FATE OF A GENETICALLY MODIFIED BACTERIUM IN THE FOREGUT OF THE GLASSY-WINGED SHARPSHOOTER

Project Leader:
Jose Luis Ramirez
Department of Entomology
University of California
Riverside, CA 92521

Project Director:
Thomas A. Miller
Department of Entomology
University of California
Riverside, CA 92521

Collaborators:
Thomas M. Perring
Department of Entomology
University of California
Riverside, CA 92521

Carol Lauzon
Department of Biological Sciences
California State University
Hayward, CA 94542

David Lampe
Department of Biological Sciences
Duquesne University
Pittsburgh, PA 19219

Reporting Period: The results reported here are from work conducted July 2005 to September 2005.

ABSTRACT
The use of genetically modified symbionts is a new approach to control the spread of insect-transmitted pathogens by reducing vector competence. A symbiont-control strategy is being developed to reduce the spread of *Xylella fastidiosa* (*Xf)* by *Homalodisca coagulata* (*H. coagulata*), glassy-winged sharpshooter (GWSS). In this study, the fate of a transformed symbiotic bacterium inside the foregut of the sharpshooter when fed on citrus and grape was assessed. TaqMan-based quantitative real-time PCR was used to detect and quantify bacterial cells remaining in the foregut at the end of four time periods. GWSS pre-exposed to the transformed bacterium (S1*Axd*) were observed to maintain an infectivity ratio of 40-50% at the end of a 12 day period. We observed a trend for lower S1*Axd* infection rate in GWSS that fed on citrus although not statistically different from the group that fed on grapevines.

INTRODUCTION
A recent approach to control the spread of insect transmitted pathogens is symbiotic control. This relies on genetically modified symbionts capable of releasing a gene product that is toxic to the pathogen (Beard *et al* 2002) to reduce vector competence.

A symbiotic bacterium, *Alcaligenes xylosoxidans* var. *denitrificans* (*Axd*), isolated from the cibarium of *H. coagulata* is currently being engineered to express anti-pathogenic products against *Xf* (Pierce's disease strain) to control Pierce's disease (PD). *Axd* was found to colonize citrus (Bextine et al. 2004) but a transformed variety of the same bacterium did not colonize grape over long periods. Here, we tested the fate of a genetically transformed *Axd* (S1*Axd*) inside the foregut of GWSS when fed on an optimal *Axd* host plant (citrus) and suboptimal *Axd* host plant (grapevines).

OBJECTIVES
1. Investigate the fate of a genetically modified *Axd* (*S1Axd*) in a population of GWSS after acquisition when fed on two host plants.

RESULTS
Field-collected GWSS adults were allowed to acquire the transformed endophyte (S1*Axd*) from an artificial acquisition system for a period of 48hr acquisition access period (AAP). The artificial system consisted of black-eyed pea stems placed in a 1.5 ml microcentrifuge tube containing about 500 µl of bacterial suspension (Figure 1). Subsequently, they were transferred to either grapevines or citrus (sweet orange). A pool of 26 sharpshooters was collected at 0hr. post-AAP and 10 sharpshooters were collected from each host plant and replicate at days 2, 4, 9 and 12th post-acquisition (Figure 2). GWSS collected were stored at -80^{o}C until processed.

After a standard surface sterilization procedure the head and eyes of each sharpshooter was removed and DNA extracted using the DNeasy Tissue Kit (Qiagen Inc.). Detection and quantitation of bacterial titers was done in a real-time quantitative PCR (qPCR) assay by using a set of primers and TaqMan probe designed for the target insert. The qPCR assay included 5 ten-fold dilution points (ranging from 115,940 to 5 copies/µl) that served as standards for our quantification purposes.

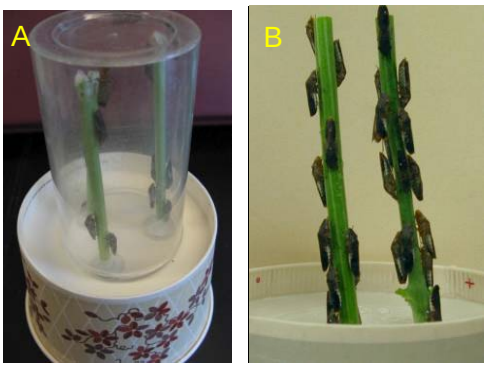

Figure 1. Acquisition of S1*Axd* by GWSS adults.
A.- Complete set-up of the acquisition system.
B.- GWSS feeding on a bacterial suspension.

Bacterial titers acquired by GWSS after the 48hr. acquisition period ranged from 3 to 28,407copies/ul of GWSS head sample. This variation declined over the next testing periods and by day 12 post-acquisition sharpshooters carried from about 1 to 14 copies/µl of sharpshooter head sample.

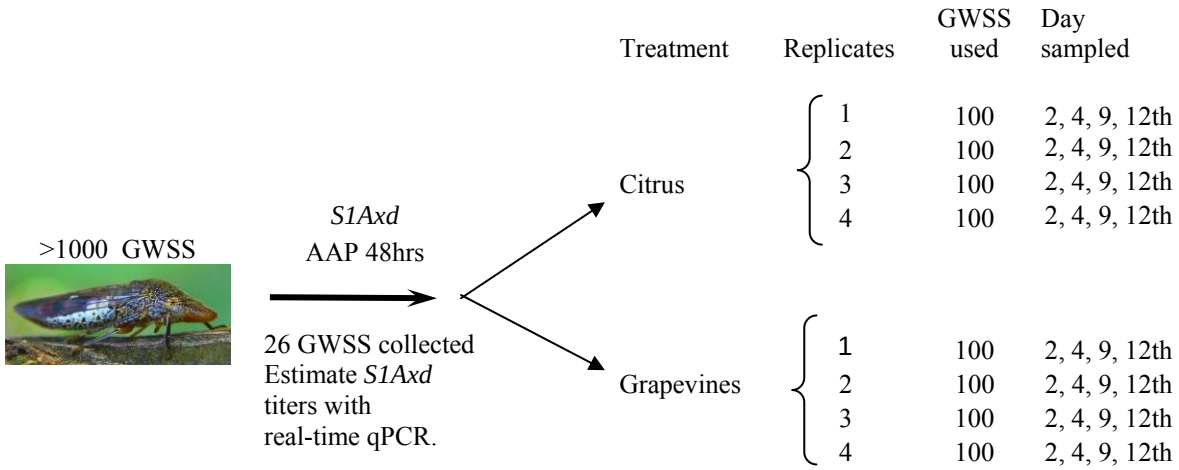

Figure 2. Partial diagram of the experimental procedure.

The infection rate (number of GWSS testing positive/total # of GWSS tested) was about 65% at 0hr. post-acquisition and decreased slightly over time with no significant difference between sharpshooters feeding on grapes or citrus. Infectivity of GWSS on day 12 remained at about 50 % for GWSS feeding on grape and 40% for GWSS feeding on citrus. These two results are not statistically significant.

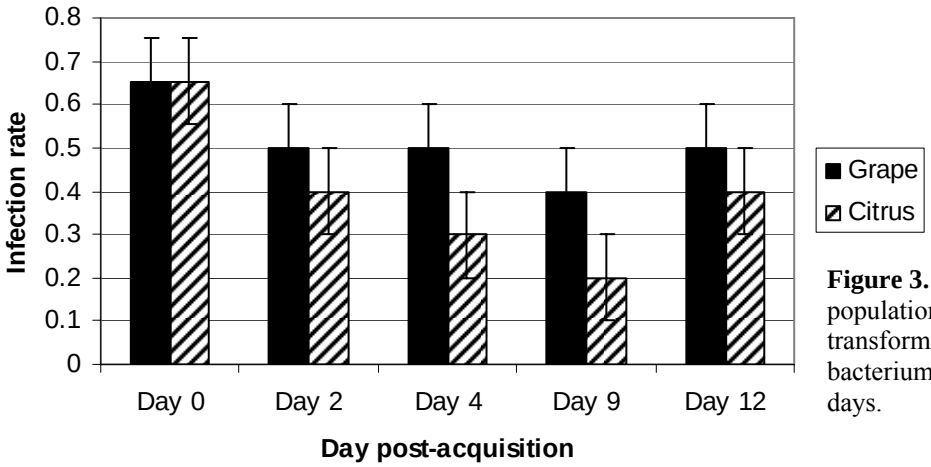

Figure 3. Infection rate of a population of GWSS by the transformed *Axd* (S1*Axd*) bacterium during a period of 12 days.

CONCLUSIONS

The wide variation in the bacterial titers acquired by GWSS might be due to feeding behavior, age difference or physiological state of the sharpshooter foregut. The infection rate data suggest that S1*Axd* was able to colonize the foregut of GWSS and maintain an infection rate of about 40-50 % independent of what host plant they fed on.

REFERENCES

Beard, C. B., C. Cordon-Rosales, and R. V. Duvarsula. 2002. Bacterial symbionts of the Triatominae and their potential use in control of Chagas disease transmission. Ann. Rev. Entomol. 47:123-141

Bextine, B., C. Lauzon, S. Potter, D. Lampe, and T. A. Miller. 2004. Delivery o a genetically marked Alcaligenes sp. to the glassy-winged sharpshooter for use in a paratransgenic control strategy. Curr. Microbiol. 48:327-331

FUNDING AGENCIES

Funding for this project was provided by the USDA Animal and Plant Health Inspection Service, and the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.