

Spatial and Temporal Incidence of *Ca. Liberibacter* in Citrus and Psyllids Detected Using Real Time PCR

Funds for this project were released on 19 March. Objective 1. Assess seasonal patterns of pathogen incidence in citrus trees and psyllids in regions of high HLB incidence. A 12 acre block of 'Valencia' orange trees was selected at a commercial grove in 2008. A sample of psyllid adults collected in the block in November 2008 and analyzed at USDA-ARS Riverside labs contained 21% HLB positive psyllids. The block was divided into 16 plots, which receive, two levels of micronutrients+SAR, insecticide treatments, or left as control. Plant and psyllid samples are being collected every four months to test for *Candidatus Liberibacter asiaticus* using PCR. Insecticide applications significantly suppressed psyllids compared to control. No significant differences in the field distribution of HLB in plants were observed using quantitative geostatistical analysis between November 2008 (40% HLB infection) and April 2009 (33% infection). A tree determined to be PCR positive and one PCR negative tree in each plot was trimmed to induce new flush. On 3 June, 10 psyllid adults from HLB negative colony on orange jasmine (*Murraya paniculata*) were released on a new shoot that had been caged immediately after trimming. An additional cage was placed on a previously uncaged and psyllid-infested shoot on the same tree. All cages along with branches and psyllids were collected 2 weeks later for PCR analysis. Nineteen percent of shoots caged with psyllids from the HLB negative colony tested positive for HLB on trees, regardless of whether trees had previously tested HLB positive or negative. In contrast, shoots that were naturally infested with psyllids when caged were 63% and 56% positive on previously HLB positive and negative trees, respectively. It would appear that infestation with infected psyllids from the field was over 2.5 times more likely to result in a positive plant sample, whereas the previous history of testing had little bearing. However, results were different when the same experiment was repeated in July-August using same plants. Shoots caged with psyllids from HLB negative colony were 64% and 42% HLB positive on previously HLB positive and negative plants, respectively. Whereas, naturally infested caged shoots were 7% and 18% HLB positive on previously HLB positive and negative plants, respectively. Adults emerging from infested shoots caged on HLB symptomatic trees at SWFREC during Feb-March 2009 and analyzed at US Sugar and SWFREC were found to be 5% HLB positive by both labs. Psyllids collected at large at the same time and location were 25% HLB positive, followed by 11% in April. Adults that emerged from infested shoots caged on HLB symptomatic trees were 23% HLB positive according to the USDA-ARS Riverside lab. Adults that emerged in the cages on HLB symptomatic trees from same location during July-August 2008 were 27% positive according to the Riverside lab. Therefore, psyllid origin and time of collection are major sources of variation on PCR results that we will need much more data to sort out. Additional psyllid samples from these experiments are being processed. As our methods for addressing the first objective are refined as described above, the second objective is being addressed by the identification of additional sites to survey for psyllids which differ with respect to tree age, variety, and rootstock, and block size as described in grant proposal. A survey to administer to growers for tracking HLB and grove management tactics in the selected sites is in preparation.