Sex pheromone perception in male pine sawflies, *Neodiprion sertifer* (Hymenoptera; Diprionidae)

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Summary. Electroantennographic and single sensillum recordings were performed on male pine sawfly, *Neodiprion sertifer*, antennae. Responses to the sex pheromone component (2S, 3S, 7S)-3,7-dimethyl-2-pentadecenyl (diprionyl) acetate (SSS:OAc), to the behavioral inhibitor (2S, 3R, 7R)-diprionyl acetate (SRR:OAc), to the six other enantiomers of diprionyl acetate, and to the biosynthetic precursor diprionol were recorded. Responses to trans-perillenal, a monoterpene identified in female gland extracts and to (2S, 3S, 7S)-diprionyl propionate (SSS:OPr), a field attractant for *N. sertifer* and some related sawfly species were also recorded.

EAG recordings demonstrated a high antennal sensitivity to SSS:OAc and to SSS:OPr. A somewhat lower response was elicited by SRR:OAc.

Single sensillum recordings revealed 8-12 different cells firing in each sensillum, corresponding to the number of cells observed in earlier morphological investigations. Out of these cells all, except one, responded to SSS:OAc and to SSS:OPr. No differences in the response to the two components could be observed. The largest amplitude cell in each sensillum was specifically tuned to the behavioral antagonist, SRR:OAc. The pheromone perception system encountered in male pine sawflies thus differs clearly from that observed in moths.

Key words: Sex pheromone – Electroantennogram – Single sensillum – Olfaction – Diprionol

Introduction

Male perception of female produced sex pheromones is a well investigated phenomenon in lepidopteran insects. The extremely high sensitivity in the male olfactory system has triggered a large volume of research (e.g. Kaissling 1986). Physiological and morphological properties of moth pheromone receptors have been described in a wide range of families, and show a great degree of consistency. From the most primitive to the most advanced moth families the pheromone receptors look and work in a very similar way (Steinbrecht 1987).

A similar system, based on long range attractive, female produced sex pheromones, has been reported in Hymenoptera, more specifically in diprionid sawflies. Coppel et al. (1960) found a very high attractivity of virgin female *Diprion similis* to the conspecific males. However, the active pheromone component was not described until 1976 when Jewett et al., after extraction of 27000 females, identified the occurrence of 3,7-dimethyl-2-pentadecanol (diprionol) in virgin females of 3 different sawfly species. In *Neodiprion lecontei* and *N. sertifer* the acetate of diprionol and in *Diprion similis* the propionate were established as the actual sex attractants.

The acetate of diprionol has 3 chiral centra, hence 8 different stereoisomers can exist, each of which might display its own biological activity. The stereoisomer responsible for attraction in the pine sawfly, *Neodiprion sertifer*, has been shown to have the (2S, 3S, 7S)-configuration (SSS) (Kikukawa et al. 1983; Kraemer et al. 1983; Lofqvist 1986; Olaifa et al. 1987). The SRR acetate, on the other hand, is a strong behavioral inhibitor if the concentration exceeds 1% in a sample of the attractive isomer (Lofqvist 1986).

A problem associated with the earlier EAG and field experiments was the low purity of the synthetic pheromone samples used. Due to the methods of synthesis employed, unwanted stereoisomers were usually present in these samples, often in unknown concentrations, leading to potentially unreliable results. We have recently developed synthetic and analytical methods by which any of the 8 stereoisomers can be prepared in high and known isomeric purities (Högberg et al. 1990).

Trans-perillenal, a furanoid monoterpene, was identified in extracts from an intertergal abdominal gland of...
female pine sawflies by Ahlgren et al. (1979). The morphology and ultrastructure of this gland were described by Hallberg and Löfqvist (1981). Trans-perillenal has, however, not yet been demonstrated to have any behavioral significance (Kikukawa et al. 1983).

As yet no traces of behaviorally active components, such as the acetate or propionate of diprionol, have been detected in insects. Diprionol is, however, present on various parts of the female body, head, thorax, abdomen and wings (Wassgren, pers. comm.). Where the production and acetylation of this alcohol take place is, however, not yet known.

The male and female pine sawfly antenna show a strong sexual dimorphism, with pronounced branching in the male, but only vestigial branches in the female. A similar sexual dimorphism is observed in many lepidopteran species. The olfactory sensilla are densely packed on the branches of the male N. sertifer antenna. The long sensilla trichodea contain 8–12 cells per sensory hair. Each sensory process splits into 2–3 branches at the distal end of the sensillum (Hallberg 1979).

In order to investigate the function of the male olfactory system involved in the pheromone perception in N. sertifer, and to compare the function of sawfly sex pheromone receptors with those of lepidopteran species, both single sensillum and electroantennographic recordings were performed with high purity stimuli. All 8 optical isomers of diprionyl acetate, the SSS of the propionate, diprionol and trans-perillenal were tested for their electrophysiological activity.

Materials and methods

Second to third instar larvae of Neodiprion sertifer were collected in the field from young scots pine (Pinus sylvestris) in plantations in southern Sweden. The larvae were held in cardboard boxes (40 × 40 × 50 cm), and fed fresh pine twigs. After cocoon formation, the males were kept at 20 °C until pupation. The stage of pupation was placed in a Ringer solution filled, grounded pipette electrode. The base of the excised antenna and den Otter 1978) recordings were obtained from single olfactory sensilla on the male antenna. The temperature in the experimental setup was 20 °C.

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The 8 different optical isomers of diprionyl acetate were synthesized by Högb erg et al. (1990), the SSS diprionyl propionate and the SSS-diprionol by Byström et al. (1981) and the trans-perillenal by Baeckström et al. (1982). The compounds and their purities are listed in Table 1.

The synthetic chemicals were diluted in hexane in decadic steps corresponding to stimulus cartridges loaded with from 10⁻² to 10⁻⁴ µg. Each dilution was applied to a piece of filter paper (5 × 15 mm) from which the hexane was allowed to evaporate. The filter paper was then inserted into a Pasteur pipette. For the EAG recordings the stimulus pipette was connected to an EAG stimulator (Syntech, P.O. Box 1547, NL-1200 Hilversum, The Netherlands), delivering short duration puffs (ca. 50 ms) of 1 ml of air containing stimulus molecules. For single sensillum recordings, 3 ml of air from the pipette atmosphere was injected during 1 s using the stimulation apparatus (Syntech, see above) to control airflow and stimulus duration precisely. Both the EAG and single sensillum stimuli were injected from the pipette into a filtered and humidified airstream (0.5 m/s) directed onto the antenna, with the airstream containing the stimulus debouching 1 cm from the antenna. The temperature in the experimental setup was 20 °C.

The EAG experiments were performed according to standard procedures (e.g., Van der Pers 1981; Hansson et al. 1989), using excited antennae. To compensate for individual variation in absolute sensitivity and antennal fatigue, the responses were normalized by using a standard stimulation before (Sb) and after (Sa) each experimental stimulation (E). The responses were normalized according to E/[Sb + Sa]/2. The standards used were 1 µg of the SSS-acetate isomer of diprionol in the Fig. 2 experiment and 3 µg of the same isomer in the Fig. 3 experiment. In the age-dependence experiment the EAG values were not normalized, as the differences between individuals was the subject studied.

Using the tip recording method (Kaissling 1974; Van der Pers and den Otter 1978) recordings were obtained from single olfactory sensilla on the male antenna. The base of the excised antenna was placed in a Ringer solution filled, grounded pipette electrode. A sensillum was cut by means of microscopic glass knives, a second pipette electrode (tip ~ 5 µm) was put over the cut surface, and contact was established with the cells in the sensilla.

Adaptation experiments were performed to establish the specificity of the different cells in the sensilla. In these experiments a strong concentration of one compound was used to adapt the cells responding to it, whereafter a second stimulus was applied. If the compounds stimulated the same cells no response could be observed in the second stimulation, if they did not, a response would be seen to the second compound.

Both EAG and single sensillum responses were transferred to a Macintosh II computer by means of a MacLab analog/digital interface with Chart software (World Precision Instruments, Quintipac Ave., MA, USA), which allows a display of the responses and voltage measurements. In order to separate the 8–12 cells present in the single sensillum recordings, a spike analysis program was developed, categorizing spikes according to amplitude and duration (Hansson et al., unpublished).

Table 1. Purity and content of SSS and SRR isomer of the 8 optical isomers of 3,7-dimethyl-2-pentadecanoyl acetate, the 3,7-dimethyl-2-pentadecanol, the trans-perillenal and the 3,7-dimethyl-2-pentadecanoyl propionate used in the electrophysiological investigations

<table>
<thead>
<tr>
<th>Compound</th>
<th>Isom. purity (%)</th>
<th>SSS cont.</th>
<th>SRR cont.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSS:OAc</td>
<td>99.0</td>
<td>99.0</td>
<td>0</td>
<td>Högb erg et al. 1990</td>
</tr>
<tr>
<td>SSR:OAc</td>
<td>&gt;98.3</td>
<td>1.5</td>
<td>0.1</td>
<td>Högb erg et al. 1990</td>
</tr>
<tr>
<td>RRS:OAc</td>
<td>&gt;97.6</td>
<td>&lt;0.2</td>
<td>0</td>
<td>Högb erg et al. 1990</td>
</tr>
<tr>
<td>RRR:OAc</td>
<td>98.9</td>
<td>0</td>
<td>&lt;0.03</td>
<td>Högb erg et al. 1990</td>
</tr>
<tr>
<td>SRS:OAc</td>
<td>97.3</td>
<td>&lt;0.03</td>
<td>2.4</td>
<td>Högb erg et al. 1990</td>
</tr>
<tr>
<td>SSR:OAc</td>
<td>&gt;97.5</td>
<td>0.4</td>
<td>&gt;97.5</td>
<td>Högb erg et al. 1990</td>
</tr>
<tr>
<td>RSS:OAc</td>
<td>&gt;97.4</td>
<td>&lt;0.05</td>
<td>0</td>
<td>Högb erg et al. 1990</td>
</tr>
<tr>
<td>RSR:OAc</td>
<td>&gt;98.1</td>
<td>0</td>
<td>0</td>
<td>Högb erg et al. 1990</td>
</tr>
<tr>
<td>Diprionol</td>
<td>86.0</td>
<td>86.0</td>
<td>0.02</td>
<td>Byström et al. 1981</td>
</tr>
<tr>
<td>trans-perillenal</td>
<td>98.0</td>
<td>–</td>
<td>–</td>
<td>Baeckström et al. 1982</td>
</tr>
<tr>
<td>SSS:OPr</td>
<td>86.0</td>
<td>86.0</td>
<td>0.02</td>
<td>Byström et al. 1981</td>
</tr>
</tbody>
</table>

*a 14% SSR isomer*