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

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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 1×10^1 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.

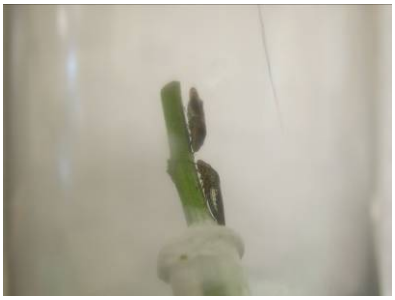

Figure 1. GWSS feeding on a cut stem infused with *Xf*.

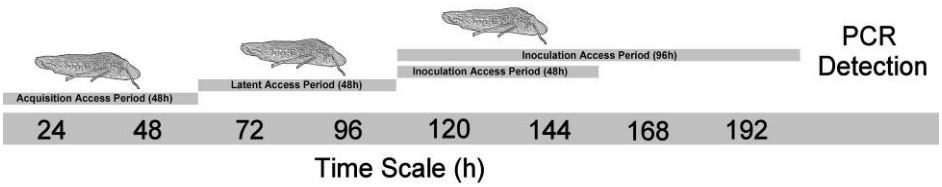

Figure. 2. Artificial Pierce's disease cycle for determination of *Xf* transmission.

OBJECTIVES

Our long-term goal is to understand quantitative aspects of the process of *Xf* transmission by GWSS in order to develop a means of reducing the efficiency with which it spreads the pathogen from an infected plant to a non-infected one. Our specific objectives for this project are to:

1. Determine relationship between time a GWSS spends on a PD-infected grapevine and titer of *Xf* they acquire.
2. Determine the relationship between time a GWSS spends in post-acquisition on a non *Xf* host and titer of *Xf* they contain.
3. Determine the relationship between time an infectious GWSS (i.e., 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 titer of *Xf* inoculated into a plant and the probability that it will become diseased by developing a transmission index.

RESULTS

Quantitative Real-Time Polymerase Chain Reaction

We developed the capacity to quantify as few as 5 *Xf* cells in both in plant tissue and insect vectors by quantitative real-time polymerase chain reaction (QRT PCR) (Bextine et al. 2006, Oliveira et al. 2002). Our QRT PCR diagnostic technique is performed in a Rotor Gene 3000 (Corbett Research, Australia) using iQ Supermix (Bio-Rad Laboratories Inc., Hercules, CA) in 20µl reactions with a new *Xf*-specific primer set (set 6) and probe (BCJ probe) based on the *gyrB* gene.

Inoculation Access Period

A bacterial suspension was made by suspending bacterial cultures scraped from plates in sterile phosphate buffered saline (PBS) by gentle shaking. 10 cm sections of cut *Chrysanthemum grandiflora* were artificially inoculated with *Xf* by forcing the bacterial suspension through by attaching a 10cc syringe to the proximal end of the stem and applying pressure until fluid was seen coming out of the distal end. Field-collected GWSS were allowed to feed from these stems for an acquisition period of 48 h. The insects were then exposed to a clean plant for a latent period of 24 h to ensure that detected *Xf* were not due to contaminated stylets. Surviving GWSS were then allowed to feed on sterile 5 cm cut *C grandiflora* stems for an IAP of 30, 60, 90, or 120 minutes. Finally, GWSS were removed from the cutting and DNA was extracted by vacuum extraction techniques followed by the Qiagen DNeasy tissue kit. Plant inoculation targets were crushed in a lytic buffer in an Agdia bag, then DNA was extracted using the Qiagen DNeasy tissue kit. QRT-PCR was run to detect *Xf* cells, using (set6 primers and BCJ probe1) and optimized run conditions. SYBR green melt curves were used to verify amplification products.

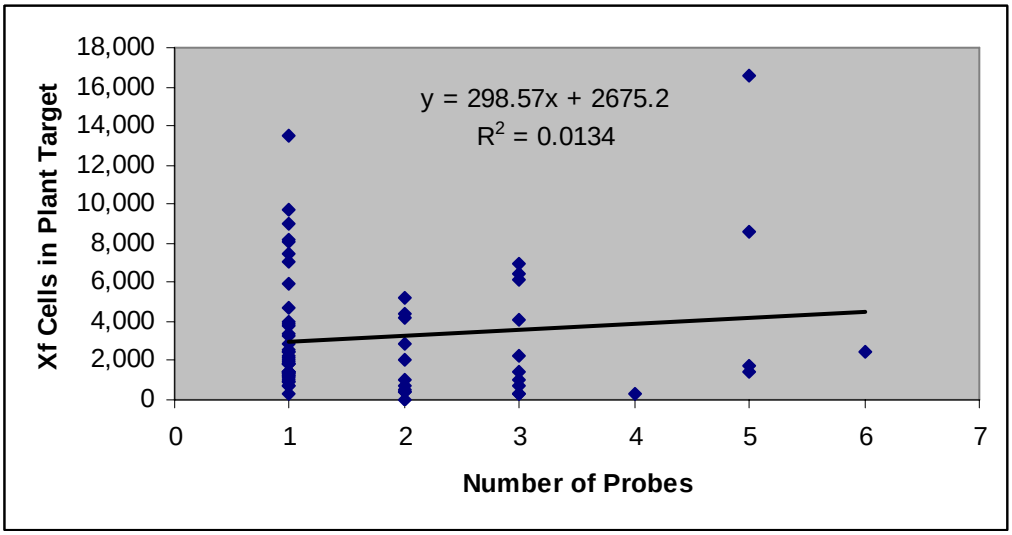

Figure 3. *Xf* cells vs. the number of probes into the plant target by GWSS.

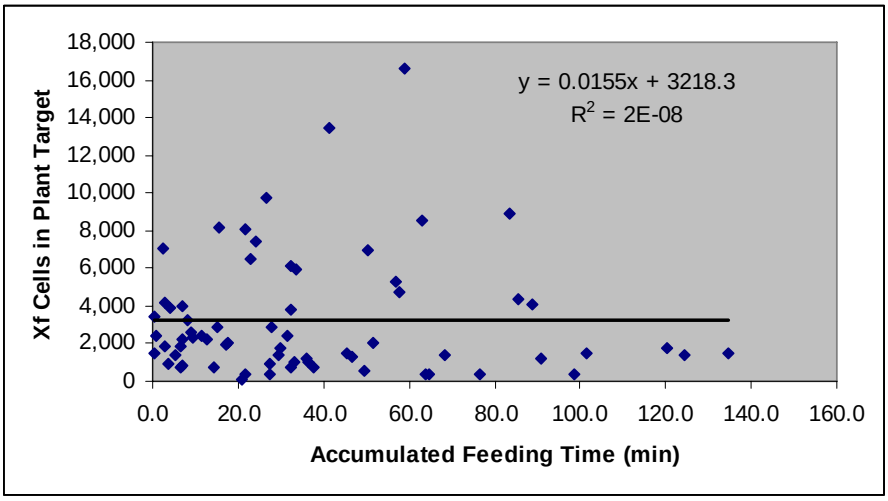

Figure 4. *Xf* cells vs. the total accumulated feeding time of GWSS on the plant target.

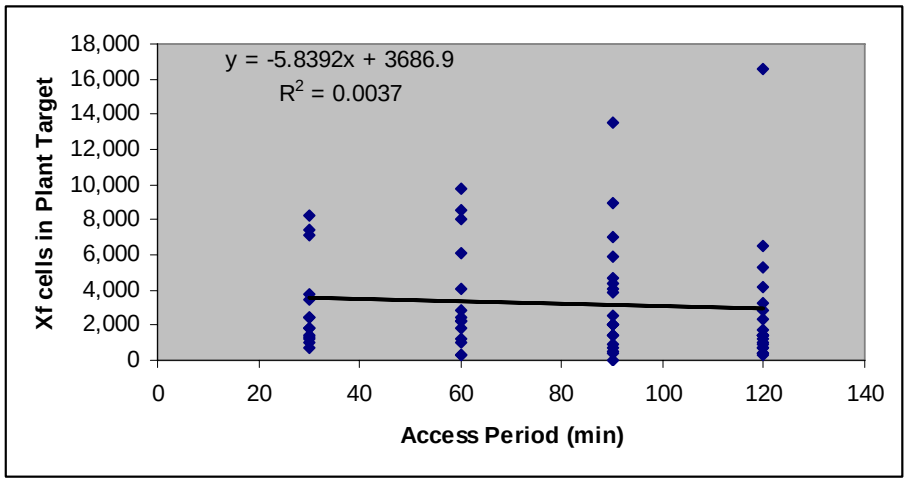

Figure 5. *Xf* Cells vs. Access Period of GWSS to plant target.

Further replicates were relatively consistent with the preliminary data. We are currently finishing the data set, so full statistical evaluation has not occurred. However, there was a positive correlation between the number of probes into a target plant and the number of *Xf* cells transmitted (Figure 3). There appears to be little correlation between accumulated feeding time and the number of *Xf* cells in the plant target (Figure 4). In other words, prolonged feeding by GWSS does not result in a greater number of *Xf* cells being transmitted. Finally, there was a slightly negative correlation between the access period and the number of *Xf* cells in the plant target (Figure 5). This is not unexpected, as the maximum access period was so short that the number of probes was not well correlated with the access period, with some insects feeding for the whole period, some probing extensively but feeding little, and some not feeding at all. Over longer access periods (hours and days), there will be more opportunities to probe, and thus greater transmission of *Xf* is expected.

CONCLUSIONS

Overall, the high degree of variability in transmission rates indicates that transmission is a complex event. Whether or not *Xf* is sheared from the foregut walls may not be easily predicted based on time feeding or number of probes alone (evidenced by low R^2 values), although trends may be shown. As we finish this data set and statistical analysis is applied to these events, we will determine if one acquisition probe is as good as 6 probes or if the positive correlation is due to an artifact of the methods used. It is also important to note that in our study, the distribution of *Xf* in stems is homogeneous, as opposed to an aggregated distribution of *Xf* in stems which is what we would expect from an diseased plant in the field. Additionally, significant amounts of bacteria may be transmitted to a plant by an insect that has probed only once, or fed for only a matter of minutes; pesticides that require insects to feed on the plant may prevent secondary transmission (acquisition from an infected grapevine and transmission to another within the same field) and lower the vector population, but will not entirely prevent transmission. With regard to number of cells vs. access period or accumulated feeding time, the critical time period might occur before 30 minutes. In our initial studies, we looked at these transmission events on a scale of 24, 48, and 96

hours. In this reporting period, we looked at shorter periods (30, 60, 90, or 120 minutes). In other words, the statistically significant slopes of the line generated if time periods might be between 1 to 10 minutes.

Disease epidemics involving *Xf* depend on the transmission of the bacterium from an infected host to an uninfected host by an insect vector. Successful transmission is a function of two major events: acquisition and inoculation. Our studies examine the interactions that involve GWSS feeding behaviors that are associated with *Xf* acquisition and inoculation, and the probability that these behaviors will lead to plant infection. The quantitative aspects of our studies are unique, due in part to technologies that allow us to quantify low numbers of *Xf* cells. Understanding these associations will allow the development of plant protection tactics that take advantage of critical "weak links" in the transmission process.

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DISPERSAL AND MOVEMENT OF THE GLASSY-WINGED SHARPSHOOTER AND ASSOCIATED NATURAL ENEMIES IN A CONTINUOUS, DEFICIT-IRRIGATED AGRICULTURAL LANDSCAPE

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ABSTRACT

A combination of field and laboratory experiments in this study have been designed to advance our understanding of the operative host-plant factors utilized by adult glassy-winged sharpshooters (GWSS) and associated natural enemies as long-range cues to locate feeding and oviposition hosts in a complex agricultural landscape. Specifically, a second year of field studies have been conducted to determine the influence of continuous deficit irrigation regimes implemented in sweet orange cv. 'Valencia' oranges on the population dynamics of GWSS and other associated natural enemies. Dispersal and population dynamics of GWSS were monitored under continuous irrigation treatments receiving 60%, 80%, and 100% of evapo-transpiration (ET_c) rates. Similar to the results obtained in our 2005 season, citrus trees irrigated at 60% ET_c had warmer leaves, significantly higher xylem matric potential, and fewer adult and immature GWSS than experimental trees irrigated with 80% and 100% ET_c. Mean numbers of adult and nymphal GWSS collected from beat samples and observed in visual inspections were numerically higher in the 80% versus 100% ET_c treatments. In caged experiments using sweet orange cv. 'Washington navel' and avocado cv. 'Hass' maintained under different continuous deficit irrigation illustrated GWSS population shifts that occurred between plants. Adult GWSS showed a preference for contact with surplus-irrigated plants of both species compared with plants under continuous deficit irrigation, with a stronger response evident in the avocado trials. During preliminary nutrition trials with overwintered adults, GWSS that landed on plants showed a slight preference for settling on plants fertilized with ammonium versus nitrate averaging over 3 trials. An olfactometer system for studying the response of GWSS to host-plant volatiles has been built and the airflow dynamics adjusted to equally integrate odor fields from humidity or volatile sources. However, evaluation of the data (number of insects landing on the target) to date shows no conclusive differences among a variety of treatments, suggesting that GWSS may not use olfactory cues during host location, or that olfaction is used only in conjunction with visual cues. Identifying how the dispersing lifestages of GWSS locate and exploit specific host species will begin to provide the necessary information required to develop strategies for control of this highly mobile insect and the spread of *Xylella fastidiosa* into susceptible crops.

INTRODUCTION

The glassy-winged sharpshooter (GWSS) is a highly polyphagous and mobile insect utilizing numerous plant species as both feeding and oviposition hosts (Adlerz, et al. 1979, Daane et al. 2003, Groves et al. 2003). Recent research has documented that different host plant species are not equally utilized by all GWSS lifestages. Mizell and Andersen (2003) report that host plant xylem chemistry plays a key role in the regulation of GWSS feeding and oviposition over a wide range of host plant species. Similarly, Daane and Johnson (2003) concluded that ornamental landscape plant species greatly influence GWSS seasonal population biology. Specifically, ornamental species which favorably support adult GWSS oviposition and feeding do not equally support comparable nymphal populations. Although significant new information has become available regarding the sequence of hosts in which GWSS populations thrive, little is understood about the host-location strategies of GWSS, which are critical behavioral responses that assist the insect in locating suitable hosts. Successful insect-host associations depend upon an insect's ability to locate a suitable host(s) in a complex, heterogeneous landscape. Mechanisms of host location in many phytophagous insects are often mediated by long-range, semiochemical cues arising from their host plant(s), which vary by plant physiological conditions including nutrition (available nitrogen and carbohydrate), xylem water potential, and plant age or developmental stage (Finch 1986). Similarly, we have an incomplete understanding of host-selection cues utilized by the mymarid egg parasitoids of GWSS, which may involve the host (GWSS egg mass), the host plant, or a combination of both. Ongoing experiments in this study will increase our understanding of the operative host-plant factors utilized by adult GWSS and associated natural enemies as cues to locate feeding and oviposition hosts in a