

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

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INTRODUCTION

Current attempts to reduce the economic impact of the glassy-winged sharpshooter, *Homalodisca coagulata*, (GWSS) to important crops in California have focused on GWSS or the causal pathogen, *Xylella fastidiosa*, (*Xf*), but little attention has been given to an essential step of *Xf*-diseases: the transmission of the pathogen by its insect vector. *Xf* has been causing diseases in California for a long time, but GWSS is apparently a more effective vector than other sharpshooters previously involved in California. Our objectives are to characterize *Xf* transmission by GWSS to grapevines, since this is a vital but understudied area of PD. Because of space limits, this report emphasizes objective #1.

OBJECTIVES

1. Characterize the transmission of *Xylella fastidiosa* to grapes by the glassy-winged sharpshooter.
2. Develop *in vitro* assays to assess vector transmission of *Xylella fastidiosa*.
3. Test the possibility of biological control of *Xylella fastidiosa* transmission through competition for attachment site in vector's foregut.

RESULTS AND CONCLUSIONS

GWSS transmission of Xf to grapes:

The basic characteristics for *Xf* transmission determined for other vectors were also found for GWSS. Transmission i) occurred without latent period, ii) was persistent over time, iii) unless molting occurred (no transtadial transmission), iv) nymphs and adults were vectors. GWSS transmitted *Xf* to 2-year-old woody tissue of grapevine cuttings with similar efficiency as that to green shoots. Transmission by nymphs had efficiency of approximately 70% (2 days inoculation access period - IAP). Inoculation efficiency increased with longer IAP, but even with 96h IAP efficiency was approximately 35%, a value lower than that obtained for an efficient *Xf* vector, the blue green sharpshooter (BGSS, *Graphocephala atropunctata*). Acquisition efficiency did not increase with longer acquisition access periods (AAP) after 6 hours. Overall transmission efficiency was 15-20% per insect per day, with large variability in transmission rates among experimental repetitions. Comparable transmission efficiency by BGSS is over 90% (Hill and Purcell 1997). Using the culture detection method for *Xf*, we found no association between *Xf* detection in the heads of GWSS and individual transmission of the pathogen to plants, similar to the findings of Hill and Purcell (1997) for BGSS. Further studies using other detection methods based on PCR may prove to be better predictors of vector infectiousness. In general, GWSS transmission of *Xf* had the same characteristics observed for other vector species, but had much lower and more variable transmission efficiency among experiments. GWSS inoculation of 2-year-old wood of grapevines in the lab suggested that summer and fall inoculations in the field may occur, and that these infections may become chronic disease because plant tissues where inoculation occurred will not be removed during regular winter pruning.

GWSS transmission of Xf to dormant grapes in the field:

Because GWSS has been found to feed on dormant vines during the winter, we tested the possibility of GWSS inoculating *Xf* into dormant vines in the field. We previously reported that GWSS transmitted *Xf* to dormant grapes under laboratory. Our field experiment with dormant plants was done in a screen cage built in Bakersfield, Kern Co. Grape 'Pinot noir' cuttings were planted within the cage in September 2001, and standard cultural practices used for the plants. In February 2002, 3 sets of inoculations were done with groups of GWSS taken on plants to Bakersfield. Briefly, adults had 4 d AAP on source plant;; we later transferred these GWSS (groups of 4) in the greenhouse to green seedlings for 4 d IAP, which served as indicators of group infectivity. Plants with insects were taken to Bakersfield and transferred to dormant plants for a 1 week IAP, seedlings returned to Berkeley for symptom development. One inoculation was done in May, as a positive control to test GWSS survival under similar conditions and transmission to green plants growing in the field. We inoculated 64 dormant plants during three dates in February, 13 on May, and left 16 un-inoculated. We verified transmission to dormant plants in the field with efficiency not much lower than that to green seedlings in the greenhouse. No negative control plants were positive for *Xf* or showed any PD symptoms. Survival of insects in the field was high (47-90% for all insects in different dates). Figure 1 summarizes the results obtained.

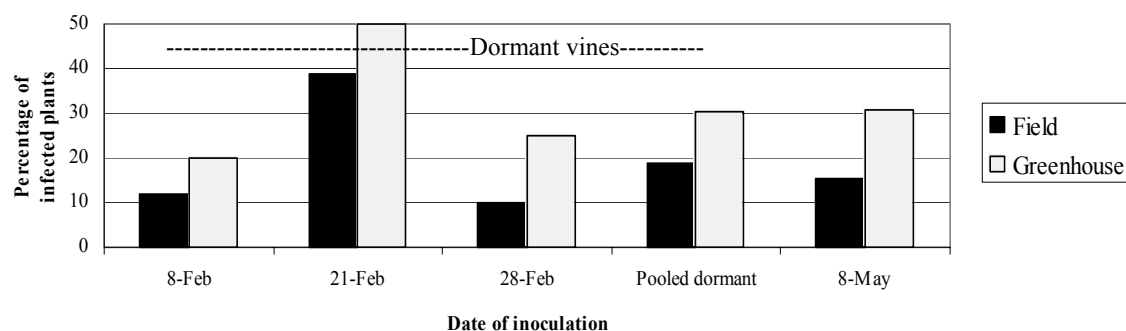


Figure 1. Percentages of dormant (in the field) and green (in the greenhouse) vines infected with *Xf* by GWSS.

Association of *Xf* in vectors' foregut and its transmission to grapes:

We used scanning electron microscopy to observe *Xf* cells attached to the foregut of GWSS and BGSS. We found the expected structures in the pre-cibarium (sensilla, pre-cibarial valve). Even though GWSS has low and variable transmission efficiency (see above), we tested if *Xf* could be found in the foregut of insects that had 4 d AAP followed by ~1 week of incubation period. We found *Xf* cells in only 1 out of 35 insects, and then decided to do a similar test with an efficient vector (BGSS). In this test, 14 BGSS had 4 d AAP on source plants, ~2 weeks on mugwort and 4 d IAP on healthy grape. All insects that transmitted to plants had large amounts of *Xf* in the pre-cibarium. Similar pictures have already been reported (Purcell et al. 1979 and Brlansky et al. 1983). In vitro assays of sharpshooter feeding through a membrane on suspensions of *Xf* in sterile xylem sap revealed that sharpshooters picked up *Xf* in larger numbers than from *Xf*-infected plants but did not subsequently transmit the bacterium to grape. We have isolated numerous bacteria from the surface-sterilized heads of GWSS fed on PD-grape but that failed to transmit *Xf*. Continuing studies will attempt to assess these bacteria as possible antagonists to GWSS transmission of *Xf* to grape. So far have not recovered any of the isolates that we sprayed onto foliage in greenhouse experiments.

Electronic monitoring of BGSS:

Because we found that GWSS general *Xf* characteristics are the same as those for other vector species, and that it has lower and more variable transmission efficiency than BGSS, we have used BGSS as a model vector to start a study on feeding behavior and *Xf* transmission. This work was done in cooperation with Dr. Elaine Backus (University of Missouri, Columbia). Although BGSS probing behavior has already been studied electronically (Crane 1970), we found that waveforms previously observed were not comparable to the system we used, mostly due to technological advances. We characterized various waveforms for BGSS probing behavior, and found that insects feed on xylem and mesophyll (may be phloem too, but data inconclusive).

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