

ULTRASTRUCTURAL CONTRIBUTIONS TO THE STUDY OF THE GLASSY-WINGED SHARPSHOOTER AND PIERCE'S DISEASE

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ABSTRACT

A variety of microscopic techniques including light microscopy, confocal scanning light microscopy, transmission electron microscopy, and scanning electron microscopy are helping to elucidate the structure and function of the mouthparts and the salivary sheath of the glassy-winged sharpshooter, a vector of Pierce's disease.

OBJECTIVES

1. Describe the morphology and ultrastructure of the glassy-winged sharpshooter mouthparts.
2. Describe stylet penetration and the function of each stylet pair during feeding.
3. Ascertain the path of mouthparts from the epidermal layer to the vascular tissue of the host plant, and to ascertain if the sharpshooter has fed in parenchymatous or phloem tissue en route to xylem tissue.
4. Determine the ultrastructure of the salivary sheath and its association with all plant tissues encountered from the epidermal layer to the xylem tissue.

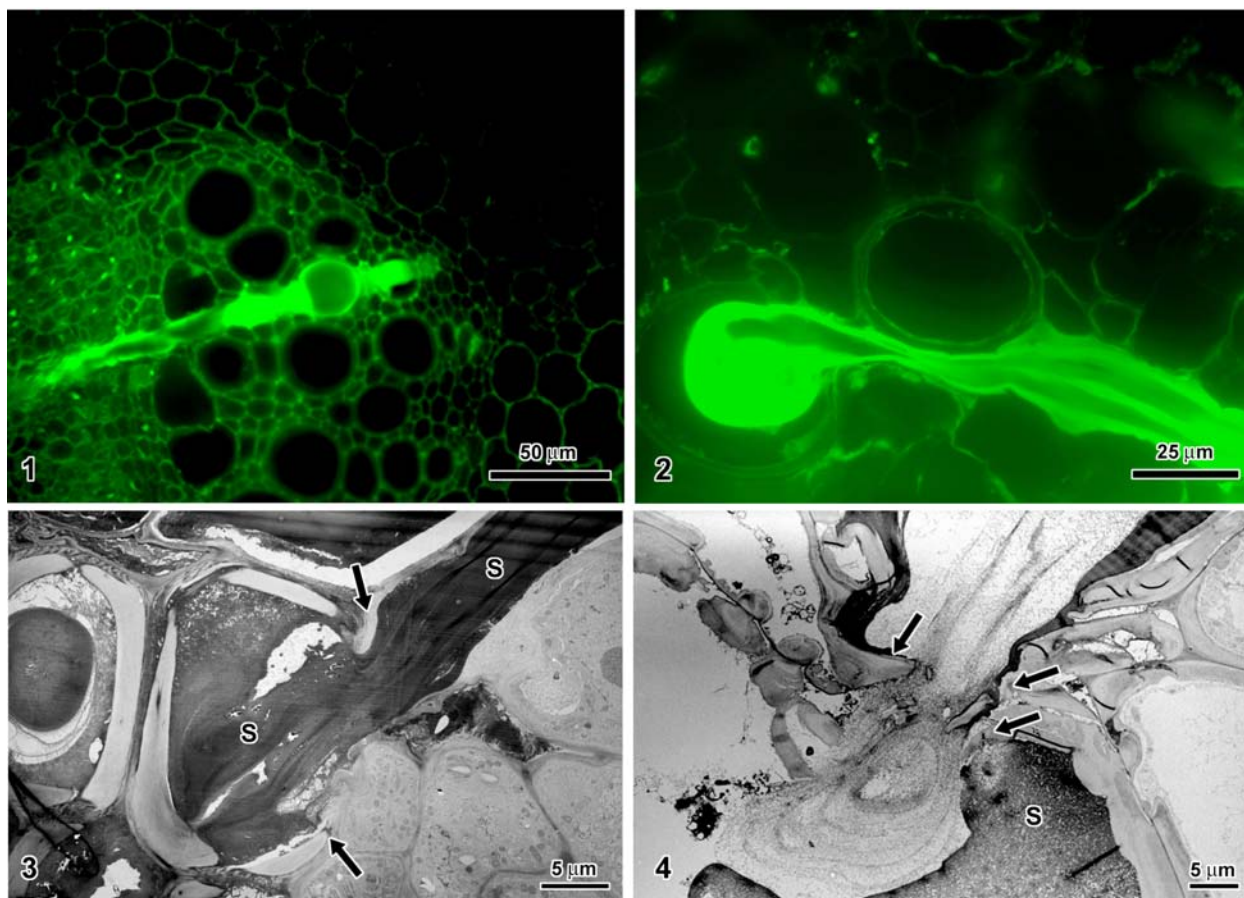
RESULTS AND CONCLUSIONS

The glassy-winged sharpshooter (GWSS) has a significant economic impact as the vector for the transmission of *Xylella fastidiosa*, which causes Pierce's disease in grapes, leaf scorch in oleander and almonds, and variegated chlorosis in citrus. Different strains of the bacterium also cause diseases of avocados, peaches, plums, apricot, cherries, and many other trees and ornamentals (Purcell and Saunders 1999, Purcell et al. 1999). The GWSS feeds primarily on the xylem fluid of more than 100 different host plants from more than 35 plant families.

In response to the tremendous economic importance of this insect, a variety of research avenues are under investigation to develop control or management strategies. One important research area that has not received adequate attention is the interaction between the GWSS and the host plants. Until very recently we knew very little regarding the structure of the GWSS mouthparts, and simply assumed that they were similar to those of other leafhoppers. During the last two years, we have provided extensive ultrastructural descriptions of the GWSS mouthparts, including several new sensory structures associated with the sharpshooter stylets and labium (Leopold et al. 2003, Freeman et al. 2002, 2003).

Many unbranched salivary sheaths and branches of very complex sheaths, formed by nymph and adult sharpshooters, do not always extend directly from the host-plant epidermis to the xylem tissue. GWSS stylets may penetrate only as far as the vessel element wall or they may actually fragment the lignified wall and enter the cell lumen (Figures 1-4). Several vessel elements in a vascular bundle or secondary xylem may be damaged during a single sharpshooter probe (Figure 1). Fragmented vessel elements (Figures 2-4) would change the dynamics of water translocation. Penetrated vessel elements are only infrequently surrounded by salivary sheath material, which raises questions as to the function of the sheath in reducing or preventing cavitation. Penetrated vessel elements can, however, become partially or completely occluded with GWSS salivary sheath material (Figures 1-3), a situation that would also disrupt water translocation even in the absence of *X. fastidiosa*.

The glassy-winged sharpshooter ingests large volumes of xylem fluid during feeding, most of which is quickly excreted. We have noted that both nymph and adult sharpshooters produce exudates during probes that do not reach the xylem, suggesting that they may be feeding in host cells located between the epidermal layer and the xylem. The transfer of *Xylella* to parenchyma cells outside of the xylem (Backus et al. 2003) might be another indicator that sharpshooters are feeding in non-xylem tissues. With a high assimilation efficiency of carbon (Brodbeck et al. 1993, 1995, 1996), there may be a nutritive advantage for even limited feeding in parenchymatous tissues. We now have preliminary data showing that first, second, and third-instar nymphs successfully feed on sunflower stems where the xylem is located too distant from the epidermis to be reached by the length of their stylets. We note that less than 50% of first and second instars have salivary sheaths terminating in the xylem even when the xylem is within the reach of their stylets. Third and fourth instars are only slightly more successful.



Figures 1, 2. Confocal scanning light micrographs. Figure 1. Several vessel elements damaged by a single GWSS stylet probe.

Figure 2. Salivary sheath material occluding a fragmented vessel element

Figures 3, 4. Transmission electron micrographs showing fragmented vessel element walls (arrows) and salivary sheath occlusions (s).

In our greenhouse and laboratory studies, host plants fed on by sharpshooters for several days to weeks begin to show symptoms similar to those of plants infected with the bacterium *X. fastidiosa*. These symptoms occur in our host plants even though the sharpshooters we are studying are free of *Xylella*. Previous reports indicated that the symptoms of Pierce's disease may occur very shortly after inoculation with *X. fastidiosa*, long before there is a significant increase in the population of the bacteria to a level believed necessary to produce symptoms (Labavitch *et al.* 2002). Many plant species infected by strains of *X. fastidiosa* show no symptoms of Pierce's disease (Purcell and Saunders 1999). Our research is ongoing to determine the correlation of mechanical damage and occlusion of vessel elements to the onset of symptoms in non-infected host plants

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