DOI:10.6660/TESFE.1998007 台灣昆蟲Formosan Entomol. 18: 65-82 (1998) 綜合論述 Review article

Formosan Entomologist
Journal Homepage: entsocjournal.yabee.com.tw

Feeding Biology of the Silverleaf Whitefly (Homoptera:Aleyrodida【Review article】

銀葉粉蝨(同翅目：粉蝨科)的取食生物學【綜合論述】

Allen Carson Cohen* Chang-chi Chu, Thomas J. Henneberr , Thomas Freeman, Dennis Nelson, James Buckner, Dennis Margosan, Patrick Vai and Louis H. Aung.
Allen Carson Cohen* 朱昌祺 Thomas J. Henneberry, Thomas Freeman, Dennis Nelson, James Buckner, Dennis Margosan, Patrick Vail and Louis H. Aung.

*通訊作者E-mail :

Received: Accepted: 1998/04/07 Available online: 1998/06/01

Abstract

Whitefly feeding biology is complex and includes the location of appropriate sites to probe leaves so that minor veins can be located. Since survival of the nymphal stage of the silverleaf whitefly Bemisia argentifolii Bellowa and Perring (Homoptera: Aleyrodidae) requires styley penetration of the smallest veins in host plant leaves , an elaborate series of behavioral acts must be performed for the insect to reach its target. Our studies, using light and electron microscopy as well as confocal imaging, reveal that successful feeding always involves probing of minor veins that contain no more than three xylem elements. There are distinct surface structures such as lamina trichomes and elongated epidermal cells that serve as guides for a first instar nymph (crawler) to begin its probing at appropriate places. The nymphs use a specialized saliva to produce a sleeve-like structure called a salivary sheath that guides the stylets (hollowed, elongated and interlocking maxillae and mandibles)to the minor veins. The sheaths are often sinuous and branched. Branching takes place both in the mesophyll and at veins. Most of the sheath material inside the leaf is extra-cellular and is built in the extensive air space between spongy. Parenchyma cells. Only a small portion of the sheath is found inside cells, that portion being in epidermal cells. We found almost no evidence of stylet or sheath penetration into parenchyma or palisade cells. When a sectioning technique was used, it appeared that some feeding sheaths led from the plant surface to sites other than vascular tissue. However, when non-sectioned leaves were stained and cleared so that entire sheaths could be viewed intact, it became apparent that nymphs that succeeded in developing beyond the first instar always made contact, via salivary sheaths, with veins. Sheaths were up to 140µm long and about 2µm in diameter at their widest dimension. Sheaths had the appearance of fused beads. The sheaths were occasionally glued to cell walls and made contiguous contact between the plant leaf surface where penetration originated, proceeding all the way to the veins. Some sheaths terminated blindly without reaching a vascular bundle, and these sheaths were invariably sealed at the end. It appeared from our studies that successful feeding always involved intact sheaths. The relative success of silverleaf whitefly on different species of host plants is, in part, attributable to the geometry of the feeding arrangement in relationship to the availability of minor veins in the host plants. This accounts for the high success of this species on cantaloupe and other cucurbit hosts and the low success on lettuce, the former having more than twice the amount of vascular bundle tissue than the latter.

摘要

銀葉粉蝨 (Bemisia argentifolii Bellows & Perring) 具有刺吸式口器，其取食習複雜。粉蝨必需在寄主植物葉片背面尋找適宜部位，使其口針容易穿刺到葉片內的細小葉脈以便取食。因為銀葉粉蝨若蟲的生存需要把口針穿刺到最細小的葉脈，它們必定採用一系列的靈巧動作才能達到目的。我們光學顯微鏡、電子顯微鏡和影像顯微鏡觀測發現，粉蝨取食都是穿刺到木質部不超過三根導管的小葉脈。第一齡爬行若蟲總是以差別明顯的表皮結構 (如葉片絨毛和細長形的上表皮細胞) 為線索，在葉片上尋找適宜場所開始進行穿刺。這些若蟲用其特殊的唾液形成一袖狀結構，稱為唾液鞘 (asalivary sheath) ，用來引導口針找到小葉脈。唾液鞘常彎曲分枝，而分枝的部位發生在葉肉和維管束內。唾液在葉片內位於細胞外面，海綿狀薄壁細胞之間空間，只有很小部分的唾液鞘位於外表皮細胞內。我們還沒有發現口針或唾液鞘探入到薄壁細胞或柵欄細胞中。應用切片技術，我們在維管束外的組織中發現過一些從植物面到取食點的取食痕跡。然而，當我們用整葉染色透明技術使唾液鞘清晰可辨時發現，凡是發育超過第一齡的若蟲，其口針都是通過唾液鞘與維管束相連。唾液鞘可長達140µm，最寬處的直徑約2µm，看起來像一連串合併的空泡，有的黏附在細胞壁上,經過鄰近的細胞表面，一直延伸到維管束。沒有達到維管束的唾液鞘，其末端總是封閉的。我們的研究證明，形成完整的唾液鞘是取食成功的關鍵。銀葉粉蝨對不同寄主植物種類的喜好，往往取決於維管束的數量。銀葉粉蝨喜好甜瓜和其它葫蘆科寄主而不喜好萵苣就是一個證明。

Key words: Bemisia argentifolii, Aleyrodidae, Homoptera, salivary sheaths, feeding biology , cotton, stylets, vascular bundles.
關鍵詞: 銀葉粉蝨、維管束、取食行為、唾液鞘

Full Text: PDF(34.53 MB)

中華昆蟲 18: 65-82(1998)
Chinese J. Entomol. 18: 65-82(1998)

Feeding Biology of the Silverleaf Whitefly (Homoptera: Aleyrodidae)

Allen Carson Cohen*, Chang-chi Chu and Thomas J. Henneberry USDA-ARS, Western Cotton Research Laboratory, Phoenix, AZ 85040, USA

Thomas Freeman Electron Microscopy Center, North Dakota State University, Fargo, ND 58105, USA

Dennis Nelson and James Buckner USDA-ARS, Biosciences Research Laboratory, State University Station, Fargo, ND 58105, USA

Dennis Margosan, Patrick Vail and Louis H. Aung USDA-ARS, Postharvest Quality & Genetics Research Laboratory, Fresno, CA 93727, USA

ABSTRACT

Whitefly feeding biology is complex and includes the location of appropriate sites to probe leaves so that minor veins can be located. Since survival of the nymphal stage of the silverleaf whitefly *Bemisia argentifolii* Bellows and Perring (Homoptera: Aleyrodidae) requires stylet penetration of the smallest veins in host plant leaves, an elaborate series of behavioral acts must be performed for the insect to reach its target. Our studies, using light and electron microscopy as well as confocal imaging, reveal that successful feeding always involves probing of minor veins that contain no more than three xylem elements. There are distinct surface structures such as lamina trichomes and elongated epidermal cells that serve as guides for a first instar nymph (crawler) to begin its probing at appropriate places. The nymphs use a specialized saliva to produce a sleeve-like structure called a salivary sheath that guides the stylets (hollowed, elongated and interlocking maxillae and mandibles) to the minor veins. The sheaths are often sinuous and branched. Branching takes place both in the mesophyll and at veins. Most of the sheath material inside the leaf is extra-cellular and is built in the extensive air space between spongy parenchyma cells. Only a small portion of the sheath is found inside cells, that portion being in epidermal cells. We found almost no evidence of stylet or sheath penetration into parenchyma or palisade cells. When a sectioning technique was used, it appeared that some feeding sheaths led from the plant surface to sites other than vascular tissue. However, when non-sectioned leaves were stained and cleared so that entire sheaths could be viewed intact, it became apparent that nymphs that succeeded in developing beyond the first instar always made contact, via salivary sheaths, with veins. Sheaths were up to 140 μm long and about 2 μm in diameter at their widest dimension. Sheaths had the appearance of fused beads. The sheaths were occasionally glued to cell walls and made contiguous contact between the plant leaf surface where penetration originated, proceeding all the way to the veins. Some sheaths terminated blindly without reaching a vascular bundle, and these sheaths were invariably sealed at the end. It appeared from our studies that successful feeding always involved intact sheaths. The relative success of silverleaf whitefly on different species of host plants is, in part, attributable to the geometry of the feeding arrangement in relationship to the availability of minor veins in the host plants. This accounts for the high success of this species on cantaloupe and other cucurbit hosts and the low success on lettuce, the former having more than twice the amount of vascular bundle tissue than the latter.

Key words: *Bemisia argentifolii*, Aleyrodidae, Homoptera, salivary sheaths, feeding biology, cotton, stylets, vascular bundles.

*Correspondence / reprint request address

Introduction

The silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring (Aleyrodidae: Homoptera) (see Bellows *et al.*, 1994 and Brown *et al.*, 1995 for a discussion of taxonomic issues in the *Bemisia* complex) is a devastating pest of numerous crops (Byrne and Bellows, 1991). Its success in feeding on more than 400 species of host plants is, in part, a testimony to its highly efficient feeding biology (Cohen, *et al.*, 1996a and b). Nearly all feeding by *Bemisia* species takes place on the underside of leaves (Lenteren and Noldus, 1990). The major factor in determining the selection of feeding sites was shown to be leaf morphology (Chu *et al.*, 1995). Although earlier analysis indicated that some feeding by *Bemisia* was directed to non-vascular tissue (Pollard, 1955; Cohen and Hendrix, 1994), it has recently been demonstrated that all successfully developing nymphs at some time use vascular tissue (Cohen *et al.*, 1996). Unlike some aphids that use major veins as feeding sites (Gibson, 1972; Klingauf, 1987), *Bemisia* uses only minor veins as a food source (Cohen *et al.*, 1996a and b).

Since *Bemisia* must reach the minor veins in order to develop, it seems reasonable that they possess some mechanism that allows them to perform a non-random search for their tiny targets. If feeding probes were completely random, the number of successful ventures would be proportional to the relative amount of space occupied by minor veins in relationship to the amount of non-vascular space within a leaf. Since cantaloupe leaves have less than 15% of their volume dedicated to minor veins, and cotton and lettuce have even less (Cohen *et al.*, 1996a), it seems that the assumption of a non-random probing is well-founded.

Over the past five years, several studies have been published on various aspects of feeding by *B. tabaci* (Genn.) and *B. argentifolii*. However, what is lacking is a comprehensive perspective that unifies the various observations and discoveries about *Bemisia* feeding. This paper's purpose is to present such a unification, and it also presents some new information about the feeding biology of this pest as studied by diverse techniques including histological sectioning / light microscopy, confocal microscopy and electron microscopy. We believe that a very clear understanding of the feeding processes of silverleaf whitefly will provide a solid foundation for future work on the control of this species and perhaps other phloem feeding insects.

Materials and Methods

Sources of Plants: Field and Greenhouse Studies. Leaf tissues from Cantaloupe (*Cucumis melo catalupensis* L.), cotton (*Gossipium hirsutum* L.), lettuce (*Lactuca sativa* L.), lantana (*Lantana camara* L.) and hibiscus (*Hibiscus rosa sinensis* L.) were collected in the field (or as urban landscape plants in the cases of lantana and hibiscus) in the Imperial Valley, CA or in Maricopa County, AZ during the summers of 1993 and 1994. Only fully expanded leaves were used for these studies. We used four methods of leaf analysis described below: 1) surface analysis by electron microscopy and dissecting microscopes, 2) sectioning and examination of cross-sections and 3) staining, clearing and examination of intact leaf disks and 4) leaf fracture and electron microscopy. Sectioning / light microscopy is currently used more widely than the clearing of intact leaves. Cantaloupe and cotton, Delta Pine 90 (DPL 90) and DPL 115 were grown during July and August of 1994 and 1995 in Phoenix, AZ.

Insects: Cantaloupe plants at the ten leaf stage and cotton at the six leaf stage were transferred into a greenhouse containing plants that were highly infested with *B. argentifolii*. All laboratory and greenhouse studies included *B. argentifolii* that were produced on cotton plants unless otherwise specified. Whitefly specimens are vouchered at the Western Cotton Research Laboratory, USDA, ARS, Phoenix, AZ.

Histological treatment: Leaves were prepared by the methods of Berlyn & Miksche (1976) with slight modifications described by

Cohen *et al.*, (1996a). Cotton leaves that were heavily infested with silverleaf whitefly were selected and the abaxial sides of leaves were embedded with a 1% agarose solution poured at 50℃ to hold nymphs in place (Walker, 1985). Leaves were fixed in FAA (0.5 ml formalin: 0.5 ml glacial acetic acid: 9 ml 50% EtOH) for 20 h then dehydrated in an alcohol series from 50–100%, cleared in xylene and infiltrated with Paraplast®. Five, 10 and 20 mm sections were made of tissues. We found the 10 mm sections to be most satisfactory for examination of salivary sheaths. Sections were stained with saffranin / fast green (Pollard, 1955) or with periodic acid (Berlyn & Miksche, 1976). Sections were placed for 5 min sequentially in each of the following solutions serially: xylene, 100%, 95%, 50%, 25% EtOH, distilled H_2O, periodic acid, H_2O, Schiff's reagent, H_2O, sodium metabisulfate, H_2O. Specimens were re-desiccated in an alcohol series as described above and returned to xylene before being mounted on slides.

Leaves used for whole-mounts were decolorized with 95% EtOH, then cleared with either 15% NaOH or lactic acid, glycerol, water (L: G: W) (1:1:1). The clearing procedure with NaOH took 20–44 h, depending upon the leaf species or variety; and the clearing with LGW took 25–45 min in an autoclave at 120℃, 1.05 kg cm^{-2}. Whole-mounts of cotton leaves that were to be used for measurements of stained stylet sheaths were first placed in McBride's solution (Backus *et al.*, 1988) for 10 to 20 h, until salivary sheaths were deeply stained. Sections were then destained in 95% EtOH for 15–30 min, and then cleared in LGW and autoclaved as described above. Sections that retained too much stain after this procedure were either further destained in 95% EtOH or placed in fresh LGW and re-autoclaved. Specimens were mounted in LGW for microscope observations. Mounted sections were viewed at 256x and measurements made with a Boecleler VIA-170 video imaging system. Specimens analyzed by compound microscopy were examined at 400x, and measurements were made with an ocular micrometer. Measurements were made on cotton leaves of the distance from the labium of the whitefly at the point of leaf contact to the point of attachment on the vascular bundle in 200 specimens from a total of 25 whole-mounts. Evaluations of stylet course were made by counting the number of sheaths that went in a direction other than one that would lead directly to the vascular bundle. Instances were counted only where contact with the vein was found.

Bright Field Microscopy: Using light microscopy at 400x, we examined 225 specimens (7500 sections) to determine placement of mouthparts. We recorded salivary sheath placement as 1) directly connected to veins, 2) appearing to approach a vascular bundle, but not visibly connected, and 3) seen approaching only non-vascular tissue. Using the same sections, we noted the frequency of stylet penetration of epidermal cells versus insertion between cells. Veins were classified as single-stranded, double-stranded, triple-stranded (all considered minor veins) and four-or-more stranded, according to the number of xylem elements that were noted.

We examined 100 stained specimens (cleared nymphs in intact feeding position on leaves) with bright field light microscopy at 400x to determine placement of mouthparts in relationship to plant structures. Veins were classified as single-stranded, double-stranded, triple-stranded (minor veins) and four-or-more stranded, according to the number of xylem elements that were noted.

Confocal Microscopy: We examined 45 cleared and acid fushin-stained nymphs situated on both cotton and cantaloupe leaves with the aid of a Leica TC-4D upright confocal laser scanning microscope. We examined specimens with 488 / 568 dual excitation, using a transmission detector with a 590 longpass filter. We used 50, 100, 200 and 400x to study the placement of sheaths throughout the plant material, recording images at 0.5, 1, 2 or 5 mm intervals from the point of contact of whitefly labia at the plant surface to the vascular bundle region beyond the reach of the salivary sheath. We used the Leica system "movie" and three-dimensional reconstruction

programs to view composite images of all focal planes containing feeding structures.

Electron Microscopy. Leaves were either fixed in glutaraldehyde, dehydrated in EtOH and critical point dried using CO_2 as a transitional fluid or fixed and dehydrated in acidified 2,2-dimethoxypropane prior to critical point drying and viewed with a JEOL JSM 6300 scanning electron microscope. About 300 fractured leaf sections were examined at 15 kV from a transverse view. Electron micrographs were used to make measurements of the epidermal cells and for assessment of epidermal surface contour as well as for assessment of the appearance of the salivary sheaths and their relationship to epidermal cells, spongy mesophyll and veins.

Behavior of whitefly nymphs: We exposed five DPL 115 cotton plants (hairy-leafed isoline) to heavy infestations of whiteflies in a greenhouse for two days. In 10-20 mm^2 randomly chosen abaxial surface sections, we counted the number of eggs within and outside of a 600 μm diameter around lamina trichomes. We chose this diameter as an extremely conservative distance that was less than the labium-ovipositor length of silverleaf whitefly (Cohen, unpublished observations). Twenty newly-hatched 1st instar nymphs (crawlers) were observed on fresh leaves and tracked with a Boecleler VIA-170 video imaging system as they moved over the surface of leaves. All measurements were made in the laboratory at 25°C under fiber optics light. Distances traveled over a 2 min interval and pattern of movement were recorded in relation to position of surface cells associated with veins. Positions of eggs were recorded in relation to vascular bundle-related surface cells, stomata and trichomes. Measurements were made to determine what percentage of total leaf surface area was within a 600 μm diameter of trichomes (the approximate distance between the labium and ovipositor). We conservatively estimated that, if a female whitefly inserted an egg within this area, she could have been in contact with a lamina trichome. Distances were measured between egg pedicels and trichomes using the video imaging system calibrated with a stage micrometer. Difference among these measurements were evaluated with χ^2 tests and ANOVA (SAS Institute, 1988).

Results

Leaf surface structure: Non-glandular leaf trichomes in cotton leaves all appeared to originate from vein-associated epidermal cells (Fig. 1A). This conclusion was based examination by light and electron microscopy. Epidermal cells in the areoles measured about 34.5 (S.E.M.=+/- 1.69) μm x 36.4 (S.E.M.=+/- 2.54) mm, and epidermal cells associated with veins were about 74.8 (S.E.M.=4.83) mm x 19.6 (+/- 1.48) μm in size. Surface cell dimensions were well within the span of the legs, antennae, and carapace of whitefly nymphs (Fig. 1B). Elongated epidermal cells (Fig. 1A) were evident wherever veins were present, including even the finest, single-stranded veins. The targets of whitefly nymphs were always the most minor veins found at the apex of the spongy parenchyma and immediately below the palisade cells (Fig. 1C). Whitefly nymphs were never observed to target the major veins (Fig. 1D).

Stomata on the adaxial surface were about half as numerous as those on the abaxial side of leaves (156.8 ± 10.28 / mm^2 versus 321.6 ± 13.51 / mm^2, mean±S.E., respectively; N=15). The width of bundles of minor vein-associated epidermal cells was 35.6 μm±0.86, (N=20), and the mean distance across areoles or between closest minor veins was 531 μm±28.3 μm, S.E. (N=100).

Leaf internal structure and salivary sheath positioning: Observation of 300 stained sheaths in cleared leaves revealed no evidence of stylet penetration through stomatal openings. In contrast to this finding, Lenteren and Noldus (1990) mentioned that some probes by *B. tabaci* penetrated stomata. In the cotton leaves examined, the lower portions were always characterized by spongy parenchyma with abundant air spaces (Figs. 1C, 1D, 2A and 2B). In cross section, the upper

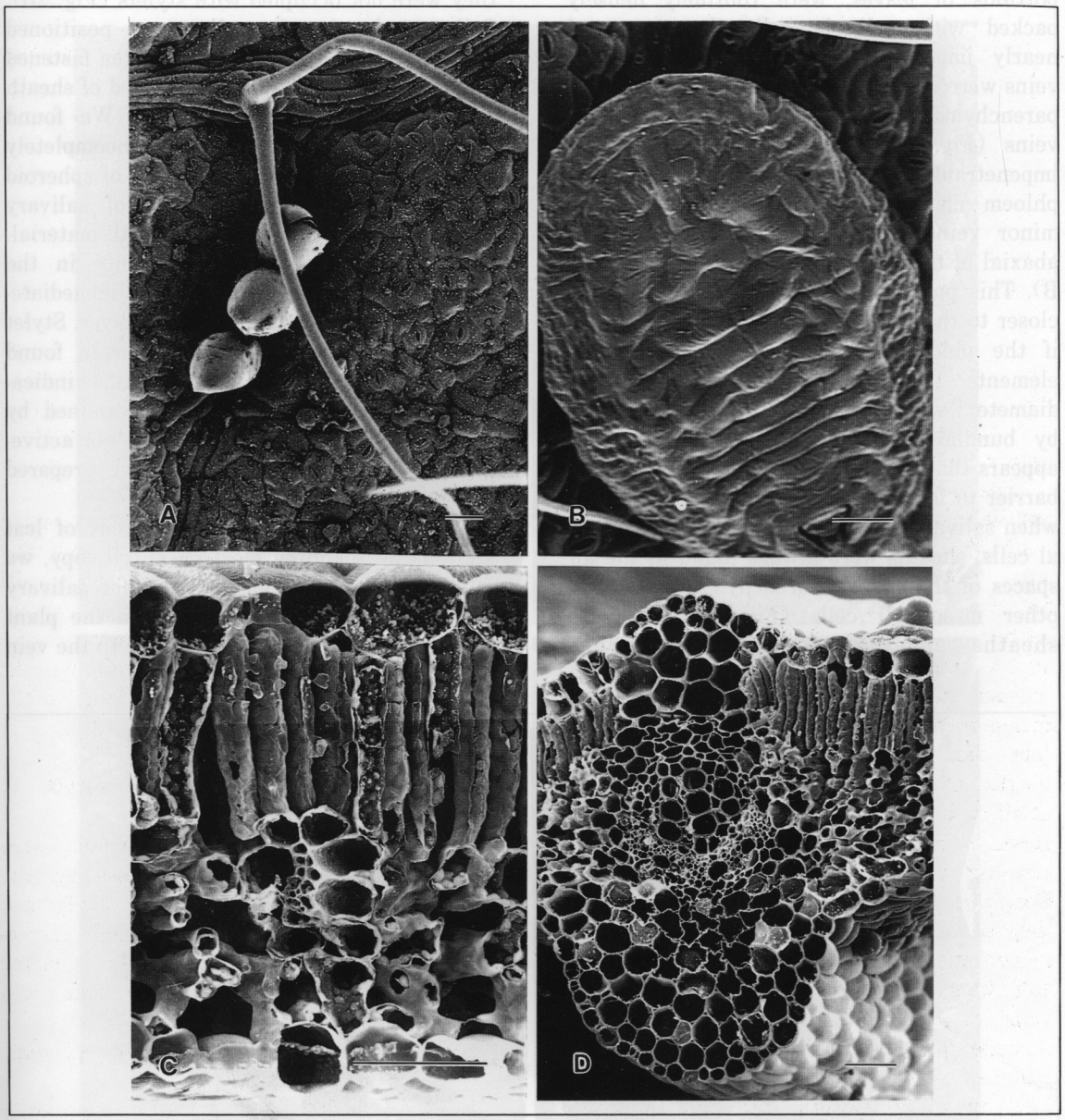

Fig. 1. A: Scanning electron micrograph (SEM) of abaxial (under) surface of cotton leaf showing 3 eggs deposited into epidermal cells associated with a minor vein. An aerial trichome is visible, and the elongated epidermal cells that are under minor veins can be compared with isodiametric cells from non vascular regions of the leaf. B: A 2nd or 3rd instar *Bemisia argentifolii* nymph on abaxial surface. SEM shows relative size of nymph and the leaf's epidermal cells. C: Section inside cotton leaf showing upper epidermis with palisade cells below, a medium sized vein surrounded by hollow bundle sheath cells. The large air spaces in the spongy parenchyma are visible here as are the lower epidermis (bottom) with salivary sheaths visible in two middle epidermal cells. D: A major vein in a cotton leaf. Note that neither of the veins in C or D is a suitable target for *Bemisia argentifolii* (see text for comments). Bars=50 μm.

portions of leaves, were routinely densely packed with palisade cells that appeared nearly impenetrable to stylets. The minor veins were usually located between the spongy parenchyma and the palisade layer. The large veins (Fig. 1D) were thick and appeared impenetrable by stylets intending to reach phloem elements. The phloem elements in minor veins were characteristically located abaxial of the xylem elements (Figs. 2A and 2 B). This positions the targeted phloem tissue closer to the lower leaf surface than it would, if the phloem were adaxial to the xylem elements. The smallest veins (10–20 mm in diameter) were the only ones not surrounded by bundle sheath cells (Fig. 2A), and it appears that the bundle sheath cells may be a barrier to feeding by whitefly nymphs. Except when salivary sheaths penetrated the epidermal cells, sheaths were always found in the air spaces of the spongy parenchyma or between other mesophyll cells (Fig. 2B). Salivary sheaths appeared to be sealed when they were not occupied with stylets (Fig. 2B). Salivary sheaths generally were positioned along the cell exterior and were often fastened to the cells with "tendrils" composed of sheath material (Figs. 3A–3D and 4A). We found several sheaths that seemed incompletely formed and appeared to be a mass of spheroid droplets (Fig. 4B), presumably of salivary gland origin, as is the formed sheath material. Spheroid droplets were found only in the epidermal cells or within the space immediately adaxial to the lower epiderm al layer. Stylet tips (Figs. 4C and 4D) were rarely found protruding from the salivary sheaths, indicating that most of the sheaths examined by scanning electron microscopy were not actively being used for feeding when we prepared the specimens for examination.

Although we examined hundreds of leaf sections by scanning electron microscopy, we found only a few completely intact salivary sheaths that could be traced from the plant surface through their entire course to the vein

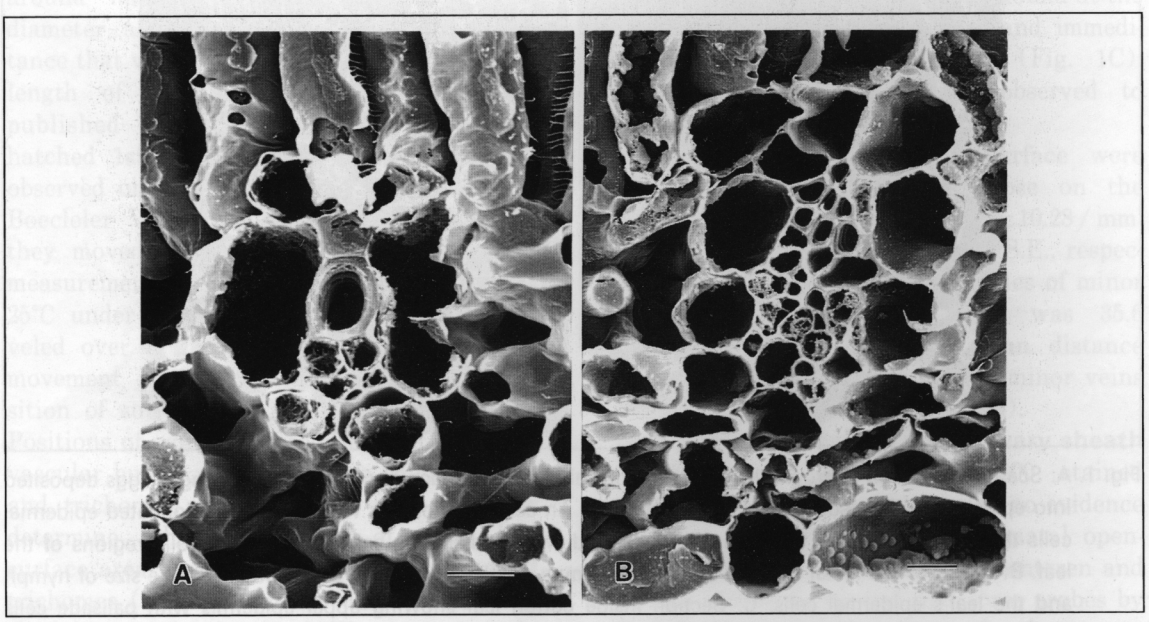

Fig. 2. A: A minor vein with a single xylem element (at the center) and three sieve elements immediately below the xylem element. B: A salivary sheath from *Bemisia argentifolii*. This sheath, with a single branch was formed in an air space in the spongy parenchyma and is seen to protrude from the lower epidermal cells. The bead-like nature of the salivary sheath is evident here. Both branches of the sheath are closed at their terminal ends. Bars=10 μm.

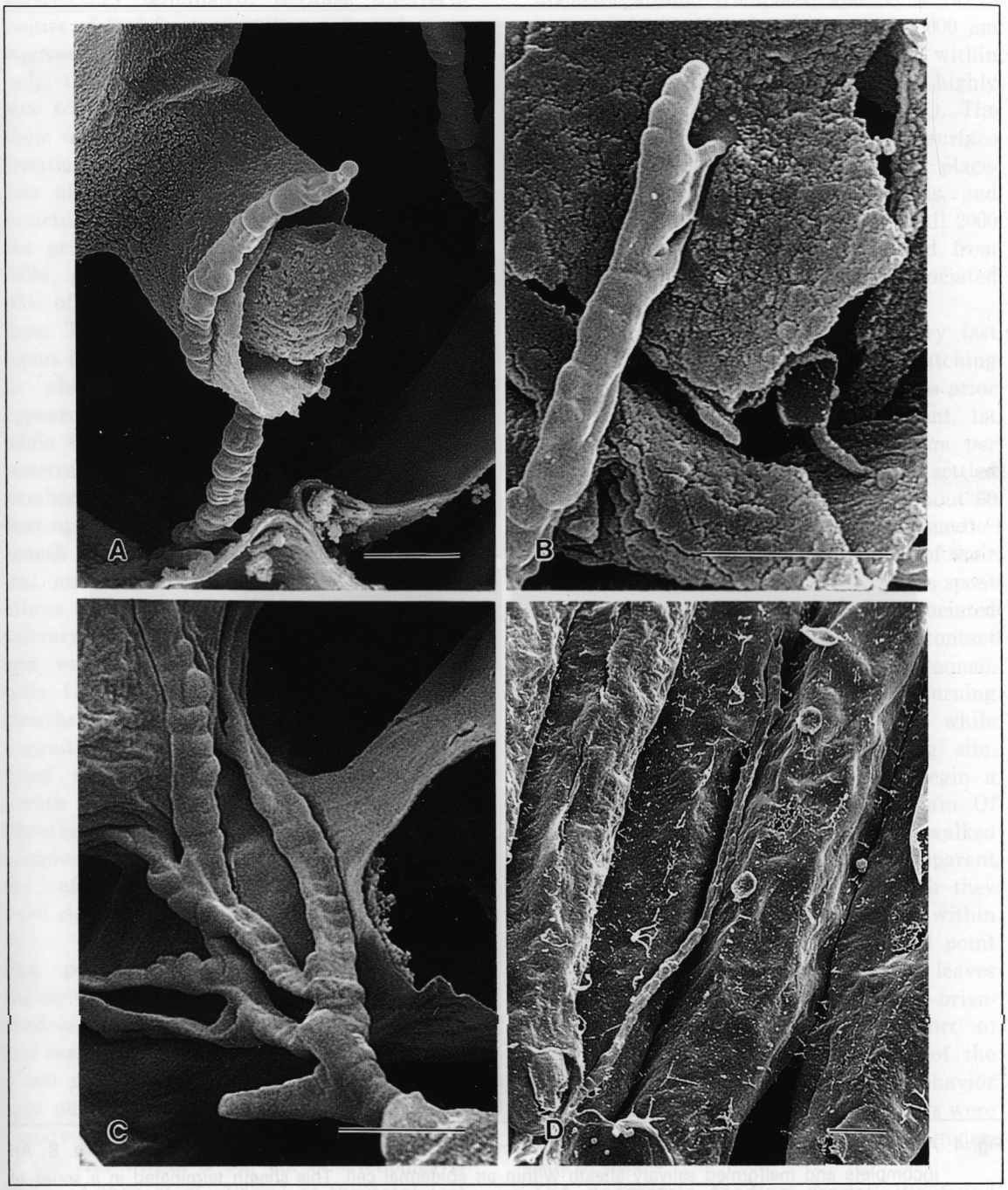

Fig. 3. A: Salivary sheath wrapping around a mesophyll cell. B: Salivary sheath attached to a mesophyll cell with "tendrils" adhered to cell surface. C: Highly branched salivary sheath with at least 6 branches visible. D: A single salivary sheath amid palisade cells. Bars=5 μm.

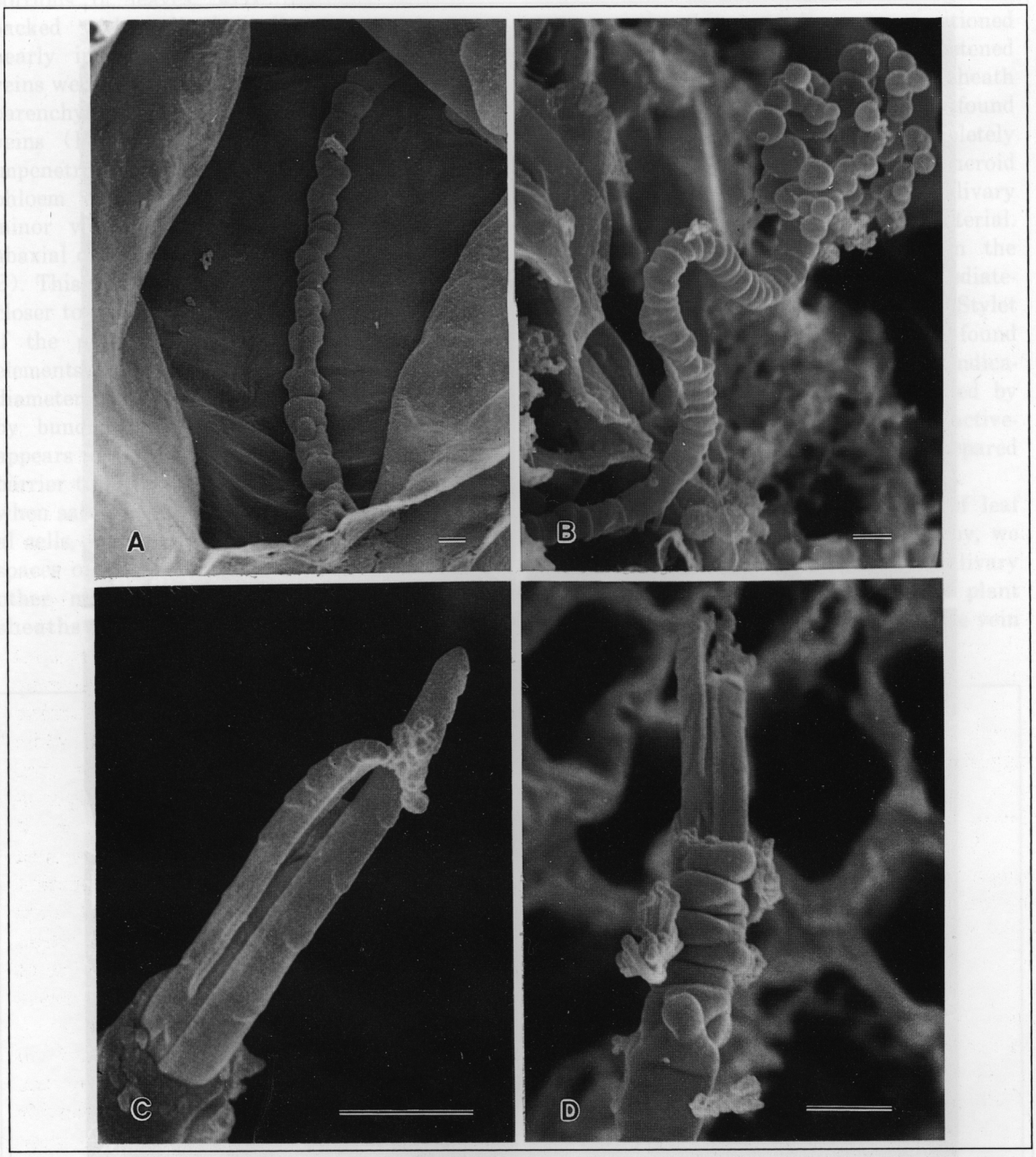

Fig. 4. A: Salivary sheath attached to spongy parenchyma cell, showing "tendrils" attached to cell walls. B: An incomplete and malformed salivary sheath within an epidermal cell. This sheath terminated in a series of "bubbles" of sheath material that hardened before a complete sheath could be formed, completing connection with a phloem element. C: The stylet tips, showing the mandibular stylets with their characteristic dentition. D: Stylet tips, showing the relationship of the stylets within the sleeve of sheath material. Note the beaded appearance of all stylet sheaths, reflecting from the manner in which the sheaths are formed. Bars=2 μm.

where they terminated. Because the techniques of leaf fracture and scanning electron microscopy permit examination of surfaces only, these procedures did not permit extensive tracking of salivary sheaths throughout their entire range. The diameters of the sheaths were consistently about 2 μm, regardless of whether the sheaths were primary structures ("main trunks") or branches from the primary structure. Sheaths, observed by SEM, penetrated epidermal cells more than 90% of the time rather than passing between them. This observation differs from a previous report from our laboratory (Cohen *et al.*, 1996a) in which about 50% of all penetrations appeared to occur between epidermal cells, while the remaining 50% were intracellular penetrations. The SEM study revealed a large number of epidermal cells that had inclusions that appeared to be partially formed salivary sheath material. These structures were spherical, and many were singular spherical while others appeared to be fused (Fig. 4B). Also, salivary sheaths frequently wrapped around, and were attached to, spongy parenchyma cells (Fig. 3A). The structure of salivary sheaths in the spongy parenchyma was clearly suggestive of their formation as a series of fused spherical bodies made of solidified sheath material (Figs. 2C, 2D, 4B and 4D). Sheaths were highly branched and very complex, the branching sometimes adjacent to the leaf surface (Fig. 3C) while at other times were closely adjacent to the target vein (Fig. 3C).

Egg placement and crawler behavior: Eighty-one eggs were counted within ten randomly selected 20 mm^2 samples of cotton leaf surface. Of that total, 57 eggs were within a 600 μm diameter of the trichomes and 24 eggs outside of this diameter. In the DPL 90 variety of cotton leaves, the amount of surface of the leaf within a 600 μm of trichomes is 10.1% (Cohen *et al.*, 1996a) of the total leaf area (excluding major veins which silverleaf whitefly avoid as oviposition sites). Assuming the hypothesis that egg deposition is totally random, we would expect that about 10% of the eggs (=8) would be placed within the 600μm diameter around trichomes, and 90 % or 73 eggs would be placed outside of the 600 μm diameter. Therefore, the 57 eggs found within the diameter around trichomes is highly significant ($P<0.005$; $\chi^2=301.2$; d.f.=1). The responses of whitefly nymphs to the surface features were evaluated by tabulating placement of eggs, the position of nymphs, and crawler (1st instar nymph) behavior. All 2000 lamina trichomes examined originated from elongated epidermal cells that were associated with veins.

Walking speed varied from a very fast pace with infrequent stops soon after hatching to a very slow pace with frequent stops prior to settling. During fast-paced movement, 1st instars walked an average of 2300 μm per minute$\pm$280 μm (N=30) until they settled upon feeding sites that were within about 60 μm from the center of the abaxial bundle-associated epidermal cells. About 80% of their searching time (=96 s, $\pm$28, N=30) was spent in contact with the elongated vein-associated epidermal cells apparently making contact with these cells with legs or antennae. Crawlers, frequently back-tracked, turning around at a given leaf surface area while searching for an appropriate feeding site. Then they would either settle and begin a feeding probe or commence walking again. Of the 30 crawlers that we observed, none walked onto the petiole or made any other apparent effort to abandon the leaf upon which they had eclosed. All the crawlers remained within an area of about 100 mm of the initial point where they were first seen. Although leaves were observed with the abaxial surface oriented upwards, crawlers made no effort to relocate to the positional underside of the leaves. This is in contrast with behavior observed by Chu *et al.*, 1995 where adults were reported to relocate to the positional underside of leaves.

The geometric feeding model: We found that whitefly feeding (and possibly feeding by other phloem exploiters) can be described parsimoniously by a "geometric model" (Cohen *et al.*, 1996). This model sets an upper limit on the number of whiteflies that a given

species (or variety) of host plant can accommodate per unit of leaf surface area. The model (illustrated in Fig. 5A) includes an imaginary plane projecting from a minor vein through the abaxial surface of the leaf (represented by the line "a"). If a whitefly begins a feeding probe with labial contact at any distance from the intersection of "a" and the leaf surface, then its successful location of the vein with the stylets forms (roughly) a right triangle. One of the triangle's legs is "a"; the second is the imaginary line from the point of labial contact with the leaf surface and the closest point on the leaf surface described by "a's" intersection with the surface. The stylet bundle of the whitefly can be thought of as the hypotenuse of the triangle. The constraints on this model, then, are the length of the stylets (about 80 μm, according to Pollard, 1955) and the depths of the minor veins (35–55 μm, according to Cohen *et al.*, 1996). This means that the maximum distances at which 1st instar nymphs (crawlers) could begin potentially successful probes are between 58 and 72 μm from the point of intersection of "a" and the lower leaf surface.

The implications of the geometric feeding model are evident in Fig. 5B, where the minor veins from six host plants have been traced in proportion to their size and distribution and the relative size of a crawler. It is evident from this figure that such host plants as cantaloupe and honeydew melon have greater densities of minor veins than does lettuce. Given the limited length of stylets in crawlers of both *B. argentifolii* from the present study and *B. tabaci*, described by Pollard (1955) and the geometric constraints depicted in Fig. 5A, it is evident that plants with more minor veins can support larger populations of these species of whiteflies, and therefore are more likely to be successfully exploited by an individual whitefly seeking a feeding site.

Histological observations of intact leaf tissues: Using both light and confocal microscopy, we found that all attached nymphs had sheaths that went to veins. Many nymphs were touching the elongated surface cells that directly below veins, but sometimes the salivary sheaths of these nymphs were attached to a vascular bundle other than the one corresponding to the elongated surface cells that they were touching. The courses of salivary sheaths were always indirect and often tortuous and multi-branched (Fig. 6A). Sheaths were never observed penetrating mesophyll cells (spongy parenchyma or palisade cells), and they rarely extended beyond the adaxial side of the veins i.e., into the palisade cells (Figs. 3A–3D and 6B–6D). Sheaths that did reach palisade tissue were never observed intracellularly.

Figure 7 is an example of the image resulting from histological sectioning, staining, and conventional light microscopy. The stylets from this nymph are seen to penetrate an epidermal cell and presumably extend to the small vein visible to the lower left of the penetration site (Fig. 7). Examination of hundreds of such sections revealed several broken or fragmented sheaths within the leaf tissue (Cohen *et al.*, 1996a). Such discontinuities led to underestimations of the extent to which *Bemisia* spp. are obliged to utilize vascular tissue (Cohen *et al.*, 1996a).

Figure 8 is a feeding model based on a diagram of a potential feeding site superimposed on a cotton leaf surface with a single areole outlined. The perimeter of this structure was about 2600 μm and the area within about 450,000 μm^2. Because a crawler's stylet tip cannot reach beyond 70–80 μm from the site of labial contact with the plant's surface, this feeding model was conservatively based on an 80 μm boundary around minor veins (Cohen *et al.*, 1996b). An area whose perimeter is about 80 μm from the minor vein surrounding the areole is demarked with another line. The area within this smaller region represented the total surface from which veins would be out of reach to a probing crawler (Fig. 8). Therefore, the defined area of about 250,000 μm^2 is the area within the areole (with a perimeter of about 2000 mm) that was unavailable as a potentially usable feeding site. In this case, roughly 55% of the areole surface and surrounding minor veins

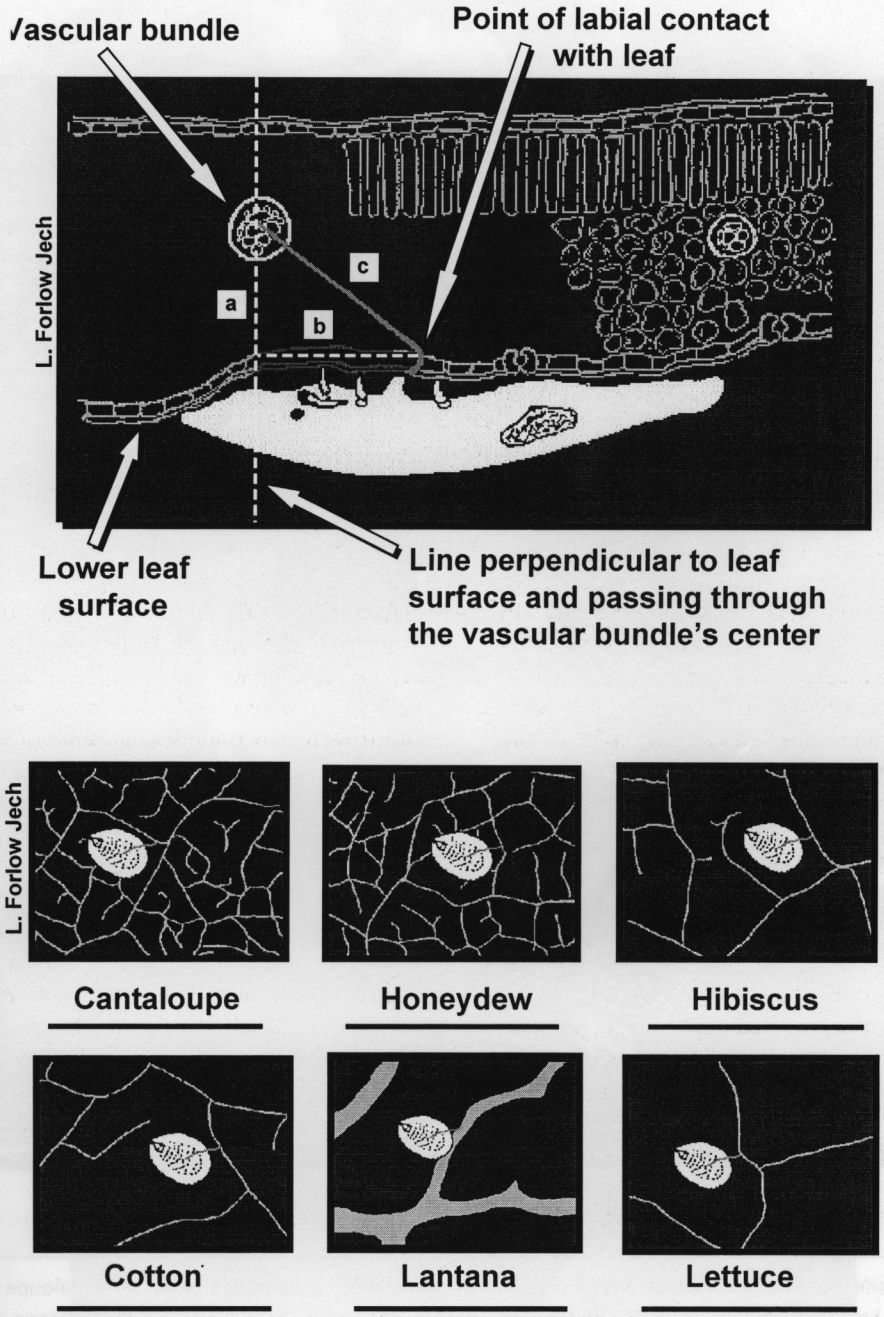

Fig. 5. A: Geometric model of whitefly nymphal feeding. The hypotenuse of the right triangle (c) represents the nymph's stylets; the distance represented by (a) is the depth of the sieve elements, and (b) is the distance from the point of labial contact with the plant surface to the intersection of line (a) with the plant surface. B: Vascular bundle (minor veins) distribution in 6 types of whitefly host plant. A crawler with its stylet bundle is superimposed to show spatial relationships.

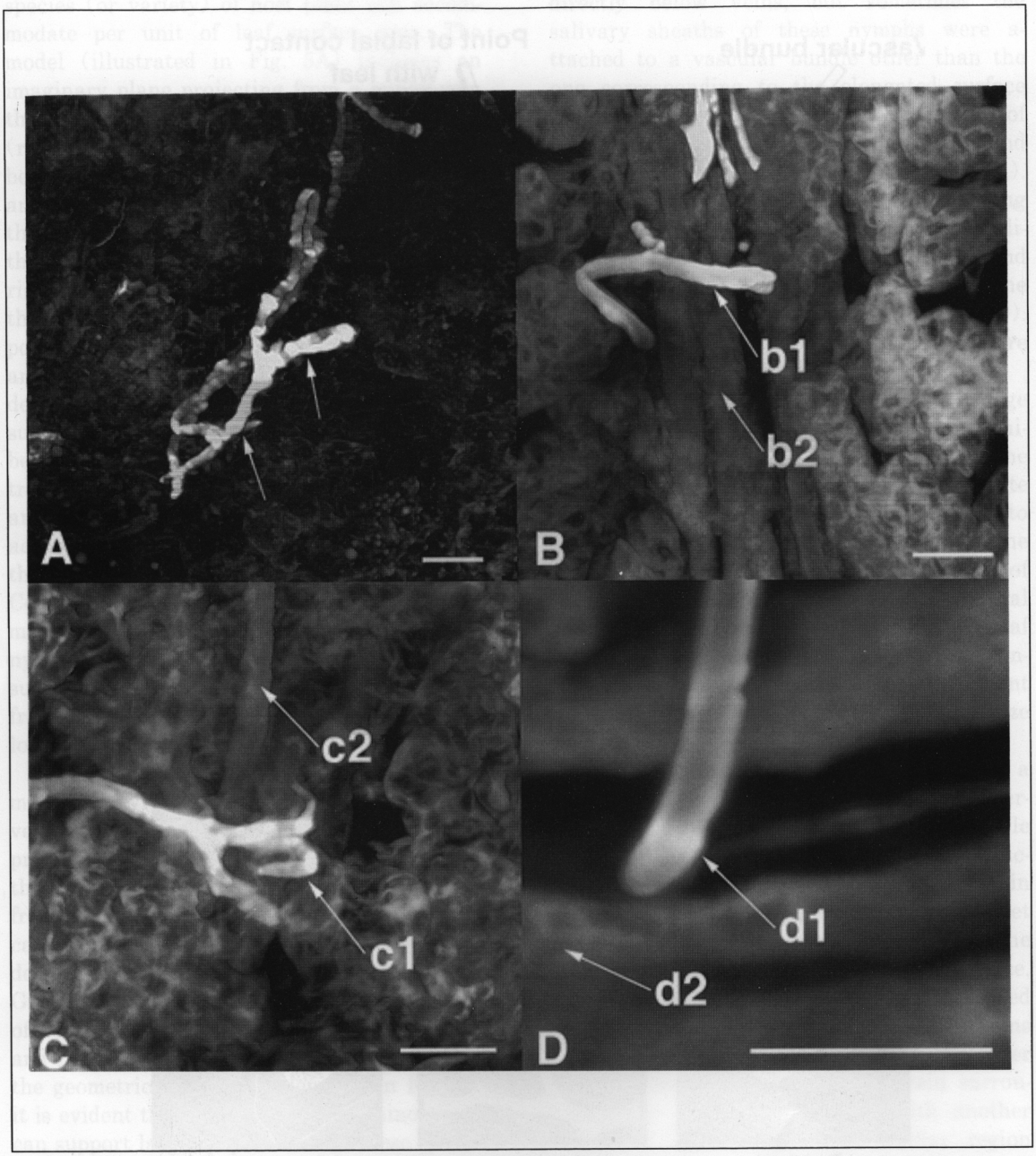

Fig. 6. Composite confocal images of whitefly stylets in association with minor veins of cantaloupe leaves. A: Highly branched salivary sheath showing the nature of "stylet wandering," a phenomenon where the whitefly nymph fails to make immediate contact with its targeted phloem element. B: a salivary sheath wrapped around a minor vein, with branch b1 evidently penetrating a phloem element. Arrow b2 points to a phloem element, and b3 points to a local mesophyll cell. C: a salivary sheath with multiple branches wrapping around and penetrating phloem elements. Arrow c2 depicts the phloem element being targeted. D: a close-up of a salivary sheath (d1) whose stylets are evidently penetrating the phloem element marked d2. Bar=5 μm.

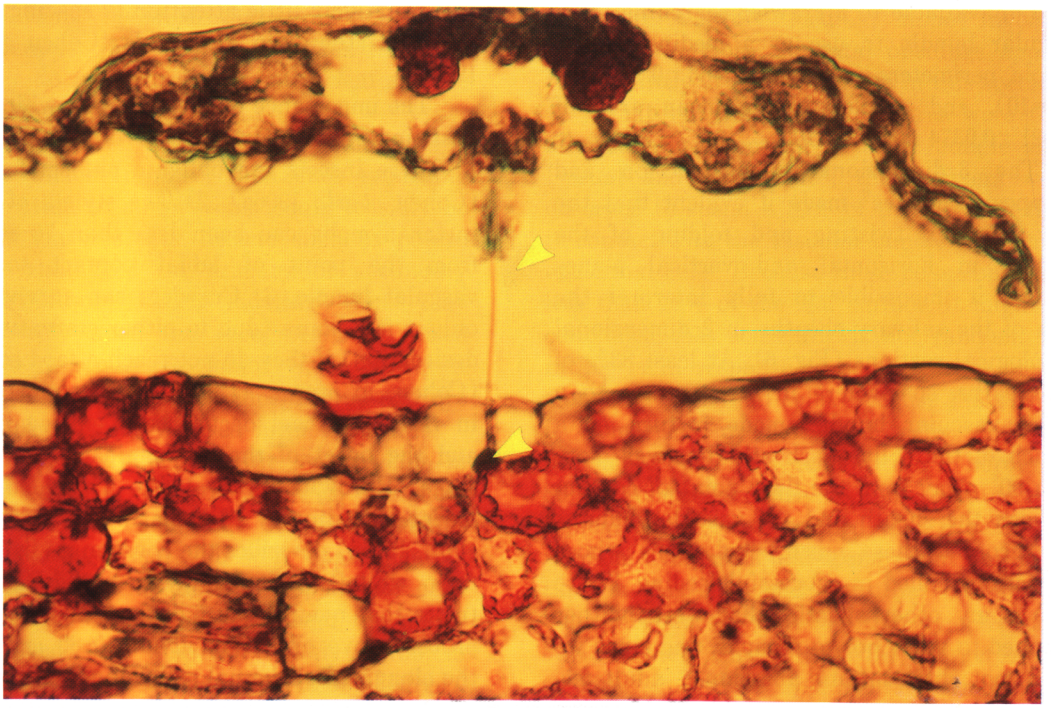

Fig. 7. A light micrograph of the stylets and sheath of a whitefly nymph penetrating an epidermal cell on their course to a minor vein. Bar=50 μm.

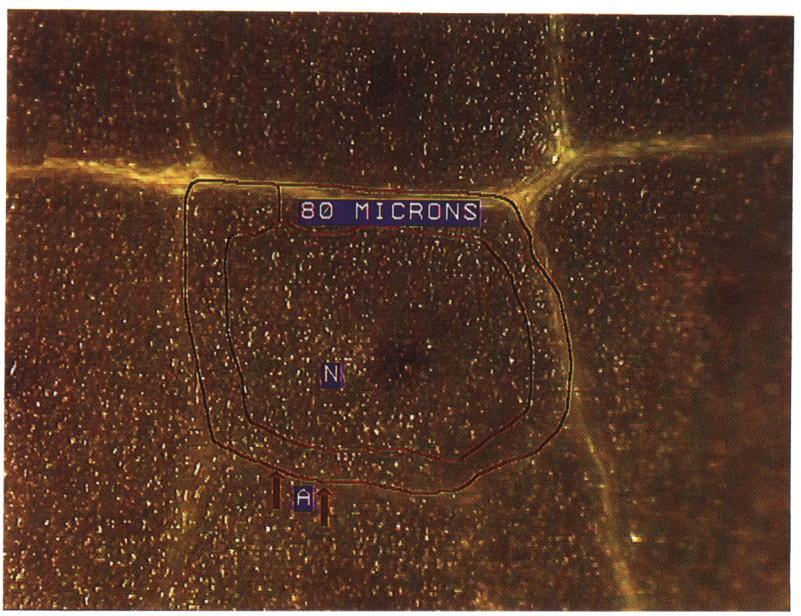

Fig. 8. Abaxial (under) surface of a DPL 90 cotton leaf showing minor veins. The areole is labeled "A," and a non-target area within the areole is labeled "N" (where stylets of crawlers cannot reach veins). The non-target zone is nearly 60% of the entire areole.

were unavailable to a crawler searching for a vascular bundle to use as a feeding target. Areole perimeters measured 2.463±0.111 mm (N=10), and areoles had a mean area of 0.3821±0.0374 mm (N=10).

The 3-dimensional reconstruction and "composite movies" made it evident that the very complex twisting and folding of the sheaths in horizontal and vertical planes renders it impossible to fully interpret the path of the salivary sheaths in 2-dimensional histological sections (Fig. 6). At least 83% of all sheaths, somewhere in their course to a target vascular bundle, made a turn or a branch of the salivary sheath that was more than 90° away from the final point of contact between the sheath and the vascular bundle. We recorded these misdirected turns whose corrections ended in successful feeding. There were many other sheaths that were neither attached to veins nor to nymphs, and since we could not differentiate abandoned adult-feeding sites from unsuccessful attempts by nymphs to locate a vascular bundle, we did not consider further sites that did not have attached nymphs. Through the use of confocal optical imaging of the feeding sites and of the associated plant tissue, detailed studies of the behavior of the salivary sheaths in relationship were possible.

Discussion

The selection of a host plant to be used by the developmental stages of aleyrodids is predetermined by the mother's ovipositional choices (Lenteren, van and Noldus, 1990; Byrne and Bellows, 1991). However, the crawler selects a specific feeding site on the leaf, apparently according to surface features that provide cues that target plant tissue is within reach of the stylets (Cohen *et al.*, 1996a & 1996b). For homopteran insects such as potato aphids that feed on larger veins, the prominence of leaf veins may be a cue to commence feeding (Gibson, 1972). While the location of feeding sites has been the subject of extensive investigation in aphids (e. g., Pollard, 1973 and Klingauf, 1987), there has been relatively little attention to specific site-selection stimuli in aleyrodids (Cohen *et al.*, 1996a).

The importance of the silverleaf whitefly's host finding cues is underscored by four facts. 1) These insects are obligate phloem feeders (Cohen *et al.*, 1996a). 2) The stylets of first instar nymphs can span less than 70-80 μm from the point of labial contact to the vascular bundle. 3) Crawlers can survive for only a few hours (due to either starvation or desiccation) if they do not reach a food source (Cohen, in prep.). 4) The greatest mortality, in *Bemisia* tabaci occurs between egg hatch and the onset of the second instar nymphal stage (Horowitz *et al.*, 1984). The geometric model of feeding by silverleaf whitefly (Cohen *et al.*, 1996a) describes the constraints imposed upon whitefly nymphal feeding and demonstrates that nymphs must initiate their feeding probes above or within about 70-80 μm from the elongated epidermal cells found on the leaf surface. These constraints are apparent in Figure 8, which represents a typical areole. The area of this areole is about 450,000 μm^2. Of this total area, less than 250,000 μm^2 is within the span of the stylets of a 1st instar nymph. Probes by a crawler in the remaining area would fail to terminate in successful contact with the minor vein.

This model shows clearly that a crawler's chances of reaching a minor vein by probing randomly would be less than 50%. If it missed a vein in the first probe, its chances of missing again in the second would also be a little more than 50% and so on in subsequent probes. Therefore, we would expect that about 50% of all crawlers would miss once; 25% would miss twice; 12.5% would miss three times, etc. Without knowing how many misses result in the death of a crawler, we cannot predict the degree of accuracy that a crawler must exhibit. However, considering the time and material investment involved in finding a suitable minor vein, a random search seems non-adaptive. Crawlers can move over the leaf surface at a rate of 2300 μm / min., indicating that the full width or length of a cotton leaf could be traversed in 2 hours. However, our

observations revealed that crawlers spend considerable time turning and back-tracking, so they do not search the entire leaf surface area before they settle.

If the search for a suitable feeding site is a non-random process, then cues regarding the location of minor veins could feasibly be surface features. For insects such as potato aphids that feed on primary or secondary veins (Gibson, 1972), locating veins may simply rely upon the recognition of raised sites corresponding to major veins. However, minor veins are far less conspicuous and are not characterized by raised surface features.

Here, we found that the minor veins in cotton leaves were associated with elongated epidermal cells and that vascular bundle-associated epidermal cells were approximately 80 x 20 μm, while the other epidermal cells were about 35 x 35 μm. Although the minor veins themselves were not readily visible in fresh leaves (ones that were not cleared), these veins were evident under about 200x magnification where they were seen to be darker green than areole regions. Under SEM, the vascular bundle-associated epidermal cells were apparent because of the elongated appearance of these cells compared to the isodiametric appearance of the other epidermal cells. Although it was not apparent from cross sections of leaves, SEM observations reveal slight depressions or ruts in the surface epidermal cells that are associated with minor bundles. While it is possible that this is an artifact resulting from differential drying, it may represent a natural contour of the surface of cotton leaves. Another characteristic feature that differentiates vascular bundle-associated epidermal cells from non-vascular associated epidermis is that trichomes were present only in the former. One other difference between the epidermal cell types (vascular-associated and non-vascular-associated cells) is that stomata only occurred in non-vascular-associated epidermal cells. There were about twice as many stomata on the abaxial surface than there were on the adaxial side of the leaf. However, out of several hundred stylet penetrations observed silverleaf whitefly feeding, none involved stomata; therefore, stomatal openings appear to be of little or no importance in this species.

Whatever the surface features were that allowed recognition of vascular bundle sites (visual and / or tactile), the crawlers were observed to spend a 80% of their search time in contact with the vascular bundle-associated epidermal cells. Because these cells constitute less than 25% of the leaf surface, it might be concluded that searching is not random, but rather is in response to some feature of the elongated epidermal cells. Because the surface is comprised of elongated cells and the proximate epidermal cells within a border of 80 μm comprises nearly 50% of the area of a typical areole, we reasoned that if search is random, nymphs should spend about half their time in this zone and the other half in the "non-target zone." The fact that nymphs spent 80% of their time within the "target zone" suggests that there is a contact stimulus to remain near bundles; and this pre-disposes the nymphs to begin their probes close to sites where successful feeding is most likely.

One of the most intriguing aspects of these studies is the internal relationship between the salivary sheaths and the plant cells. It is important to remember that the sheaths represent an exact history of the movement of the stylets. The sheaths provide a record of all of the places within the leaf that the whitefly explored as potential feeding sites. We found that the sheaths were present almost exclusively in the air spaces in the spongy mesophyll.

This study has shown that in hairy cotton leaves such as DPL 115 (hairy isoline), there is an obvious preference for oviposition sites near the lamina trichomes. While it is not apparent from this study what the advantages are in placing eggs close to trichomes, it is possible that the proximity of the trichomes to veins might favor water uptake by the eggs, which have been shown to derive much of their water from plant cells (Byrne *et al.*, 1990). The details and mechanisms of ovipositional site selection by silverleaf whitefly warrants further attention, especially in rela-

tionship to trichomes and other surface features.

We feel that it is important to understand this surface / feeding relationship in order to develop strategies alternative to pesticide treatments to control whiteflies. Several studies have shown that whiteflies have definite preferences for certain kinds of host plants, in part, in relation to certain plant physical features (Chu *et al.*, 1995; Cohen *et al.*, 1996a and b). They also show definite preferences for different varieties of host plants within a given species (Natwick *et al.*, 1995; Rao *et al.*, 1990; Puri *et al.*, 1993; Wilson *et al.*, 1993). While the reality of these differences in feeding choice are well-documented, the basis is still poorly understood. If plant breeders or genetic engineers knew which surface features might confuse the normal stimulus-response pattern of crawlers, they could use this as a first line of defense in developing a management system aimed at thwarting the impressive efficiency of colonization and host plant utilization of this important pest.

Acknowledgments

We express our appreciation for the advice on the English-Chinese translation of the Summary provided by Drs. T. X. Liu and Yuan-an Wei. Lynn Forlow Jech prepared Figure 5. We also thank Drs. J.K. Brown, D.N. Byrne, H.M. Flint, and two annonymous reviewers for their suggestions on an earlier version of this paper.

References

Backus, E. A., W. B. Hunter, and C. N. Arne. 1988. Technique for staining leafhopper (Homoptera: Cicadellidae) salivary sheaths and eggs within unsectioned plant tissue. J. Econ. Entomol. 81: 1819-1823.

Bellows, T. S., T. M. Perring R. J. Gill, and D. H. Headrick. 1994. Description of a species of *Bemisia* (Homoptera: Aleyrodidae). Ann. Entomol.Soc. Ameri. 87: 195-206.

Berlyn, G. P., and J. P. Miksche. 1976. Botanical microtechnique and cytochemistry. Iowa State University Press, Ames, Iowa.

Brown, J. K., D. R. Frolich, and R. C. Rosell. 1995. The sweetpotato or silverleaf whiteflies: Biotypes of *Bemisia tabaci* or a species complex ? Annu. Rev. Entomol. 40: 511-534.

Byrne, D. N., A. C. Cohen, and E. A. Draeger. 1990. Water uptake from plant tissue by the egg pedicel of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae). Can. J. Zool. 68: 1193-1195.

Byrne, D. N., and T. S. Bellows. 1991. Whitefly biology. Annu. Rev. of Entomol. 36: 431-457.

Chu, C. C., T. J. Henneberry, and A. C. Cohen. 1995. Silverleaf whitefly (Homoptera: Aleyrodidae): Host preference and factors affecting oviposition and feeding site preference. Environ. Entomol. 24: 354 -360.

Cohen, A. C., C. M. Newman, and D. L. Hendrix. 1993. Histochemical studies of feeding sites of *Bemisia tabaci* on cotton leaves, . In: D. J. Herber & D. A. Richter (Eds.). Proceedings, Beltwide Cotton Conferences, 9-16 February, National Cotton Council of America, Memphis, TN. p. 959.

Cohen, A. C., and D. L. Hendrix. 1994. Demonstration and preliminary characterization of a-amylase in the sweetpotato whitefly, *Bemisia tabaci* (Aleyrodidae: Homoptera). Comp. Biochem. & Physiol. 109B, 593-601.

Cohen, A. C., T. J. Henneberry, and C. C. Chu. 1996a. Geometric relationships between whitefly feeding behavior and vascular bundle arrangements. Entomol. 78: 135-142.

Cohen, A. C., C. C. Chu, T. J. Henneberry, T. Freeman, J. Buckner, and D. Nelson. 1996b. Cotton leaf surface features serve as behavioral cues to silverleaf whiteflies. Southwest. Entomol. 21:377-385.

Gibson, R. W. 1972. The distribution of

aphids on potato leaves in relation to vein size. Entomol. Exp. Appl. 15: 213–223.

Horowitz, A. R., H. Podoler, and D. Gerling. 1984. Life table analysis for the tobacco whitefly *Bemisia tabaci* (Gennadius) in cotton fields in Israel. Ecol. Applic. 5: 221–233.

Inayatullah, C. M. A. Ghani, and A. Ghaffer. 1985. Cotton whitefly, (Genn.) and its control. Lahore, Punjab, Directorate of Agricultural *Bemisia tabaci* (Information.) 90 pp.

Klingauf, F. A. 1987. Host plant finding and acceptance. In: Aphids Their Biology, Natural Enemies and Control. Vol. A. (Eds.) A. K. Minks and P. Harrewijn. Elsevier, Amsterdam. pp. 209–223.

Lenteren, J. C. van, and L. P. J. J. Noldus. 1990. Whitefly–plant relationships: behavioral and ecological aspects. In: Whiteflies: their Bionomics, Pest Status and Management. (Ed.) D. Gerling. Intercept, Andover, UK. pp. 47–89.

Mauseth, J. D. 1988. Plant Anatomy. The Benjamin / Cummings Publishing Company, Inc. Menlo Park, CA. 560 pp.

Natwick, E. T., C. C. Chu, H. H. Perkins, T. J. Henneberry, and A. C. Cohen. 1995. Pima and upland cotton susceptibility to *Bemisia argentifolii* under desert conditions. Southwest. Entomol. 20: 429–438.

Pollard, D. G. 1955. Feeding habits of the cotton whitefly *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae). Ann. Appl. Biol. 43: 664–671.

Pollard, D. G. 1973. Plant penetration by feeding aphids (Hemiptera, Aphidoidea): a review. Bull. Entomol. Res. 62: 631–714.

Puri, S. N. A. S. Ansingkar, V. N. Ajankar, R. C. Lavekar, G. D. Butler, Jr., and T. J. Henneberry. 1993. Effect of cotton leaf morphology on incidence of *Bemisia tabaci* (Genn.) on cotton. J. Appl. Zool. Res. 4: 41–44.

Rao, N. Venugopal, A. S. Reddy, R. Ankaiah, Y. N. Rao, and S. M. Khasim. 1990. Incidence of whitefly (*Bemisia tabaci*) in relation to leaf characters of upland cotton. (*Gossypium hirsutum*). Ind. J. Agri. Sci. 60: 619–624.

SAS Institute. 1988. SAS user's guide, basics. SAS Institute, Cary, NC.

Walker, G. 1985. Stylet penetration by the bayberry whitefly, as affected by leaf age in lemon, *Citrus limon*. Entomol. Exp. Appl. 39: 115:121.

Weathersbee, A. A., III, D. D. Hardee, and W. R. Meredith, Jr. 1995. Differences in yield responses to cotton aphids (Homoptera: Aphididae) between smooth-leaf and hairy-leaf isogene cotton lines. J. Econ. Entomol. 88: 749–754.

Wilson, F. D., H. M. Flint, B. R. Stapp, and N. J. Parks. 1993. Evaluation of cultivars, germplasm lines, and species of Gossypium for resistance to Biotype "B" of sweetpotato whitefly (Homoptera: Aleyrodidae). J. Environ. Entomol. 86: 1857–1862.

Received for publication December 24, 1997
Revised manuscript accepted April 7, 1998

銀葉粉蝨（同翅目：粉蝨科）的取食生物學

Allen Carson Cohen* **朱昌祺** **Thomas J. Henneberry** USDA-ARS, Western Cotton Research Laboratory, Phoenix, AZ 85040, USA

Thomas Freeman Electron Microscopy Center, North Dakota State University, Fargo, ND 58105, USA

Dennis Nelson and James Buckner USDA-ARS, Biosciences Research Laboratory, State University Station, Fargo, ND 58105, USA

Dennis Margosan, Patrick Vail and Louis H. Aung USDA-ARS, Postharvest Quality & Genetics Research Laboratory, Fresno, CA 93727, USA

摘　要

銀葉粉蝨（*Bemisia argentifolii* Bellows & Perring）具有刺吸式口器，其取食習性複雜。粉蝨必需在寄主植物葉片背面尋找適宜部位，使其口針容易穿刺到葉片內的細小葉脈以便取食。因為銀葉粉蝨若蟲的生存需要把口針穿刺到最細小的葉脈，它們必定採用一系列的靈巧動作才能達到目的。我們用光學顯微鏡、電子顯微鏡和影像顯微鏡觀測發現，粉蝨取食都是穿刺到木質部不超過三根導管的小葉脈。第一齡爬行若蟲總是以差別明顯的表皮結構（如葉片絨毛和細長形的上表皮細胞）為線索，在葉片上尋找適宜場所開始進行穿刺。這些若蟲用其特殊的唾液形成一袖狀結構，稱為唾液鞘（salivary sheath），用來引導口針找到小葉脈。唾液鞘常彎曲分枝，而分枝的部位發生在葉肉和維管束內。唾液鞘在葉片內位於細胞外面，海綿狀薄壁細胞之間的空間，只有很小部分的唾液鞘位於外表皮細胞內。我們還沒有發現口針或唾液鞘深入到薄壁細胞或柵欄細胞中。應用切片技術，我們在維管束外的組織中發現過一些從植物表面到取食點的取食痕跡。然而，當我們用整葉染色透明技術使唾液鞘清晰可辨時發現，凡是發育超過第一齡的若蟲，其口針都是通過唾液鞘與維管束相連。唾液鞘可長達140μm，最寬處的直徑約2μm，看起來像一連串合併的空泡，有的黏附在細胞壁上，經過鄰近的細胞表面，一直延伸到維管束。沒有達到維管束的唾液鞘，其末端總是封閉的。我們的研究證明，形成完整的唾液鞘是取食成功的關鍵。銀葉粉蝨對不同寄主植物種類的喜好，往往取決於維管束的數量。銀葉粉蝨喜好甜瓜和其它葫蘆科寄主而不喜好萵苣就是一個證明。

關鍵詞：銀葉粉蝨、維管束、取食行為、唾液鞘。

*抽印本索取及論文聯繫之負責人