

Research Article

# Morphometrics and distribution of antennal sensillae of both sexes of *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae)

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# Abstract

Fall Armyworm, *Spodoptera frugiperda*, is a devastating invasive pest persisting as a menace hampering the progress of Indian agriculture. The morphometrics and distribution of antennal sensilla of both sexes of *S. frugiperda* were investigated in the study. The antenna was filiform for both sexes and was composed of scape, pedicel and flagellum. Male antennas had more subsegments (65-71) in the flagellum than female (56-70) antennas. Male and female *S. frugiperda* antennae possessed eight types of sensilla: sensilla trichoidea, sensilla chaetica, sensilla coeloconica, sensilla styloconica, sensilla basiconica, sensilla auricillica, sensilla squamiformia and Böhm's bristles. Sensilla trichoidea was the most abundant sensilla found in the antenna of both sexes and was more abundant in males than in females. It was also noticed that male antenna was longer than the female antenna. The results of the present study helps to assess alternative management strategies with an electrophysiological response of the pest towards sex pheromones and in combination with plant info chemicals for monitoring and management of *S. frugiperda* in agricultural ecosystems.

Keywords: Antennal morphology, Distribution, Spodoptera frugiperda, Sensillae types

# INTRODUCTION

The fall armyworm (FAW), Spodoptera frugiperda (J E Smith) (Lepidoptera: Noctuidae), is a devastating invasive pest introduced to India in 2018 (Ganiger *et al.*, 2018; Sharanabasappa *et al.*,2018). This pest causes enormous economic loss due to its high dispersal ability, wider host range and higher egg laying capacity (Chormule *et al.*, 2019). Until 2015, the pest had not been reported other than from the Americas, its place of origin, but it wreaked tremendous economic havoc in African countries after its introduction in 2016 (Goergen *et al.*, 2016). Fall armyworm is a polyphagous pest that shows a specific preference for the Poaceae family (Casmuz *et al.*, 2010) and is reported to feed on 353 host plants widely distributed in various families (Montezano *et al.*, 2018). Crops extensively damaged

by FAW include rice, maize, sorghum, sugarcane, onion, cotton, cabbage, tomato, beet, potato, pasture grasses, peanut, soybean, alfalfa and millets (Pogue, 2002; CABI, 2019).

At the outset, it is essential to focus our attention on the crippling impact of this pest on India's maize cultivation. Maize is the third most important crop in India after rice and wheat and is cultivated in an area of approximately 9.03 million ha with a production of 27.72 million tons (FAO STAT, 2019). Invasion of fall armyworms is causing a considerable dent in maize productivity in India. It has been reported that maize production in India fell from 28.7 million tons (2017) to 27.8 million tons (2019), with a loss of 3.2 percent due to FAW attacks (Manupriya, 2019). The percentage of damage caused to plant parts ranged between 25 and 50%, and grain yield decreased by 58% (Chimweta *et al.*, 2019).

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Farming communities mostly rely upon synthetic insecticides to control invasive pestssuch as *S. frugiperda*, which always poses a great risk to the environment and the health of consumers and negatively affects non-target beneficial insects. Insecticides have been used to control this pest in Latin America (Gutiérrez-Moreno *et al.*, 2019), but their high cost and insecticide resistance development in *S. frugiperda* make them unsustainable in the long run. The absence of Bt maize in many developing countries has led to the indiscriminate use of insecticides, which has ultimately paved the way to the build-up of resistance to several synthetic insecticides (Yu, 1991). However, for most Bt proteins, FAW has developed field evolved resistance (Gutiérrez-Moreno *et al.*, 2019).

The corn strain 'C' of the fall armyworm prefers corn, sorghum, and cotton, whereas the rice strain 'R' prefers rice and turfgrass (Nagoshi and Meagher, 2016). With reports of the R-strain and C-strain in populations of fall armyworms (Unbehend *et al.*, 2013), the failure of readily available management practices and the widening host range of fall armyworms in India, it is important to develop an accurate and precise formulation of sex pheromone lures in combination with plant volatiles for efficient mass trapping and management of pests under Indian conditions.

Insect antennae play a crucial role in insect behaviour by guiding them in locating their habitats and mates (Chapman, 1998). Various types of sensory structures are present on the antennae of insects and function as chemoreceptors, thermoreceptors, hygroreceptors and  $CO_2$  receptors (Keil, 1999). The study of antennal morphology and sensillal distribution on antennae of *S. frugiperda* is fundamental to understanding the type of sensillae and their role in the perception of pheromones and plant volatiles. The study was aimed at studying the morphometrics and distribution of antennal sensilla of both sexes of *S. frugiperda*.

## MATERIALS AND METHODS

# Insects

Spodoptera frugiperda egg masses were collected from different maize growing research plots at the Tamil Nadu Agricultural University campus, Coimbatore (Tamil Nadu), and upon hatching, the larvae were reared in an artificial diet modified by Department of Agricultural Entomology, Tamil Nadu Agricultural University with lablab as a major ingredient. After pupation, the pupae were separated and kept in a cage containing sugar solution and *Nerium oleander* twigs as an ovipositional site. Freshly emerged male and female moths were used to study antennal morphology by using scanning electron microscopy (SEM).

# Sample preparation for SEM

A Quanta 250 scanning electron microscope manufac-

tured by Field Electron and Ion Company (FEI), Czech Republic with a tungsten electron source and Everhart Thornley detector in the Department of Nanoscience and Technology, TNAU, Coimbatore, was used for image capture. Sample preparation of the insect antenna for studying antennal morphology was carried out based on the methodology followed by Malo *et al.* (2004) with minor modifications.

Male and female antennae of 5 adults each were separately soaked in 2% formaldehyde followed by 70% ethanol solution for 24 hours. The scales were removed by ultrasonication with a Labman Scientific Instruments ultrasonic cleaner (LMUC-6) at 40  $\pm$  3 kHz ultrasonic frequency and 150 W ultrasonic wattage for 50 minutes. The antennal samples were then dehydrated in ethanol solutions containing 80, 90 and 100% ethanol for one hour each. The sensilla characteristics were studied using SEM after they were critical point dried and gold-coated (30 nm).

Observations such as the average length, the basal diameter of antennae and number of antennal segments of each sex were counted. Length and basal diameter of specific antennal segments, scape, pedicel, 1<sup>st</sup> flagellar subsegment, 21<sup>st</sup> flagellar subsegment, 41<sup>st</sup> flagellar subsegment, 61<sup>st</sup> flagellar subsegments, penultimate flagellar subsegments and apical flagellar subsegments were taken. Different types of sensillae were separately identified, and measurements were taken for each sex.

# Terminology and statistical analysis

The differences in the size of the whole antenna, antennal segments and number and size of sensilla of both sexes of *S. frugiperda* were analysed and compared using unpaired t- tests. Values are expressed as Mean  $\pm$  SEM. All statistical analyses were accomplished with SPSS version 16.0 software. The sensilla identification and terminology used for relating these sensilla were followed based on the scientific reports published by Schneider, 1964; Malo *et al.*, 2004.

# **RESULTS AND DISCUSSION**

#### Morphology of antenna

The antenna is composed of three constituent parts: scape, pedicel and flagellum. The male antenna was longer than the female antenna, and the antennal length varied significantly between sexes (t = 4.96; df = 18; P < 0.0001; Table 1). Male antennas had longer (t = 8.03; df = 18; P < 0.0001; Table 1) and wider (t = 14.92; df = 18; P < 0.0001; Table 1) scapes than female antennas. Pedicel was shorter (t = 13.16; df = 18; P < 0.0001; Table 1) in females with a narrow basal diameter (t = 61.73; df = 18; P < 0.0001; Table 1) than in males. Gradual narrowing of antennal segments to the apical end was noticed, which indicates the filiform na-

ture of the antenna (Table 2). Male antennas had more subsegments (65-71) in the flagellum than female (56-70) antennas (t = 1.34; df =18; P=0.1; Table 1). The present findings on the antennal morphology of *S. frugiperda* are similar to the findings of Malo *et al.*, 2004.

Antenna possesses a dense covering of scales (Fig 1. B), which might serve as a protectant against external stresses, such as mechanical damage, as seen in *Tuta absoluta* (Bawin *et al.*, 2017). However, their placement on the antennae of insects such as *Yponomeuta* spp. promote the ability to perceive volatile stimuli by trapping and concentrating odourant molecules (Van der pers *et al.*, 1980).

## Types of antennal sensilla

Male and female *S. frugiperda* possess eight types of sensilla: sensilla trichoidea (ST), sensilla coeloconica (SCo), sensilla chaetica (SCh), sensilla basiconica (SB), sensilla styloconica (SSt), sensilla auricillica (SAu), sensilla squamiformia (SSq) and Böhm's bristles (Fig. 2C and 3A-F). The antennal morphology and distribution of sensilla of *S. frugiperda* observed are similar to the findings of Malo *et al.* (2004). The basic arrangement pattern of sensilla was similar to that of other *Spodoptera* species (Jefferson *et al.*, 1970; Ljungberg *et al.*, 1993; Monti *et al.*, 1995; Seada., 2015).

#### Sensilla trichoidea (ST)

These are the most abundantly found sensillae in the antennae of both *S. frugiperda* males and females.

They are sharp-pointed structures that are generally curved at the apex region (Fig. 2C). The wider base of the sensillum inserts into the antennal socket. Sensilla trichoidea was found in both sexes but was longer (t = 9.75; df = 18; P < 0.0001; Table 3) and more frequent in male antennae (t = 23.07; df = 18; P < 0.0001; Table 4). Unlike antennae of both sexes of S. littoralis, S. descoinsi and S. latifascia, which possess two different size classes of trichoid sensilla (Ljungberg et al., 1993 and Monti et al., 1995), S. frugiperda antennae have a single class of sensilla trichoidea. Three types of trichoid sensilla were reported to be present in both male and female S. exigua and S. ornithogalli (Jefferson et al., 1970). Sensilla trichoidea is primarily associated with sex pheromone perception (Shneider, 1964 and Steinbrecht, 1995) and is sensitive to plant volatiles in female Heliothis virescens (Hillier et al., 2006) and S. litura (Zhang et al., 2013). In fruit borers, Conogethes punctiferalis, Grapholita molesta, and Spilonota albicana, they are involved in the perception of both host plant volatiles and sex pheromones (Li et al., 2018).

Sensilla chaetica (SCh) are straight sensillae with a wider base, curved apex and truncated tip. Each flagellar segment possesses six sensilla chaetica, but the apical segment has eight chaetica sensilla (Fig. 2C). Sensilla chaetica was classified into three types namely, central chaetica, lateral chaetica and dorsal chaetica, based on their position in flagellar segments and length. Central chaetica was longer (t = 10.04; df = 18; P < 0.0001; Table 3) and had a wider basal diameter (t

 Table 1. Spodoptera frugiperda antennal segment morphometrics (mean ± SEM)

Observations	Male	Female
Antennal length (mm)	8.98 ± 0.03 <sup>a</sup>	8.76 ± 0.03 <sup>b</sup>
No. of subsegments	67 ± 0.52 <sup>ª</sup>	$66.1 \pm 0.43^{a}$
Scape length (µm)	281.69 ± 0.41 <sup>ª</sup>	$276.54 \pm 0.49^{b}$
Basal diameter of scape(µm)	202.17 ± 0.41 <sup>a</sup>	197.56 ± 0.15 <sup>b</sup>
Length of pedicel(µm)	151.98 ± 0.26 <sup>ª</sup>	147 ± 0.22 <sup>b</sup>
Basal diameter of pedicel(µm)	163.46 ± 0.16 <sup>a</sup>	152.02 ± 0.09 <sup>b</sup>

Unpaired t test, P<0.05, N=10 (5 individuals of each sex), male and female values followed by the same letter within a row are not significantly different.

Table 2. Length and base	al diameter (um)	of selected antennal f	lagellar segments	(Mean ± SE)	of Spodoptera t	fruaiperda
	VI /		5 5	\ /		

Flagellum number	N	lale	Female		
	Length (µm)	Basal diameter (µm)	Length (µm)	Basal diameter (µm)	
1 <sup>st</sup>	82.78±0.14 <sup>ª</sup>	130.87±0.28ª	77.93±0.2 <sup>b</sup>	128.4±0.17 <sup>b</sup>	
21 <sup>st</sup>	95.73±0.26aª	106.29±0.14ª	94.50±0.28 <sup>b</sup>	104.66±0.25 <sup>b</sup>	
41 <sup>st</sup>	79.43±0.16ª	82.69±0.12 <sup>a</sup>	76.61±0.15 <sup>b</sup>	80.79±0.11 <sup>b</sup>	
61 <sup>st</sup>	59.13±0.17ª	72.67±0.13ª	57.65±0.14 <sup>b</sup>	71.27±0.25 <sup>b</sup>	
Penultimate	57.58±0.09ª	66.83±0.09 <sup>a</sup>	57.08±0.18 <sup>b</sup>	64.54±0.15 <sup>b</sup>	
Apical	109.16±0.17ª	57.83±0.15ª	88.33±0.26 <sup>b</sup>	56.65±0.16 <sup>b</sup>	

Unpaired t test, P<0.05, N=10 (5 individuals of each sex), male and female values followed by the same letter within a row are not significantly different.

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Sensilla types		Male	Female		
	Length (µm)	Basal diameter (µm)	Length(µm)	Basal diameter (µm)	
Trichoidea	34.68±0.16 <sup>ª</sup>	2.26±0.06 <sup>a</sup>	32.43±0.17 <sup>b</sup>	2.15±0.03 <sup>b</sup>	
Chaetica (Central)	41.22±0.24 <sup>a</sup>	5.16±0.02 <sup>a</sup>	38.28±0.16 <sup>b</sup>	4.53±0.02 <sup>b</sup>	
Chaetica (Lateral)	67.51±0.22 <sup>ª</sup>	5.75±0.02 <sup>a</sup>	56.17±0.19 <sup>b</sup>	4.69±0.02 <sup>b</sup>	
Chaetica (Dorsal)	46.48±0.24 <sup>a</sup>	3.70±0.01 <sup>a</sup>	45.69±0.16 <sup>b</sup>	3.79±0.02 <sup>ª</sup>	
Styloconica	27.58±0.10 <sup>ª</sup>	6.11±0.01 <sup>a</sup>	25.41±0.12 <sup>b</sup>	6.34±0.02 <sup>a</sup>	
Coeloconica	-	9.21±0.02 <sup>a</sup>	-	9.71±0.01 <sup>a</sup>	

#### Table 3. Morphometrics of antennal sensilla (µm) (Mean ± SE) of Spodoptera frugiperda

Unpaired t test, P<0.05, N=10 (5 individuals of each sex), male and female values followed by the same letter within a row are not significantly different

Table 4. Mean number of sensilla of male and female Spodoptera frugiperda (Mean ±SE)

	Number of sensilla		
i ype of sensina	Male	Female	
Trichoidea	3638.5±30.98 <sup>a</sup>	2734.2±24.03 <sup>b</sup>	
Chaetica (three subtypes)	404.4±2.45 <sup>a</sup>	398.6±1.33 <sup>b</sup>	
Styloconica	60.1±0.9 <sup>a</sup>	59.1±0.95ª	
Coeloconica	405.3±1.41 <sup>a</sup>	400±1.25 <sup>b</sup>	

Unpaired t test, P<0.05, N=10 (5 individuals of each sex), male and female values followed by the same letter within a row are not significantly different.

= 28.18; df = 18; P < 0.0001; Table 3) in males than in females. Males had significantly longer lateral chaetica than females (t = 39.52; df = 18; P< 0.0001; Table 3), and they also had larger basal diameters than females. (t = 38.42; df = 18; P < 0.0001; Table 3). Dorsal chaetica was shorter in females than in males (t = 2.73; df = 18; P = 0.007; Table 3), but the basal diameter was larger in females than in males (t = 4.28; df = 18; P= 0.0002; Table 3). Female antennas had fewer of the three types of chaetica sensilla than male antennas (t = 2.08; df =18; P=0.03; Table 4). The Sensilla chaetica distribution is similar to that observed in S. littoralis, with the disposition of two central chaetica, two dorsal chaetica and two lateral chaetica in each flagellar segment (Seada, 2015). They are presumed to play a role in gravid Tuta absoluta oviposition behaviour (Bawin et al., 2017).

#### Sensilla coeloconina (SCo)

Sensilla coeloconina or pit pegs are surrounded by 12-14 cuticular spines and have a peg-like structure with striations emerging from its centre (Fig. 3A). They are located from the middle to the distal portion of each flagellar segment. Females possessed sensilla coeloconica with a significantly wider base diameter than males (t = 28.52; df= 18; P < 0.0001 Table 3). Male antennae had a greater number of sensilla coeloconica (t = 2.81; df = 18; P = 0.006; Table 4). Sensilla coeloconica, having an olfactory function in *Bombyx mori*, does not respond to pheromones but responds to green leaf volatiles and a group of aromatic compounds (Hunger and Steinbrecht, 1998). In predaceous biting midges such as *Forcipomyia, Atrichopogon, Aus*- *troconops, Culicoides* and *Brachypogon*, spines surrounding pegs in sensilla maintain a high level of humidity, which aids in the capture and transport of volatile molecules through channels in the peg wall (Urbanek *et al.*, 2014).

### Sensilla styloconica (SSt)

This sensillum is always found in the upper-middle region of each flagellar subsegment and has a smooth petiole and a conic extremity with one to three apical structures (Fig. 3B). The male antenna had a longer sensilla styloconica (t = 13.62; df = 18; P < 0.0001; Table 3), but the female antenna had a wider sensilla styloconicabase diameter (t = 7.99; df = 18; P < 0.0001; Table 3). The number of sensilla styloconica present in both sexes was not significantly different (t = 0.76; df = 18; P = 0.23; Table 4). They function as gustatory receptors in *Bombyx mori* (Zhang et al., 2013), where they are involved in taste and related responses (Agnihotri *et al.*, 2016).

#### Sensilla basiconica (SB)

This is the least frequent sensilla, with a wider base and rounded apical area (Fig. 3C). These are important olfactory setae that recognize plant odours (Schneider, 1964). In *Tuta absoluta,* sexual dimorphism is observed for sensilla basiconica and is assumed to be involved in plant volatile perception (Bawin *et al.*, 2017)

#### Sensilla auricilica (SAu)

These sensilla are either dorsoventrally flattened or have a typical rabbit ear shape and are located among



**Fig. 1.** SEM images of antenna of S. frugiperda. A) Whole antenna. B) Sc, Scales. C) Scape and pedicel with Böhm's bristles; Sp, Scape; Bb, Böhm's bristles; Pe, Pedicel



**Fig. 2.** SEM images of antenna of S. frugiperda. A) Flagellar subsegment of antenna showing various types of sensilla; SSt, Sensilla styloconica; SAu, Sensilla auricillica; ST, Sensilla trichoidea; SCh, Sensilla chaetica; SCo, Sensilla coeloconica; Sc, Scales. B) Apical segment showing eight sensilla chaetica. C) ST, Sensilla trichoidea; SCh, Sensilla chaetica



**Fig. 3.** Scanning electron micrographs of S. frugiperda. A) SCo, Sensillum coeloconicum; Sp, spines. B) SSt, Sen sillum styloconicum; circle denotes its spines. C) SB, Sensillum basiconicum. D) SSq, Sensillum squamiformia. E) SAu, Sensillum auricillica. F) Bb, Böhm's bristles

scales (Fig. 3E). Sensilla auricillica are involved in plant volatile and pheromone detection, as observed in males and females of *Scoliopteryx libatrix* Linnaeus (Noctuidae) (Anderson *et al.*, 2000).

# Sensilla squamiformia (SSq)

These sensilla resemble scales in morphology with their longitudinal ridges but are narrower than scales and taper at the distal end (Fig. 3D). These are located among the scales in the dorsal part of the antenna and are also present on the scape and pedicel of the antenna. Sensilla squamiformia is presumed to act as a mechanoreceptor (Schneider, 1964) involved in the perception of air movements in *Tuta absoluta* (Bawin *et al.*, 2017)

# Böhm's bristles (Bb)

These sensilla look like short thorns in appearance and are shorter and more pointed than sensilla chaetica (Fig. 3F). These are clustered around the basal region of the scape and the intersegmental region between the scape and pedicel. It has been reported that when Böhm's bristles were surgically removed from the basal segments of *Daphnis nerii* antennae, moths were unable to bring their antennae into flight position, causing frequent collisions with the flapping wing (Krishnan *et al.*, 2012).

# Conclusion

The study of antennal morphology and sensilla distribution on antennae of S. frugiperda is fundamental in understanding the types of sensillae and their role in perception of pheromones and plant volatiles. The study revealed that the antennas of both sexes of S. frugiperda was filiform in nature and possessed eight types of sensilla, namely, sensilla trichoidea, sensilla chaetica, sensilla coeloconica, sensilla styloconica, sensilla basiconica, sensilla auricillica, sensilla squamiformia and Böhm's bristle. Sensilla trichoidea was the most abundant sensilla in the antennae of both sexes, with higher abundance in male antennae. Alternative management strategies for monitoring and managing S. frugiperda using electrophysiological response of the pest towards sex pheromones and in combination with plant info-chemicals can be made possible.

#### **Conflict of interest**

The authors declare that they have no conflict of interest.

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