INSECT-SYMBIOTIC BACTERIA INHIBITORY TO *XYLELLA FASTIDIOSA* IN SHARPSHOOTERS: PRESSURE BOMB EXTRACTION OF XYLEM FLUID TO IMPROVE BACTERIAL DETECTION OF *XYLELLA* IN PLANTS

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INTRODUCTION

The glassy-winged sharpshooter (GWSS) is the principal vector of the xylem-limited bacterium *Xylella fastidiosa* (*Xf*), which causes Pierce's disease (PD) in grapes and oleander leaf scorch. Limiting the spread of this pathogen by rendering GWSS incapable of pathogen transmission is a promising method of pathogen control.

Paratransgenic approaches to pathogen control are currently being developed to deliver single-stranded antibodies to disrupt Triatomid transmission of *Trypanosoma cruzi* (Beard et al. 2002), and to prevent colitis in mammals (Beninati 2000; Steidler, 2002). Candidate paratransgenic bacterial agents must thrive in the cibarial/foregut area where they will be in close proximity to the pathogen, *Xf* for a paratransgenic strategy to be applicable to the *Xf*/GWSS system.

Alcaligenes xylosoxidans denitrificans (Ax) was cultured and identified from the cibarium and foregut regions of GWSS's alimentary canal several times throughout the growing season indicating that it is a consistent symbiotic organism (Lauzon, in preparation). *Ax* has been described as a non-pathogenic plant endophyte and a non-pathogenic soil-borne microbe (Meade et al. 2001, Yang et al. 1999). This bacterium was genetically transformed with exogenous DNA being incorporated into the chromosome to express fluorescent markers (Lampe, in preparation). My project was to set up a disease cycle, which would offer evaluation of paratransgenic delivery. To do this it was important to improve detection of *Xf*.

OBJECTIVES

1. Develop a delivery system to re-introduce paratransgenic bacteria to GWSS and introduce *Xf* to GWSS to improve the disease cycle in the laboratory.
2. Improve detection of *Xf* in plants and insect vectors.

RESULTS AND CONCLUSIONS

Cycling of dsRed Alcaligenes xylosoxidans denitrificans in GWSS:

I have developed a plant-based bacterial delivery system for GWSS that allows bacteria to be available for consumption by the insect vector. Earlier attempts to feed GWSS on varied sucrose solutions from membrane sachets were unsuccessful because GWSS did not probe and died within 24 hr. GWSS probed from a flowing feeding system but would not sustain feeding and died within 24 hr. The manipulation of the chrysanthemum xylem fluid by forcing a bacterial suspension through a cut stem allowed a GWSS to feed on a concentrated bacterial suspension and was successful in maintaining GWSS for up to 5 days.

GWSS were exposed to the bacterial delivery system that contained dsRed *Ax* (OD600=2.85) for 48 hr. then removed and placed on "clean" chrysanthemum plants for an indefinite period of time. At 0, 7, 14, 21, 28, and 35 days post-exposure, GWSS were collected and inspected by fluorescence microscopy for presence of dsRed *Ax*. Samples from day 0 had individual *Ax* in the cibarium and foregut. GWSS sampled at 7 days and beyond had "clumps" of *Ax*, indicating colonization of the cibarium and foregut. *Ax* was present on all dates sampled and was independent of sex.

Evidence of horizontal transmission was also collected by introducing a single dsRed *Ax*-fed GWSS into a population of 10 "clean" GWSS for 14 days caged on a single chrysanthemum. *Ax* was detected in the cibarium or foregut of 17 of 42 GWSS that survived to the end of the trial.

Creating the PD disease cycle in the lab: bacterial delivery system for introduction of Xf to GWSS:
After 48 hours exposure to *Xf* offered through the bacterial delivery system, 100% of GWSS heads from exposed insects tested positive by PCR for the presence of *Xf*.

The Scholander pressure bomb is used to extract xylem fluid from a plant (Hallmann et al. 1997), allowing collection large amounts of fluid (Guo et al. 2001). The use of the pressure bomb technique for detection of *Xf* in oleander was less sensitive then traditional sample collection when used in conjunction with ELISA and PCR. Attempts at culturing *Xf* from xylem fluid collected using the pressure bomb was more prone to contamination then from traditionally collected samples. The consistency of the oleander pressure bomb xylem fluid was semi-solid making use with DNA extraction for PCR, ELISA, and culturing difficult. Therefore, pressure bomb collection of xylem fluid as a technique for early detection of *Xf* was discontinued.

Use of the pressure bomb for improving *Xf* detection in grape plants was more successful. By ELISA and PCR, pressure bomb fluid collections almost doubled the level of detection when compared to traditional sample collection. However, as with oleander culturing of *Xf* from xylem fluid collected with the pressure bomb was prone to contamination.

Development of the Pierce's disease cycle in the laboratory and the improvements in *Xf* detection provides a dependable system to test candidate paratransgenic bacteria identified by Carol Lauzon and genetically altered by David Lampe with toxins provided by Don Cooksey.

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