CITRUS ADVANCED TECHNOLOGY PROGRAM

QUARTERLY & FINAL REPORTS: Control of Citrus Greening, Canker & Emerging Diseases of Citrus

SELECT PERIOD September 2015

(●) *Quarterly Report* ( ) *Final*

Proposal Title

Field trials of Naturally Occurring Soil Microbials to combat HLB

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
10/1/15	Citrus Research and Development Foundation	Epidemiology and Cultural Control

REPORT UPDATE (500 words-It is not necessary in this public report to disclose your institution's proprietary information or intellectual property.)

This is a 3 year study to provide a side-by-side comparison of 5 soil-applied commercially available products as well as an organic mulch as recommended by growers. The impact of treatments on tree health, foliar nutrition, disease rating, HLB status, root density, yield and fruit quality will be evaluated. Trials will be located in 3 Florida sites: Central Ridge, East Coast and Southwest and will incorporate well-managed trees with HLB but that are as healthy as possible. Each trial will be large enough to be statistically significant with treatments within label guidelines to avoid need for crop destruct or permitting. Objectives of the Project: Test 5 soil-applied products, with mulch subplots, plus an untreated control (6 treatment plots) on health and HLB status of orange trees over 3 years. The 6 treatment plots of 20 trees each will be replicated 4 times plus mulch subplots at the 3 sites will include: 1. BioFlourish (Triangle Chemical).3 applications per year; 2. Ecofriendly Citrus Soil Amendment. 8 x per year; 3. Serenade Soil (Bayer CropScience) 3 x per year; 4. Aliette (Bayer CropScience) 3 x per year 5. Quantum product line 12 x per year; 6. Untreated control (3 mulched trees only 1/year) Our responsibility is to analyze microbe products, analyze root mass, Las in the root, survival or colonization of microbes in the roots, and indicator strains of microbial diversity in the soil.

We have received soil samples and root samples from all three sites and have analyzed the effect of different treatments on root mass. No significant effect was observed for all the treatments.

The DNA from rhizosphere soil was extracted and Illumina Miseq 300 bp pair end sequencing was conducted to determine the survival of bacteria in the products and the effects of the application to the rhizosphere bacterial community.

For each treatment, 50 cc soil from each of the 6 uniform trees of one replicate were combined and the roots were extracted, then the rhizosphere soil (root attached soil) was collected for DNA extraction as one sample. Thus, there were 24 DNA samples (6 treatments*4 replicates) for each field trial. For all the three sites, there were 72 DNA samples.

For 16S V3-V4 region 2*300 Miseq sequencing, the DNA from 4 replicates were pooled together and treated as one sample, so there are 6 samples (6 treatments) per site and 18 samples totally. The aim is to check the survival of applied bacteria, and the effects of application of commercial bacterial products on the native rhizosphere bacterial community. Totally 4.2 Million paired end reads were generated. The clean pair end reads were merged based on overlap information and demultiplexed based on barcode sequences.

The merged sequences were aligned to the 16S v3-v4 region based on Silva v119 database and the OTU tables as well as taxonomic annotations were generated using MOTHUR pipeline. The results suggested the bacterial community in site C (east coast trial site) was very different from the other two sites, while the site A and site B shared more similar bacterial community based on the prevalent bacterial community (OTUs relative abundance>1%) . No OTU in the prevalent bacterial community was identified to be derived from the applied bacteria containing products in this study (e.g. Bio Flourish). Based on the whole bacterial community (OTUs relative abundance>0.1%), the samples from each site were grouped together and the three sites formed three separate groups. Mg and P were suggested to contribute to the bacterial community in site A while element Fe and K contributed to the bacterial community in site C. The contribution by treatments was minimal. The result was consistent with the VPA result, which suggested site location was responsible for 93.2% of the bacterial community difference observed in this study, while the treatments contributed 0.1% of the bacterial community difference observed.

PI First Name Nian

PI Last Name Wang

Email nianwang@ufl.edu

Phone 863-956-8828

Organization University of Florida

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Total Funds (current year)

Form PR-12 Quarterly Reports **SUBMIT REPORT**

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