Huanglongbing-Control Workshop: Summary

Joseph M. Bové[1] and Michael E. Rogers[2] (Co-Conveners)
[1]University of Bordeaux Ségalen and INRA, Bordeaux, France
[2]University of Florida, CREC, Lake Alfred, USA

OVERVIEW

The present work is a summary of all contributions (listed below) performed during the HLB-control workshop held during the 12th International Citrus Congress in Valencia, Spain.

The University of Florida has estimated in 2012 that, in Florida, Huanglongbing (HLB) has resulted in the loss of 6,611 jobs from 2006 throughout 2011, $1,3 billion in revenue to growers and $3,63 billion in economic activity. The State's commercial citrus acreage has shrunk to 531,493 acres as of the fall of 2012, a 28% decline from 748,555 acres in 2004. For the 2012/2013 season, the USDA has revised the crop forecast downward twice since October, when the outlook was for 154 million 90-pound boxes of oranges, but in January the estimate predicted only a crop of 142 million boxes. The grapefruit forecast has dropped to 18 million boxes from 20.3 million. Also in São Paulo State, Brazil, HLB has taken a heavy toll. Since 2004, 18 million HLB-affected trees (~10%), representing a value of 216 million US$ at 12 US$ a tree, have been eliminated.

Can HLB be controlled, how and when? Answering these questions was the goal of the HLB control workshop! Two forms of HLB were considered: (i) high temperature-sensitive African-HLB, with *Candidatus* (*Ca*.) Liberibacter (L.) africanus and the African citrus psyllid vector, *Trioza erytreae*, and (ii) high temperature-tolerant Asian-HLB, with *Ca*. L. asiaticus and the Asian citrus psyllid vector, *Diaphorina citri* (Bové, 2009).

There is little evidence of genetic resistance to HLB in citrus. Apparently, citrus has had only a recent association with liberibacters, an association too short to have built up resistance to the bacterium. Hence, according to a general consensus, resistance to HLB is supposed be obtained by engineering, into citrus, genes with anti-liberibacter and/or anti-psyllid activity (National Research Council, 2010). However, such HLB-resistant cultivars will probably not become available to the growers before several years and, in the meantime, solutions must be developed to control HLB and hopefully save the present day citrus industry from destruction (Belasque Jr et al., 2010; Chamberlain, 2010; Timmer et al., 2011). Thus, for HLB control, short-term systems for "today" and long-term systems for "tomorrow" have been discussed. In addition, for regions or countries at risk of HLB, such as Argentina, California and Texas, contingency plans have to be considered.

RISK-BASED SURVEYS, CONTINGENCY PLANS

HLB in Argentina: a New Disease Outbreak

By P. Cortese[1], L. Santinoni[2], L. Palma[2], J. Agostini[3], C. Preussler[3], Y. Outi[3], G. Gastaminza[4], G. Perez[5] and E. Dominguez[6]
[1]SENASA, Buenos Aires, Argentina
[2]MAGyP Buenos Aires, Argentina
[3]INTA Montecarlo, Misiones, Argentina
[4]EEA Obispo Colombres, Tucumán, Argentina
[5]AFINOA, Buenos Aires, Argentina
[6]CECNEA, Concordia, Entre Rios, Argentina

Huanglongbing (HLB) caused by *Ca.* L. asiaticus was reported in 2004 in São Paulo, Brazil, and three years later in the southern state of Paraná, 300 km away from Argentina's northeastern border. In 2009, the Argentine citrus industry (AFINOA and CECNEA) and the institutions (MAGyP, SENASA, INTA, INASE, and EEA Obispo Colombres) set up a task force to develop quarantine guidelines for prevention of introduction of HLB into Argentinean citrus areas. This program was based on measures including 1) border inspections, 2) citrus nursery certification, 3) control on the production, transit and trade of citrus fruit, 4) field survey and diagnosis for the early detection of the disease in trees or the vector *Diaphorina citri* in citrus groves, 5) development of research and technology capacity, and 6) communication about the quarantine program and the disease. Diagnostic laboratories were set up in each citrus region and more than 100 inspectors were deployed in different citrus areas for: 1) survey of *Diaphorina citri* by yellow sticky traps, 2) visual inspection of *Murraya paniculata*, 3) inspection of all citrus nursery production under aphid mesh screen according to Resolution 930/09, and 4) survey of 100% of the citrus area (150,000 ha) with at least 10 surveys of the highest risk area. There were 52,000 inspection locations for 13,160 tree or Diaphorina samples.

In June 2012, a positive detection of HLB was confirmed in a backyard tree in the Northern Misiones Province across the border from Brazil. Since then, 5 surveys were carried out in the area surrounding the focus with the detection of 15 positive trees, all in backyards. The HLB positive trees included 12 tangerine trees, and 3 Rangpur lime trees from 7 to more than 10-years-old. In all cases, the trees were eradicated by the owners. The psyllid population in this area is very low and all PCR samples of the vector were negative. At present (November 2012), HLB has not been detected in commercial groves.

California

By Marylou Polek
Citrus Research Board, Visalia, California, USA

California is a fresh fruit industry with approximately 80-85% of the production industry located in the Central Valley of the State. The first Asian citrus psyllid (ACP) was detected in southern California in 2008. Industry and government officials took immediate action including the deployment of over 40,000 yellow panel traps statewide and chemical treatment of host plants within a 400 to 800 m radius around each detection site. Despite these activities, ACP has become widespread in, but is limited to, southern California and mainly in residential areas. In March 2012 one backyard tree tested positive for *Ca.* L. asiaticus. This has heightened the need to locate and eradicate infected trees before the psyllid and the disease spreads into the Central Valley production area. To maximize efforts while using minimal resources, California has implemented a “Risk-Based Residential Survey” devised by Drs. Timothy Gottwald and Weiqi Luo. The model on which the survey is based uses a geographical square-mile grid system. Each square-mile section is assigned a ‘combined risk factor’; the greater the risk factor, the more samples are collected and at more frequent intervals. Scientists and industry representatives provided input in identifying the potential risks. These include: population data based on the 2010 Census – density, ethnicity, type of residence; the number of ACP detections, number of repeated ACP detections, number of HLB detections, and traffic corridors – roads used for bulk shipments of citrus fruit. The model filters out factors such as waterways, elevation greater than 700 m, commercial properties such as factories, medical facilities, shopping malls, parking lots, and military installations. Using applications such as ‘Google Earth’, the risk map can be linked to satellite imagery. Field technicians can use GPS/GIS to identify locations for visual survey and the collection of ACP and plant material for laboratory testing for HLB. Survey data is periodically sent to Gottwald and Luo and fed back into the model. This refreshes the model, increasing its accuracy. Industry intends to expand this survey statewide and in production areas as well. Similar models have been developed for Texas and Arizona. A Texas risk-based survey has also been deployed and an Arizona survey will be deployed in early 2013.

The Texas Experience

By Mamoudou Sétamou and John V. da Graça
Texas A&M University-Kingsville Citrus Center, Weslaco, Texas, USA

The Asian citrus psyllid, *Diaphorina citri*, is an invasive pest that was first reported in Texas in 2001. Its potential as an economic pest was underestimated, and limited to no attention was paid to this pest. However, *D. citri* pest status has quickly changed with the detection of HLB in Florida in 2005, as the potential of HLB introduction can seriously compromise the sustainability of the multi-million dollar Texas citrus industry. In response, statewide surveys were launched in 2006 for early detection of HLB and for evaluating the spread of *D. citri*. A state-level task force was appointed to develop a roadmap for HLB mitigation. The Texas HLB operation and planning group was created and this group oversees all HLB mitigation efforts comprising the psyllid areawide management, clean nursery production system, surveys and detection, and the outreach and education program. From 2006 to 2011, a total of 18,000 suspicious leaf tissue and 35,000 insect samples were tested via PCR, but no HLB positive was detected. In accordance with the recommendation of the National Science Foundation, set of measures to reduce the incidence and spread of HLB were set forth to protect the Texas citrus industry. A restriction on movement of all Rutaceae plants and an implementation of an aggressive vector control were decided to protect the state citrus industry. The voluntary areawide management of the psyllid vector was launched during the dormant season of 2009-2010, and the percentage of commercial citrus under this program increased from 60% in 2010 to 85% in 2012. Concurrently a dramatic decline was observed in psyllid numbers in all citrus groves. Despite all these efforts, HLB was detected in two adjacent commercial groves in January 2012. A rapid response program including rogueing and destruction of all known HLB-infected trees, and an aggressive psyllid control program in the two groves and all commercial and residential citrus within a one-mile radius is being implemented. Such measures have thus far limited the spread of the disease as no addition tissue detection has been made in the ongoing detection surveys.

LONG-TERM SYSTEMS (ASIAN HLB AND AFRICAN HLB)

Breeding for Resistance/Tolerance to HLB within Citrus

By Ed Stover[1], T. Gregory McCollum[1], Randall Driggers[1], Richard Lee[2], Robert Shatters[1], Jr., YongPing Duan[1], Mark Ritenour[3], Jose X. Chaparro[4] and David G. Hall[1]
[1]USDA/ARS, USHRL, Ft. Pierce, FL, USA
[2]USDA/ARS, National Clonal Germplasm Repository for Citrus and Dates, Riverside, CA, USA
[3]University of Florida, Indian River Research and Education Center, Ft. Pierce, FL, USA
[4]University of Florida, Dept. of Horticultural Sciences, Gainesville, FL, USA

Huanglongbing disease (HLB) is now widespread across the southern half of the Florida peninsula and is found in every citrus producing county and it has now been found in California and Texas as well (CDFA, 2012). The disease greatly debilitates trees and sometimes contributes to tree death (Bové, 2006).

Development of citrus cultivars resistant to HLB is the best long-term control solution for endemic diseases such as HLB in Florida. Compared to other tested cultivars in experiments outside the US, lower susceptibility to HLB associated with *Ca.* L. asiaticus, has been reported for limes (Schwarz et al., 1973; Lange et al., 1985; Shokrollah et al., 2009), pummelos (Schwarz et al., 1973; Koizumi et al., 1997), lemons (Schwarz et al., 1973; Cheema et al., 1982; Nariani, 1982), some mandarin types (e.g. 'Ladu' and 'Som Pan') in Thailand (Koizumi et al., 1997) and various non-cultivated citrus or related species.

In Florida evaluations, *C. trifoliata* and its hybrid, 'Carrizo' citrange developed less severe HLB symptoms and the lowest titer of *Ca.* L. asiaticus among the genotypes evaluated in a recent greenhouse study (Folimonova et al., 2009). An analysis conducted on *C. trifoliata* and some of its hybrids suggested that some of these genotypes tolerate and/or suppress *Ca.* L. asiaticus even when grafted onto a high-titer source (Stover et al., 2010). A trial of more than 80 seedling populations from accessions of Citrus and citrus relatives has been underway for 2.5 years with intense HLB and ACP pressure in Ft. Pierce, FL. *C. trifoliata* is among the few genotypes in the citrus gene pool that continues to show substantial resistance to HLB (Lee et al., unpublished). *C. trifoliata* also displayed reduced colonization by ACP (Westbrook et al., 2011). Because of this continued evidence of HLB resistance in *C. trifoliata*, several trials are now underway using diverse trifoliates and their hybrids, including some advanced material with near commercial fruit quality. The hope is that molecular markers can be identified to facilitate introgression of resistance through conventional breeding and/or genes can be used to generate HLB-resistant standard cultivars using transgenic methods.

Diverse cultivars were surveyed for HLB and *Ca.* L. asiaticus in the Indian River area of Florida in infected commercial groves (Stover and McCollum, 2011). ‘Temple’ tangor showed the most consistently low incidence of HLB symptoms and lower *Ca.* L. asiaticus titer (25X lower than sweet orange and 30X lower than ‘Minneola’ tangelo), even when only infected trees were compared. HLB symptoms, yield and growth were assessed in a replicated trial of ‘Triumph’ (T), ‘Jackson’ (J), ‘Flame’ (F), and ‘Marsh’ (M) grapefruits established before HLB arose. HLB symptoms were severe in all trees and *Ca.* L. asiaticus titers were similar (Stover et al., 2012). However, F&M were almost completely defoliated in some years while T&J had full canopies. Cumulative fruit/tree was greater for T&J (255 & 220 kg) than for F&M (29 & 66 kg). T&J fruit met commercial standards and had normal size but F&M fruits were unacceptable with many being small and misshapen. These results suggest that useful resistance or tolerance to HLB may be found in conventional scion cultivars and further work is needed to assess this potential and its commercial value, and to mobilize such resistance into the range of commercial fruit types necessary to satisfy consumer demands.

The USDA citrus breeding program has used a large genetic base to create unique hybrids, many of which are exposed to HLB in our Florida farms. In addition to the studies described, a number of hybrids and even a few cultivars appear to have useful tolerance or resistance to HLB. A major focus of our breeding program is to characterize this resistance/tolerance and incorporate it into genotypes which also have outstanding fruit quality in a range of market phenotypes.

PRODUCTION OF GENETICALLY MODIFIED CITRUS (GMC) TREES

Agrobacterium-Mediated Transformation of Mature/Juvenile Citrus

By Viviani Marques
Fundecitrus, Araraquara, SP, Brazil

Conventional citrus breeding has important limitations due to male and female sterility, cross- and self-incompatibility, apomixis and polyembryony, high heterozygosity and quantitative inheritance of important quality traits. Consequently, programs for citrus breeding have resulted in very few commercial genotypes, most cultivars grown nowadays being derived from natural chance hybridizations and budsport or induced mutations.

Genetic transformation offers and excellent opportunity for improvement of citrus cultivars allowing the introduction of specific traits into known genotypes without altering their genetic elite background. Reinforced transgenic crops like cotton, corn and soybean are already available to growers and consumers in different countries. Within the different methodologies available for plant transformation, the one mediated by *Agrobacterium tumefaciens* infection presents many advantages, such as transferring and inserting in the plant genome long DNA segments without rearrangements, integration of low number of copies of the foreign DNA and low-cost compared to other transformation technologies. However, *Agrobacterium*-mediated transformation of citrus is considered recalcitrant because most *Citrus* species are not natural hosts of this bacterium. After many efforts in optimization, *Agrobacterium*-mediated transformation of juvenile tissues from some citrus cultivars became possible about 20 years ago. However, many years are required for evaluating the resulting phenotypes due to the juvenility of the source plant material used. Alternatively, transformation of mature tissues could represent a huge advantage for molecular breeding of citrus.

Production of transgenic plants from mature citrus material allows phenotype evaluation in 12-24 months, including characterization of fruit traits. Optimization of the transformation methodology for mature material is much more complex than that for juvenile tissues, as the developmental stage of the source material is an essential factor influencing transformation efficiency, which is clearly much lower for mature material. Thus, the first critical step is getting mature plant material in an ideal ontological stage and free of potential contaminants which could prevent transformation/regeneration during the in vitro culture phase. Different tissues and explants, including leaf segments, stem internodes, shoots internodes and other tissues can be used for transformation. Besides this, sterilization conditions of the source plant material are also important. At the bacterial inoculation step, time of incubation and bacterial concentration are critical factors. At subsequent phases, parameters such as light, temperature, nutrients (phytohormones, macro- and micro-nutrients, vitamins), antibiotics and their concentrations to prevent agrobacterial overgrowth, should be carefully assayed depending on the source material and citrus type used. Co-cultivation period ideal for favouring *Agrobacterium* infection should last 72 hours with no or very low illumination. For callus formation, explants have to be maintained at complete darkness during at least 3 weeks. To regenerate shoots, explants need to be cultured at 16 h/8 h light/dark photoperiod during a variable period of time (up to two months). At this stage, frequent evaluation (daily for some kind of explants) of buds and shoots regenerating from explants is required. Elongation of shoots from transformed events depends on the micrografting methodology. Its success relies on many factors, including the type of cut performed for scion insertion, the age and genotype of seedlings used as rootstocks, illumination and photoperiod, and composition of the culture media, among others. All the steps in the mature transformation procedure should be optimized for each *Citrus* species and even for each cultivar. Currently, we are able to transform adult material from elite sweet orange cultivars such as Pera, Navelate, Pineapple and Valencia.

Anti-Liberibacter Genes

By Ed Stover
USDA-ARS, USHRL, Ft. Pierce, FL, USA

Since no strong HLB resistance has been found in cultivated citrus scion varieties, transgenic citrus may provide the best opportunity for developing HLB-immune citrus scions. Transgenic strategies also should confer HLB resistance while maintaining known desirable traits of existing cultivars. Numerous approaches are being used.

Antimicrobial peptides (AMPs) and defensins, systemic-acquired-resistance (SAR) related genes, and a phage gene disrupting outer membranes of gram-negative bacteria (Gabriel, pers. commun.) are among the HLB-directed transgenics with greatest progress to date since these approaches are not dependent on in-depth knowledge of the HLB-pathosystem. AMPs disrupt bacterial membrane integrity, but do not damage plant cells, and confer some resistance to a wide array of disease-producing pathogens (Montesinos, 2007). There are numerous reports of AMP-transformed plants having disease resistance. In citrus, the AMP attacin has been demonstrated to confer resistance to citrus canker in sweet orange (Boscariol et al., 2006). Many citrus trees transgenically expressing AMPs (Bowman, Dutt, Grosser, Mirkov, pers. commun.), spinach defensins (Mirkov, pers. commun.), SAR-related genes (Dutt, Febres, Grosser, Moore, pers. commun.), and a phage gene from *X. campestris* (Gabriel, pers. commun.) are in field trials in Florida. Genomic data from the host (Xu et al., 2012) and the pathogen (Duan et al., 2008) and transcriptome data from infected plants (Albrecht and Bowman, 2008) are providing HLB-specific targets for transgenically derived HLB-resistance. Other strategies are being actively explored as new information and technology arise. In particular, for the first time, SCFV antibodies are now available against almost any liberibacter protein (John Hartung, pers. commun.). Candidate SCFV antibodies for resistance to HLB are those directed against proteins found to be essential for liberibacter growth and replication. Combination of the CTV gene vector (see below: 4) and the SCFV anti-HLB antibodies might become a most efficient anti-HLB system.

Anti-Psyllid Genes
By William O. Dawson
University of Florida, CREC, Lake Alfred, FL, USA

There are two different methods that are being considered to control psyllids by producing anti-psyllid products in plants, specifically in the phloem, for uptake by psyllids while feeding.

1. Antimicrobials to Prevent Endophytic Bacteria in Psyllids. One approach is to allow feeding psyllids to take up antimicrobials, probably antibacterial peptides, from phloem as they are feeding to kill endophytic bacteria that reside in psyllids. It has been shown that aphids, which are relatives of psyllids, need endophytic bacteria for production of necessary amino acids and other precursors for the survival of the aphid. This approach is based on the assumption that psyllids also need endophytic bacteria for their survival. Plant-produced antimicrobials that accumulate in the phloem would be sucked up by the psyllid. This could be at any stage of the insect. However, nymphs that grow rapidly and take up large amounts of phloem sap would likely be more susceptible.

2. RNAi Molecules to Suppress Psyllid Enzymes. Gene silencing by RNA interference (RNAi) is an innate defense mechanism of eukaryotic organisms. Double-stranded regions of RNAs are cleaved by a double-strand specific ribonuclease (dicer) into small RNAs that associate with the argonaute complex to target specific RNAs for degradation. If the target is a messenger RNA, production of the associated gene product is prevented. Thus, the objective of this strategy is to target psyllid enzymes that are necessary for its survival. Since psyllids suck up sap from plant phloem, this allows the targeting of psyllid enzymes by producing RNAi molecules in citrus phloem. Again, nymphs that take up large amounts of phloem sap would likely be the most susceptible target. The good news is that several labs are obtaining encouraging results using this approach. For instance, Nabil Killiny's laboratory has selected the abnormal disk wing gene (*awd*). Psyllid nymph-instars that acquired *awd*-dsRNA had diminished development and survival. Moreover, knockdown of *awd* gene expression was observed through malformation of adult wings.

CTV Gene Vector
By William O. Dawson
University of Florida, CREC, Lake Alfred, FL, USA

There are two major methods to express foreign genes in citrus trees. One is by transformation, which is insertion of the foreign gene into the nuclear DNA (see above II.B.1.). The advantage of this approach is that it is generally considered to be permanent. Another approach is the use of virus-based vectors. This procedure is not permanent because eventually the virus loses the foreign sequences.

Citrus tristeza virus (CTV) is an endemic virus in most citrus industries. Although some isolates of the virus cause serious economic losses, many isolates cause little damage. For example, most trees in Florida are infected with isolates of CTV that cause little damage as long as trees are not grown on the sour orange rootstock. By recombinant DNA technologies, CTV can be engineered into a transient expression vector to produce foreign proteins or RNAs in citrus trees. CTV is a phloem-limited virus, and this is an advantage when trying to control the bacterium that causes HLB because it also is limited to phloem. Additionally, the psyllid vector feeds on citrus phloem. The most remarkable feature of the CTV vector is that it is much more stable than other virus-based vectors so far examined. The CTV vector is capable of production of foreign gene products for years. The vector makes large amounts of foreign gene products in cells adjacent to sieve elements of the phloem.

The initial use of the CTV vector was in screening foreign genes for activity against HLB or the psyllid vector. The vector, which can be transmitted to other citrus plants of different varieties and ages by grafting is a much faster screening tool. However, because HLB has spread much faster than expected in Florida and because the majority of citrus is for juice, the time required to get resistant or tolerant trees in production is critical. Since juice processing plants require minimal amounts of fruit for processing to remain open and the fear is that production will start declining, it is possible that processing plants will have to close unless developments are made to stabilize fruit production. Although it is likely that transgenic citrus trees will be the long-term answer to citrus production, there is concern that they will not be available in adequate numbers in time to save the industry. For those reasons, the CTV vector is being considered as a temporary measure to protect trees until transgenic trees become available. The advantage of the CTV vector with an anti-HLB gene over transgenic plants is that the vector can be deployed sooner. Another advantage of the CTV vector is that if effective anti-HLB genes or anti-psyllid genes or RNAi molecules can be found, the vector could be used to treat trees in the field that are already infected with HLB.

Guava Effect: Production of GMC Trees that Repel Psyllids

By Berta Alquezar
Instituto Valenciano de Investigaciones Agrarias (IVIA), Moncada, Valencia, Spain

Nowadays, HLB management is based on detection and elimination of symptomatic trees, monitoring and control of the psyllid vector and resets with disease-free trees coming from insect-proof nurseries. Most of the insecticidal compositions employed for psyllid control are extraordinarily long-lived, persisting within the environment to which they are applied almost indefinitely, and excessively toxic to non-target organisms in the ecosystem. In addition, it is widely reported that insect species usually evolve resistance to most of the known insecticidal compositions. Thus, a medium-term need exists for non-toxic, shorter-lived, durable and efficient compositions to control psyllid vector of HLB-causing bacteria, such as those that may be provided by biological repellent compositions of plant origin.

It is known that in Vietnam guava grown in proximity to or intercropped with citrus has a repellent effect against the Asian citrus psyllid. This effect is likely due to volatiles produced from the guava leaves, because the protective effect is present all year round. The repellent effect of guava leaf volatiles on *Diaphorina citri* was confirmed employing an Y-tube olfactometer. In order to determine which specific compound(s) was (were) responsible for the effect, gas chromatography-mass spectrometry (GC-MS) analysis of volatiles emitted from leaves of different citrus and guava cultivars, at different developmental stages, different seasons and different hours of the day, were performed with the objective to produce transgenic citrus plants emitting these guava volatiles, in order to repel the psyllids in citrus orchards. To that end, genes responsible for the biosynthesis of these volatiles were cloned from citrus and *Arabidopsis* and the functional activity of the encoded proteins was confimed by in vitro functional assays. Selected genes were used for generating different genetic constructions, which may produce distinct levels of specific volatiles. These constructions were employed to generate transgenic *Arabidopsis* plants, some of which repelled the psyllids in olfactometric assays. Transgenic sweet orange plants carrying some of these constructions have been generated and are already available in the greenhouse to be further tested.

Blocking Psyllid Transmission of *Ca.* L. asiaticus

By Nabil Killiny
UD-CREC, Lake Alfred, FL, USA

Ca. L. asiaticus produces only one of the LuxR two-component cell-to-cell communication system: LuxR protein. The other component, Acyl-Homoserine lactone (AHL), is provided by the psyllid. LuxR protein from *Ca.* L. asiaticus has to combine with AHL from the psyllid for *Ca.* L. asiaticus to be able to colonize the insect and be transmitted to the plant. In order to block transmission (and knowing that normal citrus plants lack LuxR), *Ca.* L. asiaticus-infected citrus plants expressing LuxR protein in the phloem were produced experimentally. During feeding acquisition on these plants, the psyllid will acquire both Las and LuxR protein. AHL in the psyllid will be able to bind either LuxR on Las or LuxR from plants: in the first case transmission can occur, in the second case there will be no transmission. Indeed, *Ca.* L. asiaticus populations in the LuxR-expressing plants were significantly reduced and the acquisition by the psyllid was correspondingly reduced.

SHORT-TERM SYSTEMS (AFRICAN HLB)

H.F. Le Roux[1], S.P. van Vuuren[1], M.C. Pretotius[1] and C.H. Buitendag[2]
[1]Citrus Research International, Nelspruit, South Africa
[2]Pest & Disease Consultants, South Africa

Huanglongbing (HLB), better known in South Africa as citrus greening, has had a devastating effect on citrus production since it was first observed in 1928 (Oberholzer et al., 1965). The disease is caused by the gram negative bacterium *Ca.* L. africanus. With the exception of one area in Ethiopia where *Ca.* L. asiaticus was reported, *Ca.* L.asiaticus and americanus do not occur in Africa. *Ca.* L. africanus subsp. capense can be found in the Cape chest nut tree in the Western Cape (Garnier et al., 2000).

Although *Ca.* L. africanus can survive in a few *Rutaceae* species other than *Citrus*, citrus has always been the main source of inoculum. Work done by Garnier et al. (2000) and Pietersen (pers. commun., 2012) show that in all genera other than *Citrus* where Liberibacter could be found in South Africa, it was a subspecies of *Ca.* Liberibacter africanus and not the same as that found in *Citrus*. When HLB is detected for the first time in a citrus producing area the first option would be eradication. In South Africa the disease existed for more than forty years before it was realized that a pathogen was involved. By that time the disease had spread to such an extent through the northern citrus growing areas that it was not practical to implement an eradication programme. In spite of predictions that the whole southern African citrus industry would be destroyed by HLB in the 1970s, the industry has doubled its exports four-fold and has exported in access of 100 million 16 kg cartons in 2012.

The success of the southern African citrus industry starts with the production of HLB free citrus trees by accredited nurseries under the Citrus Improvement Scheme. This scheme has so far voluntary, but external threats such as the Asian HLB has convinced most of the citrus industry that it should be statutory. Restrictions on the movement of citrus nursery material have also been put in place prohibiting the movement of citrus trees from HLB-infested magisterial districts/provinces to HLB-free districts.

Chemotherapy

During the 1970s tree injections using tetracycline hydrochloride and PMT were developed and applied commercially (Buitendag and Bronkhorst, 1983). However, it was not sustained as a commercial treatment because the method proved expensive, remission was only temporary, treated trees were inclined to produce small fruit, phytotoxic effects occurred at the injection site and high levels of residues were found in the fruit of treated trees (Buitendag and Von Broembsen, 1994).

Removal of Branches

According to Buitendag and von Broembsen (1994) trees up to 5 years of age that show symptoms of HLB, should be removed. Trees from 6 to 10 years old: Only the infected branches should be removed. Regrowth should be prevented using NAA and these trees should be on a strict programme of systemic insecticides. Inter-planting young trees in orchards older than 10 years which have a history of HLB is not recommended.

Insecticides

Trioza erytreae is present in South Africa but not *Diaphorina citri*. *Trioza* is an excellent invader and it can readily locate isolated areas of growth flush over several hundred metres (Samways and Manicom, 1983). Although contact and systemic insecticides may not prevent the spread of HLB to an orchard especially the systemic insecticides serve to reduce the spread of the disease within the orchard (Van den Berg et al., 1990).

The industry realized that control should be aimed at keeping the orchards psylla free, thereby limiting the dissemination of HLB to a minimum. However, it was found after the development of organophosphate resistance in red scale that multiple sprays aimed at maintaining low populations of psylla were impractical and caused pest repercussions. Aldicarb (Temik®) was also applied to non-bearing trees. It moves systemically into the foliage and has a long residual action against citrus psylla. As a result of the high dosages and frequent applications, the development of accelerated degradation of aldicarb by soil micro-organisms was common. In replant situations where the citrus nematode, *Tylenchulus semipenetrans* occurred, this rendered aldicarb ineffective as a nematicide (le Roux et al., 1998). The product has since been withdrawn from the market.

In 1986 Buitendag produced a breakthrough with the registration of monocrotophos (Azodrin® 40) in a systemic trunk application. Treatment costs using this chemical were relatively low, minimal disruption of beneficial insects occurred and its application using the Calibra trunk applicator was both easy and effective. Control for 26 days was achieved using monocrotophos. The product also controls aphids, budmite, leafhopper and thrips on young flush. Unfortunately monocrotophos was withdrawn from the market in South Africa in 2003. During the early 1990s, Buitendag introduced a second chemical for use as a systemic insecticide by means of trunk applications, *viz.* methamidophos (Citrimet®). It was effective against psylla for 18 days. Authorities should rethink the use of these products, to be used in the special trunk applied formulations in areas where HLB is a crisis.

However, trunk applications can be dangerous if used incorrectly or when formulations not registered for stem applications are used. It is of extreme importance that trees should not be treated when under stress. The stomata should be open and there has to be sap flow when treatments are done. If not, the products can become phytotoxic and can cause injuries to the trunks which, in extreme cases, could result in ring barking and ultimately the death of the tree (le Roux, unpublished).

Imidacloprid (Confidor®) and acetamiprid (Mospilan®) are also used. These are not organophosphates and belong to the chloro-nicotinyl group of chemicals, thus reducing the likelihood of psylla developing resistance to the trunk-applied products. The residual effect of these products in the tree after trunk application is approximately twice that of monocrotophos or methamidophos, respectively. Initially Buitendag showed that stem-applied imidacloprid gave psylla control for up to 52 days whereas acetamiprid stem applications controlled the vector for 42 days. Recently psylla control of more than 100 days was achieved commercially after imidacloprid was applied as a soil drench through open hydroponic drip irrigation systems (I. Bruwer, pers. commun.).

Though no problems have so far occurred with regard to accelerated microbial degradation of any of the chloro-nicotinyls, this phenomenon must be kept in consideration and growers should ideally alternate systemic stem applications with soil drenches to protect the different products.

Biological Control

The major parasite of *Trioza* in South Africa is a species of parasitoid wasp, *Tetrastichus* spp., that oviposits in the psylla nymphs, and appears to limit psylla populations (Catling, 1969). A survey of predators in the country showed that none of them reduced densities low enough to control HLB because of its hyper parasites.

Advanced Production Systems

Because of the threat of HLB, South African producers tend to implement systems that will allow them to get into production at an earlier stage with the idea to remove the orchard sooner than normal if HLB becomes a problem. This resulted in higher density plantings and the so called open hydroponic irrigation systems (OHS). Because of these systems growers were able to produce up to 20 tons of exportable lemon fruit within 24 months of planting. Similar figures are available for Nadorcot mandarins under OHS systems. The OHS system is a properly managed drip system through which irrigation is applied daily, if possible using several pulses. If the OHS is managed properly there will be masses of feeder roots under each dripper. Applying the systemic insecticides through such a system will give optimal results.

The disadvantages of this system is that the trees grow profusely and if for some reason the cultivar used do not set fruit properly, the trees will be over grown within a few years resulting in heavy pruning which will impact negatively on fruit set and will advance vegetative growth once again. High density plantings will also enhance certain fruit and foliar diseases eg. *Alternaria* problems on cultivars such as Nova and Minneola and *Botrytis* on Eureka seedless lemon. High density plantings must set good crops each year and the tree size must be managed by pruning from an early age. Because pruning enhances regrowth which is attractive to *Trioza* these flushes should always be protected with systemic insecticides.

Final Comment

It is only possible to produce citrus economically in HLB infested areas if the three pronged concept is followed viz. disease free planting material, the removal of inoculum and the effective application of systemic insecticides.

SHORT-TERM SYSTEMS (ASIAN HLB)

Three-Pronged System for HLB Management

The three-pronged system (TPS) for HLB management is based on three measures: (i) surveys for identification and removal of HLB-affected trees to reduce sources of inoculum, (ii) use of healthy plants grown in covered, insect proof nurseries for resets and (iii) insecticide applications against the Asian citrus psyllid (ACP) vector.

Lessons from HLB Management in São Paulo State, Brazil

By Antonio J. Ayres[1], José Belasque Jr[1] and Joseph M. Bové[2]
[1]Fundecitrus, Araraquara, SPS, Brazil
[2]Univ. Bordeaux Ségalen and INRA, Bordeaux, France

The TPS has been applied to HLB-affected citrus orchards as soon as HLB was detected in São Paulo State (SPS) in 2004. After eight years of HLB management by the TPS in SPS, it has been learned that, under certain favorable farm characteristics, HLB-control is possible and can be achieved, while with "opposite" unfavorable characteristics, control is more difficult and can even fail. These favorable/unfavorable characteristics are: (i) HLB-incidence in the region where the farm is located; (ii) percentage of HLB-affected trees when HLB-management starts; (iii) distance between the managed farm and neighboring farms with poor or no management; (iv) size of farm; and (v) age of trees. For a given farm, these five characteristics cannot be changed: HLB control relies entirely on the TPS measures. Characteristics which are FAVORABLE (or unfavorable) for HLB control are as follows: (i) LOW (or high) HLB-incidence in the region, (ii) LOW (or high) percentage of HLB-affected trees in the farm at start of management, (iii) distance between managed farm and farm with no or poor management is GREATER (or smaller) than ca. 4 km, (iv) farm is LARGE with at least 500 ha of citrus (or small), and (v) trees are ADULT (or young) when management starts. With favorable farm characteristics, the TPS management program can be simple; with unfavorable characteristics, stronger programs with more surveys for symptomatic tree removal and additional insecticide treatments for psyllid control must be used. Thus, the HLB management method is successful not only when farm characteristics are favorable for HLB control, but even when farm characteristics are not so favorable, provided that the HLB-management programs are reinforced and become adapted to the needs.

The major handicap for HLB control comes from groves, which are not submitted to a strong HLB-management program and in which the percentage of HLB-affected trees is high. Some of these groves are abandoned, most others have insufficient HLB-management programs, allowing their psyllids to invade and contaminate trees of neighboring, well-kept farms where adequate HLB-management is practiced (see also "2.", next section). Legal tools (laws) have been developed since 2005 and reinforced in 2008, to remove these heavily infected groves, but the tools are used too softly. Eventually, farms where HLB is well managed, particularly the larger ones where restructuring occurs, will survive, and citrus groves with poor or without management, particularly small and middle size farms, will probably disappear or turn into sugar cane and other economical activities. To circumvent such unfortunate perspectives, small and middle sized farms must team up regionally and create large (1000 ha and more) citrus health management areas for successful HLB control.

Regional HLB Management by the TPS in São Paulo State, Brazil

By Renato B. Bassanezi
Fundecitrus, Araraquara, SP, Brazil

During the psyllid migration from one citrus grove to a second citrus grove, the psyllid population in this second grove is concentrated in the first 150 m from the grove's edge and, consequently, in the edge of groves there will be more pressure for new primary infections (edge effect). In a small TPS managed area, the ratio "edge area"/"total area" is much higher than the ratio in a large TPS managed area. Therefore a small TPS managed area is much more affected by external sources of bacteriliferous psyllids, resulting in a higher disease progress rate in the total managed area.

When psyllid control and inoculum removal are done in small individual areas (only local control), psyllid and inoculum sources are kept at short distances resulting in a faster re-infestation of treated areas with bacteriliferous psyllids from non-treated surrounding areas and higher disease progress in the total area. When psyllid control and inoculum removal are done in a large area (area-wide or regional control), psyllid and inoculum sources are kept at long distances. Psyllid migration from non-treated surrounding areas will achieve first the edge of treated area resulting in a slower re-infestation of the internal treated areas with bacteriliferous psyllids from non-treated surrounding areas and in lower disease progress in the total treated area. Therefore HLB progress is more a function of regional TPS management than of local TPS management. Experiments conducted in SPS clearly demonstrated that regional TPS management increases the efficiency of local HLB control, delaying the starts of epidemics and reducing the disease progress rate, because reduces the external sources of inoculum and psyllid population, consequently reduces the external bacteriliferous psyllid population and primary infections. With area-wide HLB TPS management less local intensive control becomes efficient and control costs can be reduced. As a consequence, area-wide HLB TPS management keeps the longevity and profitability of groves and, more important, allows resets or grove replanting keeping the sustainability of citriculture.

In conclusion, area-wide HLB TPS management is efficient to reduce the disease epidemics and its improvement must be the goal for the citriculture where HLB is present. It can be done by reinforcing the inoculum removal by growers, planting larger and continuous new groves, applying more intensive psyllid control on the grove edges but also on neighboring groves, and establishing coordinated voluntary groups for area-wide psyllid control.

Example of HLB Management and Farm Restructuring by the TPS in São Paulo State

By A. Tachibana
Cambuhy, Matão, SP, Brazil

Cambuhy farm has 14,123 ha, with 45% citrus, 31% forests and 24% other crops (rubber, sugarcane and cereals). The three main factors to keep HLB incidence at low rates are: (i) TPS outside the farm (on neighbors); (ii) TPS inside the farm and (iii) restructuring the farm. HLB management by the TPS inside Cambuhy farm consists of (i) 7 to 9 inspections per year of all citrus blocks with inspectors on platforms to identify HLB-affected trees and immediate eradication of these trees, (ii) several aggressive insecticide treatments against the psyllid vector on the basis of psyllid incidence as determined by weekly monitoring for the vector. The worst damage resulting from neighboring farms with no HLB management was the edge effect: the highest incidence of HLB-affected trees in Cambuhy farm was found in the peripheral blocks located closest to the “no management” farms. For this reason, it was necessary to control psyllids not only within Cambuhy farm, but also outside the farm in the “no management” neighboring farms. When permission was given, eradication of HLB-affected trees in these “no management” neighboring farms was also carried out. Restructuring of the citrus area of Cambuhy farm as a result of HLB management aimed at developing large areas (400 to 500 ha) of new citrus plantations rather than having a mixture of new and old blocks. Indeed, with such large, homogeneous areas edge effects are much reduced and HLB management becomes easier.

Finally, Cambuhy farm insists on growing not only citrus but also other, non-citrus crops: “Never work with only one crop!”. This is not solely for economic and financial issues, but also for better and easier HLB control: the non-citrus crops, sugar cane, rubber trees, cereals, are grown on the edges of the farm, thus increasing the distance between citrus in the center of the farm and the “bad” neighbors!

Using the TPS for HLB management, Cambuhy achieved the following results: 1) reduced HLB incidence to less than 1% HLB-affected trees; 2) restructured the farm; 3) increased productivity (box/ha); 4) reduced costs (US$/box); 5) had two options for the future: (i) keep HLB incidence low through HLB management and continue to produce normal, non-transgenic citrus, and (ii) grow HLB-resistant, transgenic citrus cultivars when these will hopefully become available.

The Three-Pronged System for HLB Management in Florida

By G. McCollum
USDA, ARS, USHRL, Fort Pierce, FL, USA

Huanglongbing (HLB) disease is having a devastating impact on the Florida citrus industry. Trees infected with *Ca.* L. asiaticus have been confirmed throughout all citrus production regions in Florida and some estimates suggest at least 70% of all commercial citrus trees in the state are infected with *Ca.* L. asiaticus. Immediately upon confirmation of HLB in Florida, citrus growers were encouraged to follow a three pronged system (TPS) for management of HLB. The TPS includes: 1) controlling the Asian citrus psyllid (ACP) vector by insecticide applications; 2) reducing inoculum by removal of trees confirmed to be infected with *Ca.* L. asiaticus; and 3) planting only nursery stock free of *Ca.* L. asiaticus. Based upon these recommendations, growers began to scout for trees showing signs of HLB and confirm *Ca.* L. asiaticus infection by testing in diagnostic laboratories; trees confirmed as positive were removed. The state of Florida imposed stringent regulations on the citrus nursery industry to ensure production of nursery stock free of ACP/*Ca.* L. asiaticus. Implementation of the TSP is costly, resulting in a tripling of Florida citrus production costs. When it became apparent that *Ca.* L. asiaticus infection was so widespread in Florida, some growers ceased removal of infected trees rather than suffer the costs associated with tree removal and subsequent loss of yield. However, maintaining *Ca.* L. asiaticus-infected trees not only increases the amount of inoculum, but also results in increased fruit loss due to drop and a decrease in the quality of fruit that enters the processing stream. In addition, the presence of infected trees increases the likelihood that newly planted trees will become infected with *Ca.* L. asiaticus and succumb to HLB prior to ever producing an economic return. Effectiveness of the TPS is dependent upon all three components, rejecting the importance of removing infected trees will result in a continued increase in HLB and a downward spiral that could lead to the demise of the Florida citrus industry.

Insecticidal Control of Asian Citrus Psyllid

By M.E. Rogers
University of Florida, Citrus Research & Education Center, Lake Alfred, FL, USA

1. Introduction. Use of insecticides for controlling the Asian citrus psyllid (*Diaphorina citri* Kuwayama) is one component of the three-pronged strategy (TPS) for managing Huanglongbing (HLB) disease of citrus. As a result, where both *D. citri* and HLB are present, pest management associated costs for citrus production have increased dramatically. In Florida, citrus growers currently apply an average of 8-10 insecticides per year to keep *D. citri* populations at or below detectable levels with the intent of slowing the rate of spread of HLB disease. Such increased use of insecticides is viewed as a short-term approach to maintaining citrus production until long-term solutions that are more economically and environmentally sustainable are developed. The following is a brief overview of current insecticide-based management approaches for psyllid control that should be considered when developing a HLB management program.

2. Value of Insecticide Applications. When citrus growers are selecting insecticides for use in a psyllid management program, it is important to understand the benefits provided. These benefits fall under one of two categories, either population suppression or direct prevention of pathogen transmission. The goal of population suppression is to reduce the pest population to low levels thereby reducing the rate at which the pathogen moves from diseased to healthy trees. Most insecticides currently used for psyllid management function primarily to suppress psyllid populations. The most effective insecticides for population suppression are broad-spectrum in nature (primarily organophosphates and pyrethroids), providing control of both adult and immature stages of *D. citri*. Also of value are selective insecticides with novel modes of action. While these selective insecticides are less likely to disrupt naturally occurring biological control of other pests, they typically only control *D. citri* nymphs. This is important to consider when deciding whether to use such products since they fail to control the adult stage of *D. citri* that is responsible for pathogen spread. Alternatively, some insecticides may prevent pathogen transmission by causing mortality of a vector prior to successful pathogen transmission (i.e., inoculation and/or acquisition). In the case of *D. citri*, insecticide induced mortality prior to initiation of phloem-related feeding has been postulated to preclude successful transmission of the HLB pathogen (Bonani et al., 2010; Serikawa et al., 2012). Use of an electrical penetration graph (EPG) monitor to study *D. citri* feeding behavior on insecticide-treated plants has shown that feeding disruption provided by foliar-applied insecticides is short in duration, whereas use of soil-applied systemic insecticides may disrupt *D. citri* feeding up to 6 weeks, thus providing a longer duration of protection compared to foliar applied insecticides (Serikawa, 2011; Rogers et al., 2012). However, use of soil-applied systemic insecticides is only effective for young trees due to rate of product that can be applied and significant amount of product dilution throughout the

canopy of larger trees.
3. Timing of Insecticide Applications. To provide the most effective control of *D. citri*, insecticide applications should be applied before psyllid populations reach high levels. This is important not only from the standpoint that it is easier to control pests when populations are low, but once populations reach high levels, it can be assumed that those psyllids present have spread the HLB pathogen to healthy trees upon which they are found feeding. The reproductive biology of *D. citri* is dependent on the presence of new leaf growth (flush) for egg laying and subsequent nymphal development to the adult stage. Thus, psyllid populations are lowest during periods when no new flush is available and only adult psyllids are present. Therefore, pesticide applications timed to these periods will provide the most effective suppression of psyllid populations. Thus, it stands to reason that the first applications for control of adult psyllid populations should be applied during the winter months when citrus trees are "dormant" or producing little or no new flush. Such an approach has previously been used for control of other citrus pests such as the citrus rust mite (*Phyllocoptruta oleivora* Ashmead) (Thompson, 1948). Subsequently, Qureshi and Stanlsy (2010) demonstrated that use of dormant sprays targeting *D. citri* during the winter months were effective in maintaining psyllid populations at low levels during the early part of the year.
4. Insecticide Application Methods. Recent studies have shown that the residual activity of insecticides (in terms of psyllid mortality) can be relatively short in duration, lasting from 3 weeks to as little as 3 days after applications are made (Weaver and Rogers, unpublished data). The variation in duration of efficacy is likely a result of climatic conditions (rainfall, UV radiation, and temperature) during the time when applications are made. Furthermore, *D. citri* has been found to move between adjacent groves on a frequent (if not daily) basis (Boina et al., 2009). Different pesticide application methods are available to allow rapid treatment of groves that provide control of psyllids before dispersal from treated to untreated areas can occur. These methods include aerial application (fixed-wing and helicopter), low-volume sprays (mist-type applications) and tractor-driven airblast applications. Each of these application methods has limitations in terms of level of psyllid control provided. Aerial applications provide rapid delivery of broad-spectrum insecticides to the outer canopy of large acreages with the assumption that the lack of coverage of the interior portion of the tree canopy will be offset by the fact that most psyllids are present on the outer canopy of the tree. Low-volume applications (typically applied using a sprayer mounted on the back of a truck) allow rapid treatment of groves and provides control of insects present at the time of application which are directly contacted by the pesticidal-mist. The drawback to this application method is that much of the pesticide active ingredient applied is lost to drift and the residual of that which is deposited on the leaf surface of trees has little or no residual activity beyond several days. While these three application methods by nature vary in their coverage and perceived effectiveness, the actual effectiveness of the spray has less to do with coverage and more to do with the amount of acreage treated and time required to do so. Thus, the application method used should be matched to the growing situation (i.e., the number of acres being treated). For example, use of a tractor-driven airblast application would be well suited for groves that could be treated within a 1-week period. However, if the acreage is so large that this cannot be accomplished, low-volume or aerial applications provide the means to treat these large areas in a time period that reduces the likelihood of psyllids recolonizing recently treated areas from adjacent yet to be treated areas.
5. Border Sprays. Approaches to managing psyllids with minimal pesticide inputs are needed. One potential approach is the use of border sprays. Both researchers and citrus growers have noticed an "edge effect" where both psyllid populations and incidence of HLB diseased trees are highest on the edge of a grove. It is hypothesized that the increased incidence of psyllids on the outer edge of a grove is a result of the initial colonization of psyllids from surrounding areas and/or the result of psyllids moving from the interior of a grove that settle on the outer tree rows once they can move no further

without leaving an adequate food source. Thus, it is possible that sprays targeting psyllid populations on the edges of groves might be used as a supplemental psyllid control between planned sprays of an entire grove. In a large-scale field trial, a pyrethroid insecticide was applied to the borders of individual citrus blocks within a commercial citrus planting encompassing more than 8,900 ha. Based on scouting of 272 blocks of citrus within this planting, *D. citri* populations were reduced by 79.6% using border sprays between the regularly scheduled complete grove pesticide applications. These results demonstrate that supplemental border sprays can provide effective suppression of psyllid populations where control programs have been implemented but psyllid movement from unmanaged groves continues to result in rapid reinfestation of recently treated groves.

6. Preventing Insecticide Resistance in Psyllid Populations. Due to the repeated use of insecticides for controlling psyllid populations, it is important to take steps to prevent resistance to insecticides. Thus, pesticide rotation should be practiced to ensure that the same mode of action is not used repeatedly. Development of pesticide resistance in psyllid populations is not well documented but has been observed. One such example is in India, where citrus growers have described failure of dimethoate to control psyllids after relying solely on that insecticide for both psyllid and thrips control on mandarin varieties (Rogers, pers. observ.). In Florida, while failures of insecticides to control psyllids in commercial groves have not been reported, research focused on monitoring for pesticide resistance development has demonstrated shifts in the baseline susceptibility of *D. citri* to commonly used insecticides (Tiwari et al., 2011).

7. Young Tree Care. In areas where HLB is endemic, preventing young trees from becoming HLB-affected is one of the most important problems growers must deal with. As mature tree productivity declines, due to age or disease, those trees must be replaced. Young trees however are more prone to being colonized by psyllids because of the continuous production of new leaf growth needed by psyllids for reproduction. For this reason, young trees are likely to become HLB-diseased within the first few years after planting. If a young tree becomes diseased, it is unlikely that it will ever reach its full fruit-bearing potential. In Florida, the soil-applied systemic neonicotinoid insecticides imidacloprid, thiamethoxam and clothiandin are considered the most important tools for protecting young trees from becoming HLB infected. When applied to the soil and taken up by the root system, these insecticides can provide up to six weeks of psyllid feeding disruption that prevents successful inoculation of a healthy plant with the HLB-associated bacterium (Serikawa et al., 2012; Rogers, unpublished data). Results of an ongoing field trial in Florida have demonstrated that use of soil-applied neonicotinoids every six weeks combined with foliar applications of non-neonicotinoid insecticides between soil-treatments (to prevent pesticide resistance) kept young trees HLB-free during the first 12 months after planting. This was is comparison to HLB infection rates of up to 11% in other pesticide regimes tested in the same trial (Kim and Rogers, unpublished data). While the use of soil-applied insecticides for young tree protection can provide tremendous benefits in terms of protecting young trees from becoming infected with HLB, the soil applications can be negatively affected by irrigation practices and thus may not be well suited for all growing conditions. In Florida for example, after being applied to the soil surface, the insecticide must be lightly watered into the root zone for proper uptake, which is accomplished using microsprinkler irrigation. If the product remains in the soil too long without being moved into the root zone for uptake, the insecticide may become bound to soil particles and subsequently become unavailable to the plant. Conversely, overwatering, such as use of flood irrigation, will move the product away from the root zone before the plant can take it up. These factors, along with tree size and product rate must be considered for successful protection of young trees using soil-applied systemic insecticides.

8. Area-Wide Control of Psyllid Populations. Due to the increased use of insecticides and the associated costs, new approaches are needed for managing psyllids so that citrus production can remain both economically and environmentally sustainable. The high reproductive rate of psyllids, combined with their between-grove dispersal capabilities reduces the likelihood that an individual citrus grower can successfully manage psyllids if management practices are not implemented in surrounding groves. One approach to this problem is the development of an area-wide psyllid control program. In the past, area-wide control programs have been developed and successfully implemented in both citrus (e.g. fruit fly control) and other agricultural systems. Hendrichs et al. (2007) summarizes many of these successes and also describes the characteristics of pest/crop systems that make area-wide control a feasible approach. In the case of citrus and *D. citri* these include the facts that psyllids are highly mobile and citrus is a high value crop with low tolerance for psyllid presence. Furthermore, implementation of an area-wide control program leaves no refugia for immigrants thereby reducing the need for frequent repeated pesticide applications and can also facilitate efforts to prevent pesticide resistance development. Based on the benefits that can be obtained through an area-wide control program, a report was issued by the National Academy of Sciences (National Research Council, 2010), recommending that area-wide management programs be implemented to reduce the rate of spread of HLB. The term Citrus Health Management Areas (CHMAs) was specifically used to describe areas of commercial citrus groves where growers would coordinate their HLB management efforts. In Florida the CHMA program was implemented in 2011 with the initial goal of coordinating the timing and mode of action of insecticide applications by growers to enhance current psyllid control efforts and manage the development of pesticide resistance. To date, 38 CHMAs have been developed throughout Florida encompassing more than 196,000 ha of commercial citrus groves. As part of the CHMA program, personnel from the United States Department of Agriculture and Florida Department of Agriculture conduct psyllid scouting of more than 6,000 blocks of citrus every 3 weeks to determine the success of the CHMA program in terms of reducing psyllid populations. This scouting has shown that statewide, psyllid populations have been reduced by an average of 68% since the implementation of the CHMA program thus demonstrating the success of this area-wide psyllid control program.

Three-Pronged System (TPS): Conclusions
By J.M. Bové
University of Bordeaux Ségalen and INRA, Bordeaux, France

1. TPS in São Paulo State (SPS). The TPS is a preventive control system, preventing trees from becoming infected with *Ca*. L. asiaticus through transmission by the Asian Citrus Psyllid (ACP). It was applied in SPS immediately after HLB was detected in March 2004, a time when HLB-incidence in the farms was still low. Practically all the many large farms (≥600 ha or 300,000 trees) used the TPS. From 2004 until now (November 2012), the TPS has been constantly improved and adapted to changing situations. The need to control psyllids and to remove HLB-affected trees has never been questioned. The major difficulty for well-managed farms was the presence of neighboring farms with no or poor HLB-management. The TPS has made it possible to keep the HLB-incidence below ~1% affected trees a year, meaning that ~98% trees are uninfected and healthy, thus providing a very low HLB environment. The acreage of TPS-managed farms with low (≤1%) HLB-incidence amounts to ~200,000 ha or one third (1/3) of the total SPS citrus acreage. SPS is the only region in the world where the TPS has been so successful on a large scale. It is estimated that an additional acreage of 200,000 ha of citrus with low HLB-incidence can be obtained by (i) applying the TPS to farms in regions of SPS where HLB has just arrived and is still of low incidence, (ii) using regional HLB-management, especially in the case of small and middle-size farms cooperating within so-called Citrus Health Management Areas (CHMA), and (iii) moving to more advanced citrus production systems.

The successful results obtained with the TPS have changed the perspectives of the SPS citrus industry (Bové, 2012). Until it was demonstrated in 2010 that HLB management by the TPS was successful (Belasque et al., 2010), it was believed that the

TPS was only a short-term solution to keep the citrus industry alive until such times when HLB-resistant, transgenic cultivars would hopefully become available. Today, SPS has not only the transgenic, long-term solution available, but also a second option for its citrus industry: regular, non-transgenic orchards kept at very low HLB-incidence by TPS-management. This second option will also offer most favorable conditions for the development of the young, transgenic orchards, as these will benefit of the low HLB-environment provided by the TPS.

2. TPS in Florida. The TPS has also been used successfully in Southern Florida on some large farms, but many growers were reluctant to remove HLB-affected trees. Growers have turned to enhanced nutritional programs (see below) and by 2012, the HLB-incidence in most farms was too high for the TPS to work. Florida probably has only one long-term option: HLB-resistant, transgenic trees.

ENHANCED NUTRITIONAL PROGRAMS

Vector Control and Foliar Nutrition for Management of Huanglongbing in Florida Citrus

By P.A. Stansly, A.H. Arevalo, J.A. Qureshi, M.M. Jones, K. Hendricks, P.D. Roberts and F.M. Roka
SWFREC, Immokalee, FL, USA

Huanglongbing (HLB) or citrus greening is a bacterial disease vectored by the Asian citrus psyllid (ACP), causing mottled leaves, tree decline, and yield loss. Vector control and foliar nutrition are widely employed in Florida and elsewhere to respectively slow the spread of HLB and mitigate debilitating effects of the disease. A 4 year replicated field study was initiated Feb. 2008 in a 5.2-ha commercial block of ‘Valencia’ orange trees planted in 2002 employing a factorial design to evaluate individual and compound effects of a popular foliar nutrient program and threshold-based vector management. A mixture consisting primarily of micro- and macro-nutrients was applied three times a year corresponding to the principal foliar flushes. Insecticides were sprayed once or twice in winter during tree dormancy or when psyllid populations exceeded a nominal threshold of 0.2 adults per tap for a total of 4 to 7 sprays a year. ACP numbers per stem tap on trees receiving no insecticide exceeded those on insecticide-treated trees by over 13-fold the first year and between 5- to 7-fold in successive years. Incidence of HLB, estimated using PCR from samples from every 5th tree, rose from a mean 30% at the beginning of the study, to 95% in only18 months. Apparently by chance, incidence was initially greatest in plots receiving foliar nutrition only, with area under the disease incidence curve (AUDCP) significantly greatest for that treatment as well. Furthermore, threshold cycle (Ct) values were lowest from nutrition-only treated trees through Jan 2010, indicating higher titers of the target (bacterial) DNA, with variable effects later in the trial. Both vector management and foliar nutrition decreased acid content in 2010 by 0.04 and 0.06% respectively and reduced brix:acid ratio as well. The only significant effects on juice quality in 2012 were seen with a decrease in brix of 0.37 TSS due to insecticides. The vector control factor significantly improved yields for all harvest but the first year of the experiment, while the nutritional factor only improved yields in the 4th year, possibly because of initially higher HLB incidence. However, best yields all 4 years were seen from trees receiving both foliar nutrition and vector control, with a mean of 90.6±1.8 kg per tree the 4th year, only 7 kg short of the pre-HLB regional average for 10 year old ‘Valencia’ on ‘Swingle’. Nevertheless, costs for the combined treatment exceeded profits at current juice prices.

This experiment demonstrated salutatory effects on HLB-infected trees of both vector control and enhanced foliar nutrition, especially when used together. Further research is necessary to establish economic thresholds for both insecticide and nutrient application under different market and environmental conditions.

Nutritional Treatment Effects on HLB-Affected Trees in Florida
By G. McCollum
USDA, ARS, USHRL, Fort Pierce, FL, USA

HLB disease is now endemic in Florida, with some estimates suggesting greater than 75% of commercial citrus trees are infected with *Ca.* L. asiaticus. Florida citrus growers are faced with a dilemma, whether to practice the established recommendation of removing trees infected with *Ca.* L. asiaticus or attempting to maintain productivity of HLB-affected trees with so-called "Enhanced Nutritional Programs" (ENPs). Interest in ENPs came about based on reports of a Florida citrus grower who claimed that his ENP "cocktail" resulted in a remission of HLB symptoms and reinvigoration of productivity in a citrus grove severely impacted by HLB. Based on these initial reports, a number of different ENPs have been developed both by citrus growers and agricultural chemical companies. Although there are many different ENPs in use, they are all essentially comprised of combinations of foliar applications of micronutrients, phosphorous acids, and in some cases elicitors of systemic acquired resistance. Florida citrus growers have rapidly adopted ENPs with the belief that they can maintain productivity in trees affected by HLB; however, the value of ENPs has been based on anecdotal, rather than scientific evidence. To date, there have been no published scientific reports confirming the value of such ENPs. One recently published report (Gottwald et al., 2012) indicated inconsequential effects of ENPs on health, productivity and fruit quality of HLB-affected sweet oranges. Large scale, non-replicated grower trails have failed to produce conclusive evidence supporting the value of ENPs for maintaining productivity in HLB-affected groves.

It is without question that good nutritional management is a critical component of citrus production. ENPs have significant impact on tree appearance, especially canopy color, but their value on overall tree health and productivity in both the short and long term remain questionable at best, and perhaps extremely detrimental. Because ENPs have no impact on incidence of *Ca.* L. asiaticus infection or titer in infected trees, their use sustains the HLB epidemic and in the long term may have greater negative than positive impact on the Florida citrus industry.

THREE-PRONGED SYSTEM (TPS) AND ENHANCED NUTRITIONAL PROGRAMS (ENPS): CONCLUSIONS
By Joseph M. Bové
University of Bordeaux Ségalen and INRA, Bordeaux, France

The TPS has been mainly used in São Paulo State (SPS), the ENPs mainly in Florida. Eight years of experience in SPS show that TPS is successful in large farms and when initial HLB-incidence is low, for instance at beginning of HLB outbreaks. TPS has maintained HLB-incidence at low values (≤ 1%) over 200,000 ha of citrus.

ENPs have been applied when HLB-incidence was already high because growers had been reluctant to remove HLB-affected trees even though they applied insecticides to control psyllids. In orchards where ENPs are applied, the trees look better, but in spite of psyllid control, the percentage of HLB-infected trees increases rapidly and soon, in a few years, 100% of trees are infected (HLB-incidence: 100%).

Even if productivity is increased (which remains to be demonstrated) area-wide applications of ENPs result in HLB-saturated citrus environments and high inoculum pressure, which most likely hinder, today, the development of new, young orchards and might even jeopardize, tomorrow, the establishment of orchards with genetically modified citrus.

ENPs are not substitutes for TPS!

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