PRECIBARIAL AND CIBARIAL CHEMOSENSILLA IN THE WHITEFLY, *BEMISIA TABACI* (GENNADIUS) (HOMOPTERA: ALEYRODIDAE)

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Abstract—The internal anatomy of the anterior alimentary canal of the whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) B-biotype, was examined by light, scanning, and transmission electron microscopy to elucidate the location and number of precibarial and cibarial gustatory sensilla. Elucidation of the epipharyngeal organ complex within the precibarium revealed 10 precibarial sensilla located proximal to where the paired maxillary stylets diverge on their retraction. The sensory organ complex within the cibarium consists of 8 sensilla, 6 on the epipharyngeal sclerite with 2 found within the hypopharyngeal sclerite. Fine structure investigation revealed the individual neurons to terminate at sensillar pores, which allow direct contact with passing fluids, thus supporting a chemosensory function. Ultrastructure of the neurons is similar to that of precibarial and cibarial gustatory chemosensilla found in other piercing-sucking insects. Their importance to whitefly feeding is discussed. Copyright © 1996 Elsevier Science Ltd

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INTRODUCTION

Transmission of plant pathogens by insects is intimately associated with feeding. Examination of insect anatomy, feeding behaviors, and host plant selection, elucidates the components involved, their functions, and provides clues for the development of novel management strategies. The whitefly, *Bemisia tabaci* (Gennadius), B-biotype, (syn. *B. argentifolii*, Perring et al., 1993) feeds by first piercing plant cells and sucking up their contents, and finally by inserting stylets into the phloem of plants ingesting the sap (Pollard, 1955; Janssen et al., 1989). During feeding, whiteflies may cause mechanical damage to plant tissues, extract plant nutrients, and inject phytotoxic saliva (Segarra Carmona et al., 1990; Yokomi et al., 1990; Costa et al., 1993). Furthermore, they excrete abundant amounts of honeydew which leads to problems with sooty mold (Mound and Halsey, 1978; Perring et al., 1993). The most severe problems, however, arise from the transmission of geminiviruses (Mound, 1973; Brown et al., 1995).

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Information on the internal gustatory organs of whiteflies is sparse. However, other investigations which have provided relevant information into understanding whitefly anatomy and feeding mechanisms of the adult, have been described by Weber (1935) for *Trialeurodes vaporariorum* (Westwood) and for 4th instars of *Dialeurodes eugeniae* (Maskell), the jamun whitefly, by Singh (1949), with thorough general descriptions of homopteran morphology by Snodgrass (1935), with a recent description of the stylets of *Bemisia tabaci* by Rosell et al. (1995). Also, excellent examples of external morphology can be found in Gill (1990), in English, and in Weber (1935), in German.

Like leafhoppers and thrips, whiteflies have external sensory structures on the tip of their labium, which may have dual contact chemosensory and mechanosensory functions, thus aiding in host plant selection (Walker and Gordh, 1989; Van Lenteren and Noldus, 1990). Within the Aleyrodidae, evidence from light microscopy suggested the presence of an epipharyngeal sensory organ (Weber, 1935) within the region now referred to as the precibarium (Backus, 1985), but did not elucidate the structure, or number of sensilla comprising this organ complex nor the sensory organ within the cibarium. Because the precibarial and cibarial sensory organs are likely to govern the selection of specific tissues for feeding (Backus and McLean, 1983), an understanding of them would improve our understanding about whitefly host plant selection and feeding behavior.

Internal gustatory chemosensilla have been described in insects from the Homoptera (Wensler and Filshie, 1969; Backus, 1985; Ullman and McLean, 1986), Heteroptera (Miles, 1958), Diptera (Rice, 1973; Lee and Davies, 1978; Colbo et al., 1979), Lepidoptera (DeBoer et al., 1977), and Thysanoptera (Hunter and Ullman, 1994) and may occur in all insects (Moulins, 1971). The position of these sensilla is such that their dendrites directly contact food passing through the anterior gustatory canal before entering the esophagus. The purpose of this study is thus to describe the location and number of the sensilla comprising these 2 sensory organ complexes found in the whitefly, *Bemisia tabaci*, to compare these sensilla with those found in other piercing–sucking insects, and to propose the role these sensory organs may play in host plant selection.

**MATERIALS AND METHODS**

All whiteflies examined were adults from laboratory colonies of *Bemisia tabaci*, B-biotype, maintained at the Central Florida REC in Leesburg. Confirmations of species and biotype were performed by Tom Perring, University of California, Davis. Insects were reared in cages, on collard, *Brassica oleracea* L., var. *acephala*, "Georgia Southern", at 23–27°C, with photo period of 16 L: 8 D h.

**Preparation of whiteflies for scanning electron microscopy**

Whiteflies were dehydrated in an ethanol series, 50, 70, 80, 95, and 3 changes in 100% ethanol. Specimens were then critical-point dried (BALZERS, Leichtenstein, Germany) and mounted on electron microscopy stubs with either silver conductive paint (FULLAM Inc., Latham, NY, U.S.A.), as described in Hunter and Ullman (1994), or with double sticky mounts (AVERY DENNISON, Spot-o-glue, Schaumburg, IL, U.S.A.). They were then either dissected with fine tip forceps and/or cut first with a new, Teflon® coated razor blade, then dissected. Following dissection, specimens were sputter-coated for 4 min and examined with a Hitachi S-800 Field Emission Scanning Electron Microscope (Hitachi Ltd., Japan). Both sexes were examined for a total of 96 whiteflies.

**Preparation of whiteflies for transmission electron microscopy**

Whole whiteflies, which had a cut made in their thoraces, were fixed for 8 h on a rotator in a 2% glutaraldehyde, 2% paraformaldehyde solution (in 0.1M phosphate buffer, pH 7.4). Fixation was followed by three 15-min washes in buffer with postfixation overnight in osmium tetroxide (in phosphate buffer) on a rotator. Specimens were then washed 3 times for 15 min each, dehydrated in an ethanol series (50, 70, 80, 95%) over a 3-h period and embedded through transitional stages into LR-White resin (30%, 50%, 100% resin) mixed with 95% ethanol. Embedded whiteflies were examined in thin-sections and thick-sections, sectioned with an ultramicrotome (Sorvall®, M12-
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B, DuPont Co., U.S.A.) in cross, transverse, and sagittal orientations. Thin-sections were picked up on formvar-coated nickel grids, 50 mesh, and stained with uranyl acetate (2% aqueous, 20 min) and lead citrate (6 min). Twenty whiteflies were examined in this manner (Hitachi H-600 Electron microscope, Japan). Thick-sections, 1–3 μm were picked up with a wire loop and placed on a glass slide. The slide was placed on a hot plate and left for 30 sec after the water had evaporated. Then the section was stained with Azure II for 10–15 sec, rinsed with water and then counterstained with basic fuchsin for 30–40 sec, again rinsed, let dry, and mounted with Permount*.

RESULTS

Two views of the whitefly head, one external and one internal, are provided for orientation (Figs. 1 and 2). Whiteflies have piercing–sucking mouth parts, with a stylet bundle consisting of paired mandibular and maxillary stylets (Fig. 3). The whitefly cibarium (Cib) (Fig. 2) consists of an upper and lower sclerite. The lower, hypopharyngeal sclerite (Hyp) forming the bottom, is slightly concave, and is more rigid than the flexible epipharyngeal sclerite (Epi) that forms the top of the cibarium. As the cibarial dilator muscles (Cdm), which arise on the clypeus (Clp), contract, the epipharyngeal sclerite is drawn upward, thereby creating a vacuum and drawing fluids up through the food canal (Fc) within the maxillary stylets (Mx) (Fig. 4), through the precibarium (PrC), past the precibarial valve (Pv) and into the

Fig. 1. Scanning electron micrograph (SEM) of the whitefly, Bemisia tabaci, showing the compound eye (C), clypeus (Clp), labium (Lbi), labrum (Lbr), legs (L), stylet bundle (St).

Fig. 2. Parasagittal thick-section of the whitefly, B. tabaci, showing the accessory salivary gland (Ag), clypeus (Clp), crumen (Cr), cibarium (Cib), cibarial sensilla (Cs), cibarium dilator muscles (Cdm), esophagus (Es), labium (Lbi), labrium (Lbr), leg (L), pharynx (Ph), pronotum (Pn), precibarial sensilla (Ps), precibarial valve (Pv), precibarial valve dilator muscles (Pvm), salivary duct (sd), salivary pump (sp), salivary pump muscles (spm), Stylets (st), subesophageal ganglion (SbG), supraesophageal ganglion (SpG), thoracic ganglion (TG).
lumen of the cibarium (Cib). When the precibarial valve dilator muscles (Pvm) relax, the valve is closed. Then the cibarial dilator muscles (Cdm) relax to force the food out of the cibarium (Cib) up into the pharynx (Ph) (Figs. 2 and 5).

Sensilla within the precibarium [precibarial sensilla-(Ps)] are located in the epipharyngeal sclerite (Epi) where the paired maxillary stylets (Mx) diverge (Fig. 6). The 10 sensilla each terminated at a single sensillar pore (p) (approx. 100-nm wide) (Fig. 6). The most distal sensory cavity was the only one innervated by 2 neurons, thereby having 2 sensillar pores
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Fig. 5. TEM of parasagittal section through the precibarium (PrC) and cibarium (Cib) of *B. tabaci* (see position in Fig. 2). Shows 5 of the 10 precibarial sensilla (Ps), precibarial valve (Pv), valve muscles (Pvm), cibarial sensilla (Cs), epipharyngeal sclerite (Epi), hypopharyngeal sclerite (Hyp) with ventral cibarial sensilla (Vcs), attachment point of salivary duct (sd), salivary canal (sc) through the hypopharyngeal sclerite, which connects the salivary pump (Sp) to the salivary canal formed in the maxillary stylets (Mx), salivary pump muscles (spm), mandibular stylet (Md).

(Fig. 6). All of these sensilla were located in the epipharyngeal sclerite (Epi) and positioned to directly contact food as it passed into the cibarium (Fig. 5). The majority of precibarial sensilla appeared to be innervated by 4 dendrites, while the 3rd most distal sensilla appeared to have 5 dendrites (not shown). In the cibarium (Cib), 8 chemosensilla were found; 6 of these were located proximal to the precibarial valve (Pv), in the epipharyngeal sclerite (Epi) (Figs. 5 and 7). These sensilla were paired (Figs. 7 and 8), each being innervated by 2–3 dendrites (d) and were located medially with 3 sensilla on each side (not shown). Two pairs were fully sectioned and each was shown to terminate at a single sensillar pore (p) at their tips (approx. 107 nm wide), and positioned such that direct contact with food passing through the lumen of the cibarium (Cib) was possible (Fig. 8). A single pair of chemosensilla, called ventral cibarial sensilla (Vcs), were also found in the hypopharyngeal sclerite (Hyp), each of which was found to terminate at a single pore (p) opening into the cibarium (Cib), located opposite the dorsal cibarial sensilla (Cs) (Fig. 5).

DISCUSSION

Anatomy and function of precibarial and cibarial sensilla

The evidence presented is the first to elucidate the position and ultrastructure of the precibarial and cibarial sensory organs in *B. tabaci*. A chemosensory function is supported by the structure, location, and innervation of these sensilla.
Fig. 6. SEM of the precibarial sensilla (Ps) in the whitefly, *B. tabaci*. Sensilla were located where the maxillary stylets (Mx) diverged, 9 sensory cavities are shown, the most distal (1) being the only one with 2 sensory pores (p) (arrows). Maxillary stylets dislodged from maxillary stylet channels (Mxc) during dissection. Epipharyngeal sclerite (Epi), precibarial valve (Pv). Inset. Precibarial sensilla sensillar pores (p), approx. 100 nm wide.

Consequently, fluids passing through the lumen of the precibarium and cibarium directly contact these sensory organs, which may be used to detect and ultimately to evaluate favorable and/or unfavorable stimuli during feeding. Though the presence of an epipharyngeal organ located within the precibarium has been previously illustrated by Weber (1935, Figs. 3 and 30), our examination adds important information and provides a more thorough description about this group of sensilla. Furthermore, this is the first elucidation of the cibarial sensory organ.

The 2 gustatory sensory organs found in the precibarial and cibarial regions of the anterior alimentary canal in whitely appear to be analogous to gustatory chemosensilla observed previously in aphids (Davidson, 1914; Wensler and Filshie, 1969; McLean and Kinsey, 1984), leafhoppers (Backus and McLean, 1982, 1983; Backus, 1985), psyllids (Ullman and McLean, 1986) and thrips (Hunter and Ullman, 1994).

Analogous examinations of precibarial sensilla in leafhoppers (Miles, 1958; Backus, 1985), suggest that these sensilla would enable insects, like the whitefly, to detect and respond to chemical stimuli after stylet penetration into plant tissues and as fluids began to move through the anterior alimentary tract. Whiteflies have the potential then to evaluate and discriminate between the chemicals detected in their food prior to its ingestion. This is further supported by studies that have observed whitefly feeding and noted that a short
bout of probing by the insect seemed necessary before host plant discrimination would occur (Van Lenteren and Woets, 1977; Van Sas et al., 1978; Verschoor-van der Poel and Van Lenteren, 1978). These observations indicate that discrimination may be based on
chemical plant stimuli, most likely determined in part by these 2 gustatory organs. The ability to discriminate plant chemical constituents while still held within the precibarium and/or cibarium, may not only have a direct impact on host plant selection, but may also influence the efficiency of whitefly virus transmission by directly influencing feeding behavior.

Comparisons of the gustatory organs

Previously described systems, like those found in aphids and leafhoppers, showed some similarities. As in most insect systems described herein, there are distal and proximal groups of gustatory chemosensilla. These occur on either side of the precibarial valve, and in aphids, *Brevicoryne brassicae* (L.), the cabbage aphid (Wensler and Filshie, 1969), and *Macrosteles fascifrons* Stal., the aster leafhopper (Backus and McLean, 1982), these groups of sensilla are equally divided. In the western flower thrips, *Frankliniella occidentalis* (Hunter and Ullman, 1994) and mosquitoes, *Toxorhynchites* species (Lee and Davies, 1978), the distribution of sensilla are uneven with the majority of sensilla occurring in the cibarium. However, in *Bemisia tabaci*, although the distribution of sensilla is uneven, the majority of sensilla occur within the precibarium (10 versus 8 in the cibarium).

A feature that is shared with all the insects mentioned above, is that the distal, precibarial sensory organ occurs only in the epipharyngeal sclerite, while the proximal, cibarial sensory organs are found to be in both the epipharyngeal and hypopharyngeal sclerites, the majority of these sensilla being located in the epipharyngeal sclerite just proximal of the precibarial valve (Figs. 2 and 5). For example, in *B. tabaci*, there are 6 epipharyngeal cibarial sensilla and only 2 hypopharyngeal ventral sensilla (not shown).

The anatomy of the precibarial valve in whiteflies is similar to that of aphids, and psyllids, being similar to a “piston” that is short, flattened and shallow, being closed when in the relaxed state (McLean and Kinsey, 1984; Ullman and McLean, 1986). The main function of an insect’s multi-valve ingestion system appears to be related to regulation of plant fluids during uptake and in the separation of these sensory organs to allow an independent evaluation of plant fluids (Backus and McLean, 1985). Observations herein also support Weber’s (1935) earlier, and exhaustive descriptions of the associated musculature, and anatomy found in other whiteflies.

The anatomical investigation of whitefly gustatory chemosensilla, yields insights into the sensory mechanisms being used during whitefly feeding and host selection. This knowledge provides possible explanations for observed feeding behaviors, such as short feeding bouts made during host selection. We can see that: (1) Whiteflies have internal gustatory chemosensilla, which directly come into contact with plant fluids as it passes through the anterior alimentary canal. (2) These sensilla would allow the whitefly to assess the quality of plant fluids prior to the ingestion of the material into the esophagus and foregut. (3) The function of these sensilla may influence whitefly feeding and host plant selection, possibly affecting virus transmission by influencing where and how long it takes an insect to begin feeding.

Ultimately, the knowledge of which chemical compounds are being detected by these gustatory sensory organs, and which compounds are detected as favorable or unfavorable, may allow plant growers and geneticists to manipulate such compounds to develop plant cultivars resistant to whitefly feeding, which may aid in the management of geminivirus diseases.

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