Olfactory Male Sensitivity and Its Variation in Response to Fluoranalogs of the Main Pheromone Component of Female *Mamestra brassicae*

A. A. Nikonov, T. V. Tyazhelova, Ye. A. Nesterov, V. M. Rastegayeva, F. E. Ilyasov, P. V. Mashkin and B. G. Kovalyov

Institute of Cell Biophysics, Russian Academy of Sciences, Pushchino, Moscow Region, 142292, Russia

Z. Naturforsch. **49c**, 508–515 (1994); received September 14, 1993/March 14, 1994

Electroantennogramme, Chemical Communication, Fluoranalogs, *Mamestra brassicae*

According to the EAG study and the field trapping tests, fluoranalogs of the main pheromone component of *Mamestra brassicae* females have been found to possess disguising properties for males when used together with the main pheromone component itself. In males, at low concentrations of the above substances the diminishing of attractiveness of these blends correlates with the absence of EAG responses to them. An unequal action of these substances has been revealed in different concentration ranges of odours. The mechanisms of the action of these substances are under discussion.

**Introduction**

At present, an intensive search for effective biological ways of protecting plants from insect pests is going on. One of the promising directions of this search is the use of natural pheromones as well as their analogs, because their use in traps and other preparations has a weak toxicity and results in disturbances of sex communication of insects. So, it becomes possible to regulate the number of pests (Campion, 1984).

On the other hand, some substances are known to suppress the attraction of males of certain insect species to sex pheromones or their synthesized analogs, effectively. These substances may be of the natural origin. Their presence in pheromone blend appears to be necessary in the course of interspecies communication of insects (Bogdanova, 1980; Christensen, 1990). They can be also synthesized *de novo* (Albans, 1984). The search for these substances and their synthesis are rather expensive. Investigations in this direction are performed for a long time (Roelofs, 1968). Here the possibility of a specific disturbance of the sexual chain in some insect species without any marked change in chemocommunication of the others is very attractive. Although this problem seems to be easy, it has not been satisfactorily solved yet. The main reasons for this are: (i) little evidence on the primary mechanisms of olfactory reception, including principles and mechanisms of odour information recognition; (ii) the changes in sensitivity and those of distinguishing the odours by insects in natural conditions are weakly studied.

In this work we have studied electrical responses of antennae of *Mamestra brassicae* males to the main pheromone component (Z11-16:OAc) and its fluoranalogs, as well as the attractiveness of the above substances for *Mamestra brassicae* males.

**Materials and Methods**

In the laboratory experiments the males of *Mamestra brassicae* were used for the study. Butterflies were bred out of larvae in the diapause at 25 °C and at humidity of 70%. *Mamestra brassicae* with developed and intact antennae were kept at 10 °C after emergence and used 3–5 days old.

As pheromone we used a synthesized analog of the main component (Bestmann, 1978). Haloacetate analogs of *Mamestra brassicae* pheromone were synthesized in the laboratory headed by Boris Kovalyov (Kovalev et al., 1979) (Research Institute of Biological Methods of Plants Protection, Kishenev) as follows: 1) 16-fluor, (Z)-11-hexadecenal-1-il trifluoracetate (F16,Z11-16:OCOF3); 2) 16-fluor, (Z)-11-hexadecenal-1-il acetate (F16,Z11-16:OAc); 3) (Z)-11-hexadecenal-1-il trifluoracetate (Z11-16:OCOF3).

Reprint requests to Dr. A. A. Nikonov.

0939–5075/94/0700–0508 $ 06.00 © 1994 Verlag der Zeitschrift für Naturforschung. All rights reserved.
For morphological investigations the antennae were cut and fixed in 1.5% solution of glutar aldehyde at 4°C for 2 h. Then after dehydration they were embedded into resin “Spur” (Tiras and Moshkov, 1978).

Electrophysiological measurements were performed at the Institute of Cell Biophysics (Pushchino) by using a standard electrophysiological device. According to Schneider’s method (Schneider, 1984), EAG was recorded from the ends of the cut-off antenna by capillary electrodes filled with an analog of hemolymph solution (Kaissling, 1986). Odour was pushed into the continuous flux of purified air (v = 0.5 l/min) by using the system allowing to avoid mechanical artefacts, as it had been described earlier (Minor and Vasilyeva, 1979). The exit end of the glass tube (4 mm in diameter) was at a distance of 10 mm off the antenna plane. Paper filter bands (1 cm²) with 10 µl of the substance diluted in hexane served as a source of stimulating vapours. In different experiments the excitement duration was changed from 0.1 to 0.5 sec. The time between excitements was 1–2 min. The parameters of stimuli (sequence frequency and push duration) were changed automatically. The potentials were recorded and the results obtained were statistically processed (Zacks, 1971) under automatic conditions by using an extra developed device and software programmes based on PC “Iskra-226”.

The field trapping experiments were performed in 1988–1989 in the fields near Kishenev. The traps were hung about at a height of 1.2 m around vineyards and a pea field, placed in a line by blocks (5 traps in each line). The experiment was carried out in two variants: (i) the study of attractiveness of used substances No. 1 (F16,Z11-16:OCOF₃), No. 2 (F16,Z11-16:OAc) and No. 3 (Z11-16:OCOF₃) and analog of main pheromone component (Z11-16:OAc); (ii) the study of attractiveness of used different mixture of the above-mentioned substances. For this purpose two capsules, the one with substance under study, the other with pheromone, were placed into each trap. Sometimes the capsule with substance under study was placed near the trap filled with pheromone. The traps with uncharged capsules served as a control. The distances between the traps were as follows: 15 m in the block, 30 m between the blocks, 200 m between the variants. The number of butterflies caught was counted every 3–4 days during 20 days in the summer of the second generation of *Mamestra brassicae* (Buchko, 1985). The results obtained were statistically processed and represented as tables and diagrams.

**Results**

**Electrophysiological study**

The use for stimulation of antenna led to appearance of Z11-16:OAc negative potential oscillations (Fig. 1 A), the amplitude and the shape of which depended on concentration and duration of the stimulus. The first stimulation of the antenna (10⁻¹⁰ µg per filter) always evoked a response of a greater amplitude than the subsequent ones used at the same or stronger concentrations. The concentration area of 10⁻⁹–10⁻⁷ µg per filter was characterized by small and stable amplitudes. A statistically significant increase of the amplitude of responses (1.05 ± 0.05 mV) was observed at higher concentrations (10⁻⁶ µg per filter). A further enhancement of the odour concentration resulted in a periodic increase of the amplitude of responses (Fig. 1 B). This dependence was not of a typical linear character. The curve acquired an S-shaped form, on which a plateau in the region of several concentration ranges as well as some decrease of the amplitude of EAG responses could be seen within the range between 10⁻³–10⁻¹ µg per filter.

![Fig. 1. A. Standard EAG response of Mamestra brassicae males had been recorded after analog-digital converter. B. Dose-response curve for the main pheromone component Z11-16:OAc and fluoranalog of main pheromone component F16,Z11-16:OCOF₃ tested on males (n = 10). Mean response ± SEM.](image-url)
Using fluoranalog No. 1 for stimulation, we observed an EAG reaction of the antenna of *Mamestra brassicae* males similar to the reaction to the main pheromone component. However, the concentration curve of the main component lay above that of fluoranalog No. 1 (Fig. 1 B). In this case significant differences between them ranged from $10^{-7}$ to $10^{-3}$ µg per filter while the beginning and the end of the curves did not coincide with each other. The activity of fluoranalogs No. 2 and No. 3 showed a different picture. Substance No. 2 caused two statistically distinguishable amplitudes of responses, relatively small in size, within concentration ranges of $10^{-10}$–$10^{-5}$ µg and $10^{-4}$–$10^{-1}$ µg per filter (Fig. 2). Fluoranalog No. 3 had one concentration region ($10^{-9}$ µg) where the antennogramme amplitude drastically rose. A similar increase of the amplitude of responses took place upon approach of stimuli concentration to this region from both lower and higher concentrational ranges. A further increase of the concentration of fluoranalog No. 3 did not lead to any changes in the amplitude of responses (the amplitude was constant) (Fig. 2).

To study the mechanisms of the influence of the above substances on the insect receptor system, the pheromone was investigated at the background of the fluoranalogs. In this case some differences in action of the substances were observed.

Introduction of fluoranalog No. 1 into the continuous air flux ($10^{-10}$–$10^{-5}$ µg) led to disappearance of statistically significant differences in the amplitudes of responses to the main component in the concentration ranges of $10^{-10}$–$10^{-4}$ µg per filter. In this case the amplitude increase within $10^{-3}$–$10^{-2}$ µg per filter observed (Fig. 3). It is noteworthy that introduction of the main pheromone component ($10^{-7}$ µg) into the continuous air flux blowing over the antenna resulted in the same dependence of the amplitude of responses on the concentration of fluoranalog No. 1 (Fig. 3).

Fluoranalog No. 2 at concentrations of $10^{-9}$–$10^{-5}$ µg did not make the response to the main pheromone component disappear completely, yet the amplitude was small and constant (Fig. 4).
In the continuous air flux fluoranalog No. 3 at a concentration of $10^{-10} \mu g$ changed the EAG responses to the main pheromone component (Fig. 5). A significant decrease of the amplitude of responses appeared at a concentration of $10^{-9} \mu g$ per filter. The enhancement of the concentration of fluoranalog No.3 to $10^{-6} \mu g$ changed the character of responses: the amplitude decreased and the dependence of the amplitude of responses on lgC disappeared. When the concentration of fluoranalog No. 3 increased, the EAG response of males upon injection of the main component was completely blocked in a wide range of concentrations (Fig. 5).

**Field trapping experiments**

The traps with odorants under study (2 mg of each substance on the cuts of a rubber hose) were hung about after *Mamestra brassicae* (the second generation) had started their flight. The total number of *Mamestra brassicae* caught into the traps in 1988 are presented in Tables I and II. There were mainly two species of butterflies, which were caught into the traps filled with the main pheromone component of *Mamestra brassicae*: a) *Mamestra brassicae* (its part in the total number was 49–65%) and b) *Discestra trifolii* Hufn. (28–39%). In the traps individual representatives of other species were present as well. Pure fluoranalogs No. 1 and No. 2 did not attract *Mamestra brassicae* and were practically of no interest for other species inhabiting this territory. Fluoranalog No. 3 turned out attractive for *Mamestra oleracia* L. (Table I).

Fluoranalog No. 3, used in the trap together with the main component (Table II), completely suppressed the attractiveness of trap for *Mamestra brassicae* and *Discestra trifolii* Hufn. while the attractiveness of this blend did not change for *Mamestra oleracia* L. In a similar situation fluoranalog No. 1, used like No. 3 with the main component, suppressed the attractiveness to *Mamestra brassicae*, and besides it enhanced the attractiveness of the blend for *Discestra trifolii* Hufn. Fluoranalog No. 2 changed the main component attractiveness to none of *Mamestra brassicae* (Fig. 6). Later on we paid our attention to fluoranalog No. 3 as the chief candidate for suppression of attractiveness of the main pheromone component of *Mamestra brassicae*.

In the series of 1989 we investigated the concentration dependence of the influence of fluoranalog

![Fig. 5. Dose-response curves for main pheromone component at the background of fluoranalog 3 - Z11-16:OCOF3 at different concentrations (solid line, $10^{-10} \mu g$, dashed line, $10^{-6} \mu g$). Background substance has been introduced into the continuous air flux blowing over the insect antenna. Tested 8 males. Mean response ± SEM.](image)

![Fig. 6. Attractivity of pheromone traps with blends of main pheromone component and fluoranalogs for *Mamestra brassicae* (F1, main component + F16,Z11-16:OCOF3; F2, main component + F16,Z11-16:OAc; F3, main component + Z11-16:OCOF3). The ordinate indicates the quantity of the caught insects normalized by control quantity.](image)
Table II. The number of male butterflies of different species caught in the traps filled with blends of substances. Mean data for one trap are presented in brackets.

<table>
<thead>
<tr>
<th>Noctuidae</th>
<th>Standard</th>
<th>Variants of experiments</th>
<th>No. 1</th>
<th>No. 2</th>
<th>No. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mamestra brassicae L.</td>
<td>59</td>
<td>2</td>
<td>70</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(11.8 ± 3.2)</td>
<td>(14.0 ± 2.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mamestra oleracia L.</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>144</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(29.7 ± 2.8)</td>
<td></td>
</tr>
<tr>
<td>Discestra trifolii Hufn.</td>
<td>26</td>
<td>71</td>
<td>23</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(5.2 ± 1.0)</td>
<td>(14.2 ± 1.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 7. Attractivity of pheromone traps with blend of main pheromone component and fluoranalog 3 (Z11-16:OCOF3) at different concentrations for Mamestra brassicae (control, the pure main pheromone component; +0.1 F3, added 0.1 mg of fluoranalog F3; +0.05 F3, added 0.05 mg of fluoranalog F3; +0.01 F3, added 0.01 mg of fluoranalog F3; +0.001 F3, added 0.001 mg of fluoranalog F3; +0.0005 F3, added 0.0005 mg of fluoranalog F3).

No. 3 in the field trapping experiments. Fig. 7 represents this dependence in a normalized form. It should be noted that the concentrations of fluoranalog No. 3 from 0.1 to 0.001 mg per trap are rather effective in order to suppress attractivity for Mamestra brassicae. However, this effectiveness only manifests itself if fluoranalog is in the trap. Its presence becomes entirely ineffective when it is transferred out of the trap 40 cm to the right or left or both up and down (Fig. 8).

Discussion

In conditions of our experiment with males of Mamestra brassicae we found a complicated form of dose-dependent EAG responses to the main pheromone component (Z11-16:OAc). Unlike the dependence mentioned in the work of Boeckh, Kaisling and Schneider (1965), we revealed an S-shaped character of the amplitude upon an increase of the stimuli concentration. A similar dependence of the amplitude of responses was discussed by Skirkyavichus and Skirkyavichene (1988), who explained these effects by the presence of several types of receptor cells in one sensillum. These receptor cells determined by an unequal biological role of the latter in this signal.

Sexual pheromones of Mamestra brassicae are multicomponent, polyfunctional blends. Our preliminary morphological investigations also showed a great “populatedness” of the sensillum of Mamestra brassicae with different kinds of dendrites (Fig. 9). In the olfactory sensillum we found about 9 dendrites, different in diameter. Proceeding from Schneider’s model (Schneider, 1962), an increase of the EAG response amplitude at increasing stimuli concentrations could account for a growing number of cells involved into the reaction.
In our case the periodicity of the amplitude increase might be explained by a different threshold sensitivity of individual cell communities to the main pheromone component. Olfactory receptor proteins with different constants of binding to one substance (camphor) were earlier revealed in vertebrates (Fesenko et al., 1985). The olfactory system of insects may also include several classes, differentiated by specificity of binding of olfactory receptors and/or specific pheromone-binding proteins of receptor lymph (Vogt and Riddiford, 1981; Vogt et al., 1991) that allow an effective functioning of the analyzer in a wide concentration range.

High-sensitive “distant receptors”, which are present on the antennae of males, react to a little amount of female pheromone in the air. This is suggested by behavioural reactions of insects (wing vibration), which appear at odour concentrations usually insufficient for recording the EAG responses (Kaiissling and Priesner, 1970). We succeeded in recording an electroantennographic response of the Mamestra brassicae receptor system to the first presentation of the main pheromone component at a rather low concentration, different by 4 orders from that giving rise to a statistically significant repeated threshold EAG reaction (Fig. 1B). Similar responses were also revealed upon presentation of fluoranalogs No. 1 and No. 3. We think that this response reflects the reaction of “distant receptors”, which determine the region of behavioural thresholds of the above insects ($10^{-10} - 10^{-9}$ μg).

The absence of significant EAG responses within $10^{-9} - 10^{-7}$ μg per filter may be due to autoadaptation found by Strausfeld and Kaiissling (1986) (this assumption being indirectly supported by a greater amplitude of EAG response to an odour, after the antenna had been blown through with fresh air for a long time), or due to some decrease in antenna size on amputation which can lead to changes in the threshold of olfactory reactions by 2 to 4 orders (Tamhancar et al., 1990).

The maximal amplitudes of responses were typical for the concentration range of $10^{-7} - 10^{-3}$ μg. This concentrational region was also characterized by the linear dependence of amplitudes on the stimuli concentration as described earlier (Boeckh et al., 1965). Here the majority of receptor cells of Mamestra brassicae responded to odour, and odour differentiation processes occurred in them. These responses are shown in Fig. 1. Significant differences between fluoranalog No. 1 and the main pheromone component are peculiar to this concentration range. This indicates that these substances interact with different receptors. Different effects of this odour on receptor cells in these two concentration ranges are, probably the reason for the loss of attractivity to main pheromone component.

A joint action of the above substances on the insect antenna, which is demonstrated in Fig. 3, witnesses a greater specificity of these receptor to the main component within $10^{-7} - 10^{-3}$ μg. By contrast to this, in the range of threshold concentrations they most likely interact with similar receptors. This is supported by the equal amplitudes of responses. As these substances act in a different manner within these concentration ranges, this may be a reason of different behavioural responses to them.

In the field tests fluoranalog No. 3 showed good disguising properties with respect to Mamestra brassicae. In our opinion, the mechanism of its action, however, differs from fluoranalog No. 1. This is indicated by a strong suppression of the EAG.

---

Fig. 9. Cross section of trichoid sensillum of Mamestra brassicae. Nine different outer dendritic segments are enclosed in the dendritic sheath. Ultrathin sections were obtained on Ultratom LKB-880 by using electron microscope JEN-100 B. Bar = 1.1 μm.
response in both concentration ranges (Fig. 5). Such a suppression could be interpreted as a competitive inhibition. However, the inhibition of EAG responses when fluoranalog No. 3 is used in a pure state or when this mixed with pheromone, makes such an assumption less possible. Field tests have shown that fluoranalog No. 3 is attractive for *Mamestra oleracea* L., whose main pheromone is composed of Z11-16Ac and Z11-16OH and is inactive for *Mamestra brassicae* L. (Subchev et al., 1989).

This effects might be explained by the influence of these substances on different receptor cells in sensillum. Such a way of suppressing the reactions to a sexual attractant is known and was mentioned earlier (Bogdanova et al., 1980; Christensen et al., 1990). It is not excepted that this is heteroadaptation described by Strausfeld and Kaissling (1986). This heteroadaptation is not very long-term, which is confirmed by the data obtained in our behavioural experiments (Fig. 8). But this conclusion made on the base of the study of antenna EAG responses and field tests may not be considered definitive. It requires a confirmation by a more direct method, *i.e.* by recording electric responses of single sensilla in the situations modulating field conditions.

At present almost any change in pheromone blend is known to result in a decrease of its attractiveness (Safonkin, 1991). However, the mechanism of this phenomenon still remains unclear on the cellular level (Kaissling, 1986; Christensen et al., 1989). When analyzing the responses of cells on different levels of olfactory analyzer of insects (O'Connell, 1985; Kaissling et al., 1989), the authors conclude that an odour is received and coded on the level of receptor cells. Yet recognition of odour information occurs in the highest regions of the nervous system. Most likely, different kinds of adaptation play the key role in the primary processes (Kapitsky and Gribakin, 1991). The change in EAG responses of the blend of pheromone-like substances revealed by us in different concentration ranges makes the picture highly diverse. The present study demonstrates a possibility of investigating these processes on the antennographic level.

---


