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Morphological characterization of the mouthparts of the vector leafhopper *Psammotettix striatus* (L.) (Hemiptera: Cicadellidae)

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ABSTRACT

The leafhopper *Psammotettix striatus* (L.) (Hemiptera: Cicadellidae), is one of the most significant economic pests of wheat in Western China. This insect vectors a phytoplasma that causes wheat blue dwarf (WBD), a severe disease limiting wheat production in the Western China. A microscopic analysis of the ultrastructure of the mouthparts of the adult was conducted using scanning electron microscopy and the putative functions of the mouthparts were determined. The piercing-sucking mouthparts of *P. striatus* are of the conventional type comprising a three-segmented labium with a deep groove in the anterior side, a stylet fascicle consisting of two mandibular and two maxillary stylets, and an uppermost small cone-shaped labrum. The mandibular stylets, located laterad of the maxillary stylets, have sculpture on their tips, which may function in tearing plant tissue, cutting channels into the plant tissues, and attaching the body to the host plant during molting. The maxillary stylets are interlocked to form two separate compartments, a larger food canal and a smaller salivary canal. Two dendritic canals are also found in each maxilla and one in each mandible. Four kinds of sensilla were found on the labium: s. trichodea I, s. trichodea II, s. basiconic I, s. basiconic II. These may be involved in host recognition and are likely chemo- or mechanosensory, or both.

1. Introduction

Insects have an array of different mouthpart types acquired through feeding specializations during their evolution. Often mouthpart structures are characteristic of all members of a genus, family or order of insects, such that knowledge of mouthparts is useful for both classification and identification, and for ecological or physiological study (Gullan and Cranston, 2005). Hemiptera is the single most successful order of insect phytoplasma vectors (Weintraub and Beanland, 2006). Mouthparts of Hemiptera play important roles in host location, feeding, and transmitting phytoplasmas. Abundant data are available on the mouthpart ultrastructural morphology of Hemiptera based on light and scanning electron microscopy (Anderson et al., 2006; Boyd, 2003; Boyd et al., 2002; Freeman et al., 2000, 2001; Pollard, 1973; Rani and Madhavendra, 1995; Mora et al., 2001; Rosell et al., 1995; Tavella and Arzone, 1993; Wiesenborn, 2004). A few studies also focus on the internal structures of mouthparts in Sternorrhyncha and Fulgoromorpha, using SEM and TEM to observe cross-sections of the rostral segment of adult specimens, supplying comparative analysis of the connecting systems between right and left maxillae in part of Hemiptera (Brozek, 2006; Brozek et al., 2006). However, less information is available on mouthpart morphology and the fine structure of the mouthparts in Cicadellidae, including how mouthpart structure relates to the function of locating the phloem tissue within the plant host (Leopold et al., 2003). Pollard (1972) described the structure of the mandibular and maxillary stylets of the leafhopper *Eupteryx melissae* Curtis, Leopold et al. (2003) studied mouthpart morphology and penetration in host plant tissues of the glassy-winged sharpshooter, *Homalodisca coagulata*, elucidating where the leafhopper probes in the plant and the function of its mouthparts. Moreover, a few studies on rostral sensilla have indicated their possible function and the role of sensilla in host plant selection and structural adaptation for phytophagy (Backus and McLean, 1982; Foster et al., 1983a,b; Rani and Madhavendra, 1995, 2005; Walker and Gordh, 1989).

The phytophagous leafhopper *Psammotettix striatus* (L.) (Hemiptera, Cicadellidae, Deltocephalinae) is a serious pest of wheat crops and has been reported in the arid and semi-arid region of Northern China since the early 1950s (Xiang et al., 1996). While its feeding does little direct damage, it is primary vector of the highly destructive wheat blue dwarf (WBD) phytoplasma (An et al., 1991). Wheat has been severely affected by wheat blue dwarf in Shaanxi and elsewhere in Western China (An et al., 1991; Zhang et al., 1993). Like other cicadellids, *P. striatus* has highly specialized piercing-sucking mouthparts adapted for tapping into and feeding

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upon xylem or phloem. Its mouthpart morphology, mechanism of penetration in the host and pathogen transmission is unclear, although some aspects of the biology and behavior of this pest are known (Xiang et al., 1996; Xu et al., 2005; Zhang, 1990).

Thus, the focus of this study was to examine ultrastructural morphology of mouthparts of *P. striatus*, including the labrum, labium, mandibular and maxillary stylets using scanning electron microscopy (SEM). Demonstration of the presence of these structures is critical to an understanding of the sensory system and feeding behavior of leafhoppers. Host selection by this insect is ultimately dependent upon their feeding and sensory capabilities. Since *P. striatus* is an efficient vector of plant pathogens, this knowledge is probably also important in aspects of feeding behavior relevant to plant pathogen transmission.

2. Materials and methods

2.1. Leafhopper rearing

The experiment was conducted in the laboratory of the Entomological Museum, Northwest A&F University. Adults and exuviae of *P. striatus* were obtained from a continuously reared greenhouse culture established in 2008 from field populations in Yangling, Shaanxi, China. This culture is maintained on 20 cm high seedlings of healthy wheat growing in 10 cm-diameter pots, covered by 35 cm high transparent gauze cage, in a greenhouse at ambient temperature and under 12 h photoperiod.

2.2. Sample preparation for SEM

The mouthparts of adult leafhoppers were excised and dissected under 40× magnification (Nikon SMZ 1500, stereomicroscope, Japan) and fixed with 2.5% glutaraldehyde for ~12 h at 4 °C. The mouthparts were then rinsed for 15 min four times in phosphate buffer saline (PBS, 0.1 M, pH 7.2) and cleaned in an ultrasonic cleaner for 1 min before dehydration in a graded series of 75%, 80%, 85%, 90%, and 99.9% ethanol: water and critical point drying with liquid CO_2. Thereafter, the samples were mounted on aluminum stubs with double-sided copper sticky tape and sputtered with gold/palladium (40/60) in a LADD SC-502 (Vermont, USA) high resolution sputter coater. The exuviae of leafhoppers on the leaf were air-dried and coated with gold/palladium in a sputter coater. The samples were subsequently examined with a Jeol JSM 6360LV SEM operated at 15 kV.

3. Results

3.1. Gross morphology of mouthparts

The mouthparts of *P. striatus* (L.) resemble those of other piercing-sucking insect pests, consisting of a tube-like structure including the labrum (Lm) (Figs. 2 and 4), labium (Lb) (Figs. 3 and 4) and a stylet fascicle (Sf) comprising two mandibular (Md) and two maxillary stylets (Mx). The three-segmented labium has a deep longitudinal groove on the anterior surface (Figs. 3 and 4), within which lies the stylet fascicle, consisting of two inner maxillary stylets partially surrounded by two somewhat shorter and serrate-edgesd mandibular stylets, and above which is a small cone-shaped labrum. The labium is covered with different types of setae symmetrically positioned on either side of the labial groove or arranged on the distal end (Fig. 4). The mouthparts of *P. striatus* (L.) are directed backward beneath the body and the complete length of the rostrum is appressed to the prosternum when at rest. When the leafhopper is feeding the mouthparts are perpendicular to the leaf surface with the labium bent forward with the tip anchored to the leaf surface, while the stylets enter the plant tissue (Fig. 1).

3.2. Labrum

The labrum (Lm) is short (≈100 μm) and generally conical, and is attached to the anterior margin of the anteclypeus and overlays the labial groove (Lg) of the 1st and 2nd labial segments (Figs. 2 and 4). The surface of the labrum is rugose, with some small triangular cuticular processes of <3 μm in length clearly visible. These cuticular processes are arranged singly or pairwise on the anterior area of the labrum (Fig. 2).

3.3. Labium

The modified labium (Lb) (also known as rostrum or proboscis) is cylindrical, composed of three segments (Fig. 3) and its anterior surface is bisected by a deep labial groove (Lg) extended its entire length (Fig. 4), which encases the stylet fascicle. In resting adults, the labium extends beneath the body and the length ranges from 290 to 330 μm, depending upon the size of the individual. The labial tip is flattened with an opening from which the apex of maxillary stylets are extended (Fig. 13).

All segments of the labium are covered with different types of sensilla mainly distributed on each side of the labial groove (Lg) and distally, with fewer sensilla on the lateral and dorsal surface (Figs. 3 and 4). The first labial segment is shortest and widest, partly covered by the anteclypeus. There are no sensilla on the first segment and the sculpture consists of several small denticles on the anterior surface (Fig. 4). The second labial segment is narrower than the first, with several denticles in the area close to the first segment anteriorly. There are two types of sensilla trichodea on the lateral and anterior surface, including a pair of s. trichodea I (S.t.I), two pairs of s. trichodea II (S.t.II) symmetrically located on each side of the labial groove, and several s. trichodea I located on laterally (Fig. 4). S. trichodea I are slender, slightly curved, and inserted in a cuticular sheath like a cylindrical socket, and range from 16 to 68 μm in length. S. trichodea II are oblate with a longitudinal groove in the shaft, inserted in a similar socket, and range from 45 to 60 μm in length. While the dorsum of the second labial segment is bare without any sensilla (Fig. 3). The third labial segment is the most slender and longest of the three segments and covered with the most sensilla (Fig. 4). Several pairs of s. trichodea I and s. trichodea II are symmetrically arranged on each side of the labial groove, and some s. trichodea I are randomly distributed on the anterior and dorsal of labium (Figs. 3–5). The two longest s. trichodea I of 66 μm in length, symmetrically located on either side near the tip of the labium can be clearly discerned (Fig. 4). Moreover, there are two kinds of short, stout basiconic pegs around the tip of the labium. The larger one is s. basiconic I (S.b.I) (Figs. 4 and 6) with a sharp tip, 12.6 μm in length, arising from a sunken pit or cavity on the surface. The smaller is s. basiconic II (S.b.II) on each side of the labial groove, with a bulbous base, measured 0.4 μm in length (Fig. 13).

3.4. Stylet fascicle

The stylet fascicle (Sf) is needle-like, composed of two mandibular and two maxillary stylets (Fig. 7). The diameter of the stylet fascicle is approximately 19.6 μm and the average length of the stylets is about 290 and 310 μm for the mandibular and maxillary stylets respectively. They originate in the head capsule lateral and ventral to the cibarium. The apex of the maxillary stylets protrudes from the flattened labial tip and the mandibular stylets are entirely enclosed in the labium when not in use (Fig. 13).

The mandibular stylets (Md) are abutted on each lateral side of the maxillary stylets (Mx) and slightly shorter than the maxillary