

**2007 Huanglongbing Technical Working Group  
Conference Call  
March 13, 2007**

**Executive Summary**

USDA APHIS PPQ convened a meeting of the HLB Technical Working Group (TWG) on March 13, 2007 to make recommendations on survey and regulation of this disease. The HLB TWG met because of 1) potential detections of the *Huanglongbing* (HLB) pathogen in its insect vector in Florida and Texas, and 2) to discuss experimental evidence characterizing the host status of *Murraya* spp. Several U.S. research and regulatory programs are generating methods for the detection of HLB in its insect vector, which opens the possibilities for enhancing current survey strategies. This also raises a number of regulatory questions on the significance of finding the pathogen in the vector if no infected plant host is found. In addition, a critical question asked of the TWG was to determine whether the common nursery plant orange jasmine is also a host of the HLB pathogen, and if so, determine what the risk of disease spread is to citrus through this route.

The TWG agreed that the use of the Asian citrus psyllid to detect incipient infections of HLB in areas where the disease is not known to occur is a potentially useful survey tool. However, the amount of bacteria in single infected psyllid can be near or below the current detection limits of real-time PCR technology, and confirmation of positive finds needed for regulatory actions may be problematic. In addition, diagnostic results from psyllid surveys using the current technology may have a high and possibly unacceptable false negative rate. Population studies on the Asian citrus psyllid need to be conducted to address this problem. Alternatively, awareness of the problems of false negatives can be incorporated into the survey manual SOP, so that regulatory personnel can properly take actions on survey results.

Even with these caveats, the TWG members strongly recommended that a survey of psyllids occur in Texas, both in the Rio Grande valley and in the Corpus Christi area. This survey should be done at an appropriate time when the probability of psyllids carrying the pathogen is high to best utilize resources. At this time, a validated diagnostic is available for plant samples and proficiency tests for HLB in plants will be available soon. Work is being conducted to adapt the HLB diagnostics so that a validated protocol may be available for use in psyllids.

The TWG recommended that *Murraya* spp. should be regulated. Texas state law regulates *Murraya* spp. and California also prohibits its entry. All *Murraya* spp. from Florida should be prohibited from leaving the HLB quarantine area to prevent disease (and vector) spread into unregulated areas. Sufficient scientific evidence is present for justification of restriction.

Because of the recent increase in research and regulatory efforts with the HLB pathosystem, it will be necessary to have follow-up meetings to provide recommendations as new information arises. The meeting of the TWG may become a regular event, perhaps occurring once every few months.

## Introduction

Infestation of the high-consequence disease of citrus huanglongbing in several south Florida counties was confirmed in late 2005. Since then, intensive regulatory and scientific activities have occurred in the U.S. Science-based regulatory programs have been initiated with the twin objectives of delimiting the current infestation and preventing the spread of the pathogen to citrus-producing areas still free of the disease. A Science Panel was convened in February 2006 to gather the latest scientific knowledge of the disease biology and epidemiology to provide recommendations to U.S. state and federal regulatory programs for the efficacious fulfillment of these objectives. At that time, the disease had been confirmed only in south Florida.

Since that Science Panel report, several lines of research and regulatory data have been collected to better elucidate many of the previously unknown characteristics of the disease. Because of these developments, it is appropriate to review the science behind current regulatory programs to determine if changes or adjustments are needed. The TWG was briefed on the current situation with putative positive psyllid detections in Texas and the need to determine if surveys of psyllids in Texas and Florida can be efficacious in providing data for prevention of further spread of HLB.

Recent developments in two areas of the disease biology are relevant for discussion. First, several U.S. research and regulatory programs are generating methods for the detection of the HLB pathogen (*Candidatus Liberibacter asiaticus*) in its insect vector, the Asian citrus psyllid (ACP) (*Diaphorina citri*). These developments open possibilities for enhancing current survey strategies and also raise a number of regulatory questions on the significance of finding the pathogen in the vector if no infected plant host is found. The potential for this scenario is thought to be significant in areas where the vector is found, but the disease is not known to be present, especially Texas and northern Florida.

Second, because orange jasmine (also known as orange jessamine), *Murraya paniculata*, and other species in this genus are citrus relatives, favored hosts of the psyllid, and widely distributed from commercial nurseries, there is a potential for spread of HLB through commercial trade in *Murraya* spp. (including *Murraya koenigii*; syn. *Bergera koenigii*). A critical question asked of the TWG was to determine whether *Murraya* species are also hosts of the HLB pathogen, and if so, determine what the risk of disease spread is to citrus through this route.

The TWG participants were forwarded questions from state and federal regulatory personnel based on the current regulatory situations in Florida and Texas. The questions were formatted so that science-based responses could be directly applied. These were then forwarded to the TWG (Appendix I). While some of the forwarded questions were discussed during the conference call, several were not covered. Important remaining questions will need to be addressed in future TWG meetings.

## 1. Implications of Detecting HLB Directly in the Psyllid

### A. Research Update

Several scientists in the TWG have active research programs for detecting HLB and/or elucidating the biology of the HLB pathogen in the psyllid vectors. Important questions to be addressed by the TWG included: What are the criteria for positive infection? Does the detection of infected psyllids mean that infected plants are in proximity? Is there a difference in the detection of infected adults vs. nymphs?

The consensus was that only a varying proportion of psyllids feeding on infected trees may become capable of spreading infection. Values attained to date have ranged from 0 to 40%. Scientific literature from China indicates that acquisition of the bacterium as nymphs results in high infection rates. Limited experience with nymphs indicates that high percentages (up to 40%) of infected psyllids can be obtained after feeding on infected plants as nymphs. No experiments have been conducted so far to differentiate acquisition efficiency when comparing males and females.

The biology of the HLB bacterium in the psyllid was also discussed. A few journal reports of the presence of large amounts of bacteria in haemolymph and salivary glands indicate that the bacteria may be propagating in the vector (Moll and Martin 1973<sup>1</sup>; Xu *et al.* 1988<sup>2</sup>); however, the TWG feels that this is not enough evidence to make definitive conclusions and that additional research still needs to be done in this area. (If HLB propagates in the vector, a general characteristic of this relationship is an increase in transmission efficiency over time.) The literature indicates that no transovarial transmission of the bacterium occurs (Hung *et al.*, 2004<sup>3</sup>), even though this is known to occur with the related *Ca. L. africanus* in the African citrus psyllid (*Trioza erytrae*) (van den Berg *et al.* 1992)<sup>4</sup>. However, more work in this area also is merited. If HLB is not transmitted transovarially, then eggs of the psyllid present in nurseries are not likely to be a pathway for HLB spread. This also makes it more likely that if infected psyllids are detected, infected plants are the source and likely to be relatively close to the point where the psyllids were detected. The TWG noted that nymphs are relatively sessile. Adults move, but the movement pattern is observed to be mostly to nearby hosts. However, intermediate- or long-distance movement of adults cannot be discounted, and detailed research on the movement of psyllids is still needed. It was noted that nursery plants carrying eggs meant that the plants were visited by psyllid adults. If those psyllids were

---

<sup>1</sup> Moll, J. N. and Martin M. N. 1973. Electron microscope evidence that citrus psylla (*Trioza erytrae*) is a vector of greening disease in South Africa. *Phytophylactica* 5: 41-44.

<sup>2</sup> Xu, C.-F., Xia, Y.-H., Li, K.-B., and Ke, C. 1988. Further study of the transmission of citrus huanglungbin by a psyllid, *Diaphorina citri* Kuwayama, pp. 243-248 In L. W. Timmer, S. M. Garnsey, and L. Navarro [eds.], Proc. 10th Conference of the International Organization of Citrus Virologists. Riverside, CA.

<sup>3</sup> Hung, T.H., Wu, M.L., and Su, H.J. 2004. Identification of alternate hosts of the fastidious bacterium causing citrus greening disease. *J. Phytopathology* 148:321-326.

<sup>4</sup> Vandenberg, M. A., VanVuuren, S. P. and Deacon, V. E. 1992. Studies on greening disease transmission by the citrus Psylla, *Trioza erytrae* (Hemiptera: Triozidae). *Israel J. Entomol.* 25-26: 51-56.

infected with HLB and fed on these nursery plants during ovipositing, they should be considered as compromised. It also was noted that feeding psyllids (especially nymphs) may bio-accumulate the bacterium as it is pumped from phloem, through the psyllid and excreted, with the implication that the psyllid may reach detectable bacterial levels quicker than infected hosts.

## B. Update on Survey in Texas

In February 2007, psyllids collected by Dr. John DaGraca's research group in the Corpus Christi area of Texas were sent to Dr. Keremane Manjunath, a research scientist at the USDA-ARS National Clonal Germplasm Repository for Citrus and Dates in Riverside, California. Of the six samples tested using Real-Time PCR, two citrus samples showed positive HLB reactions. The scientists realized the regulatory significance and the select agent status of this data and properly forwarded the DNA samples and other specimens connected with these two positive testing samples to the APHIS-PPQ National Plant Germplasm and Biotechnology Laboratory (NPGBL). The remaining DNA samples and unprocessed psyllids that were forwarded to the NPGBL could not be confirmed as positive, using both the test performed in Riverside or the validated test used by PPQ.

Because of the mean  $C_t$  (cycle threshold) value from Real-Time PCR obtained from putative positive psyllids, the limited numbers of psyllids collected and tested, and the failure to detect HLB in remaining specimens received from ARS, no federal confirmation of HLB presence in Texas could be made. A decision was made by Texas state and federal regulatory agencies to conduct an intensive survey by gathering samples of host plants and psyllids in the immediate vicinity (1-mile radius) of the original collection site.

Stuart Kuehn provided an update on the survey for the TWG. A one-mile survey was conducted around the potential source of the psyllids on Mustang Island (near Corpus Christi). A total of 1397 residences and one nursery were surveyed for psyllids and symptomatic citrus or orange jasmine. Suspect plant samples and any psyllids found were collected and sent to the NPGBL in Beltsville. There were very few psyllids found in the area this time of year. All samples from this collection were determined to be negative.

## C. Biology of Psyllids for Survey of HLB

Several factors of the HLB pathosystem have to be better understood before it can be determined when and how to best use psyllids as a survey tool for the presence of HLB in an area. The TWG was asked to discuss factors that are known about the pathosystem so that information gaps and areas for future research can be identified.

The first consideration discussed was the relationship between symptoms on plants and the proportion of infected psyllids. It was observed that positive psyllids have been

detected in regulated areas in Florida up to nine months before symptomatic plants were found. Other TWG members noted that symptomatic trees produced the highest proportion of positive psyllids. In early epidemics in commercial citrus groves, it is observed that positive trees tend to occur in epicenters of 20-50 tree spaces, after which much less disease is found. This indicates that psyllids may not routinely travel far from infested trees. However, longer distance spread also might occur. The eastern borders of several large commercial citrus groves on the western edge of the Everglades have high levels of HLB incidence. The most plausible sources of inoculum are coastal urban communities. At least one psyllid, *Bactericera cockerelli*, is known to migrate transcontinentally, so flights of 50 miles or so are not out of the question for other psyllid species.

Another consideration for the TWG was to determine the best seasons for sampling of both psyllids and plants. It was observed by several TWG scientists that in Florida the highest percentage of HLB positives from plant samples were collected in August and September after a flush occurred. Positive psyllid samples collected during the spring months were at a lower percentage. Periods when host plants are in active flushes of growth appear to affect the ability to detect the bacterium. A drop in bacterial titer during flush is commonly observed. This corresponds to periods of the maximum psyllid population growth. The ability to detect the bacteria in plants increased if collected just after flush, with the fall flush (August and September) more conducive than the spring flush.

The TWG discussed the most appropriate means to collect psyllids based on the biology of the organism under the various environmental conditions at likely survey sites. The Halbert and Manjunath review in the journal "Florida Entomologist" (Halbert and Manjunath, 2004<sup>5</sup>) cites research that yellow sticky traps worked best (on sunny days whereas 'brown yellow' traps worked best on cloudy days). The optimum height for capture is 1.5 meters (Aubert and Hua 1990<sup>6</sup>). As noted previously, using sticky trap specimens to assay for HLB is problematic. First, the pathogen loses detectability if the trapped psyllid specimen is not processed by DNA extraction soon after trapping. In addition, only adults will be caught in this manner and may not be directly associated with a host plant.

The regulatory meaning of a positive find from a trap was not resolved. However, consideration was given to this trapping method being used in areas where psyllids are not known to occur and where nodes of host movement occur, for example in packing

---

<sup>5</sup> Halbert, S.E., and Manjunath, K.L. 2004. Asian citrus psyllids (Sternorrhyncha: Psyllidae) and greening disease of citrus: a literature review and assessment of risk in Florida. *Florida Entomologist* 87(3):330-353.

<sup>6</sup> Aubert, B. and Hua, X.-Y. 1990. Monitoring flight activity of *Diaphorina citri* on citrus and *Murraya* canopies, pp. 181-187 In B. Aubert, S. Tontyaporn, and D. Buangsuwon [eds.], *Rehabilitation of Citrus Industry in the Asia Pacific Region*. Proc. Asia Pacific International Conference on Citriculture, Chiang Mai, Thailand, 4-10 February 1990. UNDP-FAO, Rome.

houses and where unwashed fruit shipments are stored. For this situation, yellow sticky traps would be the most appropriate type to use<sup>7</sup>.

Another suggestion for the best means of collecting large numbers of adult psyllids was to tap infested branches three times so that psyllids jump or fall onto a collection sheet (Jawwad Qureshi, unpublished data). Infested trees can accumulate massive numbers of psyllids, more than 41,000 per tree in one study (Ahmad 1961<sup>8</sup>). The TWG discussed that the most recent information collected on the HLB pathosystem is based on observations made in infested areas of south Florida, and that conditions in Texas or elsewhere could have different effects on the pathosystem.

#### D. Diagnostic Considerations of Using Psyllids for Survey of HLB

Because making diagnostic determination of HLB presence in psyllids uses a published procedure currently only used in research and has not been validated for regulatory survey purposes, it was important to evaluate the methods and procedures currently being used along with the results obtained thus far. It was recommended that one diagnostic method be established and used so that results could be compared between laboratories, as necessary. Researchers agreed to share details of extraction methods, probes and primers in use for this purpose. No single method was used by researchers to extract psyllid DNA and a variety of primers and probes were in use. Chemical degradation of DNA or accumulation of DNA reaction inhibitors can occur depending on the source and storage of samples used for DNA extraction. A diagnostic assay for regulatory purposes also needs to include a psyllid DNA internal control to verify the quality of the DNA extracted so that a negative-testing sample is not a result of the poor quality of the DNA extraction. The ability to detect the bacteria in the psyllid and diagnostic means of confirming determinations if regulatory action is going to be pursued needs to be elucidated.

The TWG agreed that bacterial infections in single psyllids can be detected using Real-Time PCR. If the  $C_t$  value from Real-Time PCR is low enough (indicating high concentrations of the target bacteria), then conventional PCR can be used as a confirmatory assay. The TWG scientists that have been studying this indicate that although a single infected psyllid can give a  $C_t$  value in the low 20's (indicating a high enough concentration of bacteria to be useful in less sensitive tests), many of the positive psyllids have a  $C_t$  value of around 28 (cycles), which indicates low concentrations of bacteria in psyllids. It was determined that obtaining consistent conventional PCR bands from single psyllids will be difficult, although it is possible in some cases. However, it was also noted that the sensitivity of conventional PCR varied with the target gene, with the Bove 16S primer pair being less sensitive than the  $\beta$ -operon pair. The TWG agreed

---

<sup>7</sup> Although blue sticky traps are reported to provide a consistent catch and catches fewer non-targets, the highest trap catch (up to 500 feet away from infected tree) occurs when using yellow traps. David Hall is reporting this find in a manuscript that will be published next month (David Hall, pers. comm.).

<sup>8</sup> Ahmad, R. 1961. Citrus psylla: Its damage, population and efficacy of some insecticides against it. Pakistan J. Science 13: 195-200.

that the Real-Time PCR currently in use is more sensitive than any current conventional PCR in use, but there is a need for an additional confirmatory assay. Compositing samples in batches of 3 – 10 psyllids was commonly done in the research laboratories. If enough of the psyllids in a composite sample are infected, these batched DNA extracts contained enough bacterial DNA concentrations to produce Ct's lower than 28 and also conventional PCR bands intense enough for DNA sequencing. In these cases, confirmation of positives would be facilitated.

Preservation of psyllids for collection and extraction is another important component of effective diagnostics. It was noted that psyllids properly stored in ethanol at -20 C. for 1 ½ years have no noticeable change in detectability. A TWG member observed that psyllids collected from sticky traps lose bacterial titer over time and couldn't be detected after seven days. Therefore, survey traps need to be checked at very close intervals (every 1-3 days).

## **2. Potential Role of *Murraya* species in the Spread of HLB**

### **A. Research Update**

Zhou *et al.* 2007 reports the successful infection of citrus and re-isolation of an isolate of the HLB pathogen from *Murraya paniculata* plants from a nursery in Miami using dodder (*Cuscuta pentagona*, a parasitic plant)<sup>9</sup>. Although this report doesn't characterize the role of the insect vector in the infection of *Murraya*, the ability to support replication of the pathogen in *M. paniculata* is significant in determining risk.

Research at ARS, Ft. Detrick is focused on the role of *Murraya* spp. and the psyllid vector in the potential spread of HLB. Vern Damsteegt presented to the TWG updates of his experiments as follows: 1) psyllids from infected citrus were given inoculation access on *Murraya* (50 psyllids/plant for 2 weeks were used), 2) psyllids were then killed with insecticide, 3) the plants were grown for up to two months prior to assay, and 4) plants were then back-inoculated to sweet orange using healthy psyllids. Infection of *Murraya* occurred as determined by both symptoms on sweet orange and by PCR. Although the infected *Murraya* do not appear to grow as vigorously as adjacent healthy plants, no HLB-like symptoms were observed. HLB-like symptoms have been observed on *M. paniculata* plants by Susan Halbert and others in Florida. In contrast, Wenbin Li reported that similar experiments where HLB-infected orange was successfully grafted onto *Murraya* or where extracted bacterial suspensions were injected into *Murraya*, no HLB bacteria were detected in plants by PCR. However, the report of Zhou et al., and data presented by Vern Damsteegt provides evidence that the modified Koch's postulates may have been fulfilled. (The inability to culture HLB makes fulfilling the traditional version of Koch's postulates impossible.)

---

<sup>9</sup> Zhou, L.J., Gabriel, D.W., Duan, Y.P., Halbert, S.E., and Dixon, W.N. 2007. First report of dodder transmission of huanglongbing from naturally infected *Murraya paniculata* to citrus. Plant Dis. 91:227.

## B. *Murraya* Speciation in the HLB Pathosystem

The TWG discussed potential botanical differences between *Murraya paniculata* and *M. exotica*. Published research out of Australia differentiates these as two species, with *M. exotica* thought to be susceptible to the HLB pathogen and *M. paniculata* reported as resistant. There is a RAPD primer that has been used to differentiate the two species (OPN19). The “Handbook to Plants of Ceylon” by Stone describes *M. exotica* as “a hybrid of unknown parentage” and that the two species were “indefinite and overlapping.” The US Agricultural Research Service GRIN database cites *M. exotica* as a synonym of *M. paniculata*, whereas the Natural Resources Conservation Service (NRCS) “Plants” database lists *M. paniculata* as a synonym of *M. exotica*. It was noted that botanists at FDACS-DPI consider *M. paniculata* to be the species in the Florida nursery trade.

In addition, *Murraya koenigii* as a psyllid host was discussed, some panel members observe that psyllids prefer this plant much more than *M. paniculata*<sup>10</sup> when these two species are placed together. *Murraya koenigii* is now placed under a new genus, *Bergera*<sup>11</sup> and is listed as such in the USDA GRIN database. Some TWG members discussed the need to study this plant as a host of HLB as well.

It is possible that *Murraya paniculata* and *M. exotica* in the U.S. being are sold in the current nursery industry, with considerable confusion in the differentiation between the two (if indeed they are distinct). It was noted by Tim Gottwald that his observations of *Murraya* in Brazil are more consistent with *M. exotica* morphology than that described for *M. paniculata*. In addition, there are at least two varieties of *M. paniculata* (designated Chakas and Lakeview), with differences in morphology. A final note on the subject was it may not only be differences in the genetics of the host that account for the apparent differences in the susceptibility of *Murraya*, but that there is no information on the genotypes of the pathogen used for these experiments. Bove’s research shows that genotypic differences occur with this pathogen. From a regulatory standpoint, if these two putative plant species cannot be distinguished readily from one another, it would be prudent to regulate all species within the genus. The TWG strongly recommended regulating species of *Murraya* because they all are preferred hosts of the psyllid and can serve as a means of distributing infected psyllids far and wide and also probably the HLB pathogens if the psyllids are infected.

---

<sup>10</sup> Beattie, G. A. C., Holford, P., Mabberley, D.J., Haigh, A., Bayer R., and Broadbent P. 2006. Aspects and Insights of Australia-Asia Collaborative Research on Huanglongbing. JIRCAS HLB Conference Japan pp. 47-64.

<sup>11</sup> Samuel, R., Ehrendorfer, F., Chase, M.W., and Greger, H. 2001. Phylogenetic analyses of Aurantioideae (Rutaceae) based on non-coding plastid DNA sequences and phytochemical features. *Plants* 77-87.

## Recommendations

### A. Survey of HLB in the Asian Citrus Psyllid

The TWG agreed that the use of the psyllid to detect incipient infections of HLB in areas where the disease is not known to occur is potentially a useful survey tool. However, the implications of diagnostics to survey situations opened many questions that still need addressing before an effective survey program should be fully implemented. The ability to detect the bacteria in a single infected psyllid appears to occur more consistently only when using Real-Time PCR. The detection of the bacteria in a single infected psyllid appears to be near the current detection limits of this technology. Confirmation of Real-Time PCR positive finds for regulatory actions may be problematic because conventional PCR may not be sufficiently sensitive to serve as a reliable confirmatory assay. In any case, actions should be considered in cases of limited detection (such as increased survey); however, without multiple positive psyllids and confirmatory diagnostics, any actions must be contemplated carefully.

This constraint may be mitigated partially using composited DNA extractions of psyllids, but the validation of the diagnostics for identification or implications of positives in a sample containing multiple psyllids will need to occur. This presents an interesting potential paradox, in that composite samples of [infected] psyllids will increase the number of DNA targets that can be amplified and detected, however, potential inhibitory components from psyllid DNA extracts may become problematic and detection of single infected psyllids in a composite sample may be reduced due to dilution of the target DNA. The parameters of detection for compositing psyllids will need to be established, as well as any potential for inhibition of PCR in batched samples.

In addition, it was noted that psyllid surveys using the current technology will likely have an unacceptably high false negative rate, which means that the failure to detect will not provide assurances that disease is absent from an area, based on the low percentage of positive psyllids found in feeding experiments. In order to determine the significance of such surveys using the current diagnostics, population studies on the psyllids need to be conducted in conjunction with infection rate, symptom expression within the plant throughout the course of the year, etc. In the absence of dramatic improvements in diagnostics, awareness of the problems of false negatives should be included in the survey manual SOP.

Even with these caveats, the TWG members strongly recommended that a survey of psyllids occur in Texas, both in the Rio Grande valley and in Corpus Christi. This survey should be conducted so that resources for the effort are maximized for the optimum time of year to collect psyllids.

The TWG also discussed the current situation in Mexico, since the Asian citrus psyllid is known to occur there. The government of Mexico reported that no symptomatic plants in the 23 citrus-producing areas in Mexico have been observed, but the psyllid is

widespread. Ted Boratynski was going to Mexico for an exchange on the current situation and updates on diagnostics.

The TWG recommended that the USDA APHIS New Pest Response Guideline incorporate changes for the survey program to include testing of psyllids for bacteria. The TWG discussed some observations on the best timing for this kind of survey, but documented studies on the characteristics of this kind of survey still are lacking. In addition, observations made by the TWG that may be currently relevant for Florida may not be applicable for Texas or California survey efforts.

It was noted that a lab certification program for HLB diagnostics used by USDA APHIS currently is being developed so that labs outside PPQ can provide regulatory determinations on most HLB samples. This program will be similar in design to the Provisional Laboratory Approval program for molecular diagnostics of *Phytophthora ramorum*. Labs that are already participating in the Provisional Approval program will not need to have a site inspection. At this time, a validated diagnostic is available for plant samples and proficiency tests for HLB in plants will be available in about June. Work is being conducted to adapt the HLB diagnostics so that a validated protocol and proficiency test can be developed for psyllids. Currently, all putatively positive psyllid DNA extracts are being considered by the pending Potentially Actionable Suspect Samples (PASS) policy. When complete, the PASS document will establish which biological and chemical samples must be forwarded to NPGBL, Beltsville, for federal confirmation for HLB.

#### B. *Murraya* Species as a Host for the HLB Pathogen

The TWG recommends that *Murraya* spp. should be regulated. Texas, California, and Arizona should prohibit entry of *Murraya* spp. from Florida. *Murraya* from Florida should be prohibited to prevent disease and/or vector spread or introduction into unregulated areas. There is sufficient scientific evidence to justify these restrictions. This can be done by amending the existing Federal Order or drafting a new rule specific to HLB.

It was noted that psyllid control is needed for nurseries that sell either citrus or *Murraya* plants, because nursery environments for these plants allow for the constant presence of psyllids. Infected psyllids could potentially bridge perpetuation of HLB between blocks of plants, even if the plants are removed and replaced. In general, current regulations are not designed for mitigation of diseases caused by vectored pathogens that are characterized by long latent periods after infection.

## **Action Items**

Although the conference call format didn't allow discussion to occur on all the questions forwarded to the TWG in the agenda, sufficient science-based information was presented for a number of recommendations. The TWG was invited to provide written responses to specific questions for program consideration and future discussion, and many of these are incorporated into this report.

Because of the recent increase in research and regulatory efforts with the HLB pathosystem, it will be necessary to have follow-up meetings to make recommendations as new information arises. The meeting of the TWG may become a regular event, perhaps occurring once every few months.

## Appendix I:

### 2007 Huanglongbin Technical Working Group

Date: March 13, 2007  
Time: 2:00 PM – 5:00 PM  
Phone#: 866-648-8079  
Passcode: 6102825#

Format: This is a 'virtual' technical working group that will take place as a phone conference. The most updated background material will be provided to the group members for preparation. After a brief introduction, the group will discuss scientific questions on the biology of this pathosystem so that responses to the main tasks can then be addressed. This conference is scheduled for three (3) hours, but the phone lines will remain open beyond this allotted time, if necessary

**Mission: To provide technical guidance to state and federal officials on regulation of asian citrus psyllid (*Diaphorina citri*) vectors of Huanglongbin (*Candidatus Liberibacter* species).**

Introduction – Housekeeping and Objectives

Phil Berger

Updates of detection if HLB in psyllids

Review of 2006 HLB Technical Working Group

#### **Tasks:**

1. Summarize the scientific meaning of detecting *Ca. Liberibacter* spp. (HLB) in psyllids?
2. What should the regulatory response to detection of HLB in psyllids be, from a technical/scientific standpoint?
3. What does it mean to find different levels of the bacteria in the psyllids?
  - a. Specificity and selectivity of diagnostics.
  - b. Epidemiological implications.
    - Is it possible to delimit an infestation of HLB-positive psyllids?
  - c. What conclusions can be made about *Murraya* as a host and/or vehicle of spread.

Specific Questions:

#### **Survey**

Psyllids:

4. Is there a practical field diagnostic for infected psyllids that can be used for survey?
5. What information would data on a psyllid survey generate?
6. When is sampling psyllids via PCR analysis appropriate / useful in the survey program?
7. Can composite samples of psyllids be made from field collections and a PCR diagnostic be applied for surveying larger growing areas?

Trees :

8. Based in the relative effectiveness of each of the detection survey strategies:
  - Which strategy is most cost-effective? (Effectiveness= defined as most likely method of detecting HLB in various settings:
    - a. Hot-zone/demographic
    - b. Sentinel residential survey
    - c. Sentinel grove survey
    - d. Current rapid delimiting HLB survey using transects/concentric annuli
    - e. Vector survey
    - f. Self inspection by industry and home owners
  - Which of these strategies provide the most useful information?
  - Are there survey data not currently being collected that could assist in long-term evaluation of the problem?
9. What state or federal regulations should be enacted prior to the discovery of HLB to provide the best means of containment?
10. What are the implications of early detection of HLB (in psyllids) and does this present an opportunity for eradication or control?

**Diagnosis**

10. Does it make sense to restrict all the testing for HLB (psyllids or plant material) to just certain federal labs?
11. What is the current situation of the laboratory approval program for local diagnostic testing for HLB?
  - What steps can be taken to increase throughput and/or decrease time needed to provide definitive diagnostic results.

**Refining research efforts:**

12. What research initiatives are needed to develop better early detection techniques for HLB?

13. Which research initiatives will speed up progress to determine vector transmission principles for use in regulatory programs?

14. Is Orange jasmine a host for HLB as well as psyllids?

### **Mitigation, Control, Regulatory Action**

15. Are different state or federal laws/regulations needed to respond to HLB in psyllids as compared to plant material?

- Should psyllid hosts be regulated in the same way as HLB hosts?

16. What is the appropriate buffer distance for quarantine on host nursery stock near a positive detection of HLB in Texas?

- Would this apply to psyllid hosts as well as HLB hosts?

- What restrictions on regulated articles would be recommended?

17. If positive trees are discovered in Corpus Christi:

a. Can a quarantine program prevent spread to the Rio Grande Valley?

b. What is the situation with residential hosts in Rio Grande?

d. What if only infected psyllids are found?

c. Is there a reasonable distance for the removal of exposed trees to (1) minimize the disease and (2) eliminate it from an area?

d. What has been applied in foreign countries to control HLB spread? How effective is it?

18. Would the response to Questions 15, 16, and 17 be different if the pathogen is determined to be *L. americanus* or *L. africanus*?

19. What are the best management practices (BMP's) for groves in Texas?

20. What are the BMP's for nurseries where HLB occurs and for nurseries where it is not known to occur? What has been applied in Florida and foreign countries to control HLB spread? How effective is it?

21. Can chemical controls for psyllids on large trees in groves be used to protect the Rio Grande industry from infection?

22. If HLB is introduced to the Rio Grande, will vector control protocols be different than current Florida situation?

### **CONCLUSIONS**

Action Items.

Adjourn

**Appendix II:****2007 Huanglongbin Technical Working Group  
Participant List**

Wayne Dixon	<a href="mailto:dixonw@doacs.state.fl.us">dixonw@doacs.state.fl.us</a>	352 372 3505 ext 118
Tim Gottwald	<a href="mailto:tgottwald@ushrl.ars.usda.gov">tgottwald@ushrl.ars.usda.gov</a>	772 462 5883
David Hall	<a href="mailto:Dhall@ushrl.ars.usda.gov">Dhall@ushrl.ars.usda.gov</a>	772 462 5897
Mike Irey	<a href="mailto:msirey@ussugar.com">msirey@ussugar.com</a>	863 902 2249
John Hartung	<a href="mailto:hartungj@ba.ars.usda.gov">hartungj@ba.ars.usda.gov</a>	301 504 6571
Susan Halbert	<a href="mailto:halbers@doacs.state.fl.us">halbers@doacs.state.fl.us</a>	352 372 3505 ext 185
Vern Damstreegt	<a href="mailto:vern.damsteegt@ars.usda.gov">vern.damsteegt@ars.usda.gov</a>	301 619 7307
	<a href="mailto:vdamsteegt@fdwsr.ars.usda.gov">vdamsteegt@fdwsr.ars.usda.gov</a>	
Tim Schubert	<a href="mailto:schubet@doacs.state.fl.us">schubet@doacs.state.fl.us</a>	352 372 3505 ext 143
Michael Rogers	<a href="mailto:mrogers@crec.ifas.ufl.edu">mrogers@crec.ifas.ufl.edu</a>	863 956 1151
Keremane Manjunath	<a href="mailto:rivmk@ars-grin.gov">rivmk@ars-grin.gov</a>	951 827 4399
Magally Luque-Williams	<a href="mailto:mlwilliams@jps.net">mlwilliams@jps.net</a>	951 782 3271
Russ Bulluck	<a href="mailto:russ.bulluck@aphis.usda.gov">russ.bulluck@aphis.usda.gov</a>	919 855 7646
Phil Berger	<a href="mailto:philip.h.berger@aphis.usda.gov">philip.h.berger@aphis.usda.gov</a>	915 855 7412
John De Graca	<a href="mailto:j-dagraca@tamu.edu">j-dagraca@tamu.edu</a>	956 968 2132
Shashank Nilakhe	<a href="mailto:Shashank.Nilakhe@agr.state.tx.us">Shashank.Nilakhe@agr.state.tx.us</a>	512 463 1145
Phillip Mason, Ph.D.	<a href="mailto:Phillip.A.Mason@aphis.usda.gov">Phillip.A.Mason@aphis.usda.gov</a>	970 494 7565
Patrick Gomes	<a href="mailto:Patrick.J.Gomes@aphis.usda.gov">Patrick.J.Gomes@aphis.usda.gov</a>	919 855 7313
Paul Parker	<a href="mailto:Paul.E.Parker@aphis.usda.gov">Paul.E.Parker@aphis.usda.gov</a>	956 586 7301
Stuart W. Kuehn	<a href="mailto:Stuart.W.Kuehn@aphis.usda.gov">Stuart.W.Kuehn@aphis.usda.gov</a>	512 916 ,5241
Ted Batkin	<a href="mailto:ted@citrusresearch.org">ted@citrusresearch.org</a>	559 738 2460
Wenbin Li	<a href="mailto:Wenbin.li@aphis.usda.gov">Wenbin.li@aphis.usda.gov</a>	301 504 7100
Laurene Levy	<a href="mailto:Laurene.Levy@aphis.usda.gov">Laurene.Levy@aphis.usda.gov</a>	301 504 7100
Joel Floyd	<a href="mailto:Joel.P.Floyd@aphis.usda.gov">Joel.P.Floyd@aphis.usda.gov</a>	301 734 4396
Mamoudou Setamou	<a href="mailto:MSetamou@ag.tamu.edu">MSetamou@ag.tamu.edu</a>	956 968 2132
Richard Lee	<a href="mailto:rivrl@ars-grin.gov">rivrl@ars-grin.gov</a>	951 827 4399
Patrick Shiel	<a href="mailto:Patrick.J.Shiel@aphis.usda.gov">Patrick.J.Shiel@aphis.usda.gov</a>	919 855 7416
Dan Fieselmann	<a href="mailto:Daniel.A.Fieselmann@aphis.usda.gov">Daniel.A.Fieselmann@aphis.usda.gov</a>	919 855 7415
Don Seaver	<a href="mailto:Donald.M.Seaver@aphis.usda.gov">Donald.M.Seaver@aphis.usda.gov</a>	919 855 7448
Lisa Jackson	<a href="mailto:Lisa.D.Jackson@aphis.usda.gov">Lisa.D.Jackson@aphis.usda.gov</a>	919 855 7549