

Recovery Plan

for

Huanglongbing (HLB) or Citrus Greening

caused by

"Candidatus" Liberibacter africanus, L. asiaticus, and L. americanus

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This recovery plan is one of several disease-specific documents produced as part of the National Plant Disease Recovery System (NPDRS) called for in Homeland Security Presidential Directive Number 9 (HSPD-9). The purpose of the NPDRS is to insure that the tools, infrastructure, communication networks, and capacity required to mitigate the impact of high consequence plant disease outbreaks are such that a reasonable level of crop production is maintained.

Each disease-specific plan is intended to provide a brief primer on the disease, assess the status of critical recovery components, and identify disease management research, extension, and education needs. These documents are not intended to be stand-alone documents that address all of the many and varied aspects of plant disease outbreak and all of the decisions that must be made and actions taken to achieve effective response and recovery. They are, however, documents that will help USDA guide further efforts directed toward plant disease recovery.

Executive Summary

Huanglongbing (HLB) of citrus, also known as citrus greening is recognized as a major threat to citrus production around the world. In the United States, the harvested citrus acreage has averaged about one million acres in the past 10 years. This includes oranges, grapefruit, lemons, tangelos, tangerines and temples. In 2004, citrus production yielded 16.4 million tons of fruit valued at 2.35 billion dollars. U.S. production is expected to be seriously affected by HLB.

HLB was detected on two homeowner trees in South Florida on August 23 and confirmed on September 2, 2005. The Florida Department of Agriculture and the USDA Animal Plant Health Inspection Service are conducting an extensive survey in Florida and other regions of the country. HLB appears well established in Florida. The State of Florida feels eradication is no longer possible and that we must shift gears to disease management.

Because of the discovery of HLB in Florida, there can be no doubt that the citrus industry in the United States is threatened. This disease effectively reduces the quantity and quality of citrus fruits, eventually rendering infected trees useless. In areas of the world affected by HLB the average productive lifespan of citrus trees has dropped from 50 or more years to 15 or less. This is an especially insidious disease because it displays few symptoms in its early stages and may escape detection by our current diagnostic methods. The arrival of the insect vector of HLB, the Asian citrus psyllid, in 1998, made it inevitable that the HLB pathogens would spread if they were introduced. It is feared that this vector, now in the presence of the HLB, has spread HLB beyond our detection capabilities and therefore the ability to practice effective eradication.

Improvement of survey methods is a key component of this recovery strategy. A basic limitation of current surveys for HLB is the lack of accurate and rapid detection techniques. Other critical research needed to effectively confront HLB includes: determining the host range of the HLB pathogens, clarifying vector-plant interactions, producing resistant citrus by transgenic or other means, developing the capacity to culture the pathogens of HLB, and clarifying our understanding of the basic biology of the Asian citrus psyllid, the disease vector.

Insecticides have been the first line of defense to manage the vectors of HLB. Unfortunately, while this strategy may be effective, we do not know how effective it is; at what rate and how often to apply insecticides; or which insecticides to apply (we do have some preliminary data). This is complicated in the U.S. by the lack of specific pesticide labeling for the Asian citrus psyllid, a situation currently being addressed by several pesticide companies.

We will depend on the effective use of disease-free nursery plants coupled with vector management and cultural controls. Citrus production areas outside of Florida will be protected by quarantines, vector control and early detection. Surveys will play an important role in managing this disease and its vector.

Huanglongbing (HLB) or Citrus Greening

(caused by "*Candidatus*" *Liberibacter africanus*,
L. asiaticus, and *L. americanus*)

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Reviewer: The American Phytopathological Society

I. Introduction

Huanglongbing (HLB) is the recognized common name adopted by the International Organization of Citrus Virologists (Da Graca and Korsten, 2004) and the American Phytopathological Society (Timmer et al., 2000). It was first described in 1956 in Chinese and described in English as the "yellow shoot disease" (Lin, 1956). It has also been called citrus greening and yellow dragon disease. HLB is probably the most serious disease of citrus worldwide. Many authors suggest that HLB originated in China or India in the late 1800s (Bove, 2006; Da Graca and Korsten, 2004; Gottwald et al., 1989; Halbert and Keremane, 2004; Timmer et al., 2000). HLB seriously affects production of citrus in India, Asia, Southeast Asia, the Arabian Peninsula, and Africa. In countries where the disease is endemic, infected citrus seedlings decline within 5 to 8 years after planting and rarely bear usable fruit. Other names for HLB are likubin in Taiwan, leaf mottling in the Philippines, and vein-phloem degeneration in Indonesia.

The current range of HLB is in Africa (Burundi, Cameroon, Central African Republic, Ethiopia, Kenya, Madagascar, Malawi, Mauritius, Nigeria, Reunion, Rwanda, Somalia, South Africa, Swaziland, Tanzania, and Zimbabwe), Asia (Bangladesh, Bhutan, Cambodia, China, East Timor, India, Indonesia, Japan, Laos, Malaysia, Myanmar, Nepal, Pakistan, Papua New Guinea, Philippines, Saudi Arabia, Taiwan, Thailand, Vietnam, and Yemen) (Appel, 2004; Bove, 2006; CAB, 2003; Halbert and Keremane, 2004). In 2004, HLB was detected in South America in Brazil (Bove, 2006; Coletta Filho et al., 2004; Lopes et al., 2005; Teixeira et al., 2005) and recently, in 2005, North America in the United States (Bove, 2006; USDA/APHIS, 2005).

The pathogen that causes HLB is a non-culturable, phloem-inhabiting, Gram-negative bacterium. Three very similar pathogens have been described that cause HLB, one each from Africa, Asia and Brazil. They have been separated into three species: "*Candidatus*"

Liberibacter africanus, "*Candidatus*" *Liberibacter asiaticus*, and "*Candidatus*" *Liberibacter americanus*, named for the continent on which they were first found.

In Florida, the Asian species of HLB was identified in August 2005. The Asian citrus psyllid (*Diaphorina citri*) has been present in Florida since 1998 and is believed to be the primary means by which HLB is transmitted there (Appel, 2004; Bove, 2006; Coletta Filho et al., 2004; Halbert and Keremane, 2004; Teixeira et al., 2005; USDA/APHIS, 2005). The Florida Department of Agriculture and the USDA Animal Plant Health Inspection Service are conducting an extensive survey throughout Florida and in other citrus states. At least 652 trees in 12 counties in Florida have proven positive by PCR tests (DNA identification of the HLB pathogen). Most of the positive trees are in dooryards (557) but commercial plantings (95) also are involved (USDA/APHIS, 2006). A team of state and federal scientists will continue to monitor the incidence of HLB in Florida and develop an integrated strategy to manage this disease. Eradication of HLB is not seen as a possibility (University of Florida, 2006).

The Asiatic and African forms of the disease have indistinguishable symptoms. However, the African form develops only under cool temperatures of 20-25°C, while the Asian form develops under cool and warm temperatures (20-35°C). Information on the newly discovered form of the disease in Brazil is preliminary, but the pathogen appears to be more closely related to "*Candidatus*" *L. asiaticus* than to "*Candidatus*" *L. africanus*. This newly discovered pathogen is not recognized by primers (DNA probes) specific to either of the previously described species (Teixeira et al., 2005).

All citrus plants are potential hosts. Historically, the most susceptible hosts are sweet oranges, tangelos, and mandarins. Moderately susceptible hosts have been grapefruits, lemons, Rangpur lime, calamondins, and pummelos. Mexican limes and trifoliolate orange have been more tolerant. Non-citrus species, such as *Murraya paniculata*, may also serve as hosts of the HLB pathogens (Appel, 2004; Bove, 2006; Da Graca and Korsten, 2004; Halbert and Keremane, 2004; Lopes et al., 2005; Timmer et al., 2000).

II. Symptoms

Symptoms alone are not diagnostic because many plant pathogens or cultural conditions (fertility, weather, etc.) cause similar symptoms (Appel, 2004; Bove, 2006). Hence, caution is needed in concluding that a plant with the symptoms shown below is infected with one of the three "*Candidatus*" *Liberibacter* species.

The initial symptoms are frequently the appearance of yellow shoots or mottled leaves on a tree (Figure 1). As the bacteria move within the tree, the entire canopy progressively develops a yellow color, retarded growth and tip necrosis (Bove, 2006; Da Graca and Korsten, 2004; Halbert and Keremane, 2004; Timmer et al., 2000).

The most characteristic symptoms of HLB are a blotchy leaf mottle and vein yellowing that develop on leaves attached to shoots showing the overall yellow appearance (Figure 2). These foliar symptoms may resemble other diseases or a zinc deficiency although the green and yellow contrast is not as vivid with HLB as it is with zinc deficiency. Leaves with HLB have a mottled appearance that differs from nutrition related mottling in that HLB-induced mottling usually crosses leaf veins. Nutrition related mottles usually are found between or



Figure 1. Yellowing of a citrus shoots, on left, caused by HLB; healthy trees on right. Photo courtesy Stephen M. Garnsey.



Figure 2. Leaf mottling caused by HLB. Photo courtesy J.M. Bové and M. Garnier. Reprinted from Timmer, L.W., Garnsey, S.M., and Graham, J.H. 2000, Compendium of Citrus Diseases, Second Edition, American Phytopathological Society, St. Paul, MN.



Figure 3. Misshapen and greenish fruit of citrus affected with HLB compared to healthy fruit shown in the lower left. Photo courtesy P. Broadbent. Reprinted from Timmer, L.W., Garnsey, S.M., and Graham, J.H. 2000, Compendium of Citrus Diseases, Second Edition, American Phytopathological Society, St. Paul, MN.

along leaf veins. Also, HLB-infected leaves may be small and upright (Halbert and Keremane, 2004).

Fruit from diseased trees are small, often misshapen, and typically some green color remains on ripened fruit (Figure 3). This is a key symptom, especially in the presence of aborted seeds, and is the origin of the common name "greening." The preferred name is currently huanglongbing or HLB. Yields are almost non-existent, and the remaining fruit are rendered worthless due to small size, poor color, and bad taste (Bove, 2006; Halbert and Keremane, 2004; Timmer et al., 2000).

HLB is an especially insidious disease because it displays few symptoms in its early stages, may escape detection with our current diagnostic methods, and may be vectored over a wide area before detection occurs. If this disease has entered the U.S. prior to the introduction of the citrus psyllid, the HLB vector, simple removal of unproductive trees probably eliminated the pathogens of HLB before they spread. After the discovery in 1998 in the U.S. of the citrus psyllid (Halbert and Keremane, 2004), it is feared that this vector, in the presence of the HLB, spread HLB beyond our detection capabilities and therefore the ability to practice effective eradication.

III. Spread

Long distance spread of HLB occurs by grafting with diseased budwood. Local spread of the disease may be due to grafting but is also attributed to two species of citrus psyllids.

Diaphorina citri is the primary carrier for the Asian form of the disease and presumably the American form of the disease. *Trioza erytreae* is the primary vector for the African form. However, both psyllids can transmit the Asian and African species that cause HLB, at least experimentally (Halbert and Keremane, 2004).

The Asian citrus psyllid (*Diaphorina citri*) is found only on citrus (crops and ornamentals) and closely related Rutaceae (citrus family). A preferred host is *Murraya paniculata*, an ornamental Rutaceae plant called jasmine orange that is often planted in the Southern United States from California to Florida. It is utilized in hedges and as Bonsai. A closely allied species, *Bergera koenigii*, is used as a backyard spice plant and is also a potential host. The Asian citrus psyllid was introduced, probably in early 1998, and spread to most citrus growing areas in Florida (Da Graca and Korsten, 2004; Halbert and Keremane, 2004). However, the bacteria that cause HLB have not been reported in the U.S. until very recently (Bove, 2006; USDA/APHIS, 2005). It has been noted that elsewhere in the world, when the vector appears, it is a matter of only a few years until HLB also appears (Appel, 2004).

Adult Asian citrus psyllids are small (3 to 4 mm) with mottled brown wings (Figure 4). Adults are active, jumping insects. Eggs are bright orange and deposited on newly emerging citrus tissue. Nymphs are green or dull orange, and feed on leaves and stems where they are difficult to see. The Asian citrus psyllid is most likely to be found on new shoots, and population increases occur during periods of active plant growth (Appel, 2004; Halbert and Keremane, 2004).

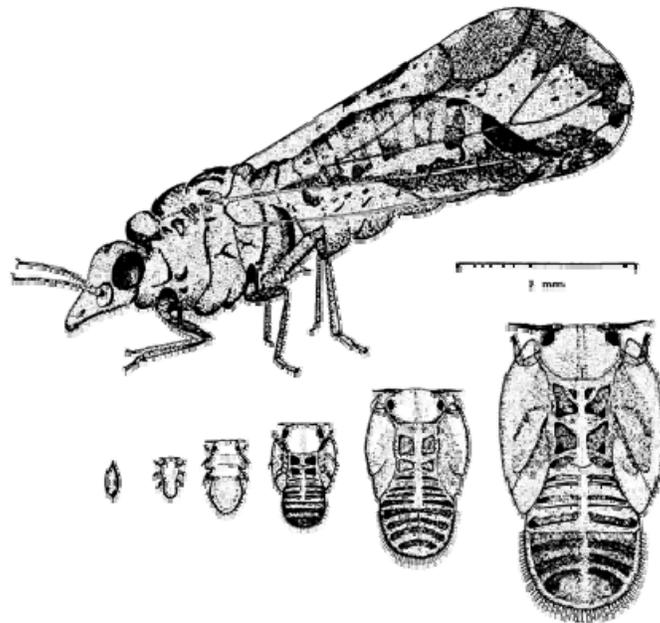


Figure 4. Adult female and nymphal instars of Asiatic citrus psyllid. Drawing courtesy of Frank Mead, Florida Department of Agriculture and Consumer Services. Reprinted from Catling, H.D., 1970, Distribution of the psyllid vectors of citrus greening disease, with notes on the biology and bionomics of *Diaphorina citri*, FAO Plant Protection Bulletin 18(1):8-15.

The interactions between the vectors and the pathogens are poorly understood. Acquisition times of between 30 minutes and 24 hours have been reported. The pathogen also may multiply in the vector. Adults and fourth and fifth instar Asian citrus psyllid nymphs can transmit *L. asiaticus* after 8-12 days. A shorter latent period of 1 day is reported for the African psyllid (Halbert and Keremane, 2004).

The African citrus psyllid and HLB are found in Africa, the Saudi Arabian Peninsula, and several islands in the Indian Ocean. The Asian citrus psyllid and HLB are found throughout Asia, in the Saudi Arabian Peninsula, and in the same islands in the Indian Ocean. The

Asian citrus psyllid also occurs in several South American and Caribbean countries, as well as in Florida and Texas in the U.S. The Asian citrus psyllid is very likely to be a vector of "*Candidatus*" L. americanus as well as "*Candidatus*" L. asiaticus in Brazil (Appel, 2004; Halbert and Keremane, 2004).

IV. Monitoring and Detection

Current Surveys

Presently, several states are conducting surveys for HLB or the psyllid vector of HLB.

Alabama – Currently Alabama is surveying for HLB in any area that has citrus, including ornamentals.

Arizona – The Arizona Department of Agriculture has a survey for HLB at the present time.

California – California has been conducting a citrus canker survey every spring that considers HLB. The survey is performed in a four-year cycle, with the intent to survey approximately 25% of the acreage each year. The State is divided into three geographical survey districts: Northern, Central and Southern and each district pathologist coordinates the survey within their district overseen by a state coordinator. In addition to surveying for citrus canker symptoms, crews are also trained in the identification of the psyllid *Diaphorina citri* (vector of HLB).

Florida – Beginning in the spring of 2004 the Florida Cooperative Agricultural Pest Survey (CAPS) team began an ongoing systematic survey for HLB in select areas within the state. Areas surveyed were chosen based on the expectation of HLB. Citrus plant samples collected in the field by inspectors were screened by departmental experts and highly suspect samples were tested by the PCR method (see the Detection and Diagnosis section below). Six rounds of the survey were completed: spring 2004, fall 2004, and spring 2005 in the greater Orlando area and the Tampa/St Petersburg area. In addition to CAPS, the Florida Division of Plant Industry completed an HLB survey from July 2004 to April 2005. Despite these extensive surveys, HLB was not found until a CAPS survey targeted likely farms in Homestead, Florida.

HLB was detected on two homeowner trees in South Florida on August 23 and confirmed on September 2, 2005 (USDA/APHIS, 2005). The Florida Department of Agriculture and the USDA Animal Plant Health Inspection Service are conducting an extensive survey throughout Florida and in other citrus states. At least 652 trees in 12 counties in Florida have proven positive by PCR tests (DNA identification of the HLB pathogen). Most of the positive trees are in dooryards (557) but commercial plantings (95) also are involved (USDA/APHIS, 2006). Southeast Florida is heavily infested, while southwest and south central Florida has a lighter infestation of HLB. A team of state and federal scientists will continue to monitor the incidence of HLB in Florida and develop an integrated strategy to manage this disease. APHIS and the State of Florida have instituted quarantines to limit the spread of HLB within Florida and to other states. Eradication of HLB is no longer seen as a possibility (University of Florida, 2006).

Louisiana – Currently surveying for HLB in any area that has citrus or its relatives, including ornamentals.

Texas – Texas is conducting surveys. The Texas Department of Agriculture inspectors are being trained to recognize HLB-like symptoms.

Quarantine interceptions at ports of entry – More than 400 seizures of citrus plants, leaves and budwood have occurred from passenger baggage since 1985. Many of those interceptions originated from countries known to have HLB. Therefore, routine passenger entry is a likely means by which HLB entered Florida.

Detection and Diagnosis

After the collection of suspect samples by surveyors, they are examined by specialists within each state or the National Plant Diagnostic Network (NPDN) labs. Any likely samples are tested by polymerase chain reaction (PCR), a DNA probe, for molecular identification of the HLB pathogens. However, the PCR primers may miss some HLB pathogens leading to false negatives. Also, the inability to culture the pathogens of HLB has limited detection capabilities. New methods of identification are being developed (Bove, 2006; Da Graca and Korsten, 2004).

The ultimate authority for confirming a diagnosis of HLB, since it is a select agent and exotic pest, rests with the Plant Protection and Quarantine (PPQ) division of USDA/APHIS. See the following section on USDA Pathogen Permits or the APHIS web site at <http://www.aphis.usda.gov/ppq>.

Biological indexing, electron microscopy, serology or some other technique is needed to provide a more robust general detection of HLB (Okuda et al., 2005). A purely molecular approach to diagnosis is not wise, since primers for “*Candidatus*” *Liberibacter africanus* and “*Candidatus*” *Liberibacter asiaticus* did not detect the new “*Candidatus*” *Liberibacter americanus* pathotype. A primer set specific for it has been prepared; however, the precedent is now established that other “*Candidatus*” *Liberibacter* variants may exist which would not be detected by any of the 3 probes.

Future Surveys

Surveys for HLB are hampered and rendered questionable by two important detection and sampling limitations. First, simple observations of the symptoms of HLB are often confused with others diseases and conditions. Second, citrus plants infected by the HLB pathogens may not show symptoms for years following infection. During this latent phase of HLB the detection technique, PCR, is ineffective.

The most likely route of HLB introduction into Florida was probably via smuggled HLB-infected plant material. For instance, in California citrus tristeza virus (CTV) was discovered in a young commercial grove in Fresno County in 1999. An investigation concluded that budwood infected with CTV was smuggled into California from Japan. Based on this experience and others, future movement of HLB by this route is a likely possibility.

In the future, surveys may be worthwhile on other hosts of the psyllid vectors of HLB, especially if those hosts are moved across state lines as are many ornamentals such as *Murraya* spp.

V. Response

While this plan is focused primarily on recovery, certain facets of the response to a new detection involve aspects of recovery with a continuum of activities from response to recovery. The response is under USDA, APHIS, Plant Protection and Quarantine’s authority delegated from the Secretary under the Plant Protection Act of 2000.

Generally, after a detection has been confirmed by a USDA, APHIS, PPQ recognized authority, APHIS, in cooperation with the State Department of Agriculture, is responsible for the response. The response is immediate in the form of advance assessment teams of experts and survey personnel sent to the site of initial detection to place holds, conduct investigations, and initiate delimiting surveys. Actions that may be taken include regulatory measures to quarantine infected or potentially infected production areas, stop the movement of infected or potentially infected articles in commerce, and control measures which may include host removal and destruction, and/or insuring adherence to required sanitary practices. APHIS imposes quarantines and regulatory requirements to control and prevent the interstate movement of quarantine-significant diseases or regulated articles, and works in conjunction with states to impose these actions parallel to state regulatory actions which restrict intrastate movement.

Huanglongbing is now considered established in Florida and is under management as part of the Citrus Health Response Plan. Once an HLB sample in another citrus growing state is confirmed positive by an APHIS recognized authority, an advanced technical team may be sent to the site as the first step in a response. A larger team would then be deployed consisting of state and federal regulatory personnel operating under a unified command within the Incident Command System. Survey teams will conduct delimiting surveys in the area using trace back and trace forward information and with various appropriate stratified delimiting sampling schemes for surveys in the area of detection. It is important as part of the response to control the movement of disease host and vector host nursery stock out of infested counties since this may serve as an avenue to infect other citrus production areas.

After the results of delimiting survey are known, if the disease is considered generally distributed through commercial and residential plant hosts in an area, options for control are very limited. In areas where the vector is present, such as in citrus growing areas of Texas, because of the lack of information about dispersal distance of the vector and what an appropriate buffer distance for tree removal around infected trees is, the response will likely be a long-term management strategy similar to the Citrus Health Response Plan in Florida. If the disease is isolated as it may be in an area where vectors are not present, there is a good chance for eradication by destroying all infected trees and any trees propagated from infected trees.

The "HLB New Pest Response Guidelines" document gives details of the response options discussed above. For more information, see the link to these guidelines at: http://www.aphis.usda.gov/ppq/ep/citrus_greening/index.html

VI. USDA Pathogen Permits

USDA/APHIS/PPQ permit and registration requirements for plant diseases and laboratories fall under two authorities, the Plant Protection Act (7 CFR Part 330) and the Agricultural Bioterrorism Protection Act of 2002 (7 CFR Part 331). Laboratories receiving suspect infected plant material or cultures are required to have PPQ permits. Laboratories possessing, using, or transferring select agents such as "*Candidatus*" Liberibacter spp., the causal agents of HLB, are required to be registered, however diagnostic laboratories that identify select agents are exempt from this requirement as long as they complete an APHIS/CDC Form 4 and destroy the culture within 7 days.

The Plant Protection Act permit requirements apply to all plant pests. This includes arthropod vectors of plant pathogens, such as the Asian citrus psyllid (*Diaphorina citri*) and infected plant material, including diagnostic samples, regardless of their quarantine status,

shipped interstate and require that the receiving laboratory have a permit. For further guidance on permitting of plant pest material, consult the PPQ permit website at: <http://www.aphis.usda.gov/ppq/permits/> or contact PPQ Permit Services at (301) 734-8758.

The Agricultural Bioterrorism Protection Act of 2002 (7 CFR Part 331) specifies requirements for possession, use, and transfer of organisms listed as select agents as are the HLB pathogens. Once an unregistered diagnostic laboratory identifies a select agent, they must immediately notify the APHIS Select Agent Program, complete an APHIS/CDC Form 4 within 7 days and either destroy or transfer the agent to a registered entity within 7 days. In compliance with this Act, if a diagnostic laboratory held back part of a screened sample or culture for voucher purposes and that sample forwarded to the USDA Beltsville Laboratory came back as positive for a select agent, the diagnostic laboratory is required to notify the APHIS Select Agent Program immediately. This must take place within seven (7) days of results notification and a PPQ Officer must have the opportunity to witness the destruction of the sample or culture within that time period. Clarification of this and other information related to adherence to the select agent regulations is available on the following APHIS website: http://www.aphis.usda.gov/programs/ag_selectagent/index.html, or call (301) 734-5960.

Researchers in the U.S. wishing to work with foreign plant pathogens that are not select agents should review the websites listed above and contact the PPQ permit unit to understand how best to comply with the permit requirements.

VII. Economic Impact and Compensation

Severe yield losses result from HLB infections of citrus trees. Trees in the orchards usually die in about 3-8 years after becoming infected and require removal and replanting. An 8-year survey conducted on Reunion Island (France: Indian Ocean) in the 1980s and 90s determined that 65% of the citrus trees were infected and unproductive within 7 years of planting (Gottwald et al., 1989). Healthy citrus trees must live 5 to 10 years to make a profit. An infected tree produces misshapen fruit that is bitter and generally unsuitable for sale as fresh fruit or for juice (Appel, 2004; Bove, 2006; Da Graca and Korsten, 2004; Halbert and Keremane, 2004; Roistacher, 1996).

A study was undertaken in the late 1990's to ascertain the number of HLB-infected trees worldwide. It was estimated that 53 million trees were infected in Asia and 10 million in Africa. A decade earlier HLB infection resulted in the destruction of 30 million trees in Indonesia alone. Four million citrus trees infected by HLB were destroyed on the island of Bali in Indonesia over the period of 1986-88 and replanted with mandarins in 1991. Of that new planting, 40% of the trees were infected by 1993 and 90% showed symptoms by 1996. These examples explain why HLB is held responsible for the marked decline in the citrus industry in many countries (Bove, 2006; Da Graca and Korsten, 2004; Halbert and Keremane, 2004).

Harvested citrus acreage in the United States has averaged one million acres in the past 10 years. This includes oranges, grapefruit, lemons, tangelos, tangerines and temples. In 2004, citrus production yielded 16.4 million tons of fruit valued at 2.35 billion dollars (Appel, 2004; USDA/NASS, 2005). Production efficiency is expected to be seriously compromised by HLB.

The reaction of the Risk Management Agency (RMA) to a loss caused by a disease of this sort is straightforward. Under the Florida Fruit Tree Pilot Crop Provisions, disease coverage is provided only for specifically listed diseases. Currently, "Asiatic Citrus Canker" is the only disease for which insurance coverage is provided.

The Florida Citrus Fruit Crop Provisions do not list loss of marketable fruit due to disease as an insurable cause of loss.

Disease is an insurable cause of loss under the Pilot California Citrus Dollar Crop Provisions, the Arizona-California Citrus Crop Provisions, and the Texas Citrus Fruit Crop Provisions. However, disease will only be an insured cause of loss if there are no effective control mechanisms. The loss of marketable fruit will generally be a covered cause of loss only for the first-year of occurrence.

Under the Nursery Crop Provisions, disease is a covered cause of loss if there are no effective control mechanisms. To be insured, nursery trees and plants must be shown on the approved plant listing for the area. In Florida, listed plants may include citrus trees and ornamental host plants grown by commercial nurseries. Disease coverage is provided only for the insured trees and plants exhibiting damage. Coverage is not provided for ordered, recommended or other voluntary removal of any neighboring insured nursery trees or plants that may have been exposed and do not exhibit the disease symptoms.

RMA expects producers to implement recommended disease control measures for subsequent crop years to maintain insurance coverage.

VIII. Mitigation and Disease Management

Production of clean nursery stock

The first step in successful management of HLB is the production of clean nursery stock. Without a clean start, there is no hope of an economic return. Best management practices (BMP) for nurseries endorsed by state and federal authorities include certification of stock, inspection, physical and chemical protection for scion trees, liners and propagations, location requirements such that nurseries are not in the immediate vicinity of concentrations of citrus, and routine testing of stock plants. Citrus nurseries should propagate trees from sources that have been certified as "pathogen free" of HLB. A program similar to that in California for citrus tristeza virus may be prudent. In that program, testing of registered nursery source trees is mandatory (Appel, 2004).

Chemical management of vectors

In countries where HLB exists, insecticides are the first line of defense to manage the psyllid vector, thereby reducing the spread of the HLB pathogens. Applying insecticide sprays at critical flushing periods in order to kill psyllid nymphs may be an effective method of HLB management. Unfortunately, we do not know how effective it is or at what rate and frequency to apply insecticides. We are not even sure about which insecticides to apply, although some preliminary data is available. The following materials in Table 1 are registered in the U.S. (availability may vary by state) for psyllid control on citrus and have some efficacy. The materials listed in Table 2 are either of unknown efficacy or not registered for use on citrus. Imidacloprid, fenprothrin, aldicarb, dimethoate, and thiamethoxam have been shown to be effective insecticides for psyllid management on citrus (Rogers, 2005).

Table 1. Insecticides registered on citrus and have efficacy for psyllid management.

| Common Name | Trade Names | Registered* | | Labeled Pest(s) on Citrus | Efficacy** on Psyllids |
|----------------------------|------------------|-------------|-----------|---|------------------------|
| | | In U.S. | On Citrus | | |
| aldicarb | Temik | Yes | FL | Asian citrus psyllid | + |
| chlorpyrifos | Lorsban | Yes | Yes | Mites, aphids, thrips, flies, scale, etc. | + |
| cyfluthrin | Baythroid | Yes | CA & AZ | Sharpshooter, thrips, grasshopper, etc. | + |
| dimethoate | Dimate | Yes | Yes | Aphids, mites, scales, thrips, etc. | + |
| fenpropathrin | Danitol | Yes | Yes | Psyllids (knock-down) | + |
| horticultural mineral oils | several | Yes | Yes | Psyllids (suppression) | + |
| imidacloprid | Admire - soil | Yes | Yes | Asian citrus psyllid (prophylaxis) | + |
| | Provado - foliar | | | | + |

* Consult your state's pesticide regulatory agency to confirm registration status.

** Efficacy based on published (Rogers, 2005) and unpublished data by Michael E. Rogers.

Table 2. Insecticides of uncertain efficacy for psyllid control and/or not registered on citrus.

| Common Name | Trade Names | Registered* | | Labeled Pest(s) on Citrus | Efficacy** on Psyllids |
|--------------------------------|----------------------|-------------|-----------|--|------------------------|
| | | In U.S. | On Citrus | | |
| acibenzolar-S-methyl | Actigard | Yes | No | N/A (plant activator against bacteria) | ? |
| <i>Beauveria bassiana</i> GHA | Mycotrol, BotaniGard | Yes | Yes | Psyllids | ? |
| buprofezin | Applaud | Yes | Yes | Scales | ? |
| capsaicin | Super Pepper Guard | Yes | Yes | Aphids, mites, scale, thrips | ? |
| carbaryl | Sevin | Yes | Yes | Leafrollers, mites, plant bugs, scales | ? |
| fenpyroximate | FujiMite | Yes | No | N/A | ? |
| kaolin | Surround | Yes | Yes | Psyllids | ? |
| malathion | many | Yes | No | N/A | ? |
| piperonyl butoxide + pyrethins | Pyrenone | Yes | Yes | Psyllids | ? |
| pyrethrins | MGK | Yes | Yes | Psyllids | ? |
| pyriproxyfen | Knack | Yes | Yes | Scales, flies, leafminer | ? |
| spirodiclofen | Envidor | Yes | Yes | Mites | ? |
| spiromesifen | Oberon, Forbid | Yes | Yes | Mites (non-bearing fruit trees only) | ? |
| thiamethoxam | Actara | Yes | No | N/A (registration sought) | + |

* Consult your state's pesticide regulatory agency to confirm registration status.

** Efficacy based on published (Rogers, 2005) and unpublished data by Michael E. Rogers.

N/A = not applicable because there are no U.S. labels for citrus use.

Biological control

Biological control should be considered in any pest management program. However, in this case little success has been experienced from biological control programs. The population fluctuations inherent in the biological system of a perennial crop diminish the effectiveness of biological control for long term management of citrus psyllids. Biological control of the two psyllid vectors of HLB apparently was achieved on Reunion Island (France: Indian Ocean) with hymenopteran psyllid parasites: *Tamarixia radiata* introduced from India against *Diaphorina citri*, and *Tamarixia dryi*, from South Africa against *Trioza erytreae* (Gottwald et al., 1989). The unparalleled success on Reunion Island is thought to be related to the fact Reunion has an unbuffered, closed island ecosystem. However, this remarkable result was not achieved anywhere else. In contrast, the parasites have been present in other locations such as India, Taiwan, Brazil, and Miami, Florida where HLB continues to spread unabated. The two parasites specific to *D. citri* were introduced into Florida in 1999, but only one, *T. radiata*, is established (Appel, 2004; Michaud, 2004).

Lady beetles, the native *Olla v-nigrum* and the exotic *Harmonia axyridis*, provide the best suppression of *D. citri* populations in Florida (Michaud, 2004). Biological control of *D. citri* may suppress vector populations in residential areas if no pesticides are used, but reliance on biological control for commercial production probably is impossible.

Biological control is expected to play a greater role in residential planting (dooryards). Dooryard plantings do not employ the management methods of commercial plantings such as insecticides and removal after PCR detection methods. They will rely heavily on biological and cultural tools that are inexpensive and more readily available to homeowners.

Cultural control

The use of cultural methods for control of HLB has had some limited success. In South Africa, removal of infected branches and trees, removal of neglected trees, and the use of HLB-free planting material are used to reduce the impact of HLB. In this regard, clean nursery programs are essential. These methods must be combined with removal of nearby ornamental citrus species that may act as a reservoir of the HLB vectors and possibly HLB (Halbert and Keremane, 2004). Reservoir species that may be especially troublesome are orange jasmine and Chinese box orange.

Severe pruning of infected trees, partially successful in South Africa, has not been useful in Brazil. After three months, 100% of pruned plants were PCR positive for HLB. Brazil now recommends that infected trees be removed within 48 hours of confirmation.

Education

Education of homeowners and the citrus community is key to recovery from this disease. Informal surveys by these groups may very well help identify infected plants. Also, if the public and citrus producing communities are educated about the threat posed by HLB, they are much more likely to support management measures. Cooperative Extension plays a key role in developing this component. There is an increased need for broadly-trained personnel to educate the citrus industry and the public about IPM and related issues.

Eradication

Early detection and removal of infected trees should be the most effective way of eradicating HLB after introduction. However, the success of such an eradication strategy depends heavily on how early identification and destruction of infected trees. Detection tools are inadequate in this regard. By the time HLB is detected by visual symptoms and confirmed by PCR, it has been resident in the infected trees for years and when a vector is present has likely spread to other trees. For this reason, identification in Florida was after

the early detection phase of this disease and eradication of HLB is no longer considered a possibility in the State of Florida (University of Florida, 2006).

Exclusion

Propagating material and plants are the primary means by which HLB is spread over long distances (Appel, 2004). Regulatory restrictions on the movement of both budwood and plants are crucial, especially if HLB vectors are present (Bove, 2006; Timmer et al., 2000). The USDA APHIS has restrictions in place for the importation of citrus germplasm (budwood, plants, etc.) into the United States. Since the outbreak of HLB in Florida, the USDA has imposed additional restrictions on the export of citrus, citrus relatives, and other host species (e.g. *Murraya*) from infested areas to other areas of Florida and other citrus-producing states.

Citrus seeds are imported routinely without any restrictions. This avenue of HLB entrance is controversial (CAB, 2003; Halbert and Keremane, 2004) and should be further investigated in order to exclude the possibility of the entrance of HLB by this route.

Another exclusionary technique would be the removal of HLB and psyllid hosts, say a mile around commercial fields, to limit transmission of HLB by psyllids.

Germplasm

Citrus germplasm is not rated routinely for resistance to the HLB pathogens, although it is routinely screened for the presence of any pathogens, such as those causing HLB. There are, however, differences in susceptibility among citrus species, although all citrus species are potential hosts. Historically, the most susceptible hosts are sweet oranges, tangelos, and mandarins. Moderately susceptible hosts reported include grapefruits, lemons, Rangpur lime, calamondins, and pummelos. Mexican limes, and trifoliate orange have been reported as the most tolerant. Surprisingly, the Florida isolates that cause HLB are especially hard on pummelos, limes and grapefruit. While conventional breeding techniques may yield resistant varieties, efficient genetic transformation (biotech) techniques, already developed for citrus, may yield results sooner (Yang et al., 2001).

Other controls

The combination of shoot tip grafting, heat therapy and indexing is an effective way to clean up infected propagation material. Heat therapy is expensive, but it is used on high value material such as "Foundation" nursery plant material. The effectiveness of heat therapy on a large-scale has been variable between the Asian and African HLB likely due to their different temperature regimes.

Other possible management techniques worthy of consideration are the use of mild strains of HLB for cross protection. This and a wide variety of other innovative management methods are discussed in the Proceedings of 2nd International Citrus Canker and Huanglongbing Research Workshop held in November 2005 (LaVigne et al., 2005).

Integration of Mitigation and Disease Management Strategies

An integrated disease management strategy is needed to limit the spread of HLB. Clean nursery programs that provide disease-free citrus are imperative in concert with the destruction of infected trees and the judicious use of insecticides to manage the psyllid vector. This includes the removal of nearby ornamental citrus relatives that may act as reservoirs of the HLB vector and possibly the pathogens. Use of vector parasites and planting in areas free of vectors also should be considered. Of course, effective scouting by well-trained personnel with practical and interdisciplinary pest biology backgrounds will be essential. Grove self-inspection should be a goal of Cooperative Education programs.

These measures should be supported by research that enhances our understanding and advances our management of this disease but they must be used in concert – individually they will have little effect. Results will be realized much sooner if a source of resistance can be identified (Appel, 2004; Bove, 2006; Da Graça and Korsten, 2004; Halbert and Keremane, 2004; Timmer et al., 2000).

IX. Infrastructure and Experts

A citrus research infrastructure exists to study citrus diseases. That infrastructure could be directed to answer several important issues of HLB listed in the next section on research priorities. If permitted, most of that research probably would be limited to Florida, where the current infestation has been found, due to quarantine concerns. However, in some instances there will be good reason to conduct research in locations that lack all three components: citrus, psyllids, and the pathogens of HLB. In Florida, the primary centers of citrus research are at the University of Florida's Citrus Research and Education Center at Lake Alfred and Ft. Myers as well as at the USDA/ARS facility at Ft. Pierce.

Research projects concerning HLB are active at the University of Florida in Ft. Myers, Gainesville and Lake Alfred; USDA-ARS facilities in Ft. Detrick Maryland, Riverside California, Ft. Pierce Florida, and Beltsville Maryland; and the U.S. Department of Energy's Los Alamos National Laboratory in New Mexico. According to the USDA/CSREES Current Research Information System, \$15 million dollars were allocated for HLB related research from 2001 to 2005. Of this amount, slightly less than \$500 thousand were spent on research projects directed specifically at HLB or its vector.

The following experts on HLB have been identified (Appel, 2004):

Joseph Bove – expert on all phases of HLB
(retired) Lab Biol Cell Mol, Inst Biol Vegetale Mol, INRS, BP 81, F-33883 Villenave d'Ornon Cedex, France, +33-5-57-122369, joseph.bove@wanadoo.fr

Ron Brlansky – expert on identification and management of HLB and vector transmission
Univ of Florida, Citrus Research and Education Center, 700 Experiment Station Rd, Lake Alfred, FL 33850, 863-956-1151 ex 1300, fax 863-956-4631, rhby@ufl.edu

Russ Bulluck – expert on biology and response to HLB
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Joel Floyd (Contact), USDA Animal and Plant Health Inspection Service – experts on biology and response to HLB, USDA, APHIS, PPQ, 4700 Riverdale, MD 20737, 301-734-4396, joel.p.floyd@aphis.usda.gov

Tim Gottwald – expert on incidence and spread of HLB (epidemiology)
USDA, ARS, US Horticultural Research Laboratory, 2001 South Rock Road, Ft. Pierce, FL 34945-3030, 772-462-5883, fax 772-462-5983, tgottwald@ushrl.ars.usda.gov

John da Graça – expert in all phases of HLB
Texas A&M University-Kingsville, Citrus Center, P.O. Box 1150, Weslaco, TX 78599-1150, 956-968-2132, FAX: 956-969-0649, j-dagraca@tamu.edu

Susan Halbert – expert on biology and management of HLB vectors and field survey for HLB
Florida Department of Agriculture and Consumer Services, Division of Plant Industry, PO
Box 147100, Gainesville, FL 32614-7100, 352-372-3505 ex 185, halbers@doacs.state.fl.us

John Hartung – expert in genomics of citrus and HLB
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Manjunath Keremane – expert on detection of HLB in psyllid vectors
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Jim Stack (Director), National Plant Diagnostic Network – experts in diagnosis and detection
of HLB, Kansas State University, 4024 Throckmorton Hall, Manhattan, KS 66506-5502, 785-
532-1388, fax 785-532-5692, jstack@ksu.edu

Hong-Ji Su – expert on detection, biology, and management of HLB
National Taiwan University, Taipei, Taiwan, 886-2-23625717, hjsu@ntu.edu.tw

X. Research, Extension, and Education Priorities

Research Priorities

The following lines of research are needed to enhance detection and management of HLB. They would improve our ability to block the entrance, detect the presence, and help manage the impact of HLB. The research priorities listed below are broken into three groups based on relative importance. Priorities within groups are considered equal. The top group is listed as Most Important, the second group is Highly Important, and the last group is Needs Evaluation – important but less so than the first two groups.

Research is active in several areas, including: biological control and IPM techniques, germplasm development, molecular approaches such as DNA probes, improved assays, maintenance of pathogen collections, and epidemiology and genetic diversity. Specifics about this research can be obtained from the USDA/CSREES Current Research Information System website at: <http://cris.csrees.usda.gov/>. The future of research on HLB was discussed in detail and a research priority list was constructed at an international meeting held in 2005 in Orlando Florida (LaVigne et al., 2005).

Most Important

- Develop **survey technique** for the HLB pathogens that is quick and inexpensive – The method should allow fast and efficient survey and detection using serology or better and cheaper PCR primers. This should help explain disease epidemiology leading to better management.

- Determine **host range** of *Liberibacter* spp. and Asian citrus psyllid – Survey design will be impacted and improved by knowledge of the host range. Hosts known to harbor the HLB vector should receive first priority. *Liberibacter* populations in hosts should be determined.
- Investigate **seed transmission** of *Liberibacter* spp. – Current open policy on international trade in citrus seeds may be allowing a potential hazard that quarantine regulations could help resolve. Determine seed and graft transmission from asymptomatic plants.

Highly Important

- Develop **transgenic citrus varieties** with resistance to *Liberibacter* spp. and a rapid screening method for resistant cultivars – Resistant varieties are a preferred means of management and probably the most promising long-term solution to HLB.
- New and/or improved **sampling and diagnosis**; sample twigs or psyllids; determine preferred sector of tree – This will enhance effectiveness and minimize cost of surveys and allow the development of a national survey standard to improve ability to compare states.
- Develop a **survey technique** for the HLB pathogens that is more accurate – Should allow recognition of all strains thereby reducing the number of false negatives and positives during survey and detection.
- Evaluate **insecticide efficacy** on psyllids – Should guide selection of insecticides from regular registrations and for Section 18 requests.
- **Culture** *Liberibacter* spp. – This is an important tool for research in many areas. Plant and insect tissue should be investigated as culture media. Successful culturing will lead to serological tests – important survey tools.
- Determine complete **genomic sequence** of HLB pathogens – Serve as a basis for other HLB research and foster a forensic approach to determine fine differences among strains or isolates.
- Investigate how **host-pathogen interactions** affect disease severity and epidemiology – This area has great potential for enhancing management.

Needs Evaluation

- Evaluate psyllid and HLB **invasive potential** – Determine the potential range of the Asian citrus psyllid and HLB in the U.S. and the expected severity of disease.
- Determine the impact of the **vector and pathogen** on each other – Culturing *Liberibacter* spp. may be more successful with insect medium. The potential impact of the pathogen on vector biology may explain disease patterns and control.
- Determine benefit of **psyllid control** – This is currently undetermined and is needed to justify psyllid management. Determining the importance of adults vs. nymphs as vectors is also important.
- Evaluate time and parameters required for **psyllid transmission** – This will help in developing effective disease management strategies based on psyllid biology and disease epidemiology.
- Determine threat of **fruit-mediated transmission** of psyllids and/or HLB – Evaluate possibility that HLB could travel in fruit shipments either in fruit or psyllids.
- **Economic impact** of HLB on the U.S. citrus industry – Communicate importance of HLB to the citrus industry to justify research priorities.
- Determine differences in **symptoms** of infected residential citrus trees vs. those infected in the field and nursery; determine factors that affect symptom expression; determine symptoms on non-citrus hosts – This should improve detection and therefore management.

Extension Priorities

The following items need development:

- Develop plans to test citrus propagation material to ensure that it is free of HLB;
- Survey citrus and alternate host growing areas and greenhouse areas in the U.S. with previous citrus history, including soil, water, crops and weed samples;

- Encourage the development of culture and germplasm collections and foster international cooperation on collections and research;
- Develop centers for the production of clean plant material using shoot tip grafting, heat therapy, and other methods; and
- Determine the differences in symptoms of infected residential citrus trees, commercial groves, and commercial nurseries. Also, determine factors that affect symptom expression and symptoms on non-citrus hosts.

Education Priorities

The following items need development:

- Educate a new cadre of plant pathologists in the epidemiology and management of bacterial diseases;
- Develop training courses on detection, monitoring, and management of HLB;
- Educate county extension, growers and crop advisors in sampling, monitoring and management of related diseases and in the utility of map-based tracking and information systems such as the Pest Information Platform for Education and Extension (PIPE);
- Provide standard HLB sampling procedures and training materials for all citrus-producing states, especially for high risk urban areas (most likely sites for initial establishment) and nurseries (potential distribution centers for infected trees), administered through CAPS citrus commodity surveys; and
- Target outreach to homeowners, growers, and pest management specialists through cooperative extension programs and the NPDN.

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Web resources

APHIS website: http://www.aphis.usda.gov/ppq/ep/citrus_greening/index.html

APS website: <http://www.apsnet.org/media/hottopics.asp>

CREC: <http://www.lal.ufl.edu/extension/greening/index.htm>

Florida Dept Agriculture: <http://www.doacs.state.fl.us/pi/chrp/greening/citrusgreening.html>

University of Florida: <http://ipm.ifas.ufl.edu/agricultural/fruit/citrus/ASP-hoy.htm>

USDA initial report: http://www.aphis.usda.gov/lpa/news/2005/09/greening_ppq.html