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## ANTENNAL SENSILLA OF *TOXOTRYPANA CURVICAUDA* (DIPTERA: TEPHRITIDAE)

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### ABSTRACT

Antennal morphology and funicular sensilla of male and female papaya fruit fly, *Toxotrypana curvicauda* Gerstaecker, were studied with light microscopy and scanning electron microscopy. Antennae were 3-segmented, consisting of a scape, a pedicel, a flagellum or funiculus that bears the arista. Antennal segments of males showed a trend toward slightly greater dimensions than those of females, but the differences were not significant. Numerous microtrichia as well as trichoid, basiconic, clavate, and styloconic sensilla were observed on the funiculus, and the sensillar characteristics were similar to those reported for other tephritid species. Males and females did not differ in total number or type of sensilla found on the funiculus, but trichoid and clavate sensilla were significantly larger in females. This difference may be related to female chemoreception of male-emitted sex pheromone and of host plant volatiles.

**Key Words:** antennae, funicular sensilla, scanning electron microscopy, papaya fruit fly, *Toxotrypana curvicauda*

### RESUMEN

La morfología antenal y los sénsulos funiculares de machos y hembras de la mosca de la fruta de la papaya *Toxotrypana curvicauda* Gerstaecker fueron estudiados usando microscopía de luz y microscopía electrónica de barrido. Las antenas consistieron de un escapo, un pedicelo y un flagelo o funículo, en éste se encontró una arista. Los segmentos antenales de los machos presentaron dimensiones ligeramente mayores que las de las hembras, pero las diferencias no fueron significativas. Numerosas microtriquias, sénsulos tricoideos, basiconicos, clavados y estilocónicos se observaron en el funículo. Las características morfológicas del sénsulos funiculares fueron muy similares a los reportados para otros tefritidos. Los machos y hembras no difirieron en el número total y tipo de sénsulos funiculares, excepto que los sénsulos tricoideos y clavados fueron más grandes en las hembras que en los machos. Esta diferencia podría estar relacionada con quimiorrecepción, por parte de la hembra, de la feromona sexual emitida por los machos y de los volátiles de la planta hospedera.

Translation by the authors.

The feeding and reproductive behavior in tephritid fruit flies depends greatly on the chemical stimulus they receive and process (e.g., pheromones and plant volatiles). The primary structures involved in the initial reception of semiochemical information are the antennal chemoreceptors (Rice 1989; Keil 1999). A better comprehension of chemoreception and associated behavioral responses may be gained through knowledge of the morphology and types of chemosensilla located on the antenna (Zacharuk 1985; Keil 1999). This knowledge, integrated with input from molecular, electrophysiological, and behavioral studies, will facilitate the development of a comprehensive model of the olfactory system for tephritid fruit flies (Zacharuk 1980; Sivinski & Calkins 1986; Rice 1989; Keil 1999).

The antennal morphology and types of sensilla have been described for several tephritid species of economic importance. Among these are *Bactrocera (Dacus) oleae* Gmelin (Hallberg et al. 1984); *Bactrocera (Dacus) tryoni* Froggatt (Gianakakis & Fletcher 1985; Hull & Cribb 1997); *Ceratitis capitata* Wiedemann (Levinson et al. 1987; Mayo et al. 1987; Dickens et al. 1988; Bigiani et al. 1989); *Anastrepha ludens* Loew, *Bactrocera (Dacus) cucurbitae* Coquillett, *Bactrocera (Dacus) dorsalis* Hendel (Dickens et al. 1988), *Eurosta solidaginis* Fitch (Vasey & Ritter 1987) and *Anastrepha serpentina* Wiedemann (Castrejón-Gómez 2006). For most of these species, the principal funicular sensilla are trichoid (2 subtypes), basiconic (4 subtypes), clavate, and styloconic. The first 3 types are single-walled sensilla with pores

and the last one is double-walled sensillum with pores (Gianakakis & Fletcher 1985; Hull & Cribb 1997). The trichoid and some basiconic sensilla are considered as sex pheromone receptors, some basiconic and clavate sensilla are involved in the reception of plant volatiles, and the styloconic sensilla seem to have hygrothermosensitive functions (Levinson et al. 1987; Mayo et al. 1987; Dickens et al. 1988).

For *Toxotrypana curvicauda* Gerstaecker, the major tephritid pest of papaya fruit (*Carica papaya* L.) in America (Landolt 2000), there is only one report on the general morphology of the antenna and a description of some antennal sensilla (Rojas et al. 2001). The number and type of sensilla found on the funiculus and potential sexual dimorphism in these structures have not been determined. This work describes the morphology of the antenna and the funicular sensilla present in male and female *T. curvicauda*.

## MATERIALS AND METHODS

### Insects

Papaya fruits infested with larvae were collected from an orchard located at Centro de Desarrollo de Productos Bioticos, IPN, in Yauatepec, Morelos, Mexico (between 18° 05' north latitude and 99° 03' west longitude). In the laboratory, the fruits were cut open and the larvae of *T. curvicauda* were collected, identified, and placed in 500-mL plastic containers with soil from the original collection site. The containers were kept inside acrylic cages (30 × 30 × 30 cm) under environmental conditions of 25 ± 1.0°C, 50-60% RH, and natural light until adult emergence. Newly emerged adults were separated by sex, and were given sugar and water in cages described previously. Microscopy studies were conducted with antennae obtained from adults that were 6-10-d-old.

### Microscopy

For light microscopy, antennae were excised and washed in 10% KOH at 80°C for 60 min, then rinsed with distilled water for 60 min. They were then put in Petri dishes and dehydrated in 70, 80, 90%, and absolute ethanol, 1 min each. The dehydrated antennae were rinsed in xylol for 10 min, then put individually in Canadian balsam and observed under a light microscope (AO Microstar, American Optical Co., Buffalo, NY). Micrographs of each antenna were obtained with a photomicroscope Tessovar (Carl Zeiss Oberkochen, Germany) with a digital camera (Pixera Professional, Pixera Co., Los Gatos, California). The length of the antennae, and the length and width of the scape, pedicel and funiculus were measured with the Image Tool 3.0 software (Wilcox et al. 2002)

based on the micrographs of 12 individuals of each sex.

For scanning electron microscopy (SEM), material was prepared according to Valdez (1991). The antennae were dehydrated in ethanol (80, 90%, and absolute), dried in a critical-point drier (SAM-DRI-780A, Tousimis, Rockville, MD, EU) and coated with gold in a metal ionizer (JEOL Ltd, Akshima, Japan) for SEM at 15 kV (JEOL-35C, JEOL Ltd). The antennae from 4 males and 4 females were mounted, with the flattened interior surface of the funiculi exposed. Sensillar types were identified according to Rice (1989) and Keil (1999). The SEM images obtained showed circular bases of the sensilla in such a way as to permit counting of individual sensilla per image, and stimulation (Image Tool 3.0) of the total number of funicular chemoreceptors. In addition, SEM images were used to calculate the basal length and width of each olfactory receptor. Measurements were taken from 20 trichoid and basiconic sensilla, and from 10 clavate and styloconic sensilla.

### Statistical Analysis

Data on antennal length, dimensions of segments, total number of sensilla, and basal length and diameter of each type of sensilla were compared between sexes by Student *t* test and SigmaStat 3.0 (Systat Software, Port Richmond, CA).

## RESULTS

### General Morphology of the Antennae

Typical of tephritid fruit flies, the antennae of *T. curvicauda* were located in a frontal depression (antennal pit) and consisted of three segments - a scape, a pedicel, and a flagellum or funiculus (Fig. 1A). Total length of the antenna and the length and width of the antennal segments are shown in Table 1. The antennal segments of males tended to be larger than those of females, but there were no significant differences. Antennal segments of both sexes had the same general organization and pattern of sensorial structures. The scape and pedicel were uniformly covered with microtrichia and had a series of larger bristles located distally, but both segments lacked olfactory sensilla. Numerous microtrichia as well as trichoid, basiconic, styloconic, and clavate sensilla were observed on the funiculus. On the dorso-proximal end of the funiculus was found the arista, a prominent bristle covered with tactile hairs (Fig. 1A). In the latero-proximal area there was a sensory pit that contained several sensilla in its interior (not shown).

### Funicular Sensilla

The total number of funicular sensilla observed in males was greater than in females, but

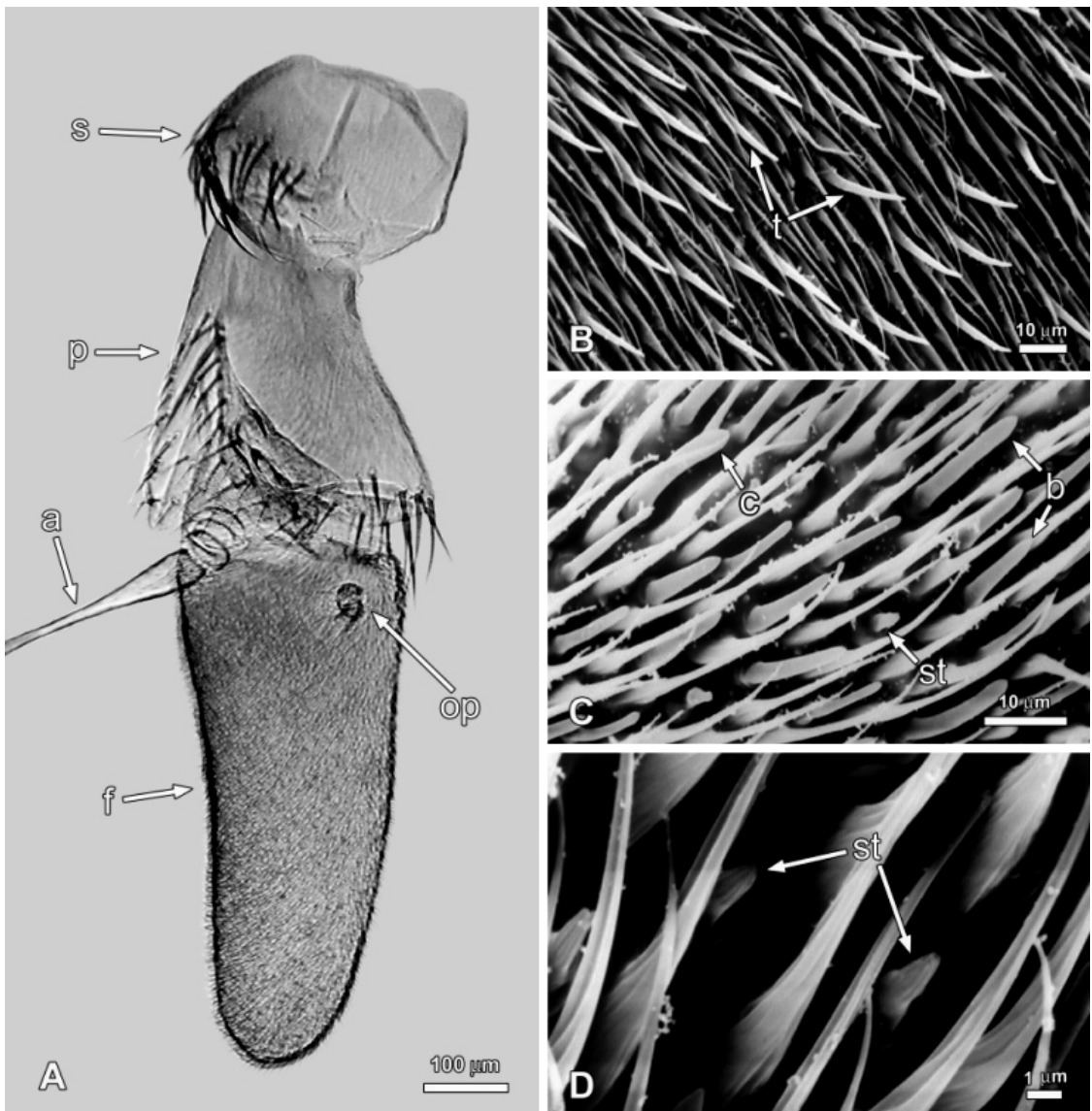


Fig. 1. Light micrograph of the antenna of *Toxotrypana curvicauda* (A) showing scape (s), pedicel (p), funiculus (f), arista (a), and the olfactory pit (op). Scanning electron micrographs of the funicular sensilla (B, C, D), showing trichoid (t), basiconic (b), clavate (c) and styloconic (st) sensilla.

the difference was not significant. The average number in males was  $3337.25 \pm 90.24$  and that in females was  $2998.00 \pm 108.97$ . The longest ( $13.28\text{--}24.44\text{ }\mu\text{m}$ ) and most conspicuous sensilla were of the trichoid type, which were thin, conic, sharply pointed, and had a smooth wall (Fig. 1B). Trichoid sensilla were significantly longer in females than in males, but were thicker in males (Table 2). The basiconic sensilla, well distributed over the funicular surface, were characterized as digitiform (finger-like) with a rounded point and a smooth surface (Fig. 1C). Basiconic sensilla showed a great variation in length ( $7.7\text{--}18.1\text{ }\mu\text{m}$ ) with no

significant differences between the sexes (Table 2). The least common receptor type was the clavate sensillum, which was localized on the proximal end of the funiculus, close to the pedicel. Clavate sensilla were similar to the basiconic sensilla but shorter and club-like (Fig. 1C). Both length and basal diameter of clavate sensilla were significantly greater in females than in males (Table 2). The styloconic sensilla were identified by the presence of distinctive longitudinal grooves in their walls, particularly over the distal half (Fig. 1D). They were distributed uniformly over the funicular surface and were the shortest ones ( $2.17\text{--}$

TABLE 1. ANTENNAL LENGTH AND WIDTH (μm) OF MALES AND FEMALES OF *TOXOTRYPANA CURVICAUDA*.

Segment	Length		Width	
	Males	Females	Males	Females
Scape	198 ± 11	195 ± 14	257 ± 14	219 ± 18
Pedicel	338 ± 11	328 ± 11	241 ± 08	237 ± 13
Funiculus	631 ± 10	623 ± 13	225 ± 04	204 ± 12
Aristae	1293 ± 27	1340 ± 35	44 ± 1.1	49 ± 1.4
Total length of antenna	1157 ± 18	1133 ± 22	—	—

Values are mean ± standard error; *n* = 12. There were no significant differences between sex for any segment parameter (Student *t*, *P* > 0.05).

3.71μm). sensilla observed. The length and basal diameter of styloconic sensilla were similar in both sexes (Table 2).

DISCUSSION

The antennae of *T. curvicauda* were very similar in terms of their general structure to those of other fruit flies studied such as *A. ludens*, *C. capitata*, *D. cucurbitae*, *D. dorsalis*, and *A. serpentina* (Levinson et al. 1987; Dickens et al. 1988; Castrejón-Gómez 2006), although they were longer and wider than those in the other species.

In *T. curvicauda*, both sexes had only trichoid sensilla on the scape and pedicel. It has been proposed that these structures, since it do not have cuticular pores, have a mechanoreceptor function related to movements of the antenna (Keil 1999). On funiculus there were 4 morphologically distinct types of the sensilla: trichoid, basiconic, clavate, and styloconic. These sensilla were similar to the ones reported in other species of tephritids (Hallberg et al. 1984; Gianakakis & Fletcher 1985; Levinson et al. 1987; Mayo et al. 1987; Vasey & Ritter 1987; Dickens et al. 1988; Bigiani et al. 1989; Hull & Cribb 1997; Castrejón-Gómez 2006). Ultrastructural and electrophysiological studies have confirmed the olfactory function of the first 3 and the hygrothermosensitive function of the last one (Levinson et al. 1987; Dickens et al. 1988; Hull & Cribb 1997).

The dimensions and the total number of the sensilla observed in *T. curvicauda* are within the interval reported for other tephritids (Dickens et al. 1988; Rice 1989). The males and females of *T. curvicauda* had the same type and total number of funicular sensilla.

The morphological characteristics of the funicular sensilla of *T. curvicauda* were also very similar to the ones observed in other tephritids (Dickens et al. 1988; Levinson et al. 1987, Hull & Cribb 1997; Castrejón-Gómez 2006). However, trichoid and clavate sensilla of the females were significantly longer than those of the males. This could indicate that these sensilla function in detection of host plant volatiles and/or sex pheromone released by males. The sensory pit observed in the funiculus of both sexes of *T. curvicauda* has also been reported in *A. ludens*, *C. capitata*, *B. cucurbitae*, *B. dorsalis* (Dickens et al. 1988), and *A. serpentina* (Castrejón-Gómez 2006). The existence of basiconic sensilla in the sensory pit has been noted (Dickens et al. 1988), and this appears to be true for papaya fruit flies as well.

With the exception of differences in the size of the trichoid and clavate sensilla, sexual dimorphism was not found in the rest of the structures studied. Although the differences found in this fruit fly could be important to sensory reception of sexual pheromones and host plant volatiles, it is necessary to prove the response to these compounds in the receptive-olfactory neurons of each

TABLE 2. LENGTH AND WIDTH (μm) OF FUNICULAR SENSILLA OF MALES AND FEMALES OF *TOXOTRYPANA CURVICAUDA*.

Sensillum	Length		Width	
	Males	Females	Males	Females
Trichoid	17.79 ± 0.54 a	19.34 ± 0.44 b	3.29 ± 0.08 a	3.75 ± 0.09 b
Basiconic	12.17 ± 0.51 a	12.43 ± 0.39 a	2.67 ± 0.12 a	2.48 ± 0.09 a
Clavate	9.12 ± 0.20 a	10.16 ± 0.25 b	1.22 ± 0.03 a	1.36 ± 0.03 b
Styloconic	2.90 ± 0.16 a	2.91 ± 0.29 a	1.76 ± 0.11 a	1.95 ± 0.12 a

Values are mean ± standard error; *n* = 20 for the first 2 rows and *n* = 10 for the last 2 rows. The values for each sensillum within a parameter by sex followed by the same letter are not significantly different (Student *t*-test, *P* > 0.05).

type of sensilla. The differences in relation to the processing of the sexual pheromone and host plant volatiles between males and females of *T. curvicauda* could be presented in other structures involved in olfactory signal processing (e.g., glomeruli of antennal lobe) (Hansson & Christensen 1999), as it occurs in other insects.

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