ROLE OF BACTERIAL ATTACHMENT IN TRANSMISSION OF *XYLELLA FASTIDIOSA* BY THE GLASSY-WINGED SHARPSHOOTER,AND OTHER FACTORS AFFECTING TRANSMISSION EFFICIENCY

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ABSTRACT
Although it is known that *Xylella fastidiosa* (*Xf*) is located on the foregut of infected insects (infective nymphs loose transmissibility after molting and there is no latent period required for transmission), the exact location in the foregut from which the bacterium *Xf* is transmitted by sharpshooter leafhoppers is not known. We examined the spatial distribution of *Xf* in the precibarium of vectors that had been fed on infected grapevines. *Xf* cells attached on end to the insect's cuticle and were distributed throughout the precibarium, with few exceptions, on both pharynges. *Xf* was not present on top of the precibarial valve, but interestingly cells were observed within the valve's pit. In a transmission experiment with long inoculation access period, all infective insects transmitted *Xf* to healthy grapevines; individuals free of *Xf* on the precibarium did not vector the pathogen. An ancillary objective was to determine if the relatively low transmission rates (5-15% daily) by the glassy-winged sharpshooter (GWSS) were influenced by the plant tissue inoculated: stem or leaf. On grape, adult GWSS feed mainly on stems. In an initial experiment with low transmission rates (less than 7% per GWSS adult) there were no differences among treatments (stem only, leaf only, stem and leaf). This will be repeated with more insects per plant and longer exposure for inoculation, but these initial results suggest that feeding site on green tissues are not a major factor.

INTRODUCTION
The vector transmission of the bacterium *Xylella fastidiosa* (*Xf*) to plants is an essential step in the spread of Pierce's disease (PD). The process of transmission would seem to be simple. Virtually any xylem sap-feeding insect can be vector (Frazier 1966). There is no – or at most 30-60 minutes -- latent period, the time required between acquisition and inoculation. Because vectors stop transmitting immediately after molting, the bacteria must be transmitted from the foregut, whose lining is shed with molting (Purcell and Finlay 1979). The hypothetical model for transmission is that the bacteria attach to the foregut during feeding on *Xf*-infected plants, and some bacteria are detached during later feeding to inoculate other plants (Purcell et al. 1979).

But this simple view is deceptive. First of all, very few live cells of *Xf* -- certainly less than 200 per insect -- are needed for efficient transmission by the blue-green sharpshooter (BGSS) (Hill and Purcell 1995) or the glassy-winged sharpshooter (GWSS) (Almeida and Purcell 2003). This makes determination of the infective status of insect vectors very difficult because even highly sensitive methods for *Xf* detection do not detect the bacterium in all transmitting insects. The small number of *Xf* needed for efficient transmission implies that the area of the foregut involved in transmission is very small, thus saturated by small numbers of *Xf*, which implies that large mats of *Xf* often seen in transmitting sharpshooters are superfluous for transmission.

The attachment (and subsequent detachment) of *Xf* to the vector foregut is thought to be a key in its vector transmission to plants (Purcell et al. 1979) and has provided an incentive to better understand this phenomenon. How these tiny bacteria remain attached to the insect foregut in such a fast-moving fluid environment of 5 to 10 cm/second is still unknown. The use of the cell signaling mutants (Newman and Lindow, in this Proceedings) has opened new ways to examine experimentally the vector transmission process. Although not an objective of this related project, it is important to learn why cell-cell signaling by *Xf* is so important to transmission.

A second transmission phenomenon we propose to evaluate using GWSS is whether access to leaf petioles, young stems, or the bases of stems affects vector transmission efficiency in grape. GWSS adults prefer feeding on stems of grape test plants, but nymphs and young adults tested on leaves (including leaf petiole) were much more efficient at transmitting than were adults (Almeida and Purcell 2003). Leaf age had a relatively small but statistically significant effect on transmission by BGSS (Purcell 1981). The procedures to test this are simple and straightforward. It is important to know how various locations on grape affect transmission performance in order to incorporate vector transmission rates in estimates of economic thresholds for control of GWSS.

OBJECTIVES

1. Determine the association of *Xf* transmission and its location in the vector's precibarium and cibarium.
2. Determine the effects of within-plant location on vector transmission efficiency.

RESULTS

The morphology of the precibarium (a canal between the food canal of the stylets and the sucking pump formed by the combined pharynges) of BGSS has been previously described (Backus and McLean 1983, Brlansky et al. 1983, Purcell et al. 1979). The channel was divided into distal and proximal regions by A flap-like valve located on the epipharynx. On the epipharynx we observed the 10 D-sensilla and 8 P-sensilla first identified by Backus and McLean (1983). The 2 H-sensilla were also observed. The proximal section of the precibarium, posterior to the valve, had sutures on the epipharynx that were not present on the hypopharynx.

Xf cells were observed on both pharynges, always attached to the insect's cuticle at each bacterium's narrow end. Probably because of the long incubation period given to insects after pathogen acquisition, we observed only cell mats rather than isolated cells/colonies. The hypopharynx had homogenous mats, with only one area always free of *Xf* (Figure 1). Based on measurements of both pharynges, this is the area where the precibarial valve or pit probably interlocks with the hypopharynx. In some cases, cells were observed only proximally to the precibarial valve; if present distally, they were always observed proximally. Cells were never found on top of the precibarial valve or the area on the epipharynx with sutures (Figure 2). Cells were often found within the precibarial valve's pit. The proximal end of the precibarium was also a location where *Xf* attached. *Xf* was also observed in the groove at the distal end of the cibarium. We also dissected 15 GWSS adults that had 4 days acquisition access period on infected plants and were transferred as a group to mugwort for 2 weeks. Only one individual was observed with *Xf*, distributed in the same manner as on GWSS.

Nineteen of the 25 insects used in our transmission experiment were adequately dissected to evaluate presence or absence of *Xf* on the precibarium. Ten insects were positive, and all of them transmitted the bacterium to grapevines. The other 9 individuals were *Xf*-free and did not transmit to plants. Location of *Xf* was the same as described above. Because of the long incubation period used, we cannot infer the location where *Xf* probably detached to inoculate the healthy plants. *Xf* transmission does not require a latent period, thus no bacterial multiplication is required for transmission. Transmission experiments using short acquisition and inoculation access periods are being conducted to determine if we can consistently locate initial *Xf* colonization areas in the foregut of transmitting insects.

CONCLUSIONS

1. Using long acquisition and inoculation access periods, we found a good correlation between vector transmission to plants and the occurrence of *Xf* on the precibarium of an efficient vector (BGSS).

2. There are specific areas on the precibarium of both pharynges where *Xf* is absent in *Xf*-colonized sharpshooters. One of these is on the distal extremity of the precibarial valve. Another is where the valve should seat against the hypopharynx. A third is the medial region of the proximal precibarium on the epipharynx. These areas might represent areas where the detachment of *Xf* cells occurs during the inoculation phase of vector transmission so that the bacteria can be expelled during feeding.

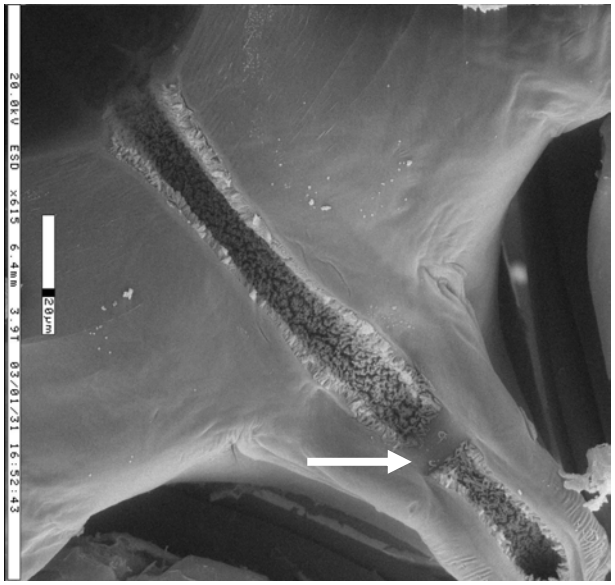

Figure 1. Hypopharynx of BGSS with *Xf* cell mat. Arrow indicates region where *Xf* was always absent.

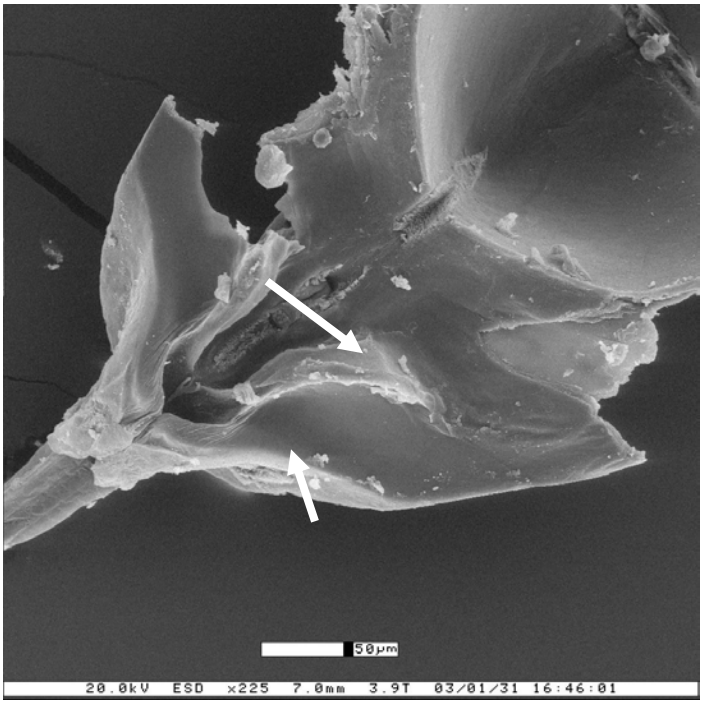

Figure 2. Epipharynx of BGSS with *Xf* cell mat. Arrows: a) precibarial valve, with no *Xf* attached on top of the flap-like structure; b) proximal region of precibarium canal free of *Xf*.

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