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Response to conspecific and heterospecific semiochemicals by *Sesamia nonagrioides* (L.) (Lepidoptera: Noctuidae) gravid females

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Abstract

The Mediterranean corn borer, *Sesamia nonagrioides*, occurs sympatrically in the northeast of Spain with other lepidopteran pests such as *Ostrinia nubilalis* and *Mythimna unipuncta*. In this study, we evaluated the electrophysiological and behavioural response of mated and unmated females and males of *S. nonagrioides* to their own complete pheromone blend, to its own four components separately, and to the pheromone components of the sympatric species *O. nubilalis* and *M. unipuncta*. Results of the electroantennogram recordings revealed that females of *S. nonagrioides* can detect their own pheromone blend and its individual components. Moreover, our results show that unmated females and males of *S. nonagrioides* are more sensitive to the female pheromone, showing higher electrophysiological response than the mated females and males. Electroantennogram recordings showed that males and females can detect the major sexual pheromone component of *O. nubilalis* (*Z*)-11-tetradecenyl acetate and the minor component of the pheromone of *M. unipuncta* (*Z*)-9-hexadecenyl acetate. When the sex pheromone stimulus was presented in the dual-choice assays, gravid females of *S. nonagrioides* were attracted to both their own complete pheromone blend and one of their own minor pheromone components, (*Z*)-11-hexadecenal, but the major sexual pheromone component of *O. nubilalis* acts as a behavioural antagonist to the females.

Keywords: electroantennogram, female, inter-intraspecific pheromones response, olfactometer

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Introduction

Insect colonization and host location are worthy of study not only for their biological and ecological interest but also with a view to implementing novel techniques for integrated crop protection. The environment of a phytophagous insect is highly complex, and includes plants of various species and different ages and shapes, with only one or a few serving as hosts. During host plant location and selection for feeding, oviposition and mating, herbivorous insects need to integrate multimodal sensory inputs (Brevault & Quilici, 2010) following three basic steps: initially, insects detect chemical plant volatiles that stimulate them to take off; then the visual stimuli such as contrast, colour and shape indicate where to land; and finally the mechano- and chemoreception indicate the host plant suitability (Finch & Collier, 2000).

In Lepidoptera, host plant selection is primarily a function of the gravid female and chemical cues play the greatest role in host selection (Renwick, 1989). Chemicals in corn plant extracts stimulate the oviposition of the corn borers *Ostrinia nubilalis*, *O. furnacalis*, *Chilo partellus* and *Sesamia nonagrioides* (Binder *et al.*, 1995; Varshney *et al.*, 2003; Konstantopoulou *et al.*, 2004; Guo & Li, 2009). However, other factors such as interspecific and intraspecific interactions could affect host selection (Gabel & Thiery, 1994; Harmon *et al.*, 2003).

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Maize is attacked by a complex of insects from the time it is planted until it is used as a food or feed (Eizaguirre & Albajes, 1989). In our study area, a number of lepidopteran caterpillars occur sympatrically and feed on different parts of the maize plant: *Mythimna unipuncta* and *Spodoptera exigua* feed on the leaves; *Helicoverpa armigera* feed on the maize ears; and *S. nonagrioides* and *O. nubilalis* are stem borers (Eizaguirre & Albajes, 1989). Except for *O. nubilalis*, the moths selected for this study belong to the Noctuidae family and share some components of their pheromone blend. The pheromone of the Mediterranean corn borer *S. nonagrioides* is a blend of four components: (*Z*)-11-hexadecen-1-yl acetate (Z11-16:Ac), (*Z*)-11-hexadecen-1-ol (Z11-16:OH), (*Z*)-11-hexadecenal (Z11-16:Ald) and dodecyl acetate (12:Ac) (Sans *et al*., 1997). The sex pheromone of *M. unipuncta* is a blend of Z11-16:Ac, Z11-16:OH and (*Z*)-9-hexadecenyl acetate (Z9-16:Ac) (Guerrero *et al*., 1986); the sex pheromone of *H. armigera* is a blend of Z11-16:Ald and (*Z*)-9-tetradecenal (Z9-14:Ald) (Tamhankar *et al*., 2003); and the sex pheromone of *O. nubilalis* is a blend of two isomers, (*Z*)-11-tetradecenyl acetate (Z11-14:Ac) and (*E*)-11-tetradecenyl acetate (E11-14:Ac) (Klun *et al*., 1973).

Interspecific interactions between lepidopteran pests and/or with other insect groups have been studied by Ofomata *et al.* (1999), Eizaguirre *et al.* (2002), Harmon *et al.* (2003), Gemeno *et al.* (2006), and Lelito *et al.* (2008). Specifically, the study of the effect of pheromone detection between different species has generated increasing interest because some pheromone compounds can act as a behavioural antagonist of males and females of other sympatric species (Stephens *et al*., 2008).

Eizaguirre *et al.* (2002) demonstrated that the densities of *O. nubilalis* in fields treated for mating disruption with *S. nonagrioides* pheromone released from PVC dispensers were always lower than those in untreated fields. In addition, Gemeno *et al.* (2006) found that rubber septa loaded with a constant concentration of the pheromone of *O. nubilalis* and different percentages of the *S. nonagrioides* pheromone cause dose-dependent antagonism in the field. In particular, the major component of the *S. nonagrioides* pheromone (Z11-16:Ac) displayed a potent antagonism in field and laboratory trials. Furthermore, in field studies, traps baited with *S. nonagrioides* pheromone attracted males of *M. unipuncta* (Albajes *et al*., 1988), while the addition of Z9-16:Ald, a minor component of *M. unipuncta* pheromone, significantly reduced the effectiveness of *S. nonagrioides* lure (Eizaguirre *et al*., 2009).

The above studies only evaluate the pheromone antagonism in males, but few studies consider whether females can recognize their own pheromone or the pheromone components of other sympatric species. If the female can detect conspecific or heterospecific semiochemicals, these chemical compounds could act as oviposition deterrents or stimulants that are of special interest in the female host location of *S. nonagrioides*, and therefore in new plant protection strategies. In this study we evaluated the electrophysiological and behavioural response of mated and unmated females and males of *S. nonagrioides* to their own complete pheromone blend, to its four components separately, and to the pheromone components of *O. nubilalis*, *M. unipuncta* and *H. armigera*.

Materials and methods

Insects

A laboratory culture was established with different populations of *S. nonagrioides* larvae collected from maize fields in the Lleida area (north-east Spain). Larvae were fed on a semi-artificial diet at 25°C, 50% RH and a 16:8 h (light: dark) photoperiod and the culture was renewed every three or four generations with larvae or pupae from the field (Eizaguirre & Albajes, 1992). For oviposition, single adult couples of males and females from different populations resulting from the laboratory culture were caged with a 5-leaf maize plant. Laid eggs were maintained on the plant till the day before hatching (larval capsule visible). Then, the eggs and a piece of leaf were transferred to a transparent cylindrical container (3.5 × 2 cm in height) with a 1 cm^3 cube of diet. Seven days later the larvae were separated, labelled according to the original population, and reared individually in a similar container until pupation. Pupae were sexed by external morphological differences, and the resulting adults separated by sex were maintained at 20°C and a 16:8 h (light: dark) photoperiod. For experiments with virgin male and female individuals, pupae coming from different populations were transferred separately by sex to a plastic container (50 × 50 × 50 cm) to avoid unexpected matings in new emerged adults. For experiments with mating females, newly emerged males and females coming from different populations were transferred to a similar plastic container at a ratio of 1♀:2♂. After 24 h the moths were collected and used for the experiments. Individuals were used only once for experiments. After the experiments, supposed mated females were then dissected to confirm the presence in the bursa copulatrix of spermatophores, which indicate successful mating.

Chemicals

Pheromone components of *S. nonagrioides* (Z11-16:Ac, Z11-16:OH, Z11-16:Ald and 12:Ac), *O. nubilalis* (Z11-14:Ac and E11-14:Ac) and *M. unipuncta* (Z9-16:AC) were purchased from Pherobank (Wageningen, the Netherlands).

Electrophysiology

The EAG apparatus was purchased from Syntech (Hilversum, the Netherlands). In summary, charcoal-filtered and humidified air with air speed between 25 and 50 cm s^{-1} was continuously directed over the insect antenna through the main branch of a glass tube (7 cm long × 5 mm diameter). Test stimulations were carried out by giving puffs of air (300 ml min^{-1}) through a Pasteur pipette for 100 ms using a TC-05 stimulus controller (Syntech). The pipette contained a small piece of filter paper (2 × 1 cm) on which the stimulus compounds were placed diluted in hexane. The solvent was allowed to evaporate before the tests.

Excised antennae of 24 h-old mated and unmated males and females were stimulated with 5 puffs of 10 μg at 30 s intervals in each of the treatments. Stimuli tested were the complete pheromone blend of *S. nonagrioides* in a ratio of 77:8:10:5 (Sans *et al*., 1997), its individual pheromone components, (Z11-16:Ac, Z11-16:OH, Z11-16:Ald, and 12:Ac) (Z11-16:ald is also the main component of the *H. armigera* pheromone), and the pheromone components of *O. nubilalis* (Z11-14:Ac; E11-14:Ac), and *M. unipuncta* (Z9-16:Ac). Experiments were conducted as in Giner *et al*. (2009). In summary, antennae of 15 different insects were used for the experiments. Control puffs with a piece of paper containing only solvent (hexane) were also intercalated before and after the 5 puffs of each pheromone stimulus to determine the baseline depolarization of the antenna. The signals were amplified (100 ×) and filtered

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(DC to 1 kHz) with an ID-2 interface (Syntech), digitized on a PC, and analysed with the EAG2000 program. The relative EAGs to a test stimulus were calculated as $R_r=R_i/((C_1+C_2)/2)$, where R_i is the absolute amplitude of the stimuli and C_1 and C_2 are the responses of the control solvents before and after the 5 puffs of each treatment stimulation. Mated males corresponded to those that were introduced in the plastic container with the females. Unmated males were those that were absent from females from pupation and unmated females were those that were absent from males from pupation. Mated females were confirmed by dissection to examine the presence of spermatophores in the bursa copulatrix.

Dual-choice bioassays

A Y-tube olfactometer and a wind tunnel assay were used to test the olfactory responses of 24 h-old adult mated females of *S. nonagrioides* to the complete pheromone blend in a ratio of 77:8:10:5 and to its individual pheromone components (*Z*11-16:Ac, *Z*11-16:Ald, *Z*11-16:OH and 12:Ac), and to the pheromone components of *O. nubilalis* (*Z*11-14:Ac; *E*11-14:Ac), and *M. unipuncta* (*Z*9-16:Ac), along with odours emitted by maize plants.

The Y-tube olfactometer (Fig. 1) consisted of two main glass tubes (diameter 20 cm, length 34 cm) linked by a glass reservoir chamber (R) (diameter 20 cm, height 34 cm) containing the test insects. The tubes diverged in a '*V*' form with an angle of 45°. At the other end of the tubes, two more reservoirs, similar in form and size to the previous one, contained the volatile source (*S*) along with a maize plant (T_*), or solvent along with a maize plant (T_0). The dimensions of the tubes and reservoirs offered the insects sufficient room to fly freely inside the olfactometer. Two round holes (4 cm diameter) at a height of 28 cm from the base of each chamber allowed connection with two small exhaust fans (3 cm diameter). Another round hole (4 cm diameter) at the same height from the base of the first chamber (28 cm) was connected to an inlet fan. The inlet fans and the exhaust fans worked concomitantly to provide an air speed of 0.22 m s^{-1}. The olfactometer was located in a climate room under a 16:8 h LD photoperiod at 25 ± 1°C and 65 ± 5% RH, illuminated by red light of 4 lux of intensity.

The amount of individual compounds deployed was related to the amount used previously in wind tunnel experiments (10 µg of pheromone) (Sans *et al.*, 1997). The solvent was allowed to evaporate and the dispensers were then ready to use. Two individual dispensers, one with the test compound and one containing hexane (10 µg) as a control, were placed inside the chambers (T_* and T_0, respectively) along with the maize plant in front of the inlet fans. After each assay, the device was thoroughly cleaned with ethanol and the plants with the treatment or the solvent were moved to different chambers to avoid a positional bias. The order of treatments was randomized. The time given to the insects to respond to the maize plants both in the olfactometer and in the wind tunnel were the three first hours after the onset of the scotophase, because during this time, gravid females of *S. nonagrioides* seek host plants (preliminary experiments).

In each assay, groups of 3 to 5 mated females were released into the common chamber (R). Female behaviour was observed every 15 min. At the end of each assay (three hours later) the number of females in T_* versus number of females in T_0 control was recorded. Only the females that performed the oviposition behaviour (oriented flight to the odour source, sweeping the plant with the ovipositor) and laid eggs, were taken into account for the statistical analysis. When a female showed the oviposition behaviour it was removed from the chamber. Females that did not show oriented flight or showed calling behaviour were removed from the olfactometer and not considered for the analysis. The assays were run until at least 35 insects responded to each compound.

The wind tunnel dual-choice assay was carried out in a laminar flow wind tunnel (length 151 cm, width 47 cm, height 44 cm) (Gemeno *et al.*, 2006) at 25 ± 2°C, 50% RH and a wind velocity of 0.3 m s^{-1} during the first and third hour of the scotophase. The tunnel was illuminated with a red light resulting in 4.5 lux intensity at the ceiling and floor.

Groups of 4 to 6 gravid females were placed at the downwind end of the tunnel at a distance of 125 cm from the two maize plants, which were located at the upwind end of the tunnel and placed 15 cm apart. Gravid *S. nonagrioides* females were offered a choice between two odour sources: maize plants along with hexane serving as a control; or the pheromone components of *O. nubilalis*, *M. unipuncta*, *H. armigera* and *S. nonagrioides*. Each treatment contained 10 µg of pheromone or hexane and was applied in a rubber septum to the middle of each maize plant. The assays were run until at least 40 insects responded to each treatment. As in the previous experiment, only the number of females that performed the oviposition behaviour and laid eggs were taken into account for the statistical analysis, and calling or non-flying females were removed from the tunnel.

Statistical analysis

All statistical analysis was done using JMP, Version 8 (2008). For all comparisons, the level of $P = 0.05$ was considered significant. Before the statistical analysis, the Shapiro Wilk test was used to check for normality distributions. For the electrophysiological recordings, depolarization means were analysed using ANOVA followed by Tukey's multiple range tests. All data obtained from the dual-choice assays (wind tunnel and dual-choice olfactometer) were subjected to a binomial test (Zar, 1996), considering that females showed no preference for either olfactometer arm (50:50 response). Responses were converted to percentages for presentation.

Results

Electrophysiology

The results show a clear difference in the electrophysiological response between males and females, the male response always being higher than the female response (ANOVA test, $F_{1,226} = 1720$; $P < 0.001$) (Figs 2A, B). Moreover, our results show that unmated females and males of *S. nonagrioides* are more sensitive to the female pheromone, showing higher electrophysiological response than the mated females and males (ANOVA test, $F_{1,469} = 5.65$; $P < 0.001$).

The response of the antenna of mated and unmated females of *S. nonagrioides* to their own complete pheromone and each component of the pheromone blend are represented in Fig. 2A. The highest response was obtained when the unmated female antennae were stimulated with the major component of their blend (*Z*11-16:Ac), the response to the full blend being lower. However, the highest responses of mated female antennae (Fig. 2A) occurred when they were stimulated with the aldehyde or the major component of their

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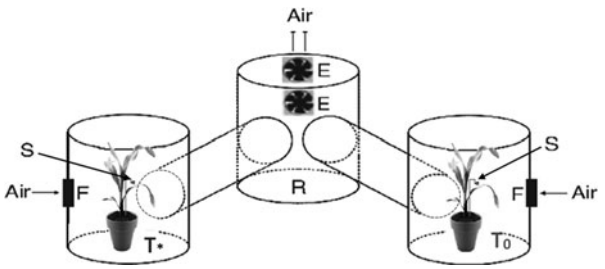

Fig. 1. Diagram of Y-tube olfactometer. *R*, common reservoir for introducing test insects; T_*, reservoir with the plant and the treatment; T_0, reservoir with the plant and the hexane (control); *E*, exhaust fan; *F*, inlet fan; *S*, volatile source (treatment or hexane).

pheromone, the responses to the full blend being lower. No significant differences were found between the electrophysiological response to Z11-16:Ald in mated and unmated females. The lowest responses in the antenna of mated and unmated females were produced by Z11-16:OH and 12:Ac, and no significant differences were found between them (ANOVA test, $F_{9,967} = 66.25$; $P > 0.001$).

The responses of the male antennae to the full blend and to each of the components are shown in Fig. 2B. As expected, the highest response was that of the antennae of unmated males to the full blend, followed by that of the antennae of unmated and mated males to the major component of their pheromone. The responses to aldehyde and to alcohol were similar in unmated and mated males. The lowest responses corresponded to 12:Ac and showed no differences between mated and unmated males (ANOVA test, $F_{9,906} = 45.8$; $P > 0.001$).

Figure 3 shows the electrophysiological response of mated and unmated females and males of *S. nonagrioides* to the major components of *O. nubilalis* pheromone and to the minor component of *M. unipuncta*. Male antennae showed a high response to the main component of the *O. nubilalis* pheromone (Z11-14:Ac) (Fig. 3A), but no differences were found between those of mated and unmated males (ANOVA test, $F_{1,113} = 1.95$; $P < 0.16$). The electrophysiological recordings in female antennae displayed a lower response to the main component and, as in males, no differences were found between mated and unmated females (ANOVA test, $F_{1,187} = 0.6$; $P > 0.43$) (Fig. 3A). There were no differences between the hexane control and the secondary component of the *O. nubilalis* pheromone (E11-14:Ac) in male and female antenna (data not shown).

The results for the pheromone components of *M. unipuncta* are shown in Fig. 3B. Males of *S. nonagrioides* exhibited noteworthy electrophysiological responses to the minor sex pheromone component of *M. unipuncta*, and differences were found between mated and unmated males (ANOVA test, $F_{1,198} = 19.41$; $P < 0.001$). Females also displayed EAG responses to Z9-16:Ac, although no differences were found between mated and unmated females (ANOVA test, $F_{1,208} = 2.03$; $P > 0.27$)

Dual-choice bioassays

Figure 4 compares the behavioural responses of mated females in both tunnel flight (A) and Y-tube olfactometer (B) to a plant without any pheromone component (control) versus a plant with the full blend or each of the components of their

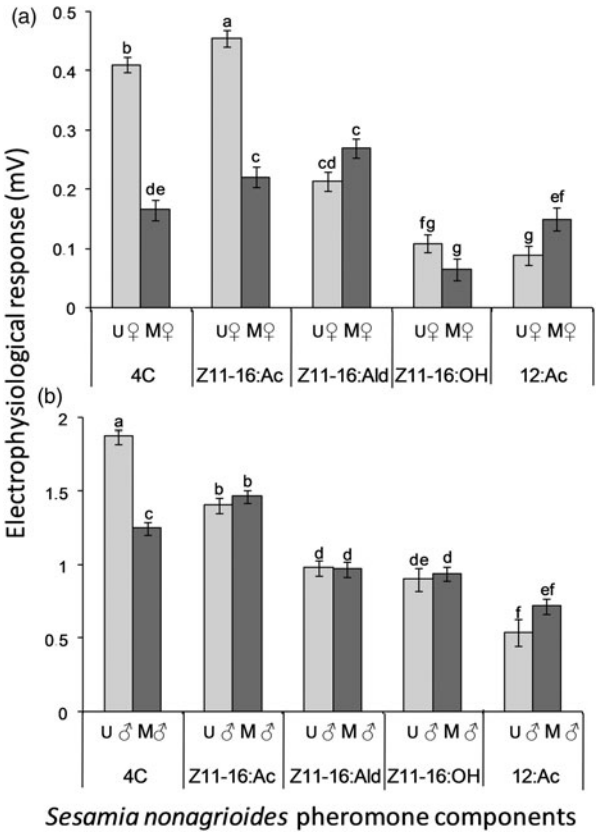

Fig. 2. Electrophysiological response of mated and unmated females (A) or males (B) of *Sesamia nonagrioides* to 10 µg of their own complete sexual pheromone blend, 4C ((*Z*)-11-hexadecen-1-yl acetate (Z11-16:Ac), (*Z*)-11-hexadecen-1-ol (Z11-16:OH), (*Z*)-11-hexadecenal (Z11-16:Ald) and dodecyl acetate (12:Ac); 77:8:10:5, respectively), and to its individual components, Z11-16:Ac, Z11-16:OH, Z11-16:Ald (also the main component of *Helicoverpa armigera* pheromone), and 12:Ac. *U*♀ = unmated female, *M*♀ = mated female, *U*♂ = unmated male, *M*♂ = mated male. $N = 30$ antennae. Bars with different letters are significantly different (Tukey's test, $P < 0.05$).

own pheromone or the major components of the *O. nubilalis* and *M. unipuncta* pheromone. Gravid females preferred the plant with aldehyde (Z11-16:Ald) in both the wind tunnel and the olfactometer. Furthermore, gravid females preferred the plant with the full blend of their own pheromone to the plant alone in the olfactometer. On the other hand, when we tested the major pheromone component of *O. nubilalis* (Z11-14:Ac), *S. nonagrioides* gravid females preferred the plant alone to the plant with Z11-14:Ac. No significant differences were found when Z11-16:Ac, Z11-16:OH, 12:Ac and the minor pheromone component of *O. nubilalis* (E11-14:Ac) and *M. unipuncta* (Z9-16Ac) were tested in the wind tunnel and Y-tube olfactometer.

Discussion

Previous studies of the pheromone interactions between adults of caterpillars feeding on maize demonstrated that *S. nonagrioides* males can detect and respond to female pheromone components and to the pheromone components of other

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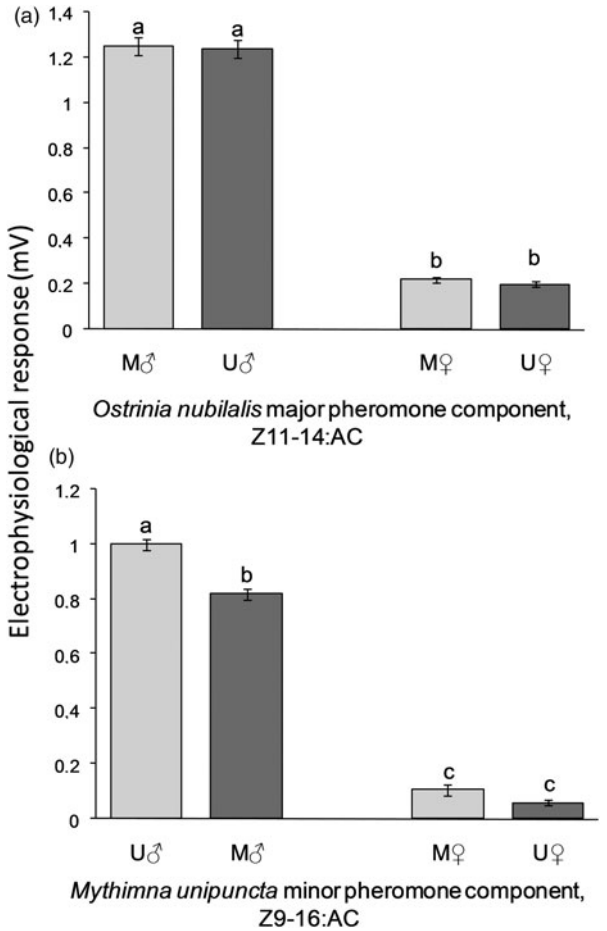

Fig. 3. Electrophysiological response of mated and unmated males and females of *Sesamia nonagrioides* to (A) the major pheromone component of *Ostrinia nubilalis*, (*Z*)-11-tetradecenyl acetate (Z11-14:Ac), and (B) the minor pheromone component of *Mythimna unipuncta*, (*Z*)-9-hexadecenyl acetate (Z9-16:Ac). *U*♂ = unmated male, *M*♂ = mated male, *U*♀ = unmated female, *M*♀ = mated female. $N = 30$ antennae. Bars with different letters are significantly different (Tukey's test, $P < 0.05$).

sympatric species such as *O. nubilalis, M. unipuncta and H. armigera* (Albajes *et al.*, 1988; Eizaguirre *et al.*, 2002, 2009; Gemeno *et al.*, 2006). The present study highlights that *S. nonagrioides* females also detect their own pheromone and pheromone components of sympatric species. Our results contradict those obtained by Acin *et al.* (2009), in which no EAG response was detected when the pheromone blend (Z11-16:Ac, Z11-16:OH, and Z11-16:Ald in a 84:15:1 ratio) was tested on female antennae of *S. nonagrioides*. The action potentials generated by activated sensory neurons (OSNs) on the insect antenna and recorded by EAG are a function of the preparation resistance, connection strength, insect vitality and position of the electrode (Nagai, 1983; Crnjar *et al.*, 1989). Therefore, the differences between our results and those of Acin *et al.* (2009) could be due to the nature of the EAG or to the lower pheromone concentration used by them (100 ng versus 10 µg) during the electrophysiological experiments. For example, to display noteworthy EAG responses, females of *S. exigua* need 1 µg (Yang *et al.*, 2009) and those of *Pandemis limitata* need 10 µg (DeLury *et al.*, 2005).

Female response in EAG, although lower than that of males, was quite noteworthy, particularly with respect to the major component and to the complete pheromone blend, the responses of male antennae being about four times higher than those of female antennae. These results are in agreement with those obtained by Acin *et al.* (2009), who found that the pheromone binding proteins (PBPs) were more than 4 times higher in males than in females. In Noctuidae the presence of PBP in female antennae has been reported to be 50 to 100% of that in male antennae (Maïbeche-Coisne *et al.*, 1997; Konstantopoulou *et al.*, 2006). Morphological characteristics of female antennae such as the number and type of the sensilla trichoidea (unpublished data) may also explain the low EAG response of female antennae in comparison with male antennae so, in species in which female are anosmic to their pheromone, antennae are less complex morphologically in comparison to males (Schneider *et al.*, 1964, 1998). Our electrophysiological experiments reinforce the 'autodetection' hypothesis discussed by Stelinski *et al.* (2006, 2014). Female pheromone detection has also been demonstrated by EAG assay in other lepidopteran species (Palaniswamy & Seabrook, 1978; Light & Birch, 1979; Ross *et al.*, 1979; Seabrook *et al.*, 1987; Den Otter *et al.*, 1996, Pearson & Schal, 1999; De Cristofaro *et al.*, 2004; Groot *et al.*, 2005; DeLury *et al.*, 2005; Stelinski *et al.*, 2006; Gökçe *et al.*, 2007; Yang *et al.*, 2009).

Unmated *S. nonagrioides* males elicited the highest electrophysiological responses to the full blend and to the major sex pheromone component and a similar response to the major component of its own pheromone was signalled for *Helicoverpa zea* by Vickers and Baker (1991). However, Linn *et al.* (1987) demonstrated, in field trials with the oriental fruit moth, *Grapholita molesta*, that the active space of a multicomponent pheromone is a function of the female's full blend rather than of the major component alone.

The electrophysiological response of male antennae decreased after mating, indicating a decrease in sexual responsiveness that is common in several insects (Barrozo *et al.*, 2010; Fischer & King, 2012). The behavioural and physiological mechanisms leading to this loss of sexual responsiveness of insect males are still unknown although many studies have attempted to determine them (Gadenne *et al.*, 2001; Barrozo *et al.*, 2010; Fischer & King, 2012). Our results showed that the antennal receptors may be involved in this drop in sensitivity to the female pheromone in mated males.

As occurred in males, the electrophysiological response of mated *S. nonagrioides* female antennae was lower than that of unmated females when the major sex pheromone component and the complete pheromone blend were tested, suggesting that the peripheral olfactory elements involved in this reduction are the same as those in mated males. Unmated females showed the highest electrophysiological response to the major pheromone component and to the complete pheromone blend. The behavioural consequences of autodetection in virgin females have not been studied for *S. nonagrioides* but Harari *et al.* (2011) showed that pheromone autodetection by virgin female of *Lobesia botrana* affected the time of calling, the number of eggs laid and the survivorship of females. The response of the antennae of mated females differed from that of unmated females. The highest response of the antennae of the mated females was obtained when they were exposed to the aldehyde (Z11-16:Ald), followed by the major

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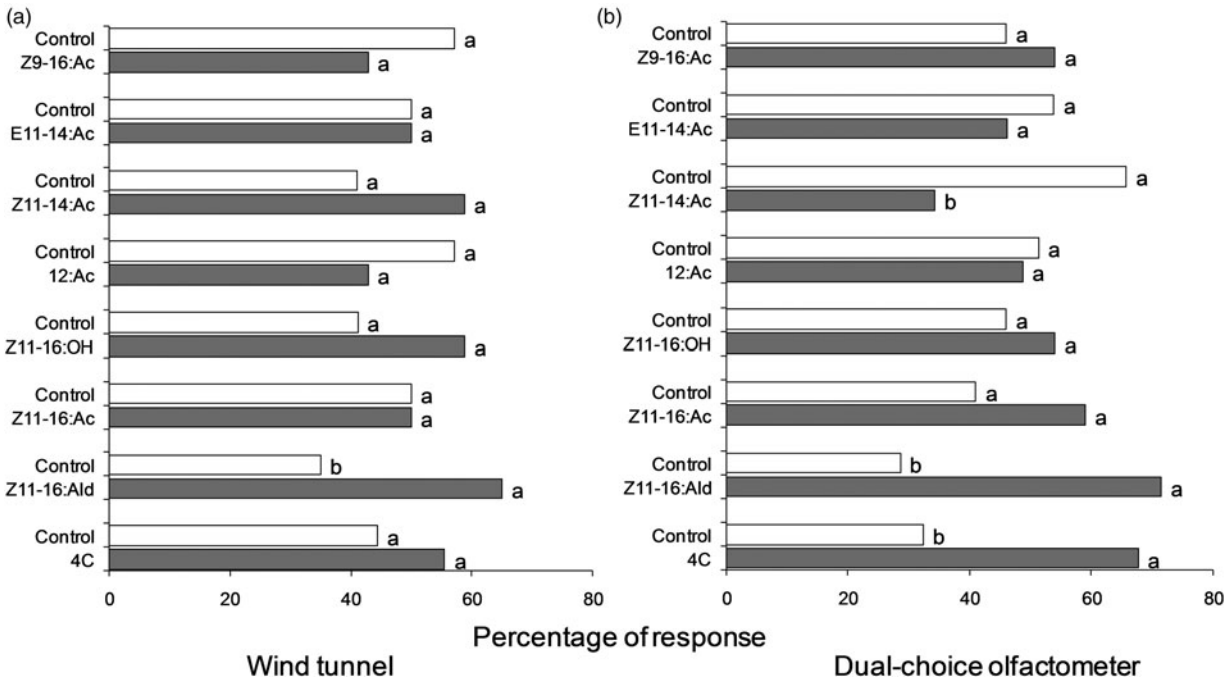

Fig. 4. Behavioural responses of gravid females of *Sesamia nonagrioides* in dual-choice bioassays (wind tunnel and a Y-tube olfactometer) to 10 µg of their own complete sexual pheromone blend, 4C ((*Z*)-11-hexadecen-1-yl acetate (Z11-16:Ac), (*Z*)-11-hexadecen-1-ol (Z11-16:OH), (*Z*)-11-hexadecenal (Z11-16:Ald) and dodecyl acetate (12:Ac); 77:8:10:5, respectively), to its individual components, Z11-16:Ac, Z11-16:OH, Z11-16:Ald (also the main component of *Helicoverpa armigera* pheromone), and 12:Ac, and to the pheromone components of *Ostrinia nubilalis*, (*Z*)-11-tetradecenyl acetate (Z11-14:Ac) and (*E*)-11-tetradecenyl acetate (E11-14:Ac), and *Mythimna unipuncta*, (*Z*)-9-hexadecenyl acetate (Z9-16:Ac). $N = 35$–40. Bars with different letters are significantly different (Binomial test, $P < 0.05$).

component of the pheromone (Z11-16:Ac). Different responses to volatiles between unmated and mated females have been demonstrated in other insects, such as *Spodoptera littoralis*, whose unmated females are more sensitive to lilac flowers and sex pheromone while mated females prefer green leaf odours (Martel *et al*., 2009; Saveer *et al*., 2012). Thus, the change in the electrophysiological response of *S. nonagrioides* females to their own pheromone depending on the mating status could be a physiological adaptation, downregulating some olfactory receptors to focus the energy on finding suitable oviposition sites. Likewise, changes in the hemolymph concentration (conductance) of proteins, inorganic ions, lipids and carbohydrates could thus also explain variations in EAG between different physiological states.

A greater proportion of gravid females responded to the pheromone components in the olfactometer than in the wind tunnel, except for those of *O. nubilalis*, possibly because the odour plumes in the olfactometer never overlap. When gravid females had to choose for laying eggs in the dual-choice experiments between maize plants without any pheromone and plants with the different pheromone components, *S. nonagrioides* females preferred plants with Z11-16:Ald in the wind tunnel and in the olfactometer and with the complete pheromone only in the olfactometer experiments. These results coincided with those obtained in the EAG assays, in which the mated female antennae showed the highest response to Z11-16:Ald, a minor component of *S. nonagrioides* pheromone and the major component of *H. armigera* pheromone, followed by the complete pheromone blend. This response could be related to aggregation egg-laying behaviour, in which detection of conspecific or heterospecific females serves to locate an appropriate oviposition site or to improve the ability to overcome plant defences (Prokopy & Roitberg, 2001). It is common that some pheromones specifically influence the females of many insects to lay eggs at the sites resulting in most egg deposition (Seenivasagan, 2009). *S. nonagrioides* gravid females showed no preference for maize plant with the rest of their pheromone component versus maize plant without any pheromone component. The main component of the *O. nubilalis* pheromone, Z11-14:Ac, elicited a high response in the male antennae and a lower response in the female antennae in the EAG. This relatively low response of the females in the EAG was enough to produce the behavioural antagonism in the olfactometer assays that was also detected in males in wind tunnel and field-trapping experiments (Eizaguirre *et al*., 2007). The pheromone detection of the sympatric species *O. nubilalis* probably helps *S. nonagrioides* gravid females to detect and avoid maize fields or plants on which *O. nubilalis* females are present in order to prevent interspecific competition of the larvae for food and space. On the other hand, the minor component of *M. unipuncta* pheromone, Z9-16:Ac, did not elicit any change in behaviour of the gravid females of *S. nonagrioides* in the wind tunnel or the olfactometer, although the high response of the males to this component in the EAG corresponds to the inhibitory effect obtained by Eizaguirre *et al*. (2009) in wind tunnel and field-trapping experiments. *M. unipuncta* has different feeding or egg laying habits to *O. nubilalis*: the distribution of the egg masses may be either in the leaves of the corn plant or in wild grasses; larvae feed on the leaves and the different generations during the year do not completely

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overlap with *S. nonagrioides*. Therefore, the overlapping niche is not large, the interspecific competition between these two species is not high, and an evasion mechanism from interspecific pheromone perception is probably not necessary.

In summary, *S. nonagrioides* females can detect their own pheromone components and the pheromone components of other sympatric species and can modulate their behaviour according to the odours detected; gravid females are attracted to their own pheromone components but some pheromone components of other sympatric species act as behavioural antagonist. Both sexes showed greater EAG responses prior to mating. These results are important because although the response of males to the different components of pheromones has been widely studied for many species, the responses to the pheromone component of the females and the influence of this response on egg-laying behaviour have been far less studied.

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References

Acin, P., Carrascal, M., Abian, J., Guerrero, A. & Quero, C. (2009) Expression of differential antennal proteins in males and females of an important crop pest, *Sesamia nonagrioides*. *Insect Biochemistry and Molecular Biology* **39**, 11–19.

Albajes, R., Canela, R., Eizaguirre, M., Riba, M. & Guerrero, A. (1988) (*Z*)-9-tetradecen-1-ol an attractant for *Athenis hospes* Freyer. Mediateurs chimiques, comportement et systematique des Lepidopteres. Applications en agronomie. *Les colloques de l'INRA* **46**, 55–58.

Barrozo, R.B., Gadenne, C. & Anton, S. (2010) Post-mating sexual abstinence in a male moth. *Communicative and Integrative Biology* **3**, 629–630.

Binder, B.F., Robbins, J.C. & Wilson, R.L. (1995) Chemically mediated ovipositional behaviors of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). *Journal of Chemical Ecology* **21**, 1315–1327.

Brevault, T. & Quilici, S. (2010) Interaction between visual and olfactory cues during host finding in the tomato fruit fly *Neoceratitis cyanescens*. *Journal of Chemical Ecology* **36**, 249–259.

Crnjar, R., Scalera, G., Liscia, A., Angioy, A.M., Bigiani, A., Pietra, P. & Barbarossa, I.T. (1989) Morphology and EAG mapping of the antennal olfactory receptors in *Dacus oleae*. *Entomologia Experimentalis et Applicata* **51**, 77–85.

De Cristofaro, A., Ioriatti, C., Pasqualini, E., Anfora, G., Germinara, G.S., Villa, M. & Rotundo, G. (2004) Electrophysiological responses of *Cydia pomonella* to codlemone and pear ester ethyl (*E,Z*)-2,4-decadienoate: peripheral interactions in their perception and evidences for cells responding to both compounds. *Bulletin of Insectology* **57**, 137–144.

Delury, N.C., Judd, G.J.R. & Gardiner, M.G.T. (2005) Antennal detection of sex pheromone by female *Pandemis limitata* (Robinson) (Lepidoptera: Tortricidae) and its impact on their calling behaviour. *Journal of the Entomological Society of British Columbia* **102**, 3–11.

Den Otter, C.J., De Cristofaro, A., Voskamp, K.E. & Rotundo, G. (1996) Electrophysiological and behavioural responses of chestnut moths, *Cydia fagiglandana* and *C. splendana* (Lep., Tortricidae), to sex attractants and odours of host plants. *Journal of Applied Entomology* **120**, 413–421.

Eizaguirre, M. & Albajes, R. (1989) Present situation of arthropod pests in maize in the Northeast of Spain. *Acta Phytopatologica et Entomologica Hungarica* **24**, 77–80.

Eizaguirre, M. & Albajes, R. (1992) Diapause induction in the stem corn borer, *Sesamia nonagrioides* (Lepidoptera: Noctuidae). *Entomologia Generalis* **17**, 277–283.

Eizaguirre, M., Sans, A., Lopez, C. & Albajes, R. (2002) Effects of mating disruption against the Mediterranean corn borer, *Sesamia nonagrioides*, on the European corn borer *Ostrinia nubilalis*. *IOBC/WPRS Bulletin* **25**, 59–68.

Eizaguirre, M., Albajes, R., Lopez, C., Sans, A. & Gemeno, C. (2007) Inhibition of pheromone response in *Sesamia nonagrioides* by the pheromone of the sympatric corn borer, *Ostrinia nubilalis*. *Pest Management Science* **63**, 608–614.

Eizaguirre, M., Lopez, C., Sans, A., Bosch, D. & Albajes, R. (2009) Response of *Mythimna unipuncta* males to components of the *Sesamia nonagrioides* pheromone. *Journal of Chemical Ecology* **35**, 779–784.

Finch, S. & Collier, R.H. (2000) Host-plant selection by insects – a theory based on 'appropriate/inappropriate landings' by pest insects of cruciferous plants. *Entomologia Experimentalis et Applicata* **96**, 91–102.

Fischer, C.R. & King, B.H. (2012) Inhibition of male sexual behavior after interacting with a mated female. *Behaviour* **149**, 153–169.

Gabel, B., & Thiery, D. (1994) Semiochemicals from *Lobesia botrana* (Lepidoptera, Tortricidae) eggs deter oviposition by the codling moth *Cydia pomonella* (Lepidoptera, Tortricidae). *European Journal of Entomology* **91**, 353–359.

Gadenne, C., Dufour, M.C. & Anton, S. (2001) Transient post-mating inhibition of behavioural and central nervous responses to sex pheromone in an insect. *Proceedings of the Royal Society B: Biological Sciences* **268**, 1631–1635.

Gemeno, C., Sans, A., Lopez, C., Albajes, R. & Eizaguirre, M. (2006) Pheromone antagonism in the European corn borer moth *Ostrinia nubilalis*. *Journal of Chemical Ecology* **32**, 1071–1084.

Giner, M., Sans, A., Riba, M., Bosch, D., Gago, R., Rayo, J., Rosell, G. & Guerrero, A. (2009) Development and biological activity of a new antagonist of the pheromone of the codling moth *Cydia pomonella*. *Journal of Agricultural and Food Chemistry* **57**, 8514–8519.

Gökçe, A., Stelinski, L.L., Gut, L.J. & Whalon, M.E. (2007) Comparative behavioral and EAG responses of female obliquebanded and redbanded leafroller moths (Lepidoptera: Tortricidae) to their sex pheromone components. *European Journal of Entomology* **104**, 187–194.

Groot, A.T., Gemeno, C., Brownie, C., Gould, F. & Schal, C. (2005) Male and female antennal responses in *Heliothis virescens* and *H. subflexa* to conspecific and heterospecific sex pheromone compounds. *Environmental Entomology* **32**, 256–263.

Guerrero, A., Canela, R., Riba, M., Eizaguirre, M., Albajes, R. & Sarto, V. (1986) A sex attractant for males of the noctuid moth *Athetis hospes*. *Entomologia Experimentalis et Applicata* **42**, 97–100.

Guo, L. & Li, G. (2009) Olfactory perception of oviposition-deterring fatty acids and their methyl esters by the Asian corn borer, *Ostrinia furnacalis*. *Journal of Insect Science* **9**, 67.

Harari, A.R., Zahavi, T. & Thiery, D. (2011) Fitness cost of pheromone production in signaling female moths. *Evolution* **65**, 1572–1582.

Harmon, J.P., White, J.A. & Andow, D.A. (2003) Oviposition behavior of *Ostrinia nubilalis* (Lepidoptera: Crambidae) in response to potential intra- and interspecific interactions. *Environmental Entomology* **32**, 334–339.

JMP, Version 8. (2008) SAS Institute Inc., Cary, NC.

Klun, J.A., Chapman, O.L., Mattes, K.C., Wojtkowski, P.W., Beroza, M. & Sonnet, P.E. (1973) Insect sex pheromones: minor amount of opposite geometrical isomer critical to attraction. *Science* **181**, 661–663.

Konstantopoulou, M.A., Krokos, F.D. & Mazomenos, B.E. (2004) Chemical composition of corn leaf essential oils and their role in the oviposition behavior of *Sesamia nonagrioides* females. *Journal of Chemical Ecology* **30**, 2243–2256.

Konstantopoulou, M.A., Pratsinis, H., Kletsas, D. & Mazomenos, B.E. (2006) Pheromone-binding protein and general odorant-binding protein of *Sesamia nonagrioides*: sex- and diel-dependent expression. *Entomologia Experimentalis et Applicata* **119**, 129–136.

Lelito, J.P., Myrick, A.J. & Baker, T.C. (2008) Interspecific pheromone plume interference among sympatric heliothine moths: a wind tunnel test using live, calling females. *Journal of Chemical Ecology* **34**, 725–733.

Light, D.M. & Birch, M.C. (1979) Electrophysiological basis for the behavioural response of male and female *Trichoplusia ni* to synthetic female pheromone. *Journal of Insect Physiology* **25**, 161–167.

Linn, C.E., Campbell, M.G. & Roelofs, W.L. (1987) Pheromone components and active space: what do moths smell and where they smell it? *Science* **237**, 650–652.

Maïbeche-Coisne, M., Sobrio, F., Delaunay, T., Lettere, M., Dubroca, J., Jacquin-Joly, E. & Meillour, P.N.L. (1997) Pheromone binding proteins of the moth *Mamestra brassicae*: specificity of ligand binding. *Insect Biochemistry and Molecular Biology* **27**, 213–221.

Martel, V., Anderson, P., Hansson, B.S. & Schlyter, F. (2009) Peripheral modulation of olfaction by physiological state in the Egyptian leaf worm *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Journal of Insect Physiology* **55**, 793–797.

Nagai, T. (1983) On the relationship between the electroantennogram and simultaneously recorded single sensillum response of the European corn borer *Ostrinia nubilalis*. *Archives of Insect Biochemistry and Physiology* **1**, 85–91.

Ofomata, V.C., Overholt, W.A., Huis, A.V., Egwuatu, R.I. & Ngi-Song, A.J. (1999) Niche overlap and interspecific association between *Chilo partellus* and *Chilo orichalcociliellus* on the Kenya coast. *Entomologia Experimentalis et Applicata* **93**, 141–148.

Palaniswamy, P. & Seabrook, W.D. (1978) Behavioural responses of the female eastern spruce budworm *Choristoneura fumiferana* (Lepidoptera: Tortricidae) to the sex pheromone of her own species. *Journal of Chemical Ecology* **4**, 649–655.

Pearson, G.A. & Schal, C. (1999) Electroantennogram responses of both sexes of grape root borer (Lepidoptera: Sesiidae) to synthetic female sex pheromone. *Environmental Entomology* **28**, 943–946.

Prokopy, R.J. & Roitberg, B.D. (2001) Joining and avoidance behaviour in nonsocial insects. *Annual Review of Entomology* **46**, 631–665.

Renwick, J.A.A. (1989) Chemical ecology of oviposition in phytophagous insects. *Experientia* **45**, 223–228.

Ross, R.J., Palaniswamy, P. & Seabrook, W.D. (1979) Electroantennograms from spruce budworm moths (*Choristoneura fumiferana*) (Lepidoptera: Tortricidae) of different ages for various pheromone concentrations. *The Canadian Entomologist* **111**, 807–816.

Sans, A., Riba, M., Eizaguirre, M. & Lopez, C. (1997) Electroantennogram, wind tunnel and field responses of male Mediterranean corn borer, *Sesamia nonagrioides*, to several blends of its sex pheromone components. *Entomologia Experimentalis et Applicata* **82**, 121–127.

Saveer, A.M., Kromann, S.H., Birgersson, G., Bengtsson, M., Lindblom, T., Balkenius, A., Hansson, B.S., Witzgall, P., Becher, P.G. & Ignell, R. (2012) Floral to green: mating switches moth olfactory coding and preference. *Proceedings of the Royal Society of London Series B: Biological Sciences* **279**, 2314–2322.

Schneider, D., Lacher, V. & Kaissling, K.E. (1964) Die reaktionweise und das reaktiosspektrum von riechzellen bei *Ahtherraea pernyi* (Lepidoptera, Saturniidae). *Zeitschrift für Vergleichende Physiologie* **48**, 632–662.

Schneider, D., Schulz, S., Priesner, E., Ziesmann, J. & Francke, W. (1998) Autodetection and chemistry of female and male pheromone in both sexes of the tiger moth *Panaxia quadripunctaria*. *Journal of Comparative Physiology A* **182**, 152–161.

Seabrook, W.D., Linn, C.E., Dyer, L.J. & Shorey, H.H. (1987) Comparison of electroantennograms from female and male cabbage looper moths (*Trichoplusia ni*) of different ages and for various pheromone concentrations. *Journal of Chemical Ecology* **6**, 1443–53.

Seenivasagan, T., Sharma, K.R., Sekhar, K., Ganesan, K., Prakash, S. & Vijayaraghavan, R. (2009) Electroantennogram, flight orientation, and oviposition responses of *Aedes aegypti* to the oviposition pheromone n-heneicosane. *Parasitology Research* **104**, 827–833.

Stelinski, L.L., Il'ichev, A.L. & Gut, L.J. (2006) Antennal and behavioral responses of virgin and mated oriental fruit moth (Lepidoptera: Tortricidae) females to their sex pheromone. *Annals of the Entomological Society of America* **99**, 898–904.

Stelinski, L.L., Holdcraft, R. & Rodriguez-Saona, C. (2014) Female moth calling and flight behavior are altered hours following pheromone autodetection: possible implications for practical management with mating disruption. *Insects* **5**, 459–473.

Stephens, A.E.A., Suckling, D.M. & El-Sayed, A.M. (2008) Odour quality discrimination for behavioural antagonist compounds in three tortricid species. *Entomologia Experimentalis et Applicata* **127**, 176–183.

Tamhankar, A.J., Rajendran, T.P., Rao, N.H., Lavekar, R.C., Jeyakumar, P., Monga, D. & Bambawale, O.M. (2003) Variability in response of *Helicoverpa armigera* males from different locations in India to varying blends of female sex pheromone suggests male sex pheromone response polymorphism. *Current Science* **84**, 448–450.

Varshney, A.K., Babu, B.R., Singh, A.K., Agarwal, H.C. & Jain, S.C. (2003) Ovipositional responses of *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) to natural products from leaves of two maize (*Zea mays* L.) cultivars. *Journal of Agricultural and Food Chemistry* **51**, 4008–4012.

Vickers, N.J. & Baker, T.C. (1991) The effects of unilateral antennectomy on the flight behavior of male *Heliothis virescens* in a pheromone plume. *Physiological Entomology* **16**, 497–506.

Yang, M., Dong, S. & Chen, L. (2009) Electrophysiological and behavioral responses of female beet armyworm *Spodoptera exigua* (Hubner) to the conspecific female sex pheromone. *Journal of Insect Behavior* **22**, 153–164.

Zar, J.H. (1996) *Biostatistical Analysis*. 3rd edn. New Jersey, USA, Prentice Hall.