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*	HLB Pathology	1/14/10

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

Peptides that have promise to control HLB have been found. Field tests are planned to begin in the spring.

TITLE and CONTACT INFORMATION

Proposal Title Identify and deliver antibacterial peptides and/or proteins for the control of citrus greening (Huanglongbing or HLB)

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Organization UF, IFAS, CREC	*Total Direct Funds (current year)* $309,194

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

This is a continuing project to find an interim control measure to allow the citrus industry to survive until resistant or tolerant trees are available. We are approaching this problem in two ways. First, we are attempting to find products that will control the greening bacterium in citrus trees. We have chosen initially to focus on antibacterial peptides because they represent one of the few choices available for this time frame. We also are testing some possible anti-psyllid genes. Second, we are developing virus vectors based on CTV to effectively express the antibacterial genes in trees in the field as an interim measure until transgenic trees are available. We think that this approach could be used beginning 2-3 years from now and until probably 15 years from now when resistant trees should be available. With effective antibacterial or antipsyllid genes, this will allow protection of young trees for perhaps the first ten years with only pre-HLB control measures. Third, we are examining the possibility of using the CTV vector to express antibacterial peptides to treat trees in the field that are already infected with HLB. With effective anti-Las genes, the vector should be able to prevent further multiplication and spread of the bacterium in infected trees and allow them to recover.

We have completed several large screenings of antibacterial peptides against Las in sweet orange trees. About 40 different antibacterial peptides have been tested in trees. We initially found three peptides that allow much better growth of trees that were grafted with HLB-infected buds. Some trees had no symptoms and no detectable Las, some trees had no symptoms and low levels of Las, and other trees had leaf symptoms but continued growth of the trees with normal levels of Las. Another result is that we found that leader peptides for the export of the peptide from the CTV-infected cell is not needed for HLB but is needed for citrus canker. Because we were concerned that graft inoculation of HLB into the trunks of small trees is a too severe challenge that might cause peptides that could work in the field to be missed, we developed a system that only allows inoculation by infected psyllids. We have established a containment plant growth room in which psyllids inoculate the plants expressing the peptides. These experiments are on-going, but we appear to have several more peptides that are protecting sweet orange.

To speed up the search for effective anti-HLB genes, Falk (UC Davis) has developed a tobacco-tomato psyllid/liberibacter model to screen for effective genes against the similar bacterium. This system is working and screening is on-going.

We also are improving the CTV-based vector to be able to produce 2-5 peptides at the same time. This will allow expression of genes against both HLB and canker or multiple of genes against HLB. We have developed a vector that can be re-added to trees if the anti-Las gene is lost or a better gene becomes available. A major objective that we are pursuing is to make a vector that cannot be transmitted by aphids.

Another major goal is to do a field test of the CTV vector with antibacterial peptides, which is an initial step in obtaining EPA and FDA approval for use in the field. We have received permission from USDA APHIS for the field test, but were delayed by EPA. We are now submitting a revised application to USDA APHIS to include EPA requirements and are expecting to establish the field test this spring.

In addition, we are screening a series of transgenic sweet orange and grapefruit expressing antibacterial genes for Erik Mirkov of Texas A&M and Mike Irey of Southern Gardens.