The Summit's afternoon session addressed the question, "Are HLB early detection technologies viable for the California citrus industry?" Featured were (left to right) Mary Palm, Ph.D., Leader of the USDA's HLB Multi-Agency Coordination Group; Victoria Hornbaker, CDFA Citrus Program Manager; Cheryl Blomquist, Ph.D., CDFA Senior Plant Pathologist; Philip Berger, Ph.D., USDA APHIS PPQ Executive Director of Science and Technology; Ed Civerolo, Ph.D., CRB Advisor and Citrograph Interim Executive Editor; Robert Atkins, Statewide Coordinator, CPDPP; and Richard Bennett, CRB Chairman.

IMMEDIATE ACTION IS NEEDED

Summary of the HLB Summit Morning Session

Beth Grafton-Cardwell, Mike Irey, David Bartels, Carolyn Slupsky and Neil McRoberts

On December 1, 2015, a meeting about huanglongbing (HLB) was conducted by the Citrus Research Board and California Citrus Mutual at the Visalia Convention Center. The conference was attended by nearly 250 citrus industry members. The goal of the morning session was to provide up-to-date information on the devastation that HLB is causing the Florida citrus industry, discuss HLB finds and the potential for spread in California, describe new technologies to detect the disease and make recommendations to the citrus industry for moving forward.

Beth Grafton-Cardwell, Ph.D., Director of the Lindcove Research and Extension Center, moderated the session. She began by describing the symptoms of HLB and showed a map of the HLB-infected trees removed in southern California. While to date, only 11 trees infected with the bacterium that is associated with HLB have been identified, it is likely there are more infected trees in California. **The spread of '*Candidatus* Liberibacter asiaticus' (CLas) from tree to tree is very rapid**, because the Asian citrus psyllid (ACP) vector lays its eggs in the same place it feeds and infects the plant. When nymphs hatch, they feed on the localized bacterial infection and take the bacteria with them when they molt into adults and fly away. Psyllid control is a temporary, but important strategy to buy time for scientists to find a cure for the disease. Grafton-Cardwell emphasized that **management focus needs to change from ACP to HLB, and the citrus industry needs to lead efforts to prevent man-made movement of psyllids that are potentially carrying CLas, the bacterium associated with HLB.**

Mike Irey, Director of Research and Business Development, Southern Gardens Citrus in Florida, provided information on the devastation that HLB has caused in that state. It is estimated that **100 percent of Florida groves have some level of HLB infection.** He reported that **costs are double to triple what they were 10 years ago** due to increased application of pesticides for ACP, nutritionals, tree removal, etc. Additionally, the state is experiencing a 50 percent reduction in yields in spite of these much greater inputs. In Florida, they are finding that maintaining optimal tree health is vital in the presence of HLB, but even with optimum tree health, growers continue to have production losses due to HLB. Growers must minimize tree stress of any type – i.e., *Phytophthora*, bicarbonates in water, frost, etc. It takes very few psyllids to spread the disease-associated bacterium, and it is very difficult to completely eliminate psyllids from the groves. However, growers cannot relax ACP control, because high psyllid numbers will infect trees with HLB-associated bacterium at a much faster rate than low psyllid numbers. The more infection sites a tree has, the faster the tree expresses the disease.

Irey recommended that **California should stay in HLB eradication mode as long as possible because it is very difficult to control the disease once it becomes**

established. He stated that "a little pain on the front end can buy you a lot of time and increased profits on the back end." Early HLB detection is the key to getting ahead of the disease spread. Having a large psyllid and plant sampling volume (large number of samples, wide area tested, etc.) is the most important factor to maximize detection of HLB. He recommended that Californians utilize both validated tests and new technologies – not relying on just one or the other.

David Bartels, Ph.D., Entomologist, USDA APHIS PPQ, Mission Laboratory, Texas, described HLB survey efforts going on in Texas and California to improve the detection of infected trees. Currently, PCR (polymerase chain reaction) is the primary tool for detecting the CLas in psyllids and citrus plants. There are two types of PCR being utilized, conventional PCR and quantitative PCR (qPCR). Both Texas and California are using qPCR for processing samples, because this method can rapidly process very large numbers of samples and potentially detect lower amounts of bacterial DNA in samples. However, conventional PCR provides the regulatory confirmation of HLB infections since the resulting product can be sequenced to provide a DNA match. Texas qPCR psyllid testing showed a shift in psyllid sample results from suspect (Ct-values 33-39) to clearly positive (Ct-values < 32) over a two-year period; then one to two years later, many trees with HLB disease were detected.

If the disease progresses similarly in California, we should expect to see additional clearly positive psyllids in the coming year. To act conservatively and get ahead of the disease spread, the California citrus industry needs to follow up on the areas of California that have had ACP with Ct-values in the suspect 33-39 range and test more psyllids and trees in those areas. Testing ACP samples is extremely useful for locating regions with HLB infection, since psyllids are accumulating bacteria as they feed on infected trees. **A CLas-positive adult psyllid doesn't tell us exactly which tree is positive, because the adults move around, but it tells us that the bacterium is in the area.** Because adult psyllids tend to be on the borders, **citrus growers should initially focus their HLB detection efforts on the borders of their orchards.**

Carolyn Slupsky, Ph.D., Professor, Department of Nutrition and Department of Food Science and Technology at the University of California, Davis, discussed the early detection technologies (EDTs) being developed to detect HLB and why growers should utilize them in addition to PCR. **To contain the disease, we need to utilize all of the detection technologies available to us,** some of which are direct detection of CLas, and some of which are indirect detection of the HLB-associated bacterium.

Direct detection technologies (such as PCR and antibody tests) detect the presence of the bacterium or its by-products. The amount of bacteria or bacterial products in these samples must exceed a specific threshold for the test to be determined positive. In the case of HLB, the bacterium is often not distributed evenly throughout the tree, and thus, sampling can be an issue – choose the wrong plant tissue, and one will miss the bacteria. For this reason, direct detection technologies can result in "false negatives."

Indirect detection technologies (such as soluble metabolites, volatile metabolites [which encompasses testing with instrumentation or testing with dogs], protein, small RNAs, spectral imaging and microbial communities) detect changes in the tree that are part of its defense against the HLB infection. Because that defense response occurs throughout the tree, indirect detection methods yield more "true positives" than direct detection, and have the potential to detect infection months to years prior to visible symptoms. However, these tests may result in some "false positives," since other conditions in the plant could mimic infection by CLas. **A combination of several early detection technology (EDT) tests would improve confidence in the results and provide more timely information on the status and spread of the disease in California.**

The room was packed for the recent HLB Summit.

Neil McRoberts, Ph.D., Associate Professor in the Department of Plant Pathology at the University of California, Davis, used an epidemiological model approach to show how factors that influence HLB disease spread are related. The California and Arizona citrus industries are focused on reducing the rate of new infections to limit bacterial inoculum by lowering psyllid densities and surveying for infected trees. However, psyllid suppression will slow down the rate of disease spread, but not stop it completely. **To shut down HLB disease spread, it is imperative to find and remove infected trees quickly.** However, there are technical problems with achieving highly accurate early diagnosis, so acceptance and removal of "false positives" is a reality.

In the meantime, while early detection is still in development, it is important to avoid contributing to the HLB problem: **respect quarantines, get involved in Psyllid Management Areas (PMAs), start monitoring groves for disease and motivate complacent neighbors.** Citrus growers need to **take the initiative to test trees in their groves and remove suspected HLB-positives, using whatever diagnostic tools are available.** This activity does not need to await regulatory confirmation of positives. Thanks to lessons learned at Florida's expense, the opportunity exists to get ahead of HLB in California, provided the appropriate cooperation occurs within the industry, and between the industry and regulatory agencies, so that resources are appropriately allocated. Immediate action is needed. **Many of the important problems in dealing with HLB aren't caused by HLB or ACP, but by people.**

Beth Grafton-Cardwell, Ph.D., is an IPM specialist and research entomologist with the Department of Entomology at the University of California, Riverside, and also Director of the Lindcove Research and Extension Center. Mike Irey is Director of Research and Business Development at Southern Gardens Citrus in Florida. David Bartels, Ph.D., is an entomologist with the USDA APHIS PPQ in Mission Laboratory, Texas. Carolyn Slupsky, Ph.D., is a professor in the Department of Food Science and Technology at the University of California, Davis. Neil McRoberts, Ph.D., is an associate professor in the Department of Plant Pathology at the University of California, Davis.

CLas (HLB) Detection Technology Descriptions

Direct HLB Detection Methods (detects CLas using psyllids or plant tissue)

- *Polymerase Chain Reaction (PCR)*
  - *Conventional* PCR detects DNA of the CLas bacteria and products that can be sequenced
  - *Quantitative PCR (qPCR)* detects CLas DNA using light and measures quantity of DNA
- *Antibody tests* use antibodies that react with CLas-derived proteins present in the phloem of the plant

Indirect HLB Detection Methods (tests the responses of the tree to infection by CLas bacteria)

- *Soluble metabolites* measure the response of the tree to infection by CLas bacteria using metabolites extracted from plant tissue
- *Volatile organic compound (VOC)* detection measures the response of the tree to infection through a VOC profile
  - *Electronic sniffer technology*
  - *Canine detection*
- *Protein tests* measure the response of the tree to infection using host plant proteins extracted from plant tissue
- *Small RNAs* measure the response of the tree to infection using small RNAs extracted from plant tissue
- *Optical imaging* measures the response of the tree to infection through measurement of reflectance spectra
- *Bacterial communities* use qPCR to measure changes in the bacterial communities in the phyllosphere (above-ground portion of the tree) or roots as a consequence of infection