CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD June 2016

○ *Quarterly Report* ● *Final*

Proposal Title

Fixed-Quat: A novel alternative to Cu fungicide/bactericide for preventing citrus canker

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
10-5-16	Citrus Research and Development Foundation	Pathogen/Disease Detection

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

In Year 3, our major focus was to further optimize the best performing Fixed-Quat material to obtain a commercially-viable product formulation. This included stability (shelf-life) and in vitro efficacy evaluation of the product that was formulated with agriculture-grade chemicals. In Year 1 and 2, the source of silica for the preparation of Fixed-Quat nanoparticle and nanogel materials was tetraethylorthosilicate (TEOS; product labeled as Fixed-Quat A gel). Previously, Fixed-Quat A was used in the 2014 Canker field trial on "Ruby Ray" grapefruit in Vero Beach, FL and was found to reduce the incidence of infection from 63% to 15% at 200 ppm spray rate, matching Kocide 3000 (0.9 lb/acre Cu) which reduced the infection to 16%. This result was promising as this is for the first time we have shown that a non-metal based non-traditional disinfectant material could be potentially used as an alternative to traditional Cu bactericide/fungicide for crop protection. The results from the 2015 canker field trial reported that the untreated control displayed an incidence of infection of 60% while our Fixed-Quat A-II nanogel reduced the infection to 26% at 200 ppm which matched Magna-Bon (0.14 lb/acre Cu) commercial product which had a reduced infection of 25%. In the Fixed-Quat A-II gel, EPA approved Quat was used at the 13,500 ppm level or higher with no issues observed with formulation stability. Although the TEOS is a well-studied chemical (in particular for proof of concept studies for making colloidal silica particles), due to its chemical reactivity and other limitations, it is not approved by the US EPA for the use as an inert at this time. Therefore, instead of using TEOS, we have used EPA approved (for "food" and "non-food" use) silica source such as Sodium Silicate which resulted in newer version of products formulations, Fixed-Quat A-II, Fixed-Quat AP-II and Fixed-Quat E. The formulation composition and interactions between the components (silica and Quat) was confirmed using Fourier Transform Infrared Spectroscopy (FTIR) in particularly looking at the Si-O stretching and SiO-H stretching frequencies of silica. The Si-O stretching frequency changed from 1039 cm (-1) to 1031 cm (-1) whereas the SiO-H frequency changed from 3391 cm (-1) to 3388 cm (-1), suggesting interaction of positively charged Quat with the silica gel. The Quat C-H bending frequency of 2923 cm (-1) was found in both controls and formulations, confirming the presence of Quat. This change also supports the interaction of Quat with silica gel. The Fixed-Quat AP-II and E nanogel's plant safety (phytotoxicity studies) was carried out in a Panasonic Environmental Test Chamber (Model MLR- 352H) which allowed for controlled day/night cycling temperatures, light intensity and humidity to simulate summer weather conditions. Studies conducted on Sour orange, a common citrus variety and Roma Tomato sp, an ornamental plant revealed no sign of plant injury when tested with Quat concentration as high as 1000 ppm. The morphology of Fixed-Quat E nanogel was examined using Scanning Electron Microscopy (SEM), displaying sub-micron to micron composites with irregular shapes in multiple layers. X-ray Diffraction (XRD) revealed that the material is primarily amorphous in nature. Antimicrobial studies of optimized Fixed-Quat E nanogel was conducted against Xanthomonas alfalfae subsp. citrumelonis (citrus canker surrogate), Pseudomonas syringae pv syringae, a gram negative causative agent of bacterial speck in citrus and tomato sp and Clavibacter michiganensis subsp michiganensis, a gram positive causative agent of canker and systemic infections in tomato. Minimum Inhibitory Concentration (MIC) was determined and compared against Kocide 3000 and copper sulfate controls. MICs of Fixed-Quat A-II and E were found to be ≤ 1.0 µg/mL for X. alfalfae, ≤ 1.0 µg/mL for P. syringae and ≤ 1.0 µg/mL for C. michiganensis. MIC results indicate no loss in efficacy with new source of silica. 2016 Citrus canker trials on "Ruby Ray" grapefruit includes both Fixed-Quat A-II and Fixed-Quat E nanogel materials. This trial results are expected sometime in January, 2017.

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