CHARACTERIZATION AND STUDIES ON THE FUNDAMENTAL MECHANISMS OF *XYLELLA FASTIDIOSA* TRANSMISSION TO GRAPEVINES BY THE GLASSY-WINGED SHARPSHOOTER

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ABSTRACT
The transmission of *Xf* to dormant grape by the glassy-winged sharpshooter (GWSS) was confirmed for a second year in outdoor cage trials in Bakersfield in January 2003, but at lower rates than in February 2002. Attempts to transmit *Xf* with GWSS from dormant grape and almond did not result in transmission. Sharpshooters fed on suspensions of *Xf* in xylem sap acquired *Xf* as determined by culture assays, but all failed to transmit *Xf* from suspensions to grape, suggesting that vector transmission requires a plant factor or environmental conditions not present in cultured cells.

INTRODUCTION
In attempts to reduce the economic impact of the glassy-winged sharpshooter (GWSS, *Homalodisca coagulata*), it is essential to better understand and evaluate GWSS's transmission of the pathogen *Xylella fastidiosa* (*Xf*). *Xf* has been causing diseases in California for a long time, but GWSS is apparently a more effective vector than other sharpshooters previously present in California in the field spread of Pierce's disease. We previously documented that GWSS transmits *Xf* with similar characteristics as other vectors (Purcell 2002). There is no or a very short (minutes) latent period; insects acquire and inoculate *Xf* with only 1 h of plant access period, although efficiency increases with larger access periods. Nymphs were as or more efficient vectors than adults, both for pathogen acquisition and inoculation, but lost infectivity after molting (Almeida and Purcell 2003). Overall, GWSS was at least 50% to 75% less efficient as a vector of *Xf* compared to the blue-green sharpshooter (BGSS) (Purcell and Finlay 1979). One difference in the feeding behavior or GWSS compared to traditional California vectors such as BGSS was that adults transmitted *Xf* with similar efficiency to green tissues as to 2-year old wood of grapevines. Similar to previous results for BGSS (Hill and Purcell 1995), we found no correlation between the amount of the pathogen detection in the head of GWSS by culture and its transmission to plants. Our objectives for the past year were to further characterize *Xf* transmission by GWSS to grapevines and to develop methods by which GWSS could transmit *Xf* from artificial diets so as to be able to better control and experimentally manipulate bacterial acquisition. The final (#3) objective of the project was addressed without success the previous year; no bacterial isolates from GWSS proved to be taken up by GWSS from surface sprays of the bacterial isolates.

OBJECTIVES
1. Characterize the transmission of *Xf* to grapes by GWSS.
2. Develop *in vitro* assays to assess vector transmission of *Xf*.
3. Test the possibility of biological control of *Xf* transmission through competition for attachment site in vector's foregut.

RESULTS
GWSS transmission of *Xf* to dormant grapevines
In a repeat of experiments conducted in an outdoor cage in Bakersfield in 2002, we confirmed that GWSS can transmit *Xf* to dormant grapevines, although rates were much lower than in 2002. We first confined GWSS on PD-grape source plants (strain STL) for 4 days in the greenhouse, then we caged groups of 4 adult GWSS per plant on grape seedlings. We transported 15 of these groups (60 insects total) on grape test plants and transferred one group per vine to a small mesh sleeve cage on field-grown Pinot Noir vines planted in an outdoor cage in Bakersfield, CA. We inoculated 15 field vines each on January 23 and 30, 2003 and removed the insects after one week. We diagnosed all plants after more than 3 months for PD symptoms and for *Xf* by culture assays (Hill and Purcell 1995) and/or by ELISA (Minsavage et al. 1994) in the CDFA Diagnostics Lab in Sacramento, using leaf petioles for diagnoses. Transmission rates in the greenhouse trials were 3 of 15 for January 19-23 inoculations and 4 of 15 for Jan. 26-30 inoculations in the lab on green vines. Only one of the 30 total groups of 4 GWSS transmitted to dormant grape in the field; this same group had also transmitted to a green grape test plant on which the insects were caged before exposure to field test plants. This rate of transmission (3%) was much lower than the 20-30% transmission achieved in identical experiments in February 2002. As we noted in our lab experiments over the previous three years, GWSS transmission to grape is quite variable among experiments (Almeida and Purcell 2003).

Electronic monitoring of sharpshooter probing behavior
This work was conducted together with Dr. Elaine Backus (USDA-ARS, Parlier CA). We used the blue-green sharpshooter (BGSS, *Graphocephala atropunctata*) as our model insect because it is more efficient than the GWSS in transmitting *Xf*. Our results were presented last year (Purcell 2002) and have been submitted for publication. Information from this work establishes benchmarks for future research addressing the mechanisms of *Xf* transmission and sharpshooter ecology.

Develop *in vitro* assays to assess vector transmission of *Xf*
Results have been already discussed in a previous report. In summary, we have demonstrated that sharpshooters can acquire planktonic *Xf* suspended in filter-sterilized grape xylem sap kept in sachets of thin flexible membranes (Parafilm M^R). But these insects did not transmit the pathogen to plants afterwards. During Spring 2003, we used BGSS that had been lab-reared on basil with frequent changes to eliminate any *Xf* attached to the foreguts of the insects. These BGSS were screened on pre-test grape plants to test for infective BGSS before using them in acquisition-feeding experiments. We suspended $\log_{10}6$ cells/ml in filter-sterilized grape sap and fed about 20 µl of suspension through a thinly stretched Parafilm^R membrane to individual BGSS adults. After a 6-8 hour access to feeding on *Xf* suspensions between membranes, we transferred the insects to grape test plants for 4 days, after which we cultured from the heads and bodies of the surviving insects. Unfortunately, half of the pre-test plants became infective, indicating that probably about 20% of the BGSS used were infective before the experiment. Of the BGSS that did not transmit to a pre-test plant, only one of over 30 BGSS transmitted after feeding on sachets of bacterial suspensions, indicating that either the insect(s) picked up *Xf* from the cell suspension or (more probably) that it was already infective but did not transmit to the pre-test plant. We recovered *Xf* from all of the BGSS we cultured except for those where contaminating bacteria made detection of *Xf* impossible. The results suggested that cultured *Xf* cells are not genetically activated in sterile xylem sap to attach to vector foreguts from which they can later be transmitted, or that plant factors not present in cultured cells are required for attachment to the vector.

Biological control of *Xf* transmission through competition for attachment site in vector's foregut
Our results on these experiments have been previously reported (Purcell 2002).

Microscopy of sharpshooter foreguts
We examined BGSS adults foreguts for *Xf* after caging them on PD-vines for 4 days, followed by at least 14 days on grape test plants. Of 19 such BGSS that were adequately dissected for SEM, we found "carpet-like" mats of *Xf* attached to all 10 BGSS examined in scanning electron microscopy that transmitted *Xf* to grape (Figure 1). We did not observe attached *Xf* in any of the 9 insects that did not transmit to grape. Not many cells of *Xf* are required for efficient transmission of *Xf*, but evidently heavy biofilms of *Xf* in the foregut also permit efficient transmission. Our previous reports (Purcell 2002) described similar findings of *Xf* biofilms in the foreguts (precibarium) of GWSS (Brlansky et al. 1983). The general distribution of *Xf* in the foregut of BGSS (Figure 1) in this transmission experiment was similar to that we described earlier (Purcell 2002).

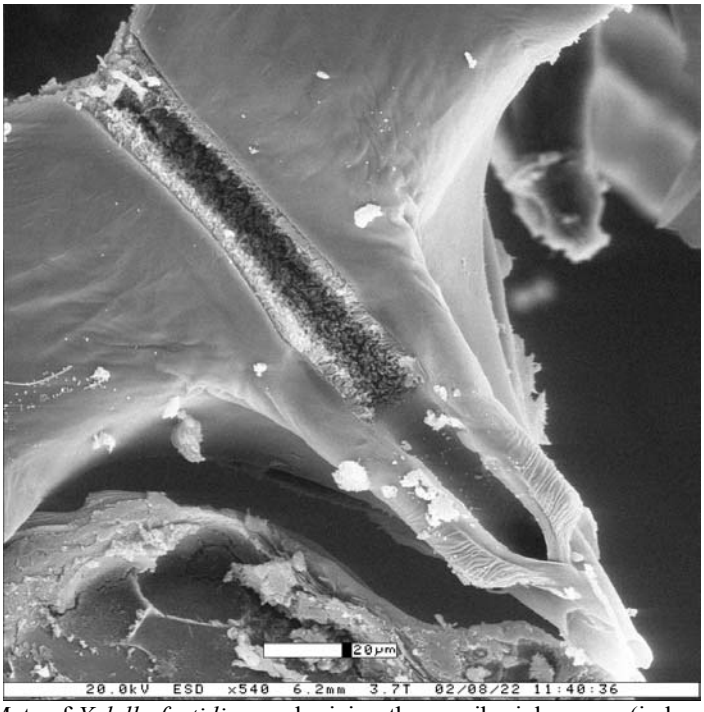

Figure 1. Mats of *Xylella fastidiosa* colonizing the precibarial groove (in hypopharynx) of blue-green sharpshooter 14 days after start of acquisition period on grape with Pierce's disease. Sucking pump chamber is at upper left.

CONCLUSIONS

We confirmed for a second year that GWSS can transmit *Xf* to dormant grapevines in the field. This implies that it is important to minimize GWSS populations feeding on vineyards in winter months as well as the growing season. Our efforts to develop a system to deliver planktonic *Xf* cells to insects were successful, but the ingested cells were not transmitted to plants afterwards, suggesting that either *Xf* responds to environmental signals to activate genes necessary for attachment or that the bacterium requires plant-derived factors for transmission.

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