

Distribution of chemo- and mechanoreceptors on the antennae and maxillae of *Busseola fusca* larvae

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Abstract

The stem borer *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) is a major pest of maize, *Zea mays* L., and sorghum, *Sorghum bicolor* (L.) Moench (both Poaceae), in sub-Saharan Africa. Like in many other lepidopteran insects, the success of *B. fusca* in recognizing and colonizing a limited variety of plants is based on the interaction between its sensory systems and the physicochemical characteristics of its immediate environment. The sensilla on the maxillary galeae of *B. fusca* larvae are typical of Lepidoptera and comprise two uniporous styloconic sensilla, which are contact chemoreceptors, three basiconic sensilla, and two aporous sensilla chaetica. The maxillary palp is two-segmented and has eight small basiconic sensilla at the tip, which were also found to be gustatory. The antennae of *B. fusca* larvae are short and simple. The sensilla of the antenna are composed of two aporous sensilla chaetica, three multiporous cone-shaped basiconic sensilla, three small basiconic sensilla, and one aporous styloconic sensillum. The basiconic sensillum located on the third antennal segment displayed a contact chemoreception response. The other basiconic sensilla did not show any action potential activity in tip-recording tests. The significant and positive dose–response curve obtained for the antennal basiconic sensillum with sucrose indicated for the first time the presence of gustatory chemoreceptors on the antennae of a lepidopteran larva.

Introduction

The stem borer *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) is an important pest of maize [*Zea mays* L. (Poaceae)] in eastern and southern Africa (Kfir et al., 2002). Like in many other Lepidoptera, the success of *B. fusca* in recognizing and colonizing a limited variety of plants [mostly maize, cultivated sorghum, *Sorghum bicolor* (L.) Moench, wild sorghum, *Sorghum arundinaceum* (Desvaux) Stapf, and *Arundo donax* L. (all Poaceae)] is based on the interaction between its sensory systems and the physico-

chemical characteristics of its immediate environment (Calatayud et al., 2006). Although host acceptance in phytophagous insects is usually viewed as solely governed by the adults, larval host acceptance is equally important in many insect species (Roessingh et al., 2007).

After hatching under the leaf sheath of the plant stem, *B. fusca* neonate larvae initially ascend to the whorl, where they either feed on the leaves or disperse to other plants via ‘ballooning-off’ (Kaufmann, 1983). The reasons for dispersal are not known, but it is possible that it is density dependent and is affected by host plant quality. After feeding in the whorl leaves, third instars descend and bore into the stem. Older instars may also migrate in search of a more suitable host plant (Kaufmann, 1983). Lepidopteran larvae display striking food preferences, which are based on a small set of chemoreceptors, thus making the larval

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stage play a role in host-plant recognition and selection (Roessingh et al., 2007). It is not known whether the number of sensillae and the inherent sensitivity to plant stimuli change with larval stage in order to enable the detection of cues emanating from different parts of the host plants fed on.

As a starting point, we studied the external morphology and distribution pattern of sensilla present on the maxillae and antennae of neonates and third instars of *B. fusca*. The putative olfactory and/or contact chemosensory functions of these sensilla were then identified from microscopic preparations using selective silver staining of the sensilla and dose–response electrophysiological tests.

Materials and methods

Insects

Neonates and third instars of *B. fusca* were obtained from the Animal Rearing and Containment Unit (ARCU) of the International Centre of Insect Physiology and Ecology (ICIPE, Nairobi, Kenya). To obtain third instars, the insects were reared on a meridic diet (Onyango & Ochieng²-Odero, 1994).

Scanning electron microscopy

Ten entire, live neonate larvae and 10 decapitated heads of third instars were used for the preparations of specimens for scanning electron microscopy. For fixation, the specimens were first placed in a 2.5% (vol/vol) glutaraldehyde 0.1 M phosphate buffer (pH 7.4) solution and kept overnight. The specimens were then dehydrated in a graded series of ethanol (70, 90, and 100%) and finally air-dried. The heads of neonates were separated from the rest of the insect body. Larval heads for both insect groups were mounted on stubs with conductive double-side adhesive tape, sputter-coated with gold, and finally examined with a JEOL JSM-T330A scanning electron microscope (JEOL, Tokyo, Japan) at 10 kV.

Silver nitrate staining

Silver nitrate staining was done to determine the presence of pores in the maxillary and antennal sensilla for each of the two larval developmental stages (neonates and third instars). Intact larvae were stained according to the method of Nayak & Singh (1983), modified as follows: larvae were first immersed in 70% ethanol containing 1 M silver nitrate for 1 h and then dehydrated in two concentrations (90 and 100%) of ethanol. Afterwards, heads of neonate larvae were separated from the rest of the body and heads of both instars were cleaned separately in xylene overnight. The specimens were mounted in Mountex (Histolab, Göteborg, Sweden) for light microscope

observations. A total of 10 head specimens were examined for each of the two developmental stages.

Electrophysiology

Putative taste sensilla on the maxillae and antennae were tested using a single-ended glass micropipette tip-recording electrode to determine if the sensilla had contact chemosensory function. The recordings were only done on sensilla of third instars, because the sensilla on neonate larvae were too small to make electrical contact possible. For the recording, a third instar was severed at the thorax and a glass micropipette filled with 10 mM NaCl was inserted into the larval body part containing the head. The micropipette was slipped over a silver wire, which served as the reference electrode. The sensilla of a total of 10 individuals were probed for electrical contact with another micropipette filled with 100 mM KCl that was sheathed over another silver wire, which was grounded to be the indifferent electrode. The action potentials were amplified using a universal AC/DC UN-06 amplifier (Syntech, Hilversum, The Netherlands) and recorded on a computer and analyzed using Autospike software (Syntech; version 2.1a). For those sensilla showing action potentials, a dose–response experiment using sucrose, a feeding stimulus for *B. fusca* larvae when reared on artificial diet (Onyango & Ochieng²-Odero, 1994), was undertaken to provide evidence for a contact chemosensory function. Sucrose concentrations of 0.01, 0.1, 1, 10, 100, and 500 mM in 10 mM KCl were used. The nervous impulses generated by different neurons in the sensilla were discriminated using Autospike software (Syntech; version 2.1a).

Data analysis

Statistical tests were performed with Statview software, version 5.0 (Abacus Concepts, Inc., Berkeley, CA, USA). For the sensilla length data, means were separated by Mann–Whitney U-test. For electrophysiological data, the spike counts during the first 150 ms for each recording were $\log(x + 1)$ transformed. A linear regression of $\log(\text{number of spikes} + 1)$ on $\log(\text{concentration})$ was done and tested using analysis of variance (ANOVA).

Results and discussion

The maxillae and antennae of both neonates and third instars had equal numbers and types of sensilla, but they were significantly shorter in neonates (Tables 1 and 2). As with several other lepidopteran families (Faucheux, 1999), the sensilla on the maxillary galeae of *B. fusca* larvae consisted of two styloconic sensilla (MS and LS), three basiconic sensilla (B1, B2, and B3), and two sensilla chaetica (C1 and C2). The maxillary palp was two-segmented

Table 1 Mean (\pm SE) lengths (μm) of maxillary sensilla of neonate and third instars of *Busseola fusca* ($n = 4$)

Larval stage	Sensillar type			
	Long sensillum chaeticum (C1)	Short sensillum chaeticum (C2)	Styloconic sensilla (LS, MS)	Maxillary palp (P)
Neonates	24.5 \pm 0.6a	12.9 \pm 1.3a	3.7 \pm 0.05a	12.1 \pm 0.9a
Third instars	113.4 \pm 0.5b	43.6 \pm 4.4b	6.1 \pm 0.6b	28.0 \pm 3.5b

Means in columns followed by different letters are significantly different at $P < 0.05$ (Mann–Whitney U-test).

Table 2 Mean (\pm SE) lengths (μm) of antennal sensilla of neonate and third instars of *Busseola fusca* ($n = 4$)

Larval stage	Sensillar type			
	Long sensillum chaeticum (C1)	Short sensillum chaeticum (C2)	Large basiconic sensilla (1, 2, 3)	Styloconic sensillum (S)
Neonates	47.4 \pm 3.7a	5.7 \pm 0.4a	8.6 \pm 0.9a	7.6 \pm 0.5a
Third instars	200.7 \pm 31.3b	35.2 \pm 2.7b	25.4 \pm 2.9b	14.8 \pm 1.1b

Means in columns followed by different letters are significantly different at $P < 0.05$ (Mann–Whitney U-test).

(Figure 1A). At the tip, the maxillary palp had eight small basiconic sensilla.

Not surprisingly, the lateral (LS) and medial (MS) styloconic sensilla were uniporous (Figure 1B). Electrophysiological tip-recordings of the neuronal activity obtained from both sensilla (Figure 2A and B) confirmed their gustatory function; this has been reported for several other larvae of lepidopteran species (Ishikawa, 1963; Schoonhoven & Dethier, 1966; Dethier & Kuch, 1971; Faucheux, 1999). In all lepidopterous larvae that have been studied, the styloconic sensilla play a major role in discriminating among plant constituents during the initial biting process and subsequent feeding (Faucheux, 1999).

The tip of each maxillary palp was clearly stained by silver nitrate, indicating their porous nature. Although the number of pores on each small basiconic sensillum of the maxillary palp could not be clearly determined, their argyrophilic properties indicated that they were porous. Three possibilities are reported in the literature on other larvae of lepidopteran species. First, all eight may be uniporous basiconic sensilla (the most frequent case). Second, they may be a combination of seven uniporous basiconic sensilla and one uniporous styloconic sensillum [e.g., in *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae)]. Third, they may consist of five uniporous and three multiporous basiconic sensilla [e.g., in *Pieris*

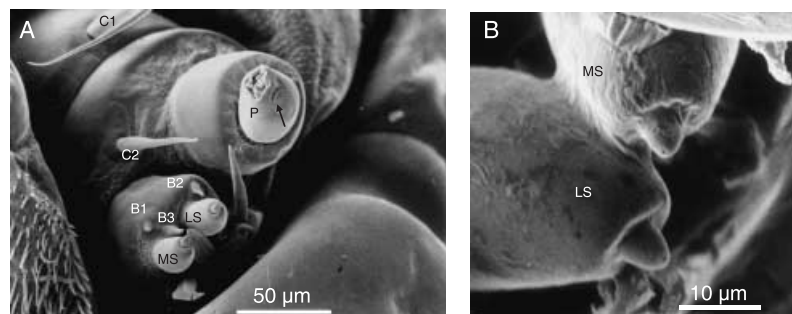


Figure 1 Maxilla of third-instar *Busseola fusca*. (A) Left maxillary galea showing uniporous lateral (LS) and medial (MS) styloconic sensilla, three aporous basiconic sensilla (B1, B2, and B3), probably playing a role in thermohygroreception and in proprioceptive monitoring the position of the styloconic sensilla (Shields, 1994; Faucheux, 1995, 1999), two aporous sensilla chaetica (C1 and C2), probably having proprioceptors that respond to mandibular movements (Grimes & Neunzig, 1986; Faucheux, 1999), and the left maxillary palp (P) with eight basiconic sensilla on the tip and laterally a sensillum digitiformium (arrow). (B) Close-up of the uniporous LS and MS, each with a terminal pore.

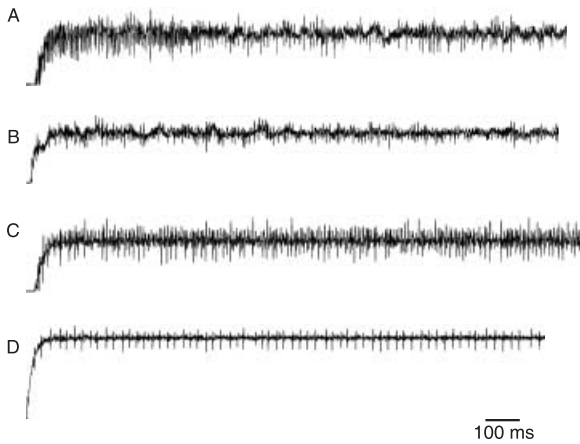


Figure 2 Electrophysiological recording of spike activity of a third-instar *Busseola fusca* in response to 100 mM KCl in (A) a lateral styloconic sensillum on the maxillary galea, (B) a medial styloconic sensillum on the maxillary galea, (C) a maxillary palp, and (D) the basiconic sensillum on the third segment of the antenna.

brassicacae (L.) (Lepidoptera: Pieridae) and *Manduca sexta* L. (Lepidoptera: Sphingidae)] (Faucheux, 1999). Because of their minute size, it was not possible to carry out electrophysiological tip-recordings on individual sensilla. However, by using this technique, the electrical contact and spike strains recorded from the tip of the maxillary palp (Figure 2C) confirmed their gustatory function, as was also reported for other lepidopteran larvae (Devitt & Smith, 1982; Faucheux, 1995, 1999; Albert, 2003).

As with larvae of other noctuid, sphingid, arctiid, and lasiocampid species (Dethier, 1937; Faucheux, 1999), the antennae of *B. fusca* larvae are short and simple, with only three segments (Figure 3A). The sensilla of the antenna

consist of two sensilla chaetica (C1 and C2), three cone-shaped basiconic sensilla (1, 2, and 3), three small basiconic sensilla (1', 2', and 3'), and one styloconic sensillum (S) (Figure 3B). On the antennae, only the three cone-shaped basiconic sensilla (marked as 1, 2, and 3 in Figure 3B) were clearly stained by silver nitrate, indicating their porous nature. These sensilla are known to be multiporous (Faucheux, 1995, 1999), suggesting that they may have an olfactory function. Olfactory function of these sensilla has been extensively reported in literature. They generally play an important role in discriminating odors that emanate from different plants and lead to location and selection of suitable hosts (Morita & Yamashita, 1961; Schoonhoven & Dethier, 1966; Dethier & Schoonhoven, 1969; Hanson & Dethier, 1973; Dethier, 1980; Faucheux, 1999). In *B. fusca*, neonate larvae oriented significantly towards maize plant volatiles in Y-tube olfactometric assays (G Juma, unpubl.). However, electrophysiological tip-recordings on the basiconic sensillum located on the third antennal segment (marked 2 in Figure 3B) indicated that it has a gustatory function as well (Figure 2D). The other basiconic sensilla (1 and 3 in Figure 3B) did not show any action potential during the tip-recording tests.

Gustatory chemoreceptors of larvae mainly occur on the maxillae and this was the first time (to our knowledge) that they were found on the antennae of larvae. To confirm the contact chemosensory function of the antennal cone-shaped basiconic sensillum of the third segment, a comparative dose-response experiment using sucrose was done on the sensilla at the tip of the larval maxillary palp. The tip of the maxillary palp was chosen for two reasons: (i) it harbors both multiporous and/or uniporous sensilla that have spike trains generated by contact electrophysiological recording (Figure 2C), and (ii) it is known to

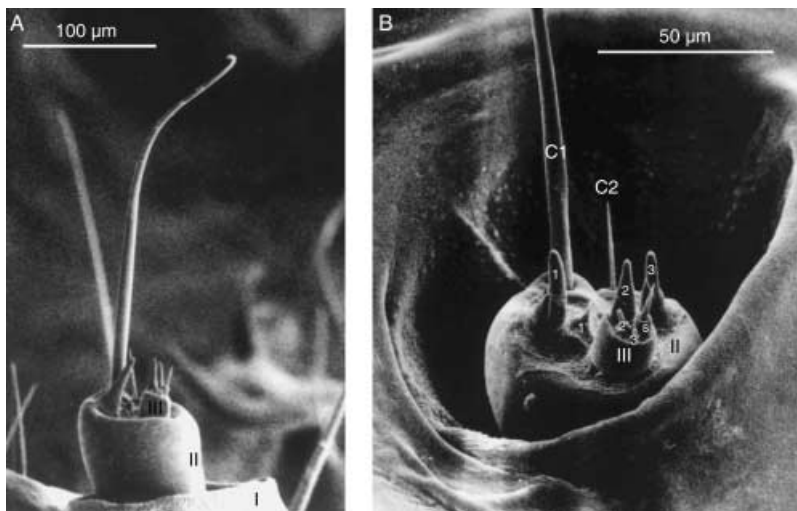
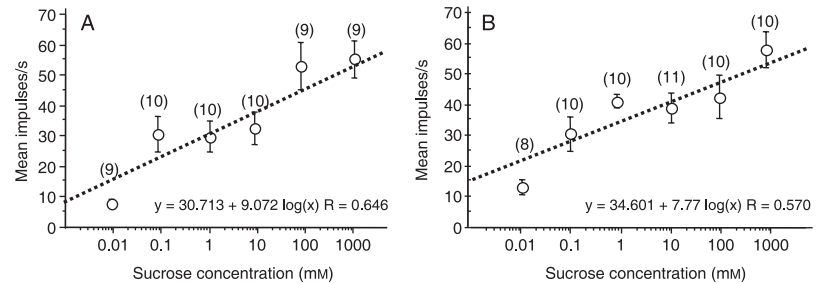


Figure 3 Antenna of a third-instar *Busseola fusca*. (A) Tip of right antenna showing the dorsal view of segments I to III. (B) Detail showing two aporous sensilla chaetica (C1 and C2), dorsally positioned on the second segment and known to detect tactile stimuli (Dethier, 1941; Faucheux, 1999); three argyrophilic cone-shaped basiconic sensilla of similar length, two located on the second segment and one on the third segment (1, 2, and 3); three small basiconic sensilla (1', 2', and 3') reported as multiporous and probably having olfactory receptor neurons (Faucheux, 1999), and one aporous styloconic sensillum (S) on the third antennal segment, reported as cold-sensitive receptors (Schoonhoven, 1967; Faucheux, 1999).

Figure 4 Dose–response curves for sucrose obtained from (A) the cone-shaped basiconic sensillum of the third antennal segment and (B) the maxillary palp of a third-instar *Busseola fusca*. Numbers of replicates are given in parentheses. Bars indicate standard error.



respond positively to sucrose in lepidopteran larvae (e.g., Albert, 2003).

When the concentration of sucrose was increased, significant positive dose–response curves were obtained for both the antennal sensillum ($F_{1,56} = 41.637$, $P < 0.0001$; Figure 4A) and the maxillary palp ($F_{1,58} = 32.124$, $P < 0.0001$; Figure 4B). These results confirm the evidence for contact chemoreception on the antenna. Preliminary observations on the behavior of second instars showed that before feeding, larvae tap the leaf surface with the tip of the maxillary palp, suggesting evaluation of the plant surface. Furthermore, the cone-shaped basiconic sensilla located on the third antennal segment frequently touch the plant surface during this tapping behavior, indicating a probable involvement of these sensilla in plant surface evaluation. However, tapping behavior on the plant surface with antennal sensilla has not been clearly observed. Additional studies are necessary to confirm these observations and to elucidate the role of the antennal sensilla in plant surface evaluation.

In conclusion, *B. fusca* larvae have sensory structures that are able to detect volatiles and surface chemical stimuli on their host plants. The olfactory receptors are mainly located on the antennae, but they are also present on the maxillary palps. Gustatory chemoreceptors occur mainly on the maxillae and their presence on the antennae of larvae has never been reported before. This is particularly interesting in view of the antennae and the maxillary palps having receptors that are important in mediating food selection in *M. sexta* (de Boer, 1993).

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