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	2/26/10

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

Principal Investigator James. H. Graham	*PI Last Name* Graham
Email jhg@crec.ifas.ufl.edu	*FDACS Contract Number* 72633; NAS-85
Phone 863-956-1151	*Project Duration (years)* 2 *Year of Project* 1
Organization UF, Citrus Research and Education Center	*Total Direct Funds (current year)* $124,906

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

Objective 1: Strains of Xanthomonas citri subsp citri (Xcc) and X. alfalfae pv. citrumelonis (Xac), the cause of citrus bacterial spot, were transformed with non-stable GFP and used to identify in situ their ability to survive and persist on leaf and fruit surfaces. When suspensions of the non-stable GFP strains were sprayed on leaf and fruit surfaces, bacterial aggregates were observed under confocal laser microscopy. Persistence of fluoresce in these aggregates confirmed the presence of metabolically active cells, but these cells had limited survivability without osmotic protection from desiccation by phosphate buffer. After inoculation of bacteria in water, scattered cells of the non-stable strain on the leaf or fruit surfaces rapidly lost fluorescence, i.e. were non-viable. Only cells in aggregates continued to fluoresce, i.e. maintained viability. These aggregates were restricted to sites on the fruit or the leaf surfaces that conserved moisture. Aggregates on leaves or fruits were located primarily in depressions where water availability was higher. The aggregates resembled what has previously been described as biofilms. In two in vitro assays, biofilm formation by canker strains Xcc strain A (X306 Brazil), Xcc strain A* (X407 Iran), X. fuscans subsp. aurantifolii strain B (X69 Argentina), X. fuscans subsp. aurantifolii strain C (X341 Brazil), X. campestris pv. campestris (1469) was compared as well as the effects of different growth media with or without glucose. Biofilm staining with crystal violet showed biofilm formation on abiotic surfaces differed among culture media used to grow the bacteria as well as among Xanthomonas strains. X. campestris had the highest and most rapid biofilm formation. Among canker strains, C strain produced more biofilm than A and B strains. Differences in biofilm production depending on the culture medium confirmed that nutrient environment can alter biofilm formation e.g. addition of glucose to the medium did not increase but sometimes reduced biofilm formation. Next experiments will evaluate expression of genes involved directly in biofilm formation (gumD) or associated phenomena (quorum sensing, motility) for biofilm induced bacteria.

Objective 2: Persistence of metabolically active cells in biofilms explains the occasional isolation of viable Xcc cells from surfaces of asymptomatic fruits infested with Xcc even after rigorous disinfection treatments with chlorine and sodium-ortho-phenyl-phenate (SOPP). Recently, Gottwald et al. 2009 demonstrated that pre-washing with a detergent was the most effective treatment for disinfection of fruit infested with Xcc. Detergents may disrupt biofilms, exposing Xcc to disinfectants, bactericides, osmotic stress agents such as SOPP, chlorine, copper sulfate and high levels of salts. Biofilm formation was evaluated for strains 306 and 341 after 24, 48 and 96 h exposure to sub-lethal concentrations of $CuSO_4$, NaOCl and NaCl. Exposure to sub-lethal concentrations did not reduce, and sometimes increased biofilm formation as a result of the stress condition. Survival of strain 306 that occurred after exposure to SOPP at concentrations less than 2% was confirmed by membrane integrity assay, detection of GumD mRNA, and growth in culture medium. Next experiments will utilize the labile gfp marked strain to assess the viability of Xcc in preformed biofilms in vitro and in vivo on plant surfaces as affected by detergents and toxic compounds.