COTTON LEAF SURFACE FEATURES SERVE AS BEHAVIORAL CUES TO THE SILVERLEAF WHITEFLY

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Abstract

We examined cotton leaves looking for correlations between surface structures and veins, using light and electron microscopy. Using placement of eggs, nymphal positions and crawler (1st instar nymph) behavior, we evaluated the responses of whitefly nymphs to the surface features. We found that all aerial trichomes originated from epidermal cells immediately lower or upper to veins, including a hairy cotton isoline DPL 115 containing 48 (+/- 2.5) trichomes/ cm^2. Leaf surface microstructures such as elongated epidermal cells were always evident wherever veins were present, including even the most minute bundles (single-stranded). Of 2000 aerial trichomes (non-glandular) that were examined, 100% originated from vein-associated epidermal cells. Eggs were generally deposited on the elongated epidermal cells associated with bundles or on cells within about 30 microns of those bundle-associated epidermal cells. Crawlers walked about 2300 microns per minute until they settled upon feeding sites that were immediately under the veins, never more than about 60 microns from the center of the lower bundle-associated epidermal cells. Crawlers spent at least 80% of their time in contact with bundle-associated epidermal cells, making contact with these cells either with legs or antennae.

Introduction

The silverleaf whitefly, *Bemisia argentifolii* (Bellows and Perring), is a devastating pest whose feeding range includes a large variety of host plants (Byrne and Bellows, 1991). The capacity of *B. argentifolii* to utilize its hosts to build huge populations depends upon the ability of individuals to find minor veins in host leaves (Cohen, et al., 1996). Cohen et al. (1996) reported that all successfully growing immatures were connected via salivary sheaths to minor veins in cantaloupe, cotton, lantana, hibiscus and lettuce leaves. Earlier studies by Pollard (1955) and by Cohen et al. (1993) indicated that some immatures of *B tabaci* and *B. argentifolii* were not connected via salivary sheaths to veins. These studies, undertaken with leaf sectioning techniques, proved to be less reliable than intact leaf staining and clearing methods (Cohen, et al., 1996).

It is now evident that successful development by *B. argentifolii* demands the ability of newly emerged 1st instars, known as crawlers, to find minor veins which occupy less than 10% of the volume of the leaf (Chu et al., 1995; Cohen et al., 1996). There is also a time window of about 3-5 h, after which crawlers will die if they do not succeed in reaching a feeding site (Cohen, pers. obs.). Furthermore, factors of host plant structure, including depth, frequency and size of minor veins impose stringent constraints upon the ability of crawlers to locate appropriate feeding sites.

One surface feature that has received extensive attention relative to insect preferentia is the presence or absence of hairs or non-glandular trichomes. Several reports indicate preferences that many homopterous insects show for hairy leaves (Wilson, et al., 1994; Butler & Wilson, 1984; Weathersbee et al., 1995). Generally, these reports explain the basis of the preference as added protection from either desiccation or from entomophages.

We report here the results of studies on the relationship between surface features of cotton (*Gossipium hirsutum* L.) leaves and minor veins. Within this context, we further report on behavioral observations that appear to be correlated with location of veins, including oviposition, nymphal settling sites and locomotory activities of crawlers.

Materials and Methods

Processing of Cotton Plants and Light Microscopy

We used cotton leaves from each of the following varieties: Delta Pine 90 (DPL 90) and DPL 115. We prepared intact leaf disks (1.0 cm dia.) by staining them with McBride's acid fuschin (1% in 90:10 ethanol glacial acetic acid) for 4 h, destaining in 95% ethanol for 2 min, then clearing by auto-claving in lactic acid, glycerol, water (1:1:1) at 121° C, 15 psi for 25 min. Disks were mounted under cover slips in fresh clearing agent. Stained, cleared disks were examined under light microscopy with a Wild 420 Macro-scope with a video imaging system and with a compound microscope with a camera. This examination included measurement of the positioning of eggs and nymphs in relation to veins, trichomes and cell surface features, including surface morphology of epidermal cells.

Electron Microscopy

Leaves were desiccated in a critical point drier, sputter-coated with gold and viewed with a JEOL scanning electron microscope at 5 kV (100x and 500x). Electron micrographs were used to make measurements of the epidermal cells and for assessment of epidermal surface contour.

Whitefly Behavior

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 labor-atory at 25° C under fiber optics light. Distances traveled over a 2 min inter-val and pattern of movement were recorded in relation to position of vein-related surface cells. Positions of eggs were recorded in relation to vein-related surface cells, stomata and trichomes. Measurements were made to determine what percentage of total leaf surface area was within a 300 µm radius of trichomes. Distances were measured between egg pedicels and trichomes using the video imaging system calibrated with a stage micrometer. These measurements wee evaluated with χ^2 tests and ANOVA (SAS Institute, 1988).

Results

Leaf Surface Structure
Leaf trichomes (non-glandular or aerial trichomes) all originated from veins-associated epidermal cells. This was evident from examination by light and electron microscopy. Epidermal cells located below areolar regions are about 35 x 35 µm, and epidermal cells that are below veins are about 80 x 22 µm. Using placement of eggs, nymphal positions and crawler (1st instar nymph) behavior, we evaluated the responses of whitefly nymphs to the surface features. All aerial trichomes originated from epidermal cells immediately lower or upper to veins. The hairy cotton isoline DPL 115 contained 48 (+/- 2.5) trichomes/ cm². Elongated epidermal cells were evident wherever veins were present, including even the most finest, single-stranded veins. Of 2000 aerial trichomes, 100% originated from vein-associated epidermal cells. Eggs were usually deposited on the elongated epidermal cells associated with bundles or on cells within 30 µm of those bundle-associated epidermal cells.

Stomata on the upper surface were about half as numerous as those on the lower side of leaves (5.8 ±0.37 versus 11.9 ± 0.59, mean ± S.E., respectively; N=15). Width of bundles of vein-associated epidermal cells was 35.6 µm (± 0.86 S.E.; N=20), and mean distance across areola or between closest minor veins was 531 µm ± 28.3 µm, S.E.; N=100).

A typical areola has a perimeter of about 2000 µm, and the area within about 450,000 µm². If a smaller area whose perimeter is about 80 µm from the minor vein surrounding the areola is demarked with another line, the area within this smaller region represents the surface that is out of reach to a probing crawler. Since the crawler cannot reach beyond 70-80 µm from the site of labial contact with the plant's surface, the 80 µm boundary conserva-tively estimates the limitations on crawler feeding potential. The defined area of about 250,000 µm² is the surface within that areola that is unavailable as a potentially usable feeding site. In this case, there is roughly 55% of the areola surface and surrounding minor veins that are unavailable to a crawler foraging for a vein to use as a feeding target.

Crawler Behavior
Crawlers walked about 2300 microns per minute (± 283 µm) until they settled upon feeding sites that were within about 60 microns from the center of the lower bundle-associated epidermal cells. Crawlers spent about 80% of their time in contact with the elongated bundle-associated epidermal cells, making contact with these cells either with legs or antennae.

Discussion

The selection of a host plant to be used by the developmental stages of aleyrodids is pre-determined 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., 1996). For insects such as potato aphids that feed on larger veins, the prominence of leaf veins is a likely cue for commencement of feeding activity (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 the specific site-selection stimuli in aleyrodids.

The importance of *B. argentifolii*'s finding cues is underscored by these facts: 1) these insects are obligate phloem feeders (Cohen et al., 1996); 2) the reach of the stylets of this species is limited to less than 70 µm from the point of labial contact to the vein; 3) crawlers can live for only a few hours before they either starve or desiccate if they do not reach a food source (Cohen, pers. obs.). The geometric model of feeding by *B. argentifolii* (Cohen et al., 1996) described the constraints of the feeding relationship. This includes the fact that whitefly nymphs must start their feeding probes on the vein-associated epidermal cells or within about 70-80µm from these elongated epidermal cells. These constraints are apparent in Figure 3 which shows that in an areola (a region of mesophyll cells surrounded by vein) of 450,000 µm², there is an area of about 250,000 µm² where initiation of a feeding probe could not possibly terminate in successful contact with the minor vein.

Using this model of feeding zones, including the target zone that includes the vein-associated epidermis and an area of about 80 µm around these bundles, and a non-target zone that includes the remaining epidermis where a probe will never result in contact with a minor vein, we have a clear picture of the decision process faced by a crawler. Based on the area of these two surface, if we assumed that the crawler made random probes on the lower surface, it would stand a less than 50% chance of reaching a target vein. Under these assumptions, if it missed the 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 craw-lers would miss once; 25% would miss twice; 12.5% would miss three times, etc. Without knowing the limits of how many misses are possible before the crawler dies, it is impossible to predict how much accuracy is needed. However, considering the time and material investment involved in finding a suitable minor vein, it is probable that a random search would be highly non-adaptive.

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

We found that the minor veins in cotton leaves are associated with elongated epidermal cells. Vein-associated epidermal cells were approximately 80 x 25 μm, while the other epidermal cells were about 35 x 35 μm. Although the minor veins themselves are not visible in fresh leaves (ones that were not cleared), these veins are evident under about 200 x magnification where they are seen to be darker green than areolar regions. Under SEM, the vein-associated epidermal cells are apparent because of the elongated appearance of these cells compared to the more-or-less square appearance of the other epidermal cells. Although it is 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. Another characteristic feature that differentiates vein-associated epidermal cells from non-vascular associ-ated epidermis is that trichomes were present only in the former. One other difference between the epidermal cells is that stomata only occurred in non-vascular-associated epidermal cells. There were about twice as many stomata on the lower surface than there were on the upper side of the leaf. However, in other studies (in preparation), we found only 2 out of several hundred stylet penetrations involved stomata; so we feel that stomatal openings are of little importance in the feeding biology of *B. argentifolii*.

Whatever the surface features were that allowed recognition of vein sites (visual or tactile), the crawlers were observed to spend a disproportionate amount of time (80%) in contact with the vein-associated epidermal cells. We found that newly-hatched nymphs crawled along the elongated surface cells with what appeared to be legs, antennae or lateral cuticular surface in contact with these surface cells. We reasoned that since the elongated cells and the proximate aerolar zone cells comprise about 45% of the area of a typical areola, nymphs should spend about half their time in this zone and the other half in the non-target zone. The fact that they spend 80% of their time in contact with the bundles 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 discovery of minor veins is most likely.

We feel that it is important to understand this surface/feeding relationship in order to develop alternative strategies to pesticide treatments to control whiteflies. There are strong evidences that whiteflies have different prefer-ences for different kinds of host plants (Chu, et al., 1995; Cohen et al., 1996) and for different varieties of host plants (Natwick et al., 1996; Rao et al., 1990; Puri et al., 1993; Wilson et al., 1993). While the reality of these differ-ences in feeding choice are well-documented, the basis is poorly understood. We feel that surface features could be the 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.

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