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

Project Leader:
Thomas P. Freeman
Electron Microscopy Laboratory
North Dakota State University
Fargo, ND 58105

Cooperators:
Roger A. Leopold
Dennis R. Nelson
James S. Buckner
USDA-ARS
Biosciences Research Laboratory
Fargo, ND 58105

Thomas J. Henneberry
Western Cotton Research Laboratory
Phoenix, AZ 85040

Reporting Period: The results reported here are from work conducted from October 31, 2002 to October 14, 2003.

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. To describe the morphology and ultrastructure of the glassy-winged sharpshooter mouthparts.
2. To describe stylet penetration and the function of each stylet pair during feeding.
3. To 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. To determine the ultrastructure of the salivary sheath and its association with all plant tissues encountered from the epidermal layer to the xylem tissue.

RESULTS
Ultrastructural studies have contributed to a more complete morphological understanding of the mouthparts of the glassy-winged sharpshooter (*Homalodisca coagulata*). Our first objective, mouthpart description, has been met. We have identified and described the morphology of specific sharpshooter mouthpart structures important for understanding stylet movement and penetration. A detailed description of these structures can be found in Leopold et al., 2003.

Mechanosensory-like sensilla cover most of the terminal segment of the labium, and are found along the margin of the labial groove in all three labial segments. A pair of short sensilla, located at the very tip of the labium, is often found in contact with the extended stylets (Figure 1). Triangular spines occur singly, in straight-line clusters, or in palmate patterns on the exposed labial surface (Figures 1, 2). The unexposed surfaces of the labial groove lateral and basal to the stylets are covered with numerous multi-lobed palmate structures (Figure 3) that appear to be in constant contact with the mandibular stylets. Larger palmate structures are located at the terminal end of the labial groove (Figure 4). These structures are located below the stylets, with only the tips of the palmate lobes apparent when the stylets are extended.

Mandibular stylet morphology varies somewhat with stage of development (Leopold et al., 2003; Freeman et al., 2002). A series of cup-shaped flanges are located along the medial surface of these stylets, with small papillae located between the flanges. Slender fingerlike projections with pointed tips are found on the ventral side near the tip of adult mandibular stylets. The projections become flattened and tab-like proximally. Each mandibular stylet has a single unbranched dendritic canal from base to tip (Figure 5).

The paired maxillary stylets interlock with each other along their length (Figure 5), except for a short distance at the apex (Figure 6). These joints, similar to mortise-and-tenon joints, keep the stylets together, forming a food canal and salivary canal, and also allow extension of either stylet individually (Figure 6). A single dendritic canal in the base of each maxillary stylet (Figure 6) branches toward the tip of the stylet (Figure 7), with neurons (Figure 8)

reaching the short row of small dentitions along the edge of each maxillary stylet (Figures 6, 9) and extending further to the very tip of the stylet.

Fractured stems reveal the course of the sheath from the flange on the outer epidermal wall well into the host-plant tissues (Figure 10). These sheaths can be dissected out of stem or leaf tissue more or less intact and examined using scanning

electron microscopy (Figures 11, 12). Ultrastructural changes related to stylet penetration of individual cells are difficult to determine. Stylet penetration ruptures cell walls, with the salivary sheath material masking much of the ultrastructure of damaged cells. The length of the sheath, the density of the sheath material, and the requirement for ultrathin sections make transmission electron microscopy arduous. Confocal scanning light microscopy provides an opportunity to examine intact salivary sheaths and assess cellular damage using optical sections and 3D reconstruction.

Salivary sheaths (Figures 13-18) formed by all developmental stages from newly hatched sharpshooter nymphs to adults were examined from 14 different host plants. Sheaths varied from unbranched (Figures 13, 16) to highly branched (Figures 13-15), and terminated in various host-plant tissues. Approximately 65% of salivary sheaths formed by adult sharpshooters terminated in host-plant xylem tissue. Parenchymal cells of the cortex or medullary ray were the next most frequent location for termination. Only a few sheaths terminated in phloem tissues or in the pith of host plants. Nymphs and second instars, with shorter stylets, preferred to feed on the mid- and lateral veins of leaves, or on very small vascular bundles along the leaf margins, rather than on stems.

Salivary sheaths commonly were found in very close proximity to one another (Figures 13, 14). It has yet to be determined whether these multiple sheaths are formed by a single sharpshooter, moving only slightly from one feeding position to another; or if each sheath is formed by a different individual. Branched salivary sheaths were found in both vascular and parenchymatous tissues. It is unclear if branched salivary sheaths represent failed attempts to locate vessel elements in the xylem tissue, or if penetrated parenchyma cells provide a nutritive advantage to the sharpshooter. Preliminary studies have shown that the sharpshooters may produce drops of exudate even without reaching the water-conducting vessel elements, suggesting that parenchymatous cell contents may be ingested.

Salivary sheaths have been found in contact with vessel elements with no indication of actual cell-wall penetration (Figure 16), and without salivary sheath material in the cell lumen. It may be possible for the sharpshooter to remove water from the vessel through the pits in the wall without actually penetrating the cell wall. However, there is clear evidence that more commonly the wall of the vessel element is ruptured by the penetrating stylets (Figures 17, 18). The large volume of salivary sheath material in the lumen of many vessel elements (Figures 15, 17, 18) is sufficient to restrict or block completely the translocation of xylem fluid within a single vessel element. The occlusion of numerous vessel elements could result in the deterioration or death of the plant even in the absence of pathogenic bacteria.

CONCLUSIONS

This ultrastructural study has provided and will continue to provide data necessary to completely understand the anatomy and morphology of the sharpshooter related to stylet penetration, salivary sheath formation, feeding behavior, transfer of the bacterium *Xylella fastidiosa*, and the nature of Pierce's disease.

REFERENCES

Freeman, T.P., R.A. Leopold, D.R. Nelson, J.S. Buckner, and T.J. Henneberry. 2002. Ultrastructural contributions to the study of the glassy-winged sharpshooter and Pierce's disease. Proceedings of the Pierce's Disease Research Symposium, December 15-18. San Diego, CA. Pages 116-119.

Leopold, R.A., T.P. Freeman, J.S. Buckner, and D.R. Nelson. 2003. Mouthpart morphology and stylet penetration of host plants by the glassy-winged sharpshooter, *Homalodisca coagulata* (Homoptera:Cicadellidae). Arthropod Structure & Development 32(2-3): 189-199.

FUNDING AGENCIES

Funding for this project was provided by the University of California Pierce's Disease Grant Program.