Sexual dimorphism of antennal, tarsal and ovipositor chemosensilla in the African stemborer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae)

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**Abstract.** The number and distribution of chemosensilla located on different organs of *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) males and females are described based on observations using scanning electron microscopy, selective staining with silver nitrate, and gustatory electrophysiological recording. The antennae and the fifth tarsomere of the prothoracic legs of both sexes bear chemosensilla: uniporous chaetica and multiporous trichoidea sensilla. However, there is a sexual dimorphism in the number and size of sensilla on these organs. The distal part of the ovipositor has uniporous gustatory chemosensilla of the chaetica type. The involvement of these sensilla in oviposition site selection by *B. fusca* is discussed.

**Résumé.** Dimorphisme sexuel des sensilles chémoréceptrices des antennes, des tarses et de l’ovipositeur du foreur de graminées africain, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae). Le nombre et la distribution des sensilles chémoréceptrices présentes sur les différents organes sensoriels des mâles et femelles de *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) ont été étudiés sur la base d’observations en microscopie électronique à balayage, par leur réponse à une coloration sélective au nitrate d’argent et à l’électrophysiologie de contact. Les antennes et le cinquième article des pattes antérieures des deux sexes possèdent des sensilles chémoréceptrices identifiées respectivement comme de type chétiforme uni-poreux et comme de type trichôide multi-poreux. Un dimorphisme sexuel est observé par rapport au nombre et à la taille de ces sensilles. La partie distale de l’ovipositeur possède également des sensilles chémoréceptrices gustatives chétiformes. Le rôle de ces structures chémoréceptrices dans la sélection du site de ponte chez *B. fusca* est discuté.

**Keywords:** Stemborer, host-plant selection, scanning electron microscopy, gustatory electrophysiological recording, Africa.

In East and Southern Africa, *Busseola fusca* (Fuller 1901) (Lepidoptera: Noctuidae) is the most important insect pest of sorghum and maize in the cooler ecozones such as the mid-altitude and highlands (Kfir et al. 2002). Various control strategies based on host plant resistance and cultural control have been tried, some with partial or local success, but none have provided a complete solution (Kfir et al. 2002). Recently, habitat-management strategies involving the use of “push-pull” or stimulo-deterrent diversionary tactics have been developed (Khan et al. 2000). Thereby, the stemborers are attracted and retained on trap plants (pull) planted as border rows, repellent intercrops (push) prevent them from infesting the crop. The effective use of such strategies requires a good understanding of the host selection and acceptance processes by the insect pest.

Given the importance of the host plant selection process for the survival of the offspring, females of *B. fusca* very likely have special organs for detection of a wide range of cues including visual, tactile, and olfactory and gustatory chemostimuli. Preliminary studies indicated that females are nocturnal and that some of these cues may play a greater role in host plant location and acceptance than others (Calatayud et al. unpublished). The role of plant volatiles as long-range chemical cues is not clearly understood in *B. fusca*, however, these chemicals may facilitate host finding by stimulating take off and flight activity in mated females. After landing on host plant, tactile and...
gustatory stimuli are likely to play a major role for the acceptance of the plant for oviposition. Physical and chemical characteristics of the oviposition site are involved in host plant acceptance for oviposition (Calatayud et al. unpublished).

The present study documents the distribution of chemosensory structures that may be involved in the process of oviposition site selection by *B. fusca* females and provides a basis for an interpretation on their role in oviposition choice. Sensilla located on the antennae and the fifth tarsomere of the prothoracic legs of both male and female *B. fusca* were compared. For females, sensilla on the ovipositor were also studied. Their physiological function was delineated based on the criteria proposed by Altner (1977) and Zacharuk (1985) with regard to morphological characteristics observed by scanning electron microscopy, penetration of dyes applied externally (Slifer 1960; Nayak & Singh 1983) and by electrophysiological tip recording (Marion-Poll et al. 1992).

**Material and methods**

**Insects**

*Busseola fusca* males and females were provided by the Animal Rearing and Containment Unit of the International Centre of Insect Physiology and Ecology (ICIPE, Nairobi, Kenya). They were reared on a meridic diet (Onyango & Ochieng’-Odero 1994). To regenerate the colony, new insects collected in the field were added three times a year.

The male and female pupae were maintained separately until emergence in a plastic box (21 cm long, 15 cm wide, and 8 cm high). A piece of wet pad maintained the moisture at > 80 % r.h. in the box. The insects were maintained in a controlled room at 26.1 ± 0.04 °C, 56.4 ± 0.4 % r.h. (means ± SE) and L12:D12 photoperiod. After three weeks of growth, the plants were placed under the same reversed photoperiod conditions. For both sexes, newly emerged adults (less than 24 h old) were directly used for microscopic preparations or anaesthetized with CO₂ for the electrophysiological experiments.

**Plants**

*Maize* (*Zea mays* L., cv. 511) seeds were provided by Simlaw, Kenya Seeds Company (Nairobi, Kenya). The plants were grown in individual plastic pots (13 cm top diameter and 12 cm high) containing peat, in a greenhouse at ICIPE. The environmental conditions were ca 31/17 °C (day/night) with L12:D12 photoperiod. After three weeks of growth, the plants were placed under the same reversed photoperiod conditions as the insects 48 h before use. A gravid female of *B. fusca* was deposited on the plant stem. The portion of the plant where the ovipositor sweep occurred, as described by Calatayud et al. (unpublished), was collected for microscopy. A portion of the same plant not touched by the ovipositing female was used as control.

**Scanning electron microscopy**

Five males and five females and the above portions of stems were fixed over-night in a 2.5 % glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) solution. Then, the insects were dissected to separate foreleg tarsi, antennae and ovipositors. The dissected organs and the stem portions were dehydrated in a graded series of ethanol (70%, 90%, and 100%) and air-dried. The specimens were mounted on stubs with conductive double-side adhesive tape, sputter-coated with gold, and finally examined with a JEOL JSM-T330A SEM.

**Silver nitrate staining**

The presence of pores in the sensilla on foreleg tarsi, antennae and ovipositors of the 10 individuals were revealed by the penetration of silver nitrate. Entire males and females were stained according to the method of Nayak & Singh (1983) modified as follows: they were first immersed for 1 h in 70% ethanol containing 1 M silver nitrate and then dehydrated in a graded series of ethanol (90% and 100%). After separation of foreleg tarsi, antennae and ovipositor, the organs were cleared in xylene over-night. Then, the samples were mounted in Mountex (Histolab) for light microscope observations.

**Electrophysiology**

Putative taste sensilla on the tarsi, the antennae and the ovipositor were probed with a tip-recording electrode to determine if the sensilla had chemosensory function. A silver wire inserted into the thorax served as a reference electrode. The sensilla were probed for electrical contact with a capillary electrode filled with 10 mM KCl, which was connected to a TasteProbe (Marion-Poll & Van der Pers 1996). The action potentials were further amplified, filtered (10-2800 Hz), recorded on a computer and analyzed for the presence of action potentials (Marion-Poll 1996).

**Data analysis**

Statistical tests were performed with Statview software (Abacus Concept, version 5.0, USA). Mann-Whitney U-test was used to compare the length and the number of antennal segments between males and females.

**Results and Discussion**

**Chemosensilla on the antennae**

The external morphology of *Busseola fusca* antennae has strong sexual dimorphism (ICIPE Annual Report 1993). Except for the basal and the fourth terminal segments, the male moth has pectinate segments along their flagellum, which are not present on female antennae (fig. 1). Antennae of males were on average 7.6 ± 0.07 mm and were significantly shorter than those of females, which measured 8.3 ± 0.08 mm (mean ± SE, n = 10) (p = 0.0002, Mann-Whitney U-test). The number of segments per flagellum did not vary with sex (62.8 ± 1.7 for males and 64.2 ± 1.0 for females; p = 0.8789, Mann-Whitney U-test). Sensilla styloconica, coeloconica, chaetica and trichoidea observed in *B. fusca* have already been described on the antennae of other noctuids such as *Trichoplusia ni* (Hübner 1802); *Helicoverpa zea* Boddie 1850; *Spodoptera ornithogalli* (Guenée 1852), *Spodoptera*
exigua (Hübner 1808), Pseudaletia unipuncta (Haworth 1809) and Copitarsia consueta (Walker 1857) (Jefferson et al. 1970; Lavoie & McNeil 1987; Castrejon-Gomez et al. 1999).

For both male and female B. fusca, one sensillum styloconicum was visible on the terminal part of each antennal segment as well as on the apical segment showing a double structure, which is frequent in noctuids (Castrejon-Gomez et al. 1999). These sensilla were argyrophilic or silver stained from their apical part (fig. 2), indicating the presence of an apical pore that suggests contact chemoreceptive function (Jefferson et al. 1970). However, this could not be confirmed by electrophysiological recording since these sensilla were not accessible to the electrode.

Sensilla coeloconica were observed on the antennae of male and female B. fusca. They also appeared to be argyrophilic. Electrophysiological recording was also not possible. Castrejon-Gomez et al. (2003) reported that these sensilla house olfactory-receptor cells in pyralids, possibly sensitive to volatile compounds.

Sensilla chaetica were observed, distributed along the antennal segments with slight differences between male and female antennae. In females, two longer (length ≈ 80 μm) and two shorter (length ≈ 60 μm) sensilla chaetica were located on the lateral and the medium part of the ventral face of the antennal segment, respectively (fig. 1a), whereas in males two longer (length ≈ 90 μm) chaetica sensilla and a shorter one only (length ≈ 54 μm) were observed (fig. 1b). Females possessed ca seven sensilla chaetica (length ≈ 83 μm) on the terminal antennal segment (fig. 1c), while male moths had six shorter sensilla chaetica (length ≈ 62 μm) (fig. 1d). For both males and females, one sensillum chaeticum (length ≈ 60 μm) was observed on the dorsal part of each segment located between the terminal and the basal segments. For both sexes, all these sensilla were silver-stained, indicating their
porous characteristic (fig. 2). Electrical contact and spike trains were recorded from long and short sensilla chaetica (fig. 3a). Such gustatory sensilla are frequently found on antennae of various species of noctuids and pyralids (Castrejon-Gomez et al. 1999; 2003). They have a basal socket, which indicates a bimodal taste/tactile function (Altner et al. 1977). During oviposition behaviour, B. fusca females walk up and down, touching the plant surface with the apical part of the antennae (Calatayud et al. unpublished) (fig. 8a and b). Since this behaviour step occurred irrespective of the plant status (host and non-host plants) and that gustatory sensilla were evident on the segments close to the terminal part of the antennae, they may play a role in the detection of plant surface chemicals that elicit host plant acceptance by the ovipositing females. For males, their function is still unclear. However, it is possible that during the evolution of the species, they

Figure 2
Silver staining impregnation of antennae of female (A, C) and male (B, D) B. fusca (A, B: middle part of the antenna; C, D: terminal part of the antenna). Both types of sensilla, chaetica (Ch) and trichoidea (Tr), were silver stained as well as the sensilla styloconica (St) and the coeloconica (Co) showing black spots distributed along the flagellum.
Chemosensory equipment of *Busseola fusca*

may have been important if mating took place on the host plant.

Sensilla trichoidea were frequently observed on the ventral face of the antennae. In females, they were 23-40 μm long while in males, the length varied between 38-46 μm on the middle part of the antennal segments and 72-83 μm on the segmental branches (fig. 1). Moreover, there were more numerous on male antennae (97-102 per segment), than on female antennae, that scored 58-61 sensilla per segment. For both sexes, the sensilla number decreases drastically with the distal position of the segment. Only 10 sensilla were count on the terminal segment. All sensilla trichoidea were argyrophilic (fig. 2), indicating that the sensilla shafts are porous. KCl solution evoked action potentials (data not shown) in these sensilla. Sensilla trichoidea are frequently described on the antennae of noctuids and pyralids (Faucheux 1990; Castrejon-Gomez et al. 2003) and, because of their multiporous nature, they are putative chemoreceptors for volatile components. The long sensilla trichoidea (72-83 μm in length) found on male antennae, were not present on female antennae. Such long sensilla trichoidea in males are frequently reported in noctuids and pyralids as dedicated to sex pheromone reception (Faucheux 1990; Castrejon-Gomez et al. 1999; 2003). For *B. fusca* females, sensilla trichoidea may play a role in the detection of volatile compounds released by the host plant. Further work remains to be done to validate this hypothesis.

**Chemosensilla on the fifth tarsomere of the prothoracic legs**

In both *B. fusca* males and females, the fifth tarsomeres of the tarsus are densely covered with scales on the dorsal and lateral sides but not ventrally (fig. 4). The tarsus is prolonged by the pretarsus, which bears two long sclerotized claws, a median arolium, and two lateral pulvilli covered by cuticular ornamental ‘microtrichia’. Two types of sensilla chaetica, short (≈ 36-50 μm) and long (≈ 57-71 μm), are distributed ventrally and laterally respectively along the fifth tarsomere (fig. 4). In *B. fusca* males, the fifth tarsomere possesses more sensilla chaetica than in females. They have about 16 sensilla chaetica: five ventral pairs, one latero-ventral pair on the distal part of the tarsomere and two lateral pairs of sensilla (fig. 4b). Females have only 8 sensilla chaetica: two ventral pairs, one latero-ventral pair on

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**Figure 3**
Electrophysiological recordings of action potentials (spikes) obtained after contact with a long lateral chaeticum sensillum of female antennae (A), a long sensillum chaeticum on the fifth tarsomere of a female prothoracic leg (B) and a short sensillum chaeticum located on the ovipositor (C) in response to dilute KCl solution. 1. Spike detected in the recording (6 ms epochs) and superposed to evaluate the distribution of their amplitude and shape; 2. Sample recordings obtained by capping the taste sensilla with a capillary tube containing KCl during 2 s. Vertical bar: 5 mV, except 3 mV for A, horizontal bar: 200 ms.

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**Figure 4**
Ventral view of the fifth tarsomere and pretarsus of the prothoracic legs of *Busseola fusca* female (A) and male (B). The fifth tarsomere bears short (a) and long (b) sensilla chaetica and the pretarsus bears long (c) and short (d) sensilla chaetica. ARO indicates the central arolium, PUL, a pulvillus; CLAW, a claw; Sc, a scale and Sp, a spine.

**Figure 5**
Silver staining impregnation of the fifth tarsomere of *Busseola fusca* prothoracic leg of female (A) and male (B). Short (a) and long (b) sensilla chaetica were silver stained.

**Figure 6**
Tip of a ventral sensillum chaeticum located on the fifth tarsomere of the prothoracic leg of *Busseola fusca* female at high magnification.
the distal part of the tarsomere and one lateral pair of sensilla (fig. 4a). All these sensilla are argyrophilic (fig. 5) and bear an apical pore as observed under high magnification (fig. 6). Trains of action potentials were recorded from these sensilla upon stimulation with KCl (fig. 3b), thus confirming their gustatory function. Taste sensilla on the fifth tarsomere have also been described in pyralids (Waladde 1983; Anderson

Figure 7
Dorsal surfaces of *Busseola fusca* ovipositor showing numerous sensilla chaetica with a basiconic form distributed along the surface of each lobe, the claws at the distal part (A, B and C) and short (a) and long (b) sensilla chaetica located in the border of the ventral surface of the distal part of each lobe (B and C). Close-up of the tip of a short sensillum chaeticum (D). Selective staining of ovipositor sensilla distributed along the distal part of the ventral surface of each lobe (E).
Typical oviposition behaviour in *Busseola fusca*: touching the plant surface with the antennae (A and B) and sweeping with the tip of the ovipositor (C and D). External surface of a maize leaf sheath after been swept by *B. fusca* ovipositor, showing several scratches (white arrowheads) left by the claws of the distal part of the ovipositor (E). External surface of a maize leaf sheath not swept by the ovipositor (=control) (F).
& Hallberg 1990; Marion-Poll *et al.* 1992; Faucheux 1995) but in *B. fusca*, their number is considerably lower. Both female and male pretarsi have two long sensilla chaetica, not argyrophilic. No electrical contact was possible for electrophysiological tip recordings on such sensilla. On the other hand, male pretarsi have an additional argyrophilic sensillum chaeticum, that is located dorsally on the pseudempodium between the two claws and overlapping above the arolium. These were not observed in females. This type of sensilla is common to many moths and has been suggested to have a contact chemo-receptive function (Marion-Poll *et al.* 1992; Maher & Thiery 2004).

**Chemosensilla on the ovipositor**

The last tergite and sternite of female *B. fusca* form two sclerotised lobes that surround the anal pore and the ovipore (fig. 7a). On the distal part of each lobe seven sclerotized claws are present. Six types of cuticular extensions are distinguishable on the ovipositor with regard to their shape, length and permeability to silver nitrate.

Numerous non-argyrophilic microtrichia are distributed over the lobe surface, similar to the pyralids (Marion-Poll *et al.*, 1992), and can be considered as simple cuticular ornamentations.

About five non-argyrophilic long sensilla chaetica (= 150-170 μm) were present on the external border of each lobe. No electrical contact was obtained for electrophysiological tip recordings, suggesting that this sensillum type is not involved in gustative function.

Shorter non-argyrophilic sensilla chaetica (= from 19 to 35 μm) are located mostly on the distal part of each lobe surrounding the claws.

Numerous other cone-shape sensilla chaetica measuring between 11 to 65 μm, with the longer ones on the ovipositor dorsal surface were also not stained by silver nitrate.

All the non-argyrophilic sensilla observed above were assumed to have mechanoreceptive function in *B. fusca*, confirming the importance of tactile input during oviposition as described by Calatayud *et al.* (unpublished).

Further six short sensilla chaetica (labelled ‘a’ in fig. 7b and c) and four long sensilla (labelled ‘b’ in fig. 7b and c), are argyrophilic and measure about 15 and 26 μm respectively. These were observed on the inner border of the ventral surface of each lobe, close to the anal and ovipositor openings. Several characteristics indicate that these sensilla possess a gustative function. A pore at the tip of each sensillum is clearly visible at high magnification (fig. 7d) and the recorded spike trains are typical of gustatory sensilla (fig. 3c).

The ovipositor of *B. fusca* bears a considerable number of mechanosensory sensilla of different lengths distributed over its surface and only about nine chemosensory sensilla are located within the inner border of the ventral surface of each lobe. These differences in number and distribution may be related to the process of probing the host plant surface for acceptance for oviposition. Prior to oviposition, *B. fusca* females were very sensitive to the shape and structure of the oviposition support (Calatayud *et al.* unpublished). The female insects usually extended the abdomen bending it ventrally, while the terminal abdominal segments protruded from their normal retracted position. This was followed by the behaviour termed as “ovipositor sweep”. It included touching the stem surface by the tip of the ovipositor followed by the dorsal extremity making broad lateral movements up and down along the stem (figs. 8c and d). It has been suggested that a combination of tactile and gustatory stimuli from the plant plays a role in the decision-making for host plant acceptance for oviposition. The insects spent much more time sweeping with the ovipositor on the more preferred plant species for oviposition (Calatayud *et al.* unpublished). It is possible that mechanoreceptors enable the female to assess the geometrical/textural configuration of the plant surface to help the placement of eggs (Hallberg & Ahman 1987). Moreover, during the “ovipositor sweep”, the claws at the distal part of the ovipositor left small injuries on the plant surface (fig. 8e). They may be deep enough to liberate inner plant cuticular compounds, which are most likely different from those present on the undamaged plant surface. The damaged plant surface may liberate both gustatory and olfactory stimuli that are detected by the ovipositor sensilla. Such a perception activated the appropriate behaviour (acceptance or rejection) depending on the nature of the chemicals.

**Conclusion**

The identification of different types of chemosensilla on the antennae and ovipositor of *B. fusca* females corroborated observation on oviposition behaviour. While the females walk down the stem of the host plant, they exhibit antennae and ovipositor movements that suggest that these organs are in contact with the stem surface. These movements can be interpreted as probing of the plant surface. The evidence of a sensory equipment allowing chemoreception on both female antennae and ovipositor confirmed that the ovipositing female evaluates the plant before deciding to lay eggs. The results suggest that during probing, the female can access plant chemicals, especially the inner chemicals.
presented on the injured part of the plant. The claws on the apical part of the ovipositor lobes injured the stem surface and the gustatory receptors located close to them enable detection of the chemicals by their apical pore. The choice of the suitable host plant is crucial for *Busseola fusca* since the neonate larvae do not have a strong propensity to migrate onto other nearby plants. Further studies are needed to identify chemical cues released by the damaged plant cuticle, that the antennae and the ovipositor perceive during the antennation and the sweeping behaviour, respectively. In addition, the role of contact chemosensilla on the tarsi remains to be elucidated.

For *B. fusca* males, apart from the possible olfactory function of the antennal trichoidea sensilla that are involved in sex pheromone detection, the role of gustatory sensilla on the antennae and the tarsi remains unclear.

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**Reference**


