

Importance of plant physical cues in host acceptance for oviposition by *Busseola fusca*

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Abstract

Plant and surrogate stems exhibiting specific combinations of physical cues were used to determine which plant-related stimuli influence the oviposition of *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae). The number of eggs and egg batches laid per female increased with an increase in diameter of both natural and artificial stems. Direct observations of the oviposition behaviour (walking, antennating, and sweeping with the ovipositor) indicated that the female moths preferred oviposition supports with a large diameter and non-pubescent or smooth surfaces over pubescent or rough ones. Pubescence and rough surfaces significantly affected the behavioural steps leading to oviposition by interfering with the ovipositor sweep process necessary to find a suitable oviposition site. Furthermore, more eggs and egg batches were laid on soft than rigid supports. The rigidity of the support affected the proper insertion of the ovipositor for egg deposition. Our results underline the importance of physical stimuli in *B. fusca*'s choice of an oviposition site, which may facilitate the identification of potential host plants or preferred oviposition sites on a plant for this species.

Introduction

In eastern and southern Africa, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) is the most important insect pest of sorghum and maize in the cooler ecozones, such as those at mid-altitudes and in highlands (Kfir et al., 2002). Various control strategies based on host plant resistance and cultural control have been attempted, 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 stem borers are attracted and retained on trap plants (pull) planted as border rows, while 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.

In contrast to polyphagous species, oligophagous species have a narrow range of potential host plants, and are strongly selective in their choice of host plants (Bernays & Chapman, 1994). Most field recoveries of *B. fusca* have been reported on cultivated cereals, specifically from maize and sorghum, but very few on wild grasses (Le Rü et al., 2006a,b; Ong'amo et al., 2006).

Given the importance of the host plant selection process for the survival of their offspring, females of *B. fusca* most likely have special organs for the detection of a wide range of cues including visual, tactile, and olfactory and gustatory chemostimuli. It has been shown that the presence of sensory hairs located on the antennae and the ovipositor are essential in host plant selection and acceptance for oviposition by *B. fusca* (Calatayud et al., 2006).

A wealth of information is available on the oviposition preference of *B. fusca* on plants. Like most noctuid borer species, it oviposits egg batches between the leaf sheath and

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the stem (Kaufmann, 1983). Furthermore, among nine cultivated and wild Poaceae species used by Haile & Hofsvang (2002) in multiple-choice experiments, *B. fusca* strongly preferred *Sorghum vulgare* Pers. variety *sudanense*, a waxy plant species. It did not oviposit at all on *Melinis minutiflora* P. Beauv., a species with glandular trichomes (Khan et al., 2000). Van Rensburg et al. (1987, 1989) reported that young maize plants are most attractive for oviposition at 3–6 weeks after planting. Thus, *B. fusca* prefers pre-tasseling plants; oviposition rarely occurs on older maize plants (Ndemah et al., 2001), but if so, the insect lays batches on the apical part of the plants where the leaf sheaths are young and soft (B Le Rü, pers. obs.). In East Africa (Kenya, Tanzania, and Uganda), *B. fusca* is rarely found on *Arundo donax* L. in the field (Le Rü et al., 2006b), while in the laboratory, it does oviposit on this plant species but deposits its eggs mostly outside the leaf sheath, probably because the leaf sheath is more rigid in this plant species than in maize or sorghum (P-A Calatayud, pers. obs.).

Although these reports and observations suggest that plant physical cues, such as surface texture (e.g., pubescence), plant size (e.g., stem diameter), and leaf sheath rigidity, strongly influence the acceptability by *B. fusca* of a host species or plant part, no study so far has shown their role in the oviposition behaviour and choice of the female moths. As *B. fusca* accepts and oviposits on artificial supports, that is, surrogate stems, under non-choice situations, without any olfactory or contact chemical stimuli (Khan & Saxena, 1997), the presence of chemical stimuli is most likely not obligatory for the acceptance process of oviposition. Within moths, a wide range of visual, tactile, and chemical stimuli are known to influence oviposition choice (Renwick & Chew, 1994). However, unlike chemical stimuli, tactile stimuli linked to the physical properties of plants that drive the oviposition behaviour are poorly documented. The aim of this study was to determine the importance of plant physical cues on the oviposition behaviour of *B. fusca* females. Various plants and artificial supports with different combinations of physical cues were used. The artificial ones were chosen to confirm the effects of the plants' physical attributes on the moth's behaviour during the post-alighting phases, before the actual egg-deposition occurred.

Materials and methods

Insects

Fourth and fifth instars were collected from maize fields in Kenya. They were reared on the artificial diet described by Onyango & Ochieng²-Odero (1994) until pupation. Male and female pupae were kept in separate plastic boxes (30 × 12 × 10 cm) containing a moist cotton pad to

maintain a relative humidity of >80%, and checked daily for adult emergence.

Pupae and adults were maintained in a controlled chamber at 25.3 ± 0.9 °C, 68.6 ± 12.8% r.h. (means ± SE), and an L12:D12 reversed photoperiod, with the scotophase lasting from 07:00–19:00 hours, herewith referred to as night. The reversed photoperiod allowed all experiments to be carried out during daytime.

One-day-old males and females were used in oviposition response experiments. For behavioural observations, 1-day-old gravid naïve females were used according to the procedure of Calatayud et al. (2007). Thereby, 1-day-old females were released in a mosquito-net cage (40 × 40 × 63 cm) at onset of the scotophase. The first females started to call 6 h thereafter. One-day-old males were introduced 1 h later. During the ensuing 1-h period, mating pairs were taken out of the cage and put individually in plastic containers (8 cm in height × 5 cm in diameter). At the end of copulation, the insects were separated and the females were used during the following night for behavioural observations.

Plants

Busseola fusca oviposition was studied on four plant species: two cultivated plants, maize and sorghum, and two wild grasses, Napier grass (*Pennisetum purpureum* Schumach) and *A. donax*. Maize (cultivar 511) and sorghum (cultivar serena) seeds were provided by Simlaw, Kenya Seeds Company (Nairobi, Kenya). Napier grass varieties, both non-pubescent and pubescent (commonly cultivated in Kenya), were provided by International Centre of Insect Physiology and Ecology (ICIPE; Nairobi, Kenya) and obtained from cuttings. *Arundo donax* has leaf sheaths that are more rigid than those of maize, sorghum, and Napier grass. Both wild grasses (*A. donax* and Napier grass) are considered to be non-hosts in Kenya (Le Rü et al., 2006a,b; Ong'amo et al., 2006). *Arundo donax* was collected directly from the field in Kenya and grown from cuttings.

All plants were grown in individual plastic pots (12 cm in height × 13 cm in diameter) containing peat in a greenhouse at ICIPE. The environmental conditions were approximately 31/17 °C (day/night) with L12:D12 photoperiod. The plants were watered three times a week and once with a complete nutrient solution.

Surrogate stems

Surrogate stems consisted of rectangular pieces of paper (17 × 9 cm). Each piece of paper was rolled helicoidally from top to bottom and held in position with a single paper clip (Figure 1). Each surrogate stem was introduced into vials with the paper clip on the upper end. The upper end was folded so that the surrogate stem could fit into the plastic vial and could be maintained in an upright position

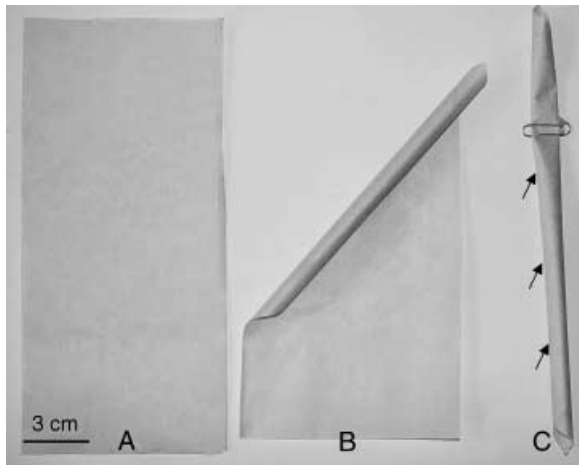


Figure 1 Steps in preparing a surrogate stem of wax paper to collect *Busseola fusca* eggs. (A) A piece of rectangular paper (17 × 9 cm) is (B) rolled helicoidally from top to bottom and (C) held in position with paper clip at the top. The eggs are laid in a linear row into the resulting grooves (see arrows) obtained after rolling the paper.

when closing the cover of the vial. Several types of grey paper simulating combinations of physical cues were chosen: (i) wax paper (40 g, 50.4 × 75.6 cm) commonly used as oviposition supports in the laboratory to rear *B. fusca* (FO Onyango, pers. comm.), (ii) crêpe paper (13 g, 50.0 × 250.0 cm) presenting a rough surface texture, and (iii) printing paper (80 g, 21.0 × 29.7 cm) with a higher sheet rigidity than wax paper.

Oviposition response experiments

Plants or surrogate stems were placed into rectangular mosquito-net cages (40 × 40 × 80 cm) and cylindrical plastic vials (16 cm in height × 10 cm in diameter), respectively. As the surrogate stems were smaller than the plants, we used smaller containers for them. Thereafter, one male and one female of *B. fusca* were introduced into each container. After 4 nights, the total number of eggs and egg batches laid on each plant or surrogate stem were counted, which indicated the number of times the females had oviposited. All experiments were replicated 10 times under the conditions described above. To ensure that all insects used had successfully mated, each female was dissected to check for the presence of spermatophore(s) in the bursa copulatrix. Only replicates with females bearing spermatophore(s) were considered in the following experiments.

Experiment 1: influence of the diameter of natural or artificial oviposition supports. We tested three different diameters, namely, 2.5–3.8, 9.2–10.2, and 17.0–19.5 mm, of sorghum and surrogate stems made of wax paper. Three sorghum

plants at the 3rd, 5th, and 7th leaf stage, exhibiting distinctly different stem diameters, were presented to *B. fusca* females in a triple-choice situation. In another experiment, three surrogate stems rolled to produce three distinct diameters were also presented in a triple-choice situation to *B. fusca* females.

Experiment 2: influence of the surface texture of natural or artificial oviposition supports. Both pubescent and non-pubescent Napier grass plants at the 5th–6th leaf stage with a stem diameter of ≈7 mm were used. Similarly, two surrogate stems consisting of either crêpe paper, with rough surface texture, or wax paper, with a smooth surface, were chosen. The diameter of both types of surrogate stems was approximately 10 mm. Pubescent vs. non-pubescent or rough vs. smooth surrogate supports were tested in dual-choice tests.

Experiment 3: influence of the rigidity of natural or artificial oviposition supports. To test the influence of leaf sheath rigidity on *B. fusca* oviposition, a no-choice situation forcing the female moths to oviposit on the single support offered was chosen. Two plant species were used, maize and *A. donax*. For each plant species, one potted plant at the 5th–6th leaf stage with a stem diameter of ≈7 mm was presented to *B. fusca* in a no-choice situation. To confirm the influence of rigidity of the support on *B. fusca* oviposition preference, two surrogate stems consisting of either wax paper with low rigidity and printing paper with high rigidity were presented together to *B. fusca* in a dual-choice test. The diameter of both types of surrogate stems was approximately 10 mm.

The rigidity of the plant leaf sheath or the paper sheet was measured with a Serigraph CYG008 (Technology Development Company, Chinese Academy of Agricultural Sciences Co. Ltd, Shanghai, China). Thereby, either a piece of leaf sheath or paper was fixed between two clamps and stretched to its breaking point. The force in Newton (N) necessary to break the material was measured.

Behavioural observations

To determine why treatments with plants or surrogate stems of a particular diameter, surface texture, and rigidity received more eggs, we compared the post-alighting pre-ovipositional behaviours of *B. fusca* females on 'stimulatory' and 'non-stimulatory' oviposition treatments. Each plant species or surrogate stem was used individually and placed in an open cage for observations. A single naïve gravid female was placed on the plant or surrogate stem and observed, for a maximum of 5 min, until the insect oviposited or took off. Illumination was provided by a 85-W incandescent red light tube. Treatments were replicated 15 times and

conducted under the conditions described above. They were performed 0–4 h after onset of the scotophase, which corresponds to the period of oviposition (Calatayud et al., 2007).

The behavioural steps included the following: (i) walking and antennating (WA), that is, the female walked on the plant stem, moving the antennae in front of the head and touching the plant surface with the distal segments of the antennae; (ii) ovipositor sweeping (OS), whereby the ovipositor touched the stem surface with the extremity and the dorsal part, making large lateral movements; (iii) attempting to insert the ovipositor (TIO), whereby the female tried to insert the ovipositor between the leaf sheath and the stem of the plants or inside the grooves that result after rolling the papers in the surrogate stems; (iv) oviposition (O), that is, actual egg deposition. The latency time taken by females before moving on the support at the beginning of each observation (i.e., duration of standing-still), the time taken to walk and to antennate (WA), and to sweep the ovipositor (OS) were recorded.

Data analysis

For oviposition responses, the data were $\log(x + 1)$ transformed when parametric tests were used for mean comparisons [i.e., Fisher's protected least significant difference following analysis of variance (ANOVA); two samples or paired t-test for no-choice or dual-choice situations]. For multiple-mean comparisons under triple-choice situations, the ranks were generated following a Friedman test, using proc Rank of SAS 9.1 (SAS Institute, 2003), and then means were separated using a Tukey–Kramer test (Proc GLM; SAS Institute, 2003).

For behavioural observations, the post-alighting pre-ovipositional behaviours were analysed using Proc Catmod (SAS Institute, 2003). Similarly to Parr et al. (1996) and Hora & Roessingh (1999), analysis of behaviour was conducted on the total number of transitions between

all possible pairs of behavioural steps and the data were pooled for all females. Transitional frequencies were used to form a contingency table.

Log-linear models were constructed, based on specific combinations of interactions to provide expected values of transitional frequencies for the contingency table (Parr et al., 1996; Hora & Roessingh, 1999). The goodness-of-fit of the log-linear model was assessed by likelihood ratio statistic (G-tests), and where the expected values of a model were not significantly different from the observed values, the assumptions of that model were accepted as necessary and sufficient to explain the observed data. In addition, an index was calculated to quantify the dissimilarity between the expected values under the assumptions of each model and the observed values. The probability of each transition given the preceding behaviour was calculated. To identify transitions that were a significant part of a sequence of behavioural steps, standardized residuals of the observed transitions were calculated and compared to a model that did not include the assumption of dependence of following and preceding behavioural steps. Significant positive transitions (those for which positive standardized residuals were obtained, which were greater than the calculated threshold) were used to construct kinetograms of the post-alighting pre-ovipositional behaviours.

For analysis of the durations of behavioural steps, the ranks were generated using a Kruskal–Wallis test, using proc Rank of SAS 9.1 (SAS Institute, 2003). Means were separated using Tukey–Kramer comparison procedure (Proc GLM, SAS Institute, 2003).

Results

Experiment 1: influence of the diameter of the oviposition support

The number of eggs laid per *B. fusca* female increased significantly with stem diameter of the plant ($F_{2,18} = 15.5$, $P = 0.0001$; Table 1). A similar trend was observed for the

Table 1 Influence of the diameter of a natural (sorghum) or artificial (rolled wax paper) oviposition support on oviposition by *Busseola fusca* in a triple-choice situation (means \pm SE, $n = 10$)

Oviposition support	Diameter (mm)	Number of eggs laid	Number of egg batches
Sorghum plant	2.5 \pm 0.2c	24.4 \pm 16.3b	0.2 \pm 0.1b
	9.2 \pm 0.8b	86.4 \pm 29.5a	1.2 \pm 0.2a
	17.0 \pm 1.0a	303.4 \pm 78.7a	2.6 \pm 0.4a
Rolled wax paper	3.8 \pm 0.5c	0.6 \pm 0.4c	0.2 \pm 0.1c
	10.2 \pm 0.8b	54.5 \pm 15.3b	2.2 \pm 0.3b
	19.5 \pm 0.5a	173.9 \pm 33.6a	3.7 \pm 0.4a

Means within a column and experiment followed by a different letter are significantly different at $P \leq 0.05$ [Fisher's protected least significant difference (PLSD) test following analysis of variance (ANOVA) for diameters; Tukey–Kramer test after generating ranks by Friedman test for the number of eggs and egg batches].

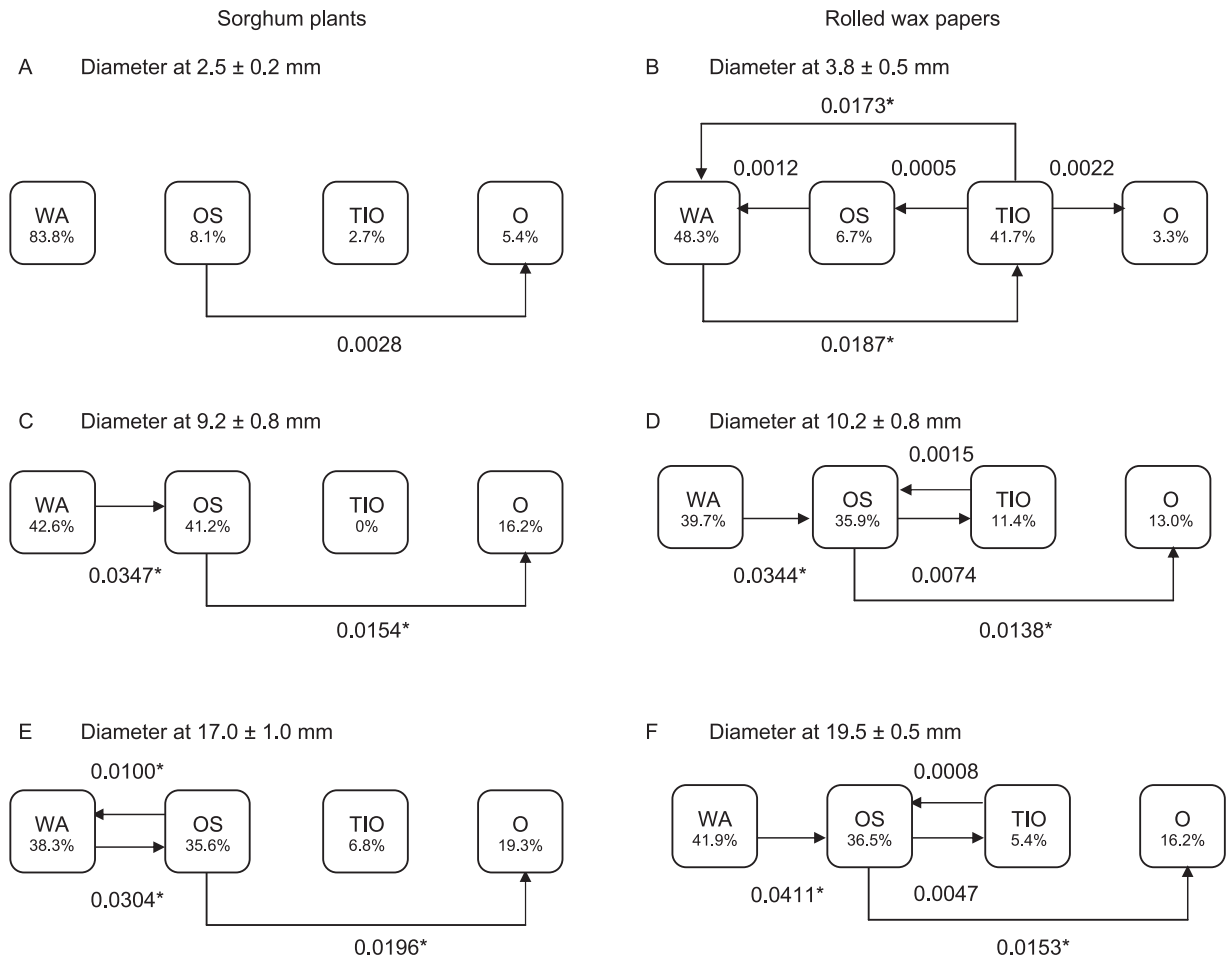


Figure 2 Pre-ovipositional behaviours of mated females of *Busseola fusca* on sorghum plants or rolled wax papers at three different diameters. Decimal numbers with the arrows represent the probability of each significant transition given the preceding behaviour. *Probability of transitions >0.0100. The percentage occurrence of each behavioural step is given below its abbreviation (WA, walking and antennating; OS, ovipositor sweep; TIO, attempting to insert the ovipositor; O, oviposition).

number of egg batches laid per female ($F_{2,18} = 15.6$, $P = 0.0001$). The oviposition behaviour of the female moths when offered sorghum stems suggested that they preferred to oviposit on larger stem diameters (Figure 2A,C,E). The probabilities of transitions between WA and OS and between OS and O increased with the stem diameter, and they were very low or absent between WA and OS on sorghum with a stem diameter of 2.5 mm. Moreover, the females spent significantly less time on walking, antennating, and ovipositor sweeping on plants with the smallest diameter than the largest (Table 2). The latency time was also significantly reduced. They walked faster on the stem of the lowest diameter before take off.

Similarly, with surrogate stems, the number of eggs and egg batches laid per female increased significantly with support diameter ($F_{2,18} = 36.6$, $P < 0.0001$ and $F_{2,18} = 114.3$,

$P < 0.0001$ for numbers of eggs and egg batches, respectively; Table 1). Although the kinetograms on artificial supports showed more transitions than those on plants, the observations confirmed that the oviposition preference of females increased with diameter of the support (Figure 2B,D,F). The probabilities of transitions between WA and OS and between OS and O increased with the diameter and they were very low or absent with paper rolled to 3.8 mm in diameter. In contrast, the probability of transitions between WA and TIO was highest on supports with the smallest diameter, where TIO occurred more frequently. Moreover, not only did fewer females exhibit OS behaviour on the support with the smallest diameter, they also spent significantly less time on walking, antennating, and sweeping of the ovipositor (Table 3).

Table 2 Duration (in s) of behavioural steps observed on plants in a no-choice situation (means \pm SE, n = 15)

	Behavioural steps		
	Standing still	Walking and antennating	Ovipositor sweeping
Plants			
Sorghum at 2.5 mm	1.1 \pm 0.3c	14.3 \pm 1.2b	3.8 \pm 1.2d
Sorghum at 9.2 mm	12.7 \pm 2.5bc	36.6 \pm 4.8a	30.3 \pm 3.7ab
Sorghum at 17.0 mm	12.9 \pm 3.1bc	34.9 \pm 3.4a	44.5 \pm 5.4a
Napier grass, non-pubescent	16.2 \pm 3.7b	39.8 \pm 3.0a	11.6 \pm 3.5cd
Napier grass, pubescent	80.9 \pm 6.4a	12.7 \pm 1.8b	4.1 \pm 1.7d
Maize	16.5 \pm 2.3b	30.0 \pm 3.7a	31.5 \pm 5.2ab
<i>Arundo donax</i>	15.8 \pm 3.5b	36.7 \pm 3.4a	24.4 \pm 5.4bc

Means within a column followed by a different letter are significantly different at $P \leq 0.05$ (Tukey–Kramer test after generating ranks by Kruskal–Wallis test).

Experiment 2: influence of the surface texture of the oviposition support

The number of eggs laid was significantly higher on the non-pubescent than pubescent variety of Napier grass ($t = 5.5$, d.f. = 9, and $P = 0.0004$; Table 4). A similar trend was obtained for the number of egg batches laid per female ($t = 4.5$, d.f. = 9, and $P = 0.0015$). Female moths preferred to oviposit on the non-pubescent Napier grass variety (Figure 3A,C). Similarly, the probabilities of transitions between WA and OS and between OS and O were higher on the non-pubescent than the pubescent variety. Furthermore, females spent more time on walking, antennating, and ovipositor sweeping on the non-pubescent Napier grass, and the latency time before initiation of the behavioural steps was higher on the pubescent than the non-pubescent variety (Table 2). Finally, on the pubescent variety, most females walked faster along the stem to the upper part of the plant and took off.

Similarly, the smooth wax paper received a significantly higher number of eggs and egg batches than the rough crêpe paper ($t = 10.6$, d.f. = 9, and $P < 0.0001$ and $t = 4.0$,

d.f. = 9, and $P = 0.0029$ for numbers of eggs and egg batches, respectively). This was verified with observations of the oviposition behaviour of the female moths (Figure 3B). The insects were most likely to leave the crêpe paper after walking rapidly on it. No significant transition was observed after WA. Moreover, as with females on the pubescent Napier grass variety, they spent significantly more time standing still before walking on the crêpe paper (Table 3). In contrast, they spent a shorter time walking, antennating, and sweeping the ovipositor on such paper.

Experiment 3: influence of the rigidity of the oviposition support

Maize exhibited lower leaf sheath rigidity than *A. donax* ($t = 6.8$, d.f. = 18, and $P < 0.0001$; Table 5). In a no-choice situation, a higher number of eggs and egg batches were laid on maize than on *A. donax* ($t = 6.4$, d.f. = 18, and $P < 0.0001$ and $t = 9.0$, d.f. = 18, and $P < 0.0001$ for numbers of eggs and egg batches, respectively). Moreover, with maize, all eggs were found between the leaf sheath and the stem while with *A. donax*, 34.0 ± 14.8 eggs (i.e., about

Table 3 Duration (in s) of behavioural steps observed on artificial supports in a no-choice situation (means \pm SE, n = 15)

	Behavioural steps		
	Standing still	Walking and antennating	Ovipositor sweeping
Artificial supports			
Rolled wax paper at 3.8 mm	40.3 \pm 6.2b	11.2 \pm 2.0bc	9.1 \pm 0.5c
Rolled wax paper at 10.2 mm	37.9 \pm 5.5b	23.1 \pm 2.8a	39.1 \pm 3.2a
Rolled wax paper at 19.5 mm	35.9 \pm 5.9b	41.7 \pm 7.2a	31.1 \pm 6.3b
Rolled crêpe paper	87.7 \pm 15.2a	6.2 \pm 0.9c	1.4 \pm 0.8d
Rolled printing paper	38.9 \pm 5.7ab	13.1 \pm 2.0b	28.5 \pm 4.8b

Means within a column followed by a different letter are significantly different at $P \leq 0.05$ (Tukey–Kramer test after generating ranks by Kruskal–Wallis test).

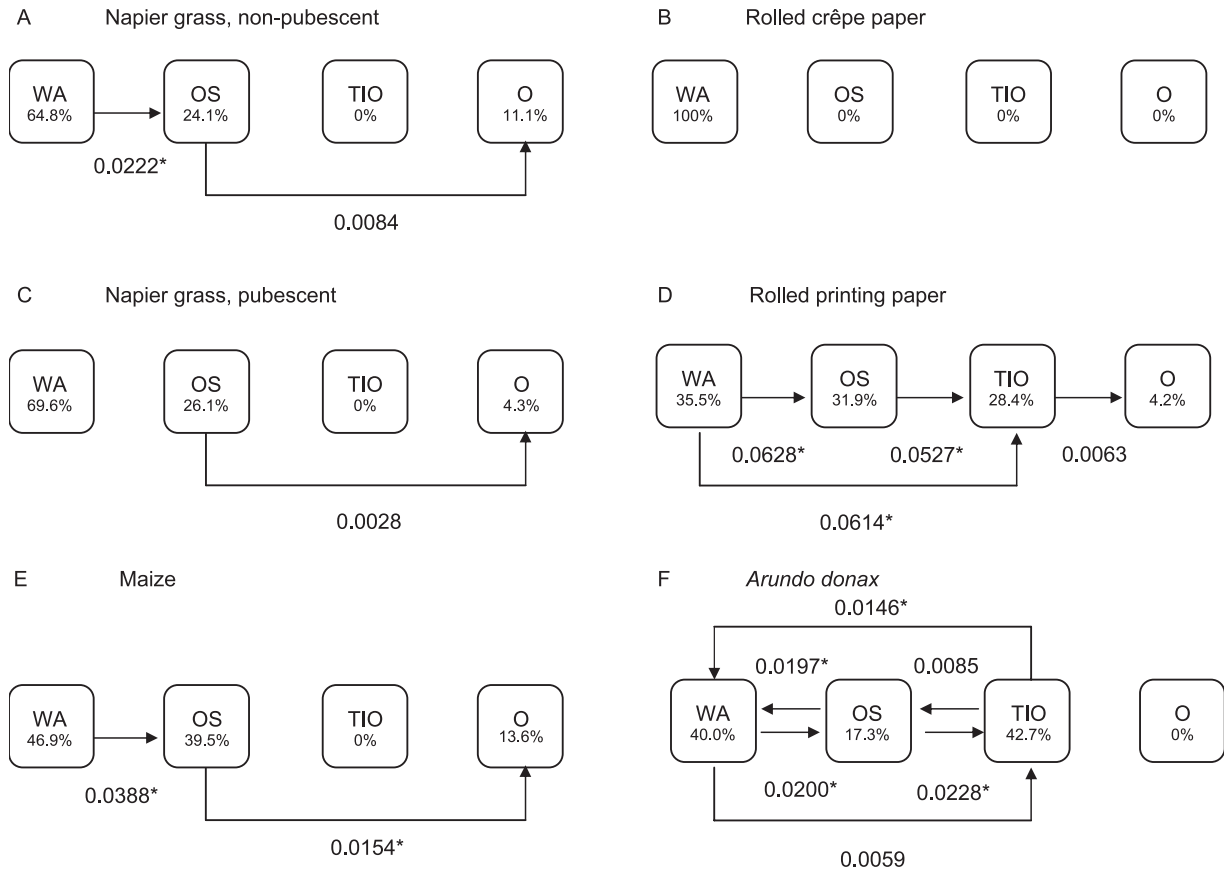


Figure 3 Pre-ovipositional behaviours of mated females of *Busseola fusca* on several oviposition supports: Napier grass non-pubescent, napier grass pubescent, maize, *Arundo donax*, rolled crêpe paper, or rolled printing paper. Decimal numbers with arrows represent the probability of each significant transition given the preceding behaviour. *Probability of transitions >0.0100. The percentage of occurrence of each behavioural step is given below its abbreviation (see legend of Figure 2).

54.5% of the total eggs laid) were found outside the plant and even on the soil close to the stem. The rigidity of the leaf sheath clearly disturbed the oviposition behaviour of the female moths. On *A. donax*, the females tried to insert the ovipositor between the leaf sheath and the stem,

but egg deposition was not successful. They frequently switched from WA to OS to TIO, from OS to WA, and from TIO to WA but without a single transition to O (Figure 3F). In contrast, the females on maize switched more frequently from WA to OS and to O (Figure 3E).

Table 4 Influence of the surface texture of natural (Napier grass) or artificial (rolled paper) oviposition support on oviposition of *Busseola fusca* in a dual-choice situation (means ± SE, n = 10)

Oviposition support	Number of eggs laid	Number of egg batches
Napier grass, non-pubescent	200.9 ± 50.3a	3.1 ± 0.4a
Napier grass, pubescent	13.3 ± 6.4b	0.7 ± 0.3b
Rolled wax paper	171.4 ± 41.9a	2.7 ± 0.6a
Rolled crêpe paper	6.5 ± 6.5b	0.4 ± 0.4b

Means within a column and experiment followed by a different letter are significantly different at P ≤ 0.05 (paired t-test).

Wax paper exhibited lower sheet rigidity than printing paper (t = 6.5, d.f. = 18, and P < 0.0001). In a dual-choice situation, the surrogate stem made of wax paper, which had low N-values, received a significantly higher number of eggs and egg batches than the more rigid printing paper (t = 4.6, d.f. = 9, and P = 0.0012 and t = 4.7, d.f. = 9, and P = 0.0012 for numbers of eggs and egg batches, respectively). As with *A. donax*, egg deposition on printing paper was less successful than on wax paper (Figures 2D and 3D). On printing paper, they frequently switched from WA to OS to TIO and from WA to TIO but with a lower probability of transition from TIO to O. Moreover, the females spent less time on walking, antennating, and ovipositor sweeping than on wax paper rolled at 10.2 mm of diameter (Table 3).

Table 5 Influence of the surface texture of natural (a) or artificial (b) oviposition support on oviposition of *Busseola fusca* in no-choice and dual-choice situations for experiments (a) and (b), respectively (means \pm SE, n = 10)

Oviposition support	Force (in N)	Number of eggs laid	Number of egg batches
(a) Maize	62.0 \pm 1.3b	273.3 \pm 40.9a	3.8 \pm 0.2a
<i>Arundo donax</i>	99.8 \pm 6.9a	28.3 \pm 19.3b	0.4 \pm 0.2b
(b) Rolled wax paper	108.1 \pm 3.8b	98.5 \pm 29.3a	6.1 \pm 0.8a
Rolled printing paper	137.4 \pm 1.2a	16.0 \pm 7.8b	1.4 \pm 0.5b

Means within a column and experiment followed by a different letter are significantly different at $P \leq 0.05$ [two-sample t-test for forces and for the numbers of eggs and egg batches of experiment (a) and paired t-test for the numbers of eggs and egg batches of experiment (b)].

Discussion

Combinations of various physical and chemical factors are generally involved in the evaluation of a potential host plant for oviposition by both butterflies and moths (Renwick & Chew, 1994). Thereby, physical factors (e.g., plant surface texture) play a more critical role for nocturnal moths than for diurnal butterflies. Although *B. fusca* is oligophagous and specialized on a few plant species only, the chemical plant characteristics are most likely not obligatory in the plant acceptance process, because gravid females oviposit on artificial supports, without any olfactory or contact chemical stimuli, when they have no choice. Therefore, *B. fusca* is easy to rear under artificial conditions compared to other specialized stem borers, such as *Manga melanodonta* (Hampson) and *Manga nubifera* (Hampson) (Lepidoptera: Noctuidae) (Moyal & Le Rü, 2006). In choice situations, physical stimuli associated with plants are known to play an important role in the host acceptance for oviposition of gravid female moths (e.g., Ramaswamy, 1988; Renwick & Chew, 1994; Foster et al., 1997). The importance of physical stimuli was verified for *B. fusca* in the present study. In the field, female noctuid stemborers very rarely lay more than one egg batch per plant. They avoid plants already having egg batches and oviposit at a distance from an occupied plant, leading to a regular distribution of egg batches in the field (Sétamou & Schulthess, 1995; Chabi-Olaye et al., 2005). More than one egg batch per plant could lead to early destruction of the food source, causing cannibalism, forced emigration, and exposure to predators, resulting in increased larval mortality. Sétamou & Schulthess (1995) suggested that the female moths mark the oviposition site to prevent oviposition of a second egg batch on the same plant or a nearby plant, in order to increase the chances of survival of its offspring. As shown by Ndemah et al. (2001), multiple egg batches can be the result of overcrowding. In our oviposition response experiments, although only one female was used per treatment, overcrowding occurred as

each female was ovipositing more than one egg batch per support or plant. Hence, while the lack of differences in egg batches between treatments in overcrowding situations would not automatically be evidence of a lack of preference, significant differences obtained in this study would indicate a very strong preference or deterrence. Direct observations indicated that the apparent oviposition deterrence observed in 'non-stimulatory' oviposition treatments (very small oviposition support diameter, pubescent or rough texture, and rigid leaf sheath) under our experimental conditions seemed to be the results of ovipositional constraints. As also shown for *Chrysophtharta bimaculata* (Olivier) (Coleoptera: Chrysomelidae) (Howlett & Clarke, 2003, 2005), oviposition deterrence in *B. fusca* is very likely caused by evolved mechanisms of oviposition site selection, that is, suitable oviposition sites are restricted to the gaps between the leaf sheath and the stem, and, hence, rigidity and pubescence of the stem or leaf sheath will affect oviposition.

Busseola fusca prefers not to lay eggs on plants with very small stem diameters, when they have a choice. The female moths seemed to be able to assess host diameter by walking on the support and touching the support with the tip of their antennae. When the smallest plant stem or surrogate diameters were used, the antennae surrounded the supports, allowing the females to walk faster up to the tip of the support and then fly away. On surrogate stems with the smallest diameters, females tried to insert their ovipositor more frequently, but with unsuccessful egg deposition. This indicates that supports with smaller diameters were more tightly rolled, which affected insertion of the ovipositor. These results explain partly previous field observations, in which larger stems received higher egg loads than thinner ones (van Rensburg et al., 1987; Moyal, 1998). Similar results were reported by Sétamou & Schulthess (1995), Sémeglo (1997), and Schulthess & Ajala (1999) for the noctuid stemborers *Sesamia calamistis* Hampson in West Africa and for *B. fusca* by Ndemah et al. (2001) in Cameroon. In addition, the latter found that once maize plants

started tasseling, oviposition by noctuid stemborer species became rare. This is probably linked with the ‘mother knows best’ theory (Schoonhoven et al., 1998): the female will choose a plant age for oviposition that guarantees a high survival of her offspring.

Another stimulus that influences oviposition of *B. fusca* was the surface texture, with females preferring to lay on smooth than rough surfaces. The pubescent and rough surfaces disturbed oviposition of *B. fusca*. The females showed strong difficulties in sweeping the ovipositor, a typical behavioural process to find a suitable oviposition site (Calatayud et al., 2006), on such surfaces and this limited the proper insertion of the ovipositor. Moreover, the examining behaviours (walking and antennating, and ovipositor sweeping) were almost avoided or inhibited on such surfaces, as expressed by the highest latency times and shortest times spent for WA and OS before taking off. Similarly, the resistance of maize cultivars to *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) has been attributed to the high density of trichomes on the leaf surface (Kumar, 1992). In addition, at tasseling maize plants start to remobilize nitrogen from stem and leaves to the ear (Bonato et al., 1999) and senescent plants become increasingly rougher. Thus, the decrease in acceptability of plants older than tasseling [reported by Sétamou & Schulthess (1995), Sémeglo (1997), Schulthess & Ajala (1999), and Ndemah et al. (2001)] might, besides decreasing nitrogen, be partly explained by changes in surface texture of leaf sheaths. Therefore, plant texture may be a good indicator when seeking for potential host plant species and plant parts accepted by *B. fusca*. In fact, among the identified host plant species of *B. fusca* in the field, none is pubescent and they all have a waxy texture (Le Rü et al., 2006a,b). It is well known that the chemical and physical properties of the plant cuticular wax play a crucial role in the interactions between plants and phytophagous insects (Müller & Riederer, 2005). In *B. fusca*, the wax composition of the plant surface seemed to influence the host plant acceptance process by the ovipositing females (Juma, 2005).

The leaf sheath rigidity also influenced oviposition of *B. fusca*. *Arundo donax*, having higher leaf sheath rigidity than maize, received fewer eggs than maize and most of them were laid on the plant surface or soil. Observations of the oviposition behaviour showed clearly that the rigidity of the leaf sheath affected the proper insertion of the ovipositor. *Busseola fusca* is rarely found on *A. donax* in Kenya where the main hosts are maize and sorghum (Le Rü et al., 2006a). This corroborates results from oviposition preference studies with the noctuid *S. calamistis*, a stem-boring pest often occupying the same niche as *B. fusca*, where no eggs were laid on *Imperata cylindrica* (L.) P. Beauv., which has thin stems and stiff tight leaf sheaths

(F Schulthess, pers. obs.). Similar observations were made with *Sesamia nonagrioides* (Lefèbvre) (Lepidoptera: Noctuidae) (B. Frérot, pers. obs.). The two *Sesamia* species share with *B. fusca* a similar morphology of ovipositor, which is covered with mechanoreceptors; they express the same oviposition behaviour (Robert & Frérot, 1998; Calatayud et al., 2006) and both species oviposit between the leaf sheath and the stem. Thus, a more rigid leaf sheath may render ovipositor insertion difficult in noctuid stem borers.

The present findings underline the importance of physical stimuli in the host plant selection and oviposition performance of *B. fusca*, and they corroborate field observations (Le Rü et al., 2006a): in Kenya, *B. fusca* avoids *Panicum deustum* Thunb., which is non-pubescent but thin stemmed, *Panicum maximum* Jacq., which has a large stem diameter but which is frequently pubescent, and *Phragmites mauritianus* Kunth., which has a large stem diameter but rigid leaf sheaths. In contrast, the most preferred wild host, *Sorghum arundinaceum* [Desv.] Stapf, as well as cultivated sorghum and maize are waxy plant species and have soft leaf sheaths (Le Rü et al., 2006a,b).

The present findings are important for future applications. For example, in a ‘push–pull’ strategy it would be preferable to choose as a trap plant a non-pubescent species with lower leaf sheath rigidity. At the same time, farmers should preferably plant pubescent varieties of maize. However, this study does not rule out the importance of plant chemistry on plant selection and acceptance for oviposition by *B. fusca*.

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