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

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ABSTRACT

Transmission of *Xylella fastidiosa* (*Xf*) by the glassy-winged sharpshooters (GWSS) involves a series of events from acquisition of the bacterium to inoculation of *Xf* to a new host. While this process is often over-simplified, certain insect/pathogen interactions may be necessary to achieve a successful transmission event and the number of *Xf* cells acquired or inoculated may govern whether or not transmission will occur. In our preliminary studies, neither higher titers of *Xf* nor longer feeding periods by GWSS result in higher rates of transmission nor a greater number of bacteria transmitted.

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 *Xf* transmission with high sensitivity, unlike 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 5 X 10^1 cells) of *Xf* in plant and insect tissue [2]. Second we have developed a low-cost method to rapidly extract DNA from GWSS and plant tissue in 96-well micro-titer plates.

Species of sharpshooters differ widely in their transmission efficiency, which ranges from a high of over 90% for the blue-green sharpshooter (*Graphocephala atropunctata*) to 1% for several others including *Oncometopia facialis, Acrogonia virescens,* and *Homalodisca ignorata* [3]. Recently, rates of *Xf* transmission efficiency for the GWSS from grapevine to grapevine were found to be as high as 20% [1]. These observations bring up 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.

OBJECTIVES

Our long-term goal is to understand quantitative aspects of the process of *Xylella fastidiosa* (*Xf*) transmission by *Homalodisca coagulata* (glassy-winged sharpshooter, GWSS) in order to develop a means of reducing the efficiency with which the pathogen is spread from an infected plant to a non-infected one. Our specific objectives for this project are to:

1. Determine relationship between the time a GWSS spends on a PD-infected grapevine and titer of *Xf* they acquire.
2. Determine the relationship between the time a GWSS spends in post-acquisition on a non- *Xf* host and titer of *Xf* they contain.
3. Determine the relationship between the time an infectious GWSS (ie, one that had acquired *Xf*) spends on a non-infected grapevine and the titer of *Xf* it inoculates into the grapevine.
4. Determine the relationship between the titer of *Xf* inoculated into a plant and the probability that it will become diseased.

RESULTS

Our preliminary laboratory experiments show that we can quantify the titer of *Xf* delivered to a stem by a single infectious GWSS immediately after a 24hr inoculation access period (IAP). In this experiment, field-collected GWSS adults were allowed to acquire *Xf* from grapevines showing Pierce's disease symptoms for a 72 hr acquisition access period (AAP). GWSS were then allowed access to cut chrysanthemum stems for 2, 4, 6, or 8 h. During this IAP, time lapse video was used to determine the amount of time GWSS feed on the stem and number of times the insect left the stem (indicating multiple

probing activities). In preliminary experiments, longer feeding durations did not influence the number of cells transmitted. Other data are too preliminary to present at this time.

CONCLUSIONS

We have the tools in place to determine transmission rates at the molecular level. Experiments are underway to determine the number of *Xf* cells that are transmitted under certain conditions. Until recently the molecular tools were not available to monitor the movement of single cells in the manner that QRT PCR allows. Almeida et al. [1]encountered difficulty in detecting levels of *Xf* in GWSS that can successfully inoculate a grapevine. That is, they found GWSS that were able to inoculate plants with *Xf* that did not test positive for the pathogen. The most reasonable explanation for these "false negatives" is that these GWSS harbored a titer of *Xf* that can cause infection in grapevines, but were below detection limits. Theoretically, one cell can cause a chronic infection; however, the probability is very low. We suspect the number of cells that are likely introduced into plants is greater than a single cell, but lower than the detection threshold of the method used by Almeida et al. [1], which is 10^2 cells. We need to embrace the molecular tools that are available to accomplish our objective.

REFERENCES

1. Almeida RPP, Purcell AH (2003) Transmission of *Xylella fastidiosa* to grapevines by *Homalodisca coagulata* (Hemiptera : Cicadellidae). J. Econ. Entomol. 96:264-271
2. Bextine B, Blua MJ, Miller TA (2004) A quantitative real-time PCR protocol and novel DNA extraction technique to detect *Xylella fastidiosa* in glassy-winged sharpshooters. J. Econ. Entomol.*:Submitted*
3. Redak RA, Prucell AH, Lopes JRS, Blua MJ, Mizell III RF, Andersen PC (2003) The biology of xylem sap-feeding insect vectors of *Xylella fastidiosa* and their relation to disease epidemiology. Ann. Rev. Entomol. *In press*

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