3.12 Molecular approach for early detection of *Candidatus Liberibacter* species in Texas Citrus

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North America was first invaded by the Asian citrus psyllid (AsCP), *Diaphorina citri* (Hemiptera: Psyllidae) in 1998 (3) and has spread from Florida to Texas (2). However, *Candidatus* Liberibacter asiaticus, *Can*.La, the pathogen associated with the development of Huanglongbing (HLB) in citrus has not been reported in Texas citrus. On June 12, 2008, the Animal and Plant Health Inspection Service Plant (APHIS), Plant Protection and Quarantine (PPQ) Molecular Diagnostics Laboratory and the PPQ Center for Plant Health Science and Technology (CPHST) National Plant Germplasm and Biotechnology Laboratory in Beltsville, Maryland, confirmed the identification of *Can*.La in citrus in a lime tree leaf sample from a residential property in Algiers, Orleans Parish, Louisiana (APHIS report DA-2008-24). The initial symptoms of HLB are leaf yellowing and development of small, misshapen, unpalatable fruit. Currently, the vector of *Can*.La, AsCP, occurs in the southeastern USA, from Florida to Texas. With a recent report from California. The development of HLB has been reported to take from 2-6 years to show symptoms (1). Early detection of this pathogen is a crucial part of a HLB management plan to maintain a viable citrus industry. Automated detection systems such as PCR and real-time PCR have improved the detection of plant pathogens, but, several problems still exist with these methods, mainly stemming from low titers of bacteria in samples. *Liberibacter* collection methods also hamper detection techniques used for pathogen management and study. The most common method of collection, extraction of the bacterium directly from leaf tissue has several limitations, such as low cell numbers collected and high amounts of plant DNA and organic matter that can interfere with molecular diagnostics, hindering early detection and often resulting in false negatives. Improving the consistency of plant pathogen detection has been the cornerstone of the Bextine lab and will enhance the study of the interaction between vectors, host plants, and the pathogen.

The point of our diagnostic program is to determine if *Can*.La has crossed the Texas border and is currently infecting plants prior to symptom expression. As a preliminary assessment of our capabilities, citrus leaves were collected from trees in 4 citrus regions in southern TX, [Beaumont (on the LA border), northern Houston, Pearland (southern Houston), and McAllen] and analyzed for presence of the bacterium. DNA was extracted from leaves using the DNeasy tissue extraction kit (Qiagen Inc., Valencia, CA). Detection of *Can*.La by quantitative real time PCR was done using two 16S rDNA primer sets (RS-HLBr 5'-GCGTTATCCCGTAGAAAAAGGTAG-3'/RS-HLBas 5'-TCGAGCGCGTATGCAATACG-3' and USHRL-CL1-R 5'-CTTACCAGCCCTTGACATGTATAGGA-3'/USHRL-CL1-L 5'-TCCCTATAAAGTACCCAACATCTAGGTAAA-3'. For both primer sets an initial 3 minute melt at 94ºC, followed by 35 cycles of 95ºC for 15 sec, 58ºC or 54ºC for 30 sec, and 72ºC for 30 sec (4). Only positive values with a crossing threshold of less than 30 were considered possible positive samples using both primer sets. If a sample tested positive, the amplicon was sequenced and compared to known *Candidatus* Liberibacter species sequences. Extra care must be taken when analyzing samples due to the highly sensitive real time PCR methodology, as it is possible that some samples may produce 'false' positives resulting in a closely related bacterium being

detected that does not cause disease. In this case, sequencing the PCR product is the best way to confirm the identity of the amplicon. All samples collected from three of the areas of Texas (Northern Houston, Pearland, and McAllen) tested negative for the presence of *Can*.La. One non-symptomatic, dooryard lemon tree in Beaumont, TX initially tested positive for the presence of *Can*.La using both of our primer sets; however this sample tested negative in subsequent assays by the USDA-APHIS and thus has not been confirmed. From this tree, 48 leaf samples were collected and only one sample resulted in a positive reaction (cycle time value of 28) and the 180 bp amplicon from the RS-Las long primer set was sequenced and matched 100% *Can*.La. A second sample from this tree was considered "questionable" because it had a cycle time of around 31. We were unable to sequence this sample and thus it is now considered to be negative. All 46 of the other samples collected from this tree tested negative for the presence of *Can*.La.

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