Florida Citrus Advanced Technology Program

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

Instructions Complete the fields based on your project specs. When finished, save the form to your local disk using a unique name. Then, go to **http://research.fcprac.com**, and log in with your user name and password using Researcher Login in the lower left. Find this project title and click on **Submit a Report**. Update your profile information if needed, then upload this report as directed.

2009-2010 REPORT	CATEGORY (drop-down)	TODAY'S DATE (m/d/yr)
◉ *Quarterly Report* ○ *Annual Report* ○ *Final*	Canker Pathology	8/2/10

WHAT IS THE "HEADLINE" FOR THIS REPORT (e.g. a one-sentence "newspaper headline" describing what you accomplished)

Citrus canker strains labelled with GFP survive in biofilms on leaf and fruit surfaces

TITLE and CONTACT INFORMATION

Proposal Title Survival of Xanthomonas citri ssp. citri (Xcc) to estimate risk of citrus canker transmission by infected fruit

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Phone 863-956-1151	*Project Duration (years)* 2 *Year of Project* 2
Organization UF, Citrus Research and Education Center	*Total Direct Funds (current year)* $114,264

REPORT UPDATE (650 words; provide details about your headline)

Under Objective 1 (biofilm formation by Xanthomonas citri subsp. citri (Xcc) in comparison with other bacteria that are known to form biofilms). Different assays are being performed to establish how different bacteria form biofilms on abiotic surfaces (glass slides) and whether the aggregates on glass surfaces contain metabolically active bacteria. Development of aggregates was visualized on glass surfaces with confocal microscopy and the use of green fluorescent protein (Gfp) expressing strains. Viability of the bacteria in aggregates is confirmed using strains transformed with a plasmid expressing a labile Gfp protein that breaks down when the bacterium dies. Similar studies on leaf and fruit surfaces indicated that all the bacterial cells in aggregates attached to the surface did not show the same metabolic activity as revealed by comparing stable and labile Gfp variants. Only bacteria that formed biofilms were viable while scattered individual cells died in the initial stages. Differences in bacterial aggregation and biofilm formation were observed between Xcc strains with different host range, A and A*, as well as X. campestris and X. alfalfae susp. citrumelonis. Preliminary results with scanning electron microscopy showed differences in biofilm structure between A strain (wide host range) and A* strain (narrow host range) on fruit and leaf surfaces that confirmed initial results in vitro with violet crystal staining of biofilm formation on plates. These results indicate reduced ability of the narrow host range strain A* to form biofilm. In addition, there were differences in bacterial motility between A and A* strains. A* strain had higher swimming and lower swarming capacity than A strain that may be related to their differences in ability to form biofilm. Under Objective 2 (studies in vitro and in vivo using transformed strains of Xcc subjected to different environmental conditions). Two different types of studies are in progress. One is the effect of different bactericides on the initial stages of biofilm formation and the other is the effect of these compounds as biofilm agents once the biofilm is formed. Aggregates were stable and Xcc in them remained viable after water washes which explains why fruits harbor residual Xcc after disinfection treatments. Fruit wash treatments with toxicants and disinfectants (CuSO4, NaCl, NaOCl and SOPP) were tested for their effect on biofilm formation. Sublethal concentrations of NaCl and SOPP in the initial stage of surface colonization increased aggregation of viable Xcc. This effect was less clear with NaOCl or CuSO4 at the sublethal concentrations tested thus far. Greater aggregation is explained as a bacterial resistance response to chemical stresses. Experiments are in progress to confirm these results. Assays to measure bacterial aggregation and biofilm formation in a quantitative manner using violet crystal staining after exposure to bactericides are in progress in order to repeat experiments performed last year. In addition total RNA is being extracted from treated biofilm forming and planctonic bacteria to evaluate differential expression of genes involved in biofilm formation and aggregation and possibly those related to motility and quorum sensing.