Identification of *Spiroplasma citri-induced* Small RNAs for Early Diagnosis of Citrus Stubborn Disease

Georgios Vidalakis and Hailing Jin
Plant Pathology & Microbiology, UC/Riverside

The fundamental control method for the stubborn disease, like most of the graft-transmissible diseases of citrus, is the use of disease tested propagative material. Modern diagnostic techniques that increase the capacity of quarantine programs such as the Citrus Clonal Protection Program (CCPP) to test source plants of citrus germplasm and improve the overall scheme of diagnostics necessary for the safe release of new varieties to the industry are essential for a sustainable and viable California citriculture.

The existing diagnostic protocols for the stubborn disease are targeting the causal agent *Spiroplasma citri*. The current project aims to an alternative approach for the stubborn diagnosis using early host responses to the spiroplasma infection.

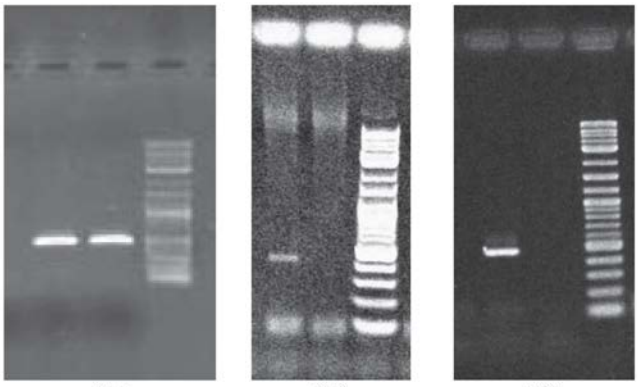

Figure 1. PCR detection of s. citri *isolate C189 in me- chanically inoculated sweet orange seedlings in frozen (A) and fresh tissue 5-7 weeks-Early point (B) & 10 weeks- Late Point (C) post inoculation.*

During the past year we have fine-tuned our experimental system off mechanical inoculation of Sweet orange (*Citrus sinensis* var Pineapple) with pure culture of *S. citri* (preliminary results were reported in the International Citrus Conference in China, October 2008) and verified the presence of the *S. citri* in the tissue collected and stored for the small RNA analysis of the early and late points of infection (fig. 1). Extraction of RNA was also performed in Hailing Jin's lab. The small RNA libraries of both early and late time points from both untreated and infected plants are under construction.

In addition, in preparation of hybridization experiments for cross interaction between host signals induced by stubborn and other diseases i.e. Huanglongbing (Hailing Jin's project "Identification of *Candidatus* Liberobacter-induced Small RNAs for Early Diagnosis of HLB…") we initiated the full testing-VI (biological indexing and laboratory, completion expected by summer of 2009) of the *S. citri* isolates of the CCPP disease collection for the presence of other graft-transmissible pathogens of citrus that might affect the expression of the host small RNAs and generate cross interactions.