DETECTION OF *XYLELLA FASTIDIOSA* IN INSECT VECTORS IN CALIFORNIA

Project Leaders:

M. Francis
Dept. of Plant Pathology
University of California
Davis, CA 95616

J. Cabrera
Dept. of Entomology
University of California
Riverside, CA 92521

H. Lin and E. L. Civerolo
USDA, ARS, SJVASC
Parlier, CA 93648

ABSTRACT

Recent spread of *Xylella fastidiosa (Xf)* to several agricultural commodities and ornamental plants in California has prompted great interest in understanding the comparative interactions between *Xf* and native and recently introduced insect vectors. The generally low titer of *Xf* in insect vectors limits the use of serological techniques, such as ELISA, for qualitative and quantitative analyses of *Xf* associated with different insect vectors. *Xf* detection by molecular techniques, such as PCR, can potentially overcome this limitation. The objective of this study was to compare standard PCR for detection of *Xf* in field-collected insects as well as in greenhouse-reared insect vectors using primers RST31/RST33 with newly developed primers HL5/HL6 in standard PCR and in Real Time PCR using the system HL5/HL6 and a probe labeled with FAM. Two native species the green sharpshooter (*Draeculacephala minerva*) and the red-headed sharpshooter (*Xyphon fulgida*), and the recently introduced glassy-winged sharpshooter (*Homalodisca coagulata)* were included in this study. Field-collected insects were obtained from *Xf*-infected grapevines and almonds in the San Joaquin Valley, California. Greenhouse-reared green and red-headed sharpshooters were also obtained from cultures maintained on a non-host of *Xf* in Parlier, California. Five-10 *Xf* cells per µL of insect head DNA sample were detected with the HL5/HL6 primer pair-FAM system. Also, using this system, the number of *Xf*-cells detected in field-collected and greenhouse reared insect was between 10^2-10^3/µL sample/reaction. This concentration of *Xf* cells was detected by visualization of the *Xf*-specific amplicon (221 bp) in gels following standard PCR with the HL5/HL6 primers. This level of pathogen in insect heads was below the limit of detection in standard PCR with primers RST31/RST33. Using Real-Time PCR quantification with the system HL5/HL6-FAM, the total amount of *Xf*-cells per insect head was estimated to be between 10^4-10^5. Implications of these results on the epidemiology of the disease are discussed.

EVALUATION OF A NOVEL, FIELD DEPLOYABLE, ELECTROCHEMICAL DETECTION SYSTEM FOR THE DETECTION OF *XYLELLA FASTIDIOSA* WITHIN GRAPEVINE PETIOLES

Project Leaders:

Vien Lam
University of Houston-Downtown
Houston, TX 77002

Lisa Morano
University of Houston-Downtown
Houston, TX 77002
moranol@uhd.edu

ABSTRACT

We have tested a new electro-chemical detection (ECD) system designed by AnzenBio, Inc. for the quick detection of *Xylella fastidiosa* within grapevine petioles. Like standard ELISA this detection method relies on antibodies against the bacterium, but unlike ELISA it detects movement of electrons through the final product conversion, measuring current rather than color change. Using a hand-held meter and pre-coated chips the test can be done in a fraction of the time (1.5 vs. 5 hrs.). Comparison of 18 Cabernet Sauvignon petioles from a vineyard with Pierce's disease (PD) to 18 petioles guaranteed PD free showed the ECD readings per gram of tissue to be higher for PD petioles (31.3 vs. 6.2 microamps). This difference is statistically different using a t-test ($p<0.0001$). In another trial in South Texas, ECD was used to evaluate the petioles from three different varieties, Blanc du Bois, Black Spanish and Cynthiana, which have been shown to carry differing levels of *Xylella fastidiosa* within this area of high PD pressure. Petioles were also categorized into those from leaves with low, medium and high PD symptoms. Analysis of variance on ECD data from the 9 symptom variety categories with 6 replications showed that ECD could detect distinct significant differences between several of the categories ($p<0.0001$). Analysis of variance on ELISA data run on the same 54 samples found no significance between categories ($p=.43$). ECD appears to give more sensitive readings over a range of bacterial levels, potentially giving fewer false positives.

Section 5:
Control Strategies