Competitive binding influences *XF* vector load: Confocal and SEM images of GFP expressing *XF* in SWSS foreguts

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Research in my lab is focused on understanding the mechanisms of acquistion and inoculation of *Xylella fastidiosa* (*Xf*) in grape by the glassy-winged sharpshooters (GWSS), *Homalodisca vitripennis*. Like all hemipteran vectors of plant pathogens, GWSS acquire their *Xf* bacterial load via ingestion of fluids containing bacteria. Nearly 30 years ago (Purcell et a. 1979), microscopic examination revealed that, unlike for any other plant pathogen, *Xf* colonizes the anterior regions of the vector's foregut, the cibarium (or sucking pump) and precibarium (Backus 1985). Properties of *Xf* inoculation support that bacteria are dislodged from the foregut during feeding, and are expelled directly into the plant during inoculation.

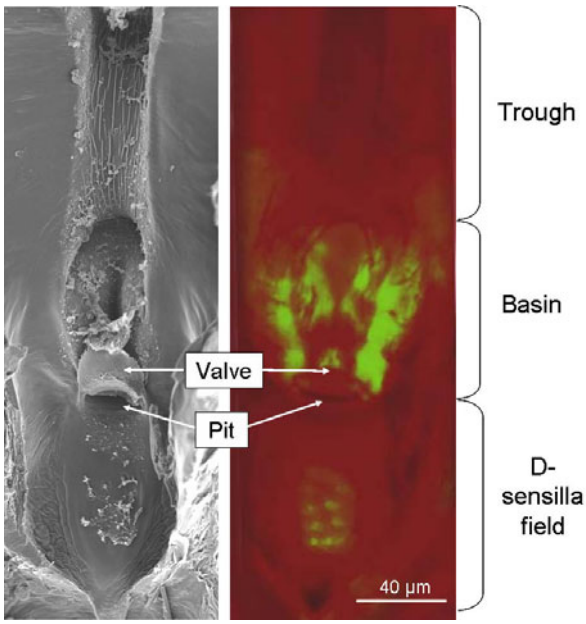

Figure 1. Scanning electron (left panel) and confocal (right panel) micrographs of the outer half of the precibarium, from the same insect, labeling its structures. Green fluorescence at right is GFP-*Xf* colonies. This is a mildly dirty, topped-off, pre-inoculation foregut (see below).

We have now perfected a confocal microscopy technique originally developed by Newman et al. (2003) to view Xf cells tranformed to express green fluorescent protein (GFP). Optical sectioning of whole, undissected foreguts (Fig. 1) reveals bright green Xf in the precibarium (Fig. 1) and cibarium. Using this technique, we performed a preliminary comparison (n = 6) of foregut colonization of lab-reared vs. field-collected GWSS, before acquisition of GFP-Xf (Fig. 2A), after acquisition (Fig. 2B), and after inoculation (Fig. 2C). For acquistion, insects were caged on symptomatic grapevines that had been mechanically inoculated with GFP-Xf four months before the test. Insects were allowed to feed for 7 – 8 days, an acquisition access period that had previously been demonstrated to be optimum to develop dense accumulations of Xf biofilm in the foregut (data not shown). For inoculation, acquired insects were allowed to make a few probes on either healthy grapevines or artificial diet; discharge of Xf was later confirmed by confocal microscopy and/or PCR.

Experiments are being repeated for higher sample sizes prior to further publication; however, results were consistent. All lab-reared ('clean') insects had unoccupied cuticular surfaces in all parts of the foregut (Fig. 2A, left). After acquisition, clean insects had become 'maximally loaded' with GFP-*Xf* in all areas of the foregut (Fig. 2B, left). After successful inoculations, insects given this treatment showed basins with significantly reduced bacteria (Fig. 2C, left). In contrast, GWSS collected from the field were almost always contaminated ('dirty') to varying degrees, before acquisition, with diverse microbes both *Xf* and non-*Xf* (Fig. 2A, right). During acquisition, there appeared to be little opportunity for GFP-*Xf* to bind to unoccupied cuticle in the precibarium, although there was ample room in the cibarium (Fig. 2B, right). Inoculations by these 'topped-off' insects were almost never successful, and what few *Xf* were present in the precibarium were stripped away (Fig. 2C, right).

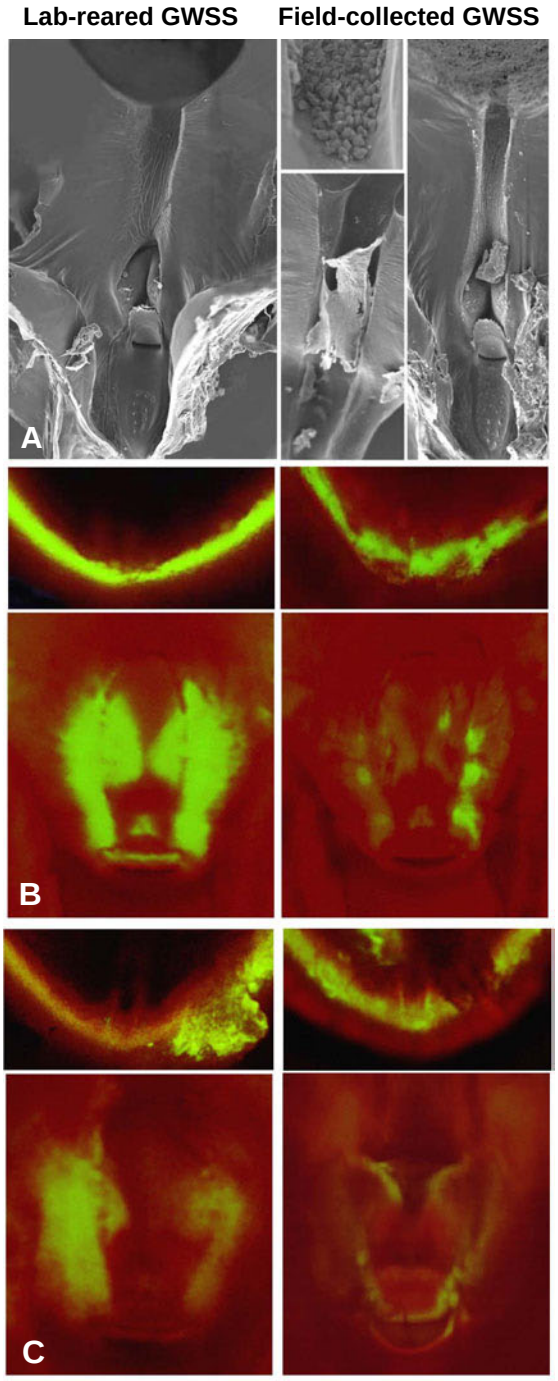

Figure 2. SEM (whole view) and/or confocal (optical slice) micrographs of the outer half of the foregut. Confocal images show the cibarium (top) and basin (bottom). Green fluorescence is GFP-*Xf*. Virtually no obstructions were seen in the trough or the D-sensilla field, so they are not shown in confocal images.

A. Pre-acquisition foregut (SEM).

Left side: Clean foregut without debris, biofilm, or blockage.

Right side: Dirty foregut (Composite image from 2 insects) blocked by a mass of *Xf* biofilm, pulled off the outer half of the precibarium during dissection (except a chunk); most is now on the inner half (lower inset) Upper inset shows a different type of microbial accumulation (possibly yeast) from another insect.

B. Post-acquistion, pre-inoculation foregut (confocal).

Left side: Originally clean, maximally loaded foregut shows large amounts of GFP-*Xf* in both the cibarium and basin.

Right side: Originally dirty, topped-off foregut shows patchy but large amounts of GFP-*Xf* in the cibarium, but only a small amount in the basin. Other microbes did not fluoresce, but were presumed present in areas where green *Xf* did not bind.

C. Post-inoculation foregut (confocal).

Left side: Originally clean, loaded, discharged foregut shows moderate to large amount of GFP-*Xf* in cibarium, only moderate, greatly reduced amount in the basin.

Right side: Originally dirty, topped-off, discharged foregut shows patchy but large accumulation of GFP- *Xf* in cibarium, very small amount in the basin.

Thus, both acquisition and inoculation appear to be influenced by availability of binding sites for *Xf* within the precibarium, and GWSS in the field accumulate many types of microbes. We hypothesize that competitive binding of microbes in the precibarium is crucial for acquisition and inoculation success, and vector efficiency.

We thank David Morgan (CDFA) for providing lab-reared, clean GWSS, and Steve Lindow (UC Berkeley) for providing GFP-expressing *Xf*. This research was partially funded by grants from the UC Pierce's Disease Research Program.

References

1. Backus, E. A. 1985. Anatomical and sensory mechanisms of leafhopper and planthopper feeding behavior. pp. 163-194 in: The Leafhoppers and Planthoppers, eds. Nault, L. R. and Rodriguez, J. G. John Wiley & Sons, New York.
2. Newman, K. L., R. P. P. Almeida, A. H. Purcell and S. E. Lindow. 2003. Use of a green fluorescent strain for analysis of *Xylella fastidiosa* colonization of *Vitis vinifera*. Applied and Environmental Microbiology. 69: 7319-7327.
3. Purcell, A. H., Finlay, A. H. and McLean, D. L. 1979. Pierce's disease bacterium: mechanisms of transmission by leafhopper vectors. Science 297: 839-841.