Temporal patterns of emergence, calling behaviour and oviposition period of the maize stem borer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae)

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Abstract. Sexual behaviour in most of the noctuid Lepidoptera follows a diel periodicity and is limited to a precise period of either the day or the night. The periodicity and the maturation periods are specific as well as the onset of oviposition behaviour. The knowledge of these traits of biology is an essential prerequisite for all the studies in chemical ecology. The periodicity of adult emergence, calling behaviour and oviposition of *Busseola fusca* (Fuller) was studied under laboratory conditions. Most males emerged before onset of the scotophase whereas most females did so one hour later. The virgin females started to exhibit calling behaviour few hours after emergence and there is no sexual maturation time. The calling behaviour starts the fourth hour after the onset of the scotophase but is lightly delayed for females having emerged the same night as compared to older females. Sexual and oviposition behaviours occur during the dark period. Oviposition started the first night after the mating night, peaked during the second night and then gradually decreased until the fifth night. The first night of oviposition, females laid eggs during the first three hours and after the 5th hour of the scotophase with a maximum during the 8th and 9th hour. During the second night, oviposition peaked during the second hour and tended to decrease thereafter.

Résumé. Chronologie de l'émergence, du comportement d'appel et de l'oviposition chez la mineuse du maïs *Busseola fusca* (Fuller) (Lepidoptera : Noctuidae). Le comportement sexuel de la plupart des lépidoptères Noctuidae est cyclique et limité à une période bien précise du jour ou de la nuit. Les rythmes et les délais de maturation sexuelle sont propres à chaque espèce. L'initiation du comportement de ponte est aussi une des composantes de l'espèce. La connaissance de ces points de biologie est un préalable indispensable à toutes études d'écologie chimique qui traitent de la reconnaissance du partenaire sexuel et de la plante-hôte. Les périodes d'émergence, du comportement d'appel et de ponte de *Busseola fusca* (Fuller) ont été étudiées dans des conditions de laboratoire. La plupart des mâles émergent avant le début de nuit et les femelles lors des premières heures. Le comportement d'appel des femelles vierges débute quelques heures après l'émergence et il n'y pas chez *B. fusca* de délai de maturation sexuelle. La période d'appel commence quatre heures après le début de nuit, et est un peu plus tardive pour les femelles qui viennent d'émerger. L'insecte présente une activité nocturne tant pour l'accouplement que pour la ponte. L'oviposition débute la nuit qui suit celle de l'accouplement et la quantité d'œufs déposés augmente la deuxième nuit de ponte pour ensuite décroître progressivement jusqu'à la cinquième nuit. La ponte se répartit sur toute la durée de la scotophase avec deux pics : l'un se situe en début de nuit et l'autre dans la seconde moitié de la scotophase.

Keywords: Africa, Kenya, sorghum, Poaceae, pupae, moth, surrogate stem.

Busseola fusca (Fuller) (Lepidoptera: Noctuidae) is a serious pest of maize and grain sorghum in sub-Saharan Africa (Kfir *et al.* 2002). This insect species is mostly found on cultivated habitat and rarely on wild grasses (Le Rü *et al.*, 2006; Ong'amo *et al.*, 2006a; Ong'amo *et al.*, 2006b; Otieno *et al.*, 2006, Wale *et al.*, 2006). Crop losses occur as the consequences of plant death, stem tunnelling, early leaf senescence and grain feeding (Bosque-Perez & Mareck 1991; Kfir 1998). To control this pest, cropping and habitat management strategies have been recently studied (Chabi-Olaye *et al.*, 2006; Matama-Kauma *et al.*, 2006; Ndemah *et al.*, 2006).

Busseola fusca oviposit up to 600–800 round and flattened eggs in batches of 30–100 eggs inside leaf sheaths (Unnithan 1987; Harris & Nwanze 1992). The larvae hatch about a week later and migrate to the whorl where they feed on the leaves or balloon off to

other plants. Older larvae migrate to the lower parts of the plant where they penetrate into the stem. The larval stage, consisting of six to eight intars, lasts between 24 and 45 days depending on temperature. Adults emerge about 13-14 days after pupation (Harris & Nwanze 1992; Onyango & Ochieng'-Odero 1994; Ratnadass *et al.* 2001).

Emergence of adults mostly occurs between sunset and midnight (Unnithan 1987; Harris & Nwanze 1992). Only *B. fusca* females emit pheromones. Thereafter, males and females exhibited a very simple and rapid courtship behaviour, without any particular characteristic events or male pheromone emission (Frérot *et al.*, 2006). Males start mating within a few hours after emergence and live slightly longer than the females (Unnithan 1987). Mating occurs generally during the first six hours of the night. The males can mate several times with equal effectiveness, fertilising eggs each time, but they transfer only one spermatophore during copulation and mate only once per night (Unnithan & Paye 1990). A single spermatophore is sufficient to have all eggs fertilised by the female throughout its life span (Unnithan & Paye 1990), indicating that polyandry is not obligatory and not necessary for *B. fusca*. The female moths, soon after mating, disperse in search of suitable host plants for oviposition. The ensuing oviposition period continues over 3–4 successive nights (Unnithan 1987; Harris & Nwanze 1992).

Although, much information on the biology of *B. fusca* is available (Harris & Nwanze 1992), there exists no detailed report on the temporal pattern of the major events constituting the reproductive behaviour that takes into account the age of the insect. Most of the noctuid moth living several days presented a diel periodicity in calling behaviour and emergence timing determined the onset of reproductive behaviour. Such an information was of interest for further studies on sex pheromone communication and on host plant choice. The present study reports details on adult emergence, calling behaviour and oviposition period of *B. fusca*.

Materials and Method

Insects

Busseola fusca pupae were provided by ICIPE mass rearing unit (Nairobi, Kenya), where they were reared on artificial diet under laboratory conditions following the method described by Onyango & Ochieng'-Odero (1994). To regenerate the colony, new insects collected from the field were added three times per year.

The pupae and adults were maintained at 25.3 ± 0.9 °C, 68.6 ± 12.8 % RH (means ± SE) and L12:D12. One room was kept under a reversed photoperiod, with the scotophase lasting from 7:00 to 19:00, herewith referred to as night, and another under normal conditions with the photophase lasting from 7:00 to 19:00. This allowed to carry out all the observations during day time. For the reversed photoperiod, the observations were made using a red 80 W fluorescent tube as a light source.

Pupae were sexed and males and females were kept separately until emergence together with moist vermiculite (80 g/100 ml) in a plastic box (30 cm length, 12 cm width, 10 cm height). Inside the box, two square pieces of folded plastic mesh (10 × 10 cm) were placed on the vermiculite to facilitate wing deployment process of emerging adults.

Adult emergence period

For a more precise estimation of emergence time, the time to full wing expansion was first assessed for each sex. Then, emergence was recorded hourly from 7:00 to 19:00 in both experimental rooms through the aforementioned plastic box. The experiment was run for ten consecutive nights and days.

Calling time

Females begin calling by extruding the ovipositor, usually to full length and cease calling by retracting it slowly. Calling was defined as 50% of full extension of the ovipositor. The calling females remained still and no puffing behaviour neither wing vibrations were observed. To determine the periodicity of the calling behaviour, the females were placed just after emergence in a mosquito-net cage (30 × 30 × 30 cm).

Calling postures were observed at hourly intervals for two different moth ages, i.e. for females that emerged during the same night they started calling and for those that had emerged during the previous night. In addition, the onset of calling according to moth age was recorded. Since no calling activities were observed during the photophase, the experiments started at the onset of scotophase and ended with onset of photophase.

Oviposition period

None of the females laid eggs the night of mating. Thus all the females used for determining the temporal pattern of oviposition had mated the night before the observations started.

To obtain a maximum proportion of mated females (96%) the following method was used. One-day old females were placed into a mosquito-net cage (30 × 30 × 30 cm) at onset of the scotophase. The first females started to call 6 h thereafter. One-day old males were introduced one hour later. During the ensuing 1-h period, mating pairs were taken out of the cage and put individually in plastic boxes (8 cm high, 5 cm diameter). When copulation was completed, the sexes were separated and the females used for oviposition experiments the following night.

Gravid females were placed individually in transparent plastic jars (10 cm diameter × 20 cm high) together with an oviposition substrate consisting of a wax paper cut rectangularly (15 cm × 6 cm) and rolled helicoidally from top to bottom to form a cylindrical surrogate stem (Khan & Saxena 1997). No eggs were laid on surrogate stems during the photophase. Thus, oviposition experiments started at the onset of scotophase and ended with onset of photophase. To evaluate the oviposition rate per night, the number of eggs was counted each night over 5 successive nights, renewing the surrogate stem each night.

In addition, oviposition rates were monitored at hourly intervals

during the first two nights after mating. The surrogate stems were renewed every hour.

After each experiment, each female was dissected to verify the presence of spermatophore(s) into the bursa copulatrix indicating that they were mated. Only females with spermatophore(s) were included in the analysis.

Data analysis

Statistical tests were performed with Statview software (Abacus Concept, version 5.0, USA). Chi-square tests were used to compare proportions of moths emerged and calling. Calling time and relative proportions of eggs laid were subjected to F-test for homogeneity of variances and to Kolmogorov-Smirnov method for data normality. Data for relative proportions of eggs laid were normalised by arcsine transformation. Means were separated by Student's *t*-test or by Fisher's PLSD (Protected Least Significant Difference) following one-way analysis of variance (ANOVA).

Results and discussion

Adult emergence

Just after emerging from the nymphal moult, the moth wings remained wrinkled alongside the body during 12.8 ± 0.9 min. (n = 7) and 13.6 ± 1.3 min. (n = 5) for males and females, respectively (means ± SE). Thereafter, the wings were deployed and remained perpendicular to the body during 28.8 ± 1.1 min.

(n = 7) and 29.2 ± 1.4 min. (n = 5) for males and females, respectively. Thus, their emergence time was estimated to have occurred about 13 or 29 minutes previously for, respectively, moths showing wrinkled wings or wings kept perpendicular to the body. For individuals showing yet wings well extended alongside the body, their emergence time was about 42 (i.e. 13 + 29) minutes before.

Males began to emerge about two hours before the females (fig. 1). Significantly more males (36%) than females (9%) emerged before onset of the scotophase (χ^2 = 14.481, df = 1, p = 0.0001). Significantly more females (86%) than males (53%) emerged during the first three hours of the scotophase (χ^2 = 18.885, df = 1, p < 0.0001). Thus a higher proportion of males emerged before females within the same night. Earlier emergence for males was also observed for *Sesamia inferens* (Walker) (Lepidoptera: Noctuidae) (Nagayama *et al.* 2004).

However, protandry does not occur in *B. fusca*. As previously reported by Ratnadass *et al.* (2001), for both sexes pupal and larval development times were not significantly different.

Approximately 5 min. after the onset of scotophase and photophase both male and female moths became active and exhibited vigorous and erratic flight, often
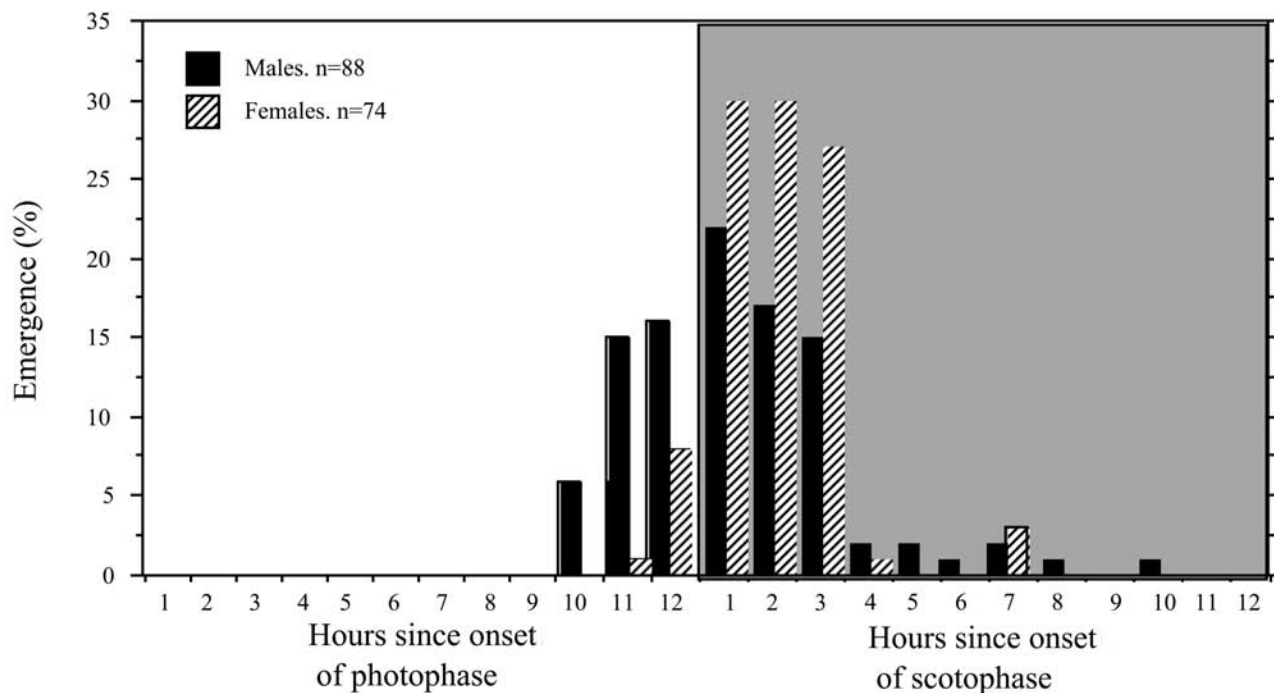
Figure 1
Emergence of *Busseola fusca* recorded at hourly intervals.
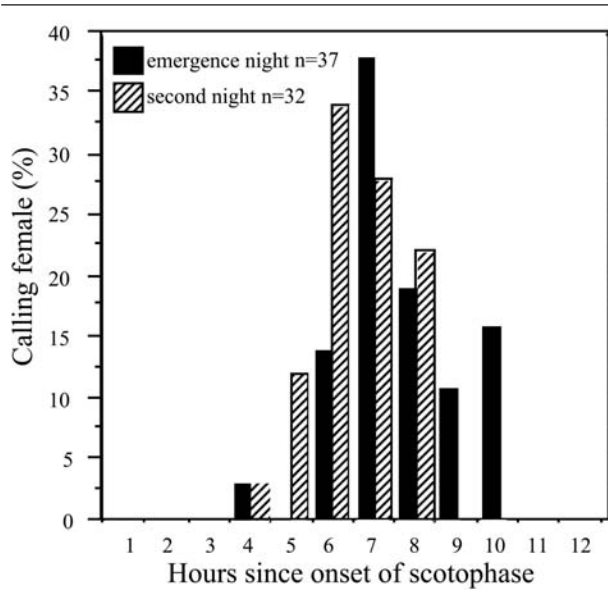
Figure 2
Calling behaviour of *Busseola fusca* recorded at hourly intervals.

bumping into the cage roof. However, in contrast to natural conditions, the switch was abrupt and not gradually. This behaviour is similar to the dispersal flight of *Heliothis virescens* F. and *Helicoverpa* (*Heliothis* sensu auct.) *armigera* (Hubner) (Topper 1987; Fitt 1989; Ramaswamy 1990).

Calling time

The virgin females started calling four hours after the onset of scotophase and the calling behaviour
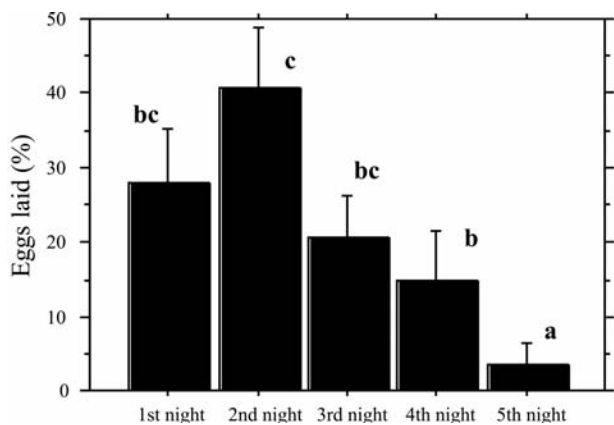
Figure 3
Relative proportions (in %, means ± SE, n=14) of eggs laid by *Busseola fusca* female laid during the first five nights after emergence. Bars headed with the same letters are not significantly different at P < 0.05 (PLSD's Fisher test).

appeared to be related with age of the female (fig. 2). The maximum proportion of calling occurred during the 6th hour and none after the 8th for one-day old females. During the night they emerged, the maximum proportion of calling was observed at the 7th hour and about 25 % of the females were still calling after the 8th hour. Considering the average onset of calling, one-day old females began to call at 6.1 ± 0.2 (mean ± SE, n = 32), significantly one hour earlier than younger females that had emerged the same night, beginning to call at 7.5 ± 0.2 (n = 37) (t = 4.631, df = 67, p < 0.0001, Student's *t*-test). The calling behaviour of *B. fusca* varied with age as already reported for other noctuids such as *Trichoplusia ni* (Hübner) (Sower *et al.* 1972), *Sesamia nonagrioides* (Lefebvre) (Babilis & Mazomenos 1992), *S. calamistis* Hampson (Fiaboe *et al.* 2003) and *S. inferens* (Nagayama *et al.* 2004). Calling activity of *B. fusca* occurred mostly during the middle of the scotophase but was delayed for females emerging the same night (fig. 2). Such delay in calling behaviour for females emerging the same night as compared to older females was also reported for *S. inferens* (Nagayama *et al.* 2004). This indicates that females need a minimum time before mating (a such of a minimum maturation time), assuming that calling behaviour and pheromone production are synchronous such as in many other lepidopterous species (Coffelt *et al.* 1978; Raina *et al.* 1986; Babilis & Mazomenos 1992).

Oviposition time

Similar to other noctuid species such as *Heliothis zea* (Boddie) and *Spodoptera ornithogalli* Guenée (Adler *et al.* 1991), oviposition peaked shortly after mating and then rapidly decreased (fig. 3). The average number of eggs laid per day was 105, 116, 45, 22 and 4 during, respectively, the 1st, 2nd, 3rd, 4th and 5th night after the mating occurred. About 90 % of the eggs were laid during the first three nights following the mating night, with a peak of oviposition on the second night. After the 5th night, most of the females died.

Observing the oviposition posture, regardless of the female age, 59% started to oviposit during the first three hours, 24% during the 6th and 8th hour of the night and 17% during the 10th and 11th hours (data not shown). Similar to *H. zea* (Callahan 1958), *B. fusca* laid eggs continuously throughout the scotophase (fig. 4). However, the oviposition pattern varied with the age of the moths. For two-day old females, most eggs were laid during the 8th and 9th hours (fig. 4a). The three-day old females laid most eggs during the first two hours (fig. 4b). Oviposition thereafter decreased gradually with a second small increase at the 6th hour.

In conclusion, the present study on the temporal

patterns of the reproductive behaviour of *B. fusca* revealed the coordination of reproductive events during the adult brief lives. The study evidenced that reproductive and oviposition behaviours in *B fusca* are nocturnal. The sexual behaviour, calling and mating, occur during the middle of the scotophase whereas oviposition last during all the dark period over several days. Reproductive behaviour characterised by female calling behaviour and male capacity to mate occurred few hours after the emergence, indicating that *B. fusca* males and females did not need a maturation period as observed in most of the European tortricid and noctuid moths (Frérot, unpublished data). The readiness to mate increases the efficiency of the reproduction in short life span insect. However, as for most of the moth, oviposition in *B. fusca* occurs the night after mating night. No information are available on the place where *B. fusca* moths mate in the wild but the oviposition delay, can have physiology origins
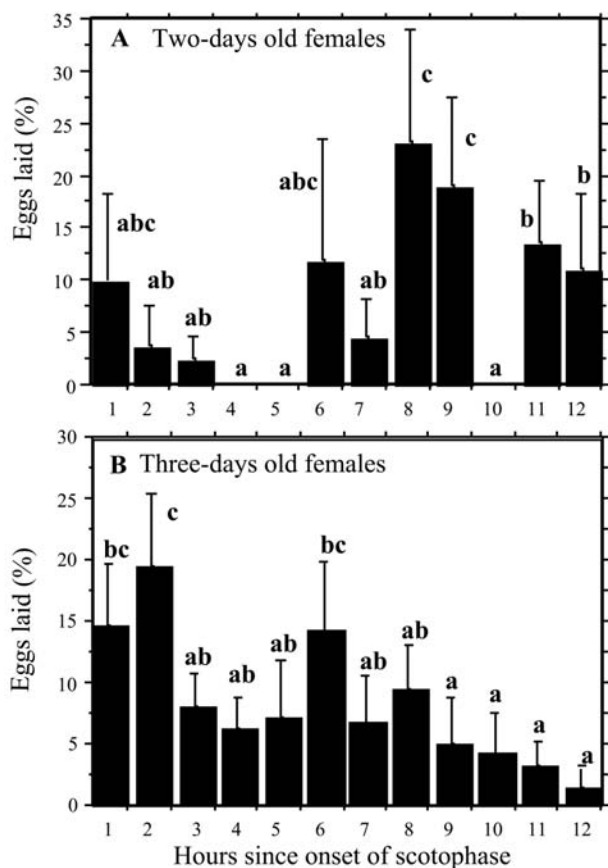
but can also favour the seeking for suitable host plant. Information on temporal patterns of reproductive behaviour brings knowledge on the biology of this species but are also the important premise for the design of experiments on sex pheromone and host plant choice. Thereby, the collection of sex pheromone and the evaluation of the host plant choice by the females should be undertaken during the optimum calling and oviposition periods respectively. This knowledge also contributes to understand underlying processes that control reproductive isolation between sympatric or closely related species whose pheromone can be chemically similar. However, the data presented were obtained under laboratory conditions and should be cautiously transposed for field study, as abiotic conditions might lightly modified diel periodicity of sexual and oviposition behaviour.

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Figure 4
Relative proportions (in %, means ± SE) of eggs laid by *Busseola fusca* female within hourly intervals during the first (A, n = 7) and the second (B, n = 12) night after the night of mating. Bars headed by the same letters are not significantly different at P < 0.05 (PLSD's Fisher test).

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