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# CARIBBEAN FOOD CROPS SOCIETY

## 46

**Forty Sixth  
Annual Meeting 2010**

**Boca Chica, Dominican Republic  
Vol. XLVI – Number 2  
T-STAR Invasive Species Symposium**

PROCEEDINGS  
OF THE  
46<sup>th</sup> ANNUAL MEETING

Caribbean Food Crops Society  
46<sup>th</sup> Annual Meeting  
July 11-17, 2010

Hotel Oasis Hamaca  
Boca Chica, Dominican Republic

**“Protected agriculture: a technological option for competitiveness of the Caribbean”**

**“Agricultura bajo ambiente protegido: una opción tecnológica para la competitividad en el Caribe”**

**“Agriculture sous ambiance protégée: une option technologique pour la compétitivité de las Caraïbe”**

**United States Department of Agriculture,  
T-STAR Sponsored Invasive Species Symposium**

**Toward a Collective Safeguarding System for the Greater Caribbean Region:  
Assessing Accomplishments since the first Symposium in Grenada (2003)  
and Coping with Current Threats to the Region**

**Special Symposium Edition  
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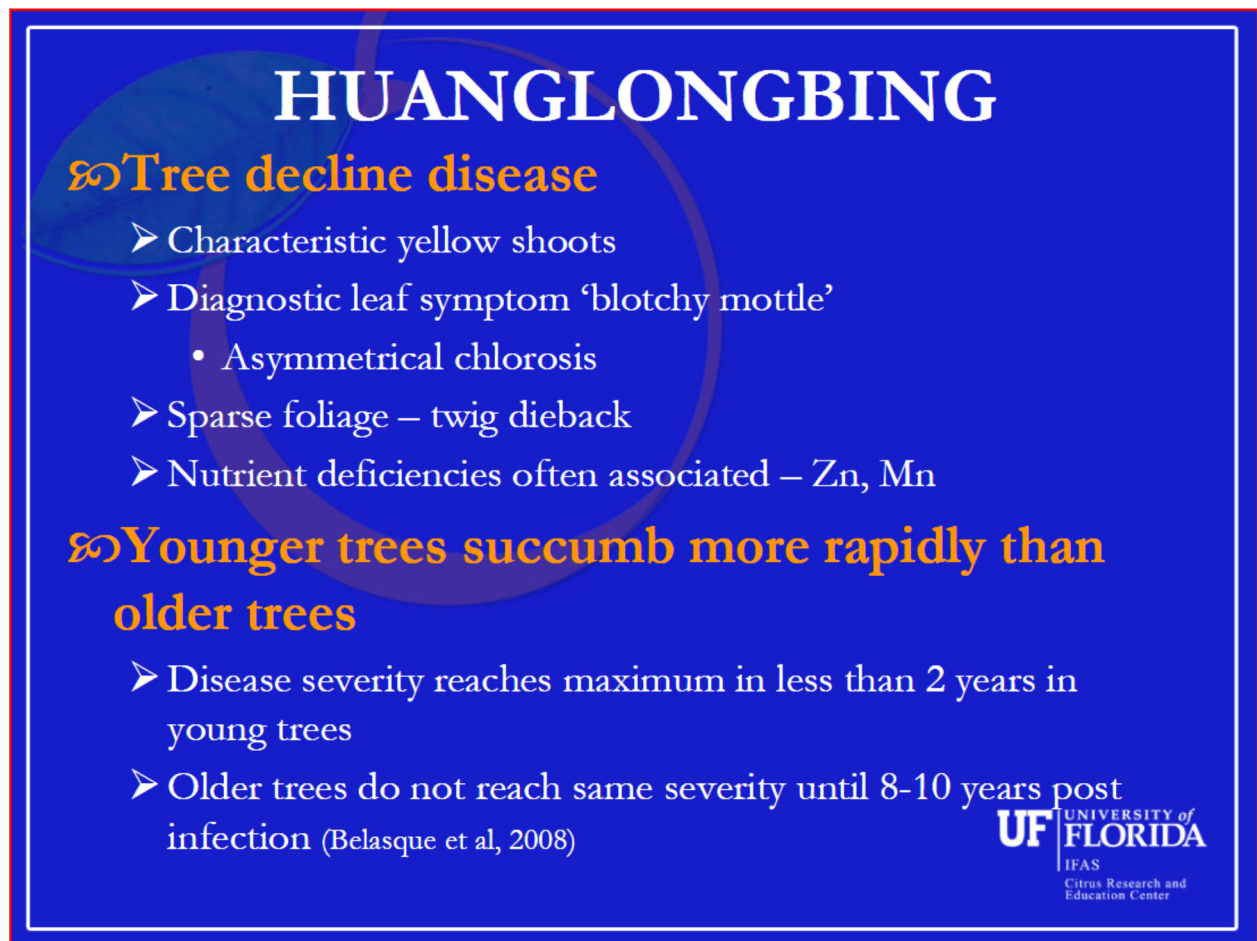
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**SESSION 3: CITRUS GREENING- ADVANCES IN RESEARCH AND PRACTICAL EFFORTS TO PREVENT, MITIGATE AND CONTAIN THE DISEASE**

**CITRUS GREENING DISEASE: THE PATHOGEN, THE DISEASE, ITS TRANSMISSION AND HORTICULTURAL APPROACHES TO MITIGATING REDUCTIONS OF YIELD AND QUALITY**

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# HUANGLONGBING

## ☞ Tree decline disease

- Characteristic yellow shoots
- Diagnostic leaf symptom ‘blotchy mottle’
  - Asymmetrical chlorosis
- Sparse foliage – twig dieback
- Nutrient deficiencies often associated – Zn, Mn

## ☞ Younger trees succumb more rapidly than older trees

- Disease severity reaches maximum in less than 2 years in young trees
- Older trees do not reach same severity until 8-10 years post infection (Belasque et al, 2008)

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Figure 1. Responses of citrus trees to infection with *Candidatus Liberibacter asiaticus*, causal agent of huanglongbing, or citrus greening disease in Florida.

# Introduction

- ⌘ HLB infection results in small, misshapen, lopsided fruit that drop prematurely
- ⌘ Juice from fruit displaying these symptoms is similar in quality to juice from less mature fruit (Dagulo *et al.* 2010)
- ⌘ Groves can become unproductive in as little as 2-4 years (Ke *et al.* 1988)
- ⌘ Yield can be reduced by 10 to 80% depending on percent of canopy affected (Bassanezi *et al.* 2006)
- ⌘ What happens to yield and quality over time when trees are “managed”?



Figure 2. HLB-infection of citrus trees can have devastating effects on the economic viability of citrus operations; however, a key question is whether trees can be managed in a manner to greatly mitigate the detrimental effects of the disease.

# Methods

- ⌘ 10 HLB and 10 healthy trees harvested
- ⌘ Fruit sized, counted and weighed
- ⌘ 1 sack of small fruit and 1 sack of “average size” fruit sampled from each tree
  - °Brix, acid, ratio and color
- ⌘ Yield per tree – pieces of fruit and total weight



Figure 3. Replicated studies were conducted to gain a quantitative understanding of the effects of HLB infection on fruit size and yield per tree as well as on juice quantity and quality.

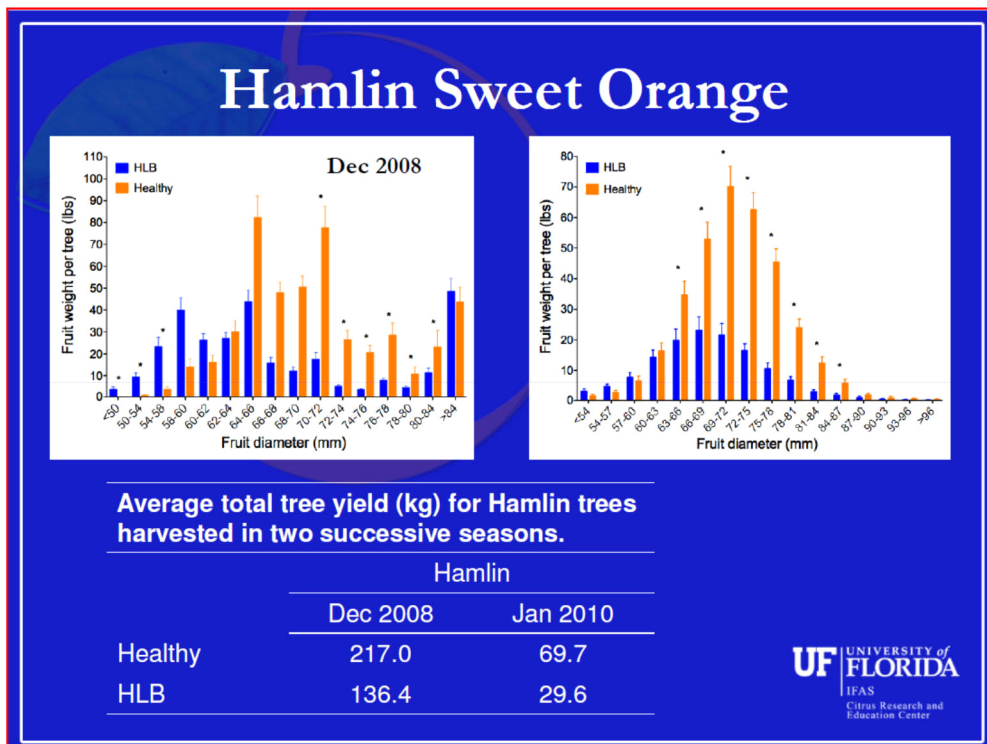




Figure 4. HLB infection has a strikingly great impact on Hamlin sweet orange fruit yield.

## HLB Fruit Effects

- ☞ **Fruit are small and misshaped**
  - Lopsided fruit
  - Center axis curved
  - Seeds often aborted
  - Abnormal color break – orange on peduncle end first
- ☞ **Affected fruit often fall from the tree before harvest**
  - Staining of vascular bundle below peduncle
- ☞ **Off-flavors in affected fruit**
  - Similar to less mature fruit

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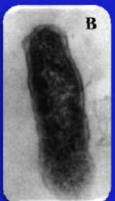
Figure 5. Effects of huanglongbing disease on citrus fruit.

Hamlin Juice Analysis						
December 2008 Hamlin juice quality parameters						
		°Brix	Acid	Ratio	Color	Juice Yield (ml/fruit)
HLB	Small	9.25 c	0.95 a	9.87 b	34.38 c	29.45 d
	Average	10.98 a	0.70 b	15.65 a	35.47 b	75.49 b
Healthy	Small	10.37 b	0.67 bc	15.73 a	36.11 a	47.05 c
	Average	9.58 c	0.64 c	14.97 a	36.32 a	92.75 a
January 2010 Hamlin juice quality parameters						
		°Brix	Acid	Ratio	Color	Juice Yield (ml/kg)
HLB	Small	11.27	0.57 a	19.88 ab	35.44 ab	336.1 b
	Average	10.88	0.57 a	19.13 b	35.13 b	368.0 a
Healthy	Small	11.54	0.54 ab	21.60 a	35.63 a	382.6 a
	Average	11.27	0.52 b	21.65 a	35.55 ab	387.5 a

Figure 6. This slide shows the properties of juice from small- and average-sized Hamlin fruits harvested in December 2008 and January 2010 (the acidity of juice from small-sized fruits from infected trees tends to be more acidic than that of average-sized fruits regardless of the disease status of the tree, and the yield of juice from fruits from infected trees is greatly reduced).

### 3 Species of Gram Negative Bacteria Cause HLB

- Ca. Liberibacter asiaticus (Las)**
  - only known species in Florida
- Vectored by 2 psyllid species**
  - Diaphorina citri* and *Trioza erytreae* (experimentally)
- Heat tolerant**
- Found on the Indian Subcontinent, Southeast Asia, Arabian peninsula, Brazil, Louisiana and Florida**






Figure 7. Three *Candidatus Liberibacter* species cause huanglongbing, but only *Ca. L. asiaticus* has reached Florida. The asian citrus psyllid, *Diaphorina citri* is the vector in Florida, and the African citrus psyllid, *Trioza erytreae*, does not occur in the Western Hemisphere. *Ca. L. asiaticus*, a fastidious, heat tolerant bacterium, is found on the Indian Subcontinent, Arabian peninsula, Southeast Asia, Brazil, Florida, and Louisiana.



# Causal Agent Can Be Ephemeral

## ⌘ Historically not understood to be bacteria

- Originally thought to be caused by nutrient deficiencies, nematodes, viruses and mycoplasmas (phytoplasmas)

## ⌘ Las is unevenly distributed throughout tree

- As expected found in phloem dense tissues
- In single tree, distribution patchy
  - Asymptomatic branches no bacteria were found
  - Symptomatic leaves up to  $10^7$  bacteria/gram of tissue

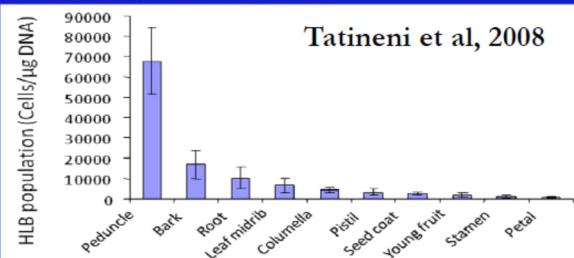


Figure 8. The determination of the causal organism of huanglongbing, *Candidatus Liberibacter* spp., has been very difficult, since the symptoms have much in common with nutrient deficiencies, and infections of nematodes, viruses, and mycoplasmas (phytoplasmas). The *Liberibacter* bacteria are found in the phloem of dense tissues. The distribution of infected phloem is patch. By far the highest concentrations of the bacteria are found in the peduncles, with much lower concentrations in the bark, roots, midribs of leaves, and columnella, the central column-like structure found in the citrus fruit.

# Ca. Liberibacter asiaticus genome sequenced

☞ **Original sequence from psyllid**

- Confirmatory sequence from citrus phloem
  - Only organism with complete sequence found in phloem
  - As close to Koch's postulates without culture
  - Estimate of 1.7 cells / phloem cell in sample

☞ **Circular 1.23 Mb circular genome**

- Confirmed part of  $\alpha$ -proteobacteriaceae
- Closest relatives in the Rhizobiaceae
- Lacking Type III and Type IV secretion systems

Duan et al., 2009 and Tyler et al., 2009



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Figure 9. The sequence of DNA of *Candidatus* Liberibacter asiaticus isolated from the Asian citrus psyllid was determined and a confirmatory DNA sequence was obtained by analyzing the DNA of the bacterium present in the citrus phloem. *Ca. L. asiaticus* has a 1.23 MB circular genome. The taxonomic position of this organism is as follows: Kingdom: Monera; Phylum: Proteobacteria; Class: Alphaproteobacteria; Order: Rhizobiales; Family: Rhizobiaceae.

# Pathogen-vector interaction

☞ **Why continue to study basic psyllid / pathogen interaction given the large amount of literature?**

☞ **The published “FACTS WE KNOW” include:**

- Psyllids quickly acquire and transmit HLB
- Once a psyllid is infected it is always infected
- No discernable effects of pathogen on psyllid
- Etc...



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Figure 10. Previous studies concluded the following: (i) psyllids quickly acquire and transmit *Candidatus* Liberibacter asiaticus; (ii) infected psyllids never become free of the bacterium, and (iii) *Candidatus* Liberibacter asiaticus has no adverse effects on the vigor, life span or reproductive capacity of the psyllid, *Diaphorina citri*. However, these findings are incorrect.

# Pathogen-vector interaction

Acquisition of Las by ACP greatest when reared on infected plant

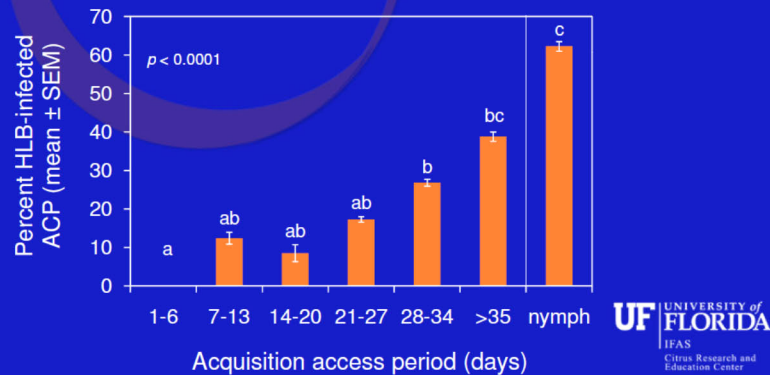


Figure 11. Asian citrus psyllids that develop on an infected citrus tree acquire much more *Candidatus Liberibacter asiaticus* bacteria than those that first encounter infected tree as adults; further the immature psyllids pick up progressively more bacteria with the passage of time.

## Psyllid retention of HLB pathogen

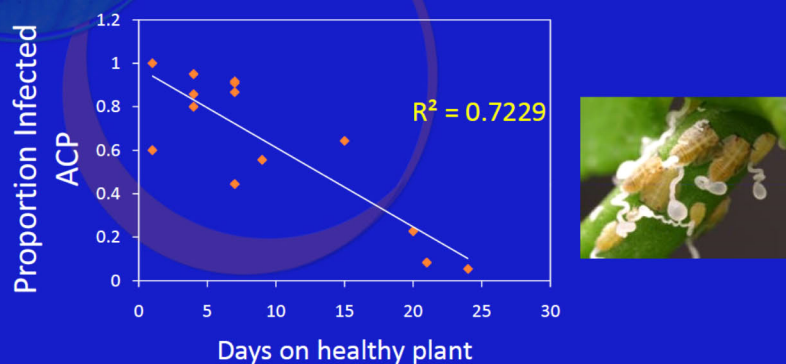


Figure 12. The retention of *Candidatus Liberibacter asiaticus* by infected psyllids that have transferred to an uninfected tree decreases steadily over about four weeks.

# Does the HLB pathogen affect fitness of psyllids?

Newly-emerged healthy and infected ACP placed on HLB- plants

Assessed:

☞ Egg production

☞ Adult survival

☞ Nymphal survival

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Figure 13. We assessed the egg production, nymphal survival, and adult survival of newly emerged healthy Asian citrus psyllids on uninfected citrus trees, and the newly emerged psyllids placed on HLB-infected trees.

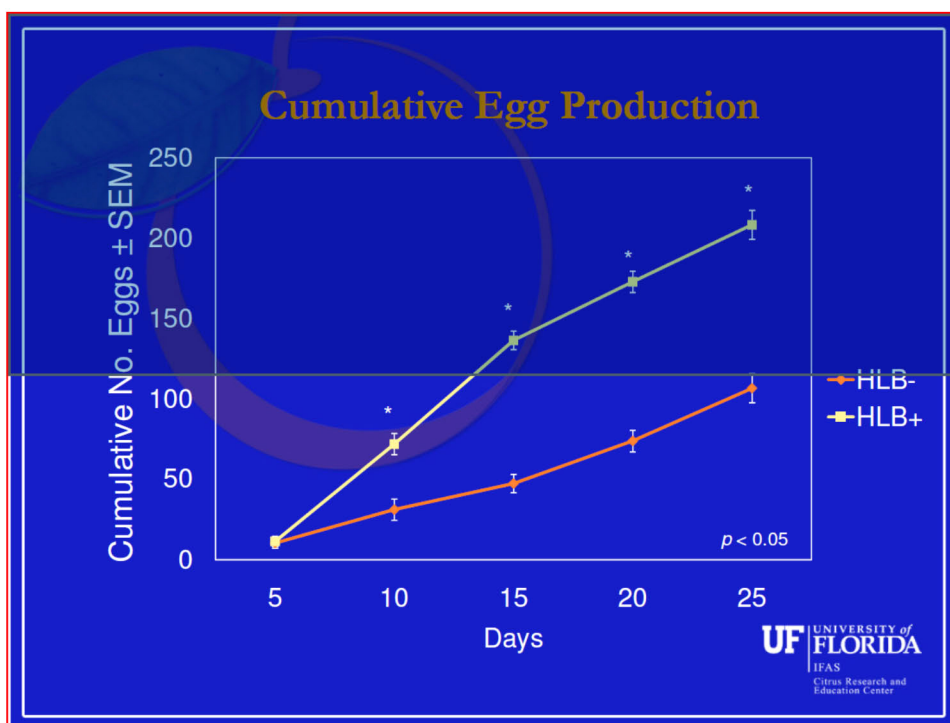


Figure 14. Surprisingly, HLB-infected Asian Citrus Psyllids deposit about two-fold more eggs over their life spans than uninfected psyllids.



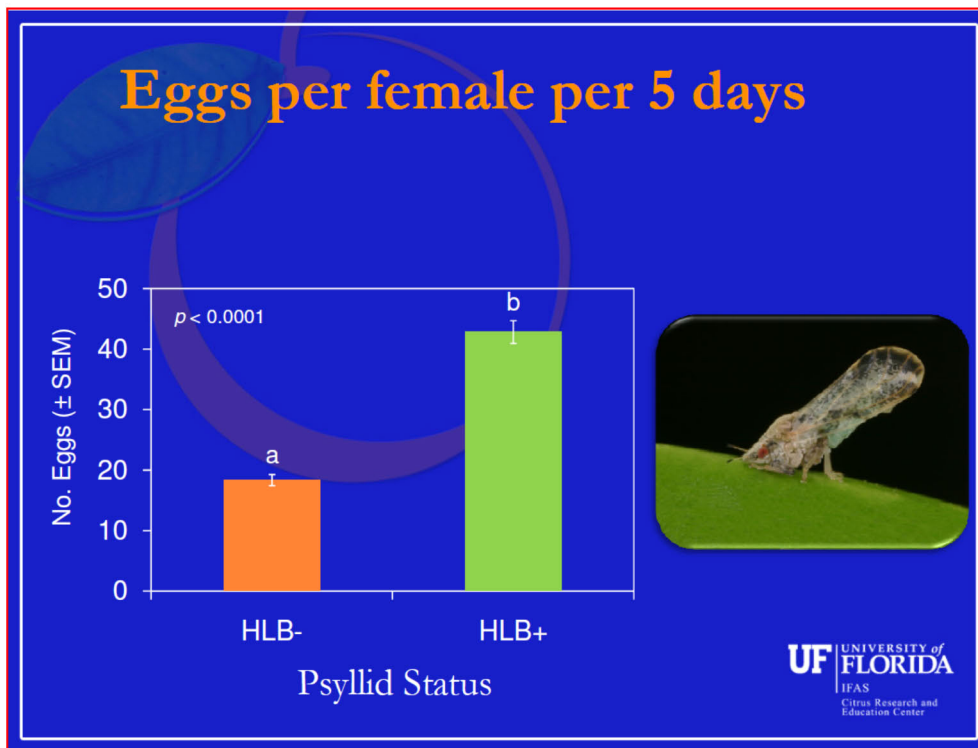


Figure 15. Indeed, HLB-infected Asian Citrus Psyllids deposit more than two-fold more eggs during a five-day period of peak egg laying than uninfected psyllids.

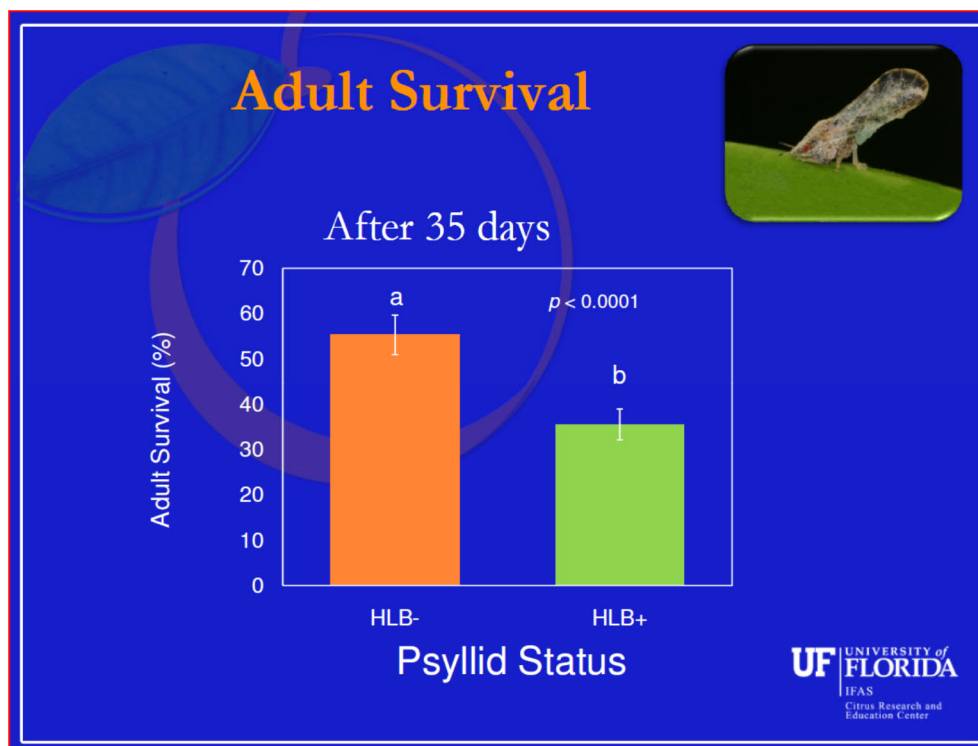


Figure 16. The percent survival of uninfected adult Asian citrus psyllids through 35 days is substantially greater than the survival of infected adult psyllids.

## Nymphal survival

### Preliminary results:

- No differences in the number of nymphs emerged from HLB+ and HLB- ACP
- Work ongoing



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Figure 17. Survival of HLB-infected nymphs has proven to be no different from that of HLB-free nymphs.

## Conclusions

- ⌘ We didn't really know what we thought we knew!
- ⌘ Acquisition greatest by nymphs
- ⌘ The "fact" that adults retain pathogen their entire lives is not necessarily true
- ⌘ Presence of HLB+ trees will increase the likelihood of HLB+ psyllids (continued feeding)
- ⌘ HLB pathogen does affect fitness of adult psyllids: increased egg laying, shorter lifespan

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Figure 18. Much of the information in the scientific literature is unreliable. The acquisition of the bacterium from infected trees is greater by nymphs than by adult psyllids. Not all adults retain the bacterium for the duration of their lives. The presence of the bacterium in the phloem increases the probability of prolonged feeding by the psyllids. The bacterium causes psyllids to increase the number of eggs laid and it shortens their lifespans.

## Conclusions

- ⌘ **HLB infected trees have more smaller fruit**
- ⌘ **Only small, symptomatic fruit show quality changes**
  - Similar to immature fruit
- ⌘ **Due to normal year-to-year variation in fruit sizes and yield it is too early to know for sure the long term effects**



Figure 19. HLB infection causes citrus trees to increase the proportion of small fruit produced. It is the small symptomatic fruits that undergo unequal ripening and have poor or unacceptable quality. The long-term effects of HLB infection on fruit quality and yield have not been characterized quantitatively.

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Figure 20. We are grateful to the Florida Citrus Production Research Advisory Council and to many colleagues without whose assistance this study could not have been undertaken.