A Sex Attractant for Male Orthosia gothica L. (Lepidoptera: Noctuidae)

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In field trapping tests male Orthosia gothica (Lepidoptera: Noctuidae) were captured in traps baited with blends of (Z)-9-tetradecenyl acetate, (Z)-9-tetradecenol, (Z)-5-tetradecenyl acetate and (Z)-11-tetradecenyl acetate. The highest catches were recorded in traps baited with a 20:20:1:1 blend. The above quaternary blend is a potent sex attractant of this species, and it can be recommended in dosages of 10–100 µg per dispenser for monitoring purposes.

Orthosia gothica L. (Lepidoptera: Noctuidae) belongs to the group of noctuids with only one flight in early spring each year. Larvae of this species feed on the leaves of various trees in late spring / early summer and are known to cause damages to orchard trees [1].

Sex pheromone traps would be a useful tool to detect the occurrence of this species. In the present paper we report on a potent sex attractant that can be used for monitoring of O. gothica.

Materials and Methods

Field trapping tests

Field tests were conducted in a mixed oak forest bordered by an apple orchard at the Julianna major Experimental Station of the Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary. In the tests sticky traps similar in shape and size to those described earlier [2], but made from polyethylene sheets were used. Traps were suspended from the branches of trees at a height of 1.5 m. Traps containing different baits were set up in rectangular blocks. The distance of traps within a block was 4–5 m. The distance between blocks ranged between 100–1000 m. Traps were moved one position forward within a block at each occasion when the traps were checked. At the same time, captured males were recorded and sticky inserts changed to new ones. In statistical analyses capture data were transformed to log (x + 1) and differences between means were tested for significance by Duncan’s New Multiple Range Test (DNMRT).

Bait dispensers for traps were prepared using pieces of rubber tubing (Taurus, Budapest, Hungary; No. MSZ 9691/6; extracted 3 times in boiling ethanol for 10 min, then also 3 times in methylene chloride overnight, prior to usage). Test compounds were applied to the surface of the dispensers as hexane solutions and dispensers were stored at –65 °C until use.

Compounds used were purchased from S. Voerman (Wageningen, The Netherlands), and were >95% pure according to gas chromatographic analysis [3]. (Z)-5-decenal, (Z)-7-decenal, (Z)-5-dodecenal, (Z)-7-dodecenal, (Z)-9-dodecenal, (Z)-5-tetradecenal, (Z)-9-tetradecenal, (Z)-5-hexadecenal and (Z)-11-hexadecenal were synthesized from the corresponding alcohols according to standard procedures [4]. The aldehydes were >99% isomerically and chemically pure and contained <0.1% of the corresponding alcohols as checked by gas chromatography (25 m OV-240-OH capillary column).

Electroantennograms

Electroantennograms (EAG) were recorded from excised male antennae, with platinum electrodes set at the tip and the basal part of the antenna. Connection between the electrodes and the insect tissues was maintained by an electrically conducting gel (Valleylab, Boulder, C.O., U.S.A.).

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Responses were amplified by a high impedance amplifier [5], and displayed on an OH 850 chart recorder (Radelkis, Budapest, Hungary).

Acetate, alcohol and aldehyde series with 10, 12, 14 and 16 carbon atoms in the chain, and with a (Z) unsaturation at positions 5, 7, 9 or 11, were screened on the male antenna. Such or similar compounds have been frequently found as pheromone or attractant components in noctuids [6]. One μg of the test compound applied to a 10 x 10 mm piece of filter paper inside a Pasteur pipette was used as an odour source. Stimuli were provided by injecting 1 ml of air through the Pasteur pipette into an air stream (80 l/h) flushing over the antenna. The interval between stimuli was at least 1 min. Responses were normalized against a common standard, which was administered before, and after the stimulus of the test compound. In the tests feral male moths were used, which were captured by a live-capturing light trap or sex attractant traps.

**Results and Discussion**

The composition (μg) of baits used in preliminary trials is shown in Fig. 1. Although a small number of male *O. gothica* were caught in traps baited with (Z)-9-tetradecenyl acetate (Z9-14Ac; abbreviation of names of compounds according to [6]), higher catches were generally recorded in traps containing mixtures of Z9-14Ac and the corresponding alcohol (Z9-14OH) (Fig. 2A). The addition of the corresponding aldehyde (Z9-14Al) or (Z)-11-hexadecenyl acetate (Z11-16Ac) as third components, did not increase catches.

In a complementary test (Fig. 2B), the addition of (Z)-11-tetradecenyl acetate (Z11-14Ac) or (Z)-5-tetradecenyl acetate (Z5-14Ac) to Z9-14Ac, increased catches. A blend of Z9-14Ac and Z11-14Ac has earlier been described as an attractant for *O. gothica* males [7, 8].

The role of (Z)-9-tetradecenyl compounds in the sexual communication of *O. gothica* was further corroborated by results of electroantennogram (EAG) recordings (Fig. 3). The highest EAG response of all compounds tested was evoked by Z9-14Ac. Also in the alcohol and aldehyde series, the (Z)-9-tetradecenyl compounds gave relatively the highest responses (Fig. 3). A sizeable response was also recorded by (Z)-9-dodecenyl acetate (Z9-
Fig. 3. Electroantennogram (EAG) responses of male *Orthosia gothica* antennae to a series of compounds frequently found as sex pheromone components in Noctuidae (5 replicates. Responses were normalized against the response to Z9-14Ac).

12Ac), while responses to other compounds were medium to low. Our results confirm a recent report on Z9-14Ac being highly EAG active in *O. gothica* [9].

A field test (Table I) was designed to study the relative importance of all compounds which had been active in the preliminary trials. In this test, binary blends did not differ significantly from zero catch (Table I; baits 1, 2, 3 and 11). Catches increased, when either Z5-14Ac or Z11-14Ac were added as third components to a blend of Z9-14Ac/ Z9-14OH (baits 4, 5). The highest catch was obtained with a blend of all four compounds (Table I, bait 8).

Interestingly, the addition of Z11-16Ac decreased catches if Z11-14Ac was also present in the blends (Table I, baits 8 vs. 9; 5 vs. 10). However, no decrease was observed with addition of Z11-16Ac to baits not containing Z11-14Ac (baits 4 vs. 7; 1 vs. 6). The reason for this phenomenon is unknown to us. A possible explanation could be that Z11-16Ac and Z11-14Ac compete for the same perception site on the pheromone sensillum, and Z11-16Ac interferes with the perception of Z11-14Ac, without evoking a similar behavioral response. On the other hand, in the case of at least one other noctuid, *Panolis flammea* Denis & Schiffermüller, it has been shown that Z11-14Ac and Z11-16Ac can substitute each other in the attractant blend [10].

The above tests show that males of *O. gothica* are best attracted to a four-component blend containing Z9-14Ac, Z9-14OH, Z5-14Ac and Z11-14Ac. A dosage test of this combination gave higher catches at the 10 and 100 \( \mu \)g dose levels, than at higher or lower dosages (Table II).

Our results confirm earlier indications [7, 8] on the attractivity of Z9-14Ac and Z11-14Ac. However, a mixture of Z9-14Ac and the corresponding aldehyde (1:9) has been reported to catch a total of 6 specimens of *O. gothica askoldensis* Staudinger in Japan [11]. The lack of response to a similar bait in the present study (bait 3 in Table I) may indicate differences in chemical communication between the Japanese form and the European form of *O. gothica*.

Table I. Catches of male *O. gothica* in traps baited with blends of Z9-14Ac, Z9-14OH, Z9-14Al, Z5-14Ac, Z11-14Ac and Z11-16Ac in Hungary (March 22 – April 1, 1989, 5 replicates).

<table>
<thead>
<tr>
<th></th>
<th>Z9-14Ac</th>
<th>Z9-14OH</th>
<th>Z9-14Al</th>
<th>Z5-14Ac</th>
<th>Z11-14Ac</th>
<th>Z11-16Ac</th>
<th>Total catch</th>
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<td>[( \mu )g]</td>
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<tr>
<td>Bait 1.</td>
<td>100</td>
<td>100</td>
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<td>10 ef</td>
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<tr>
<td>Bait 2.</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
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<td>0 ef</td>
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<tr>
<td>Bait 3.</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>2 ef</td>
</tr>
<tr>
<td>Bait 4.</td>
<td>100</td>
<td>100</td>
<td>-</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>73 b</td>
</tr>
<tr>
<td>Bait 5.</td>
<td>100</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>-</td>
<td>71 b</td>
</tr>
<tr>
<td>Bait 6.</td>
<td>100</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>36 cd</td>
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<tr>
<td>Bait 7.</td>
<td>100</td>
<td>100</td>
<td>-</td>
<td>5</td>
<td>5</td>
<td>100</td>
<td>52 bc</td>
</tr>
<tr>
<td>Bait 8.</td>
<td>100</td>
<td>100</td>
<td>-</td>
<td>5</td>
<td>5</td>
<td>-</td>
<td>161 a</td>
</tr>
<tr>
<td>Bait 9.</td>
<td>100</td>
<td>100</td>
<td>-</td>
<td>5</td>
<td>5</td>
<td>100</td>
<td>17 de</td>
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<tr>
<td>Bait 10.</td>
<td>100</td>
<td>100</td>
<td>-</td>
<td>5</td>
<td>5</td>
<td>100</td>
<td>5 ef</td>
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<tr>
<td>Bait 11.</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>1 ef</td>
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</tbody>
</table>

Catches followed by same letter are not significantly different at \( P = 5\% \) by DNMRT.
Table II. Catches of male *O. gothica* in traps baited with different dosages of a quaternary blend of Z9-14Ac, Z9-14OH, Z5-14Ac, and Z11-14Ac in Hungary (April 1–27, 1989, 4 replicates).

<table>
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<tbody>
<tr>
<td>1000</td>
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<td>100</td>
<td>5</td>
<td>5</td>
<td>43 A</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>0.5</td>
<td>0.5</td>
<td>52 A</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>0.05</td>
<td>0.05</td>
<td>10 B</td>
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</table>

Catches followed by the same letter are not different significantly at P = 5% by DNMRT.

Many male attractants have been described in the genus *Orthosia* [6], but so far no pheromone component has been identified from females. Some of the components found attractive to *O. gothica* in this study, were also described for other *Orthosia* spp. For example, male *O. carnipennis* Butler [11] and *O. cruda* Denis & Schiffermüller [12] are attracted to blends of Z9-14Ac and Z11-16Ac, and E. Priesner (personal communication, cited in [6]) mentions *O. miniosa* Denis & Schiffermüller to be attracted to a mixture of Z9-14Ac and Z11-14Ac. However, Z9-14OH and Z5-14Ac have not been found in other *Orthosia* spp. as sex attractant components. For other noctuids, mixtures of Z9-14Ac and Z11-14Ac have been frequently described as attractants [6]. The co-occurrence of Z5-14Ac and Z9-14Ac has been reported in attractants of *Diarsia dahlii* Hübner [13], *Euxoa auxiliaris* Grote [14], *Xestia ditrapezium* Schiffermüller, *X. rhomboidea* Esper [13], *Fishia evelina hanhami* Smith [15], and in the female produced pheromone of *Agrotis exclamationis* Linné [16]. Z9-14Ac together with Z9-14OH have been described to attract *Ochropulea signifera* Denis & Schiffermüller [17], *Allophyes oxyacanthae* Linné [18], *Sesamia cereica* Led. [19], and their presence has been indicated in female produced pheromone of *Mamestra biren* Goeze [20]. The joint occurrence of Z5-14Ac, Z9-14Ac and Z9-14OH has been reported in the pheromone of *Euxoa ridingsiana* Grote [21], while the presence of all four compounds, Z5-14Ac, Z9-14Ac, Z9-14OH and Z11-14Ac (together with some other components) have been identified in the female produced pheromone of *E. maines* Smith [21]. In these species the addition of Z5-14Ac did not increase trap catches, while the addition of Z9-14OH was inhibitory [21].

In conclusion, a 20:20:1:1 quaternary blend of Z9-14Ac, Z9-14OH, Z5-14Ac and Z11-14Ac at dosages of 10–100 μg per dispenser can be recommended as a practically applicable attractant for male *O. gothica*. At present it is not known whether this blend represents components present in the female sex pheromone.

**Acknowledgements**

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