Identification of the Sex Pheromone of the Oriental Tobacco Budworm, *Heliothis assulta* (GUENÉE) (Lepidoptera: Noctuidae) 1

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The oriental tobacco budworm *Heliothis assulta* is distributed in Japan and is known as a serious pest of greenhouse green peppers. Understanding of this pest’s population dynamics are important for control. The sex pheromone trap can be used to monitor population trend, so we began to isolate and identify the sex pheromone of this species.

Larvae of *H. assulta* were collected in green pepper greenhouses in Higashi (Ibaraki Prefecture) and reared on an artificial diet (KAWASHI et al., 1987) for successive generations at 23°C, 16L-8D. Pupae obtained were sexed and placed in plastic eclosion pots (21 cm I.D. x 12 cm ht.). Sugar solution (10%) in a piece of absorbent cotton was supplied as food.

Females were kept under 16L-8D for 3 days after emergence. The abdominal tips of the females were cut at 2 hr after light off and extracted with hexane in a glass bottle. A total of 4,000 female equivalents (FE) of the crude extract was collected.

The extract was purified by column chromatography with Florisil (100/200 mesh, 30 g) according to the class-separating method for lipids (CARROLL, 1961). A HP 5970 gas chromatograph-mass spectrometer (GC-MS) was used for analysis. The temperature of the separator and the ion source were 210°C and 250°C, respectively. A capillary column (CPS-1, 0.25 mm I.D. x 50 m, J & W Scientific) was used under a programmed oven temperature condition of 80°C for 1 min increased to 160°C at a 5°C/min rate. Samples were injected to GC-MS in the splitless mode (purge delay time, 1 min) and helium gas was used for a carrier at a flow rate of 20 cm/sec. From the fraction eluted with 5% ether in hexane (5% fraction) in Florisil column chromatography, 7 peaks with retention times of 28.17 min (peak A), 29.42 min (peak B), 29.72 min (peak C), 30.27 min (peak D), 37.00 min (peak E), 37.50 min (peak F) and 38.49 min (peak G) were found. From the mass chromatogram (MC), peak A was estimated to be hexadecanal (16: Ald). Chemical structures of peaks B, C and D were estimated to be hexadecanols and those of peaks E, F and G were thought to be hexadecenyl acetates from MC. These retention times were well-fitted with those of authentic hexadecanal (28.20 min), (Z)-7-hexadecanal (Z7-16: Ald, 29.25 min), (Z)-9-hexadecanal (Z9-16: Ald, 29.68 min), (Z)-11-hexadecanal (Z11-16: Ald, 30.41 min), (Z)-7-hexadecenyl acetate (Z7-16: Ac, 37.28 min), (Z)-9-hexadecenyl acetate (Z9-16: Ac, 37.46 min) and (Z)-11-hexadecenyl acetate (Z11-16: Ac, 38.57 min), respectively. To confirm the position of double bonds, derivatization with dimethyl disulfide (DMDS) of a portion of the 5% fraction was carried out according to the method of BUSER (1983). Six DMDS adducts were detected in a selected ion monitor (SIM) with a capillary column (DB-23, 0.25 mm i.d. x 30 m, J & W Scientific) under a programmed oven temperature condition of 60°C for 1 min increased to 200°C at a 4°C/min rate. Diagnostic M+, RCH2CH2S+ , OHCRCRSCHS+ and AcORCRRSCHS+ ions were selected for monitoring. The retention times of these peaks were 60.35 min (m/z 322, 173, 159), 61.25 min (m/z 322, 145, 187), 62.80 min (m/z 322, 117, 215), 66.02 min (m/z 376, 173, 203), 67.25 min (m/z 376, 145, 231), and 69.82 min (m/z 376, 117, 259). Therefore the chemical structure of peaks A, B, C, D, E, F and G were identified to be 16: Ald, Z7-16: Ald, Z9-16:...
16: Ald, Z11-16: Ald, Z7-16: Ac, Z9-16: Ac and Z11-16: Ac, respectively. The ratio of these compounds were quantified to be about 8:2:100:3:2:15:3. In the 25% ether in hexane fraction from Florisil column chromatography in which authentic alcohols were eluted, any peaks estimated to be C12-C16 alcohols were not detected with GC-MS.

A behavioral study was carried out with a laboratory wind tunnel (30 cm O.D. × 155 cm). A rubber septum loaded with a sample was placed on a piece of cardboard (10 cm × 20 cm) at a height of 10 cm near the upwind end of the tunnel. Male moths were kept under continuous light and moved to a dark place 1.5-3.5 hr before use. Male moths were released individually from the downwind end and behavior was observed for 2 min after flight began. As shown in Table 1, 4 components (16: Ald, Z9-16: Ald, Z11-16: Ald and Z9-16: Ac) were necessary for landing and contacting with the source.

Field tests were carried out at Ami (Ibaraki Prefecture) using chemicals (Shin-Etsu Chem. Co. Ltd.) which were purified with columns of silicic acid impregnated with 16.7% silver nitrate (isomeric purity 99.9%). Chemicals were loaded on rubber septa (Takeda F) as a hexane solution.

Table 1. Behavioral responses of male H. assulta to various synthetic mixtures in a laboratory wind tunnel

<table>
<thead>
<tr>
<th>Source</th>
<th>Males showing response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O.F.</td>
</tr>
<tr>
<td>1-component</td>
<td>6.7</td>
</tr>
<tr>
<td>2-component</td>
<td>76.7</td>
</tr>
<tr>
<td>4-component</td>
<td>83.3</td>
</tr>
<tr>
<td>7-component</td>
<td>86.7</td>
</tr>
<tr>
<td>Control</td>
<td>0.0</td>
</tr>
</tbody>
</table>

- 1-component: Z9-16: Ald, 2-component: mixture of Z9- and Z11-16: Ald, 4-component: mixture of 16: Ald, Z9-, Z11-16: Ald and Z9-16: Ac, 7-component: mixture of 16: Ald, Z7-, Z9-, Z11-16: Ald, Z7-, Z9- and Z11-16: Ac. The ratio of the components was the natural ratio. The amount of Z9-16: Ald was fixed at 1 mg/septum.
- O.F.: upwind orientation flight.
- A: approach to the source within 20 cm.
- L: landing on the cardboard.
- C: contact with the source.

Table 2. Field trapping of H. assulta with 7-component and 6-component mixtures (Ami, Sep. 5-Oct. 30, 1989)

<table>
<thead>
<tr>
<th>Sources</th>
<th>Number of males caught</th>
</tr>
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<tbody>
<tr>
<td>7-component</td>
<td>15</td>
</tr>
<tr>
<td>6-component</td>
<td></td>
</tr>
<tr>
<td>16:Ald deleted</td>
<td>5</td>
</tr>
<tr>
<td>Z7-16: Ald deleted</td>
<td>18</td>
</tr>
<tr>
<td>Z9-16: Ald deleted</td>
<td>0</td>
</tr>
<tr>
<td>Z11-16: Ald deleted</td>
<td>2</td>
</tr>
<tr>
<td>Z7-16:Ac deleted</td>
<td>15</td>
</tr>
<tr>
<td>Z9-16: Ac deleted</td>
<td>5</td>
</tr>
<tr>
<td>Z11-16: Ac deleted</td>
<td>14</td>
</tr>
</tbody>
</table>

- mixture of 16: Ald (240 μg), Z7-16: Ald (60 μg), Z9-16: Ald (3000 μg), Z11-16: Ald (80 μg), Z7-16: Ac (50 μg), Z9-16: Ac (440 μg), Z11-16: Ac (90 μg) and BHT (400 μg).
- one of the components of 7-component mixture was deleted.
with 400 μg of BHT. The septa were attached to 2 sticky entrance traps (sticky surface, 25 cm × 20 cm) (Koshihara et al., 1978). These traps were placed in the field 1.2 m above the ground at intervals of more than 5 m. All 4 of the components were shown not to be necessary for attraction (Table 2). Then the attractiveness of the 4-component mixture (16:Ald, Z9-16:Ald, Z11-16:Ald and Z9-16:Ac) was compared with that of virgin females in a field screen cage (4.3 × 7.3 m, 2.6 m h.) where 100 of 3-day old male moths which emerged under natural light condition were released every day. The activity of the 4-component mixture was shown to be stronger than that of virgin females. During 3 days, the 2 traps of the 4-component mixture caught 149 male moths while those of 5 virgin female moths caught 25 male moths.

From these data, 16:Ald, Z9-16:Ald, Z11-16:Ald and Z9-16:Ac were identified to be the sex pheromone components of *H. assulta*.

**REFERENCES**


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Differences in Esterase Zymograms between Non-diapausing and Diapausing Individuals of the Hawthorn Spider Mite, *Tetranychus viennensis* Zacher

(Acari: Tetranychidae)¹

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The hawthorn spider mite, *Tetranychus viennensis* Zacher, is a pest of deciduous fruit trees such as apple, peach and pear. It overwinters as an adult female in a state of diapause (Emara and Shinjaki, 1975; Jeppson et al., 1975). Diapausing females are characterized by a suppression of reproductive activity and bright red body colour distinct from the dark red in non-diapausing females (Gotoh, 1984; Veerman, 1985). Diapause in adult insects is also manifested as a suppression of reproductive function (Beck, 1980). There are quantitative as well as qualitative differences in many physiological aspects between non-diapausing and diapausing individuals. The latter usually exhibit relatively low enzyme activities because protein metabolism is reduced during diapause (Sluss et al., 1975; Beck, 1980). In non-diapausing insects, ovary development is accelerated by a female-specific lipoprotein. Biosynthesis of this protein is indirectly regulated by esterase activity (Kunkel and Nordin, 1985). In this paper, we determined differences in esterase isozymes between non-diapausing and diapausing individuals of the hawthorn spider mite.

**MATERIALS AND METHODS**

Three strains of *T. viennensis* were used. One was collected from the apple *Malus pumila* var. *dulcisima* Koncz. at Morioka, Iwate Pref., on 31 July 1989 (apple strain), a second from the cherry *Prunus yedoensis* Matsum. at Inzai, Chiba Pref., on 8 May 1989 (cherry strain), and the third from a deciduous oak *Quercus mongolica* var. *grosseserrata* (Blume) at Tsukuba, Ibaraki Pref., on 15 May 1989 (deciduous oak strain). Laboratory stocks were maintained on leaf discs of host plants placed on a water-soaked cotton bed in petri dishes (9 cm dia.) at 16L–8D, 25 ± 1°C and 60–70% RH. Under long-day conditions, no diapause occurred (Gotoh, 1984). Diapausing individuals were obtained by rearing them at a short photoperiod of 10L–14D and 18 ±