USDA AGRICULTURAL RESEARCH SERVICE
CITRUS GREENING WORKSHOP SUMMARY
April 24, 2008
Fort Pierce, Florida

Citrus Greening disease (Huanglongbing; HLB) undoubtedly poses the most serious threat that the Florida citrus industry has ever faced. It was first detected in Florida in 2005, and has been identified in most of the citrus growing counties in the state. In spite of the best efforts to date, HLB literally threatens the survival of the Florida citrus industry, and is a potential threat to the entire U.S. citrus industry.

Several factors make this disease especially difficult to manage successfully:

- Commercial citrus cultivars are highly susceptible to the disease, and there are no resistance sources for use in conventional breeding.
- Transgenic approaches offer promise for resistance, but transformation efficiency is low and the search for resistance genes is just beginning.
- The psyllid vector flies long distances and is already widespread throughout Florida.
- While members of the Rutaceae family, to which citrus belongs, are the only known hosts of HLB and are preferred hosts of the vector, some ornamental Rutaceous species are widely planted in Florida and harbor the HLB-associated '*Candidatus* L. asiaticus'.
- '*Candidatus* L. asiaticus' has not been isolated in culture, so the cause of the disease has not been established conclusively.
- The long latent period, several months to a few years, makes it impossible to identify a diseased tree so it can be removed before the pathogen spreads to healthy trees.
- No systemic bactericides are available for management of plant diseases, and chemical controls are lacking.

Since the disease was first found in Florida, ARS scientists have been actively engaged in research with university and industry partners focused on the critical components of this disease: i.e., etiology and epidemiology; the insect vector; and the plant host's response to HLB infection. In February 2008, with the goal of Florida citrus industry survival, members of the citrus industry requested additional input from ARS scientists outside of the citrus field, to elicit ideas about research needs, gaps, priorities and opportunities from the most diverse expertise available.

To meet this urgent request and fill a critical need, ARS convened a workshop at the U.S. Horticultural Research Laboratory in Fort Pierce, with ARS scientists who are leaders in their scientific fields but are not engaged in citrus research. These ARS scientists brought to the workshop their collective expertise in animal and plant entomology, animal and plant pathology, genetics, horticulture, and microbiology. The workshop was coordinated with the National Academy of Sciences (NAS), because the NAS had been contracted by the Florida Department of Citrus to develop a Request for Proposals to manage grant funds in research leading to HLB management. Key researchers from the University of Florida Citrus Research and Education Center were included in the Workshop to present their research progress on HLB.

The Workshop was a 2-1/2 day information sharing session, with free exchange of ideas, information and plans for future research activities, similar to the approach applied to critical

problems like Pierce's Disease of grapevine and Colony Collapse Disorder of honeybees. The charge to the assembled non-citrus ARS experts was to capitalize upon their diverse expertise to identify researchable topics/themes/areas that may not be currently addressed, in order to maximize the likelihood of research to provide potentially effective, new solutions to the citrus greening disease problem.

After a thorough review of current literature and reports from Research Leaders at Fort Pierce and key University of Florida researchers, followed by two days of intense deliberations, the ARS team developed a series of researchable items (see attached table). Each item was rated according to length of time to complete the research, priority for having potential impact on mitigating or managing HLB, and likelihood of success (i.e., achieving research objectives). In addition, an attempt was made to identify research that would help maintain a viable industry over the next 5 years, and to sustain the industry in the longer term, (i.e., >5 years). The ARS team felt that the current research programs conducted by ARS and the University of Florida have laid an excellent foundation on which to build. A few areas were identified by the team that are not currently being addressed. In order to maximize the impact of future research on mitigating or managing HLB in the short- and long-term, the ARS team recommended that emphasis be placed on interdisciplinary, interagency collaborative team research. The research topics or areas identified during this process provide excellent opportunities for this.

The ARS team recommended that an overarching plan be developed and funded to address the primary targets of the disease triangle:

- Crop improvement through transgenic and conventional approaches;
- Vector management and IPM; and
- Research targeting the agent(s) that cause or are presumed to cause HLB.

Interdisciplinary and multi-institutional research must be pursued and broadened to include additional scientists not currently working in this area. Collaborating scientists would benefit from visiting scientists to Fort Pierce, and Fort Pierce scientists should spend time in other labs to gain new insights and methodologies that can be brought back to benefit the HLB research program in Florida. The main points of the discussion with ARS scientists are summarized below. Details of these research areas are provided in the attached table.

Crop Improvement: A comprehensive, thoroughly planned, and well-staffed program to develop transgenic citrus with HLB resistance was viewed as essential to the HLB response. With excellent coordination and management from the onset, this program can identify the most promising genes for genetically-engineered resistance and find solutions to technical, regulatory and intellectual property obstacles so that transgenic plants can be developed and tested in the field in a timely fashion. The most important technical obstacles identified by the team were the lack of efficient and effective transformation systems for citrus and screening methods for HLB resistance. Improved methods for early disease detection are essential to develop the efficient screens needed to evaluate large numbers of transgenic plants with candidate genes for HLB resistance. In addition to genetic transformation for resistance, the current ARS germplasm improvement program should continue to provide new rootstocks through conventional means. Cold tolerance is also an important trait to be incorporated, particularly if the industry moves farther north out of the psyllid range.

Vector Biology and Management: Psyllid management is critical to management of HLB. It poses a particular problem because *Citrus* spp. in abandoned groves and backyards, and other members of the *Rutaceae* that are popular landscape ornamentals throughout Florida serve as preferred hosts of the vector. Populations of several important insect pests that have been adequately controlled by natural predators are now on the rise because of heavy insecticide applications targeted at psyllid populations. Thus, IPM methods, with attention to resistance development by the vector and maintenance of beneficial insects must be considered. There may be opportunities for specific, novel pesticide development for vector control. Different microclimates in Florida may impact disease incidence by limiting vector movement and establishment. Mating disruption through sound or pheromones and insect bait and trap techniques, as used for experimental mosquito control, should be investigated. Regardless of control method, emphasis should be placed on area-wide suppression strategies to reduce refuges for the pest and re-invasion of treated areas.

Pathogen and disease biology and detection: There is compelling data associating '*Candidatus* L. asiaticus' with HLB, but culturing the bacterium and proving that it causes the disease is a critical research need. The genomic sequence of the bacterium will provide invaluable information about the disease, including leads that will enable scientists to culture the bacterium and detect it in Citrus and psyllids. Therefore, obtaining the genomic sequence of the bacterium is of high priority. The long latency period for symptom expression, uneven distribution of the presumed causal agent in the plant, and low levels of the pathogen in plant tissues makes this disease difficult or impossible to diagnose prior to symptom development. Reliable and efficient methods are needed to diagnose the disease soon after infection for disease management programs, epidemiological studies, and for screening plants for resistance, as mentioned above.

Epidemiology/Disease Management: Creative approaches are needed to develop strategies for managing HLB in citrus in the future, and several promising tactics are already being evaluated in ongoing research programs. Interplanting guava with citrus suppresses HLB in Viet Nam, and volatile compounds from guava may limit psyllid movement or transmission. Research in this area may provide new avenues for disease management, especially if repellants can be identified. New methods of pesticide delivery to trees by kaolin deposits or application to physical barriers should be pursued. Cultural methods to adjust the timing of flushes through nutrient delivery or and to control fruit abscission are important areas for research. Systems management of citrus production in the field may be extremely valuable for maximizing production. For the short term, evaluation of the cost:benefit ratio of prolonging the life of trees should be determined. Important areas of research are cultural methods that (i) influence host tree attractiveness to the psyllids that vector '*Candidatus* L. asiaticus' [e.g., adjusting the timing of flushes through nutrient delivery and open hydroponic systems, control of fruit abscission, and systemic acquired resistance (SAR)-inducing agents as reported by some through the application of imidicloprid-insecticides], and (ii) alter the chemical environment of the phloem sap in which the bacteria live and reproduce (e.g., antibiotics, bacteristatic and/or bactericidal micronutrients or trace elements, secondary metabolites, and SAR agents). Efforts to manage citrus production in the field through systems management may be extremely valuable for maximizing citrus production.

Final Comments: The citrus seedling nursery industry is currently at a very diminished capacity, thus the re-establishment of groves will be difficult. Even if an HLB-resistant cultivar was

available today, it would b several years before it could be increased in numbers to have an impact for industry needs. Although the research plans outlined in the workshop are needed, some measures need to be taken quickly before HLB becomes endemic in Florida, and renders the industry nonproductive. In addition to research, a strong HLB response program will require cooperation between and among citrus growers, which should be encouraged through public education and incentives to destroy abandoned groves that serve as inoculum. Elimination of abandoned trees, infected tree removal, clean nursery stock production at an appropriate distance from production areas, insect control through IPM and judicious use of pesticides, are all important aspects of a best management education and extension program that will help the industry survive over the next several years until viable control methods are developed.

This narrative touches on only some of the highlights listed in the accompanying table. The increased industry funds made available through the "citrus box tax" will enable some of the research described above to be initiated and expanded, building upon the research that is in place with base funding. However, in order for the research to attain the goal of controlling HLB so that the industry can survive, the overall research program should be coordinated to maximize progress and sharing of information. A yearly HLB meeting for progress reports was suggested as a means of information sharing among scientists and industry members. Construction of a web site that is open to communicate information directly to industry members will be pursued.