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R&D in Agriculture
A Bulletin on Information Resources

Citrus Greening / Huanglongbing

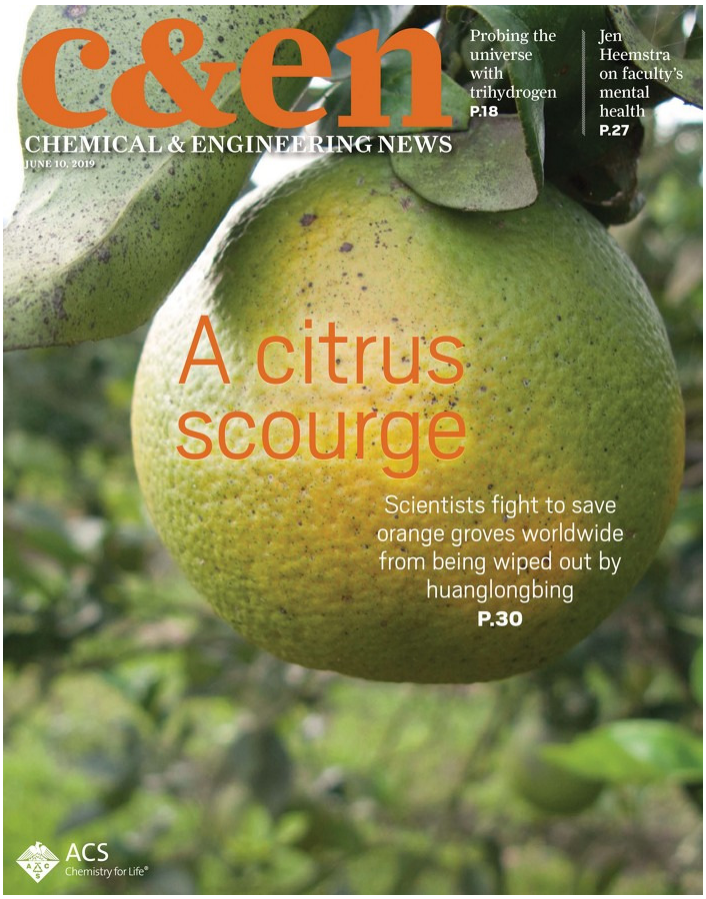

Photo Source: **Chemical & Engineering News (C&EN), American Chemical Society 97 (23).** Cover image: A citrus fruit infected with huanglongbin. Credit: Shutterstock ***https://cen.acs.org/magazine/97/09723.html***

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R&D in Agriculture: a bulletin on information resources, July 2019 issue
Theme: CITRUS GREENING / HUANGLONGBING

CONTENT

GENERAL INFORMATION - INTERNATIONAL

CABI The Invasive Species Compendium (ISC)
www.cabi.org/isc
An encyclopedic resource that brings together a wide range of different types of science-based information to support decision-making in invasive species management worldwide.

- **Citrus huanglongbing (greening) disease (citrus greening) Datasheet**

  CABI, 2019. C*itrus huanglongbing (greening) disease (citrus greening)* In: Invasive Species Compendium. Wallingford, UK: CAB International. Last modified 04 June 2019
  Partial Contents: Identity, Description, Distribution, Hosts/ Species affected, Growth Stages, Symptoms, Biology and Ecology, Means of Movement and Dispersal, Vectors, Impact, Diagnosis, Detection and Inspection, Prevention and Control
  https://www.cabi.org/isc/datasheet/16567

EPPO Global Database
is maintained by the Secretariat of the European and Mediterranean Plant Protection Organization (EPPO). The aim of the database is to provide all pest-specific information that has been produced or collected by EPPO
https://gd.eppo.int/

- ***Liberibacter asiaticus*. Datasheet**

  EPPO (2019) *Liberibacter asiaticus*. EPPO datasheets on pests recommended for regulation. Available online. Last updated: 2019-03-21
  Partial Contents: Identity, Hosts, Geographical Distribution, Biology, Detection and Identification, Pathways for Movement, Pest Significance, Phytosanitary Measures
  https://gd.eppo.int/taxon/LIBEAS/datasheet

REVIEW OF RESEARCH

Citrus Greening is Killing the World's Orange Trees. Scientists are Racing to Help
Cici Zhang
2019. Chemical & Engineering News (C&EN), American Chemical Society 97 (23)
To save a billion-dollar industry from the infectious disease, also known as huanglongbing, researchers are turning to gene editing, RNA interference, and other advanced techniques
Contents

- Creating a better tree
- More immediate tools:
- Harnessing nature: So some scientists are fighting HLB by harnessing nature rather than engineering it. These researchers have their sights set on anti-CLas natural products.
- Prevention, prevention, prevention

https://cen.acs.org/biological-chemistry/biochemistry/Citrus-greening-killing-worlds-orange/97/i23

A Review of the Citrus Greening Research and Development Efforts Supported by the Citrus Research and Development Foundation: Fighting a Ravaging Disease
National Academies of Sciences, Engineering, and Medicine
2018. The National Academies Press, Washington, DC
Description
Huanglongbing (HLB) or citrus greening, first observed more than a hundred years ago in Asia, is the most serious disease threat to the citrus-growing industry worldwide due to its complexity, destructiveness, and incalcitrance to management. First detected in Florida in 2005, HLB is now widespread in the state and threatens the survival of the Florida citrus industry despite substantial allocation of research funds by Florida citrus growers and federal and state agencies.

As the HLB epidemic raged in 2008, Florida citrus growers began allocating funds for HLB research in hopes of finding short-, medium-, and long-term solutions. This effort created the Citrus Research and Development Foundation (CRDF), an organization with oversight responsibility for HLB research and development efforts in Florida. This report provides an independent review of the portfolio of research projects that have been or continue to be supported by the CRDF. It seeks to identify ways to retool HLB research—which, despite significantly increasing understanding of the factors involved in HLB, has produced no major breakthroughs in controlling the disease—and accelerate the development of durable tools and strategies that could help abate the damage caused by HLB and prevent the possible collapse of the Florida citrus industry.
Partial Contents
Summary https://www.nap.edu/read/25026/chapter/3
2 Current knowledge on Huanglongbing (HLB) and the interactions of the Pathogen, Vector and Host
3 HLB Research and Development Efforts
4 Notable Outcomes, Pitfalls and Future Directions
https://doi.org/10.17226/25026
https://www.nap.edu/read/25026/chapter/1#ii

HUANGLONGBING DISEASE PYRAMID

Tale of the Huanglongbing Disease Pyramid in the Context of the Citrus Microbiome
Nian Wang, Lukasz L. Stelinski, Kirsten S. Pelz-Stelinski, James H. Graham, and Yunzeng Zhang
2017. Phytopathology 2017 107:380-387
Abstract
The Huanglongbing (HLB) disease pyramid is composed of Liberibacters, psyllid vectors, citrus hosts, and the environment. The epidemiological outcomes for Liberibacter-associated plant diseases are collectively determined by the inherent relationships among plant−Liberibacters−psyllids, and how various environmental factors affect plant−Liberibacter−psyllid interactions. Citrus−Liberibacter−psyllid interactions occur in a complex microbiome system. In this review, we focus on the progress in understanding the HLB disease pyramid, and how the microbiome affects the HLB disease pyramid including the interaction between HLB and the citrus microbiome; the interaction between Liberibacters and psyllids; the interaction between Liberibacters and gut microbiota in psyllids; and the effect of HLB on selected above- and belowground citrus pathogens. Their implications for HLB management are also discussed.
Keywords: Asian citrus psyllid, Candidatus Liberibacter asiaticus, Citrus Greening
https://apsjournals.apsnet.org/doi/10.1094/PHYTO-12-16-0426-RVW

MANAGEMENT AND CONTROL

Developing Citrus Huanglongbing (HLB) Management Strategies based on the Severity of Symptoms in HLB-Endemic Citrus-Producing Regions

Jinyun Li, Lei Li, Zhiqian Pang, Vladimir G. Kolbasov, Reza Ehsani, Erica W. Carter, and Nian Wang

2019. Phytopathology 109:582-592

Abstract

Citrus Huanglongbing (HLB), also known as greening, is a destructive disease caused by the fastidious, phloem-colonizing bacteria *Candidatus* Liberibacter spp.; '*Ca*. Liberibacter asiaticus' (Las) is the most prevalent of the species causing HLB. The Asian citrus psyllid (ACP, *Diaphorina citri*) transmits Las. HLB is threatening citrus production worldwide, and there is no cure for infected trees. Management strategies targeting diseased trees at different stages of colonization by Las are needed for sustainable citrus production in HLB-endemic regions. We evaluated the effect of the combinations of plant defense elicitors, nitrogen (N) fertilizer, and compost on mildly diseased trees. We tested thermotherapy on severely diseased trees and assessed tree protectors to prevent feeding by ACP, thus preventing Las from being transmitted to new plantings that replaced HLB-moribund trees. After four applications over two consecutive growing seasons we found that the combination of compost, urea, and plant defense elicitors β-aminobutyric acid, plus ascorbic acid and potassium phosphite with or without salicylic acid, slowed down the progression of HLB and reduced disease severity by approximately 18%, compared with the untreated control. Our data showed no decline in fruit yield, indeed treatment resulted in a higher yield compared with the untreated control. Thermotherapy treatment (55°C for 2 min) exhibited a suppressive effect on growth of Las and progress of HLB in severely diseased trees for 2 to 3 months after treatment. The tree protectors prevented feeding by ACP, and therefore young replant trees remained healthy and free from infection by Las over the 2-year duration of the experiment. Taken together, these results may contribute to a basis for developing a targeted approach to control HLB based on stage of host colonization, application of plant defense elicitors, N fertilizer, compost, thermotherapy, and tree protectors. There is potential to implement these strategies in conjunction with other disease control measures to contribute to sustainable citrus production in HLB-endemic regions.

Keywords: Citrus Disease Control, Huanglongbing, HLB, integrated management, Liberibacter

https://apsjournals.apsnet.org/doi/10.1094/PHYTO-08-18-0287-R

Control of Citrus Huanglongbing via Trunk Injection of Plant Defense Activators and Antibiotics

J. Hu, J. Jiang, and N. Wang

2018. Phytopathology 108:186-195

Abstract

Citrus huanglongbing (HLB) or greening is a devastating disease of citrus worldwide and no effective control measure is currently available. Plant defense activators environmentally friendly compounds capable of inducing resistance against many plant pathogens. Earlier studies showed that foliar spray of plant defense inducers could slow down HLB disease progress. In this study, eight plant defense activators and three antibiotics were evaluated in three field trials for their effect to control HLB by trunk injection of young and mature sweet orange trees. Results showed that four trunk injections of several activators, including salicylic acid, oxalic acid, acibenzolar-S-methyl, and potassium phosphate, provided significant control of HLB by suppressing 'Candidatus Liberibacter asiaticus' titer and disease progress. Trunk injection of penicillin, streptomycin, and oxytetracycline hydrochloride resulted in excellent control of HLB. In general, antibiotics were more effective in reduction of 'Ca. L. asiaticus' titer and HLB symptom expressions than plant defense activators. These treatments also resulted in increased yield and better fruit quality. Injection of both salicylic acid and acibenzolar-S-methyl led to significant induction of

pathogenesis-related (PR) genes PR-1 and PR-2 genes. Meanwhile, injection of either potassium phosphate or oxalic acid resulted in significant induction of PR-2 or PR-15 gene expression, respectively. These results suggested that HLB diseased trees remained inducible for systemic acquired resistance under field conditions. In summary, this study presents information regarding controlling HLB via trunk injection of plant defense activators and antibiotics, which helps citrus growers in decision making regarding developing an effective HLB management program.
https://apsjournals.apsnet.org/doi/10.1094/PHYTO-05-17-0175-R
https://apsjournals.apsnet.org/doi/pdf/10.1094/PHYTO-05-17-0175-R

2018–2019 Florida Citrus Pest Management Guide: Huanglongbing (Citrus Greening)
M. M. Dewdney, M. E. Rogers, and R. H. Brlansky
2018. Original publication date November 2005. Revised September 2013, April 2016, and May 2018
University of Florida, Institute of Food and Agricultural Sciences UF/IFAS Extension
Publication #PP-225
https://edis.ifas.ufl.edu/cg086

CABI Plantwise Knowledge Bank

- Pest Management Decision Guide: Green List - **Citrus greening (or Huanglongbing, HLB)**

  Created/updated: July 2014
  https://www.plantwise.org/KnowledgeBank/pmdg/20147801168

MANAGEMENT AND CONTROL - CARIBBEAN

Training manual for management of Citrus Greening (Huanglongbing) and its insect vector the Asian Citrus Psyllid (Diaphorina citri) in Jamaica
Ministry of Agriculture and Fisheries, Jamaica
2012. Ministry of Agriculture and Fisheries, Jamaica. Prepared under *The Citrus Greening FAO TCP Project*. Printed in 2012 Ministry of Agriculture and Fisheries in collaboration with Food and Agriculture Organization of the United Nations
http://www.moa.gov.jm/PlantHealth/data/Training%20Manual_Citrus%20Greening.pdf
Contents

WEBPAGE

CITRUS GREENING by the Ministry of Industry, Commerce, Agriculture and Fisheries, Jamaica
http://www.micaf.gov.jm/content/citrus-greening

- **Field Identification Guide for Citrus Greening and Its Insect Vector in Jamaica**. 2012. Ministry of Agriculture and Fisheries, Jamaica

  Printed in 2012 Ministry of Agriculture and Fisheries in collaboration with Food and Agriculture Organization of the United Nations
  http://www.moa.gov.jm/PlantHealth/data/Field%20Guide-manual_Citrus%20Greening.pdf

- **Citrus Greening symptoms poster**
  http://www.moa.gov.jm/PlantHealth/data/Symptoms%20Poster.jpg
- **Citrus Greening Residential Flyer**
  http://www.moa.gov.jm/PlantHealth/data/Residential%20Flyer-corrected-Feb1.jpg
- **Citrus Greening Management Poster**
  http://www.moa.gov.jm/PlantHealth/data/Management%20Poster-PRINTFILE.jpg

VECTOR: Asian citrus psyllid - JAMAICA, CARIBBEAN

Identification and Distribution of Haplotypes of *Diaphorina citri* (Hemiptera: Liviidae) in Jamaica and the Caribbean

Sasha-Kay V Clarke, Sherline E Brown

2018. Journal of Economic Entomology 111: 2401–2408

Abstract

Diaphorina citri Kuwayama (Hemiptera: Psyllidae), also known as the Asian citrus psyllid, is a vector of the citrus huanglongbing (HLB) disease. *D. citri* transmits all three known strains of the HLB pathogen: *Candidatus* Liberibacter africanus, *Candidatus* Liberibacter americanus, and *Candidatus* Liberibacter asiaticus. The study involved 92 psyllids representing the Caribbean Basin and reference samples representing countries within Asia, North America, and South America. This study was aimed at characterizing *D. citri* on a molecular level in order to determine the haplotype diversity and uniqueness within Jamaica and the Caribbean Basin. *D. citri*-specific primers were used to amplify an 821 bp gene fragment from the mitochondrial cytochrome *c* oxidase subunit I gene (mtCOI). The statistical parsimony program, TCS, was used to determine the 12 haplotypes found within the Caribbean, with haplotypes H2 and H7 being the most prominent. The H2 haplotype was found to belong to the South Western Asia group originating from India. H2 represented 54% of the sequenced samples and dominated the Greater Antilles, 22% were grouped as H7, dominating the Lesser Antilles, while the remaining 24% of the sequences were grouped in the remaining 10 haplotypes and were variants seen within the Greater and Lesser Antilles.

Keywords: Asian citrus psyllid mtCOI, Haplotype, Huanglongbing, Jamaica, Caribbean

https://doi.org/10.1093/jee/toy194

https://academic.oup.com/jee/article-abstract/111/5/2401/5055236?redirectedFrom=PDF

PATHOGEN: '*Candidatus* Liberibacter asiaticus'

Progress and Obstacles in Culturing '*Candidatus* Liberibacter asiaticus', the Bacterium Associated with Huanglongbing

Marcus V. Merfa, Edel Pérez-López, Eber Naranjo, Mukesh Jain, Dean W. Gabriel, and Leonardo De La Fuente

2019. Phytopathology109:1092-1101

Abstract

In recent decades, '*Candidatus* Liberibacter spp.' have emerged as a versatile group of psyllid-vectored plant pathogens and endophytes capable of infecting a wide range of economically important plant hosts. The most notable example is '*Candidatus* Liberibacter asiaticus' (CLas) associated with Huanglongbing (HLB) in several major citrus-producing areas of the world. CLas is a phloem-limited α-proteobacterium that is primarily vectored and transmitted among citrus species by the Asian citrus psyllid (ACP) *Diaphorina citri*. HLB was first detected in North America in Florida (USA) in 2005, following introduction of the ACP to the State in 1998. HLB rapidly spread to all citrus growing regions of Florida within three years, with severe economic consequences to growers and considerable expense to taxpayers of the state and nation. Inability to establish CLas in culture (except transiently) remains a significant scientific challenge toward effective HLB management. Lack of axenic cultures has restricted functional genomic analyses, transfer of CLas to either insect or plant hosts for fulfillment of Koch's postulates, characterization of host-pathogen interactions and effective screening of antibacterial compounds. In the last decade, substantial progress has been made toward CLas culturing: (i) three reports of transient CLas cultures were published, (ii) a

new species of *Liberibacter* was identified and axenically cultured from diseased mountain papaya (*Liberibacter crescens* strain BT-1), (iii) psyllid hemolymph and citrus phloem sap were biochemically characterized, (iv) CLas phages were identified and lytic genes possibly affecting CLas growth were described, and (v) genomic sequences of 15 CLas strains were made available. In addition, development of *L. crescens* as a surrogate host for functional analyses of CLas genes, has provided valuable insights into CLas pathogenesis and its physiological dependence on the host cell. In this review we summarize the conclusions from these important studies.

Keywords: Asian citrus psyllid, Bacteriology, '*Candidatus* Liberibacter asiaticus', Citrus Greening, Huanglongbing, *Liberibacter crescens*, Plant Pathogen, Unculturable Bacteria
https://apsjournals.apsnet.org/doi/10.1094/PHYTO-02-19-0051-RVW

Targeted Early Detection of Citrus Huanglongbing Causal Agent '*Candidatus* Liberibacter asiaticus' Before Symptom Expression

Sheo Shankar Pandey and Nian Wang

2019. Phytopathology 2019 109:952-959

Abstract

Citrus Huanglongbing (HLB) is the most severe disease of citrus plants caused by '*Candidatus* Liberibacter asiaticus' and transmitted by the insect vector Asian citrus psyllid (ACP). No effective curative measure is available against HLB. For citrus production areas without HLB or with low HLB disease incidence, removal of '*Ca.* L. asiaticus' inoculum is critical to prevent HLB spread. Such a strategy requires robust early diagnosis of HLB for inoculum removal to prevent ACP acquisition and transmission of '*Ca.* L. asiaticus'. However, early diagnosis of HLB is challenging, because the citrus trees remain asymptomatic for several months to years after '*Ca.* L. asiaticus' transmission by ACP. In this study, we report a new method for targeted early detection of '*Ca.* L. asiaticus' in cultivar Valencia sweet orange (*Citrus sinensis*) before HLB symptom expression. We take advantage of the fact that '*Ca.* L. asiaticus' remains around the ACP feeding site immediately after transmission into the young flush and before flush maturation. ACPs secrete salivary sheaths at their feeding sites, which can be visualized using Coomassie brilliant blue staining owing to the presence of salivary sheaths secreted by ACP. Epifluorescence and confocal microscopy indicate the presence of salivary sheaths beneath the blue spots on ACP-fed leaves. Quantitative real-time polymerase chain reaction (PCR) and conventional PCR assays are able to detect '*Ca.* L. asiaticus' in the ACP feeding surrounding areas as early as 2 to 20 days after ACP feeding. This finding lays a foundation to develop much-needed tools for early diagnosis of HLB before symptom expression, thus assisting '*Ca.* L. asiaticus' inoculum removal and preventing HLB from spreading.

Keywords: Citrus Greening, Phytopathogenic Bacteria, Plant Disease Control, Psyllids Salivary Sheath
https://apsjournals.apsnet.org/doi/10.1094/PHYTO-11-18-0432-R

An effector from the Huanglongbing-associated pathogen targets citrus proteases

K Clark et al

2018. Nature Communications volume 9, Article number: 1718

Abstract

The citrus industry is facing an unprecedented challenge from Huanglongbing (HLB). All cultivars can be affected by the HLB-associated bacterium *'Candidatus* Liberibacter asiaticus' (*C*Las) and there is no known resistance. Insight into HLB pathogenesis is urgently needed in order to develop effective management strategies. Here, we use Sec-delivered effector 1 (SDE1), which is conserved in all *C*Las isolates, as a molecular probe to understand *C*Las virulence. We show that SDE1 directly interacts with citrus papain-

like cysteine proteases (PLCPs) and inhibits protease activity. PLCPs are defense-inducible and exhibit increased protein accumulation in *C*Las-infected trees, suggesting a role in citrus defense responses. We analyzed PLCP activity in field samples, revealing specific members that increase in abundance but remain unchanged in activity during infection. *SDE1*-expressing transgenic citrus also exhibit reduced PLCP activity. These data demonstrate that SDE1 inhibits citrus PLCPs, which are immune-related proteases that enhance defense Responses In Plants.

Keywords: Biotic, pathogens, plant immunity

https://www.nature.com/articles/s41467-018-04140-9

PLANT BREEDING

Reciprocal influences of rootstock and scion citrus cultivars challenged with *Ca*. Liberibacter asiaticus

Ute Albrecht, Kim D. Bowman

2019. Scientia Horticulturae 254:133-142

Abstract

The devastating citrus disease Huanglongbing (HLB), associated with bacterial pathogens of the genus *Candidatus* Liberibacter, has spread across many citrus production areas worldwide causing devastating economic losses. In Florida, infection rates in most commercial citrus orchards approach 100 percent by the time trees are 3–4 years old. Most scion cultivars are highly susceptible to *Ca*. Liberibacter asiaticus (CLas), the pathogen prevalent in Florida, but significant tolerance has been identified within the species *Poncirus trifoliata* (trifoliate orange) and some of its hybrids that are commonly used as rootstocks. In this study we investigated the relative influence of rootstock and scion in citrus plants composed of susceptible and tolerant cultivars in both the scion and the rootstock position on HLB disease progression and expression of stress and disease-related genes. Experiments were conducted in the greenhouse using the susceptible cultivars 'Valencia' orange (*Citrus sinensis*) and 'Cleopatra' mandarin (*C. reticulata*), and the tolerant trifoliate hybrid cultivars 'US-802' (*C. grandis* × *P. trifoliata*), 'US-897' (*C. reticulata* × *P. trifoliata*), and 'US-942' (*C. reticulata* × *P. trifoliata*). Plants were either mock-inoculated or graft-inoculated with CLas. The incidence of CLas infection at 6 and 12 months after inoculation and the severity of the disease was different among scion/rootstock combinations. Typically, trees with a tolerant cultivar in the scion position contained a lower number of bacteria and were less damaged by infection than trees with a susceptible cultivar in the scion position, regardless of rootstock. Previous observations of significant influence from rootstock on field tree tolerance to CLas may be partially the result of differential rootstock ability to tolerate other stresses, or respond favourably to therapeutic treatments, under the weakened condition caused by the infection. Differential expression of defense-related and other genes found largest fold-differences between non-infected and infected plants when a susceptible cultivar was in the scion position. Fewer genes responded in roots than in leaves, and genes associated with starch metabolism responded strongly and in an opposite direction when comparing roots and leaves, probably indicative of carbon depletion occurring in roots of infected plants.

Keywords: Citrus greening, Sweet orange, Breeding, Gene expression, Starch

https://www.sciencedirect.com/science/article/pii/S0304423819303565

Apparent Tolerance to Huanglongbing in *Citrus* and *Citrus*-related Germplasm

Godfrey P Miles, Ed Stover, Chandrika Ramadugu, Manjunath L. Keremane and Richard F. Lee

2017. HortScience 52, no 1: 31-39

Summary

In a Fort Pierce, FL, field planting, plant growth, and Huanglongbing (HLB) severity were assessed as indicators of HLB tolerance on progenies of 83 seed-source accessions of *Citrus* and *Citrus* relatives mainly from the Riverside, CA, genebank. The HLB-associated pathogen [*Candidatus*Liberibacter asiaticus (*C*Las)] and vector [asian citrus psyllid (ACP), *Diaphorina citri*] were abundant, and trees were naturally challenged for 6 years before metrics (leaf mottle, percent canopy mottle, overall health, canopy density, canopy width, canopy height, and trunk diameter) were collected in Oct. and Nov. 2015. The healthiest trees with low or no HLB symptoms were distant citrus relatives: *Balsamocitrus dawei*, *Bergera koenigii*, *Casimiroa edulis*, *Clausena excavata*, *Murraya paniculata*, and one accession of *Severinia buxifolia*. Within *Citrus*, most of the healthiest trees with densest canopies, little leaf loss, and greater growth were those with pedigrees that included *Citrus medica* (citron). These included progenies of *Citrus* hybrid ('Limon Real'), *Citrus limetta*, *Citrus limettioides*, *Citrus limonia*, *C. medica*, *Citrus volkameriana*, and some *Citrus limon* accessions. Trees in this category exhibited distinct leaf-mottle characteristic of HLB and substantial pathogen titers, but maintained dense canopies and exhibited good growth. Trees from seed-source accessions in the genus *Citrus* without citron in their background were generally among the least healthy overall with less dense canopies. The exceptions were progenies of two *Citrus aurantium* accessions, which were markedly healthier than progenies of other *Citrus* seed-source accessions not derived from citron. Linear regression analysis, between metrics collected and pedigree of seed parent, indicated that percentage of citron in the pedigree significantly correlated with measures of tolerance. Although no commercial *Citrus* genotypes yielded progenies with strong HLB resistance, in this field experiment several progenies maintained dense canopies and good growth, and may be useful for breeding HLB tolerant cultivars.

Keywords: Asian citrus psyllid, Aurantioideae, Citron, Citrus Breeding, Citrus Greening, Rutaceae

https://doi.org/10.21273/HORTSCI11374-16

PLANT BREEDING – Puerto Rico

Performance of Two Citrus Species Grafted to Different Rootstocks in the Presence of Huanglongbing Disease in Puerto Rico

Rebecca Tirado-Corbalá, Dania Rivera-Ocasio, Alejandro Segarra-Carmona, Elvin Román-Paoli and Agenol González

2018. Horticulturae 4(4):38

(This article belongs to the Special Issue Horticultural Plant Pathology and Prevention).

Abstract

Since Huanglongbing (HLB) disease was detected in 2009 in Puerto Rico, a steady drop in citrus production has been experienced, forcing farmers to abandon their land or switch to other crops. Between 2015 and 2016, we used grafted trees from two experimental orchards (Tahiti lime and Nova mandarin), each on five rootstocks, to collect soil and plant tissue samples from each scion–rootstock combination to determine soil fertility, tissue nutrient content, and yield. The tree growth parameters (height, diameter, and canopy volume) and efficiency of the two orchards were also measured. These orchards, growing in Coto series (Typic Hapludox), were planted in 2009 and reported as heavily infested with HLB by 2011. Our results showed that soil and tissue samples from the Tahiti lime orchard exhibited benefits for tree growth parameters when grafted on Carrizo and Cleopatra rootstocks. Lower tree mortality (13%) was

observed for Tahiti lime grafted on Carrizo, HRS 812, Carrizo and Rough lemon rootstocks, while 25% of the Nova mandarin trees perished on the same rootstocks. Yield was higher for Tahiti lime grafted on Swingle rootstock (35.6 fruit m^{-3}) as compared to the other rootstocks. In general, HLB appears to have caused poor development and low production in the Nova mandarin orchard.
Keywords: Huanglongbing; Tahiti lime; Nova mandarin; rootstocks; scions.
https://www.mdpi.com/2311-7524/4/4/38/htm
https://www.mdpi.com/2311-7524/4/4/38/pdf
https://doi.org/10.3390/horticulturae4040038

RNA SUPPRESSION TECHNOLOGIES

Emerging RNA Suppression Technologies to Protect Citrus Trees from Citrus Greening Disease Bacteria
Wayne B. Hunter, Xiomara H. Sinisterra-Hunter
IN: Crop Protection edited by Guy Smagghe. Advances in Insect Physiology vol. 55
2018. London: Academic Press, pages 163-197
Abstract
Research on the development of RNA-suppressing technologies, which include RNA interference, (RNAi), and the application of RNA suppression using cell-penetrating peptide Morpholinos, PPMOs, for suppressing pests and pathogens are briefly presented. Due to the extensive nature of RNAi and antisense oligonucleotide research, readers are directed to this review to obtain a more in-depth understanding of each field of study. Genomic resources and websites for dataset downloads and free access are listed to facilitate a broader community collaboration to develop solutions for this complex Liberibacter pathosystem. Strategies for gene drive and psyllid population modification or replacement, with *Wolbachia* or CRISPR/Cas9-based alterations, have continued to gain interest and are being developed (Champer et al., 2016, Gantz and Akbari, 2018, Peters et al., 2016).
Keywords: Antisense oligos, *Diaphorina citri,* Huanglongbing, Psyllid, RNAi
https://www.sciencedirect.com/science/article/abs/pii/S0065280618300171

PLANT NUTRITION

Ground Application of Overdoses of Manganese Have a Therapeutic Effect on Sweet Orange Trees Infected with *Candidatus* Liberibacter asiaticus
Flavia T. Zambon, Davie M. Kadyampakeni and Jude W. Grosser
2019. HortScience 54:1077–1086
Abstract
There is accumulating evidence that root system collapse is a primary symptom associated with Huanglongbing (HLB)-induced tree decline, especially for commercial sweet orange and grapefruit trees on Swingle and Carrizo rootstocks. Maintaining root health is imperative to keep trees productive in an HLB-endemic environment. Preliminary greenhouse and field studies have shown that HLB-impacted trees had secondary and micronutrient deficiencies that were much greater in the roots than in the leaves, and that treatments containing three-times the recommended dose of manganese (Mn) improved tree health and growth and increased feeder root density in greenhouse trees. These results suggested that trees in an HLB-endemic environment have higher specific micronutrient requirements than those currently recommended. To test this hypothesis, established Vernia sweet orange grafted onto rough lemon rootstock trees were divided into eight supplemental CRF nutrition treatments (including two-times and

four-times the recommended doses of Mn and boron) using a randomized complete block design in a commercial grove in St. Cloud, FL. The following supplemental nutrition treatments were used: no extra nutrition (control); Harrell's–St. Helena mix 0.9 kg per tree; Harrell's with 32 g of Florikan polycoated sodium borate (PSB) per tree; Harrell's with 90 g of TigerSul® Mn sulfate (MS) per tree; Harrell's with 32 g of PSB and 90 g of MS per tree; 180 g of MS per tree; 64 g of PSB per tree; and 180 g of MS plus 64 g of PSB per tree applied every 6 months since Fall 2015. Leaf and soil nutritional analyses were performed in Mar. 2017, Sept. 2017, and May 2018; a quantitative polymerase chain reaction was performed for *Candidatus* Liberibacter asiaticus (*C*Las) titer estimation in Nov. 2017. Significantly higher cycle threshold (Ct) values indicating reduced *C*Las bacterial populations were observed in trees that received the higher doses of Mn, especially those receiving four-times the recommended dosage of Mn (180 g Mn). Many trees exhibited Ct values of 32 or more, indicating a nonactive infection. Fruit yields of these trees were also increased. No significant differences in juice characteristics, canopy volume, and trunk section area were found between control plants and plants treated with 180 g Mn. Soil and leaf nutrients B, K, Mn, and Zn were significantly different among treatments at various times during the study. Our results strongly suggest that overdoses of Mn can suppress *C*Las bacterial titers in sweet orange trees on rough lemon rootstock, thus providing a therapeutic effect that can help restore tree health and fruit yields. This response was not observed when Mn and B were combined in the overdose, suggesting an antagonistic effect from B on Mn metabolism. When an overdose of Mn is used, biological functions and tree tolerance lost due to nutritional imbalances caused by HLB might be restored. Further studies are needed to elucidate which metabolic pathways are altered by comparing overdosed and conventionally fertilized HLB-impacted trees and to determine if the observed therapeutic effects can be achieved in trees grafted to other important commercial rootstocks.

Keywords: Citrus sinensis; Controlled-release fertilizer, Huanglongbing, Macronutrients, Micronutrients, Soil Acidification

https://journals.ashs.org/hortsci/view/journals/hortsci/54/6/article-p1077.xml

WEATHER

Regional spatial-temporal spread of Citrus Huanglongbing is affected by rain in Florida

M. M. Shimwela, T. S. Schubert, M. Albritton, S. E. Halbert, D. J. Jones, X. Sun, P. D. Roberts, B. H. Singer, W. S. Lee, J. B. Jones, R. C. Ploetz, and A. H. C. van Bruggen

2018. Phytopathology 108:1420-1428

Abstract

Citrus huanglongbing (HLB), associated with '*Candidatus* Liberibacter asiaticus' (Las), disseminated by Asian citrus psyllid (ACP), has devastated citrus in Florida since 2005. Data on HLB occurrence were stored in databases (2005 to 2012). Cumulative HLB-positive citrus blocks were subjected to kernel density analysis and kriging. Relative disease incidence per county was calculated by dividing HLB numbers by relative tree numbers and maximum incidence. Spatiotemporal HLB distributions were correlated with weather. Relative HLB incidence correlated positively with rainfall. The focus expansion rate was 1626 m month^{-1}, similar to that in Brazil. Relative HLB incidence in counties with primarily large groves increased at a lower rate (0.24 year^{-1}) than in counties with smaller groves in hotspot areas (0.67 year^{-1}), confirming reports that large-scale HLB management may slow epidemic progress.

Keywords: Diaphorina citri, Citrus Greening, ordinary *kriging*, area under the disease progress curve, Frontal Movement, Weather Effects

https://apsjournals.apsnet.org/doi/10.1094/PHYTO-03-18-0088-R

ORGANIZATIONS

CARIBBEAN

CARIBBEAN AGRICULTURAL HEALTH AND FOOD SAFETY AGENCY – (CAHFSA)
https://www.cahfsa.org/
CAHFSA is mandated to perform a coordinating and organizing role for the establishment of an effective and efficient regional sanitary and phytosanitary (SPS) regime and to execute on behalf of Member States such SPS actions and activities that can be more effectively and efficiently executed through a regional mechanism.

- **FAO Jamaica Training Manual Citrus Greening https://www.cahfsa.org/publications/manuals**

INTERNATIONAL

USAPHIS - ANIMAL AND PLANT HEALTH INSPECTION SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE

- **Citrus Greening** Last Modified: May 8, 2019

https://www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/pests-and-diseases/citrus-health-response-program/ct_citrus_greening

UNIVERSITY OF FLORIDA, INSTITUTE OF FOOD AND AGRICULTURAL SCIENCES UF/ IFAS

- **Citrus Greening (Huanglongbing, HLB**) webpage https://edis.ifas.ufl.edu/topic_citrus_greening
  Features Related Websites, Publications

UF/IFAS Citrus REC: Citrus Greening (Huanglongbing)

UF/IFAS Solutions: Citrus Greening

UF/IFAS Southwest Florida REC: Huanglongbing (HLB) Database
https://swfrec.ifas.ufl.edu/programs/entomology/hlb-db/
The Citrus Greening Database is a cooperative effort between the University of Florida/IFAS and the Florida Center for Library Automation (FCLA) and is made possible by funding from the Florida Citrus Production Advisory Council (FCPRAC). Our objective is to collect information from around the world related to huanglongbing disease (Citrus greening) and compile and centralize it in a user friendly database that can be easily accessed by everyone.

FDACS Huanglongbing (HLB)/Citrus Greening Disease Information

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