Malaysian Journal of Science **21**: 37-44 (2002)

Ultra structure of the antennal sensilla of the fruit fly *Bactrocera carambolae* (Insecta: Diptera: Tephritidae)

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ABSTRACT The antennal sensilla of *Bactrocera carambolae* (Diptera: Tephritidae) were examined using Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM). SEM has revealed, morphologically three main types and five sub types of antennal sensilla. The main types are microtrichia (m) having longitudinal cleft, sensilla chaetica (ch) and sensilla basiconica (Sb). The two types of sensilla basiconica observed and identified were multiporus double walled sensilla (MpDs) and multiporus single walled sensilla (MpSs) together with its own three sub types; innervated with 1 to 2 sensory cells (S1) and innervated with 2 to 4 sensory cells (S2) and MpSs having pimping on the cuticular surface (S3). Microtrichia was found distributed along the whole length of the antennae with its density more in the funiculus and probably functions as mechanoreceptors. MpDs having thick wall and lesser number of pores must be regarded as tactile in functions and MpSs having thin membranous wall and numerous pores are usually regarded as receptors of taste and odour. MpDs and MpSs were distributed among the microtrichia on the funiculus alone. Sensilla chaetica (ch) were seen only on the scape and pedicel, which probably functions as tactile receptors. One sensory pit on the funiculus and 2 to 3 antennal cuticular pores on the pedicel were observed. These results are compared with previously published studies on Tephritidae.

ABSTRAK Sensila sesungut bagi *Bactrocera carambolae* (Diptera: Tephritidae) telah diperiksa menggunakan mikroskop electron pengimbas (SEM) dan mikroskop elektron transmisi (TEM). Secara morfologi SEM telah mengesahkan kehadiran tiga jenis sensila sesungut utama dan lima sub jenis. Jenis utama adalah mikrotrikia (m) yang mempunyai celah memanjang, sensila keta (ch) dan sensila basikonika (sb). Dua jenis sensila basikonika yang telah dikenalpasti adalah sensila berbilang liang berdinding dua (MpDs) dan sensila berbilang liang berdinding satu (MpSs) bersama tiga sub jenis yang masing-masing dibekalkan dengan 1 hingga 2 sel deria (S1) dan 2 hingga 4 sel deria (S2) dan MpSs melekat di atas permukaan kutikel (S3). Mikrotrikia ditemui tersebar sepanjang sesungut dan kepadatannya lebih di bahagian funikulus dan fungsinya sebagai mekanoreseptor. MpDs yang mempunyai dinding tebal dan bilangan liang yang kurang dianggap berfungsi sebagai taktil dan MpSs yang mempunyai dinding nipis bermembran dan liang yang banyak biasanya berfungsi sebagai reseptor rasa dan bau. MpDs dan MpSs tersebar di antara mikrotrikia di atas funikulus sahaja. Sensila keta (ch) dilihat hanya pada skap dan pedisel, kemungkinannya berfungsi sebagai reseptor taktil. Satu pit deria pada funikulus dan 2 hingga 3 liang kutikel sesungut pada pedisel telah diperhatikan. Keputusan yang diperolehi telah dibandingkan dengan hasil kajian terdahulu ke atas kajian Tephritidae yang telah diterbitkan.

(olfaction, pheromones, chemoreceptors, antennae, tephritidae, receptors)

INTRODUCTION

The perception of chemicals is important in many aspects of the life of insects. Antennal excision experiments and electrophysiological investigations of antennal olfactory responsiveness show that, the primary structure of insect olfaction is the antennae. The sense of olfaction plays a role in orientation behaviours, including oviposition and feeding [1,2,3]. Furthermore pheromones are perceived via olfactory sensilla. The shape and size of the antennae, and the number, types and location of olfactory receptors on them determine olfactory sensitivity to pheromones [4]. The importance of olfaction in the behaviour of tephritids is well known [5].

Bactrocera carambolae attacks a wide range of fruits and vegetables and can be considered as one of the most destructive of insect pests. It is widely distributed in Oriental Asia and South America [6,7]. Many of the monitoring and control techniques for tephritid flies use olfactory-based behavioural manipulation [8]. In spite of extensive research on the techniques of behavioural manipulation, no research work is reported on the physiology of the antennal sensory apparatus of *B. carambolae*. This paucity of information may impede the development of new control strategies [9].

The antennal sensilla of other tephritids that have been investigated includes *B. tryoni, B. dorsalis, B. oleae, B. cucurbitae, Ceratitis capitata* and *Anastrepha ludens* [10,11,12,13,14].

Knowledge of the morphology of the antennae will form a basis for further research on the electrophysiology and will add to the potential of modern genetics and molecular biology to the investigation of olfactory signal transduction and processing [15,16]. The purpose of the present study is to determine the innervations and wall structures of the funicular sensilla of *Bactrocera carambolae* in preparation for electrophysiological studies.

MATERIALS AND METHODS

Flies were obtained from infested starfruits collected from the project farm at Universiti Putra Malaysia. For scanning electron microscopy (SEM) the flies were vacuum dried for two days and fixed overnight by exposing to osmium tetroxide. The flies were attached to stubs using carbon black. It was then sputter-coated with gold and examined under a JEOL 6400 JSM scanning electron microscope (SEM) at 15 kV.

For transmission electron microscopy (TEM), antennae were removed using fine tweezers. The removed antennae were fixed overnight in 4% glutaraldehyde in 0.1 M cacodylate buffer, postfixed in 2% osmium tetroxide, blocked and embedded in uranyl acetate for 15 minutes, and dehydrated in ascending series of alcohol, starting from 30% to absolute alcohol (100%), keeping in each series for 15 minutes and embedded in epoxy resin. Thin sections were cut using Reichert ultra microtome and stained in uranyl acetate and Reynold's lead citrate stain. The preparation was viewed under Philips CM 12 TEM.

RESULTS

The antennae of *Bactrocera carambolae* consist of three well-developed segments, scape (s), pedicel (pe) and funiculus (f). The remainder of the flagellum is reduced, forming the feathered arista (a) (Fig.1a). It rises from the dorsoproximal region of the funiculus. The scape articulates with the antennal socket between the large compound eyes (c) (Fig.1b). The pedicel is enlarged distally and dorsally evaginated and joints funiculus. A single sensory pit (sp) on the medial side of the funiculus (Fig.1c) and two antennal cuticular (ac) pores on the pedicel could be seen (Fig.1d). The antennae of females are 943 μm long, the males 896 μm long.

The sensory organ on the scape and pedicel are restricted to two distinctive structures.

Microtrichia (m), located distally, are longer along the ventral margin of each of their segment (Fig. 1a). These sensilla are distally pointed with longitudinal ridges (Fig. 1e) and socketed base.
Sensilla chaetica (ch) arises from a flexible socket, with spine like bristles (Fig. 1f) having thick cuticular walls and their lumen is devoid of dendritic processes. No pores were seen on the walls (Fig. 3a).

The funiculus is broadly triangular in cross section with an inner and outer surface. The arista arising from the funiculus is covered with many non-sensory bristles. The surface of the funiculus is covered with microtrichia (m) (Fig. 2a). They are curved structures with longitudinal ridges (Fig. 3b). No pores are seen on the cuticular walls and only one or two sensory cells are seen (Fig. 3c). There is a single sensory pit on the external lateral surface of the third segment (Fig. 1c).

Interspread among the microtrichia are the following types of sensilla basiconica (Fig. 2b). In addition to the wall thickness, pore morphology and the number of the branching patterns of the receptor cells are taken into account.

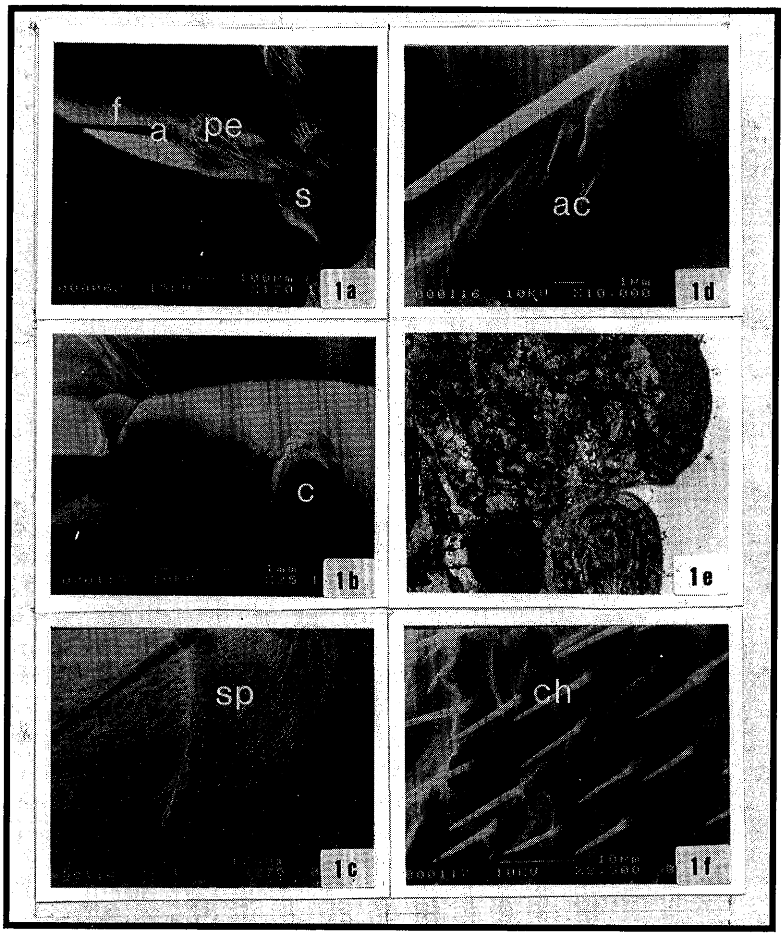

Figure 1.

1a. Antenna with scape (s), pedicel (pe), funiculus (f) and aristae (a); **1b**. antennae seen between the compound eyes (c); **1c**. opening of the sensory pit (sp) on the funiculus; **1d**. antennal cuticular pore (ac) seen on the pedicel; **1e**. ×25000 cross section of (m) showing longitudinal ridge (lg); **1f**. Sensilla chaetica (ch) seen on the pedicel.

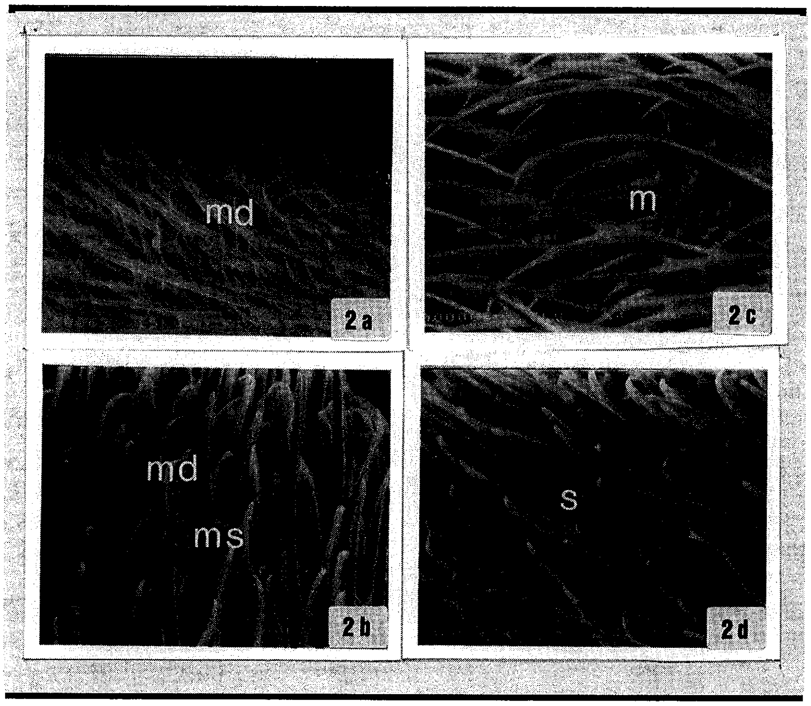

Figure 2.

2a. Sensilla on the surface of the funiculus showing microtrichia (m), multiporus double walled sensilla MpDs (md) and multiporus single walled sensilla MpSs (ms); **2b.** funiculus showing md & ms; **2c.** microtrichia (m) with longitudinal ridge on the (f); **2d.** SEM of funiculus showing one subtype (S) of MpSs.

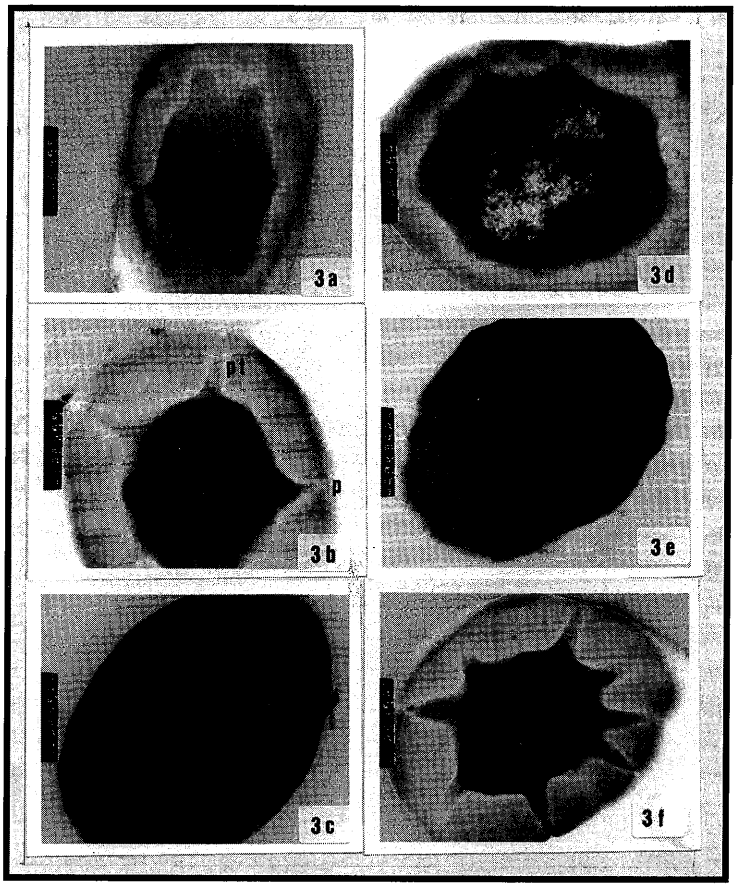

Figure 3.

3a. × 10000 c.s of sensilla chaetica (ch); **3b.** × 17000 multiporus single walled sensilla MpSs (S1) with 4 to 5 pores and pore tubules (pt); **3c.** × 45000 microtrichia with thin wall, no pores and with one sensory cell; **3d.** × 60000 multiporus double walled sensilla MpDs with lesser number of pores, thicker cuticular walls and 1 to 2 sensory cells; **3e.** ×45000 Cross section (c.s) of MpSs (S3) showing pimping on the cuticular surface; **3f.** × 35000 MpSs (S2) with 8 pores also showing the wide pore kettle (k) and 3 dendrites (d).

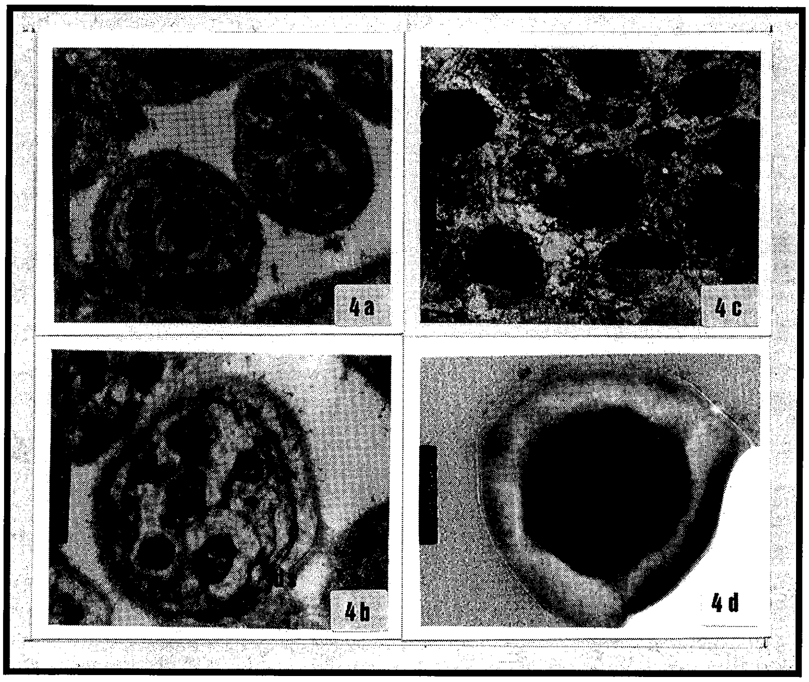

Figure 4.

4a. × 25000 multiporus single walled sensilla MpSs which have 1 to 2 sensory cells and branched dendrites; **4b & 4c.** × 22000 & × 63000 MpSs with 2 to 4 sensory cell showing extensive branching of dendrites (db) and dendritic sheath (ds); **4d.** × 25000 c.s of one of the sensilla basiconica seen on the sensory pit.

Multiporus double walled sensilla (MpDs) (Fig. 2b).

Distributed among the microtrichia are short thick-walled sensilla (Fig. 2b) MpDs. The cuticle is thicker than any other sensilla. The pores are approximately 29 nm wide and connected to sensillar cavity through gradual widening gap in the sensillar wall. The pore tubules are seen towards the constriction of pores. They have only 1-2 sensory cells leading to 1-2 dendrites, which remain unbranched (Fig. 3d).

Multiporus single walled sensilla (MpSs) (Fig. 2b)

Thin-walled multiporus pitted sensilla are more in number than MpDs. They are found mainly in the proximal and ventral region of the funiculus. These sensilla are relatively long with blunt, taper and smooth walls. They are the most abundant chemosensory receptors found on the funiculus. The wall thickness of the MpSs is about 282 nm and is constant along the whole length of the hair. The cuticle is perforated with holes (Fig. 3d, 3e and 3f). The pores have a mean diameter of 9 to12 nm, widening into a small chamber, the pore kettle (k). From the whole of the chamber walls emanates a cluster of fine tubules (Fig. 3b), the pore tubules (pt) extend towards the lumen.

There are three morphologically different subtypes of MpSs.

i) *S1* (Fig. 2c) - MpSs having thinner cuticular wall and 4 to 5 pores. They are innervated with 1 to 2 sensory cells (Fig. 3b) and the dendrites are branched (Fig. 4a).

ii) *S2* (Fig. 2c) - MpSs having thinner cuticular walls and 8 to 9 pores. They are innervated with 3 to 4 sensory cells (Fig. 3f) and the dendrites are extensively branched (Fig. 4b and 4c).

iii) *S3* - MpSs having pimping on the cuticular surface (Fig. 2d). They are innervated with 1 to 2 sensory cells and have only 1 to 2 pores. No well developed pore tubules or pore kettles could be seen (Fig. 3e).

The sensory pits seen on the funiculus consist mostly multiporus double-walled sensilla and multiporus single-walled sensilla. The cross section of one of the hairs shows the presence of 1 to 2 pores (Fig. 4d).

DISCUSSION

The external morphology and the ultra structure of the antennae of *Bactrocera carambolae* is similar to that of other tephritid flies including *B. tryoni, B. dorsalis, B. oleae, Ceratitis capitata* and *Anastrepha ludens* [10,13,12,11,17]. Each of the tephritid species so far investigated has similar antennal morphology with regard to the number of segments, types of sensilla and the presence of a single sensory pit. The size of the various sensillar type varies among species, but their structure is consistent. The only significant difference is the total length of the antennae between male and female [13]. In *Bactrocera carambolae*, the female antennae are also longer than the male antennae.

Scape and Pedicel

Two types of antennal sensilla are found on these two segments along with antennal cuticular pores. They are microtrichia and sensilla chaetica.

Microtrichia found distributed along the whole length of the antennae are commonly found throughout other higher Dipteran flies, e.g. *Delia antiqua* [18], *Ceratitis capitata* [19], *Drosophila melanogaster* [20]. Due to their free mobility on the base membrane, and longitudinally ridged surface, they probably act as mechanoreceptors [21]. Tactile hairs are of common occurrence in arthropods; in insects they are distributed over most parts of the body and the appendages. The provision with innervated movable hairs offsets the loss of surface sensitivity in animals having a sclerotized integument and enables animal to become aware of the approach or nearness of an external object before coming into actual body contact with it [21,22]. Therefore it may help in the orientation behaviour of the flies.

Sensilla chaetica, which are spine like and thick walled evidently preclude any possibility of penetration by odour or taste substances. They are therefore probably tactile in function. These are similar in external appearance to those in other species of Tephritidae, which have been shown to function as mechanoreceptors [13].

Antennal cuticular pores observed in this study may be a dermal gland. The glands occur in the epidermis and discharge the cement layer, which is poured over the surface of the wax layer just before or just after molting [23]. In most insects, these glands are found scattered all over the surface [23].

Funiculus

Two main types and five sub types of receptors are found on the funiculus. Seen rising from the funiculus is the arista, which does not have any sensilla. The absence of sensilla on the arista has been reported in other dipteran species [24,25, 26,27].

The two main types of receptors are microtrichia and sensilla basiconica. Microtrichia can be regarded as mechanoreceptors. Sensilla basiconica has two sub types, MpDs and MpSs. MpDs having thick walls and lesser number of spores and sensory cells respond to mechanical stimuli and therefore regarded as tactile in function [28]. MpDs have also been found in *C.capitata* [19], *Drosophila melanogaster* [29], *Aedes aegypti* [30], and *Delia radicum* [18,10].

MpSs is the most common type of sensilla basiconica found in a wide variety of insect groups [17]. The thin walls are presumed to be pervious to chemical stimuli and such organs are usually regarded as receptors of taste or odour [31]. The possible chemoreceptive function of this kind is suggested, by the thinness and apparent permeability of the cuticular walls of the external process and by the presence of large vacuoles surrounding the fascicle of sense cell process [31]. These sensilla respond to environmental odours and to pheromones [32,33].

The different subtypes of MpSs relate to the difference in the number of pores and the branching of the dendrites. Olfactory sensilla investigated histologically so far, have cuticular surfaces perforated with numerous pores. Perforations or pores seen on the surface of the hairs are remarkable in possessing a fine structure, which perfectly illustrates the concept of multiple receptor sites [25]. Associated with each cuticular pore is a pore cavity or pore kettle from which extends four to eight pore filaments or pore tubules into the lumen of the sensillum [4]. Pore tubules form a connection between pores and dendrites for the passage of molecules. The branching of the dendrites in *B. carambolae* differs from both *B. dorsalis* [34] and *C. capitata* [19], where no branching was found. But branching of dendrites among tephritids was found in *A. ludens* [13] and *B. tryoni* [10]. Their functional significance is yet to be determined. However, Mellor and Anderson [35] suggested that the increased surface area of branched dendrites might be an adaptation to increase the sensitivity of the sensory cells. Also, Lewis [25] has suggested that dendrite branches functions as imperfect cables in which receptor potential is conducted decrementally towards electrogenic region where action potential may be initiated. Nevertheless multiple branching of olfactory sensory dendrites has apparently evolved several times in different orders of insects, and this would be surprising if the system were functionally less efficient [25].

A single sensory pit is seen on the funiculus. Cross section has revealed the presence of sensilla basiconica, which probably relates its function to chemoreception [27]. The number of pits present in flies differs from species to species. The function of these pits in *B. carambolae* and other higher Dipterans could be a way of increasing the receptive surface of the antennae and concentrating the stimuli in the locality of the sensilla. Some of the pit sensilla may be olfactory receptors and some could be hygro or thermo-receptors. Only electrophysiological studies could give an accurate result of their functions.

These results will form the basis for the electrophysiological experiments to determine the function of the sensory hairs and for further research on the olfactory perception of *B. carambolae.*

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