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

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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 (S1Axd) were observed to maintain an infectivity ratio of 40-50% at the end of a 12 day period. We observed a trend for lower S1Axd 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 (S1Axd) 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 (S1Axd) 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°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.
Bacterial titers acquired by GWSS after the 48hr. acquisition period ranged from 3 to 28,407 copies/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.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Replicates</th>
<th>GWSS used</th>
<th>Day sampled</th>
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<tbody>
<tr>
<td>Citrus</td>
<td>1</td>
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<td>2, 4, 9, 12th</td>
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<td></td>
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<td>4</td>
<td>100</td>
<td>2, 4, 9, 12th</td>
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<tr>
<td>Grapevines</td>
<td>1</td>
<td>100</td>
<td>2, 4, 9, 12th</td>
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<td></td>
<td>2</td>
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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.
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 S1Axαd 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

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