ANTENNAL SENSILLAE OF THE CONFUSED ADULT BEETLE, *TRIBOLIUM CONFUSUM* (DUVAL) (COLEOPTERA: TENEBRIONIDAE).

MOHAMED S. SALEM; SALAH A. El- MASSARAWY AND REHAM A. M. EL-WASSEF

Department of Economic Entomol. & Pesticides, Fac. Agric., Cairo Univ., Giza, Egypt

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INTRODUCTION

Confused flour beetles attack stored grain products such as flour, cereals meal, crackers, beans, spices, pasta, cake mix, dried pet food, dried flowers, chocolate, nuts, seeds, and even dried museum specimens (Via 1999, Weston and Rattlingourd 2000). These beetles have chewing mouthparts, but do not bite or sting. The red flour beetle may elicit an allergic response (Alanko *et al.* 2000), but is not known to spread disease and does not feed on or damage the structure of a home or furniture. These beetles are two of the most important pests of stored products in the home and grocery stores.

Insect antennae are mobile, segmented, paired appendages. The entire antenna typically has three main divisions: The first segment, or scope, is generally larger than the other segments and is the basal stalk; the second segment, or pedicel, responds to movement of the distal part of the antenna; the remainder of the antenna, called the flagellum, is often filamentous and multisegmented (with many flagellomeres), but may be reduced or variously modified (Gullan and Cranston, 2003).

Numerous sensory organs, or sensillae, in the form of hairs, pegs, pits or cons, occur on the antennae and function as chemoreceptors (the senses of smell- olfaction and taste- gestation), mechanoreceptors, thermoreceptors and hygroreceptors and bearing sometimes sensors for CO_2 (Gullan and Cranston,2003; Hansson, 1999).

Favourable environmental conditions in flour lead confused flour beetles to their preferred habitats, but several different behavioural mechanisms help beetles find or remain in suitable habitats. Several of trichoid, basiconic, chaetica, coeloconica and campaniformia sensillae have been found on the confused flour beetles antennae. A comparison of sense organs in confused beetles may give knowledge of how certain structures of the sense organs serve their different functional demands.

MATERIAL AND METHODS

Tribolium confusum were reared on media consisting of white flour and5% brewers yeast. Cultures and test insects were kept at 25 ±5C° and 65±5% RH. The flour was sterilized at 60 C° for 10h to eliminate possible contaminants (El- Kady, 1978).

The heads of five adult males and females were cut with fine scissors, immediately fixed in paraformaldehyde, and kept one night at 5C° then they were rinsed in phosphate buffer three times for 15 min and bathed in ethyl ether for 15 min. The antennae were cleaned in 20% detergent aqueous solution for 90 min.

Finally the antennae were dehydrated in increasing seria of alcohol baths 30, 50, 70, (10 min), 90(twice for 10 min) and 100% (twice for 5 min). Immediately before examination, the antennae were fixed on a stub and were sputter – coated with gold, (Imen *et al.*, 2003). Five antennae of both sexes were examined by scanning electron microscopy. In classifying sensillae, the terminology of Schneider (1964) and Zacharuk (1985) was used.

RESULTS AND DISCUSSION

The filiform antennae of both sexes of the confused beetles *T. confusum* are composed of 11 distinct segments: the scape,the pedicel and the nine segments (flagellomeres) of the flagellum, wherever its end is gradually club-like, the "club" consisting of four segments as described by Walter, 1990. Based on external morphology of sense organs, sensilla chaetica, sensilla trichodea, sensilla basiconica, sensilla coeloconica and sensilla campaniformia were distinguished.

The length of both male and female antennal scape measured 300μm long and 220 μm diameter; the pedicel 120μm long and 240 μm diameter; the flagellum segments 950 μm long and 200 μm diameter. The antennae of males and females, including the dorsal and ventral surfaces, were compared for differences in number and types of sensillae. The approximate total numbers of all sensillae per antennal segment in both sexes have been summarized in Table (1). The club contains ca more than 80% of the sensillae in both sexes.

Three types of sensillae were considered as olfactory, sensilla trichodea 2 (Fig. 1C, 2A), and sensilla basiconica occur in males and females, length was 9.5 μm. (Fig 1D, 2A) in both male and female. While Sensilla trichodea type 1 occurs primarily on all segments of antenna, it ranged from (12-15μm) in length (Fig.1A) and observed on the male antenna. In male moths, trichoid sensilla respond to

female sex pheromone related compounds (Keil and Steinbrecht, 1984; Zacharuk, 1985; Hansson *et al.*, 1986; Hallberg *et al.*, 1994). Striking differences exist in the number of these trichoid sensilla, for example Merivee, (1992) and Merivee *et al.*, (1999) suggest that they probably function as sex pheromone receptors in male of some click beetles. In contrast, no considerable sexual differences in the number of immovable blunt- tipped trichoid sensilla were found on the ground beetles *Bembidion lampors, B. properans* and *Platynus dorsalis,* indicating that these sensillae respond to aggregation pheromone produced by some ground beetles (Moore and Walbank, 1968; Wautier, 1970, 1971).

TABLE (I)

Total number of sensillae on the club (4 segments), the remainder flagellum segments (with many flagellomeres), pedicel and scape in male and female *T. confusum.*

Antennal segment		**Total No. of sensillae (Each number of sensillae represent the different kind of sensilla).**	
		No. in Male	**No. in Female**
Flagellum segments		530±50	350±50
Club(4 segments)			
Flagellomeres	5	22	18
	6	18	16
	7	10	20
	8	10	20
	9	14	10
Pedicel	10	18	12
Scape	11	8	8
Total		608±50	436±50
Percent of total on club		87.1%	80.2%

Olfactory sensilla trichodea type 2 from clear dorsal and ventral sensillar fields on the flagellomeres, which seems to be common in confused beetles males, ranged from 18 -20μm in length, and occur on all segments of club (on 4 segments) (Fig. 1C), the basal diameter of this sensilla approximated 3 μm. In the coleopteran,

similar have been reported for *Tenbrio molitor* (Harbach and Larsen, 1977)and *Curculio caryae* (Hatfield and Frazier, 1976).

The length of the sensilla cheatica type 1 was 42.5 μm with the basal diameter of 4 μm, while sensilla cheatica 2 occur in pairs with length of 9.5 μm usually behind sensilla cheatica 1 on club segments in males (fig. 1B).

Sensillae coeloconica are 4.5 μm in length and 1.7μm in width at the point of insertion (fig.2 B) in antennae of both sexes; they are slightly sunken in a depression and surrounded by a raised ring of cuticle. Although the wall appears smooth at the base, following a gradual reduction in width, several finger- like projections extend toward the distal tip. Broeckling and Salom (2003) found similar sensillar type on *Laricobius nigrinus* , but sensilla trichodea type3 occur primarily on the club (Fig. 2B) was observed on the female antenna. The length of the longest sensilla was 25 μm, the basal diameter was 2 μm. Bland, (1981) reported that the sensible trichodea and basiconica probably function as chemoreceptors.

Sensilla campaniformia (Fig. 2D) is area of thin cuticle, flattened and oval in shape. It is mostly found in clusters and act as a unit and always has a proprioceptive function and located at the tip of the ninth flagellomere in antennae. Sensilla campaniformia was described in *B. lampros, B. porperans* and *P. dorsalis* (Merivee *et al.*, 2000, 2002) and they are similar to those found in some other beetles, for instance click beetles (Merivee,1992; Merivee *et al* ., 1998, 1999). The electrophysiological recordings of campaniformia sensilla on the antenna of ground beetle *Pterostichus aethiops* were distinguished as cold receptors (Merivee *et al.*, 2003). Thermoreceptors are probably involved in habitat selection of confused flour beetles and enable to avoid hyperthermia of insects.

Campaniform sensilla may act as a sensible innervated to chemo- and mechanosensory function for ultrasound response in the beetles. They are present in great numbers on the halteres of Diptera (Pflugtaedt, 1912). Almost all authors agreed in regarding the Campaniform sensilla as being sensitive to mechanical stimuli. Alternatively, they have been thought to respond to bending of the cuticle. Hence they are radially symmetrical they will react to flexion equally in all directions, where they are elliptical, as in Hicks papillae. Thus, they will be affected only by bending in their long axis, *i.e.* in the directions, of the stiffening rod. The campaniform organ on the antennae of caterpillars is stimulated by pressure upon the adjoining cuticle (Dethier, 1914).

Their numbers on the flagellomeres increased towards the tip of the antenna, the number of sensilla trichodea 2 per flagellomeres, growing towards the tip of the antenna more rapidly in the dorsal sensillar fields than in the ventral ones. Most of basiconic sensillae are located on the ventral side of the antenna (Fig.2A). Similar asymmetry in the distribution of antennal olfactory sensillae has been observed in click beetles *Agriotes obscurus* (L.) and *Melanotus villosus* (Geof.) (Merivee *et al.*, 1997, 1999). It has been suggested by Merivee *et al.*, (1997) that asymmetries in the distribution pattern of the olfactory sensilla on insects antennae may be due to the peculiarities of their search behaviour (waiting, walking, flying , antennal movements), which causes certain areas of the antennal surface to catch the wind – borne odour molecules more effectively.

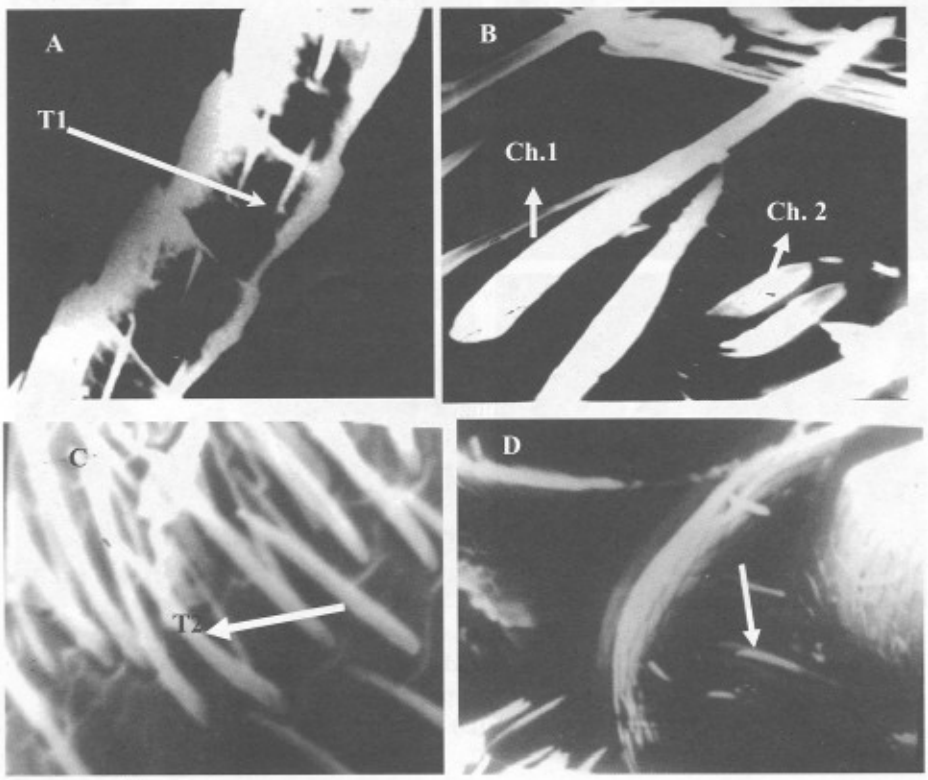

Fig.1. Antennal sensillae in males of *T. confusum* (A) Sensilla trichodea type (T1) occurs primarily on the club. (B) Sensilla cheatica 1 (Ch.1) and Sensilla cheatica 2 (Ch.2) respectively. (C) sensilla trichodea type 2 (T2).(D) sensilla basiconica.(B).

The typology, number and distribution pattern of antennal sensillae of two sexes in confused flour beetles *T.confusum* was elucidated by the scanning electron microscopy.

Based on morphological features, there were distinguished eight types of sensillae. No sexual dimorphism was found in the structure, but distribution of the antennal sensillae. Some differences were found in the number of antennal sensillae. Three types of sensillae were considered as olfactory- sensilla tricodea type 1, 2 and sensilla basiconica. Olfactory - sensillae from two separate, dorsal and ventral, fields of sensillae on the flagellomeres.

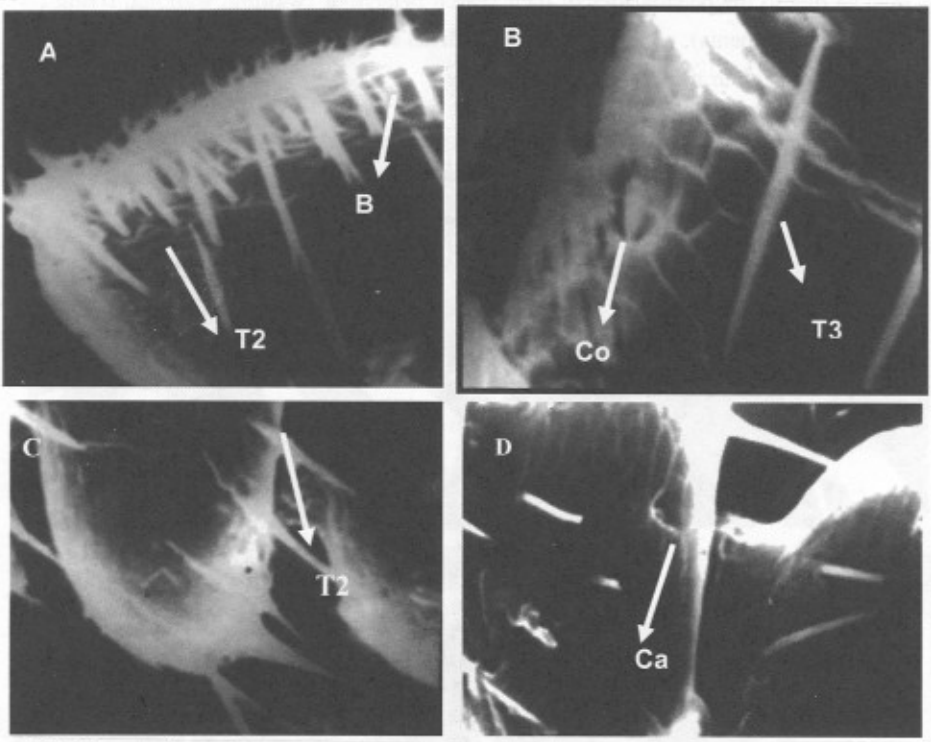

Fig.2. Antennal sensilla in female of *T. confusum* : (A) Distal part of the ninth flagellomere ,sensilla trichodea type 2 (T2) arrowheads show tiny cuticular pores and sensilla basiconica (B).(B) sensilla trichodea type3 (T3) and sensillum coelocoonicum (Co).(C) Sensilla trichodea type 2 (T2). (D) Sensillum campaniformium (Ca).

SUMMARY

The antennal sensillae of male and female confused flour beetle *T. confusum* (Coleopteran:Tenebrionidea) are investigated by electron microscopy. The filiform antennae of confused beetles consist of scape, pedicle and 9 flagellomeres. There are 3

types of sensilla trichodea, one type of sensilla basiconica, two types of sensilla cheatica, pit–organs, sensilla coeloconica and small domes campaniformia. Sensilla chaetica occur only on the antennae of males and sensilla coeloconica, campaniformia only on the antennae of females. Sexual differences in the types of sensillae are found on the antennae. The possible functions are discussed and three types of sensillae are considered as olfactory, sensilla trichodea 2 and sensilla basiconica.

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