INTERNATIONAL RESEARCH CONFERENCE ON HUANGLONGBING

Session 3: HLB Detection and Diagnostics

Orlando, Florida • December 2008

3.1 Lessons learned from a comparison and evaluation of multiple HLB testing laboratories employing common and different testing methodologies applied to a common set of samples.

Irey M.[1], Sieburth P.[2], Brlansky R. [3], DaGraça J. [4], Graham J.[3],Gottwald T. [5], Hartung J.[6], Hilf M.[5], Kunta M. [4], Manjunath K.[7], Ling H.[8],Ramdugu C.[7], Roberts P.[9], Rogers M.[3], Shatters R.[6], Sun X.,[10] and Wang N.[3]

[1]United States Sugar Corporation/Southern Gardens Citrus, Clewiston, FL.; [2]FDACS, Bureau of Budwood Registration, Winter Haven, FL.;[3]Univ. of Florida, CREC, Lake Alfred, FL.;[4]Texas A & M University, Weslaco, TX.;[5]USDA-ARS, Fort Pierce, FL.;[6]USDA-ARS, Beltsville, MD.; [7]USDA-ARS, Riverside, CA; [8]USDA-ARS, Parlier, CA., [9]Univ. of Florida, SWFREC, Immokalee, FL.;[10]FDACS-DPI, Gainesville, FL.

Several laboratories in Florida and other places are now engaging in detection of Huanglongbing (HLB) disease. To ensure reproducibility of test results from various laboratories using different instruments and methodologies, a total of 276 DNA samples, including known positives, known negatives (e.g. from screened and tested budwood trees, or from states not known to have HLB), samples that tested as questionable in preliminary testing, samples from citrus-relatives, and water blanks containing no DNA were sent for comparative analysis to thirteen labs in FL, CA, TX, and MD. The participating labs were: Southern Gardens, UF/CREC, UF/SWFREC, FDACS DPI, FDACS DPI-Bureau of Budwood Registration, USDA-ARS Fort Pierce FL, USDA-ARS Parlier CA, USDA-ARS Riverside CA, USDA-ARS Beltsville MD, and Texas A&M University, Kingsville. The DNA samples were from plants from California, FDACS-DPI Bureau of Budwood Registration, UF/SWFREC, USDA-ARS Ft. Pierce, and from commercial orchards from multiple counties in Florida. Samples were run blind by all labs and the identity and HLB status of some of the samples were blind to all groups.

Several different PCR procedures were tested: conventional and real time, different types of real time PCR (Taqman®, SYBR Green® and EvaGreen®), multiplex vs. single primer sets for PCR, different master mixes, different primer sets, different machines and different systems for determining cycling threshold values. With the exception of labs that had specific reagent or equipment problems, all of the labs correctly identified the HLB status of >93% of the samples. However, most of the labs missed one or more of the positive samples and several of the labs had what were considered false positive results (i.e. positive results from the known negative samples). Between labs using real time PCR systems and the same primers, no differences were observed between machines, reagents or detection systems. Similar detection sensitivity was found for tests using primers based on different genome regions, eg. 16S rDNA, β-operon, or DNA polymerase nucleic acid sequences. Also no differences in results were seen between labs using multiplex vs. single primer sets for PCR. As expected, conventional PCR was slightly less sensitive than real time PCR.

Several issues were identified that will require more analysis and further testing. However, based on these data, growers and researchers, particularly in Florida, should be confident that results will be comparable for samples submitted to the different labs testing commercial samples.