

## MODELING SHARPSHOOTER TRANSMISSION OF *XYLELLA FASTIDIOSA*

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**Reporting Period:** The results reported here are from work conducted July 2006 to September 2006.

### ABSTRACT

The dynamics of vectored diseases are governed by the interplay of a variety of biological and ecological factors including: vector behavior and demography, pathogen acquisition and inoculation efficiency, host resistance, and the role of environmental factors in mediating these processes. We are studying some of the ecological and biological traits that influence the transmission efficiency of *Xylella fastidiosa* (*Xf*) to grapes by the blue-green and glassy-winged sharpshooters. In particular, we are examining how sharpshooter abundance, acquisition and inoculation periods on plants, and sex affect transmission efficiency for each of these species. This work will contribute to a more mechanistic understanding of *Xf* transmission that will be used to develop biologically realistic models of disease dynamics, providing a platform for evaluating the efficacy of different Pierce's disease management strategies.

### INTRODUCTION

Pierce's disease (PD) epidemiology is complex because of the interplay among several *Xylella fastidiosa* (*Xf*) insect vectors and host plant species - which likely contribute to variability in transmission efficiency and patterns of disease spread in the field. To date the only quantitative description of *Xf* transmission is that of Purcell (1981). Our goal is to further refine this model via experimental estimation of additional ecological and biological parameters likely to govern transmission efficiency of blue-green and glassy-winged sharpshooters. We are especially interested in how vector abundance, acquisition and inoculation periods, temperature, and vector species, and sex may contribute to heterogeneous transmission efficiencies. Results from the new experiments will be used in conjunction with previously published results on the biology of *Xf* transmission to refine models of *Xf* transmission.

### OBJECTIVES

1. Determine the effect of temperature, vector numbers and time on sharpshooter transmission of *Xf*.
2. Develop a model to describe sharpshooter transmission of *Xf* as a function of variables that affect efficiency.
3. Determine if *Xf* colonization of vectors affects their fitness.

### RESULTS

This project is being initiated. We will first focus our studies on how sharpshooter abundance and inoculation period affect, independently, transmission – these two variables were treated as interchangeable by Purcell (1981). In this experiment we varied sharpshooter number (1, 2, or 4 adults - each species separately) fully crossed with inoculation access period (0.5, 1, 2, 4 days - access acquisition period constant at 4 days) and measured the probability of transmission to grape seedlings. Data are currently being collected, therefore we have no results to report at this time.

### REFERENCES

Purcell, A. H. 1981. Vector preference and inoculation efficiency as components of resistance to Pierce' disease in European grape *Vitis vinifera* cultivars. *Phytopathology* 71: 429-435

### FUNDING AGENCIES

Funding for this project was provided by the University of California Pierce's Disease Grant Program.

# QUANTITATIVE ASPECTS OF THE TRANSMISSION OF *XYLELLA FASTIDIOSA* BY THE GLASSY-WINGED SHARPSHOOTER

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**Reporting Period:** The results reported here are from work conducted July 2004 to September 2006.

## ABSTRACT

In this report, we describe quantitative aspects of *Xylella fastidiosa* (*Xf*) transmission by the glassy-winged sharpshooter (GWSS). In previous studies, we discovered correlations between the number of *Xf* cells acquired by GWSS and acquisition access period, and total ingestion time on *Xf* sources. On the other end of the disease cycle, correlations were detected between the number of *Xf* cells inoculated into plant stems and the length of inoculation access periods (IAP), and the number of probes. In the study reported here, correlations between the number of cells inoculated into a plant and IAP or number of probes were consistent when the IAP was restricted to 30, 60, 90, or 120 minutes.

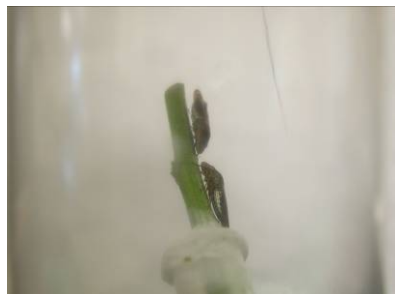
## INTRODUCTION

Solutions to Pierce's disease (PD) are coming out of an understanding of basic biological aspects of the vector, the pathogen, their hosts, and especially the interactions among these three divergent organisms that culminate in a disease epidemic. The most important of these interactions is the transmission of the pathogen by the vector to a non-infected plant. Transmission is a product of vector acquisition of the pathogen from an infected plant, and inoculation of the pathogen into a non-infected plant. It is a complex process involving sharpshooter host finding and feeding behaviors, and probabilities that a critical titer of bacterium will be acquired from an infected host by a feeding sharpshooter, and once acquired, will be inoculated into an uninfected host. In addition, for an inoculation event to lead to infection, a critical titer of bacterium must be inoculated into plant tissue that supports reproduction and movement.

Recent advancements in technology allow us to examine quantitative aspects of *Xylella fastidiosa* (*Xf*) transmission with greater sensitivity and at lower titers of cells than with traditional means. This includes two techniques we have mastered in our laboratories. First, we are currently using a quantitative real-time (QRT PCR) technique in conjunction with commercially available DNA extraction kits to detect and quantify low titers (currently ca  $1 \times 10^1$  cells) of *Xf* in plant and insect tissue. Second, we have developed a low-cost method to rapidly extract DNA from the glassy-winged sharpshooter (GWSS) and plant tissue in 96-well micro-titer plates. In preliminary laboratory experiments *Xf* titer was quantified in plant tissues following inoculation by single infectious GWSS.

It is intriguing that species of sharpshooters differ widely in transmission efficiency. Transmission efficiency ranges from a high of over 90% for the blue-green sharpshooter (*Graphocephala atropunctata*) to 1% for several other including *Oncometopia facialis*, *Acrogonia virescens*, and *Homalodisca ignorata* (7). Recently, rates of *Xf* transmission efficiency for the GWSS from grapevine to grapevine were found to be as high as 20%. These observations beg two questions: First, what aspects of *Xf* transmission by sharpshooter vectors vary in ways that cause a wide range in efficiencies among vectors? Second, can we exploit an understanding of transmission efficiency to reduce PD spread? We seek to understand quantitative aspects of *Xf* transmission by GWSS. We are hopeful that this unique approach to investigating the transmission of an insect-vectored plant pathogen will lead to new tactics to manage disease spread.

In the pursuit of better understanding the interactions between GWSS and *Xf* during transmission events, we have developed a model system. *Xf* bacterial cultures were scraped from plates and suspended in a sterile suspension. This bacterial suspension was infiltrated into cut *Chrysanthemum grandiflora* stem (Bextine et al. 2004). GWSS were caged in snap cap vials on stems (Figure 1). Survival through the acquisition access period (AAP) indicated effective feeding because starving these insects for 48 h resulted in 100% mortality (Bextine et al. 2004). After the AAP, GWSS were placed on *Xf*-free chrysanthemums for 48 h, so that any detection of bacteria in subsequent inoculation assays would be associated with transmission and not stylet contamination (Figure 2). Surviving GWSS were transferred to sterile vials containing a fresh chrysanthemum stem cutting. The insects were exposed to a stem for an inoculation access period (IAP). GWSS and stems were tested for the presence of *Xf* by QRT PCR. While the rate of *Xf* transmission was higher than previously reported (Almeida and Purcell 2003-a, b, Costa et al. 2000), we feel this is a fair assessment of the insects' ability to move the bacterium from an infected stem to a non-infected one.



**Figure 1.** GWSS feeding on a cut stem infused with *Xf*.