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46th Annual Meeting
July 11-17, 2010

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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

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CITRUS GREENING DISEASE IN BRAZIL: ADVANCES IN RESEARCH AND CONTROL

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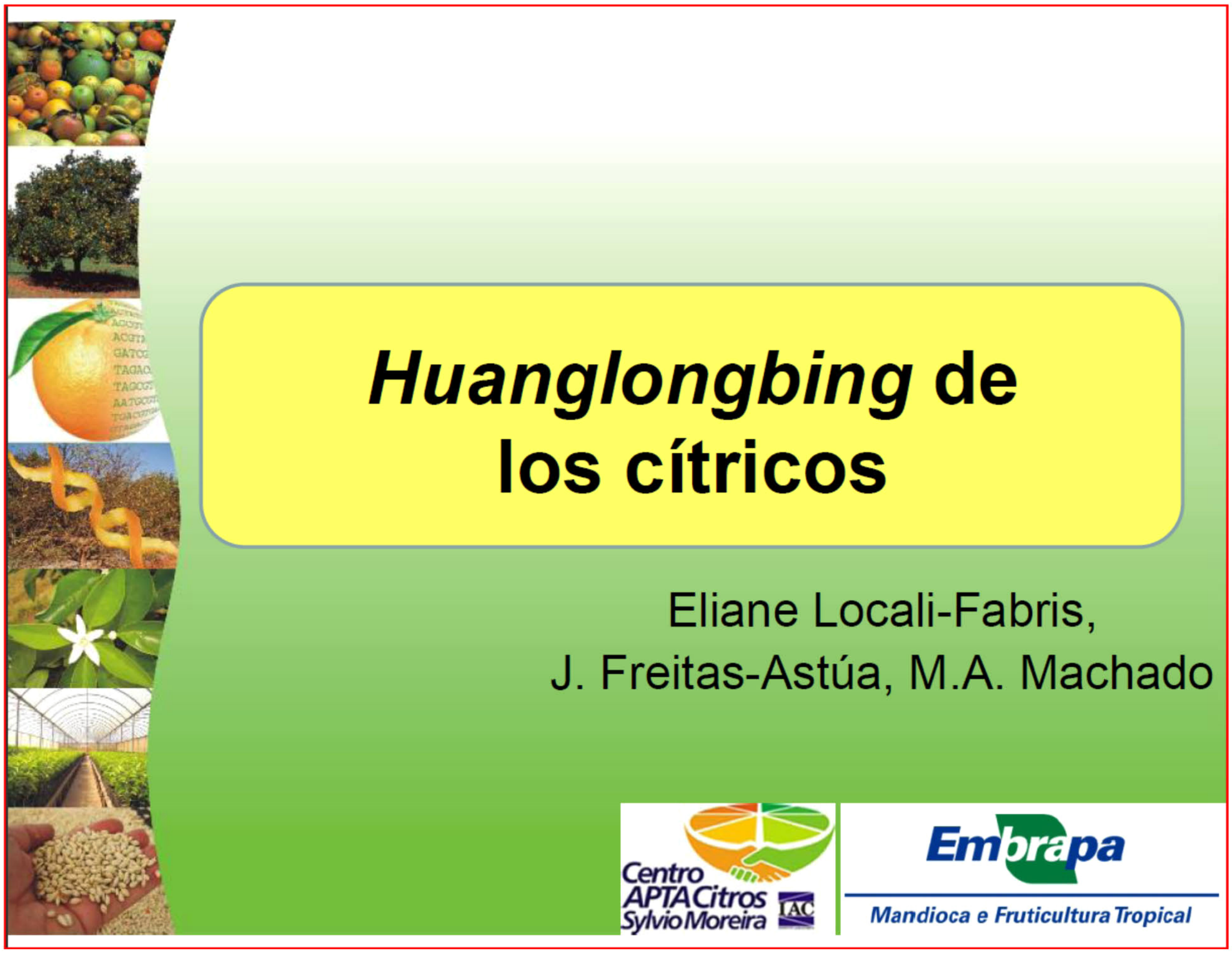


Figure 1. Symptoms of huanglongbing (HLB) were reported in São Paulo State (SPS), Brazil, in March 2004. Leaves with blotchy mottle symptoms characteristic of HLB were sampled in several farms of SPS and tested for the presence of liberibacters. '*Ca.* L. asiaticus' was detected in a small number of samples with PCR. This liberibacter was restricted to the sieve tubes of the citrus host, and also found was *Diaphorina citri*, the psyllid vector of '*Ca.* L. asiaticus'. Elimination of symptomatic trees has been mandatory since 2005, one year after the first report of HLB in the State, but has not been adopted rigorously by all farmers. Consequently, the disease has spread and today is present in all commercial citrus growing regions of SP. The last of a series of field surveys carried out in 2009 indicates that HLB is present in 24% of the 96 thousand blocks, and affects 0.87% of the estimated 214 million trees growing in the State. The Center and South of SP are the most affected regions with higher rates of disease progress.

Figure 2. Peaceful co-existence with this disease seems impossible. The citrus industry is at risk.

Figure 3. Damage includes dropping of the leaves and fruit, reduction in the productivity and quality of the fruit, general decline of the tree, and death.

Figure 4.HLB is a rapidly spreading disease. Thus it is imperative to quickly and enduringly reduce the inoculum. Neighboring growers need to take a collective security approach. It is imperative to use only uninfected propagative materials. Inspection is essential!

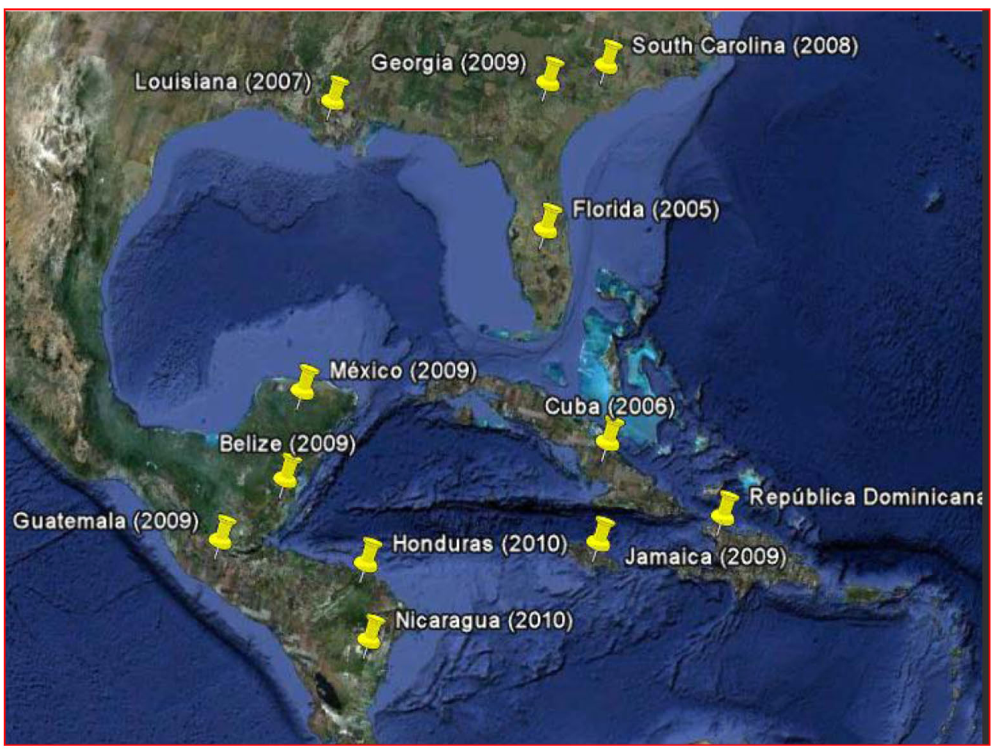


Figure 5. Originally from southeast Asia, HLB appeared in Brazil in 2004, it has been found in Florida (2005), Cuba (2006), Louisiana (2007), Dominican Republic (2008) Belize (2009), Guatemala (2009), Mexico (2009), Honduras (2010) and Nicaragua (2010).

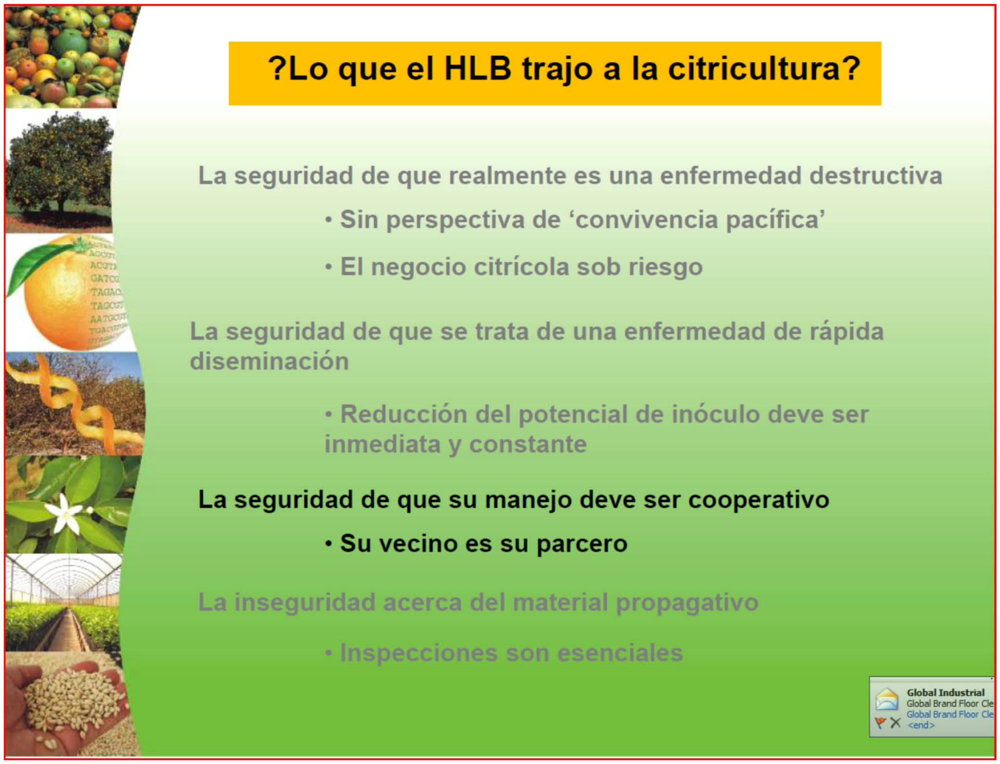


Figure 6. Security in managing HLB requires cooperation among growers. Each grower must regard his neighbor as his partner.

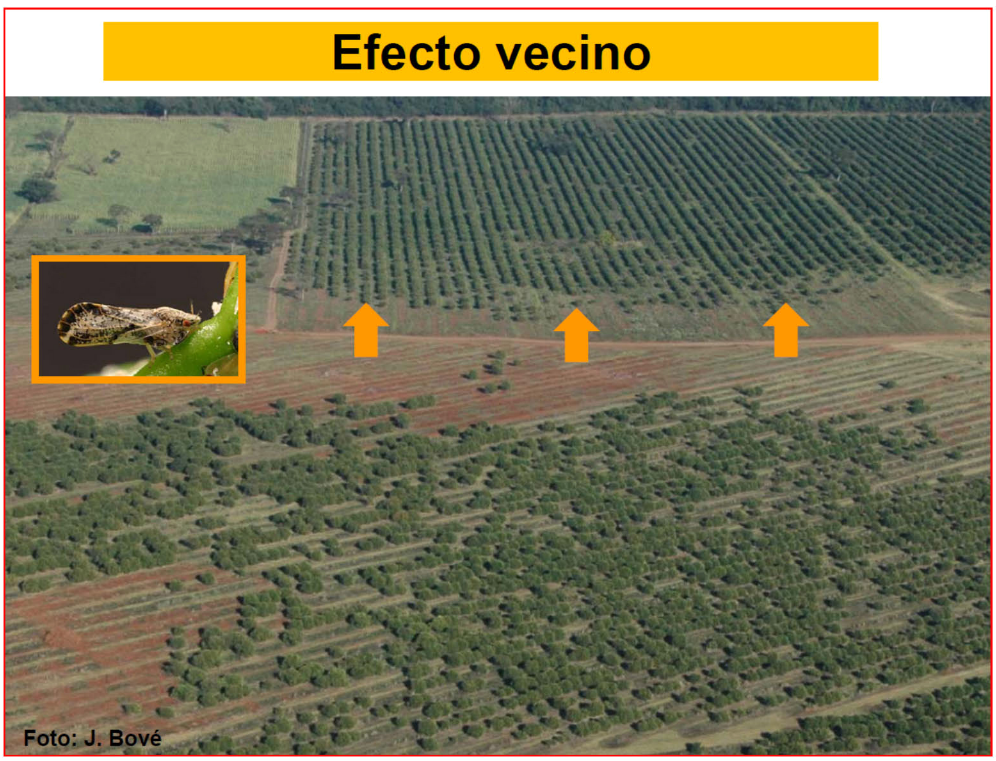


Figure 7. This figure depicts the effects that the practices of one grower can have on a neighboring grower. In the citrus grove at the bottom of the figure, HLB is not being controlled effectively and the contagion is clearly spreading into the grove at the top of the figure.

Figure 8. Security against HLB concerning propagative material requires inspection.

Figure 9. Budwood, rootstock, and other citrus propagative material must be in quarantine greenhouses.

Figure 10. Propagative material grown out-of-doors is readily accessible to the Asian citrus psyllid, the vector of *Candidatus* Liberibacter sp. Hence there is a high risk that propagative material grown out-of-doors has been infected.

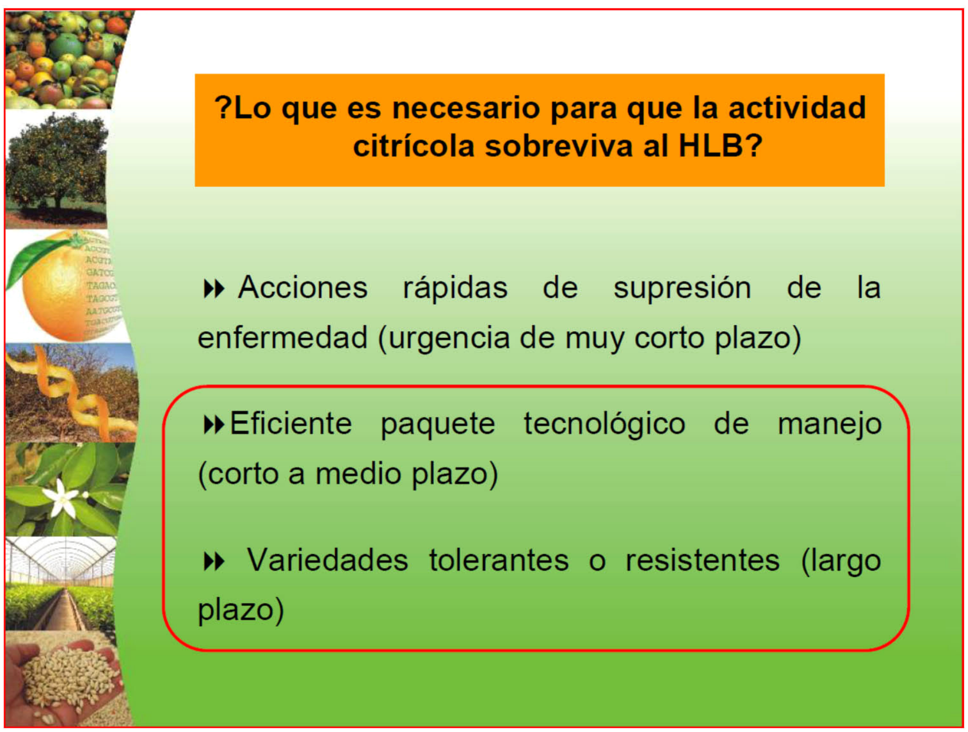


Figure 11. What is necessary for citrus to survive HLB?: (1) Rapid actions to suppress the disease to meet the short-term emergency, (2) a technological package for efficient disease management over the short to medium term, and (3) tolerant or resistant varieties in the long term.

Figure 12. The tetrahedron of the illness: (1) change the production inputs, (2) control the vector, (3) control the bacteria with antibiotics and inoculum reduction, and (4) select or develop disease resistant citrus plants. Always begin with non-infected plantlets, eliminate symptomatic plants, and control the vector.

Figure 13. Knowledge is needed for management of the disease; thus investigations are needed.

Figure 14. Objective: Develop alternative management strategies that are more efficient, less costly, and least harmful to the environment.

Figure 15. Etiology of HLB disease of citrus. Three species of *Candidatus* Liberibacter can be involved. Complex interactions involving possible new vectors, etc. present the biggest challenge.

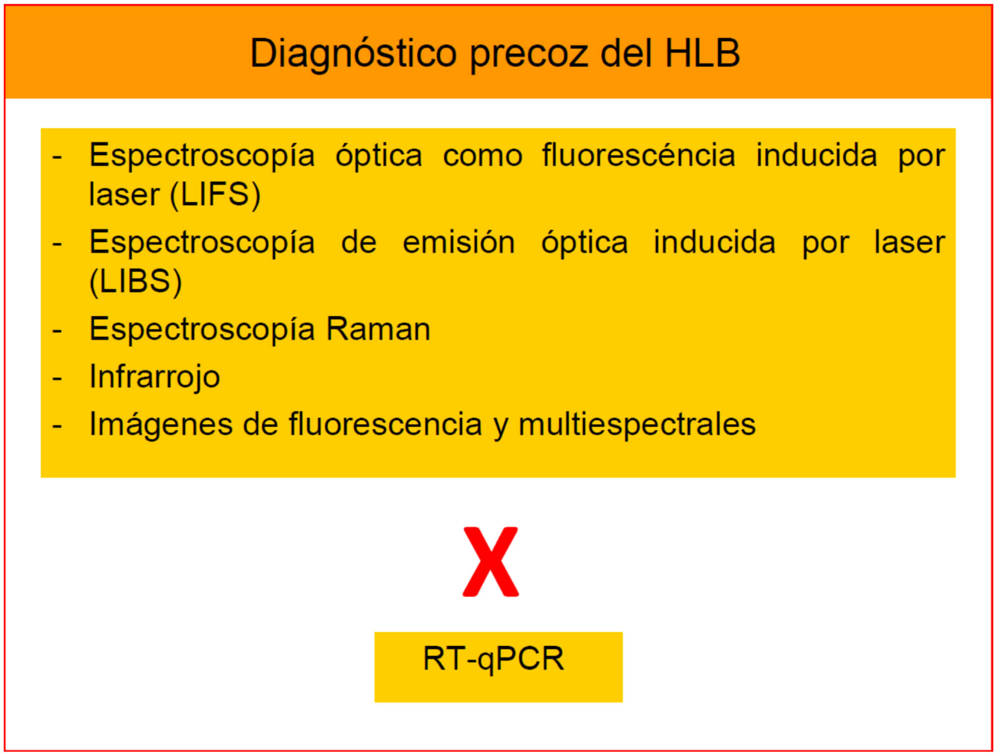


Figure 16. Early diagnosis of HLB: optical spectroscopy with laser-induced fluoresence; optical laser-induced emission spectroscopy; Raman spectroscopy; and infrared, fluorescent, and multispectral imaging X RT – qPCR.

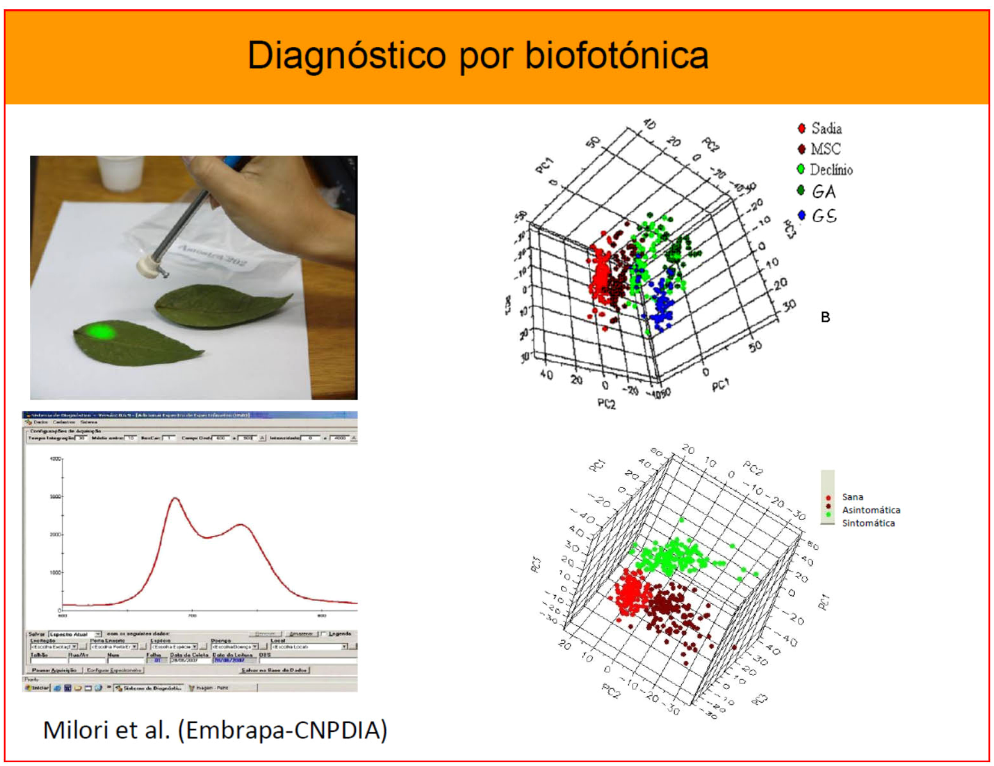


Figure 17. Diagnosis by biophotonics.

Figure 18. Control of Liberibacter by means of transgenic citrus. Various genes that confer resistance against different bacteria have been identified in rice. 'Valencia' has been genetically transformed.

Figure 19. Antimicrobial microbes such as the antimicrobial peptide Attacin, which suppresses gram-negative bacteria, have been isolated from diverse insects, including *Hyalophora cercropia, Trichoplusia ni*, and *Drosophila melanogaster*. Enhanced resistance against Erwinia amylovora in genetically modified apples. Reduction in the number of canker lesions in genetically modified orange.

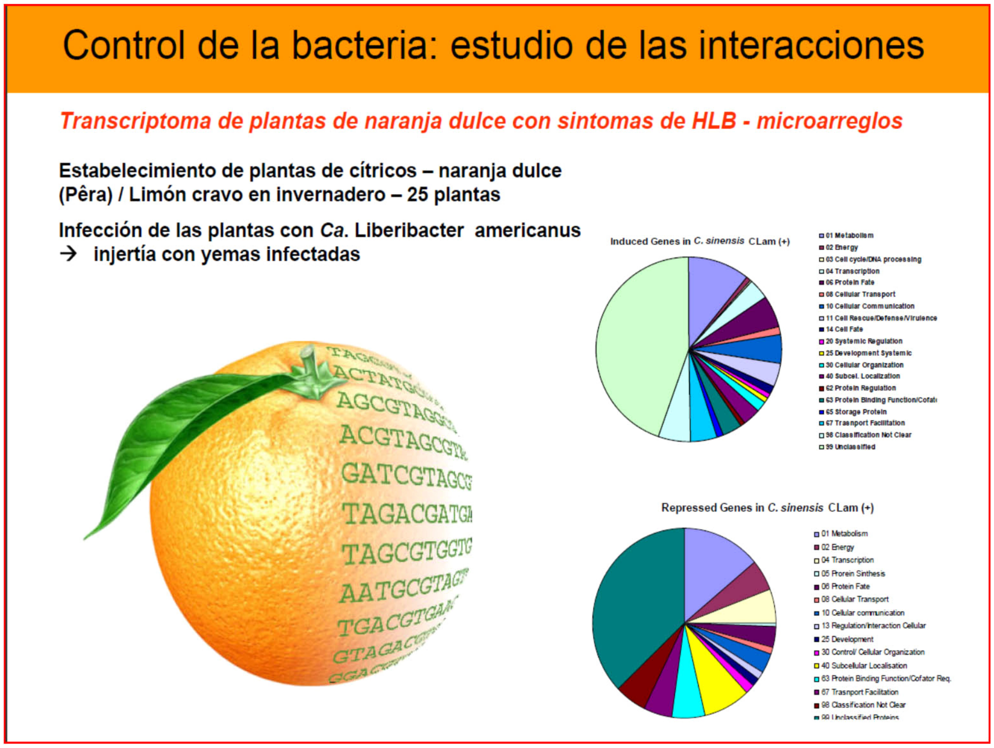


Figure 20. Control of bacteria: study of interactions with focus on transcription of citrus plants — sweet orange with HLB symptoms — microarrays.

Control de la bacteria: estudio de las interacciones

Estabelecimiento de plantas de cítricos – naranja dulce (Pêra) / Limón cravo em invernadero – 54 plantas (27CLam/27CLas)

Infección de las plantas con *Ca.* Liberibacter spp.→ injertía con yemas infectadas

Cítricos especies					Coletas			
	CLam	CLas	Poda	Infección	48h	1 sem	1 mes	Síntomas
Hamlin	08	08	06/09	07/09	ok	ok	ok	
Ponkan	08	08	08/09	09/09	ok	ok	ok	
Trifoliata	08	08	08/09	09/09	ok	ok	ok	
Azeda	08	08	08/09	09/09	ok	ok	ok	
Tahiti	13	13	05/09	07/09	ok	ok	ok	
Galego	08	08	06/09	07/09	ok	ok	ok	
Cravo	13	13	05/09	07/09	ok	ok	ok	
Lima doce	09	10	06/09	07/09	ok	ok	ok	
Sunki	08	08	06/09	07/09	ok	ok	ok	

Especies infectadas

Figure 21. Control of bacteria: Study of interactions of nine GM orange cultivars injected with *Candidatus* Liberibacter spp.

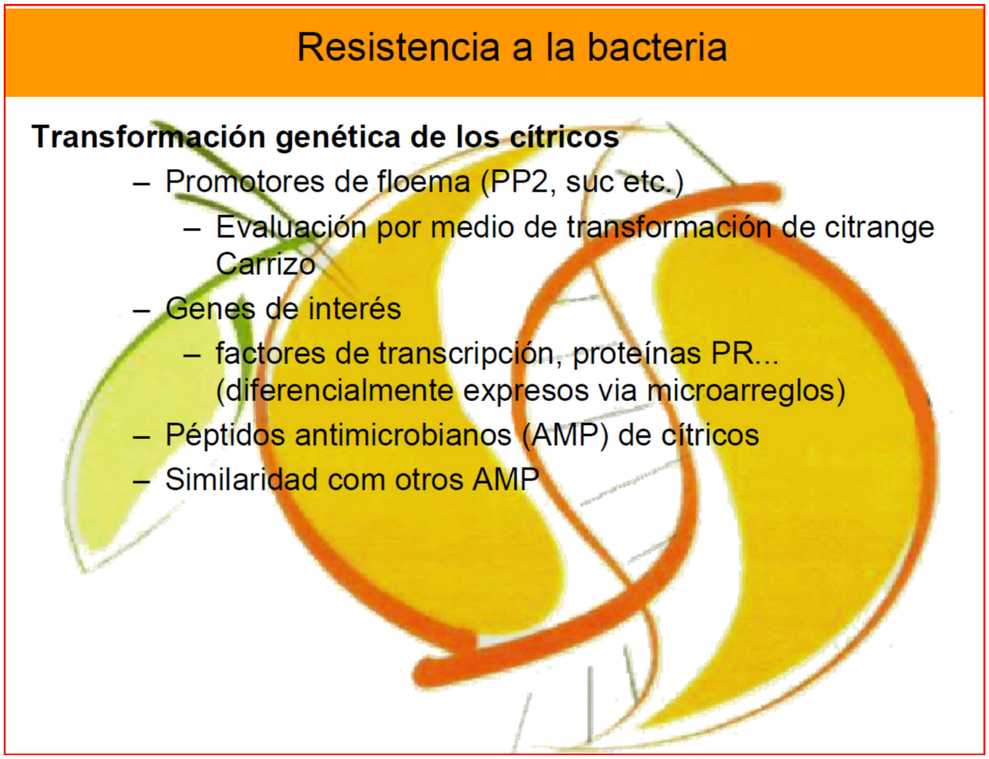


Figure 22. Resistance against bacteria. Genetic transformation of citrus using phloem promoters.

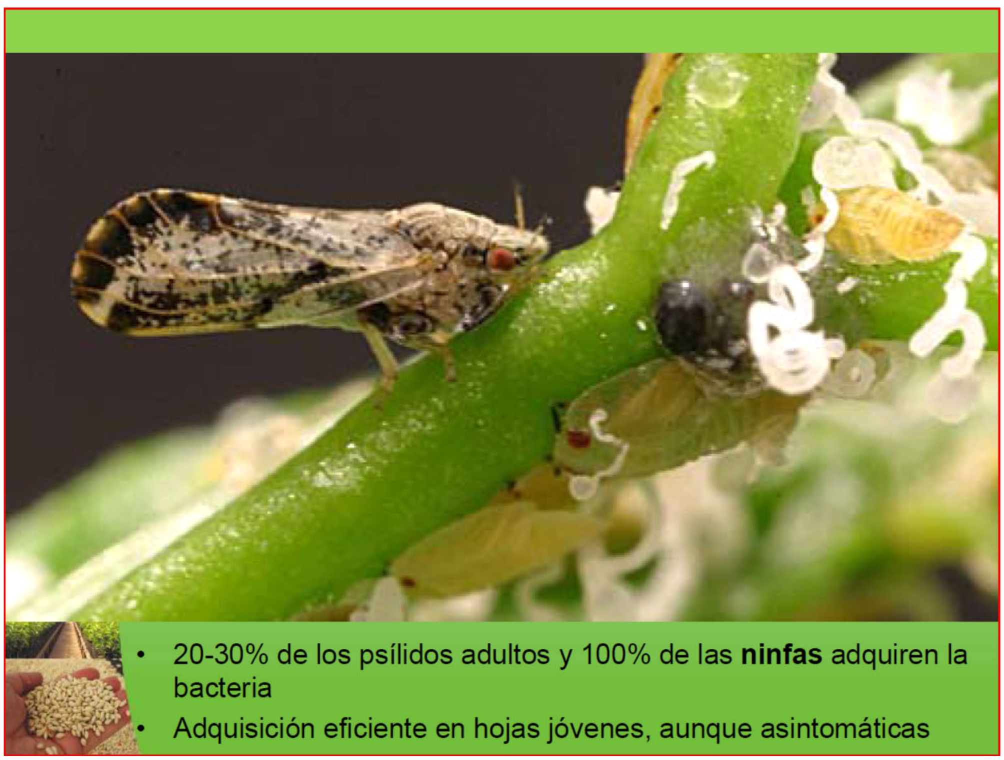


Figure 23. It is known that 20–30% of psyllid adults and 100% of nymphs acquire the bacteria. Efficient acquisition from young leaves, although asymptomatic.

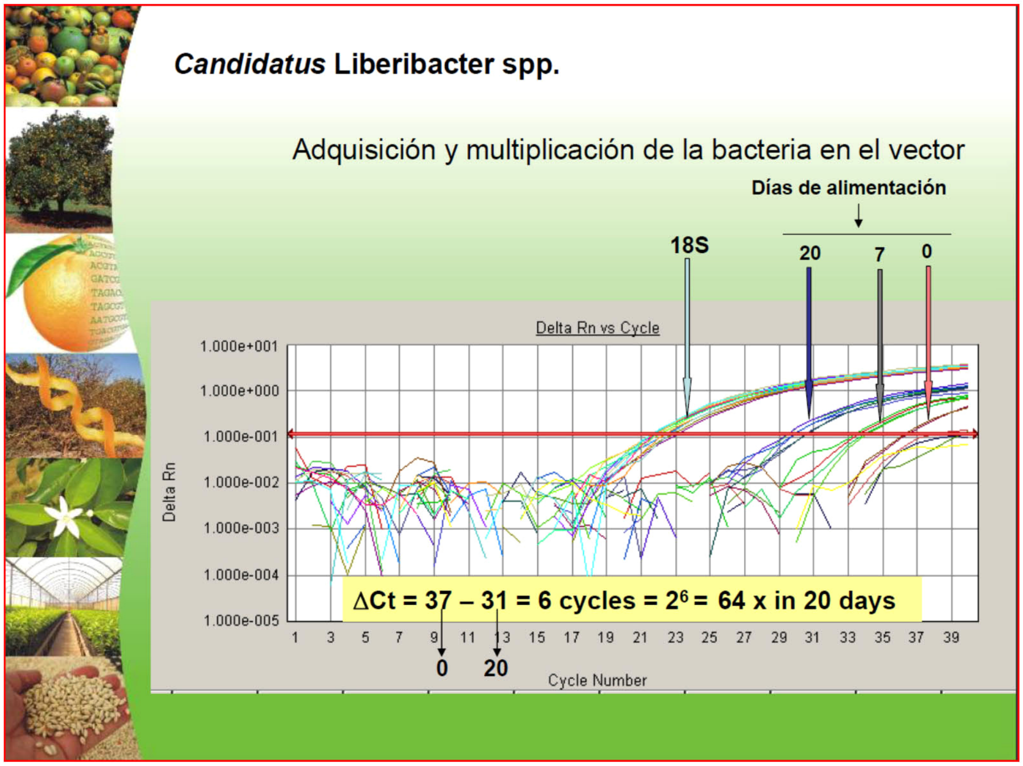
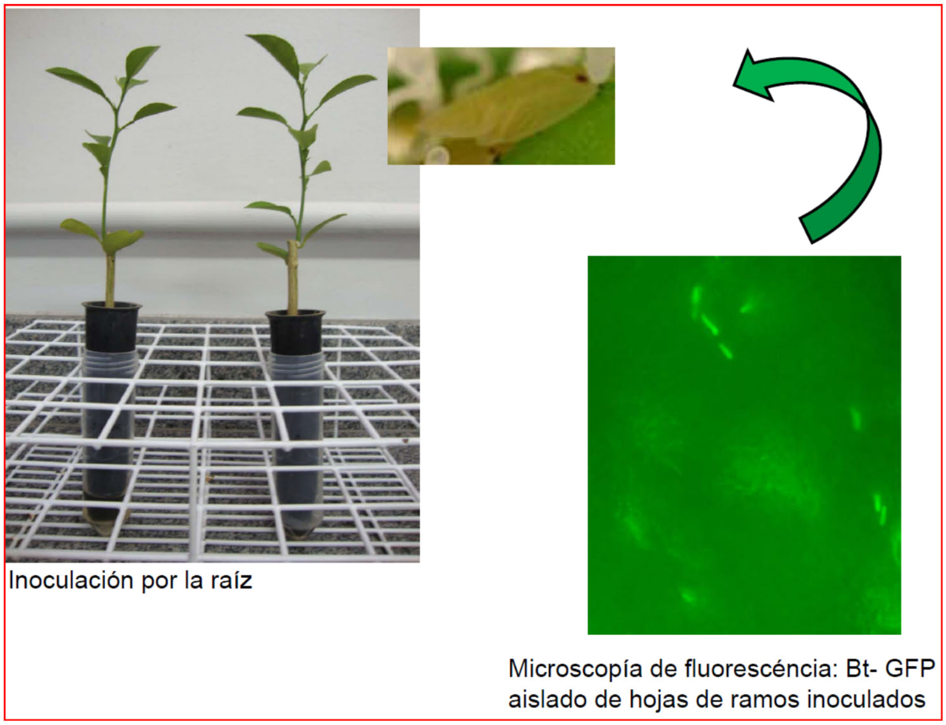


Figure 24. Acquisition and multiplication of *Candidatus* Liberibacter spp. in the vector, *Diaphorina citri*.

Figure 25. Control of the HLB vector: *D. citri*. *Bacillus thuringiensis* peptides are used to control *D. citri*. The gene that codes for the Bt peptide toxin has been cloned and used to transform citus. However, the project is still in the initial phase.



Figures 26. Citris seedlings are being inoculated through their roots. Fluorescent microscopy of Bt-GFP in isolated palm leaves.

Figure 27. Increasing resistance of the host plant. Increase systemic acquired resistance (SAR). When SAR-inducers (actigard, salicylic acid, isonicotinic acid, and imidacloprid) were applied to citrus plants, they were without effect against HLB. An experiment is under way to augment systemic-acquired resistance by means of a SAR regulator from *Arabidopsis*.

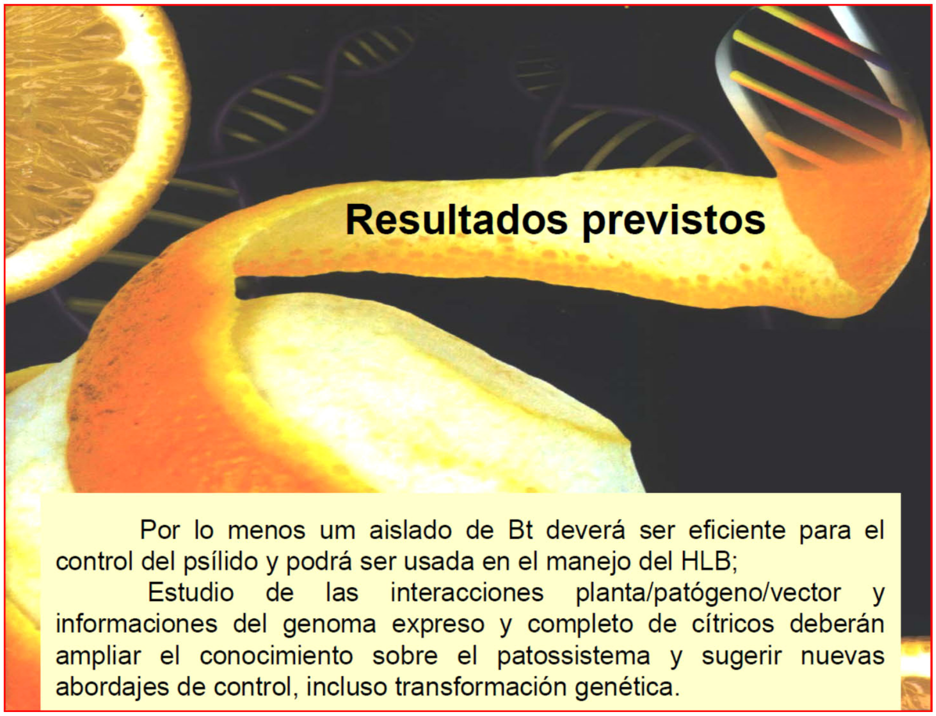


Figure 28. Results obtained: One isolate of Bt provides very efficient control of the psyllid, and it will be used to manage HLB. The study of plant/pathogen/vector, with information on gene expression and knowledge about the pathosystems, has provided new approaches to HLB control, including use of genetic transformation.

Figure 29. Many thanks for your attention!