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	7/30/10

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

Cybrids inherit resistance via transfer of mitochondria and chloroplast genes

TITLE and CONTACT INFORMATION

Proposal Title Characterization of canker resistance in citrus plants created by 'Somatic Cybridization' without citrus transformation

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Organization UF, Citrus Research and Education Center	*Total Direct Funds (current year)* $98,045

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

Objective 1: A comparative study of two susceptible hosts, Duncan grapefruit (DG, C. paradisi), and Rough lemon (RL, C. jambhiri) and two resistant cultivars of kumquat (Fortunella spp.), 'Meiwa' and 'Nagami', evaluated the mechanisms involved in the resistance of kumquat to the citrus canker. MK and Nagami NK developed a hypersensitive response (HR), with necrotic lesions with population of Xanthomonas citri subsp. citri (Xcc) < 5 log units after 168 h in detached leaf and attached leaf assays. Early expression of genes related to programmed cell death associated with HR were identified in MK and NK. The resistance in kumquats has several characteristics associated with HR: 1) Rapid necrosis of leaf tissue in 48-72 h post inoculation in vitro or 72-96h in planta; 2) Disruption of epidermal and mesophyll cells by 72 h; 3) Xcc bacterial ingress limited to few cell layers below the epidermis; 4) Xcc population growth arrested at 72 h coincident with the cellular disruption; 4) Light microscopy and TEM, show death of the cells adjacent to the inoculation site with very few bacteria proliferating; 6) 5) HR-related genes and other putative resistance-related genes expressed early in resistant KN but not in susceptible DG. Behavior of susceptible DG and RL was: 1) No symptoms are detect in susceptible until 72 hr after inoculation and water soaked developes at 168 hr; 2) pustular callus-like lesions erupted through the cuticle by 10-16 days post inoculation (dpi); 4) Xcc populations reach 6 log cfu of Xcc per inoculation site at 168 hr; 5) Xcc population increases up to 15 dpi. Objective 2: Validate the inheritance or resistance for cybrids with susceptible Red grapefruit (RG) and RL with Valencia orange (VO). The putative RL cybrid has been recently been fully analyzed and determined using Single stranded repeat (SSR) analysis to be a cybrid from a mislabeled callus line of Valencia orange and not Meiwa kumquat. Hence the inheritance of resistance is not the HR type but is one of moderate susceptibility compared to high susceptibility in RG. Evidently, there is a definite expression of resistance in the cybrid inherited as a result of presence of the heterologous mitochondrial or chloroplast genome from the VO callus line. Evidence for this is as follows: Intermediate lesion symptoms are observed for RL+VO cybrid in vitro and in-planta. In contrast to development of callus, the inoculated area develops necrosis by 10 dpi. Xcc population plateaus by 10 dpi below the bacteria populations susceptible RL or Red GF. Two types of lesion were observed: necrotic and also callus, suggesting that cell death occurs and arrests the proliferation of Xcc. Expression of HR-related genes is intermediate between MK and RL, further substantiating that some yet to be determined elements of resistance have been inherited in the cybrids. Finally, the current set of Ruby red grapefruit cybrids with VO planted in canker-affected locations on the east coast continue to be more resistant than Red grapefruit trees around them. These RG+VO cybrids will continue to be observed for vigor and eventually fruiting characteristics, as well as resistance to canker.