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Differential morphology of the sensory sensilla of antennae, palpi, foretarsi and ovipositor of adult *Tribolium castaneum* (Herbst) (Coleoptera:Tenebrionidae)

Mervat A. Seada[a,*], Amal M. Hamza[b]

[a] Entomology Division, Zoology Department, Faculty of Sciences, Tanta University, Egypt
[b] Department of Stored Product Pests, Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt

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ABSTRACT

The red flour beetle, *Tribolium castaneum*, is an invasive pest of a variety of stored products that is found in most tropical and subtropical countries. To further understanding of the mechanism of insect-host chemical communication, we examined the morphology, distribution and abundance of various sensilla associated with antennae, palpi, foretarsi and ovipositor by scanning electron microscopy. No sexual dimorphism was found between male and female antennae, palpi and foretarsi. In both sexes, four types of antennal sensilla were characterized: two subtypes of trichoid sensilla (st1-2), four subtypes of basiconic sensilla (bs1-4), multiporous grooved peg (mgp) and styloconica sensilla (sst). Overall, the olfactory and gustatory sensilla were clustered mainly on the three antennal club flagellomeres. The sensory field of both maxillary and labial palps was concentrated on the distal segment forming the chemosensory field. Three types of sensilla on both maxillary and labial palps were characterized: trichodea, basiconica, and two subtypes of sensilla styliconica. Maxillary palps carried higher numbers of all types of sensilla than labial palps. Two types of sensilla were characterized on the fore tarsi: trichoidea and cheatica. Only one type of sensilla was found on the ovipositor, trichodea. The functional roles of these sensilla are discussed in relation to their external structure and distribution. Further anatomical, physiological and behavioral studies on identifying the functional types of these sensilla and their role in the selection of an appropriate food source can provide a basis for the development of semiochemically based control strategies.

Introduction

Red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is a major pest of stored products worldwide, especially in warmer climates (Nakakita, 1982; Angelini et al., 2009). These beetles have chewing mouthparts, their growth is optimum in flour and other processed cereal products (Aitken, 1975). Economic losses include reduced weight and product quality of infested products and an accompanying unpleasant smell associated with microbial contamination.

Insects such as Red flour beetle***,*** *T. castaneum*, depend on olfaction, gustation, and contact to detect nutrient-rich foods, to avoid consuming toxic substances, and to select mates and hospitable media to deposit eggs. Insects perceive such stimuli through their contact and chemosensory receptors distributed on antennae, legs, mouthparts, and ovipositor.

Insects are covered with sensory structures known as sensilla, where sensory neurons responsible for perception of smell, taste, sound, touch, vision, proprioception, and geo-, thermo-, and hygroreception are found (Shields, 2011). Traditionally, sensillum types have been classified on the basis of the morphology of their cuticular parts, as well as location on the insect (Zacharuk, 1985; Zacharuk and Shields, 1991). Chemosensory sensilla are present on the antennae, mouth parts, legs, wings and the ovipositor (Zacharuk and Shields, 1991). Most of the olfactory receptor neurons are located on the antennae; whereas most gustatory neurons are present on mouth parts, legs, and ovipositor (Zacharuk and Shields, 1991). Sensilla on the labial and maxillary palps and legs enable insects to sample potential foods without consuming it (French et al., 2015). However, much less attention has been directed toward the sensory structures associated with the mouthparts of *T. castaneum*. In insects, mandibulate mouthpart morphology is characterized by a robust pair of unsegmented mandibles followed by two

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* Corresponding author.
E-mail address: Mervat.seada@science.tanta.edu.eg (M.A. Seada).

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pairs of multi-branched appendages, the maxillae and labium (Snodgrass, 1928; Grimaldi and Engel, 2005). The maxillary and labial appendages have outer sensory palps and inner branches called endites (Angelini et al., 2012).

As distribution of *T. castaneum* is in confined spaces with no particular need for rapid locomotion therefore the beetle possesses very short legs. The legs of insects are used to make contact with their potential foods substrate. It is unknown how important the legs of *T. castaneum* are in the stored product selection process for feeding or oviposition. Little attention has been paid to the sensory structures associated with the tarsi of *T. castaneum*.

The ovipositor of *T. castaneum* consists of membranous telescoping body segments, which remain retracted beneath the seventh sternal and tergal plates (Aspiras et al., 2011). The more anterior of these are regarded as derivatives of abdominal segments eight through ten. The anatomy of the female ovipositor varies greatly among insect groups (Scudder, 1961; Chapman, 1998; Seada et al., 2015). *Tribolium castaneum* as a Coleopteran insect has a terminal ovipositor which telescopes out to deposit eggs on the substrate and retracted when at rest (Aspiras et al., 2011). No studies have been done on the structure or function of the sensory structures associated with the ovipositor of *T. castaneum.*

The main aim of the current study was to study the differential distribution and structure of the different types of the sensilla that were found on the antennae, palpi, fore tarsi and ovipositor of *T. castaneum* by using scanning electron microscopy (SEM) techniques. This might provide the base line information to study their function in relation to their location, which may help to elucidate their significance in the behavior and chemical ecology of *T. castaneum.*

Materials and methods

Insect rearing

Approximately, 100–300 *T. castaneum* adults were placed in 850 mL glass jars containing 400 g of wheat flour as a food source. The openings of jars were covered with muslin cloth, kept in position with rubber bands. *Tribolium castaneum* was maintained in the laboratory at 28 ± 2 °C, 70 ± 5% relative humidity (RH) and a light: dark photoperiod of 16: 8 h. To obtain adult beetles, the flour was sieved by a sieve with a mesh size of 710 μm.

Fine structure and scanning electron microscopy

For light microscopy, head with antennae, fore legs, head with mouth parts, and terminal abdominal segments with the ovipositor were dissected from beetles. Then they were mounted for temporary storage onto microscope slides with a piece of double sided sticky tape. Thereafter, antennae, fore legs, mouth parts, and ovipositors and their associated sensilla were examined and photographed (Canon 1145, Canon Co. USA) with an Olympus CX31 compound microscope with bright field illumination. For scanning electron microscopy (SEM), heads with antennae and mouth parts, fore legs, and terminal abdominal segments with ovipositor were excised with fine scissors and immersed in 70% ethanol overnight at 4 °C. Specimens were then dehydrated in 80%, 90% and 100% ethanol, mounted on SEM stubs and sputter coated with gold–palladium (3:2) in a JEOL ion sputter coater, JFC-1100E (JEOL Ltd, Tokyo, Japan). The specimens were visualized using a scanning electron microscope (JSM-5300, JEOL Microscopy, Tokyo, Japan). The number of antennal and tarsal sensilla was recorded from light micrographs from 10 individuals, while the number of the palpi and ovipositor sensilla was recorded from SEM micrographs of five individuals. The lengths of all types of sensilla were then analyzed from SEM micrographs. All data were reported as means and standard error of means.

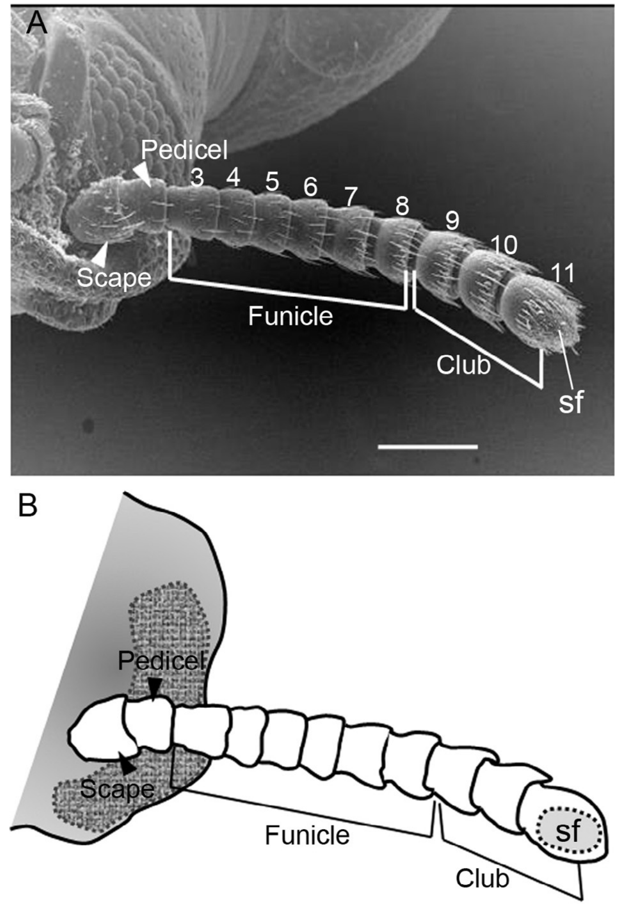

Fig. 1. Dorsal view of the whole antenna of *Tribolium castaneum* with the sensilla. (A) Scanning electron micrographs (SEM) showing 11 segmented antenna with scattered sensilla trichodea (st1) distributed throughout all antennal flagellomeres and sensilla basiconica (sb) on the distal three flagellomeres; scale bar, 50 μm. (B) Schematic drawing of the organization of the antennal flagellomeres showing in more details the proximal funicle and distal club with the apical sensory field (sf).

Results

Sensory organs of antennae

Adult male and female antennae of *T. castaneum* were physically nearly identical. The antenna was composed of a scape, a pedicel and the flagellum which was composed of two parts: the funicle (the proximal six flagellomers), and the club (the three distal flagellomers) (Fig. 1).

The antenna was 239.7 ± 8.9 μm in length. The sensory field with sensilla of various types was concentrated at the club flagellomers. The density of sensilla increased from proximal to distal ends but all types of sensilla were found only on the club (Fig. 1).

The most abundant type of sensilla was the long trichoid sensilla (st1), which were on all antennal flagellomers, but especially on the club (Figs. 1–3). These sensilla were straight with longitudinal warts and their cuticle appeared thick, suggesting mechanosensory or olfactory functions. The long sensilla trichodea (st1) varied in length from ~8 to 12 μm. The bases of these sensilla were inserted slightly depressed into the cuticle (Fig. 2). On the club of the antenna of both sexes, four additional sensillum types were found: short trichodea (st2), basiconica (bs), multiporous grooved peg (mgp), and styliconica (sst) (Fig. 3). A circular row of sensilla basiconica was arranged at the periphery of each flagellomere of the club.