

Project #	Project Area	Team (Affiliations)	Title	Main Goal & Significance	1st Year Deliverable	Budget/Year (\$K); for 2 years
1	Genome (Liberibacter/Psyllid) Sequencing	Cliff Han (LANL)- PI Hong Lin (USDA-ARS, Parlier, CA) Duan YongPing (USDA-ARS, Fort Pierce, FL)	Complete sequencing of HLB-associated Liberibacter – Liberibacter asiaticus, Liberibacter africanus, and Liberibacter americanus	Comparative HLB-associated Liberibacter genomes to identify genetic signatures common to all of them and unique to each of them. These signatures will be used for Liberibacter detection using psyllid and citrus samples.	Comparative genomes of two Liberibacter asiaticus strains and identification of unique/specific genetic signatures	
2	Genome (Liberibacter/Psyllid) Sequencing	Wayne Hunter (USDA-ARS, Fort Pierce, FL)- PI Cliff Han (LANL)	Finishing the psyllid genome sequencing	While the draft sequencing so far covers ~85% of the psyllid genome, additional end sequencing of the BAC library will be performed to enable sequencing of the whole psyllid genome. The availability of the psyllid genome sequence will allow identification of the Liberibacter by subtractive genomics. This will also allow genome-scale studies of psyllid-Liberibacter interactions.	Complete sequencing of the psyllid genome	
3	Discovery and Validation of Pathogen and Host Biomarkers	Abhaya Dandekar (UC Davis)- PI Luiz Goulart (UC Davis, Visitor)	Functional mapping of host responses by transcriptome analysis	Discovery of host biomarkers by deep sequencing of the transcripts from healthy and Liberibacter-infected (symptomatic and asymptomatic) citrus trees. The mRNA biomarkers specific for both symptomatic and asymptomatic stages will be validated by real-time qPCR and if needed, the presence of the markers at protein level will be confirmed by immuno-assay using specific single-chain antibodies.	A set of 10-20 biomarkers (RNA or Protein) specific for symptomatic and asymptomatic stages of Liberifacter infection.	

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4	Discovery and Validation of Pathogen and Host Biomarkers	Fred Gmitter (UF-CREC)- PI Chunxian Chen and Qibin Yu, (UF-CREC)	Proteomic Comparison of HLB-tolerant and HLB-susceptible citrus species	Determination of temporal protein expression by isobaric tagging and 2D LC-MS in HLB-tolerant and HLB-susceptible citrus trees to identify the host genes/proteins that confer resistance or tolerance. The relative expression level of these genes/proteins can be used as markers for HLB manifestation. Finally, over-expression of these genes/proteins in transgenic citrus may offer HLB resistance.	A set of 2-5 host factors for HLB resistance.	
5	Discovery and Validation of Pathogen and Host Biomarkers	Hailing Jin (UC Riverside, CA)- PI	Host microRNA as viable biomarkers for HLB	Differential expression of host microRNA in Liberibacter infected <i>Nules clementine</i> (a sensitive variety) and <i>Sun Chu Cha mandarin</i> (a resistant variety) by deep sequencing. This will help identify and validate HLB-specific host microRNAs for diagnosis on luminex or lateral flow microarray based platforms (projects 7 & 8).	A set of at least 3 host microRNAs as HLB biomarkers.	
6	Discovery and Validation of Pathogen and Host Biomarkers	Wenbo Ma (UC Riverside)- PI	Identification specific host-pathogen interaction during Liberibacter infection in citrus	Analysis of specific interaction by yeast-two hybrid between Liberibacter virulence factors and their protein targets in host citrus. This will help identification of specific host interactors as HLB markers. These markers can be utilized on a protein-based platform (Project 9)	A set of 5 host interactors in citrus as HLB markers	
7	Detection of Liberibacter and Diagnosis of HLB	R. Bruce Cary (Mesa Tech, NM)-PI	Low-cost and sensitive detection of Liberibacter in field and laboratory environments: design of a multiplexed device for sample-to-detection capability.	Design of integrated system to process sample, amplify DNA or RNA, and detect specific DNA or RNA signature on a lateral flow microarray patterned in a machine-readable format. The detection can be done by a cell phone and data can be analyzed by a laptop or a cell phone.	A prototype platform validated by detecting Liberibacter asiaticus 6 specific signatures and 3 negative controls and 3 backgrounds signatures (similar to but not identical to the Liberibacter)	

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8	Detection of Liberibacter and Diagnosis of HLB	P. Scott White (LANL)- PI	Deployment of Liberibacter screening assays in Florida and other Labs.	Identification of validated Liberibacter signatures from the completed genomes and development of MOL-PCR assay for species-specific detection on a luminex platform. The assay technology for the detection of validated signatures will be transferred to various laboratories.	Transfer of assay technology and reagents for the detection of Liberibacter asiaticus to regulatory agencies and research labs.	
9	Detection of Liberibacter and Diagnosis of HLB	Luiz Goulart (UC Davis, CA, Visitor) Abhaya Dandekar (UC Davis, CA)	Biosensor for rapid in field detection of HLB specific pathogen and host responses	Development of an electrochemical ceramic-based biosensor for the detection of Liberibacter proteins and validated citrus protein markers for HLB infection using specific single-chain antibodies. Using this approach, it would be possible to detect intact & live Liberibacter by targeting surface proteins.	Detection of intact Liberibacter asiaticus.	
10	Therapy by Generating Transgenic Citrus	Ed Stover (USDA-ARS, Fort Pierce, FL)- PI Abhaya Dandekar (UC Davis, CA) Goutam Gupta (LANL)	Production of chimeric AMPs to maximize efficacy of HLB control in transgenic citrus	Generation of transgenic citrus with enhanced HLB suppression by building upon the discovery of antimicrobial peptides (AMP) with high expected anti-Liberibacter efficacy and established demonstration of the utility of AMP-elastase chimera.	A validated set of 3 AMP and 3 AMP-Chimera as candidates for constructing transgenic citrus	