OPEN ACCESS Freely available online

Journal of Citrus Pathology

Research Article

Huanglongbing in Texas: Report on the first detections in commercial citrus.

J. V. da Graça[1*], M. Kunta[1], M. Sétamou[1], J. Rascoe[2], W. Li[2], M. K. Nakhla[2], B. Salas[3], and D. W. Bartels[3]

[1] Texas A&M University-Kingsville Citrus Center, Weslaco, TX 78599, USA; [2] USDA APHIS PPQ CPHST, Beltsville Laboratory, Beltsville, MD 20705, USA; [3] USDA APHIS PPQ CPHST, Mission Laboratory, Edinburg, TX 78541, USA.

*Correspondence to: john.dagraca@tamuk.edu

Citation: da Graça JV, Kunta M, Sétamou M, Rascoe J, Li W, Nakhla MK, Salas B, Bartels DW. 2015. Huanglongbing in Texas: Report on the first detections in commercial citrus. J Cit Pathol. iocv_journalcitruspathology_27939.

Abstract

Huanglongbing (HLB), also known as citrus greening, is a destructive citrus disease associated with 3 α-proteobacteria species of *Candidatus* Liberibacter. The first report of HLB in the USA was from Florida in 2005 and *Ca*. L. asiaticus (Las) is the only species currently confirmed in the USA. In January 2012, a Valencia sweet orange tree in a commercial orchard in San Juan, Texas, tested positive for Las by real-time and conventional PCR assays and by the sequence of its partial 16S rRNA gene. The sample tested negative for *Ca*. L. americanus and *Ca*. L. africanus. All 4 Valencia sweet orange seedlings that were graft-inoculated using budwood from the first Texas HLB-infected tree showed typical HLB symptoms 3 months post-inoculation and tested positive for the pathogen. Such HLB typical symptoms as leaf blotchy mottle, twig die-back, veinal chlorosis, lopsided and greening fruits were observed on the Las-positive tree in the orchard, which immediately triggered an intensive survey of the disease in the area. Typical HLB symptoms were found on 54 Valencia sweet orange trees in the same orchard and 18 Rio Red grapefruit trees in an adjacent orchard. All these symptomatic trees tested positive for Las by PCR and sequencing.

Keywords: citrus, huanglongbing, Liberibacter, Texas

Introduction

Citrus huanglongbing(HLB), citrus greening disease, is a highly destructive citrus disease caused by 3 α-proteobacteria species of *Candidatus* Liberibacter which are transmitted by citrus psyllids, and it has been the subject of several extensive reviews (Zhao 1981; da Graça 1991, da Graça and Korsten 2004; Gottwald et al. 2007; Bové 2006). It is considered to be the most destructive disease of citrus and since its appearance in the Americas (Coletta-Filho et al. 2004; Halbert 2005), it has been the subject of intensive research, and significant research funding has been allocated by governments and industries. While much has been learned in recent years about the pathogen and its interactions with its plant and insect hosts and with the environment, it is apparent that it is a complicated interaction, and there are many unanswered questions.

The origins of HLB are part of the mystery. While early reviewers cited observations in southern China as the center of origin of the Asian form of the disease (Zhao 1981; da Graça 1991; Bové 2006), reports and data from the Indian sub-continent (Husain and Nath 1927; Capoor 1963) had been overlooked, and it is possible that this is where citrus was first infected by *Ca*. L. asiaticus (Las), with subsequent movement of infected plant material to other Asian countries (Beattie et al. 2008). No native rutaceous plant species have been positively identified as a likely source, but *Severinia buxifolia*, *Murraya paniculata*, *M. exotica*, and *Clausena lansium* are all recorded hosts (Hung et al. 2001; Damsteegt et al. 2010; Deng at al. 2007) and one or more could be the original plant host. An alternative source could be a non-rutaceous species with transmission to citrus occurring via dodder (Beattie et al. 2008).

The African form of the pathogen probably originated from native African Rutaceae, and several have been found to be infected with variants of *Ca*. L. africanus (Laf) (Roberts et al. 2015), but thus far transmission to citrus has not been proven.

The initial confirmation of HLB in Brazil was associated with a previously unknown species, *Ca*. L. americanus (Lam), but it is being displaced by Las probably because the latter is more heat tolerant and reaches higher titers in citrus (Gasparoto et al. 2012), and Lam is now largely limited to *Murraya* spp. (Lopes et al. 2010). While no evidence has been found of its origins, the recent identification of a variant in psyllids in Texas

(da Graça et al. 2013) suggests it may be indigenous to the Americas.

Las was first reported in the USA in Florida in 2005 (Halbert 2005), 7 years after the discovery of the Asian citrus psyllid (ACP) *Diaphorina citri* (Knapp et al. 1998). Surveys in Florida showed that the disease had already become widespread in the south of the state (Gottwald et al. 2007), and in the following 5 years spread to all counties with commercial citrus (Brlansky et al. 2012).

The situation in Texas has been somewhat different from other recently infected areas in that the first detection appears to have occurred earlier in the epidemic than in Florida and Brazil, for example. In 2001, *D. citri* was discovered in the Lower Rio Grande Valley of Texas (French et al. 2001). Following the discovery of HLB in Florida, USDA-APHIS contracted the Texas A & M University-Kingsville Citrus Center to conduct surveys starting in 2006 to determine where the psyllids had become established in Texas and whether HLB was present. In 2008, it was reported that psyllids were present in 32 counties, but no HLB had been detected (da Graça et al. 2008). A USDA APHIS PPQ Citrus Survey was started in September 2008 in the Lower Rio Grande Valley and a USDA APHIS-certified diagnostic laboratory was established at the Citrus Center in the same year, and since then it has analyzed approximately 20,000 leaf samples and 36,000 psyllid samples from commercial orchards and residential sites for the causal bacterium, Las by quantitative real-time PCR (qPCR). In January 2012, the laboratory detected Las in a leaf sample collected by USDA-APHIS from a 6-year-old Valencia orchard in San Juan, Texas, (Kunta et al. 2012; Sétamou et al. 2012). San Juan is in Hidalgo County in the middle section of the commercial citrus area of the Lower Rio Grande Valley.

This paper is a description of the initial HLB finding in a Texas sweet orange tree orchard and subsequent 4-month survey until May 2012 in this orchard and a neighboring 5-year-old grapefruit orchard.

Materials and methods

Field sample collections

Teams of USDA-APHIS inspectors have been sampling sentinel trees and psyllids in residential properties in south Texas beginning in September 2008, and commercial orchards beginning in February 2011, for detection of Las and Lam. Prior to the first identification of an HLB-symptomatic tree, USDA APHIS had made over 40,000 site visits and collected over 36,000 psyllid samples and 20,000 plant tissue samples. Following the detection of the first infected sweet orange tree in San Juan, Texas, a tree by tree census in that 6-year-old orchard was conducted by TAMUK Citrus Center monthly from January to May 2012. A tree by tree census was also conducted in an adjacent 5-year-old grapefruit orchard separated by a paved road. Leaves with suspected HLB symptoms were collected, placed in double Ziploc® bags, and transported to the diagnostic laboratory for analysis.

DNA extractions and conventional and quantitative real-time PCR assays

DNA was extracted from leaf samples using DNeasy Plant Mini Kit (Qiagen, Valencia, CA). Small chopped leaf midribs of 0.2 g or 5 ACPs were pulverized for 3 min with a Mini-Beadbeater-96 (Biospec Products Inc, Bartlesville, OK) in a 2 ml lysing matrix A (MP Biomedicals, Santa Ana, CA) tube in the presence of appropriate extraction buffer. The extract of genomic DNA was eluted in 100 µl nuclease-free water.

For the detection of Las and Lam, multiplex qPCR assays (Li et al. 2006) were performed on 2 µl DNA extract in a 25 µl reaction using a portable SmartCycler (Cepheid, Sunnyvale, CA). A plant cytochrome oxidase (COX)-based primer-probe set (Li et al. 2006) was used as a positive internal control in qPCR tests for plant tissue. The presence of the target sequences in the DNA extracts was determined based on the Ct values obtained in the tests. Healthy plant DNA and non-template water control were included in all the qPCR assays.

The qPCR results were confirmed at USDA APHIS Beltsville laboratory by conventional PCR using OI1/O12C primers and GB1 /GB3 primers that amplify 16S ribosomal DNA sequences to detect Las and Laf (Jagoueix et al. 1996) and Lam (Teixeira et al. 2005), respectively. Additionally, the PCR amplification of ribosomal protein genes of β-operon using A2/J5 primers was conducted (Hocquellet et al. 1999). The PCR amplification products were separated by electrophoresis on 1% agarose gels, ethidium-bromide stained, visualized under UV light, and photographed using Biospectrum imaging system (UVP, Upland, CA). Thin slices of agarose gel containing the amplicon DNA were cut; DNA was purified using Qiaquick Gel Extraction Kit (Qiagen), and sequenced at GENEWIZ (GENEWIZ, South Plainfield, NJ). The nucleotide sequences were analyzed for similarities at NCBI database using the Blastn program.

Graft inoculation

Graft transmission of *Ca*. L. asiaticus was carried out at an insect-proof containment facility of USDA CPHST, Beltsville Laboratory, Beltsville, MD. Four Valencia sweet orange seedlings were graft-inoculated by side grafting with a 5 cm budwood from the initial HLB-infected sweet orange tree to ensure a high transmission rate of Las. The budwood piece was covered with plastic tape for 3 weeks. The inoculated seedlings were maintained at a constant temperature of 26°C and the plants were watered and fertilized when necessary. The presence of Las in the inoculated plants was inferred from observed symptoms and confirmed by qPCR.

Results

HLB symptoms in Valencia sweet orange

The initial Valencia sweet orange tree located on the south border showed leaves with asymmetric blotchy mottle symptoms which are typical for citrus huanglongbing (Fig. 1A-C). Other foliar symptoms included yellow and enlarged lateral veins and midribs. Color inversion was observed in fruits with yellowing from the stem end and fruits were small, lopsided, had stained vascular bundles at the end of the fruit axis, and contained aborted brownish-black seeds (Fig. 1D-F). Only 1 of 54 additional Valencia sweet orange trees that were found to be HLB-positive showed yellow shoot symptoms (Fig. 2). In addition, 18 Rio Red grapefruit trees showed symptoms similar to those observed in the initial Las-positive Valencia sweet orange tree.

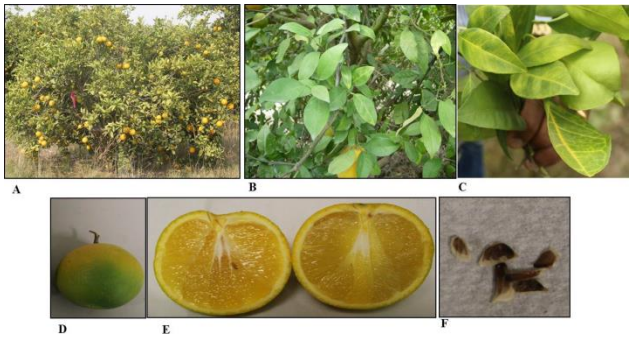

Fig. 1. Typical HLB symptoms were observed on an infected Valencia sweet orange tree in Texas. (A) Canopy of the tree; (B-C) foliage with asymmetric blotchy mottle, and yellow lateral veins and midrib; (D) lopsided fruit with color inversion; (E) brown staining in the vascular tissue of the fruit columella; and (F) aborted brownish-black seeds.

Fig. 2. HLB-infected grapefruit tree showing yellow shoot symptoms.

Detection of Ca. L. asiaticus DNA in leaf samples by conventional and quantitative real-time PCR

The DNA extracted from the initial Valencia sweet orange yielded Ct values of 23.41 and 23.42 in 2 separate reactions and COX positive internal control Ct values (17.07 and 17.24) confirmed the quality of the DNA (Fig. 3). Amplification with the 16S primers (OI1/O12C) and β-operon primers (A2/J5) resulted in 1160 bp and 703 bp fragments, respectively (Fig. 3). The nucleotide sequences for these amplicons (GenBank accessions KC481587 and KC481586 respectively) were identical to several NCBI GenBank entries of these sequences for Las.

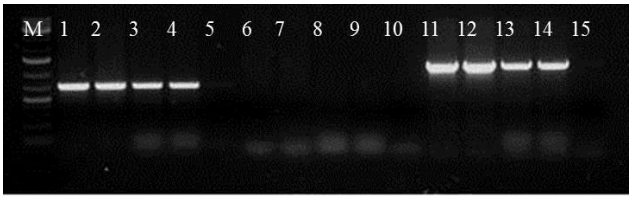

Fig. 3. Agarose gel showing PCR amplification products specific for *Candidatus* L. asiaticus. Lanes 1-5: PCR amplification products obtained using OI1/O12C primers, 1 = *Ca*. L. asiaticus positive control DNA, 2-4 = DNA from HLB-infected Valencia sweet orange, 5 = Non-template water control (NTC); Lanes 6-10: PCR amplification products obtained using GB1/GB3 primers, 6-9 = Valencia sweet orange DNA, 10 = NTC; Lanes 11-15: PCR amplification products obtained using A2/J5 primers, 11 = *Ca*. L. asiaticus positive control DNA, 12-14 = DNA from HLB-infected Valencia sweet orange, and 15 = NTC.

Graft transmission of Ca. L. asiaticus

After 3 months, all 4 grafted Valencia sweet orange seedlings displayed chlorotic young leaves and severe chlorotic yellow mature leaves with an enlarged, corky midrib and lateral veins (Fig. 4). Four months post inoculation, qPCR analysis of the foliar samples from all 4 inoculated plants produced positive results (Ct values 22.56, 21.88, 23.21, and 24.26).

HLB status in initial sweet orange and adjacent grapefruit groves from January to May 2012

A tree by tree census for foliar HLB symptoms and qPCR analysis of symptomatic samples, collected from the initial Valencia sweet orange grove and an adjacent grapefruit orchard, revealed 54 infected sweet orange and 18 infected grapefruit trees (Fig. 5). A total of 360 trees showing potential foliar HLB symptoms were not infected with Las, based on qPCR assays. No symptoms were present in 3,596 trees while 28 trees were missing.

The edge or border effect where a majority of infected trees are in border rows (Gottwald and Irey 2008) can be seen in the grapefruit orchard, but in the Valencia orchard, there is a concentration in rows 13 to 16 from the east (Fig. 5).

Discussion

The first confirmed HLB positive Valencia sweet orange tree was smaller in size compared to the other trees in the orchard. Aerial imagery from 2011 indicates that the tree had a diameter of 2.59 m compared with 3.58 m, 3.47 m, and 3.90 m for the west, north, and east adjacent trees respectively. The stunting of the tree was evident in aerial imagery back to 2008 (Fig. 6) and could have been the first tree to be infected, serving as a source of inoculum for the other trees. The tree showed classic symptoms of blotchy mottle, a corky vein, lopsided fruit with aborted seed and vascular browning, and twig die back on all sides. Delimiting surveys of all the orchards as well as residential citrus in the quarantine zone was conducted by USDA-APHIS and the Texas Department of

Agriculture. By 25 May 2012, a total of 54 additional infected trees were identified in the Valencia sweet orange orchard, as well as 18 HLB-infected Rio Red grapefruit trees in a 5-year-old grapefruit orchard immediately to the east.

Fig. 4. Three months post-inoculation, a Valencia sweet orange plant side grafted with budwood from the initial HLB-infected Valencia sweet orange tree shows yellow leaves with enlarged and raised lateral veins and midrib.

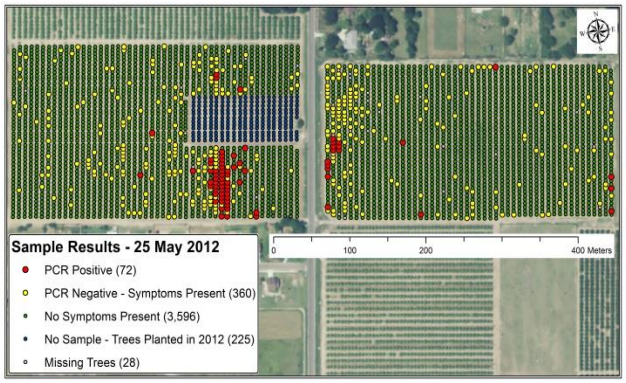

Fig. 5. HLB census map of citrus trees in the adjacent groves of Valencia sweet orange (left) and Rio Red grapefruit (right) separated by a paved road for a 5-month sample period ending 25 May 2012. The red circles = 72 trees that were qPCR positive for *Ca*. L. asiaticus, yellow circles = 360 trees that were qPCR negative but symptomatic, green circles = 3,596 trees without symptoms, blue circles = 225 young trees planted in 2012 from which no samples were collected.

Fig. 6. Aerial imagery from the 2011 International Boundary Water Commission Imagery (6 in pixel resolution), showing the first confirmed HLB positive Valencia sweet orange tree (yellow dot) in the Lower Rio Grande Valley, Texas.

Gottwald and Irey (2008) have reported that initial infections are frequently found in border rows, because these are the first trees encountered by incoming psyllids. The grapefruit orchard in San Juan displayed a border effect, but the Valencia orchard had most of the infected trees clustered 4 rows away from the eastern edge of the border. Discussions with the grower revealed that when the orchard was prepared, it was not possible to have the correct slope for water drainage because of the rectangular section identified by blue dots in Fig. 5. At the time this was a natural gas field, and was only planted with trees shortly before the HLB detection. Water from irrigation and rainfall therefore accumulated in a low lying area (rows 13 to 16) resulting in frequent flushing which would be more attractive to psyllids.

The initial detections of HLB in Florida (Halbert 2005), Louisiana, California (Kumagai et al. 2013), and Mexico (Moreno-Enriquez et al. 2014) were all in trees growing in residential settings. In spite of the fact that majority of the samples were collected and tested from residential trees, the first detection in Texas was in a commercial orchard, similar to Brazil (Anon 2004). There is a time gap of at least 5 to 10 years in discovering HLB since the introduction of the ACP into Florida and Brazil (Halbert 2005; Teixeira et al. 2005).

Manjunath et al. (2008) emphasized the importance of testing psyllids, as the disease movement in an area can be identified in insects one to several years prior to appearance of symptoms in a tree. The incidence of *Ca*. L. asiaticus in the ACP populations derived from HLB-infected field grown Florida citrus plants varied from 10% to 100% (Li et al. 2008). In the US, HLB has been detected in Florida, Georgia, Louisiana, South Carolina, Texas, and California (Gottwald 2010; Halbert 2005; Kunta et al. 2012; Kumagai et al. 2013).

Acknowledgments

This work was supported by the Citrus Commodity Pest Survey Cooperative Agreement from the Citrus Health Response Program, USDA-APHIS-PPQ. The authors sincerely thank the field operation inspectors who played a leading role in finding HLB in Texas.

This material was made possible, in part, by a Cooperative Agreement from the United States Department of Agriculture's Animal and Plant Health Inspection Service (APHIS). It may not necessarily express APHIS' views.

References

Anon. 2004. Greening chega aos pomares paulistas. Rev Fundecitrus Ano XX. 123:8-12.

Bové JM. 2006. Huanglongbing: A destructive, newly-emerging, century-old disease of citrus. J Plant Pathol. 88:7-37.

Beattie GAC, Holford P, Mabberley DJ, Haigh AM, Broadbent P. 2008. On the origins of citrus, Huanglongbing, *Diaphorina citri* and *Trioza erytreae*. In: Gottwald TR, Graham JH, editors. Proceedings of the 2008 International Research Conference on Huanglongbing. APS Crop Protection and Management Collection. Plant Management Network. p. 25-57.

Brlansky RH, Dewdney MM, Rogers ME. 2012. 2012 Florida Citrus Pest Management Guide: Huanglongbing (Citrus greening). University of Florida IFAS EDIS Publication #PP-225. http://edis.ifas.ufl.edu/cg086

Capoor SP. 1963. Decline of citrus trees in India. Bull Nat Inst Sci India. 24:48-64.

Coletta-Filho HD, Targon MLPN, Takita MA, De Negri JD, Pompeu J Jr, Machado MA. 2004. First report of the causal agent of Huanglongbing ("*Candidatus* Liberibacter asiaticus") in Brazil. Plant Dis. 88:1382.

da Graça JV. 1991. Citrus greening disease. Ann Rev Phytopathol. 29:109-136.

da Graça JV, Korsten L. 2004. Citrus huanglongbing: review, present status and future studies. In: Naqvi SAMH, editor. Diseases of fruits and vegetables. Dordrecht (Netherlands): Kluwer Academic Publishers. p. 229-245. (Vol. 1).

da Graça JV, French JV, Haslem PS, Skaria M, Sétamou M, Salas B. 2008. Survey for the Asian citrus psyllid, *Diaphorina citri*, and citrus huanglongbing (greening disease) in Texas. Subtrop Plant Sci. 60:21-26.

da Graça, JV, Sétamou M, Li W, Nakhle M, Rascoe J, Kunta M. 2013. Huanglongbing in Texas. In: Program and Abstracts of the 19th Conference of the International Organization of Citrus Virologists. Kruger National Park/Mpumalanga, South Africa. p. 47-50.

Damsteegt VD, Postnikova EN, Stone AL, Kuhlmann M, Wilson C, Sechler A, Schaad NW, Brlansky RH, Schneider WL. 2010. *Murraya paniculata* and related species as potential hosts and inoculum of '*Candidatus* Liberibacter asiaticus', causal agent of Huanglongbing. Plant Dis. 94:528-533.

Deng X, Zhou G, Li H. 2007. Detection of *Candidatus* Liberibacter asiaticus from wampee (*Clausena lansium* Skeels) by nested PCR. Plant Health Prog. doi:10.1094/PHP-2007-0419-01-BR.

French JV, Kahlke CJ, da Graça JV. 2001. First record of the Asian citrus psylla (*Diaphorina citri* Kuwayama (Homoptera: Psyllidae) in Texas. Subtrop Plant Sci. 53:14-15.

Gasparoto MCG, Coletta-Filho HD, Bassanezi RB, Lopes SA, Lourenço SA, Amorim L. 2012. Influence of temperature on infection and establishment of '*Candidatus* Liberibacter asiaticus' in citrus plants. Plant Pathol. 61:658-664.

Gottwald TR. 2010. Current epidemiological understanding of citrus huanglongbing. Annu Rev Phytopathol. 48:119-139.

Gottwald TR, da Graça JV, Bassanezi RB. 2007. Citrus huanglongbing: The pathogen and its impact. Plant Health Progr. doi: 10.1094/PHP-2007-0906-01-RV.

Gottwald TR, Irey M. 2008.The plantation edge effect of HLB: A geostatistical analysis. In: Gottwald TR, Dixon WN, Graham JH, Berger P, editors. Proceedings of the 2nd International Citrus Canker and Huanglongbing Research Workshop. Orlando, Florida. p.305-308.

Halbert S 2005. The discovery of huanglongbing in Florida. In: Gottwald TR, Dixon WN, Graham JH, Berger P, editors. Proceedings of the 2nd International Citrus Canker and Huanglongbing Research Workshop. Orlando, Florida. p. 50.

Hocquellet A, Toorawa P, Bové JM, Garnier M. 1999. Detection and identification of the two *Candidatus* Liberobacter species associated with citrus huanglongbing by PCR amplification of ribosomal protein genes of the beta operon. Mol Cell Probes. 13:373-379.

Hung T-H, Wu M-L, Su H-J. 2001. Identification of the Chinese box orange (*Severinia buxifolia*) as an alternative host of the bacterium causing Huanglongbing. Eur J Plant Pathol. 107:183-189.

Husain MA, Nath D. 1927. The citrus psylla (*Diaphorina citri* Kuw.) (Psyllidae: Homoptera). Mem Dept Agric India. 10:1-27.

Jagoueix S, Bové JM, Garnier M. 1996. PCR detection of the two *Candidatus* Liberobacter species associated with greening disease of citrus. Mol Cell Probes. 10:43–50.

Knapp J, Halbert S, Lee R, Hoy M, Clark R, Kessinger M. 1998. The Asian citrus psyllid and citrus greening disease. Citrus Ind. 79:28-29.

Kumagai LB, LeVesque CS, Blomquist CL, Madishetty K, Guo Y, Woods PW, Rooney-Latham S, Rascoe J, Galindo T, Schnabel D. 2013. First report of *Candidatus* Liberibacter asiaticus associated with citrus Huanglongbing in California. Plant Dis. 92:283.

Kunta M, Sétamou M, Skaria M, Li W, Nakhla MK, da Graça JV. 2012. First report of citrus huanglongbing in Texas. Phytopathology. 102:S4.66.

Li W, Hartung JS, Levy L. 2006. Quantitative real-time PCR for detection and identification of *Candidatus* Liberibacter species associated with citrus huanglongbing. J Microbiol Methods. 66:104-115.

Li W, Duan Y, Brlansky RH, Twieg E, Levy L. 2008. Incidence and population of "*Candidatus* Liberibacter asiaticus" in Asian citrus psyllid (*Diaphorina citri*) on citrus plants affected by Huanglongbing in Florida. In: Gottwald TR, Graham JH, editors. Proceedings of the 2008 International Research Conference on Huanglongbing. APS Crop Protection and Management Collection. Plant Management Network. p.231.

Lopes SA, Frare GF, Caamargo LEA, Wulff NA, Teixeira DC, Bassanezi RB, Beattie GAC, Ayres AJ. 2010. Liberibacters associated with orange jasmine in Brazil: incidence in urban areas and relatedness to citrus liberibacters. Plant Pathol. 59:1044-1053.

Manjunath KL, Halbert SE, Ramadugu C, Webb S, Lee RF. 2008. Detection of '*Candidatus* Liberibacter asiaticus' in *Diaphorina citri* and its importance in the management of citrus huanglongbing in Florida. Phytopathology. 98:387-396.

Moreno-Enriquez A, Minero-García Y, Ramírez-Prado JH, Loeza-Kuk E, Uc-Varguez A, Moreno-Valenzuela OA. 2014. Comparative analysis of 16S ribosomal RNA of '*Candidatus* Liberibacter asiaticus' associated with Huanglongbing of Persian lime and Mexican lime reveals a major haplotype with worldwide distribution. Afr J Microbiol Res. 8:2861-2873.

Roberts R, Steenkamp ET, Pietersen G. 2015. Novel lineages of '*Candidatus* Liberibacter africanus' associated with native Rutaceae hosts of *Trioza erytreae* in South Africa. Int J Syst Microbiol. 65:723-731.

Sétamou M, da Graça JV, Prewett R. 2012. HLB in Texas: Steps and challenges to curb the threat. Citrograph 3(5):32-38.

Teixeira DC, Danet JL, Eveillard S, Martins EC, de Jesus WC Jr, Yamamoto PT, Lopes SA, Bassanezi RB, Ayres AJ, Saillard C, Bové JM. 2005. Citrus huanglongbing in São Paulo State, Brazil: PCR detection of the '*Candidatus* Liberibacter' species associated with the disease. Mol Cell Probes 19:173-179.

Zhao XY. 1981. Citrus yellow shoot disease (Huanglongbing) – a review. Proc Int Soc Citricult. 1:466-469.