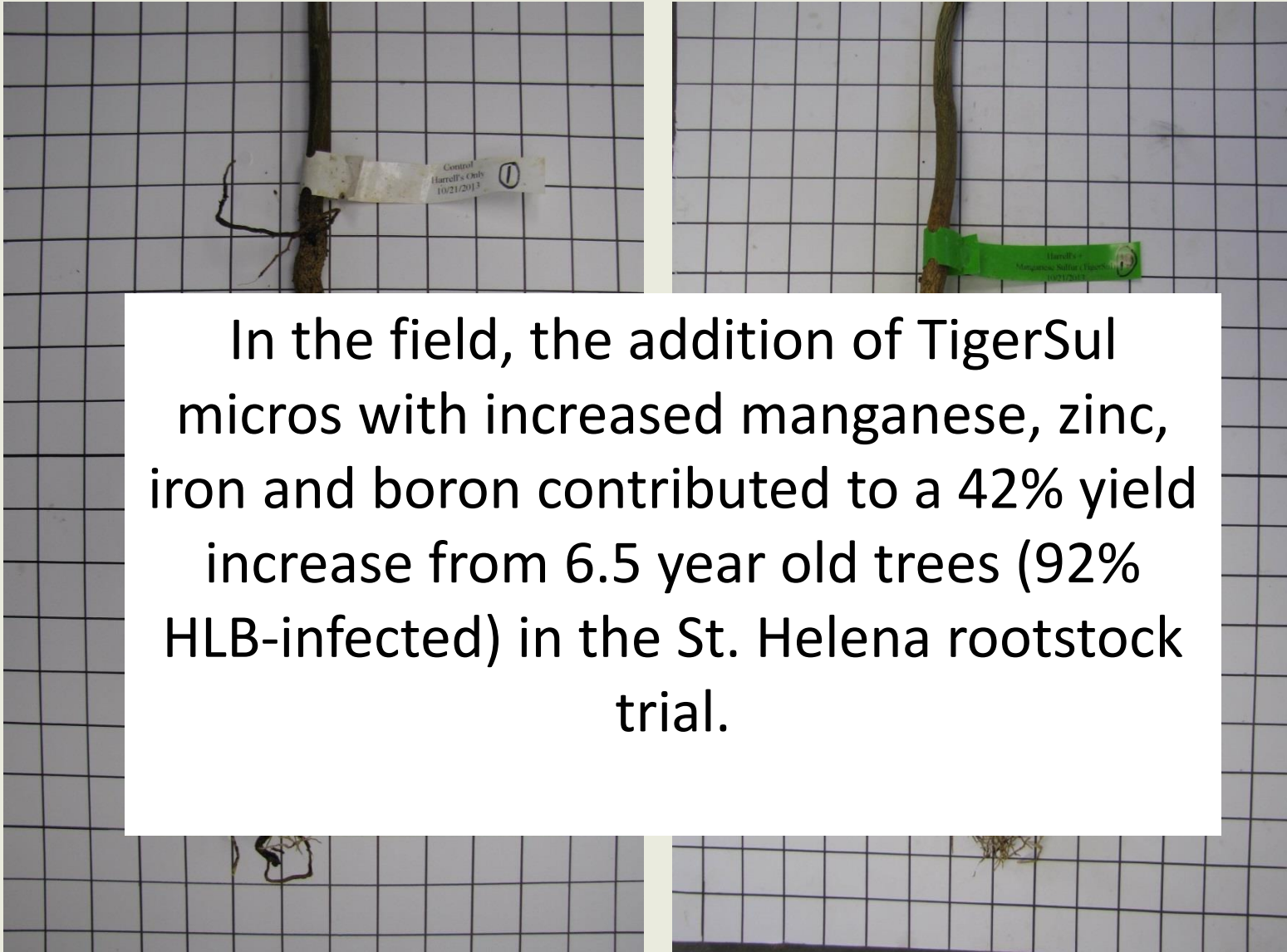
Soil-applied controlled release fertilizer (CRF) treatments impact the health and growth of HLB-infected trees – Results from greenhouse and field experiments

Jude Grosser, Gary Barthe, Jim Baldwin, Ahmad Omar
and Tripti Vashisth

Need to get nutrients to the roots

Minor element deficiencies are greater in HLB-impacted roots than in HLB-impacted leaves! Foliar sprays temporarily help the leaves, but don't get minors to the roots! What about xylem function ??? GROUND NUTRITION IS IMPORTANT! (quantity and type – constant supply is needed!)

Variety	Type of Sample	Tree Condition	Nitrogen	P	K	Mg	Ca	S	B	Zn	Mn	Fe	Cu
			%	%	%	%	%	%	ppm	ppm	ppm	ppm	ppm
CZO	Leaf	non-symptomatic	4.08	0.18	4.04	0.25	1.42	0.37	223.4	45.33	452.5	109.7	164.6
CZO	Leaf	Symptomatic	3.61	0.2	3.52	0.29	1.18	0.3	178.2	22.32	192.7	77.91	93.93
CZO	Root	non-symptomatic	2.67	0.21	1.57	0.32	1.33	0.97	29.65	1600	6913	2084	38.93
CZO	Root	Symptomatic	2.63	0.24	1.38	0.16	0.8	0.29	23.3	291	1308	496	16.46



Harrell's CRF Control #1

Harrell's+TigerSul Mn #10



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.

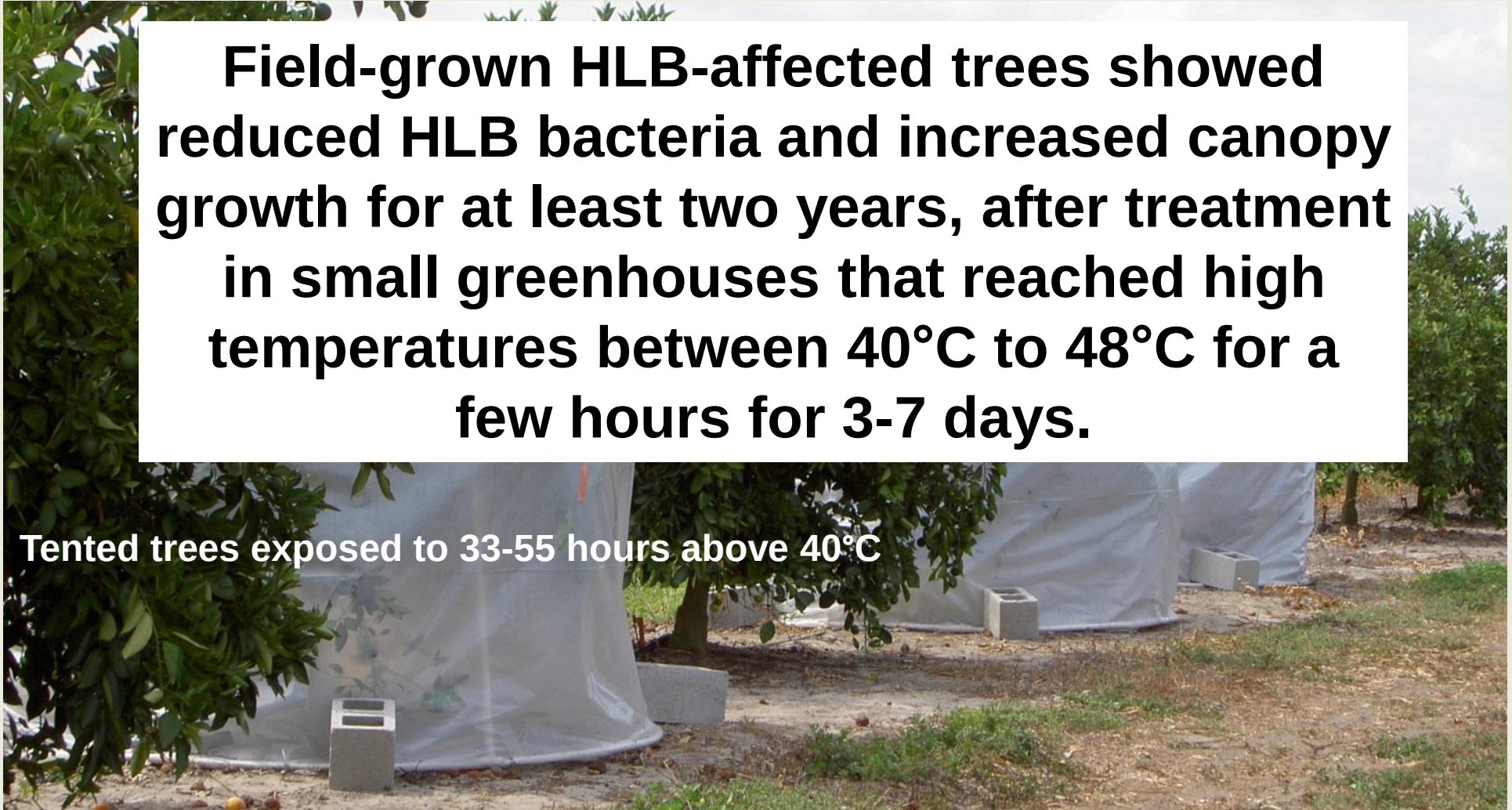


Professor Bove reminded us that he and colleagues were investigating thermo-therapy as early as 1981 with success.

Doud et al. Promising results with thermotherapy in Florida

Field-grown HLB-affected trees showed reduced HLB bacteria and increased canopy growth for at least two years, after treatment in small greenhouses that reached high temperatures between 40°C to 48°C for a few hours for 3-7 days.

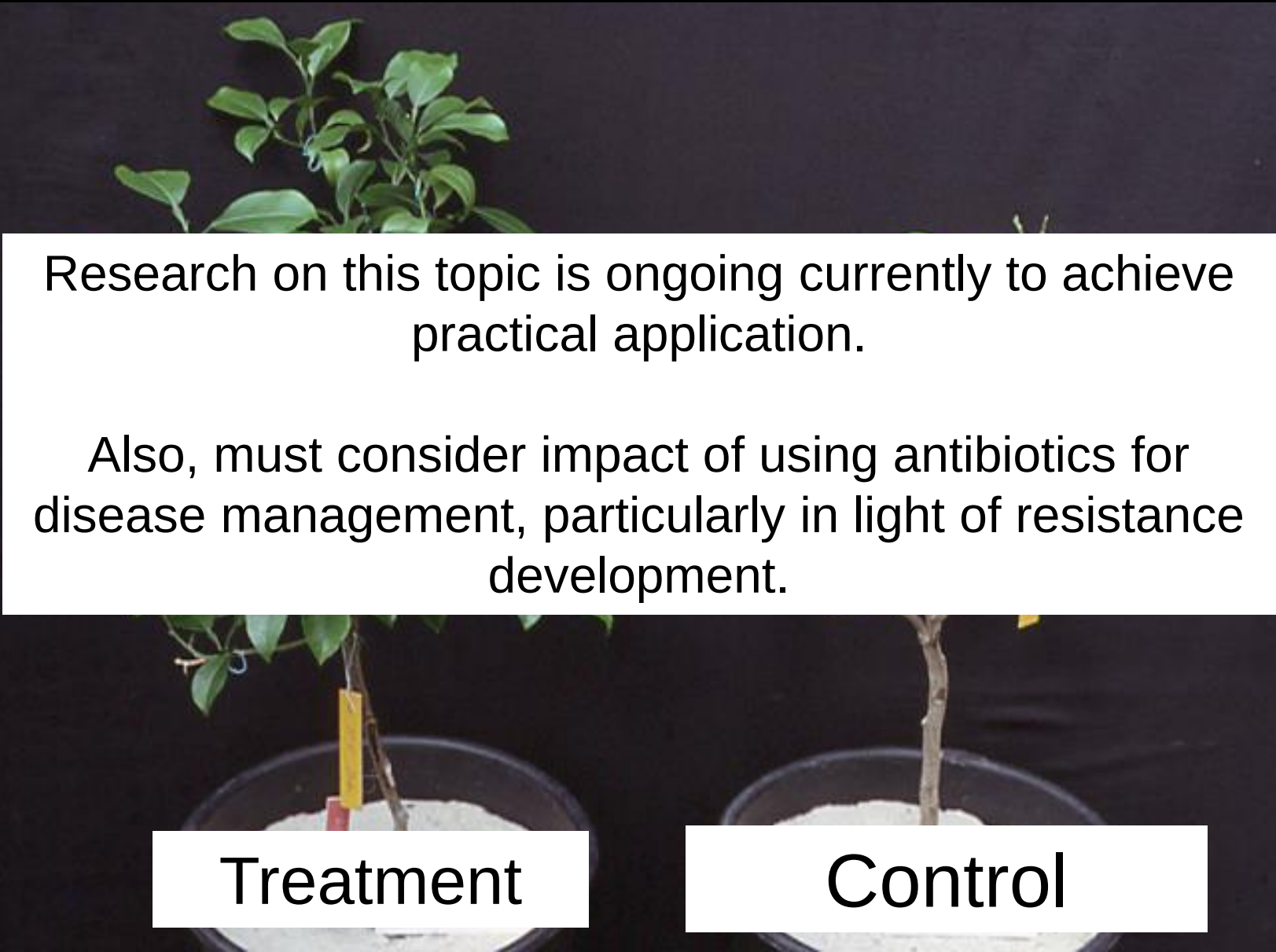
Tented trees exposed to 33-55 hours above 40°C



What about antibiotics? Professor Bove reminded us of his previous research



Antibiotics can cause remission of HLB symptoms, and this result supported the bacterial nature of the HLB agent (*Aubert and Bové, 1980; Bové et al., 1980*).



Research on this topic is ongoing currently to achieve practical application.

Also, must consider impact of using antibiotics for disease management, particularly in light of resistance development.

Treatment

Control

Summary

- Area-wide management is a must for protecting trees (no matter the age).
- Soil-applied systemic insecticides are still the best tools for vector management in young trees.
- Seems like we have insecticide resistance for ACP under control currently.
- Psyllids move and the associated epidemiological data seem to indicate that without inoculum removal, even large-scale ACP management may not prevent escalating spread of disease.
- New insecticide tools and non-insecticide 'in progress' alternatives were presented with the possibility for incorporation into vector management.
- Promising results were presented with new tolerant/resistant variety development, treatment of roots with appropriate fertilizers, and thermotherapy.

QUESTIONS?

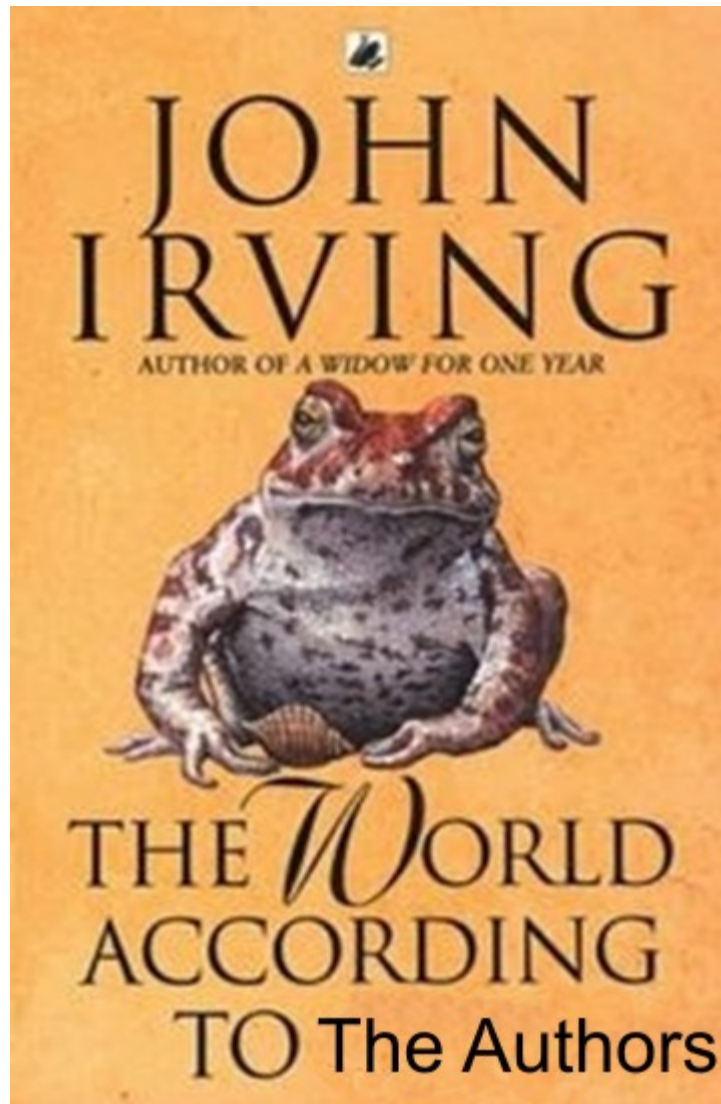
Mature tree care in the context of current management practices

- Team 2 - Mike Irey, Evan Johnson, David Hall
- 34 presentations and posters

Mature tree care in the context of current management practices: 7+ year old trees

David Hall, Evan Johnson, Mike Irey

Interpretation of the work



- The conclusions will be those of the author(s)
 - Regardless of whether or not I agree with them....
- I took the liberty of selecting what I thought were the most important points in the conclusion slides

Breakdown of talks

- Disease development on mature trees
 - Effects of disease as it affects management strategies
 - Yield and fruit quality issues
- Disease mitigation
 - Antimicrobials
 - Thermotherapy
 - Resistance
- ACP management on mature trees

Excess bicarbonate in soil and irrigation water increases fibrous root loss and decline of HLB-affected citrus trees in Florida

*Jim Graham, Kayla Gerberich
Diane Bright, Evan Johnson*

IRCHLB IV
February 9, 2015



Management implications: Reduce soil stresses first

- Balanced, lower and more frequent application of water and nutrients to the reduced root system (“spoon feeding”)
- Reduce soil pH/bicarbonate stress to sustain root function in nutrient uptake and root longevity
- To assess bicarbonate stress :
 - check soil pH (wetted zone)
 - test well water for pH, bicarbonates, salinity, cations, anions
- Water conditioning: Inject N-furic acid or sulfuric acid (40%) to reduce irrigation water to 100 ppm bicarbonates
- Soil conditioning: broadcast sulfur in wetted zone to reduce root zone pH



Management implications: Reduce soil stresses first

- Balanced, lower and more frequent application of water and nutrients to the reduced root system (“spoon feeding”)
- Reduce soil pH/bicarbonate stress to sustain root function in nutrient uptake and root longevity
- To assess bicarbonate stress :
 - check soil pH (wetted zone)
 - test well water for pH, bicarbonates, salinity, cations, anions
- Water conditioning: Inject N-furic acid or sulfuric acid (40%) to reduce irrigation water to 100 ppm bicarbonates
- Soil conditioning: broadcast sulfur in wetted zone to reduce root zone pH



HLB-induced fruit drop, fibrous roots and the interaction with *Phytophthora* spp.

*Jim Graham, Kayla Gerberich
Diane Bright, Evan Johnson and John Taylor*



IRCHLB IV
February 10, 2015



Management implications: Reduce soil stresses first

- Balanced, lower and more frequent application of water and nutrients to the reduced root system (“spoon feeding”)
- Reduce soil pH/bicarbonate stress to sustain root function in nutrient uptake and root longevity
- To assess bicarbonate stress :
 - check soil pH (wetted zone)
 - test well water for pH, bicarbonates, salinity, cations, anions
- Water conditioning: Inject N-furic acid or sulfuric acid (40%) to reduce irrigation water to 100 ppm bicarbonates
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After correcting water/soil, then manage root pathogens

- Phytophthora should be managed more aggressively to sustain root health especially in flatwoods
- Phytophthora count >10-20 propagules/cm³
- Maximum label rates of fungicides recommended

Rotation of fungicides:

- Aliette/phosphite after spring shoot flush
- Mefenoxam after spring-early summer rains begin
- Aliette/phosphite after midsummer shoot flush
- Mefenoxam after fall shoot flushes
- Root flushes follow shoot flushes



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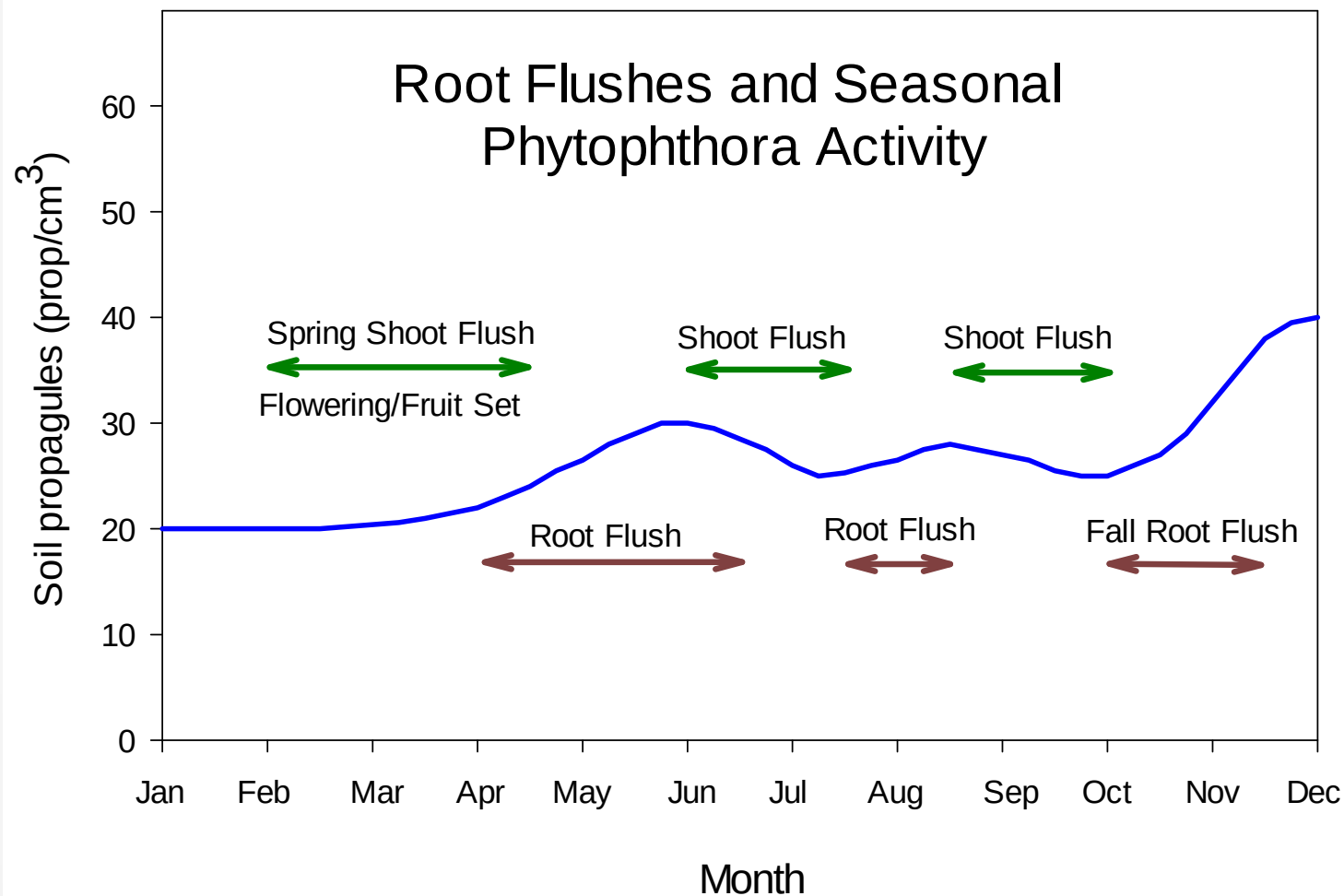
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Target fungicide applications to protect root flushes



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Citrus Huanglongbing stimulates root growth while causing overall root loss

*Evan Johnson, Kayla Gerberich
Jian Wu, James H. Graham*

IRCHLB IV
February 9, 2015



Root Growth is not inhibited

Presumed Healthy



New
Growth



Root
Density



Thinning trees



Conclusions

Two phases of root loss

- Early phase
 - 30-50% root loss
 - Before foliar symptoms
- Late phase
 - 70-80% loss
 - As leaf drop begins (canopy thinning)

Mechanism of root loss

- HLB increases root growth
 - Direct effect or response to root dieback?
 - Local Liberibacter infection?
- Reduced longevity of roots leads to root loss
 - Mechanism remains unknown
 - Not carbohydrate starvation
 - No phloem plugging

Huanglongbing reduces the effectiveness of Phytophthora fungicide control

*Evan Johnson, Jian Wu, Diane B. Bright,
James H. Graham*

IRCHLB IV



Conclusions

- HLB reduces root health by directly causing root loss and increasing *Phytophthora* infection
- HLB reduces the effectiveness of Aliette and Ridomil against *Phytophthora*
- While the effects of these treatments on yield in groves with both diseases is still being investigated, these findings emphasize the need to use *Phytophthora*-free planting material and drainage management to reduce the incidence of *Phytophthora spp.* in HLB-affected areas

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Soil-applied controlled release fertilizer (CRF) treatments impact the health and growth of HLB-infected trees – Results from greenhouse and field experiments

UF UNIVERSITY of
FLORIDA
IFAS
Citrus Research and Education Center

Jude Grosser, Gary Barthe, Jim Baldwin,
Ahmad Omar and Tripti Vashisth



UF-CREC Citrus Genetic Improvement Team
2015

My interpretation of the results:

1. **Foliar nutrition of HLB trees is important, but providing proper nutrition to the roots may be more important. Recommended levels of micronutrients are probably incorrect for citrus trees in the HLB world.**
2. Results from the greenhouse study indicate that overdoses of micronutrients in the TigerSul (clay prill) form increase feeder root density, and thus overall tree growth. Manganese appears to be the most important micronutrient in this regard.
3. There is an apparent synergy between the TigerSul products and the Harrell's CRF. **Acidification of the root zone by the slow release of the sulfate could be increasing the efficiency of CRF nutrient uptake by the roots – leading to improved tree health.**
4. **In the field, the addition of TigerSul micros with increased manganese, zinc, iron and boron contributed to a 42% yield increase from 6.5 year old trees (92% HLB-infected) in the St. Helena rootstock trial.**
5. There is clearly a tolerance/genetics/nutrition interaction.

Practical Considerations and Questions

1. A 100% CRF program on mature trees is quite expensive. Hybrid programs combining traditional dry fertilizer + CRF can improve the economics and are proving successful in some commercial operations (Duda, etc.). Significant improvements in tree health and cost efficiency are possible with additional fine-tuning.
2. **Preliminary evidence suggests that applications of CRF (including overdoses of TigerSul micros and poly-coated sodium borate) significantly reduce fruit drop from mature trees.**
3. An optimal CRF program should be tested with tree recovery following thermotherapy – this could bring trees back into production more quickly and extend their productivity.
4. Can such CRF programs slow the effects of HLB and improve overall tree health if implemented at the time of planting??? Research is underway to determine this.
5. **WE STILL HAVE A LOT TO LEARN ABOUT HLB AND NUTRITION!**



High incidence of pre-harvest colonization of *Diplodia* in HLB-symptomatic orange, its exacerbation of postharvest fruit decay and implication in HLB-associated pre-harvest fruit drop

Wei ZHAO, Jinhe BAI, Elizabeth BALDWIN*, Greg
McCOLLUM, Tim GOTTWALD
USDA-ARS, US Horticultural Research Laboratory

Background

- Multiple symptoms of Citrus HLB:
 - Reduce tree vigor and fruit yield
 - Excessive pre-harvest fruit drop
- The reason for the HLB-related fruit drop is uncertain
 - it has been proposed that the fruit drop result from:
 - a. Less efficiency in absorbing water due to loss of roots
 - b. Nutritional deficits
- Efforts have been made to reduce pre-harvest fruit drop by using
 - Plant Growth Regulators (Gene Albrigo)
 - Soil and water acidification (Jim Graham)
 - enhanced nutrition





Background

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- ❖ **The fungal pathogen- Diplodia was found consistently present in HLB-affected juice, but not in juice from healthy fruit.**

About Diplodia natalensis

- A pathogen of woody plants in tropical and subtropical area:
a wide range of hosts
 - Infection of trees: weakens the strength and toughness of the wood
 - Infections of fruits: soft brown rot
- The development of diseases in trees is usually associated with stress due to factors other than Diplodia infection itself.



- High incidence of *Diplodia* colonization in HLB-affected fruit may contribute to the excessive pre-harvest fruit drop:
 - a. Inducing fruit to produce ethylene, the plant hormone that is known to promote fruit abscission.
 - b. Producing the cell-wall digesting enzymes: directly act on calyx AZ
- Fungicide application might be an effective strategy to control the excessive fruit drop in HLB-affected groves (although may lead to resistance and fungicide-induced toxic effects)

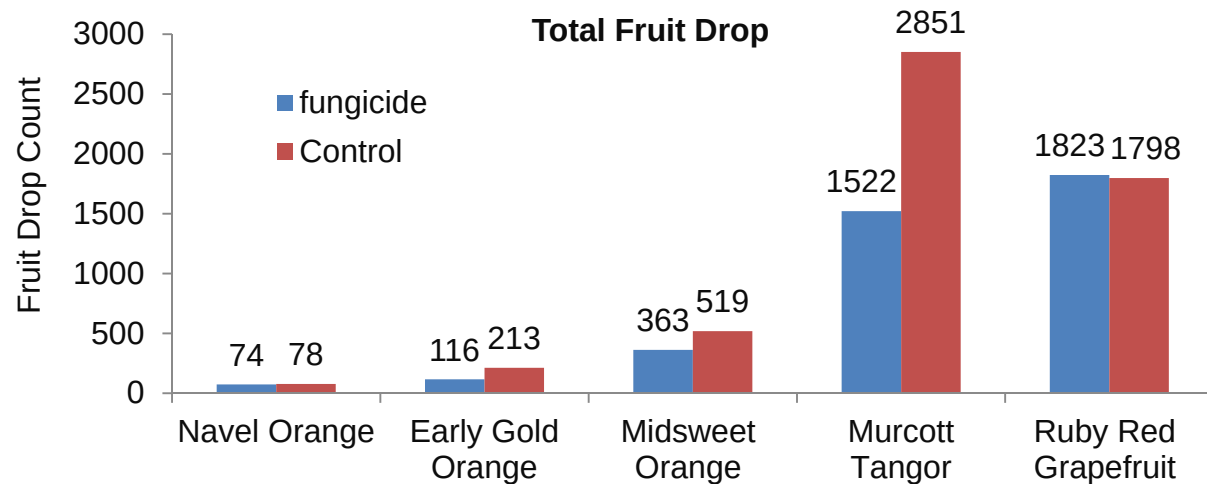
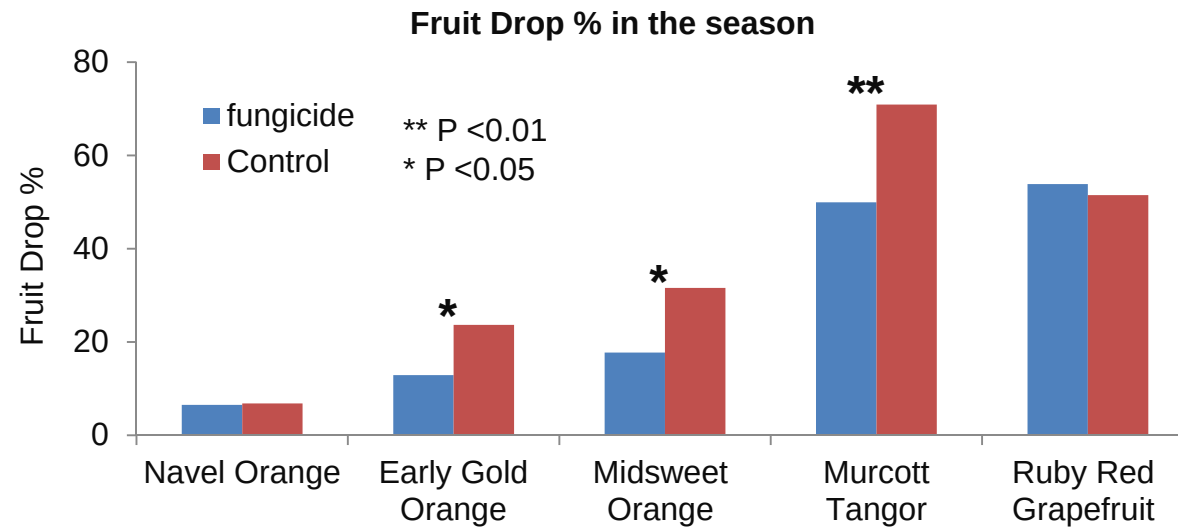
Zhao W, Bai J, McCollum G, Baldwin E. 2015. High incidence of preharvest colonization of huanglongbing-symptomatic *Citrus sinensis* fruit by *Lasioidiplodia theobromae* (*Diplodia natalensis*) and exacerbation of postharvest fruit decay by that fungus. *Appl Environ Microbiol* 81:364 –372. doi:10.1128/AEM.02972-14.



Fungicide spray trial

- Five citrus cultivars: Navel Orange, Early Gold Orange, Midsweet Orange, Murcott Tangor, and Ruby Red Grapefruit
 - 20 trees used for each cultivar
- Two treatments: fungicide spray group (10 trees) and non-sprayed group (10 trees)
- QUADRIS TOP fungicide spray: four times (15.4 oz/35 gal of water, sprayed until runoff)
 - 4/4/14, 5/30/14, 8/29/14, and 10/31/14 (for Navel and Early Gold)
 - 4/4/14, 5/30/14, 9/19/14, and 12/12/14 (for Midsweet, Murcott and Ruby Red)
- Fruit sampled 10-14 days after each spray for fruit detachment force and Diplodia titer analysis
- Fruit drop count
 - once about every three weeks
 - started from 9/11/14 (for Navel and Early Gold) and 10/2/14 (for the other three)
- Fruit left on the tree was estimated except for Early Gold, which got harvested before data could be obtained.

Effects of Fungicide QUADRIS Spray



Fruit Drop reduced (%)

45% ↓

30% ↓

46% ↓

Total fruit saved per 10 trees

+4

+108

+156

+1329

-25

HLB infected citrus tree physiology and plant growth regulator effects on preharvest fruit drop

Gene Albrigo

Conclusions

- Neither PGRs nor Headline consistently reduced fruit drop

Costs and benefits of foliar nutritional amendments – evidence from a 5-year trial

IRCHLB IV

8 February 2015

Orlando, FL

Robert Rouse & Fritz Roka



TREATMENTS

TREATMENT	TREATMENT PRODUCTS
1	KNO3+Oil+Micro+(Macro/KPhite)+SAR+H2O2
2	KNO3+Oil+Micro+(Macro/KPhite)
3	KNO3+Oil+Micro
4	KNO3+Oil
5	KNO3+Oil+SAR
6	KNO3+Oil+(Macro/KPhite) +SAR
7	KNO3+Oil+Micro+SAR
8	KNO3+Oil+(Macro/KPhite)
9	KNO3+Oil+Micro+ (Macro/KPhite)+ SAR

Cost Matters

- Cum yield 2008-12
 - #1 783 bx/ac
 - #3 712 bx/ac
- Material Cost #1 Boyd: \$368/ac-yr
- Material Cost #3 Micro: \$129/ac-yr
- Cum NPV 2008-12 (Revenue – material cost)
 - #1: \$3,720/ac
 - #3: \$4,078/ac
 - #3 more than #1: \$359/ac

Concluding Comments

- Results do NOT suggest that abandoning an inoculum control program is always correct.
- Results DO suggest that production from HLB infected trees can be enhanced.
- More is not always better.
 - Opportunity for growers to decrease costs by more critically analyzing nutritional needs of tree.
- More citrus horticulturalists needed at UF/IFAS.

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Nutritional Sprays to Manage HLB in the Field: Can they Mitigate Orange Juice HLB-induced Off-flavor?

Anne PLOTTO¹, Elizabeth BALDWIN¹, Jinhe BAI¹, John MANTHEY¹, Jan NARCISO¹, Bill WIDMER¹, Sophie DETERRE¹, Sharon DEA¹, Smita RAITHORE¹, Wei ZHAO¹, Gary LUZIO¹, Randy CAMERON¹ and Mike IREY²

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Objective

- To study the effect on fruit flavor of 3 foliar nutritional treatments (N1, N2, N3) compared with a conventional spray program for oranges from healthy and HLB-infected trees

Materials

- Hamlin**: 8 harvests, December 2009 to January 2014
- Valencia**: 5 harvests, April 2012 to April 2014 & March 2015
- 50-15 trees (500-600 fruits) per treatment
- Fruit juiced with BT extractor and pasteurized
- Trees and juice tested for CLas filter by qPCR

Technical Summary

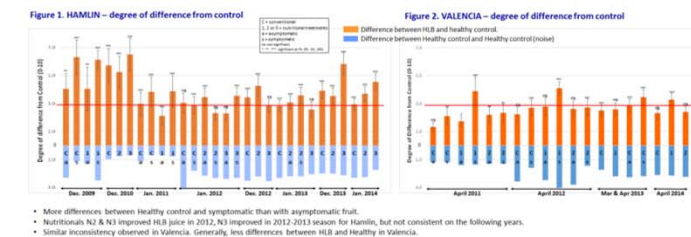
- Orange juice made with fruit from groves that are managed by nutritional treatments was tested by taste panels during 5 seasons.
- Overall, there was no effect (positive or negative) of the nutritional treatments on juice flavor.
- Off-flavor is mostly perceived in early-season Hamlin juice extracted from symptomatic fruit.

Consumer Taste Panels

Difference-from-Control tests

- Control - Healthy juice
- Control-presented with HLB, or with control (placebo effect)
- Panelists rated the difference in taste between coded sample and control
- 35 untrained panelists, staff of USDA-ARS (10/14)

Test required for statistical analysis



Trained Taste Panels

Descriptive Analysis

- 13 highly trained panelists
- 8 flavor, 5 taste, 4 mouthfeel, 9 aftertaste
- 10-point intensity scale: 0 = none; 1 = low; 15 = high
- Reference standards presented at each testing session

Figure 3: HAMLIN and VALENCIA descriptive profiles

Figure 4: Effect of Nutritionals on HAMLIN flavor profile

Figure 5: Effect of Nutritionals on VALENCIA flavor profile

Conclusion

- Juice made with HLB symptomatic fruit has more typical "HLB flavor", is more bitter, sour, metallic, tingy and grapefruit-like than juice from HLB asymptomatic fruit.
- Off-flavor is mostly perceived in early-season Hamlin juice from symptomatic fruit.
- Some of the nutritional treatments seemed to alleviate HLB off-flavor, increase sweetness and decrease bitterness early in the study. That effect did not persist in the following years.

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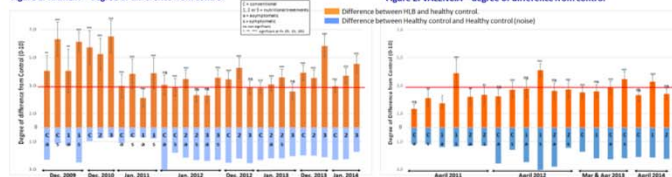
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Test repeated for statistical analysis

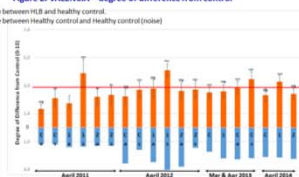


Figure 1. HAMLIN – degree of difference from control



- More differences between Healthy control and symptomatic than with asymptomatic fruit.
- Nutritional N2 & N3 improved HLB juice in 2012, N3 improved in 2012-2013 season for Hamlin, but not consistent on the following years.
- Similar inconsistency observed in Valencia. Generally, less differences between HLB and Healthy in Valencia.

Figure 2. VALENCIA – degree of difference from control



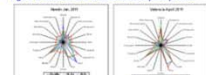
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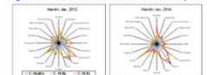


Figure 3: HAMLIN and VALENCIA descriptive profiles



- Healthy juice has greater sweet, orange, fruity flavor
- HLB juice has greater grapefruit, green, stale, oxidized and typical HLB flavor, less sweet, more sour, bitter, umami, tingling. More so in Hamlin than Valencia.

Figure 4: Effect of Nutritionals on HAMLIN flavor profile



- Nutritional N2 & N3 improved HLB Hamlin juice in 2012: decreased typical HLB flavor, bitterness and astringent
- N2 increased sweetness and orange flavor in HLB 2012
- In 2014, N3 had the highest HLB flavor and bitterness, and lowest sweetness. N2 sweetness lower than control.

Figure 5: Effect of Nutritionals on VALENCIA flavor profile



- In 2012, HLB juice from N2 treatment had the highest sourness, bitterness, metallic, tingling, and HLB flavor.
- N2 increased sweetness and orange flavor in HLB 2012
- In 2014, no difference between healthy and HLB juices, and no differences between Nutritional treatments.

Conclusion

- Juice made with HLB symptomatic fruit has more typical "HLB flavor", is more bitter, sour, metallic, tingly and grapefruit-like than juice from HLB asymptomatic fruit
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Cost and benefit analysis of severe pruning to rejuvenate HLB infected trees

IRCHLB IV
8 February 2015
Orlando, FL

Robert Rouse & Fritz Roka



February 2010



Conclusions

- HLB infected trees recover from severe pruning.
- Pruning experiment as designed (buckhorn):
 - Not Cost Effective,
 - hard to make up for the loss of fruit first year after buckhorning.
- Growers, however, looking at alternatives...
 - Cut out dead top of tree

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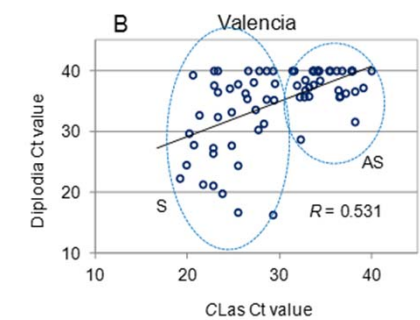
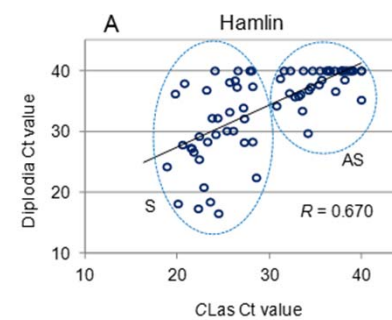
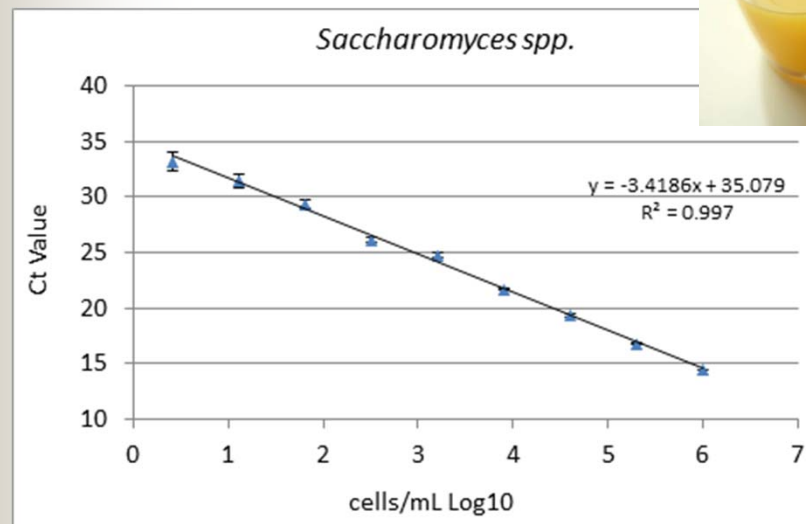
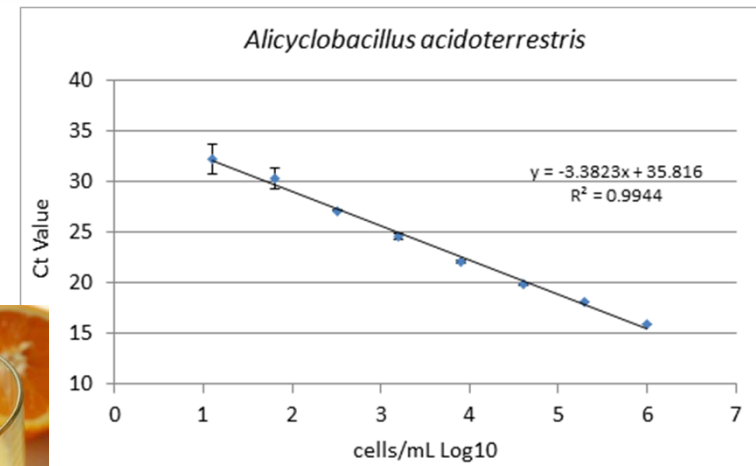
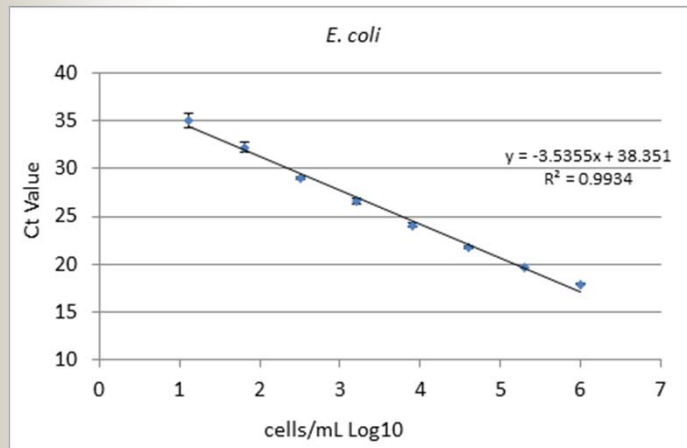
Use of qPCR to predict quality of orange juice affected by HLB



Elizabeth Baldwin, Wei Zhao, Jinhe Bai, Anne Plotto,
John Manthey, Smita Raithore and Mike Irey

US Horticultural Research Laboratory , Ft. Pierce, FL





Corr Li/HLB	Corr LJ/HLB	Li\orange	LJ/orange	Li/burning	LJ/burning
-0.650221308	-0.788697173	0.527810667	0.705207427	-0.450891717	-0.679466371
Corr Li/Bitter	Corr LJ/Bitter	Li /green	LJ/green	Li/aft bitter	LJ/aft bitter
-0.665778254	-0.679466371	-0.54042317	-0.693573863	-0.665778254	-0.679466371
Corr Li/metallic	Corr LJ/metallic	Li/orange peel	LJ/orange peel	Li/aft astring	LJ/after astring
-0.679974119	-0.704863099	-0.548996016	-0.438095888	-0.639675817	-0.640706474
Corr LI/sweet	Corr LJ/sweet	Li/oxid oil	LJ/Oxid oil	Li/aft burn	LJ/aft burn
0.61662541	0.712966687	-0.645452635	-0.724286668	-0.47504825	-0.425505407
Corr LI/sour	Corr LJ/sour	Li/umami	LJ/umami	Li/Sum aft	LJ/sum aft
-0.194126855	-0.097221665	-0.645510022	-0.771602072	-0.648777106	-0.638515573
CorrLI/stale	Corr LJ stale	Li/body	LJ/body	LI/sum pos	LJ/sum pos
-0.495466167	-0.688194752	0.445981827	0.595169395	0.588213854	0.727153998
Corr grape/LI	Corr grape/LJ	Li/tingly	LJ/tingly	Li/sum neg	LJ sum neg
-0.605449742	-0.621003765	-0.600287878	-0.555725211	-0.696179869	-0.791868269
fruity non cit/LI	Fruity non cit/LJ	Li/astringent	LJ/astringent		
0.565460735	0.681861145	-0.625057855	-0.606409874		

Conclusions for qPCR of juice

- Useful to determine infection of tree by Clas
- Useful to determine Clas titer in juice for fruit batches coming in from infected groves
- Useful to predict quality of juice based on Clas titer for classification of juice for different products
- Useful to determine titer of other off-flavor microbes for juice quality, or human pathogens for juice safety



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Progress on the development of methodology to measure and detect fruit that might contribute to HLB-associated off flavors in juice

Michael Ireys¹, Claudia Kaye², Greg Thelwell¹, Hangxin Hou², Doug Van Strijp¹, Ping Sun¹, Liz Baldwin³, Wei Zhao³, Anne Plotto³, Jinhe Bai³, and Smita Raithore³

¹ Southern Gardens Citrus, ² United States Sugar Corporation, ³ USDA-ARS

What do we know (or what do we think we know...)?

Effect of Liberibacter Infection (Huanglongbing or “Greening” Disease) of Citrus on Orange Juice Flavor Quality by Sensory Evaluation

ANNE PLOTTO, ELIZABETH BALDWIN, GREG MCCOLLUM, JOHN MANTHEY, JAN NARCISO, AND MIKE IREY

Evaluation of Bitterness Caused by Huanglongbing Disease in Orange Juice

Sharon Dea,
Anne Plotto, John Manthey,
Mike Irey, Elizabeth Baldwin



An Update on the Effect of Citrus Greening on Flavor Components of Orange Juice Over Several Seasons

Elizabeth Baldwin, Anne Plotto,
Greg McCollum, John Manthey

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY ARTICLE

Journal of
proteome
research

Article

pubs.acs.org/jpr

Metabolomic Analysis of Citrus Infection by ‘*Candidatus Liberibacter*’ Reveals Insight into Pathogenicity

Anne M. Slisz,[†] Andrew P. Breksa, III,[§] Darya O. Mishchuk,[†] Greg McCollum,^{||} and Carolyn M. Slusky^{*†,‡}

Effect of Liberibacter Infection (Huanglongbing Disease) of Citrus on Orange Fruit Physiology and Fruit/Fruit Juice Quality: Chemical and Physical Analyses

Proc. Fla. State Hort. Soc. 121:265–269. 2008.

Effect of Early Detection Huanglongbing on Juice Flavor and Chemistry

ANNE PLOTTO^{1*}, ELIZABETH A. BALDWIN¹, T. GREG MCCOLLUM², JAN A. NARCISO¹, AND MIKE IREY³

Preliminary Information on Effect of Greening on Juice Flavor and Chemistry

Anne Plotto, Greg McCollum, Elizabeth

8.2 Effect of Greening Plant Disease (Huanglongbing) on Orange Juice Flavor and Consumer Acceptability

Goodrich-Schneider R.¹, Sims C.A.¹, Span T.², Danyluk M.D.² and Rouseff R.L.²

What do we know (or what do we think we know...)?

- Juice from HLB fruit typically has:
 - Generally lower sugars
 - Sometimes higher acids
 - Higher levels of bitter limonoids (limonin and nomilin)
 - Higher oil
- Juice from asymptomatic fruit generally better than from symptomatic fruit
- Off flavors more apparent in Hamlin than in Valencia
- Off flavors more apparent early in the harvest than late in the harvest (Hamlin)

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 - Generally lower sugars
 - Sometimes higher acids
 - Higher levels of bitter limonoids (limonin and nomilin)
 - Higher oil
- Juice from asymptomatic fruit generally better than from symptomatic fruit
- Off flavors more apparent in Hamlin than in Valencia
- Off flavors more apparent early in the harvest than late in the harvest (Hamlin)

General consensus.....

- So as the infection in the state has progressed, there have been some measureable changes
- With that being said, the differences are subtle and in most cases an untrained person might not detect the off flavors

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- With that being said, the differences are subtle and in most cases an untrained person might not detect the off flavors

However, many (most) would agree that some of the quality parameters have been lower in recent years compared to pre-HLB days

Conclusions

- Using a combination of analytical methods it appears that predictions can be made as to the organoleptic qualities of the juice that would be extracted from fruit from different groves
- The prediction quality is higher in Early/Mids than in Valencia
- The ability to predict organoleptic quality now gives processors options that they did not have in the past to continue to ensure that high quality juice is produced in the presence of HLB

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Effect of HLB on volatile components in peel oil of 'Valencia' oranges

Huqing YANG, Elise BOURCIER, Wei ZHAO, **Jinhe BAI**, Anne
PLOTTO, Elizabeth BALDWIN, Mike IREY

Summary

- HLB disease altered the volatile profiles of 'Valencia' orange peel oil.
- Many terpene compounds were accumulated in a higher level in the diseased peel oil, indicating that disease stress up-regulated the terpenoid pathways.
- In contrast, some aldehydes were suppressed in diseased peel oil which may negatively impact peel oil quality.

Summary

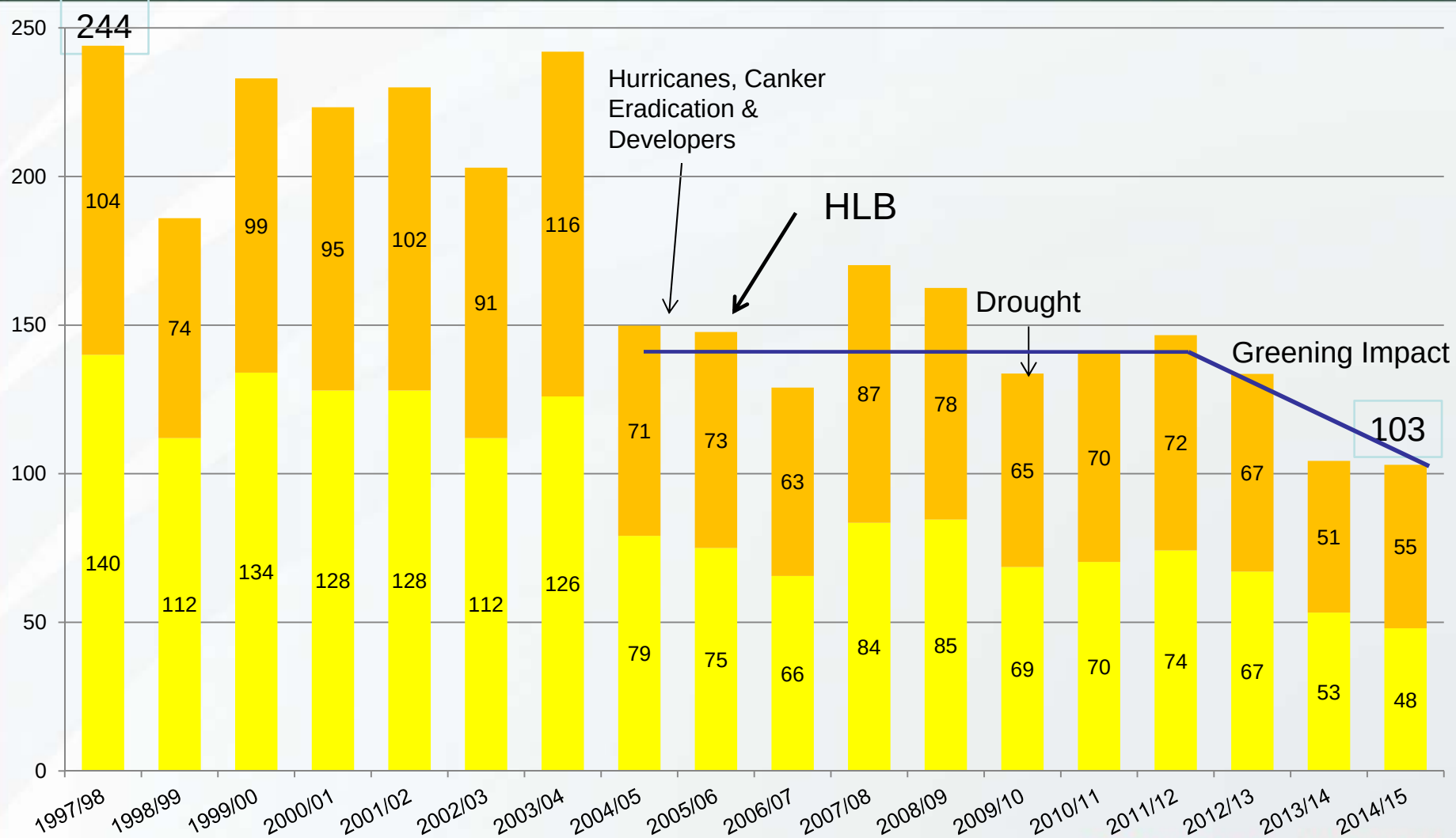
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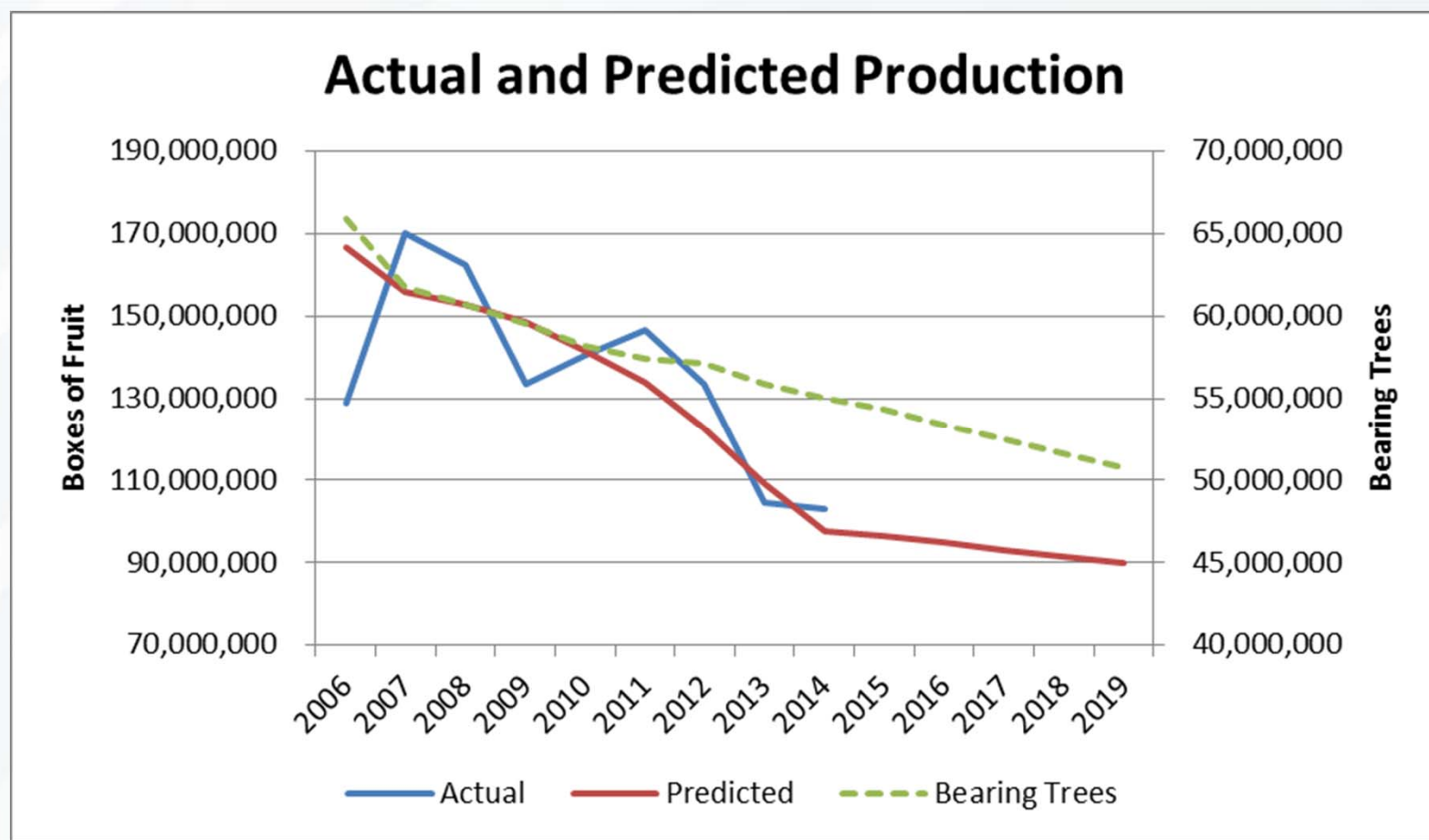
**Have the HLB associated production losses
in Florida bottomed out or are we in for
harder times?**

Michael Irey and Greg McCollum

Florida Round Orange Production



Scenario 1 – 5 year average replant (2.1M trees/year)



Predicted 2014-2015 Crop 97,690,433

Approximately a -1.7% loss/yr going forward (1.6M boxes)



Summary

- Significant production losses are not observable until a significant number of trees:
 - Have been infected
 - And have been infected long enough
- Infection in Florida is widespread enough and has been around long enough that yields should begin to stabilize as long as planting keeps up with attrition
 - Breakpoint appears to be about 3,100,000 trees a year

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Mitigation of Disease

Thermotherapy Trials and the Molecular Mechanism behind the Success of Heat Treatment for the Control of HLB



MS Doud[‡], F Luo, Y Wang, L Zhou, E Stover, and Y Duan

[‡]USDA ARS USHRL

Fort Pierce, FL

Summary

- HLB-affected citrus exposed to high temperatures respond favorably with increased growth
- Improvement in tree growth has been observed for 3 years
- Heat decreases Las bacterial levels for a period of time (6 - 18 months)
- **Severely sick trees** may **NOT** respond well to the heat treatment
- Fluctuations in results could be attributed to Las weather, initial Las load, Las strain variation, grove maintenance, etc
- Thermotherapy may buy time
- Field trials to track changes in fruit production are ongoing
- Proteomics and RNA-Seq both identify heat-induced changes in citrus

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Update on Antimicrobial Therapies for HLB Mitigation

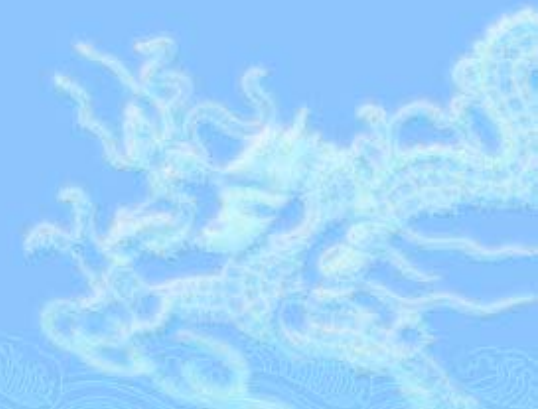
Dr. Charles A. Powell

University of Florida



Using graft-based screening system, more than 100 molecules were evaluated against HLB bacterium

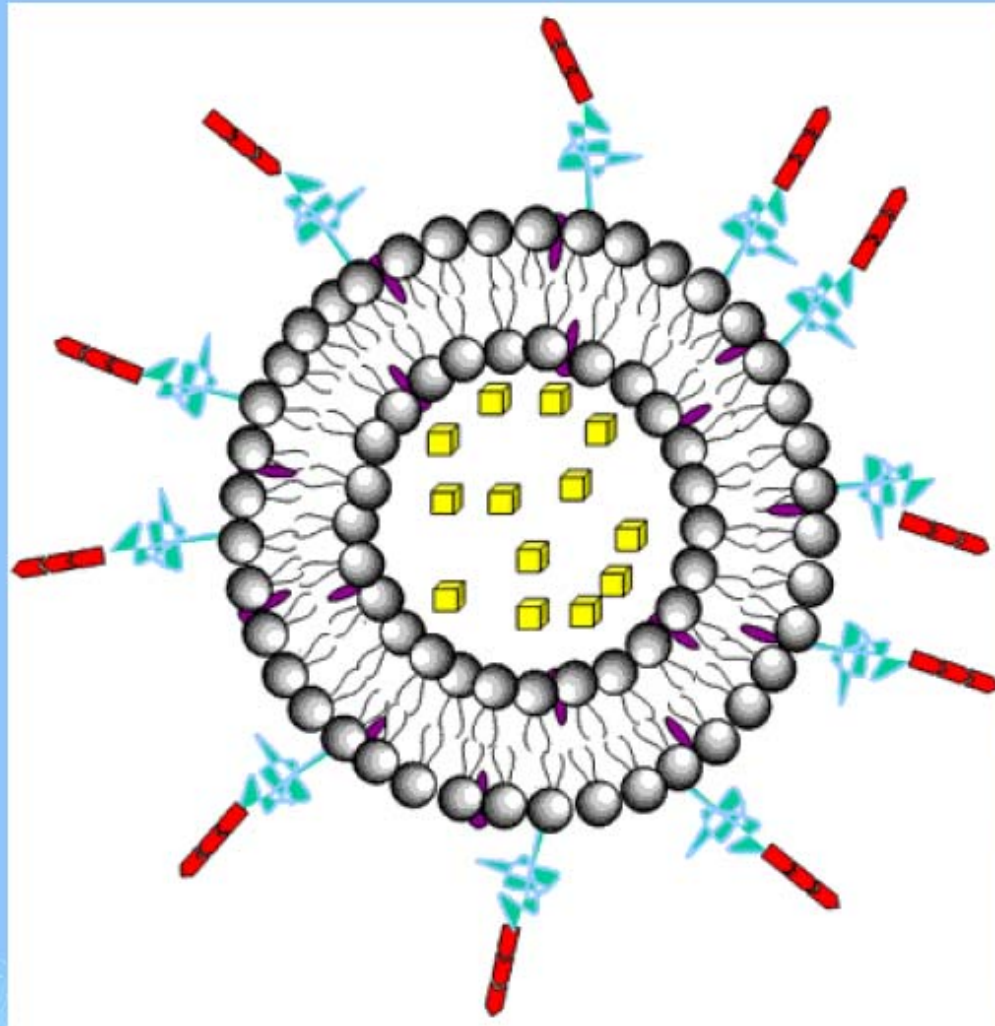
1. Antibiotics: **31**
2. Biocides: **25**
3. Peptides: **5**
4. Fungicide: **6**
5. SAR substances: **6**
6. Others: **29**



Effective compounds evaluated by graft-based screening method

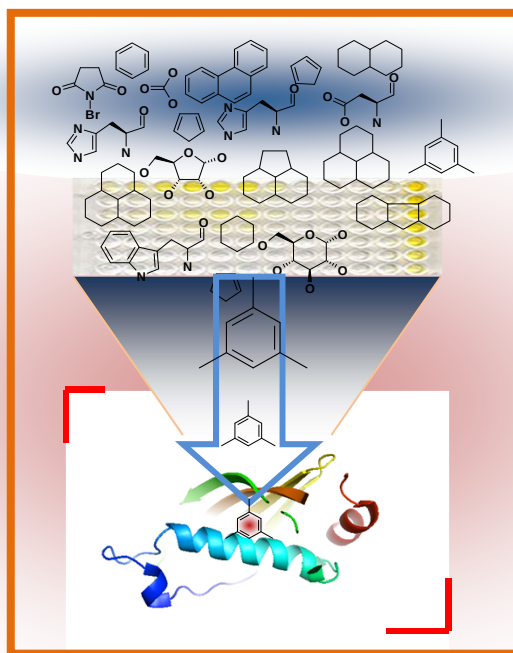
Chemical compounds	Ct value	Phytotoxicity(Y/N)
Ampicilin or Pennicillin (Antibiotic)	39.7±0.1	N
Actidione (Fungicide)	39.2±0.8	Y
EBI-601 (Antibiotic)	37.8±0.7	N
Sulfadimethoxine sodium (Antibiotic)	36.8±1.7	N
Nicotine (Biocide)	36.3±0.4	Y
80WG (SAR)	35.8±1.1	N
Sulfathiazole sodium (Antibiotic)	35.7±1.5	N
DL-buthionine-sulfoximine (Antibiotic)	35.6±0.3	N
Silver collidal (Others)	35.0±1.8	N
Poly-l-arginine (Peptide)	34.8±2.1	N
Surfactin from bacillus subtilis (Biocide)	34.8±0.7	N
EBI-602 (Antibiotics)	34.7±1.6	N
Poly-D-lysine (Peptide)	34.5±1.3	N
P-cymene (Others)	34.0±1.1	N
Carvacrol (Others)	33.9±3.1	N
Zhongshengmycin (Agri-Antibiotics)	33.7±1.0	N
Silver nitrate (Others)	32.9±1.6	N
Control	25.3±0.8	N

EFFICIENT NANO- DELIVERY SYSTEM



Claudio F Gonzalez

Drug repurposing: New chemicals and targets to combat citrus greening disease



Claudio Gonzalez and Graciela Lorca
Microbiology and Cell Science - UF

Conclusions

- Several proteins from *Liberibacter asiaticus* (CLas) selected as potential antibiotic targets were successfully purified.
- Using novel screening methods, two new chemicals with bactericidal activity against *Liberibacter crescens* were selected.
- Both chemicals demonstrated a bactericidal effect against CLas in a laboratory test using infected leaves.
- A pilot treatment using CLas infected seedlings (8-10 months old), also demonstrated to be effective against CLas.
- The compounds selected for further plant treatment were not phytotoxic and both of them are FDA approved to be used in humans albeit with different purposes.
- Next step in our research is the direct evaluation of the effects of these compounds on orchards in production infected with CLas (On going assays)

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Novel tetracyclines designed specifically for activity against the causative agent of HLB

Mark L. NELSON, Jianxing ZHANG, Eric W. TRIPLETT, Kristen
RUSSOLF, Jennie FAGEN, Robert SHATTERS

Summary

- New compounds from the tetracycline family were found to be active through foliar and bark application.
- These compounds do not have activity against human bacterial infections, and are considered “non-antibiotic” in standards set previously by the FDA.
- It is now possible to create new tetracyclines specifically used for agriculture, without the ability to cause antibiotic resistance.
- These new compounds are more active than oxytetracycline, will be inexpensive to manufacture and hold promise to control HLB in the field, by eradicating it in infected citrus plants.

Bactericides as HLB therapy: Getting them where they need to go, when they need to be there

Robert Shatters¹, Ed Stover¹, Kent Morgan¹, John Ramos¹, Taw Richardson²
and Mark Trimmer²

¹USDA, ARS, USHRL, Fort Pierce, FL

²AgroSource, Inc.



&



Summary

- Literature supports the effective activity of a number of bactericides if injected into the tree (penicillin, tetracycline derivatives, streptomycin as examples).
- Need to define what we mean when we say a bactericide is effective or not. Bottom line is does it keep trees healthy and productive in producing abundant and high quality fruit.
- Failure of an antibiotic to “work” can occur for one of several reasons:
 - Not active against *Liberibacter* or phytotoxic to the plant.
 - Not able to move systemically and/or get to the phloem.
 - Rapidly broken down in the plant.
- We have shown that systemic movement of antibiotics can be manipulated in citrus.
- These results open up the possibilities to use research to improve the efficacy of available bactericide compounds by manipulating one or several of these factors.
- We have improved uptake and systemic movement of bactericides currently used in fruit and nut crop industries.
- We are now testing in large scale commercial grove studies.

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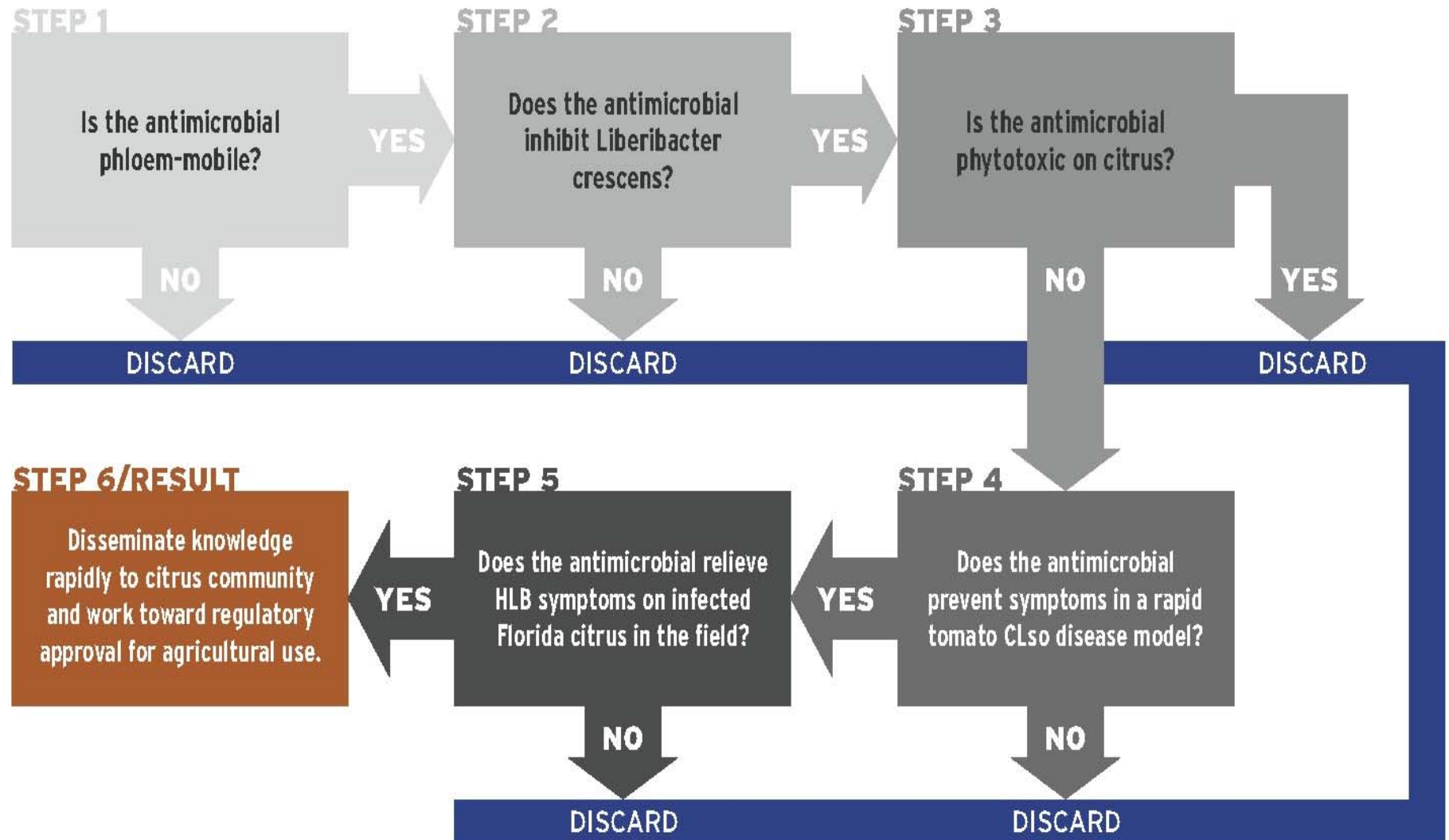
Evaluating phloem mobility and using *Liberibacter crescens* to assess efficacy of possible treatments for citrus greening

Kristin T. Rusoff¹, Janelle F. Coyle¹, Kelvin Chiong²,
Jennie R. Fagen¹, Erik Mirkov², Manuel Campos² and
Eric W. Triplett¹

¹Microbiology and Cell Science Department, Institute of Food and
Agricultural Sciences, University of Florida, 1355 Museum Rd., Gainesville,
FL 32611-0700 USA

²Department of Plant Pathology and Microbiology, Texas AgriLife Research at
Weslaco, Texas A&M University, Weslaco, TX 78596 USA

Our decision tree



CHMA Design: Performance Review, Concerns, and a Risk-based Optimization for Florida, California, Texas, and Arizona

Tim Gottwald



Weiqi Luo



Tim Riley



1/28/2015

Conclusions of CHMA forensic analysis and Potential new Treasure Coast CHMA formation:



- **Concerns:**

- We need to be concerned by the dramatic and significant increase in ACP and HLB in the Treasure Coast!
- Without CHMA-wide control HLB will quickly infect.
- New plantings may not make it into production.
- **Implications for new tree planting program not good!**

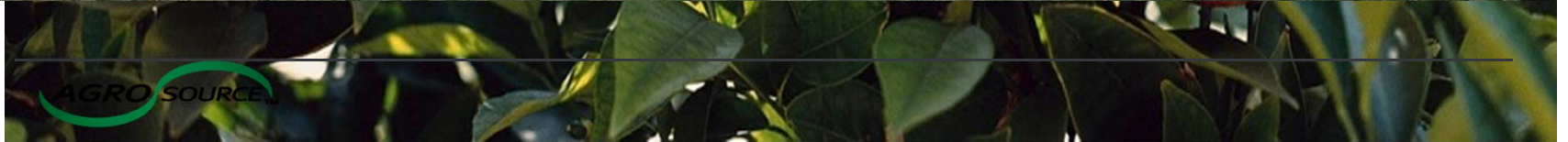
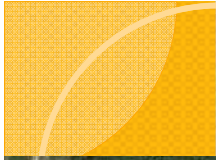
- **Moving forward:**

- Has prompted serious discussion among IRCL growers.
- Developing New **Optimized CHMAs** for Treasure Coast Citrus
- Working with growers to finalize ownership, management, and logistics concerns



Within-Grove Edge Effects of the Azimuth of the Sun on *Diaphorina citri*

Daniel J. Anco





Non-Technical Summary

- Asian citrus psyllids (ACP) are attracted to light.
- Studies were conducted to see if ACP localize in different areas of a grove in relation to time-of-day and time-of year, since the angle of incoming sunlight changes according to those factors.
- More ACP were observed during summer sampling periods, but substantial changes in their localization within groves in relation to time was not observed.
- Results are beneficial in the sense that ACP scouting protocols and management methods do not need to be spatially adapted within groves depending upon the time-of-day or time-of-year of their employment.



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Absence of windbreaks and solid set plantings increase the density of ACP in citrus

Xavier Martini and Lukasz Stelinski



Conclusions

- ACP densities were lower in the presence of windbreaks
- ACP densities were lower in mixture of mature and young trees compared to solid set young plantings
 - Solid set plantings will require more intense vector control than resets within a mature grove
- Windbreaks may help reduce edge effect

Role of Vector Management and Nutrition in Mitigation of HLB



Phil Stansly, P. Vanaclocha, J. Tansey, F. Roka, M. Jones



Summary and Conclusions

- Highest yields from Nutrition+insecticide all 6 years
- Insecticide alone significantly better than untreated last 3 years
- Nutrition alone significantly better than untreated 1 year only
- Main effects on yield: Insecticide only
 - “Enhanced” foliar nutrition beneficial only when combined with ACP control
 - Maintain optimal nutrient levels in leaves enough
- Yield benefits from vector control even if HLB incidence 100%.
 - Reduces reinfection?
 - Economic Threshold?

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**Frequent low volume applications of
horticultural mineral oil for management
of Asian citrus psyllid *Diaphorina citri*
Kuwayama (Hemiptera: Liviidae)**

James A. Tansey, Moneen M. Jones, Pilar Vanaclocha,
Jacqueline Robertson, and Philip A. Stansly

Summary and Conclusions

- Frequent, low volume applications of horticultural mineral oil were shown to provide control of Asian citrus psyllid
- The psyllid control associated with treatments did not significantly reduce the incidence of huanglongbing.
- Based on observed production gains relative to untreated trees, repetitive HMO applications and the standard grower program were equally effective.
- Frequent low-volume applications of HMO provided ACP control and, although HLB symptoms were unaffected, yield and production gains were achieved.

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Conclusions

- We need to take care of the trees and the little things count
 - Fertility
 - Tree health
 - Soil conditions
 - Disease (other) control
- More is not necessarily better
 - Need to consider cost
- Reduction in ACP population is possible
 - But it more than likely will not reduce spread of HLB
 - But it may increase production
- Juice (and oil) quality is affected
 - Working on determining methods to quickly determine quality

Conclusions

- Therapies: may buy time
 - Thermotherapy
 - Time
 - Antibiotic
 - Which one
 - Delivery
- Fruit drop still an issue
 - No clear cut remedies
- Need to keep planting
 - More than we are now
 - Jury still out as to whether we will get the trees into production
- Need to re-evaluate what we are doing
 - Buckhorning
 - Nutritionals

Bottom Line

- Lots of good work being done

No silver bullet yet!

- We need to keep the trees in as good a shape as possible to extend productive tree life.....

QUESTIONS?

Research from Outside Florida that Contributes to Current Management Practices

- Team 3 – Jim Graham, Tim Schubert, Greg McCollum
- 60 presentations and posters

Research from outside Florida that contributes to current management practices

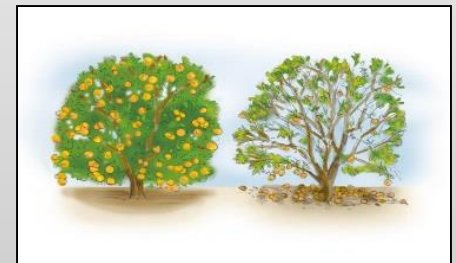
Jim Graham, UF-CREC

Greg McCollum, USDA-ARS-HRL

Tim Schubert, DPI-FDACS

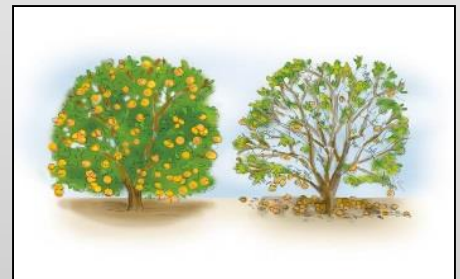
Authors of selected presentations

IRCHLB IV Grower Day
March 12, 2015



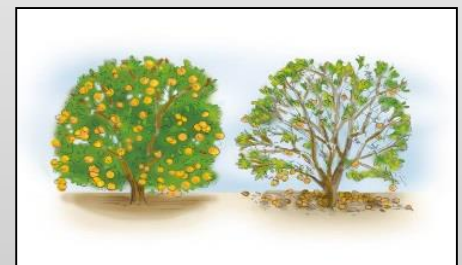
66 presentations from CA, TX, Mexico Brazil, Argentina, Japan, Vietnam, Philippines

- Early detection
- Epidemiology
- Genetics/Genomics
- ACP survey and detection
- ACP biology and management
- Disease management – 4 selected studies
 - 2 from collaborations Texas A&M, USDA-APHIS
 - 2 from Fundecitrus, Agronomic Institute, Sao Paulo State, Brazil and INRA, France



Early detection of CLas

- Detection methods, whether direct (PCR-based technology) or indirect (host plant responses) are important in citrus growing regions that have ACP but not detected symptoms of HLB (CA, AZ)
- Sampling of citrus roots is confirmed to be more reliable for detection of CLas infection than sampling of leaves (TX)
- Several novel detection technologies reported could impact efforts to prevent development of an HLB epidemic, but they are not currently relevant for FL



Evaluation of potential management tools

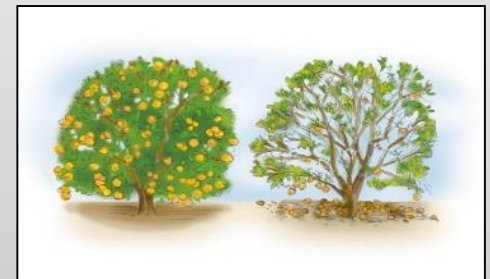
- Effects of acibenzolar S-methyl (Actigard™) and “Bio-products” (trade names Fitoplazmix™/Fitoxxit™) on incidence of CLas and bacterial titer were reported (Brazil and MX)
- No significant impact on the incidence of CLas infection or titer in infected trees indicating they have no value for mitigating HLB
- Several MX research reports confirmed the failure of biological control of ACP for slowing the spread of the HLB epidemic



Colima, MX Sept. 2014
2 yr-old Mexican limes
100% HLB infected,
ACP causing flush damage!

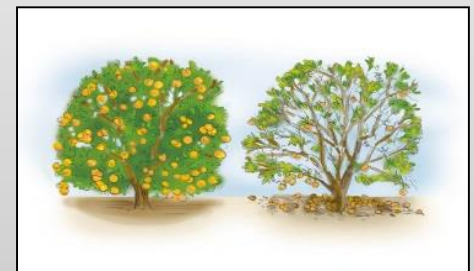
Genetics / Genomics

- Efforts continue to identify host changes induced by CLas
- Effects of CLas infection on defense responses regulated by salicylic acid were investigated (Brazil)
- Hybrids containing trifoliate orange are frequently less likely to succumb to HLB than are non-trifoliate hybrids (Brazil)



Epidemiology

- In Sao Paulo State, HLB may be spreading slower in hotter (North) than in cooler areas (South)
- Higher temperature reduces CLas titer in the tree and acquisition by ACP; anecdotal support for thermotherapy
- HLB has been confirmed in 8/9 citrus producing states in MX
- In Colima, 100% of the Mexican lime trees are symptomatic which has reduced production by 40-60%
- In MX, a federal program to slow the HLB spread into newly affected areas has been implemented based on the three pronged approach (clean stock, tree removal, chemical control of ACP)



ACP trapping

- In Brazil, refresher training for inspectors of yellow sticky cards has improved ACP detection by 10-28%, reducing overall monitoring costs
- In an effort to improve on yellow sticky cards for detection, studying attractants for ACP trapping
- ACP searching behavior is very sophisticated
- No pheromone component, however volatiles emitted from flush are attractive
- Plant based attractant will have to also work during citrus flushes
- Most active components identified are not commercially available
- Should be coupled with improvements in color attraction and trap design

Photo: M. Setamou
Texas A&M



ACP Spread & Area-Wide Management (AWM)

- In CA, tracking the invasion of ACP into residential areas implicates long-distance, human-mediated transport along roads
- In Veracruz MX, 2 years of sampling reveals that ACP are present year round, populations are highest in May, corresponding to the seasonal rise in temperature and the start of the rainy season
- In South Sonora MX (no HLB detected) , AWM programs have reduced ACP populations, this after ACP rapidly colonized all MX citrus production areas starting in 2002
- The AWM plan consists of two area-wide sprays per yr to both commercial and residential areas, one in the fall and one during the dormant season
- AWM plan also includes treatments of hot spots detected in ACP surveys

Photo: M. Setamou
Texas A&M



ACP survey and 'eradication' in CA

- In the San Joaquin Valley (Kern and Tulare counties) ACP finds are increasing, mostly in residential citrus (60% of households have citrus!)
- So far, employing an ACP 'eradication' approach (2 ground sprays in 0.5 mile radius around new finds)
- Citrus Management Areas (CMAs) are being organized
- Many small growers, including organic, are located in CMAs
- Expectation is to 'buy time' by reducing ACP spread, and eventually, HLB establishment

Photo: M. Setamou
Texas A&M



ACP Biocontrol with *Tamarixia radiata* (*Tr*)

- Provides for ACP suppression where chemical applications are not feasible to treat areas that serve as reservoirs of ACP
- In Southern CA, *Tr* releases in residential areas since 2008 are in the early stages of evaluation for effectiveness
- In high *Tr* release areas, very low levels of 1st and 2nd instar larvae and no 4th and 5th instars are observed
- In CA, an effort to identify insecticides effective against ACP but less toxic to *Tr*; all proved to be less toxic to *Tr* than to the ACP
- Spinosad + neem oil was the best performer
- In MX, mass production of *Tr* follows the FDACS-DPI protocol
- Releases target parks, residential citrus, areas with high orange jasmine populations, abandoned groves

Photo: Jeff Lotz
DPI-FDACS



Microbial Control of ACP (Japan)

- Potential for biocontrol with entomopathogenic fungi (*Purpureocillium lilacinus* and *Isaria javanica*)
- Doses of 1-10 million spores/ml was applied to orange jasmine plants under field conditions
- Fungi killed almost 100% of the adult ACP, but it took 3 days
- Slow death of adult may mean flight-capable ACP could aid in dispersal of entomopathogen and possibly reduce CLas transmission

Photo: Drion Boucias,
UF-Gainesville





Texas A&M University-Kingsville, Citrus Center
www.tamuk.edu



Edge Effects in the Spatial Distribution of Asian Citrus Psyllid in Citrus Groves: *Consequences for the Development of Best Management Practices*

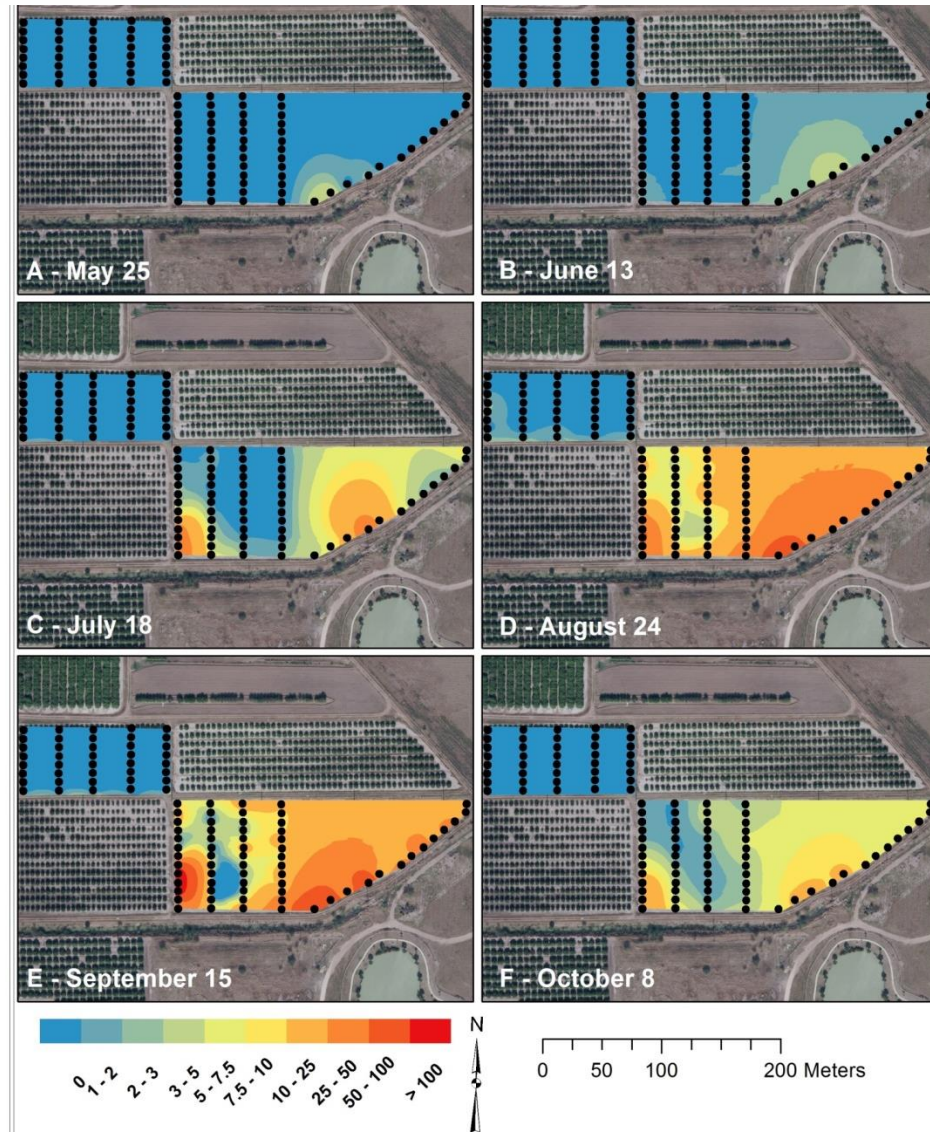
Mamoudou Setamou¹ and David W. Bartels²

¹Texas A&M University-Kingsville Citrus Center, Weslaco TX 78599

²USDA APHIS PPQ CPHST Mission Laboratory, Moore Air Base,
22675 N. Moorefield Rd., Mission, TX



ACP niche occupation in groves

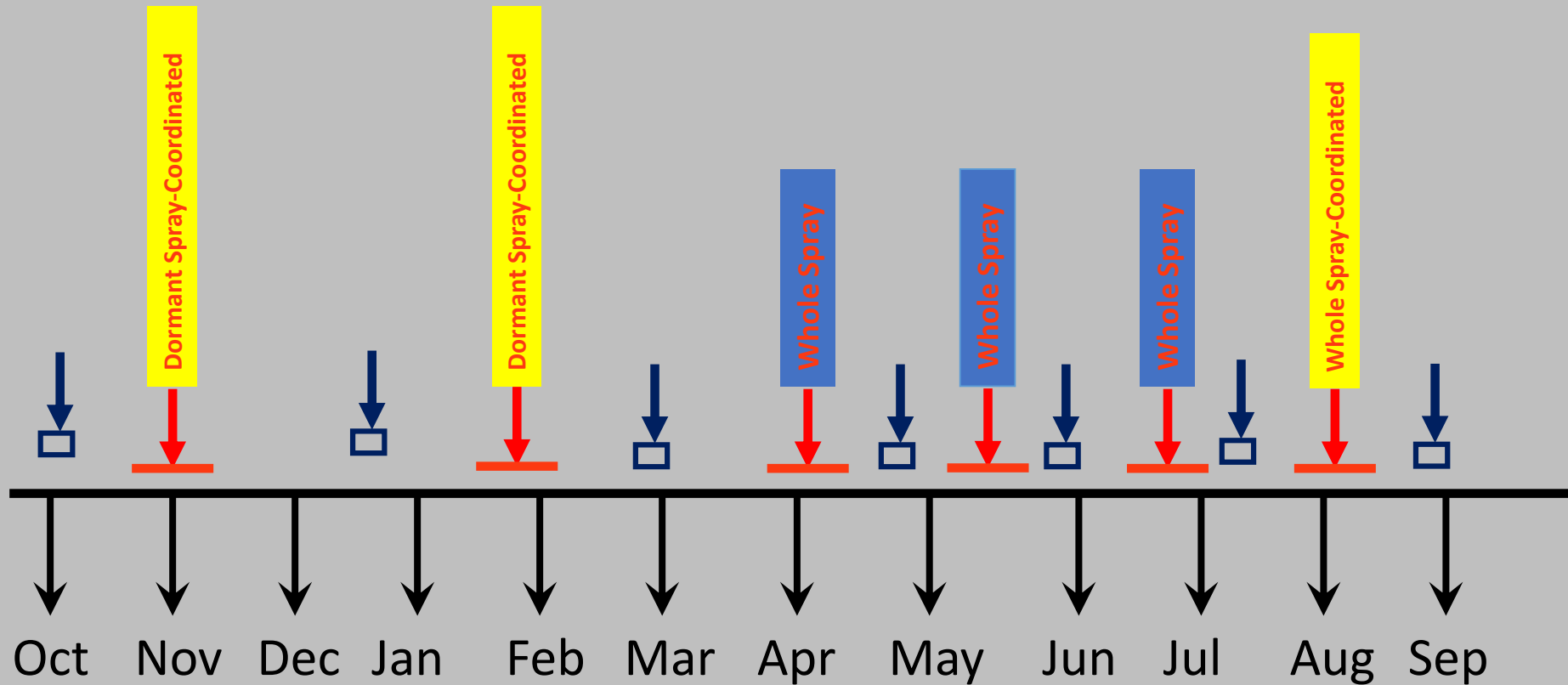


- Border trees (perimeter and adjacent) remain the preferred feeding sites for ACP
- Young groves are more infested than mature ones

Suggested Treatment for Mature Groves

Coordinated whole grove sprays

Border sprays sandwiched



Edge effects & effectiveness of border sprays

- ❑ Border trees in groves preferentially colonized by ACP
- ❑ Spray border trees first even during whole grove sprays
- ❑ Each border spray saves up to 80% of pesticides relative to whole grove sprays and reduces non-target effects of pesticides on beneficial arthropods

Nutrient profiles of citrus trees in relation to HLB disease: a case of deficiency or toxicity?

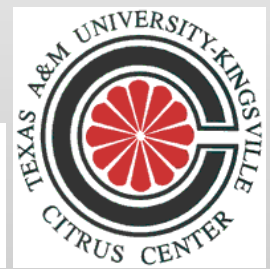


John Jifon, TX A&M AgriLife, Weslaco, TX

jjifon@ag.tamu.edu; (956)969-5643

Mamoudou Sétamou, TAMUK-CC Weslaco, TX

Olufemi Alabi, TX A&M AgriLife, Weslaco, TX

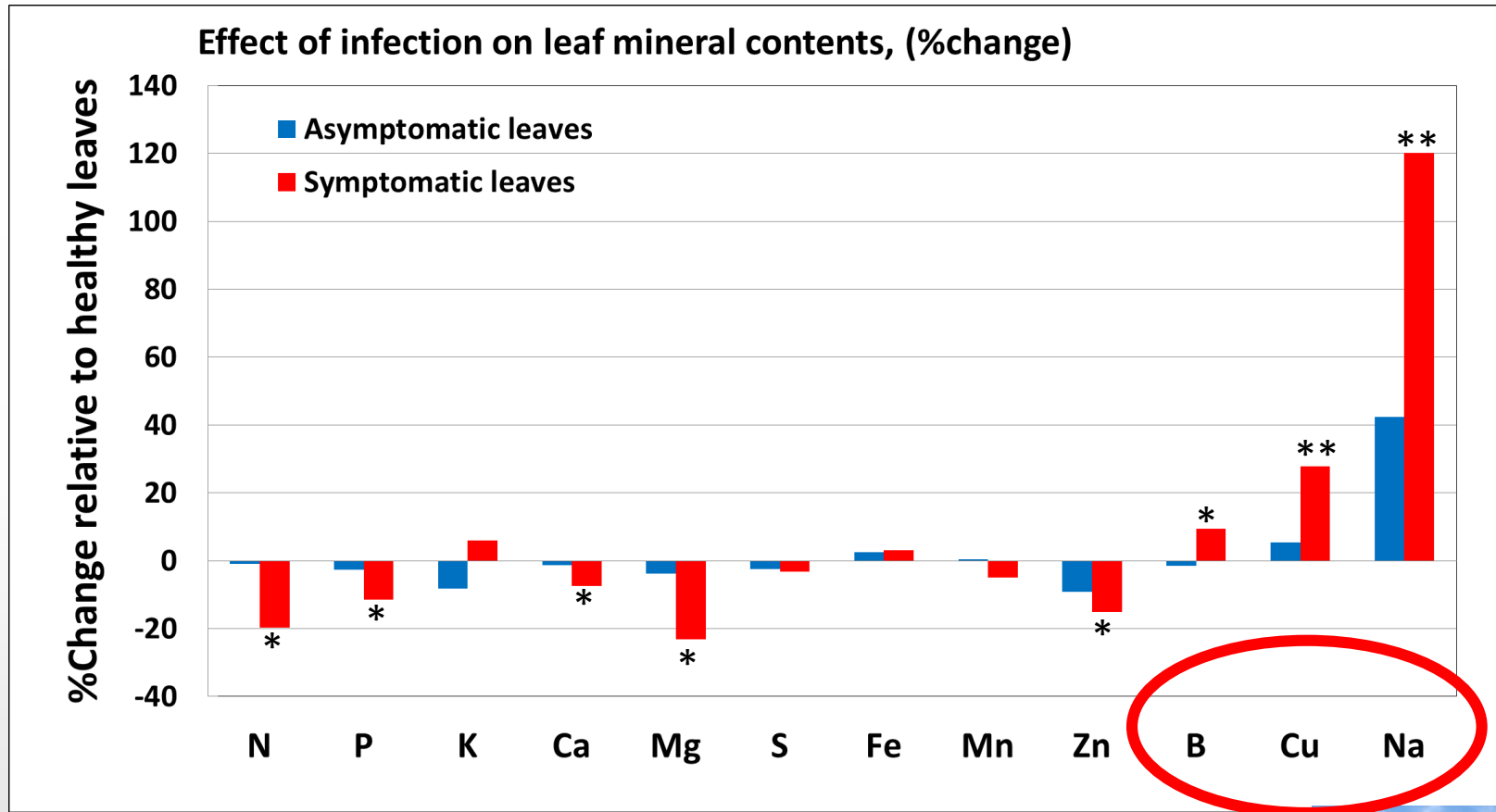


Sampling strategy

- Area-wide leaf/soil sampling across the LRGV from grapefruit and orange groves
- Tissue samples from HLB-ve & HLB+ve trees and classified as:
 - H: Healthy (uninfected trees)
 - A: Asymptomatic leaves (infected trees),
 - S: Symptomatic leaves (infected trees)
- Analyzed for minerals, TNC others



Tissue composition: HLB+ groves



	N	P	K	Ca	Mg	S	Fe	Mn	Zn	B	Cu	Na
A	-1.0	-2.7	-8.2	-1.4	-3.7	-2.6	2.6	0.5	-9.1	-1.4	5.4	42.4
S	-19.7	-11.5	6.0	-7.5	-23.2	-3.2	3.2	-4.9	-15.2	9.4	27.8	120.1



Water Quality: (95% groves flood irrigated, very high Phytophthora soil populations in a 2013 survey)

	EC	pH	Na	Ca	Mg	K	HCO3	SO4	Cl	NO3	B	Mn	Zn	Cu	Fe
			ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
Average	1.2	7.9	150.1	51.8	25.4	5.0	132.2	295.8	181.1	0.83	0.46	0.08	0.03	0.01	1.75
stderror	0.1	0.1	8.0	1.8	1.7	0.17	5.6	38.7	17.9	0.10	0.01	0.01	0.01	0.00	0.02



Summary

- Nutrient imbalances observed in HLB-affected trees
- Excess accumulation of B, Cu, Na could lead to toxicity and accelerate tree decline
 - e.g. in soils with high Cu content HLB could accelerate Cu uptake
- ENPs for HLB-affected groves should target nutrient imbalances by correcting for:
 - Observed deficiencies and toxicities



HLB epidemics and orange yield after four years of nutritional programs



Renato B. Bassanezi – Fundecitrus

Luiz H. Montesino - Fundecitrus

Dirceu Mattos Jr. – IAC

José A. Quaggio – IAC

Rodrigo M. Boareto – IAC

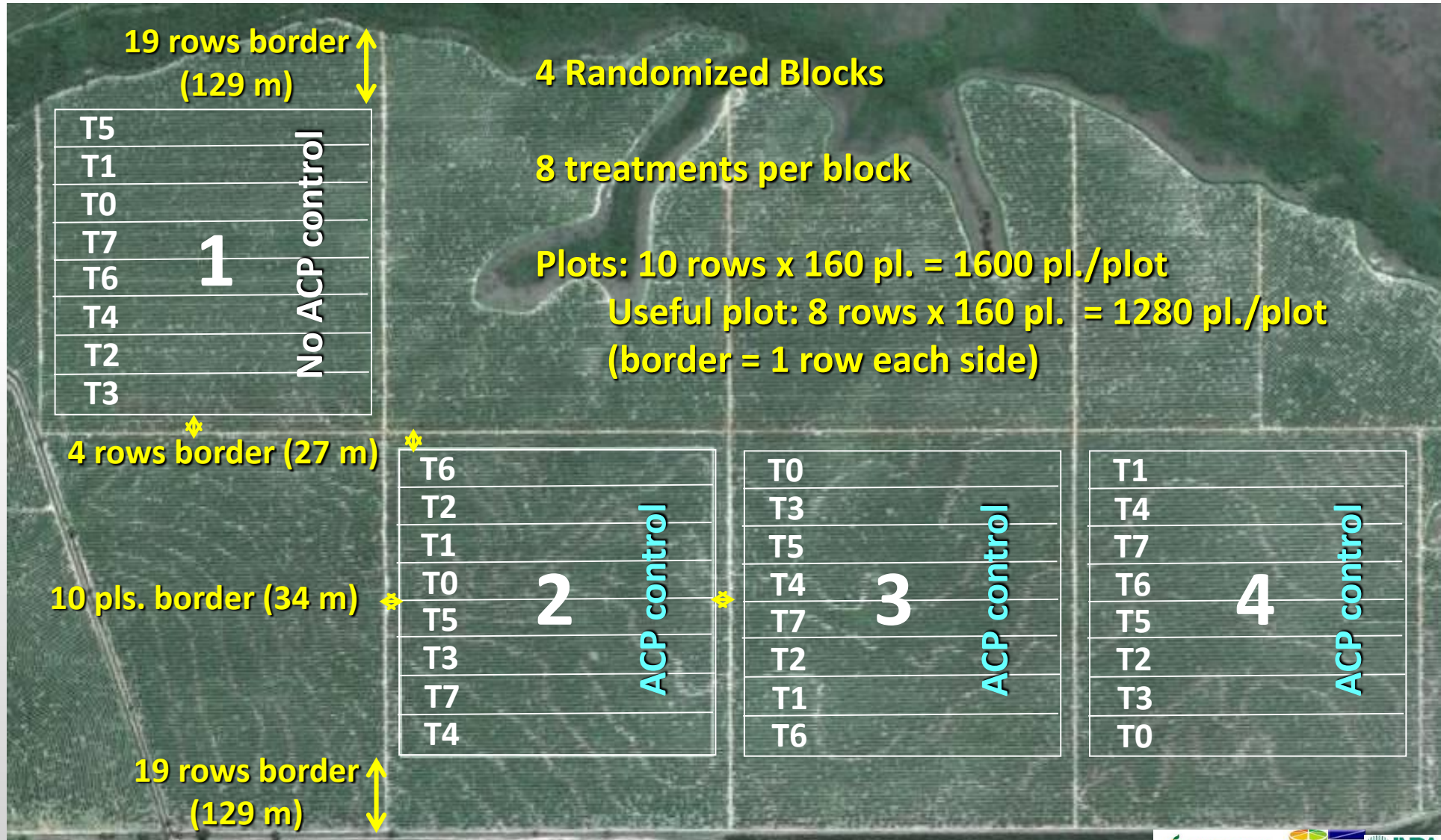
Joseph M. Bové - INRA

Objective

- To assess the effect of leaf sprayed nutrients (K, Zn, Mn, and B) and putative resistance inducers (phosphite and salicylate) on the progress of HLB incidence and severity, and yield sustainability in adult sweet orange trees

Experimental Design

- Valencia or Natal Swo/Rangpur lime – Jul-Sep/2002 – 10 x 22 ft spacing
- Non-irrigated**, good nutrition, and high productivity
- Last HLB-symptomatic trees eradication in April/2010**



Treatments	Nutrients	Amount	# Applications
T0 ('Control')	NPK dry (ground applied)	according to soil analysis	3x
T1 (T0 + 'SP' micronutrients)	T0 Boric acid Boric acid foliar Zinc sulfate (20%) Manganese sulfate (31%)	2.0 Kg/ha 3.0 Kg/2000 L 7.5 Kg/2000 L 3.2 Kg/2000 L	2x 4x 4x 4x
T2 (T1 + KNO ₃)	T1 Potassium nitrate	7.0 Kg/2000 L	4x
T3 (T0 + 'FL' micronutrients)	T0 Boric acid Boric acid foliar Zinc sulfate (20%) Manganese sulfate (31%)	2.0 Kg/ha 3.0 Kg/2000 L 7.5 Kg/2000 L 12.0 Kg/2000 L	2x 4x 4x 4x
T4 (T1 + phosphite)	T1 K-Phosphite 00-28-26	5.0 L/2000 L	4x
T5 (T1 + salicylate)	T1 Ammonium salicylate	0.1 Kg/2000 L	4x
T6 (T3 + KNO ₃ + phosphite + salicylate) 'Complete'	T3 Potassium nitrate K-Phosphite 00-28-26 Ammonium salicylate	7.0 Kg/2000 L 5.0 L/2000 L 0.1 Kg/2000 L	4x 4x 4x
T7 (T0 + 'Cocktail')	T0 'Cocktail'	according to manufacturer*	4x

*1st year – Escudo Citrus (5L/2000L) and Escudo Master (2L/2000L); 2nd and 3rd years – Coquetel (5 L/2000L)

Assessments

- **HLB progress**

- Incidence of symptomatic trees in the plot (Dec and Jun)
- Severity of symptoms on previously selected trees (monthly)

Group 1 = 20 diseased trees selected in the 1st year

Group 2 = 10 diseased trees selected in the 2nd year

- Frequency of HLB induced symptoms presence (monthly)

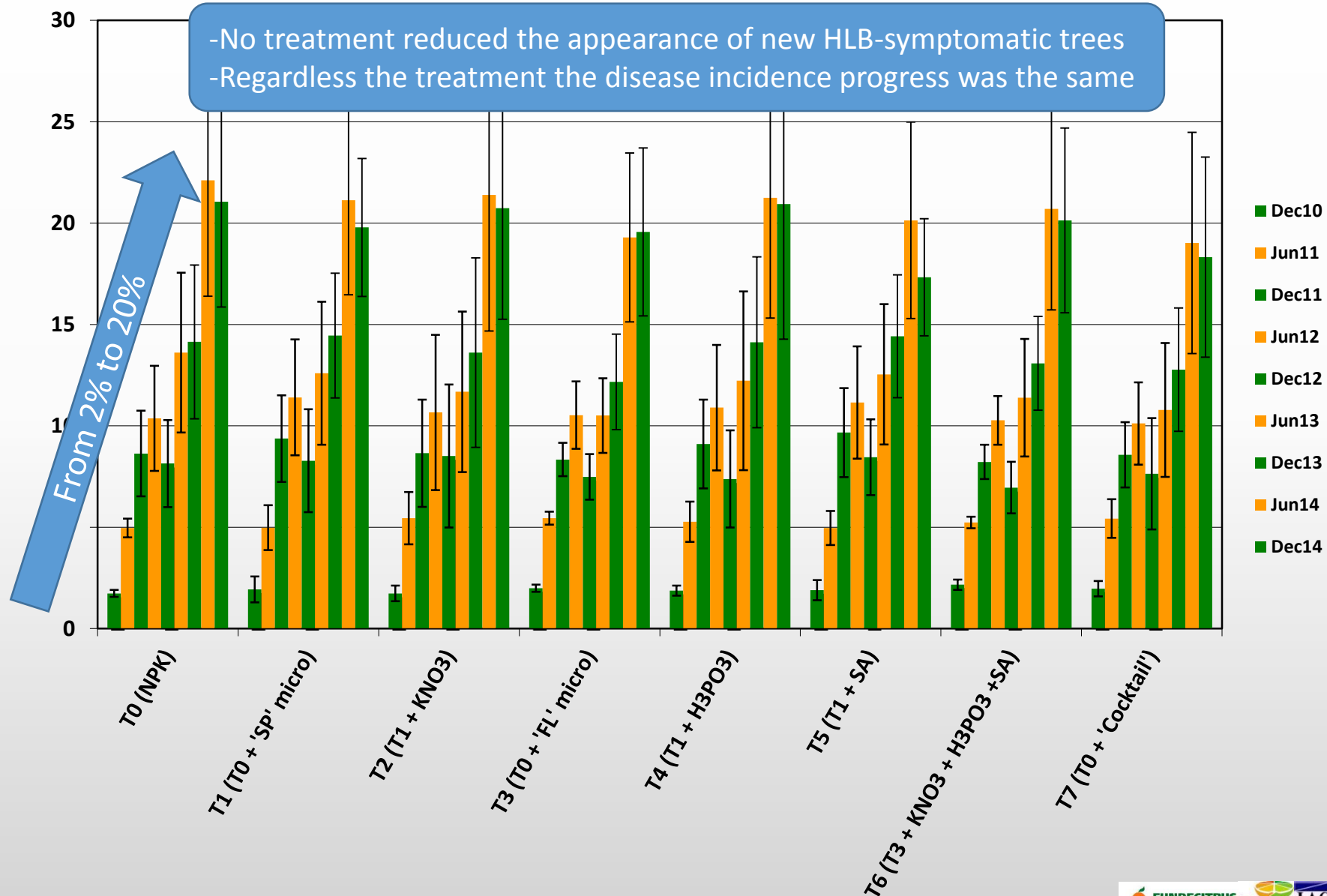
- **Yield**

- Total plot yield ('healthy' + diseased trees)
- Individual tree yield: 20 'healthy' trees

Group 1 = 20 diseased trees selected in the 1st year

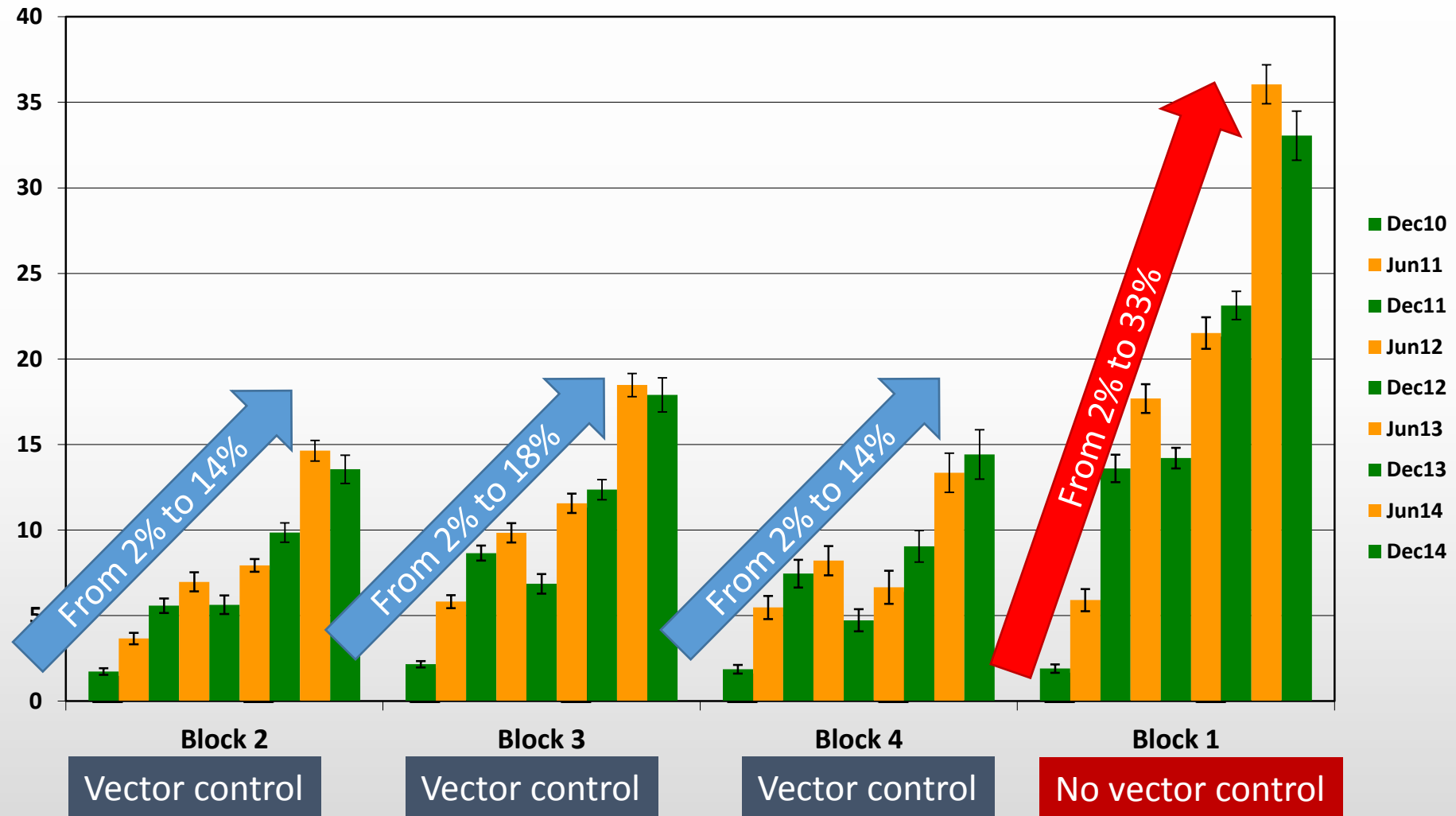
Group 2 = 10 diseased trees selected in the 2nd year

Cumulative HLB incidence (% of symptomatic trees)



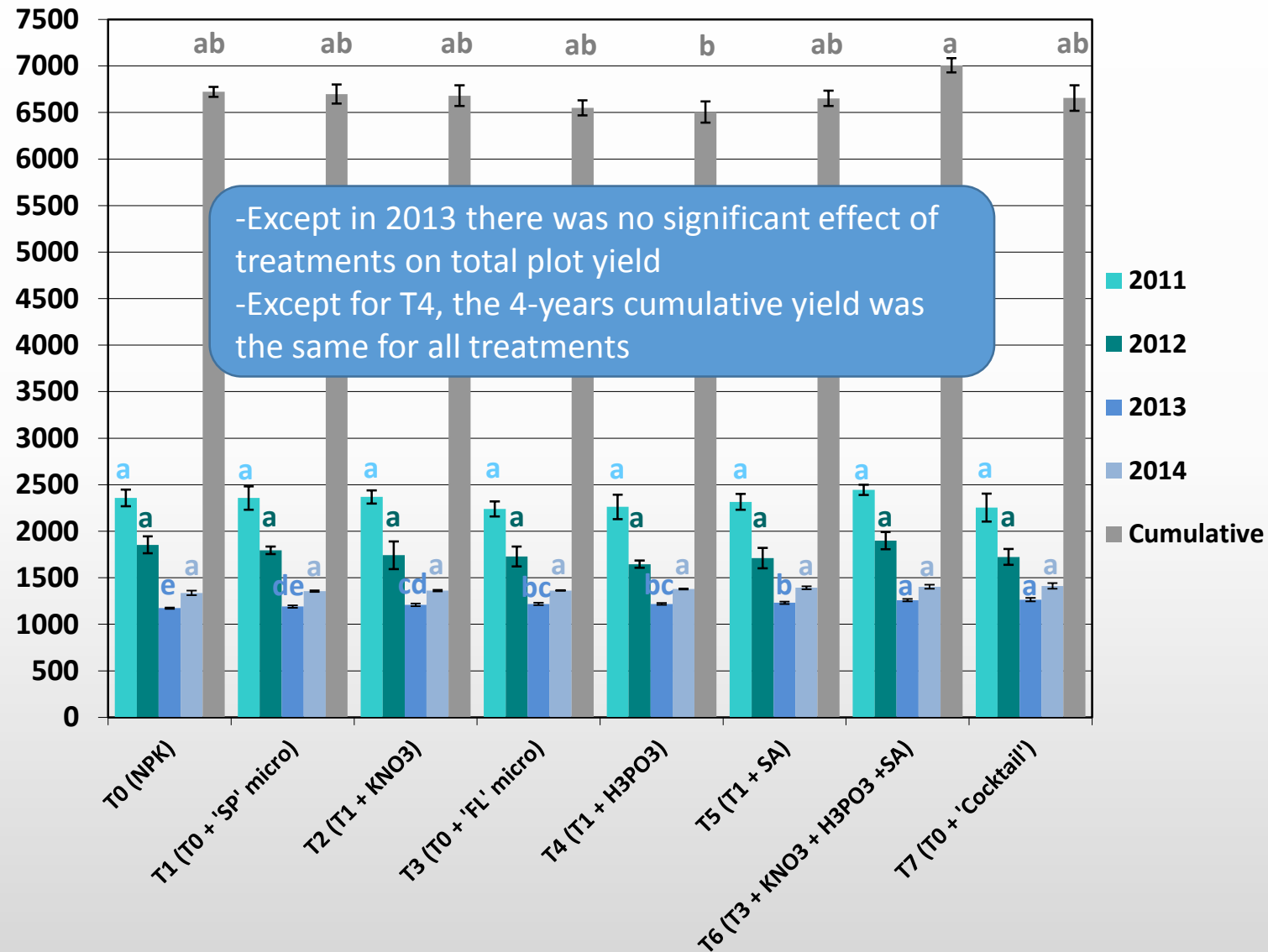
Cumulative HLB incidence (% of symptomatic trees)

Effect of ACP control



Monthly insecticide sprays + 7 aerial sprays / year

Total Plot Yield (90 lb-boxes/ha)



Means with the same letter in the same year did not differed by Tukey test ($P>0.05$)

T0 (NPK)



Diseased



Oct/2013

T6 (NPK+'FL' micro+KNO₃+H₃PO₃+SA)



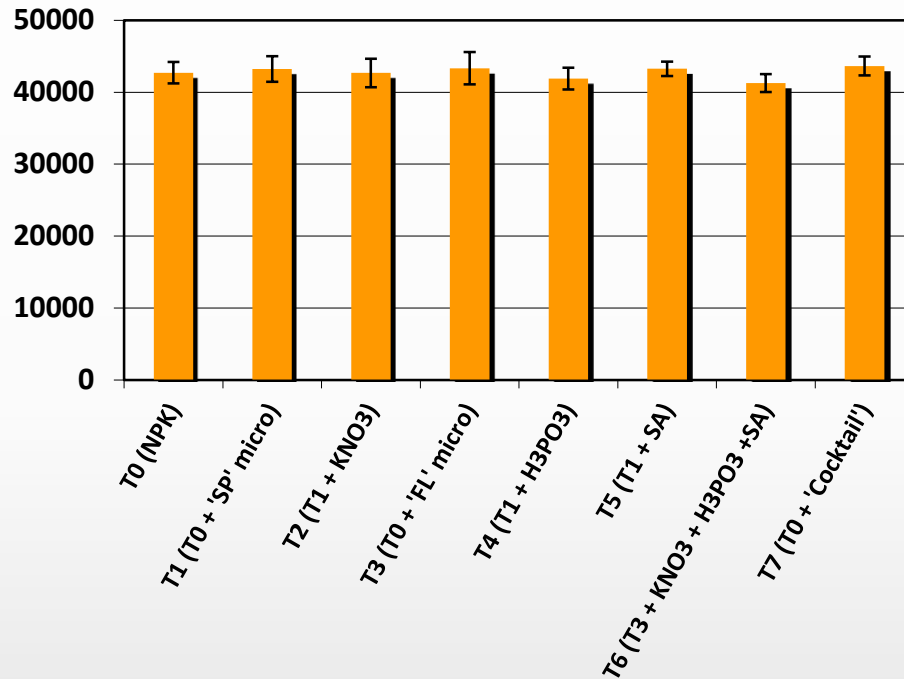
Diseased



Area Under HLB Severity Progress Curves

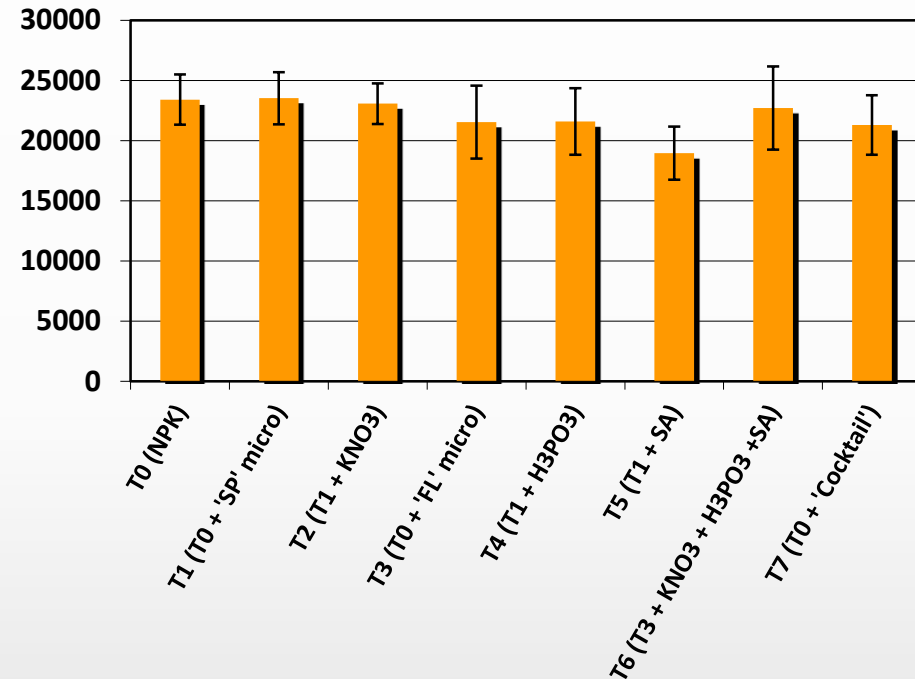
Group 1

(mean of 4 blocks with 20 trees)



Group 2

(mean of 4 blocks with 10 trees)



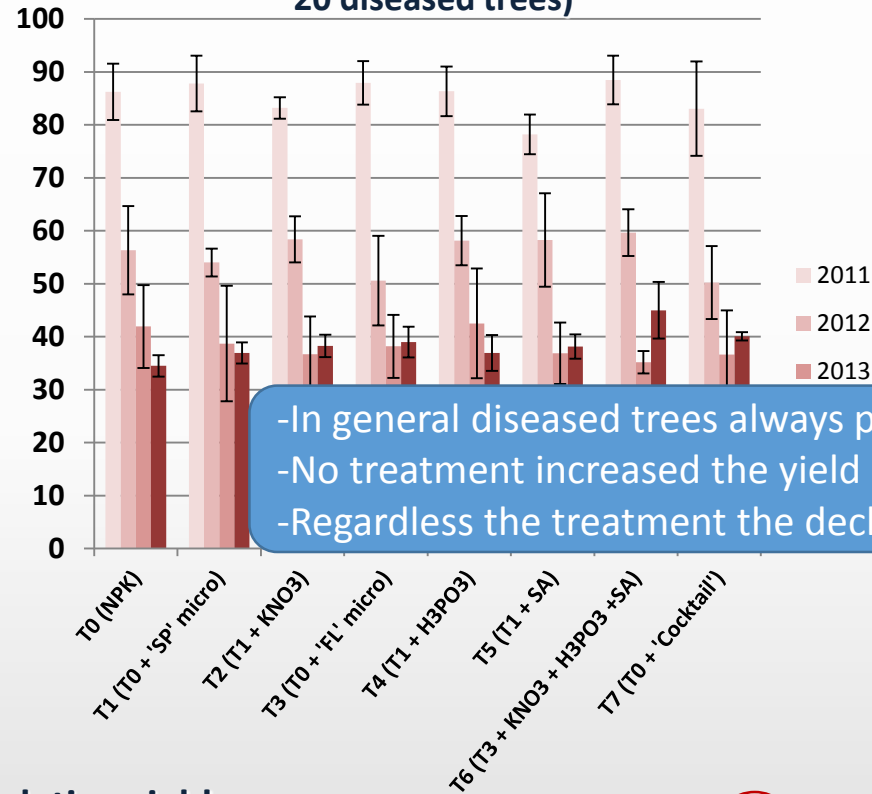
- No treatment reduced the disease severity
- Regardless the treatment the disease severity progress was the same

Means did not differed by F test (Group 1 $P=0.5128$; Group 2 $P=0.5164$)

Diseased Trees Yield in Relation to Healthy Trees (%)

Group 1

(mean of 4 blocks with 20 healthy and 20 diseased trees)



Group 2

(mean of 4 blocks with 20 healthy and 10 diseased trees)



- In general diseased trees always produced less than healthy trees
- No treatment increased the yield of diseased trees
- Regardless the treatment the decline on diseased trees yield was the same

Relative yield

2011	2012	2013	2014
85%	55%	36%	38%

Disease severity at harvest

20%	53%	53%	68%
-----	-----	-----	-----

2012	2013	2014
86%	68%	59%

12%	32%	51%
-----	-----	-----

Treatments in the same year did not differed by F test ($P>0.05$)

Non-technical summary

- Boron acid, potassium nitrate, zinc sulfate, manganese sulfate, potassium phosphite and salicylic acid were leaf sprayed 4 times/year during flushing periods to productive well-fertilized non-irrigated Sweet orange trees, both healthy and HLB-affected
- After 4 years evaluation, there was no difference among enhanced nutritional/resistance inducer treatments and the soil NPK treatment (control) for all assessed variables
- None of tested treatments:
 - Reduced the progress of HLB-symptomatic trees incidence
 - Reduced the disease severity in symptomatic trees
 - Slowed the yield decline
 - Improved the yield of HLB-symptomatic trees

Simulation of the impact of HLB in the production of citrus grove under disease management scenarios, disease incidences and grove ages at the beginning of the epidemic



Monica B. Neves*

Renato B. Bassanezi – Fundecitrus

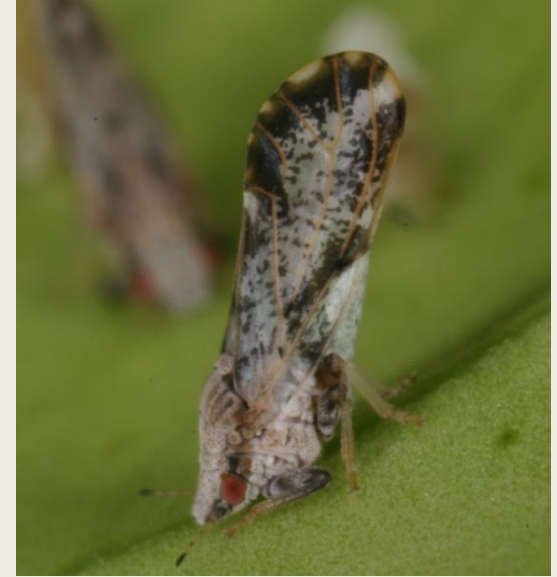
Non-technical summary

- The impact of HLB in the production of citrus groves under different disease management scenarios, initial disease incidence and age at the beginning of the epidemic was simulated
- For high expected yield of healthy groves and moderate annual rate of HLB infection**, it can be concluded that:
 - Groves younger than 6 years-old at the beginning of epidemics would have good productivity (≥ 400 90 lb.-boxes/acre) in any term, independent of initial disease incidence, **only if** managed with ACP control, eradication of symptomatic trees, and reset with healthy trees
 - Groves older than 6 years-old with lower initial disease incidence ($\leq 1\%$) could have a good productivity (≥ 400 90 lb.-boxes/acre) in a medium term (up to 10 years) only by controlling ACP well enough to prevent the secondary spread of the disease within the grove
 - For adult groves, the complete management (vector control, tree eradication and reset) will produce more than management only with vector control only in a long term (>15 years)
 - However**, the impact on fruit quality of symptomatic trees kept in the field was not considered in this study

QUESTIONS?

HLB Management: Developing New Solutions for HLB

- Team 4 – Kirsten Stelinski, Siddarama Gowda, Nian Wang
- 49 presentations and posters



Developing new solutions for HLB management: synopsis of progress from 2015 IRCHLB conference

Kirsten Pelz-Stelinski, Nian Wang, and
Siddarme Gowda

Progress towards solutions for long-term management of HLB

- Methods to enhance plant/psyllid defenses against HLB
- Rootstocks, resistance genes, and transgenics
- Las transmission by the Asian citrus psyllid
- Predicting the spread of HLB

Development of long term, effective, and sustainable solutions for HLB

METHODS TO ENHANCE PLANT/ACP DEFENSES AGAINST HLB

RNA interference (RNAi) technology for management of ACP and Las

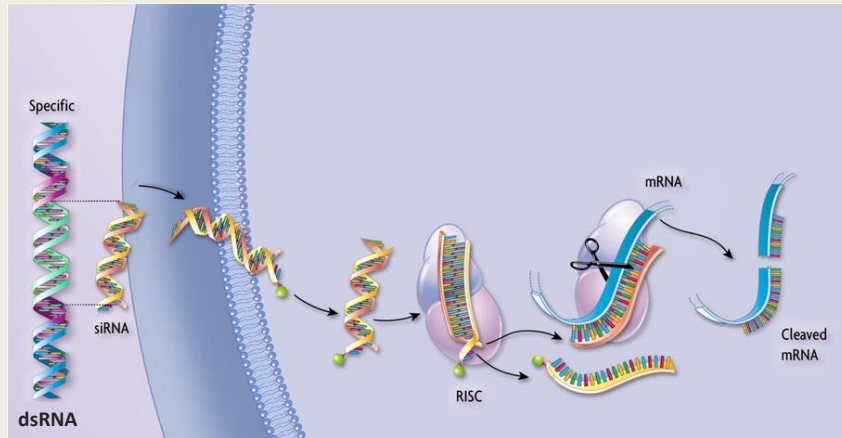
- **Exogenous dsRNA spray has been known to be effective for gene silencing of insect pests**
- **dsRNA degrade inside the plant = limited side effect**
- **Psyllids not repelled by plants treated with dsRNA**
- **Products can be designed to be safely used around honey bees and other pollinators because it uses the specific genetic code of the insect that is targeted (such as the Asian citrus psyllid)**

Hunter et al.

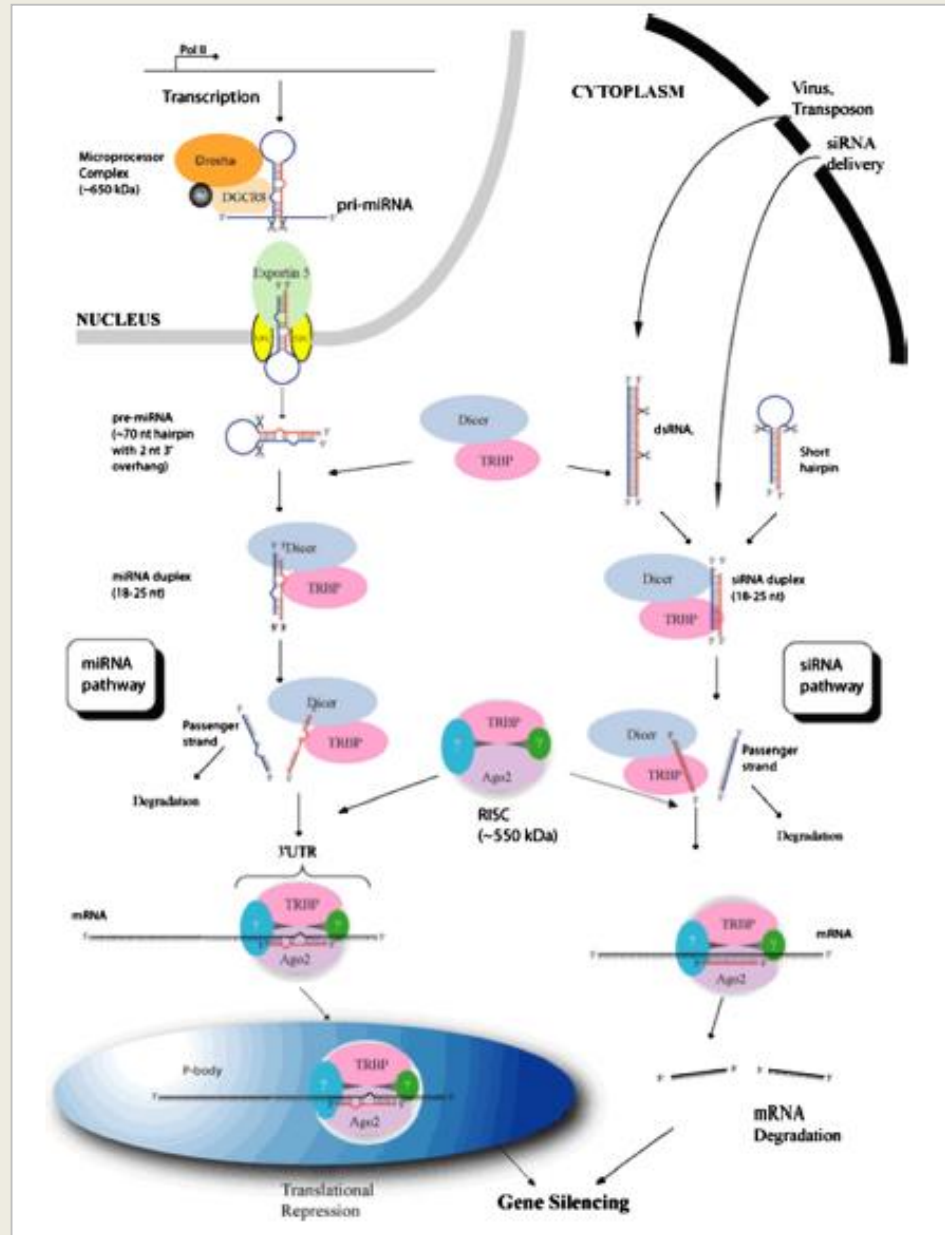
RNA interference (RNAi) technology for management of ACP and Las

RNAi - mechanism

- Natural mechanism in eukaryotic cells for gene regulation and antiviral defense;
- Mode of action: sequence specific RNA degradation

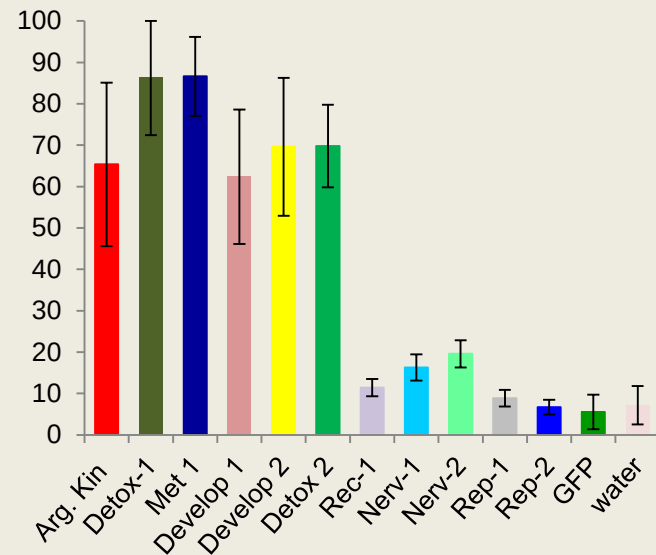


- Enables silencing genes in a specific way (surgically) using dsRNAs

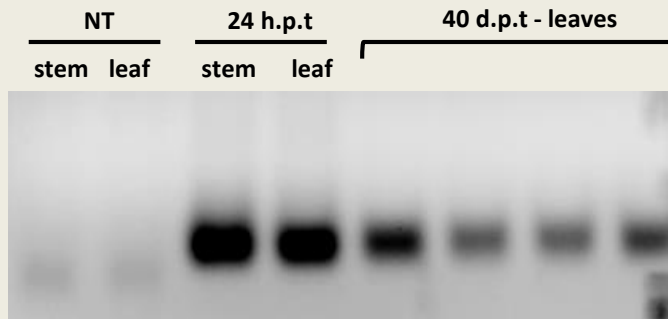
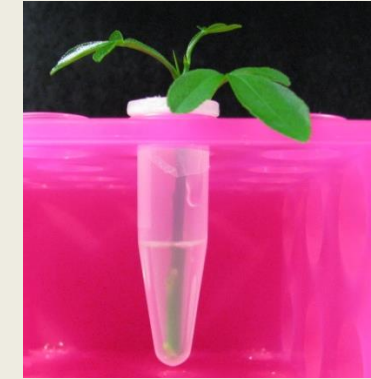
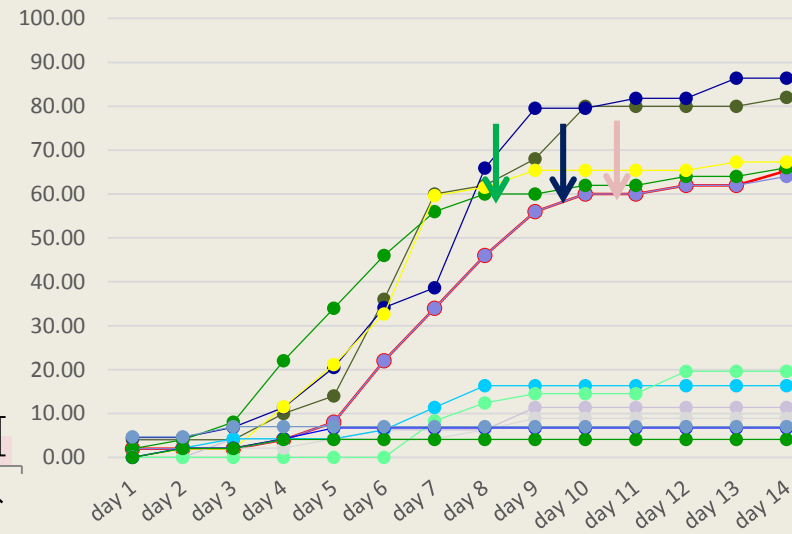


Successful development of *in plant* bioassay for screening dsRNA targetting ACP genes

ACP Mortality



ACP Mortality – over time

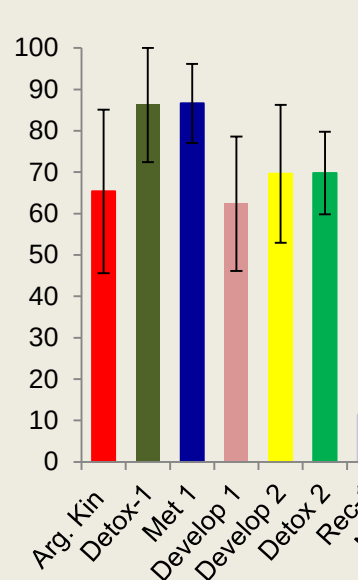


Days pos feeding

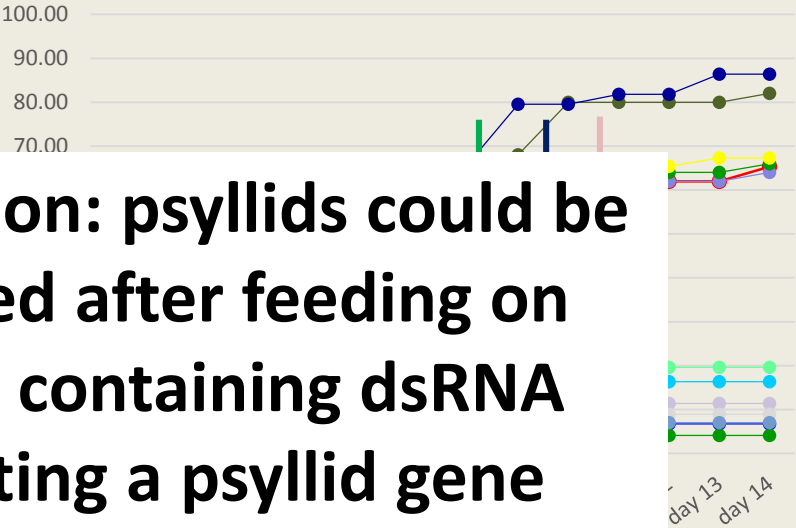


Successful development of *in plant* bioassay for screening dsRNA targetting ACP genes

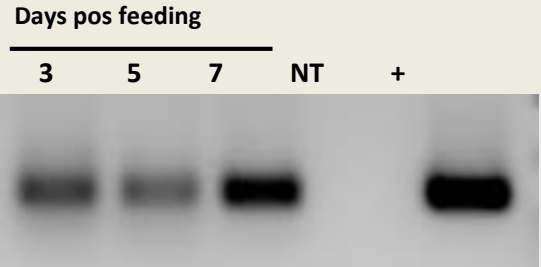
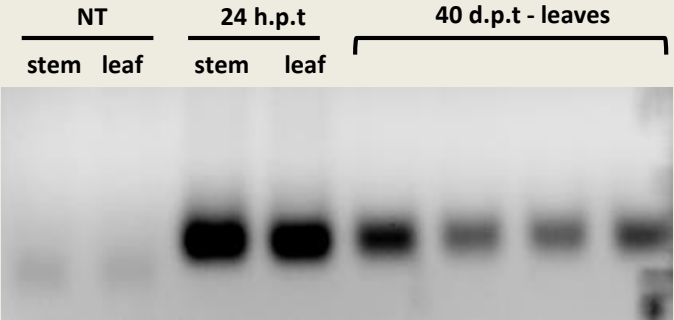
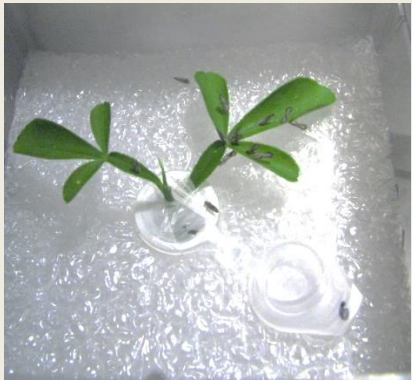
ACP Mortality



ACP Mortality – over time

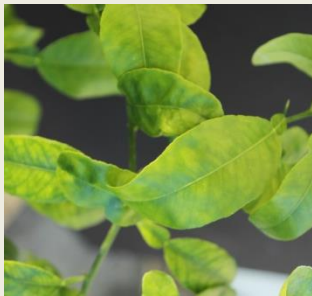


Conclusion: psyllids could be reduced after feeding on plants containing dsRNA targetting a psyllid gene



RNA interference (RNAi) technology for management Las in citrus host

- Progress: inoculated citrus plants do not show phloem plugging yet, although leaves are beginning to show symptoms.



3037 / HLB



3038 / HLB



3040 / -



Cmac / HLB

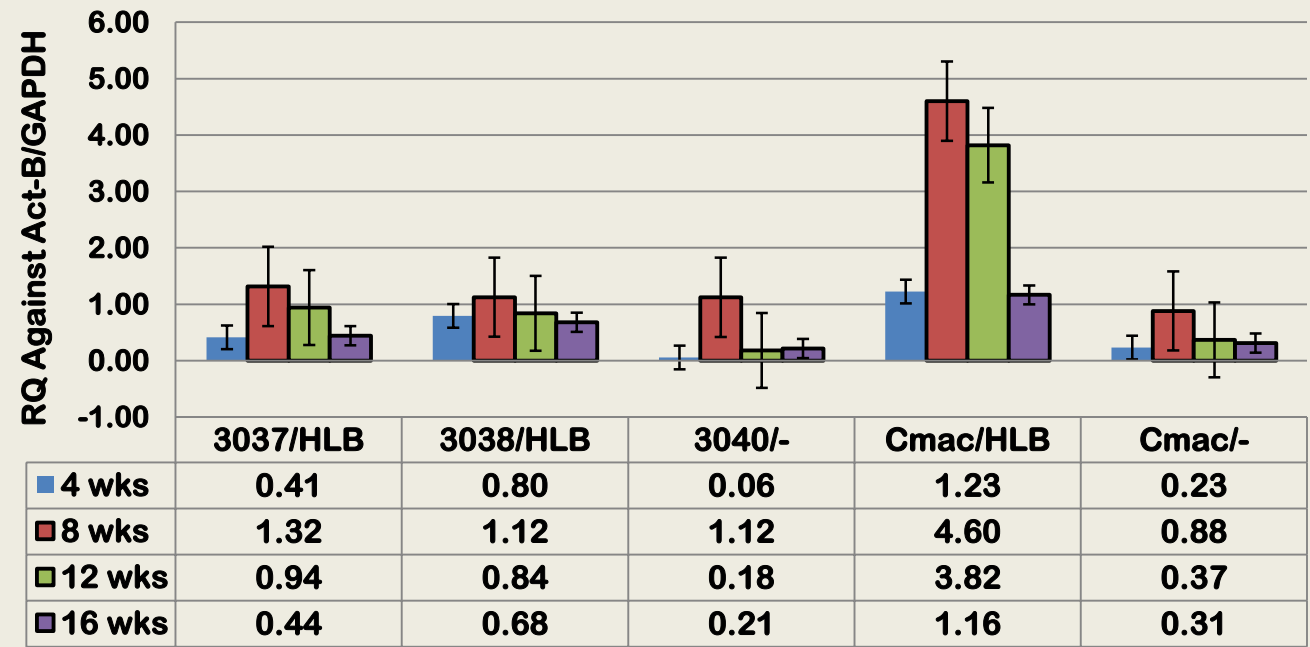


Cmac / -

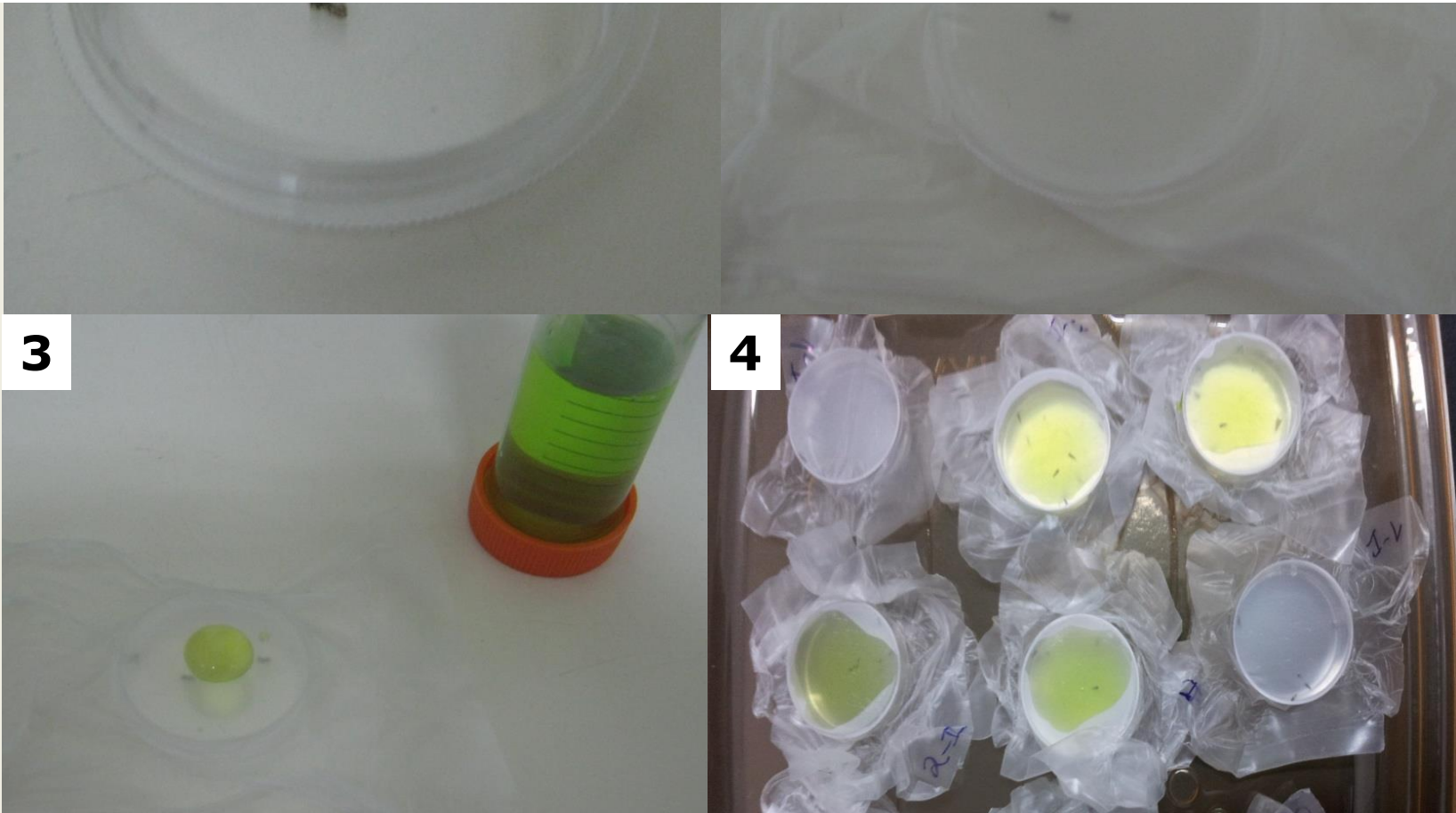
RNA interference (RNAi) technology for management of Las in citrus host

- Prevent plugging of phloem due to HLB: Induce RNAi in citrus host to down regulate citrus endogenous callose synthase and phloem proteins (PP2)

Changes in PP2 Expression with respect to Time



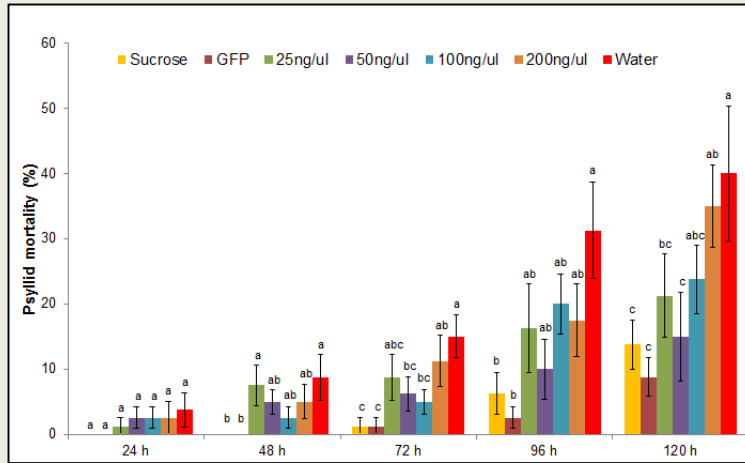
Finding optimal targets for RNAi: Screen dsRNAs targeting two psyllid genes in artificial diet (oral delivery)



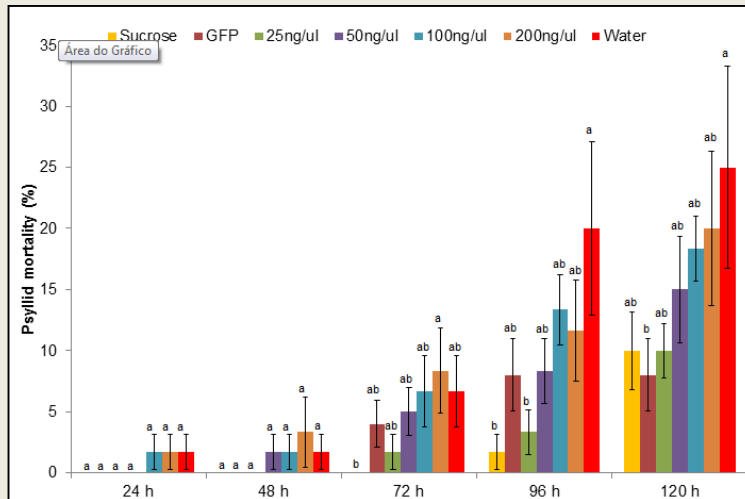
Galdeano et al.

RNA interference (RNAi) technology for management of ACP and Las

Psyllid mortality induced by feeding on decreasing concentrations of dsRNA targetting ACP genes



V-ATPase (cell maintenance gene)



Trehalase (insect blood sugar enzyme)

Galdeano et al.

RNA interference (RNAi) technology for management of ACP

Using CTV engineered to produce dsRNA to kill ACP

- Kill infected psyllids and their progeny using CTV that produces dsRNA against enzymes that participate in development and digestion (Shatters, Powell, Dawson, Gowda, **Borovsky**)
- Crowdsourcing-based identification of targets for use in development of dsRNA for ACP management

RNA interference (RNAi) technology for management of Las in citrus host

Modeling the impact of RNAi treatments on the spread of HLB among asymptomatic trees

- Mathematical models to analyze the effectiveness of potential ACP control strategies, most notably, a new biological control using RNAi
- Recent experiments show that the time to infectiousness in flush shoots is as short as 22 days

**Lee, Halbert, Dawson, Ptacek, Singer and
Keesling**

Development of novel control strategy to citrus HLB by targeting critical traits of *Candidatus Liberibacter asiaticus*
Wang et al.

- *Ca. L. asiaticus* uses salicylate hydroxylase to degrade salicylic acid (SA) to suppress plant defenses
- Counteract strategy: Apply SA or SA analogs or other plant defense inducers: 6 small molecule inhibitors have been identified.
- Spray treatment of HLB diseased trees with defense inducers BABA, INA, AA, 2-DDG, BTH could slow down the increase of Las population in citrus and HLB disease severity, but not decrease Las titer and HLB disease severity.
- Trunk injection of SA reduced the Las population in citrus.
- Trunk injection of antimicrobials, e.g., SA inhibitors, has potential to reduce Las population in citrus.

Development of long term, effective, and sustainable solutions for HLB

ROOTSTOCKS, RESISTANCE GENES, AND TRANSGENICS

Identification of variables associated with HLB development: for use in resistance screening

Important Variables:

- Budwood source: even when source trees have similar levels of the HLB pathogen, Valencia or Rough Lemon transfer more pathogen through grafting than Volk or Citron
- Moderate water or nutrient stress accelerated HLB, but more severe stress didn't
- Preliminary: some rootstocks delay HLB development

Not important:

- Graft vs. psyllid inoculation
- 24-hr light did accelerate HLB
- Girdling trees had little effect on HLB advancement
- Host source for ACP colony did not markedly affect ACP transition to different genotype and transmission of Las

Evaluation of rootstocks/scions and genes for HLB resistance

Omar, Atlaf and Grosser

- Mandarin/ *Citrus latipes* somatic hybrid root stocks have potential for improved tolerance to HLB
- *Citrus latipes* has shown to possess tolerance to HLB in other counties
- Have developed hybrids through protoplast fusion of W Murcott suspension culture derived/*Citrus latipes* leaf protoplasts
- Horticultural performance and ability to mitigate or prevent HLB in grafted commercial scions being evaluated

Evaluation of rootstocks/scions and genes for HLB resistance

Consumer friendly HLB tolerant plants

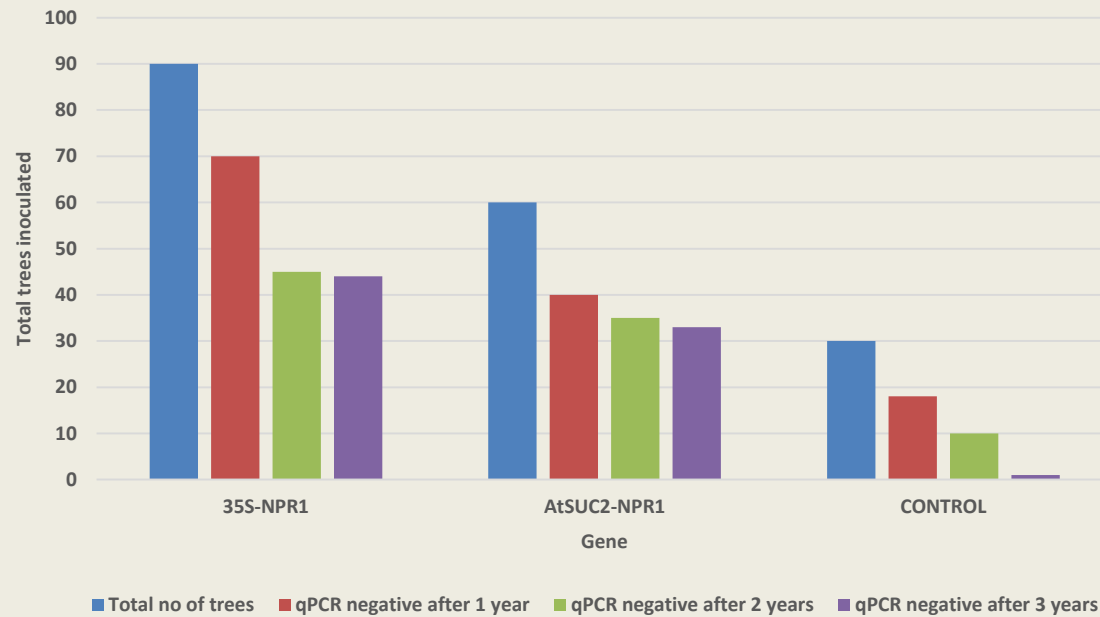
Rawat, Gmitter and Deng

- Comparison of genome organization in HLB resistant and susceptible plant resistance regions
- Analysis of the role of resistance genes (*R*-genes) in HLB resistant and susceptible plants

Dutt, Barthe and Grosser

- Development of citrus transformation system using Ubiquitin and Rubisco derived constitutive promoters and endogenous Phloem Protein 2 derived phloem specific promoters, and plant-based visual reporter
- Used for evaluation of citrus and other plant derived genes are currently being evaluated.

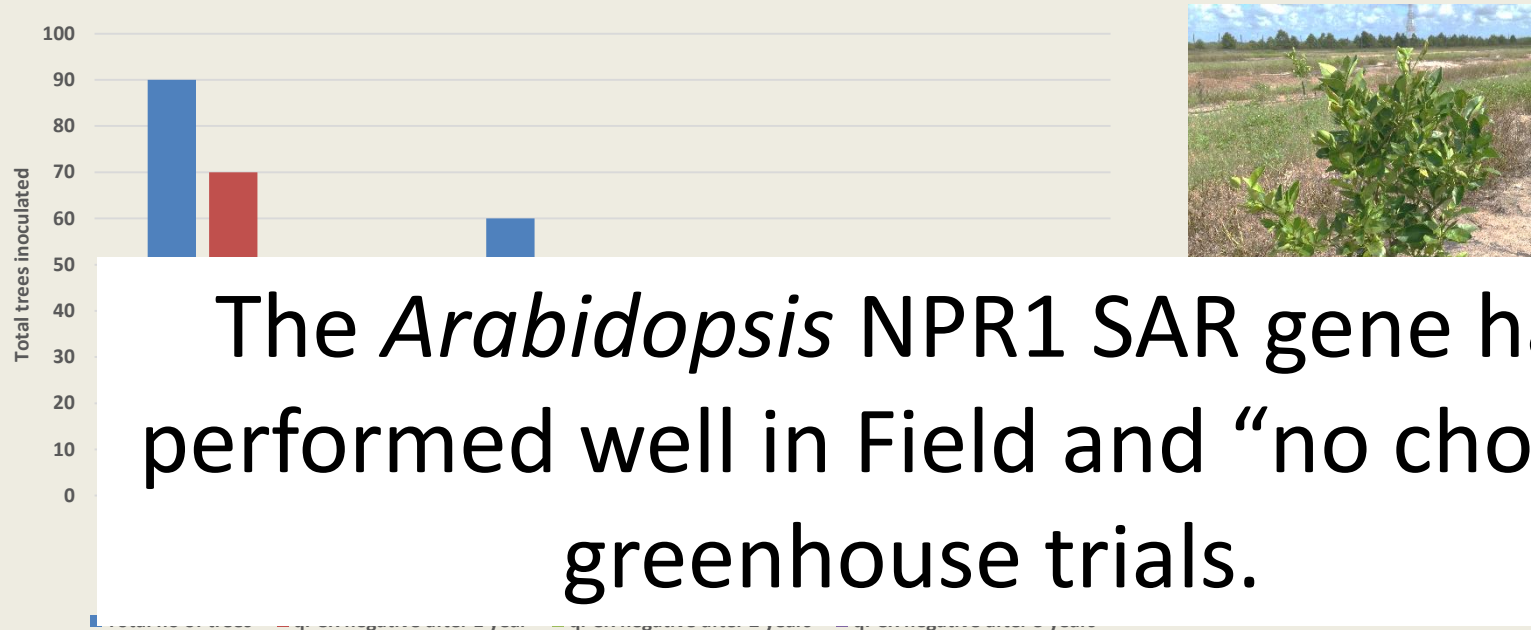
HLB infection rates on trees expressing *Arabidopsis* NPR1



A NPR1 (HLB-) tree planted in the field after 2 years in the greenhouse

- Transgenic trees and controls were planted in two sites, both in South Florida counties with a 90+ HLB infection rate.
- Samples were collected at yearly intervals and analyzed using qPCR.

HLB infection rates on trees expressing *Arabidopsis* NPR1



The *Arabidopsis* NPR1 SAR gene has performed well in Field and “no choice” greenhouse trials.

- Transgenic trees and controls were planted in two sites, both in South Florida counties with a 90+ HLB infection rate.
- Samples were collected at yearly intervals and analyzed using qPCR.

Transgenic citrus for management of Las

Engineering mobile RNA element to enhance plant defense response in rootstocks and non-transgenic scions

- Developed transgenic Carrizo root stock lines that are silenced for programmed cell death and defense response.
- The silencing signal should be able to move into scions and potentially induce long-lasting resistance to HLB

Transgenic citrus for management of Las

Developing site specific gene integration technology to introduce genes at specific sites in transgenic plants to avoid random integration

- A novel technology useful for engineering genes in transgenic citrus plants that target HLB

Citrus genome modification

Hongge Jia and Nian Wang

- Development of HLB resistant or tolerant citrus will provide a long term, effective and sustainable solution to HLB.
- Targeted genome engineering is expected to contribute significantly to future varietal improvement.
- Cas9/sgRNA technology has been developed to generate HLB resistant or tolerant citrus.
- Cas9/sgRNA is being used to specifically modify the putative targets of the virulence factor of *Candidatus Liberibacter asiaticus*.

Development of long term, effective, and sustainable solutions for HLB

LAS TRANSMISSION BY THE ASIAN CITRUS PSYLLID

Alternative hosts of ACP

- Psyllids can survive, but not reproduce, on *Zanthoxylum fagara*, an endangered relative of citrus in Florida; monitoring of this host plant important for ACP management

Nupsyllid project

Progress towards
the development of
psyllids that cannot
transmit Las

- Identification of
virus, bacteria, or
development of
chromosomally-
transformed
psyllid



Falk et al. identification of psyllid virus for delivery of RNAi

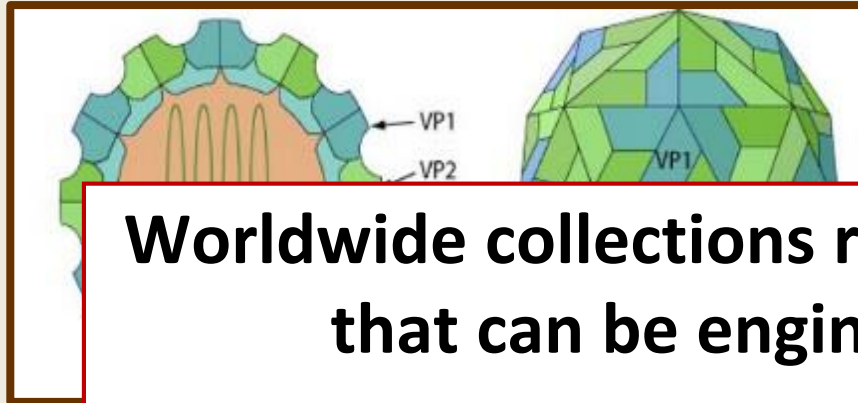
Non-Plant based RNAi delivery

Can we achieve specific, systemic RNAi effects directly in hemipteran vectors without using plants to deliver the interfering RNAs?

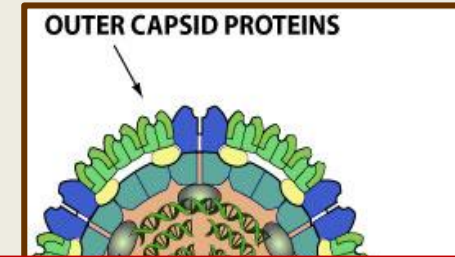
Maybe we can use insect-infecting viruses...

Newly discovered
D. citri viruses

Picorna-like virus (ssRNA)

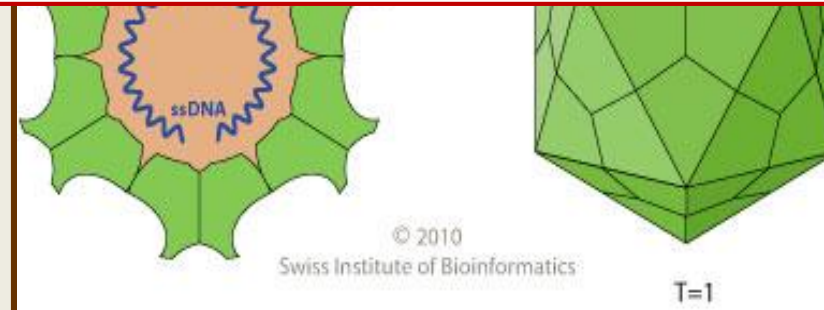


Reovirus (dsRNA)

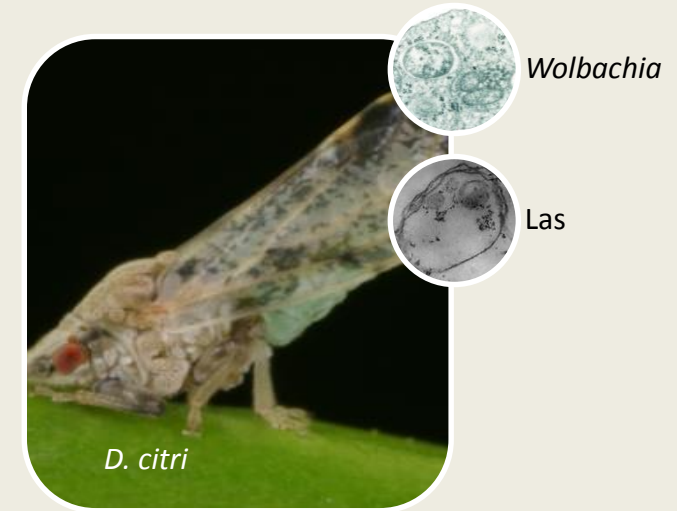
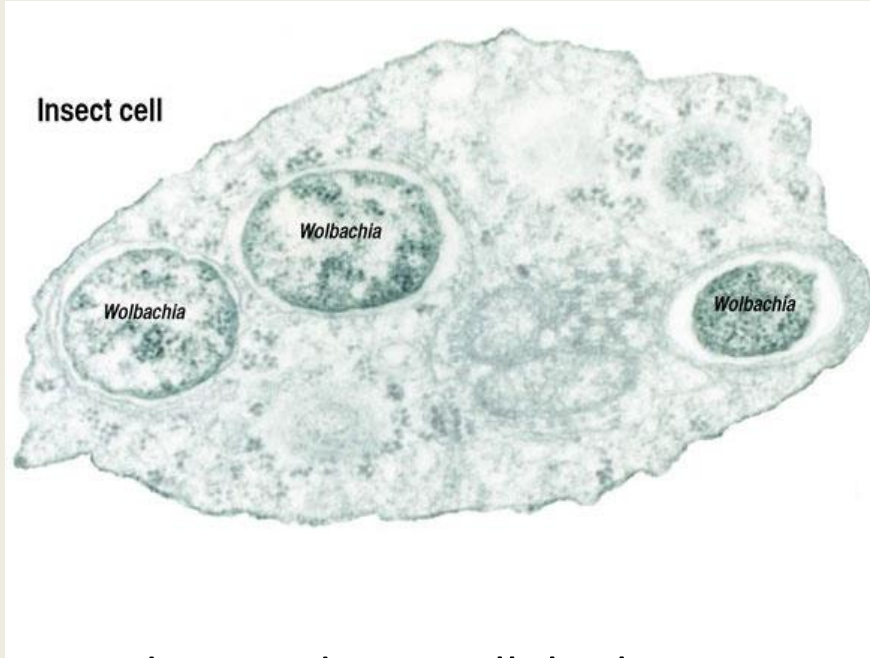


Worldwide collections revealed three psyllid viruses that can be engineered to deliver RNAi

DPCLV does not appear to be common in U.S. *D. citri* populations- an excellent candidate for engineering

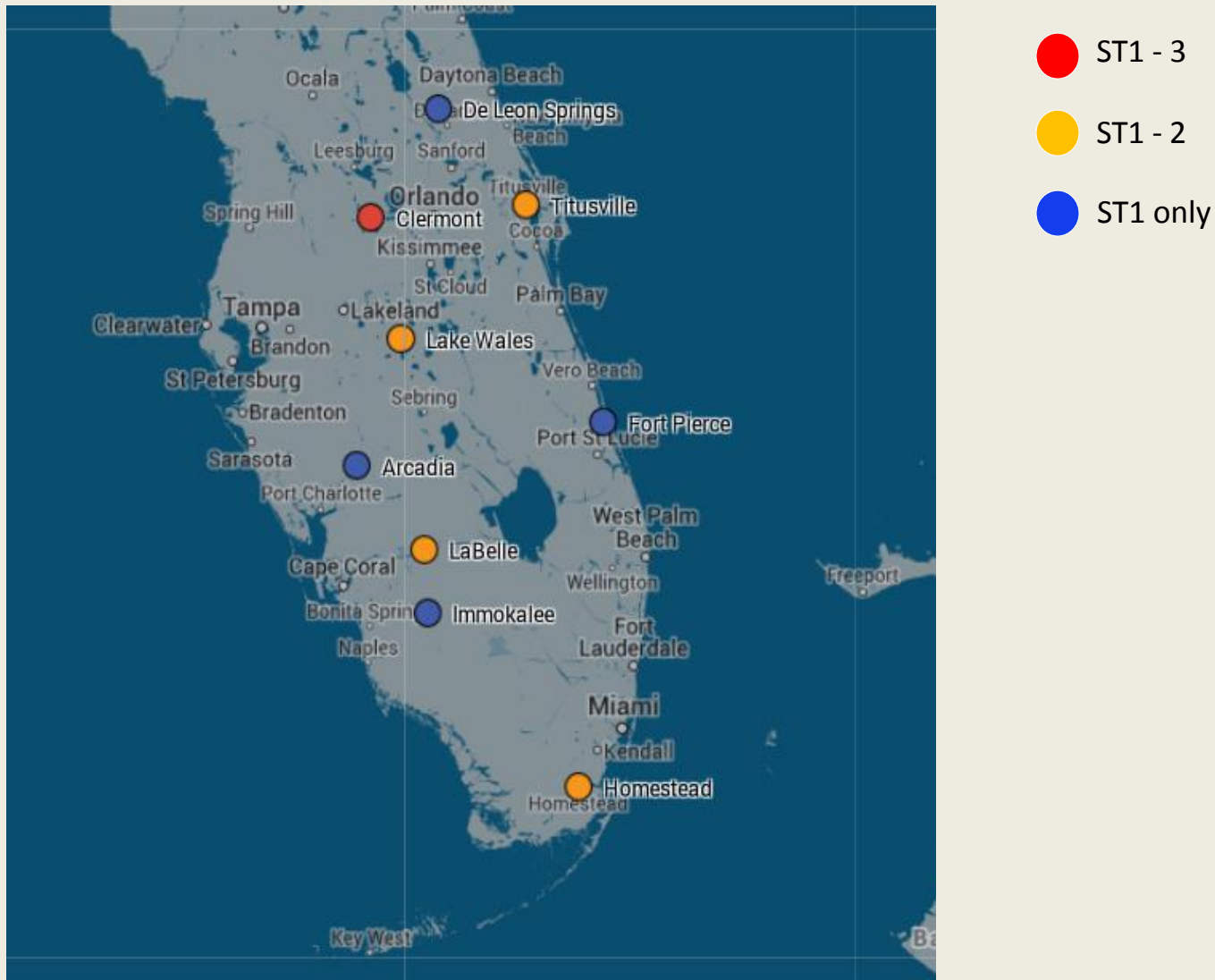


Hoffman et al. *Wolbachia*

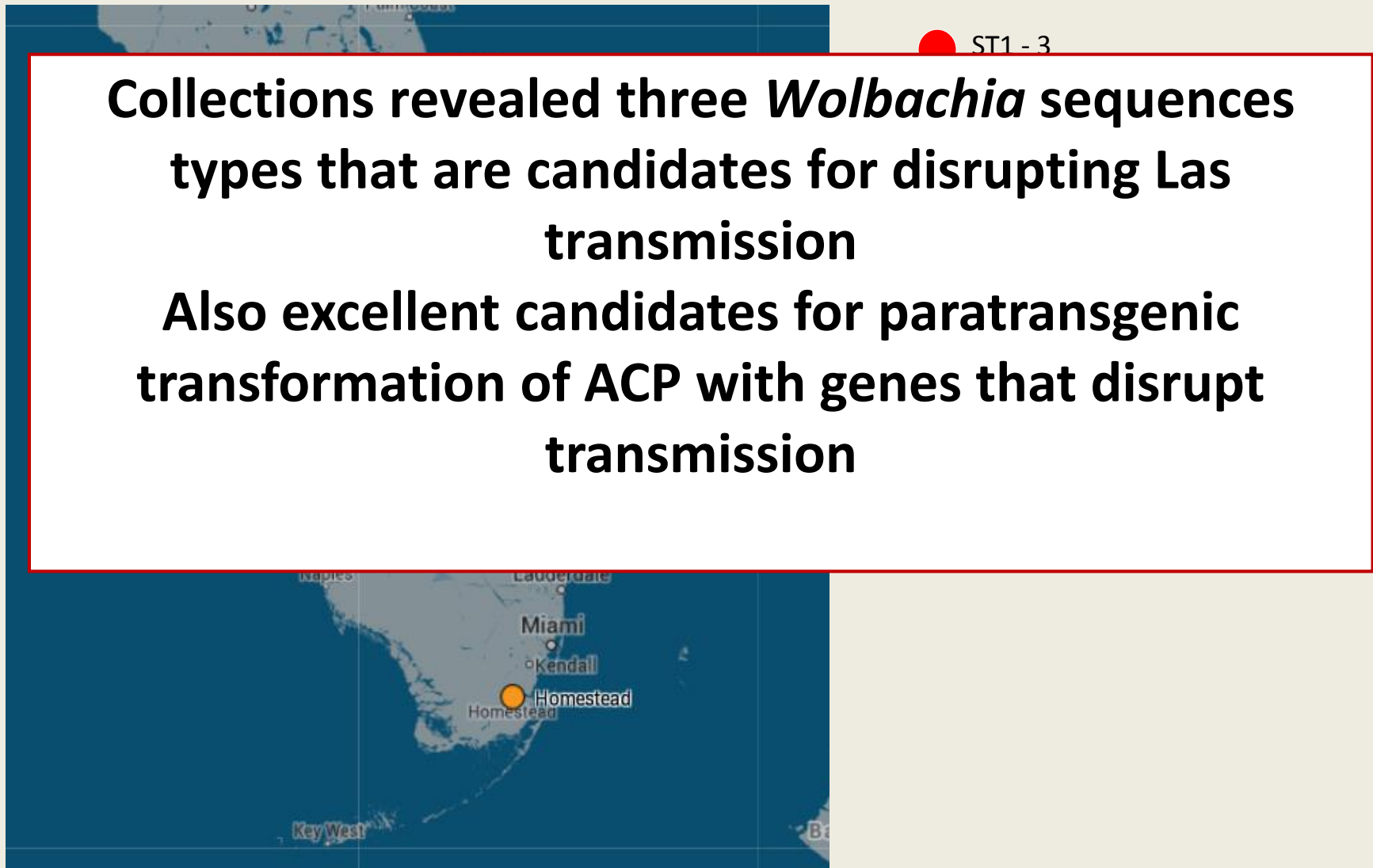


- A widespread intracellular bacterium, carried by an estimated 40% of insect spp.
- May interact with pathogens, effecting the probability of transmission (e.g. competitive exclusion, immune activation)
- Approach used in insect vectored human pathogen systems

Occurrence of *Wolbachia* sequence types in Florida *D. citri* populations



Occurrence of *Wolbachia* sequence types in Florida *D. citri* populations



Understanding Las transmission

- Ammar et al., Halbert et al.

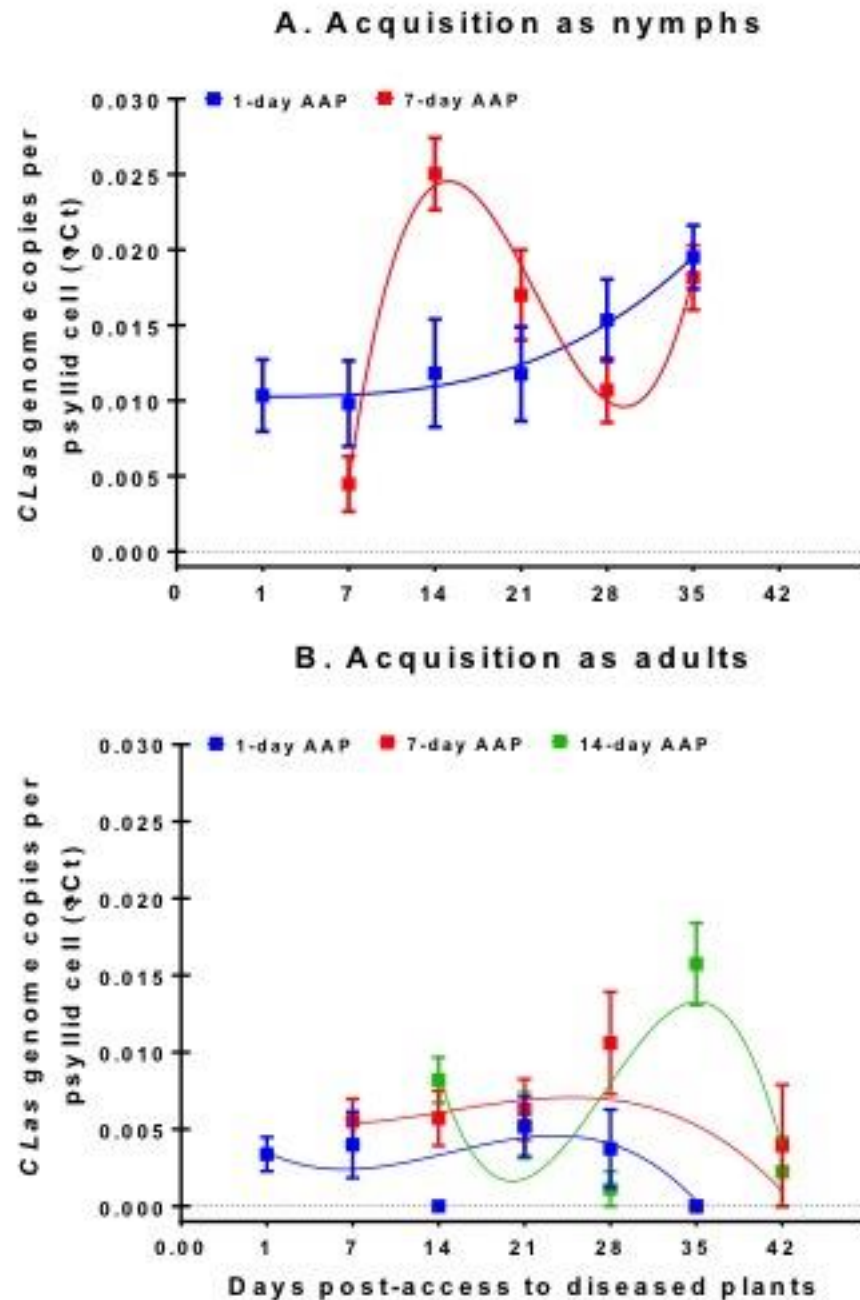
Is replication important for Las transmission?

Conclusions

- Las acquisition increases with length of feeding time in adults (1-14 days) but not in nymphs (1-7 days).
- Las can be transmitted by nymphs and adults, but adults must acquire it during the nymphal stage (important for HLB management)

- **Las replicates in ACP nymphs and adults BUT,**
 - it reaches higher titer in nymphs than in adults
 - Adults require longer feeding times

Ammar et al.



Halbert et al. shared surprising results from the Florida psyllid testing project

Symptoms found nine months after positive psyllids were detected



Photos: Susan Halbert

A new understanding of transmission

Las increases in new citrus flush immediately after initial infection, infecting the next generation of psyllids.

Since a female psyllid can lay 800 eggs, the increase is fast. It is possible to have billions of infected psyllids in a grove months or even years before any symptoms of HLB become evident.

This new mechanism has major implications for disease spread and grove management.

Standard model for vectored pathogen

- Vector infects a plant
- After a latent period, plant becomes a source for another generation of psyllids
- New psyllids acquire pathogens and transmit to another plant
- Disease cycle is at least 6 months in the field

Photo: Jeff Lotz & David Ziesk



Xiao-an Sun

Photo: Jeff Lotz & David Ziesk



How it works

- Immediate acquisition from flush shortens the latent period to the lifespan of the bugs
- Co-opts the vast reproductive capacity of the bugs
- Possible to have billions of positive psyllids after a few cycles before HLB symptoms are evident



Implications: Long range movement

- Human-assisted movement of even a single positive female Asian citrus psyllid can have serious consequences.



Photos: Susan Halbert

Implications: Can the lengthy incubation period work in our favor??

- Long incubation period indicates that plants are fighting



Implications: Rapid asymptomatic disease spread

- A single positive female psyllid can initiate a successful infective colonization event
- Positive psyllids can be distributed all over a grove years before any manifestation of disease

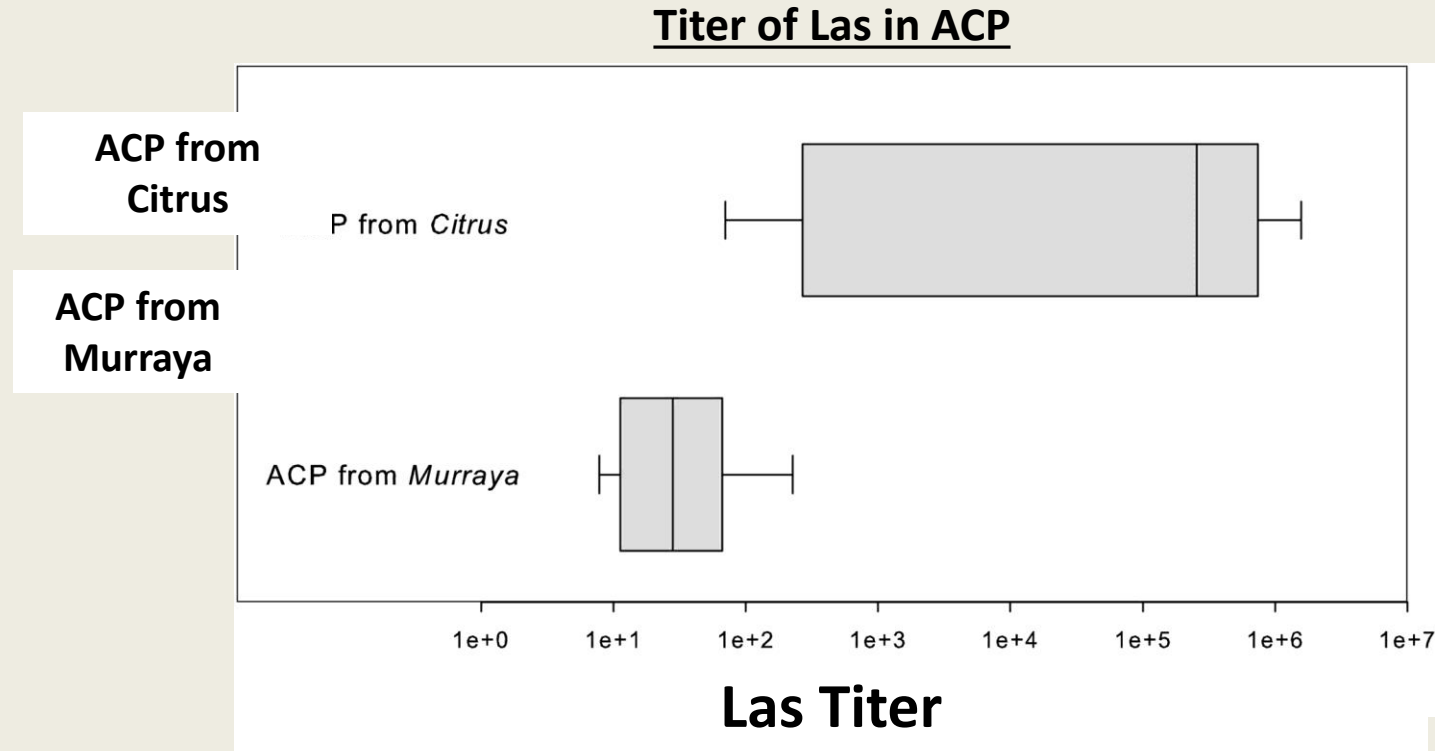


Map from Google

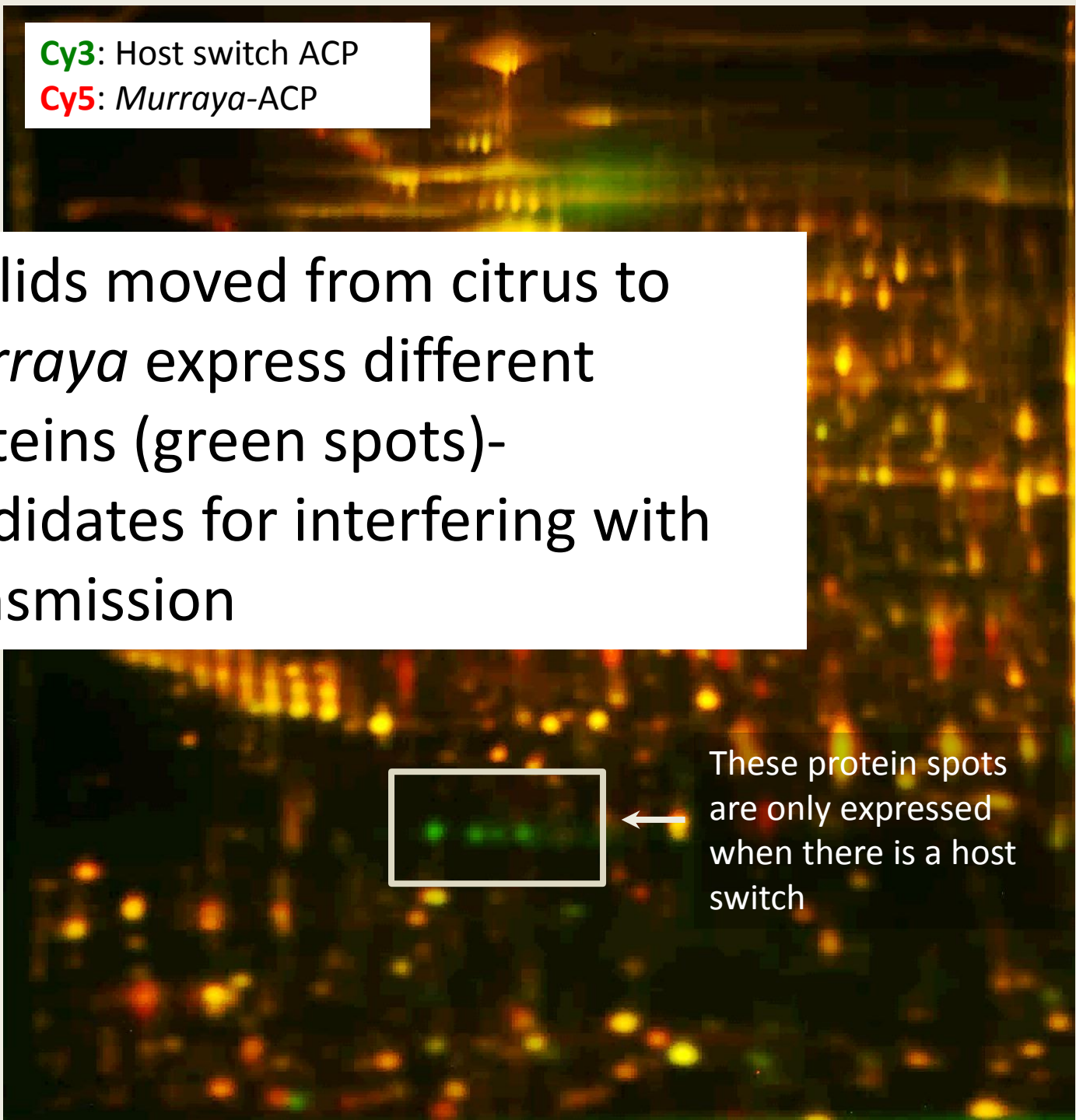
Identifying novel protein targets for controlling transmission in the psyllid

- Hu and Killiny: comparisons of Las-infected and uninfected psyllids to identify targets for management
- Mahoney: host plant influences transmission
- Ramsey: PIR to evaluate gut-Las interaction

Hall et al. previously showed that psyllids reared on citrus are better Las vectors than those reared on **Murraya**



Mahoney et al.: Are there protein changes that can explain how a good vector of Las becomes a bad vector just by living on an alternate host plant?

A fluorescence microscopy image showing a host switch ACP. The image displays a dense field of yellow and orange spots, representing the ACP, with a few green spots indicating the host switch. A white box highlights a specific region of the image.

Cy3: Host switch ACP

Cy5: *Murraya*-ACP

Psyllids moved from citrus to *Murraya* express different proteins (green spots)- candidates for interfering with transmission

These protein spots are only expressed when there is a host switch

Identify proteins involved in Las transmission

- Las must circulate through insect for successful transmission
- Las must cross multiple barriers to transmission within insect
 - Interacting psyllid and Las proteins at salivary gland and gut membranes, hemocoel

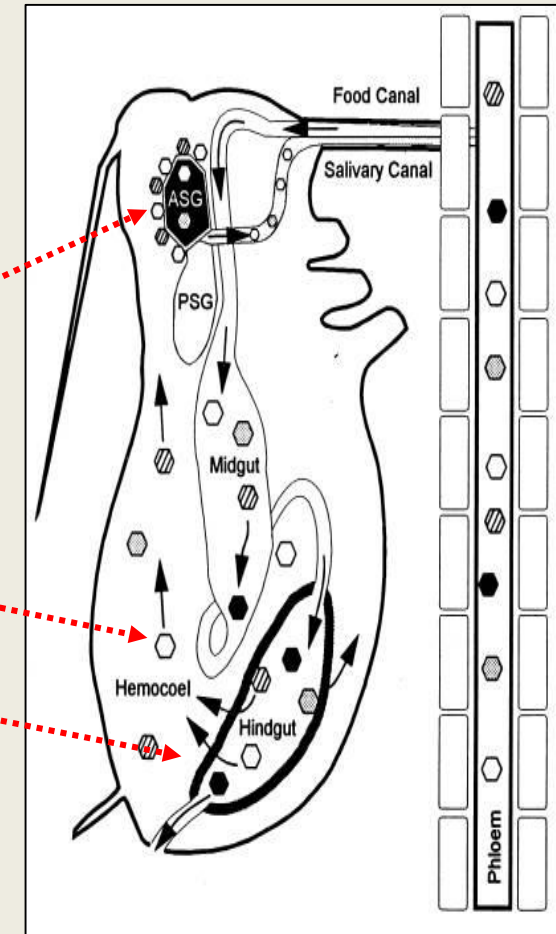
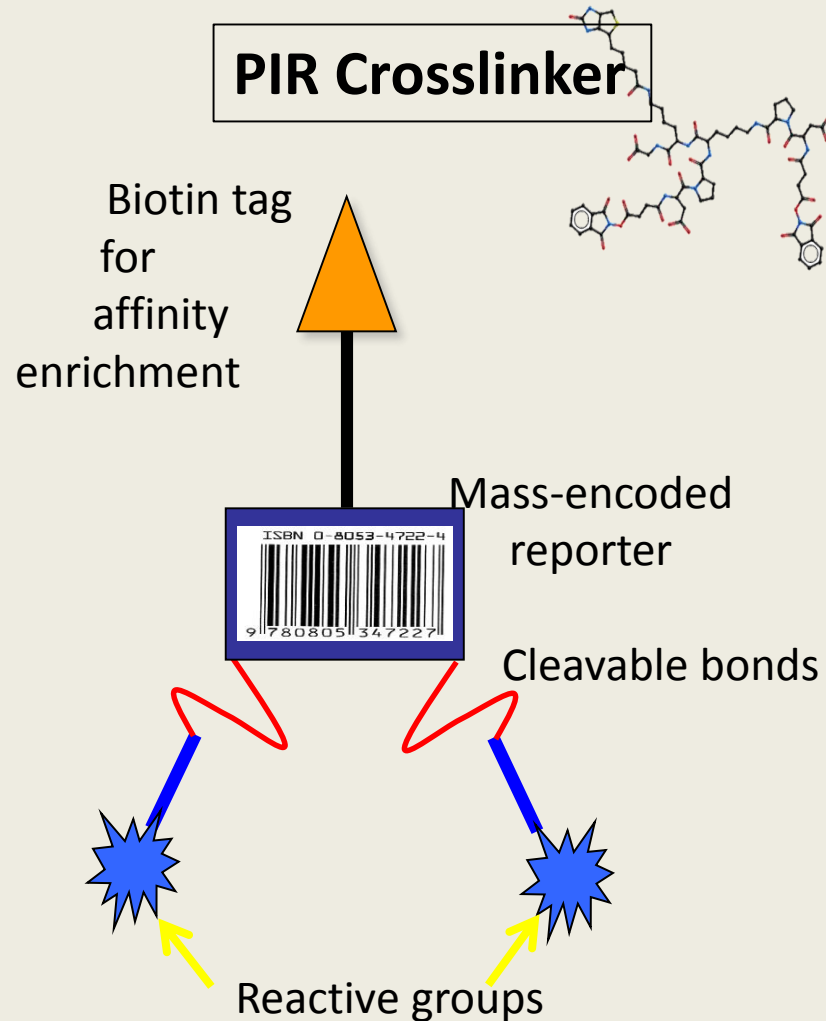
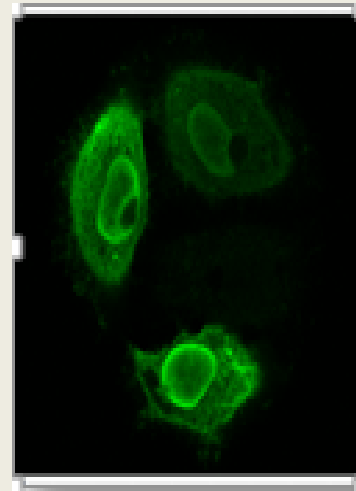


Image credit: SM Gray, 1999

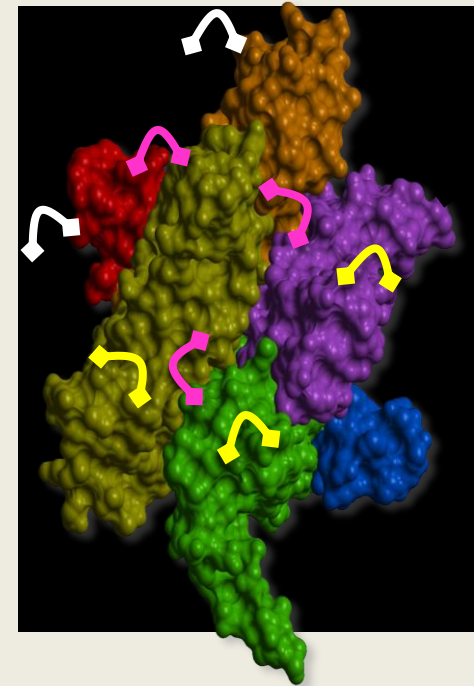
Ramsey et al. used a technique called PIR to map protein interactions in cells



Crosslinker penetrates live cells to capture protein interactions



Biotin staining in PIR labeled HeLa cells

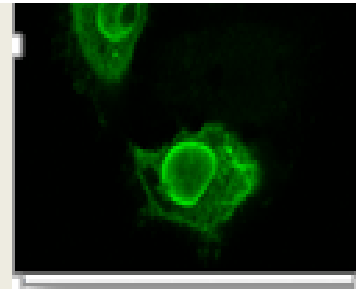
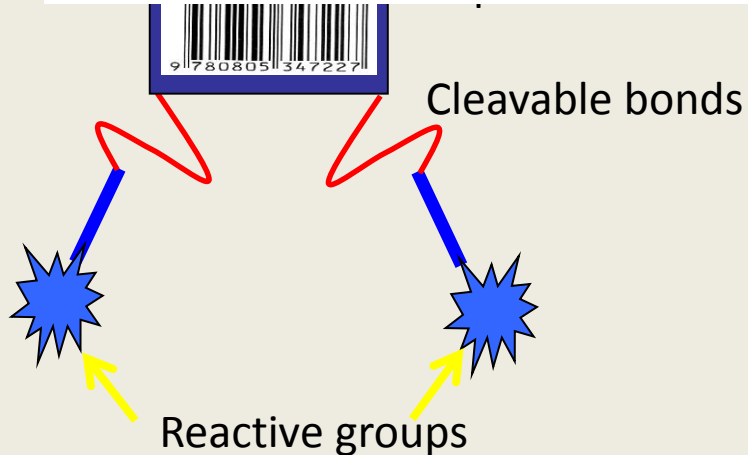


Ramsey et al. used a technique called PIR to map protein interactions in cells

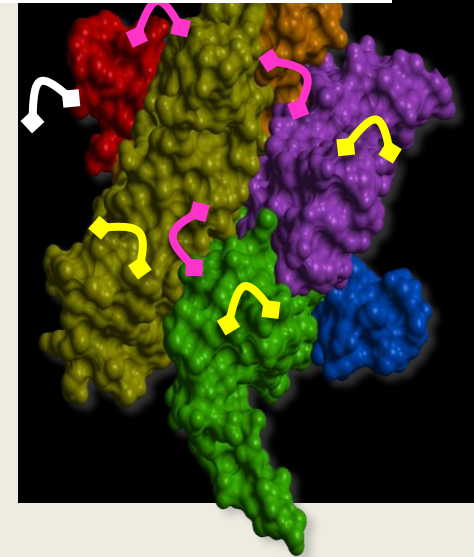
PIR Crosslinker

Crosslinker penetrates

Identification of Las bacteriophage protein that may be produced during Las transmission



Biotin staining in PIR labeled HeLa cells



Reducing transmission via ACP management

Organic insecticides for ACP management (Quereshi et al.)

- Pyganic with 435 oil in winter Pyganic maintained ACP at or below 0.1 adults per tap sample for 4-5 weeks
- Significant suppression lasted two months after the January application.
- Rotations of microbial insecticides with oils in organic programs, or synthetics in conventional programs, suppressed ACP populations for 2-4 weeks during growing season following winter treatments
- 435, Citru-Soy, and Pongamia oils alone provided additional reduction for 3 weeks during growing season
- *T. radiata* in organic programs provided additional reduction

Development of long term, effective, and sustainable solutions for HLB

PREDICTING THE SPREAD OF HLB

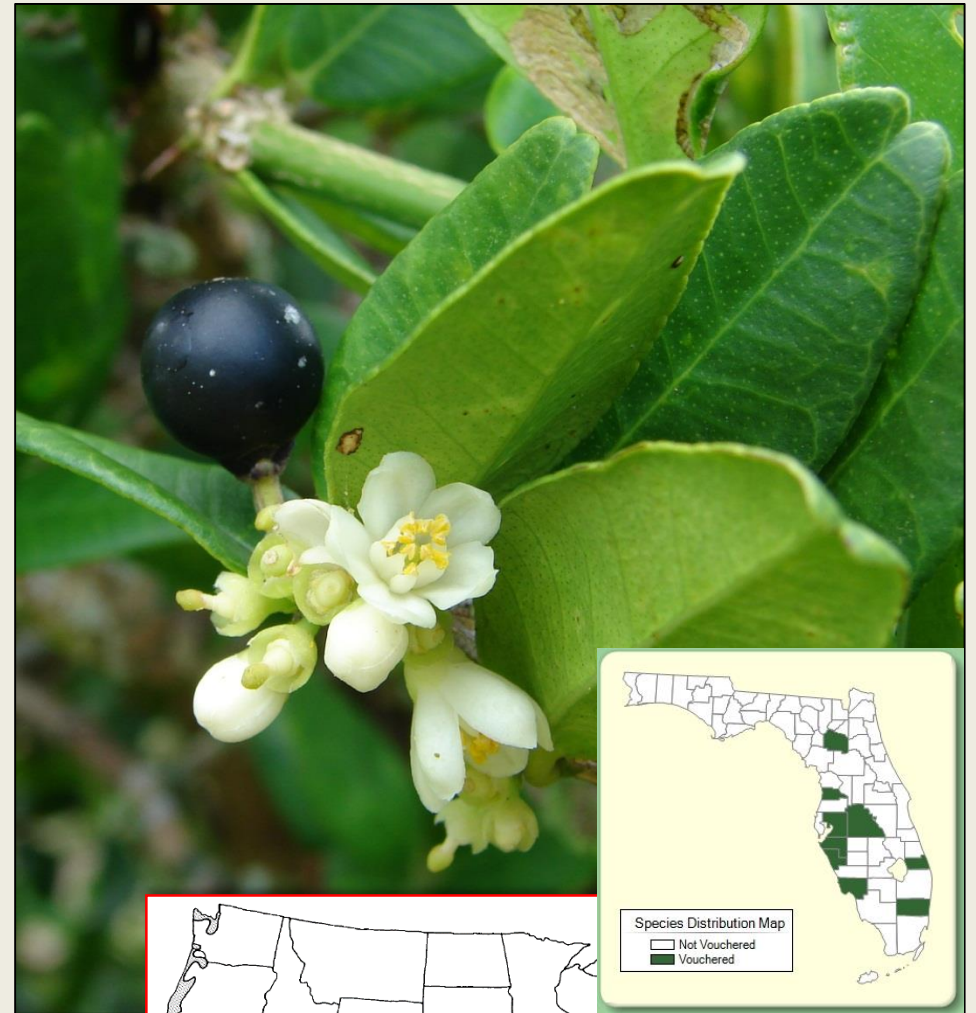
Alternative hosts for citrus diseases of quarantine concern in Florida

Severinia buxifolia

- present in parks of Miami-Dade and Broward counties
- A major threat **since citrus HLB has been identified on this host** and attractive to ACP

Swinglea glutinosa

- present in the Broward county.
- persistent presence of Canker symptoms found on stems and leaves
- the native host of Citrus leprosis



Gomez, Riley, and Robl

Cultural Control: Las Transmission

- Citrus plants grown from seeds from Las-infected, show abnormal growth phenotypes resembling symptoms expressed by infected trees, yet are not infected
- Suggested that substance(s) produced by bacteria translocates from the infected mother tree into the seedling and dramatically affects seedling growth.

Cultural control: Models

Modeling by Parnell et al. to find:

- an efficient spatial sampling scheme to detect the disease as soon as possible,
- to know how many times one should scout a given orchard in order to find 100% of symptomatic plants
- optimize the sampling frequency and intensity in order to maximize the detection probability before the disease reaches a given incidence.
- Best sampling procedures: include evaluation of plants on the orchard's edges or a fraction of the planting rows
- As the detection efficiency increases, a steep decrease in required re-inspections occurs
- 3 re-inspections are needed to find 100% of the symptomatic plants

Cultural control: Models

- Luo and Gottwald developed a model based on travel/trade data and the U.S. Census to predict initial introduction points for HLB, as well as other citrus diseases.
- The model is being integrated into existing risk-based early detection surveys in California, Texas, Arizona, and Florida to optimize regulatory/commercial disease intervention/control.

Cultural control: Models

- Luo et al. Modeling indicates ACP spread more frequently and quickly in high density commercial citrus compared with low density or residential citrus areas
- Confirms coordinated sprays slow ACP spread

Developing Solutions for HLB Management:

Some conclusions

Rootstocks, Resistance Genes, and Transgenics

- Identification of variables associated with HLB development for use in resistance screening: budwood source, moderate stress, some rootstocks delay HLB development
- Mandarin/ *Citrus latipes* somatic hybrid root stocks have potential for improved tolerance to HLB
- Progress toward development of consumer-friendly, transgenic, HLB tolerant plants



A NPR1 (HLB-) tree planted in the field after 2 years in the greenhouse

Developing Solutions for HLB Management:

Some conclusions

Methods to enhance plant/psyllid defenses against HLB

- RNAi technology for management of ACP and Las: progress on screening, delivery (CTV), and identification of targets
 - Kill ACP
 - Reduce phloem plugging caused by HLB
 - Models indicate these tactics could be successful



Developing Solutions for HLB Management:

Some conclusions

Reducing transmission

- Monitor *Zanthoxylum fagara*, alternative host of ACP
- Nupsyllid project: Identification of 3 bacterial and 3 viral candidates that can be used to develop a non-transmitting psyllid
- Improved understanding of transmission/importance of ACP management:
 - adults must acquire Las during the nymphal stage to transmit efficiently (important for HLB management)
 - Las increases in new citrus flush immediately after initial infection, infecting the next generation of psyllids

Developing Solutions for HLB Management:

Some conclusions

Reducing transmission

- Identification of targets for reducing transmission (possible candidates for RNAi/transformation):
 - identification of proteins associated with host plant-associated differences in Las transmission
 - identification of targets in psyllid gut associated with Las transmission

Developing Solutions for HLB Management:

Some conclusions

Predicting HLB spread

- Two models for improving detection of HLB/ACP:
 - confirmation that coordinated sprays slow ACP spread
 - 3 inspections needed to find 00% of infected plants
- Identification of alternative hosts of quarantine concern in Florida: *Severinia buxifolia* and *Swinglea glutinosa*



QUESTIONS?

Wrap-up

Thank you!



CRDF is proud to provide support to the Florida citrus industry