

# Courtship behaviour of the African Maize Stem Borer: *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) under laboratory conditions

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**Abstract.** *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) is the most important African stem borer damaging maize and sorghum. Chemical mediators play an essential role in all life cycle of this moth, especially for mating recognition and host plant choice. The female sex pheromone, courtship and mating behaviours act on the reproductive isolation within insect populations. *B. fusca* courtship behaviour was studied to decipher each step that could account as a process for reproductive isolation. *B. fusca* males and females presented a very simple and fast courtship behaviour, without any particular events or male pheromone emission.

**Résumé.** Comportement de cour du foreur africain de graminées : *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) en conditions de laboratoire. *Busseola fusca* (Fuller) (Lepidoptera : Noctuidae) est le plus important ravageur des cultures de maïs et de sorgho en Afrique. L'écologie chimique est essentielle pendant toute la vie de ces Lépidoptères nocturnes et intervient pour la rencontre des partenaires sexuels et le choix de la plante hôte. La phéromone sexuelle produite par la femelle et le comportement précopulatoire sont des facteurs importants du maintien de l'isolement reproducteur au sein des populations de cet insecte. Le comportement de cour de *B. fusca* a été étudié pour préciser chaque événement comportemental, qui pourrait avoir un rôle dans l'isolement reproducteur. Le comportement de cour s'avère être très simple et rapide, sans événement particulier ni émission d'une phéromone mâle.

**Keywords:** Stem borer, *Busseola fusca*, Noctuidae, Africa, mating and calling behaviours.

Moth reproduction i.e. mate location, courtship behaviour and copulation, mostly relies on the use of chemical signals. Long range behaviour is steered by the female produced sex pheromone perceived by the male that flies upwind to where the female is standing in calling posture. This scheme is, with few exceptions, common in all moths from the more primitive to the more evolved species. In contrast, the characteristic close range behaviour of courtship is very diverse between species and even within the same genus, with no relation between complexity and evolutionary stage. In some species, courting males release chemicals and engage a chemical dialogue with the females, that seems to be involved in sexual selection (Birch *et al.* 1989), whereas some species exhibited a courtship behaviour reduced to copulation attempts. Male produced scents are chemically very diverse (Blum 1987), as well as the

specialized scent structure (Birch & Hefetz 1987).

The specific mate recognition system expressed between males and females (SMRS) is constituted by a complex set of adaptive traits and thus can be considered as an essential element in the evolution of moth populations. Specifications of each SMRS depend in part on the phylogenetic history of each species and on complexity and stability of the environment in which the insects develop. In insects and especially moths, SMRS plays a major role in reproductive isolation and speciation processes. Thus, the chemically disturbed environment produced by sympatric species and interspecific competition has to be taken into account for understand means implemented by our insect to realize its reproductive isolation. Achievement of reproductive isolation is linked to different processes such as the chemistry of the blend, the difference in dial periodicity of sexual behaviour and the possible adult sympatry. At least, when two species share the same pheromone blend and when they behave in sympatry with the same timing in sexual activity, courtship behaviour is the last protection against interspecific copulation.

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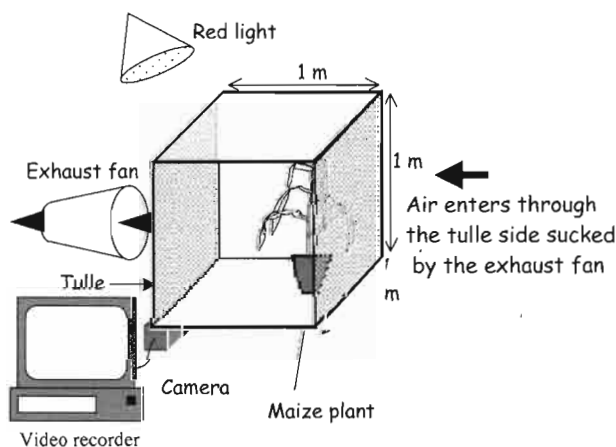
*Busseola fusca* (Fuller 1901) (Lepidoptera: Noctuidae) is the most important stem borer species damaging cultivated gramineae: sorghum (*Sorghum bicolor* Moench 1794) and maize (*Zea mays* L. 1753) in Africa (Ratnadass *et al.* 2001). The larvae cause yield losses ranging from region to region from 20% to 80%. *B. fusca* populations have moved from native wild sorghum to cultivated cereal plants and developed a preference for them (Le Rü *et al. in lit.*). As populations can be adapted to the local requirements of the habitat, host plant selection by an insect might partly be explained by the phylogeny (Wyatt 1997).

The objective of this study was to describe qualitatively and quantitatively pre-courtship and courtship behaviours in *B. fusca* on cultivated host plants under laboratory conditions. Such knowledge provides information on the process involved in reproductive isolation and also contributes to improve the trap design adapted to the male landing behaviour.

### Materials and Methods

#### Insect rearing

Pupae originated from the mass rearing unit of the International Centre for Insect Physiology and Ecology (ICIPE-Nairobi-Kenya). Individuals were sexed and each sex was kept in separate close rectangular crystal polystyrene containers (27x12x8 cm) on vermiculite until emergence. Adults were collected daily and males were housed together in boxes as described above. Adult females were placed individually into a cylindrical crystal polystyrene box (ø 3x6 cm) until they were used for experiments when two or three days old. Adults were kept under the following conditions: 25 °C, 85±10% R.H. with a 12:12-h light-dark-reversed photoperiod.



**Figure 1**  
Experimental set up for the courtship behaviour study.

#### Description and analysis of courtship behaviour

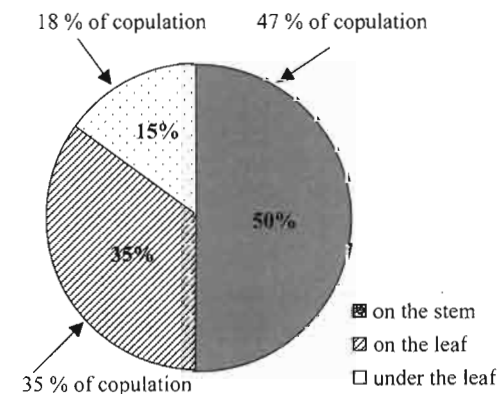
Female calling behaviour is necessary to induce male attraction. The ovipositor of *B. fusca*, like in most moths, is protractible and females initiate calling by extruding the ovipositor and cease calling by retracting it slowly. In our study calling behaviour was defined as 50% of the full ovipositor extension.

According to Calatayud *et al.* (2007), the onset of female calling behaviour is related to the diel periodicity and age. Thus observations of the pre-courtship and courtship behaviours were conducted from the fifth hours after the beginning of the 12h-scotophase. Courtship behaviour was recorded by placing a two to three day-old calling female on a maize plant in a mosquito-net cubic cage (1 m<sup>3</sup>). Two sides were made of Plexiglas and the other two sides were covered by tulle. A wind fan allowed a constant air flow to pass through (fig. 1). This airflow set up was necessary to avoid pheromone permeation and for the males to succeed in locating the calling female. Once the female was in calling posture, a male was carefully deposited on the bottom of the cage, downwind from the female. Courtship behaviour was tape recorded (Panasonic AG-7330) until copulation with a Hitachi KP 161 CDD black and white camera equipped with a Nikon objective AF Micro Nikkor 60 mm 1:2.8 D. Each experiment lasted five minutes under 20-25 °C, 45-50% R.H. and red light. The tapes were transcribed in video folders on a computer and analysed with The Observer 5.0 software (Noldus, Wageningen, The Netherlands, 2004) linked to a Psion Workabout.

### Results

#### Female pre-courtship behaviour

Most of the females started to call after the 5<sup>th</sup> hour of the scotophase. The female remained motionless in calling position. No wing fanning or abdomen puff movements was observed. Females called on the maize plant: 50% were observed on the stem, 35% on the leaf and 15% under the leaf (fig. 2).



**Figure 2**  
Relation between calling female position and percentage of mating: the percentages on the pie represent the different female positions out of all the tested females. The percentages outside of the pie represent the percentage of copulation out of the total copulation recorded.

## Male pre-courtship behaviour

With airflow, moths could sense the dimension and the gradient of the pheromone plume and used this information to steer a course to the source (Cardé & Charlton 1984). As soon as a *B. fusca* male encountered a female pheromone plume, the first behaviours observed were wing fanning, antennae rising up and moving up and down. This step was considered as the activation process before take-off. After taking-off, males flew toward the female in a zigzagging upwind flight in the pheromone plume. This step was clearly an oriented displacement towards the female and lasted an average of 13 seconds.

## Courtship behaviour

The observation of 17 complete successful behaviours led us to divide of the courtship behaviour into three steps:

1. The landing. The male landed at different places: either on the stem under the female or on the female side or directly on the female;

2. The attempt to copulate. After landing the male exhibited a wing fanning walk to reach the female and displayed an eversion of the genitalia claspers bearing hair-pencils. Then, the male bent the abdomen to contact the extremity of the female abdomen, trying to hold the female genitalia with the genitalia claspers in copulation attempts;

3. Copulation: the male and female genitalia joined and the male wing fanning ceased. The male spun around 180° staying opposite the female frontal orientation in a tail-to-tail posture typical in Lepidoptera mating. When copulation was successful, male and female remained linked for *ca* three hours.

66% of experimented pairs ( $n = 26$ ) copulated and the courtship behaviour from the take-off to the contact between male and female genitalia lasted an average of 19.73 seconds ( $\text{sem} \pm 19.75$ ). 46% of the males landed on the plant underneath the female and then walked while wing fanning to reach the female side; 27% landed on the side near the female and 27% on the female (fig. 3). Landing underneath the female leads to 48% copulation success whereas 28% and 24% ( $P = 1.52$ ) success rates were recorded for landing on the female side or directly on the female respectively. When the male landed on the female, calling behaviour stopped and the male could not again locate the female.

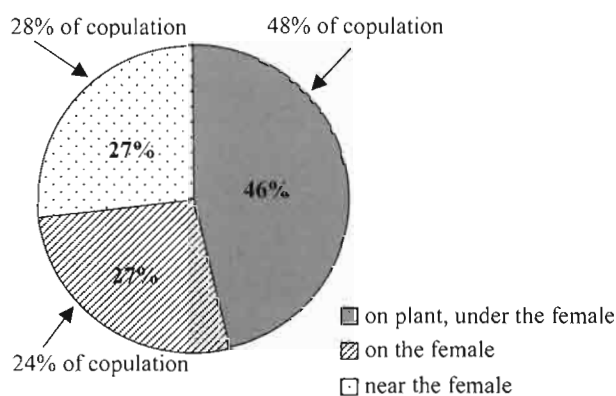
Observed unsuccessful copulations ( $n = 9$ ) were due to female rejection ( $n = 5$ ), female escaping ( $n = 2$ ) or a cease of calling ( $n = 2$ ).

Copulation percentages varied according to the site of calling behaviour: 47% of mating occurred when females called on the stem, 35% when on the leaf and 18% when under the leaf.

## Discussion

The *Busseola fusca* courtship behaviour is relatively short and simple. The pheromone modulates the male upwind flight to locate the female. Once the male encounters the female pheromone plume, it usually lands on the stem underneath the female. Despite the fact that our results are not significant certainly due to low number of experiments, it appears that landing on the stem underneath the female is the most efficient for successful copulation and must be taken into account for optimizing the design of the pheromone trap in a way that the males can land underneath the pheromone source and then walk up toward it. Sometimes, the male lands brutally on the female which exhibits a rejection motion and stops calling. Female rejection remains the main reason for unsuccessful mating. In most Lepidoptera, the mate choice is the decision of the females (Zagatti & Castel 1987).

*B. fusca* males do not present a highly elaborated mating behaviour contrary to other noctuid species such as *Mamestra brassicae* L. 1758, which exhibit a sophisticated mating behaviour with the release of a male sex pheromone produced by the abdominal brushes acting as an attractant for the calling female (Birch *et al.* 1989; Jacquin *et al.* 1991; Noldus & Porting 1989). Male pheromone release is necessary for copulation success and increases female recognition and acceptance at short range. The female are supposed to choose the courting male releasing the higher quantity of male pheromone and a sort of "sexual selection" based on female choice and male pheromone occurs (Frérot unpublished data).



**Figure 3**  
Relation between landing places and percentage of mating: the percentages on the pie represent the different male landing places out of all male tested. The percentages outside of the pie represent the percentage of copulation out of the total copulation recorded.

Clasper hair pencils of *B. fusca* males do not seem to release any sex pheromone. *B. fusca* females seem to select the males on their landing behaviour and to reject copulation, using two means: escape or stop calling. Sexual selection plays an essential role in most of Lepidoptera which copulate only once; only the most efficient moths will copulate. *B. fusca* is a polyandrous species (Calatayud *et al.* unpublished data) and this may account for no mate selection in this species. Thereby, very simple *B. fusca* courtship behaviour and the apparent absence of sexual selection lead to draw some hypothesis. With a sex-ratio favouring males (Ratnadass *et al.* 2001) and with polyandrous females, a mate choice by the female would be a waste of energy and would be detrimental for the species fitness. Sperm selection could be another way for intra specific selection but no information is available on the occurrence of such a process in *B. fusca* female egg fertilization.

The entire pheromone blend functions as a species-specific unitary signal for long range attraction, courtship and copulation (Linn *et al.* 1984). In the case of *B. fusca*, the female sex pheromone is a mixture of three compounds: the (Z)-11-tetradecenyl acetate (main), the (E)-11-tetradecenyl acetate and (Z)-9-tetradecenyl acetate (minors), 70:15:15% respectively (Nesbitt *et al.* 1980; Hall *et al.* 1981). Diversity of moth species developing on maize and sorghum crops in Kenya is reduced. The other main populations developing on these plants are *Sesamia calamistis* Hampson 1810 (Lepidoptera: Noctuidae) and *Chilo partellus* (Swinhoe 1885) (Lepidoptera: Crambidae), which produce a sex pheromone whose main components are characterized by 16 carbons ([www-pherolist.slu.se](http://www-pherolist.slu.se)) preventing interspecies attraction.

Many stem borers are associated with their host plants. Phytophagous insects often meet, court and mate on plants (Landolt & Phillips 1997). To date, how host plants influence *B. fusca* SMRS remains unresolved. Maize plants in our study allow the female to be in a good calling position and the male to spin after copulation. Does the plant only represent a calling stand or does it affect sexual behaviour remains to be answered.

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