# HOW IMMATURE WHITEFLIES FEED ON COTTON LEAVES T.P. Freeman Electron Microscopy Center, Dept. of Plant Pathology North Dakota State University Fargo, ND J.S. Buckner and D.R. Nelson Biosciences Research Laboratory USDA-ARS Fargo, ND

### Abstract

Does cotton leaf morphology influence the ability of the newly hatched whitefly crawler, to locate, penetrate, and reach phloem tissue in order to feed? Our earlier theory (Cohen et al., 1998) proposed that whiteflies feed and oviposit on the abaxial surface (underside) of leaves due to the closer proximity of phloem tissue to that leaf surface. The length of the stylet bundle at that time was believed to be 80  $\mu$ m (Pollard, 1955). We have shown for both the silverleaf whitefly, *Bemisia argentifolii*, adults and nymphs that the length of the stylet is not a limiting factor (Freeman et al., 2001).

#### **Introduction**

There are similarities in the feeding characteristics of silverleaf whitefly adults and nymphs. During feeding (Fig. 1) the adult places the end of the labium against the leaf surface and then by lowering its head the stylet is pushed down through the labium (Fig. 2) and into the leaf. When not feeding the adult stylet bundle is contained within a deep groove in the 4-segmented labium. The adult stylet (Fig. 3) which averages 214  $\mu$ m in length is more than long enough to penetrate the leaf and reach a phloem bundle from almost any position on either the abaxial or adaxial surface of the cotton leaf. The stylet bundle consists of two mandibular and two maxillary stylets. The length of the adult stylet bundle can be determined indirectly by measuring the length of the labium. The depth of insertion of the stylet bundle into the leaf is equal to the distance from the junction of labial segments 1 and 2 to the point at which the stylet bundle enters the labial groove (Freeman et al., 2001). The adult whitefly develops a salivary sheath which can be used to study the course of stylet movement (Fig. 4) within the host leaf.

### **Results**

The crawler (Fig. 5) is the only immature stage that is mobile. After hatching from the egg (Fig. 6), it must quickly find a suitable site on the leaf and probe the leaf tissue until a phloem bundle is found. All subsequent stages of the nymph feed at or near this site.

Nymph stylet insertion into the leaf is difficult to observe as it occurs below the body of the nymph. Stylets (Fig. 6) range in length from a minimum of 110  $\mu$ m for the crawler to over 200  $\mu$ m for the fourth instar.

The stylet bundle of the immature whitefly appears to be looped or coiled within the nymph. It enters a groove in the rostrum and is extended beyond the rostrum tip in order to penetrate a host leaf (Fig. 7). A salivary sheath (Fig. 8) similar to that formed by the adult marks the course of the stylets through the host leaf.

As nymphs molt between the first and fourth instars the stylets increase in length (39%) but not in proportion to the increase in body size (168%) (Fig. 5). The average length of the stylet bundle increased from 114 m in the first instar (crawler) to 158 m in fourth instar (nymphs) (Freeman et al., 2001). The stylet length is more than sufficient to reach phloem tissue which is within 53 to 127 m of the abaxial surface of leaves found on the top 20 nodes of field grown cotton (Chu et al., 2000).

Probe and feeding sites (Fig. 9) for silverleaf whitefly nymphs and adults on host leaves can be determined by staining the flange material that anchors the rostrum and labium to the leaf at the time of stylet penetration. The point of penetration of nymph stylets and the branched characteristics of the salivary sheath can be observed by treating the nymph and leaf with clearing agents (Fig. 10). Using similar clearing techniques the network of minor veins in cotton leaves can be observed (Figs. 11, 12). It is even possible to follow the course of the salivary sheath all the way from the abaxial surface of the leaf to the phloem tissue (Fig. 11). If a cotton leaf and cleared nymph are examined at the same magnification (Fig. 12) it is easy to determine that a silverleaf whitefly nymph can reach a phloem bundle from essentially any position on the leaf surface.

Thus, stylet length is not a limiting factor to account for either adults or nymphs feeding primarily on the abaxial surface of cotton leaves.

# **Summary**

The length of the stylet bundle is sufficient to allow the crawler and subsequent nymphal stages to reach a phloem bundle from any position on either the abaxial or adaxial surfaces of the cotton leaf.

# **References**

Chu, C.-C., T. P. Freeman, J. S. Buckner, T. J. Henneberry, D. R. Nelson, G. P. Walker and E. T. Natwick. 2000. *Bemisia argentifolii* (Homoptera: Aleyrodidae) colonization on upland cottons and relationships to leaf morphology and age. Ann. Entomol. Soc. Am. 93, 912-919.

Cohen, A. C., C.-C. Chu, T. J. Henneberry, T. Freeman, D. Nelson, J. Buckner, D. Margosan, P. Vail and L. H. Aung. 1998. Feeding biology of the silverleaf whitefly (Homoptera: Aleyrodidae). Chinese J. Entomol. 18, 65-81.

Freeman, T. P., J. S. Buckner, D. R. Nelson, C.-C. Chu and T. J. Henneberry. 2001. Mechanism of stylet penetration by the silverleaf whitefly, *Bemisia argentifolii* (Homoptera: Aleyrodidae) into host leaf tissue. Ann. Entomol. Soc. Am. 94, 761-768.

Pollard, D. G. 1971. Some observations on the mouth-parts of white-flies (Hem., Aleyrodidae). Entomologist Monthly Magazine 107, 81-88.



Figure 1. Adult silverleaf whiteflies feeding on the abaxial surface of a cotton leaf.



Figure 2. Stylet bundle (Arrow) partially contained within the fourth segment of the labium (L) and extended 110 mm beyond the tip.



Figure 3. Adult silverleaf whitefly stylet bundle (Arrow) extended 196 mm beyond the tip of the labium (L).



Figure 4. Adult salivary sheaths (S) within the mesophyll of a host leaf.



Figure 5. Silverleaf whitefly egg (E), crawler (C), and fourth instar nymph (N) on the abaxial surface of a leaf.



Figure 6. Third instar nymph removed from a cotton leaf showing the rostrum (Arrow) and an extended stylet (Arrowhead).



Figure 7. Ventral surface of a nymph removed from a host leaf. Note the rostrum (R) and extended stylet bundle (Arrow).



Figure 8. Fracture of a host leaf with several nymphal salivary sheaths (Arrow Heads) in the space between mesophyll cells.



Figure 9. Cleared and stained leaf showing the position of nymphs (N), vascular tissues (V), and probe feeding sites (Arrows).



Figure 10. Cleared leaf showing the position of nymphs (N) and the highly branched salivary sheaths (Arrows).



Figure 11. Cleared leaf showing a single nymph with salivary sheath (Arrows) extending from the leaf surface to the vascular tissue (V).



Figure 12. Cleared cotton leaf and overlayed nymph (N) at similar magnifications. Note the number of small vascular bundles within range of the nymph stylet.