Sex Pheromone of the Rice Stem Borer, Chilo suppressalis (WALKER) (Lepidoptera: Pyralidae): the Third Component, Z-9-Hexadecenal1,2

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The sex pheromone of the female Chilo suppressalis was previously identified as a mixture of Z-11-hexadecenal (Z-11-HDAL) and Z-13-octadecenal (Z-13-ODAL) (OHTA et al., 1976; NIBBETT et al., 1975). Field tests using the synthetic compounds showed that these two components were essential for male attraction, although less attractive than live virgin females (TATSUKI et al., 1977, 1979). These results suggested the presence of additional pheromone component(s) as known in other Lepidoptera (e.g., ROELofs et al., 1975; TAMAKI et al., 1979; KLEN et al., 1980). We preliminarily report here Z-9-hexadecanal (Z-9-HDAL) as the third pheromone component identified in the hexane extract of ovipositor tips of C. suppressalis.

Insects used were obtained from laboratory stocks successively reared on rice seedlings (UCHIUMI, 1974). For extraction, ovipositor tips of 1-2-day-old virgin females were carefully removed with fine eye-surgical scissors so that other body fragments such as scales and hairs were not included. The removed tips were then soaked in a minimum volume of redistilled n-hexane for 0.5-1 hr at room temperature. This extraction procedure allowed analysis of the crude extract by capillary-gas chromatography (GC) without any purification. Combined and concentrated extract (ca. 2,000 F.E.) was analyzed with a Shimadzu CC-Mini® gas chromatograph using a glass capillary column (0.28 mm I.D., 50 m, CHDMS-WCOT, split ratio 1:99) at 170°C isothermally. This column had proven performance in separating various isomers of the pheromone components and related compounds. With 9 major peaks obtained (Fig. 1a), retention times (RT) of P1, P3, P5, P6 and P7 were quite similar to those of authentic hexadecanal (HDAL), Z-11-HDAL, Z-11-hexadecen-1-ol (Z-11-HDOL), octadecanal (ODAL) and Z-13-ODAL, respectively (Fig. 1b). Exact coincidence of RT among corresponding peaks was further obtained by cochromatography of the extract with the above authentic compounds under the same GC conditions. P2 was expected to be one of the isomers of hexadecanal since it was located in the chromatogram between peaks of HDAL and E-11-hexadecenal (Fig. 1). Comparison of RT of P2 and several isomers of hexadecanal including Z-7-, Z-9-, E-10- and E-11-isomers was made by means of cochromatography at both 170°C and 150°C (incomplete separation of Z-9- and E-10-isomers was shown at 170°C), indicating that RT of P2 always coincided completely with that of Z-9-HDAL.

Further characterization of suspected compounds was conducted by GC-mass spectrometry (GC-MS) with a JEOL DX-300® mass spectrometer interfaced to a JEOL MS-GCGOS® gas chromatograph. A fused-silica capillary column (0.35 mm
I.D. × 25 m, PEG-20M-WCOT) with a splitless injection system was used unless otherwise stated. Both mass spectra obtained with two peaks corresponding to P2 and P3 showed the same pattern as that obtained with authentic hexadecenal (Z-11-HDAL), including a molecular ion peak at m/z 238 (M+) and a typical fragment ion peak at m/z 200 (M+−18). Therefore, P2 and P3 were identified as Z-9-HDAL and Z-11-HDAL, respectively. The peaks corresponding to P1, P6 and P7 also gave respective characteristic mass spectra of HDAL, ODAL and octadecenal, confirming the structures suspected from the GC data. The peak corresponding to P5 was not identified in the above GC-MS analysis due to some impurities. However, a mass spectrum characteristic of hexadecenal-1-ol was obtained with the TLC-purified extract using a packed column (PEG-20M, 3 mm × 2 m). Thus, P5 was suggested to be Z-11-HDOL and this has been tentatively assumed in the following. The ratio of HDAL, Z-9-HDAL, Z-11-HDAL, Z-11-HDOL, ODAL and Z-13-ODAL in the extract was estimated from GC peak area to be approximately 26:5:48:5:1:6.

A field test was conducted during the second flight of C. suppressalis in Okayama Prefecture in 1982 to compare the attractiveness of the mixture of 6 components so far detected with those of the 2 primary components and of virgin females. The mixed ratio of components in each synthetic mixture was similar to the natural one (Table 1). The purity of the synthetic compounds was determined by the capillary GC described above as follows; HDAL (>99.2%), Z-9-HDAL (>99.4%), Z-11-HDAL (>99.7%), Z-11-HDOL (>99.4%), ODAL (>97.9%), Z-13-ODAL (>99.6%). The synthetic compounds dissolved in ethyl acetate were placed on rubber septa (Takeda, f type). Either the rubber septum or a small cage with 2 virgin females was placed in a trap (Takeda, water pan type). The traps were spaced in paddy fields about 30 cm above ground level at 50 m intervals. Results are shown in Table 1. Although the moth population was low, it was clearly shown that the 6-component mixture was more attractive to male moths than either the 2-component (primary pheromone) mixture or virgin females. However, unfortunately, further field tests could not be
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Table 1. Attractiveness of mixtures of 2 primary components, 6 components and virgin females against male C. suppressalis in field test

<table>
<thead>
<tr>
<th>Source</th>
<th>Total No. males caughta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z-11-HDAL (250 µg) + Z-13-ODAL (30 µg)</td>
<td>2 b</td>
</tr>
<tr>
<td>do. + HDAL (150 µg) + ODAL (5 µg) + Z-9-HDAL (25 µg) + Z-11-HDOL (25 µg)</td>
<td>43 a</td>
</tr>
<tr>
<td>Virgin females (2)</td>
<td>11 b</td>
</tr>
<tr>
<td>Control (solvent)</td>
<td>2 b</td>
</tr>
</tbody>
</table>

a Data from 3 replications for 9 successive nights. Traps were rotated every night. Data followed by the same letter are not significantly different by DUNCAN's new multiple range test (p = 0.05).

Fig. 2. Behavioral responses of male C. suppressalis to various synthetic mixtures and to the ovipositor tip extract in the laboratory wind tunnel. O.F.: upwind orientation flight, S.R./H.: speed reduction and hovering, Z.F.: zigzag flight, L./M.D.: landing and mating dance, C.: contact with the source, see text for details. Ordinate represents response index, '3': 2 or more males responded simultaneously, '2': 2 or more males responded, although separately, '1': 1 male responded. '0': no response. Averaged data of 5 replications. Data followed by the same letter within each behavioral step are not significantly different by DUNCAN's new multiple range test (p = 0.05).

conducted due to the decrease in the number of moths.

A supplemental behavioral study was carried out with a laboratory wind tunnel (TatsuKi and Kanno, 1981). Either the rubber septum or the tip of a piece of filter paper treated with the crude extract (10 F.E.) was placed on a white piece of cardboard (19×27 cm) 20 cm high near the upwind end of the tunnel. For one or two test series, about 50 male moths preconditioned as described earlier (TatsuKi et al., 1975) were introduced into the tunnel from the downwind end. Observation was made for 1 min of their behavioral responses to each source given in random order followed by more than a 10 min interval with no source offered. The mating behavior of the
males elicited by the crude extract in the wind tunnel was composed of the following components: 1) upward orientation flight to within 20-50 cm of the pheromone source, 2) reduction of flight speed followed by hovering and/or backing up, 3) zigzag flight while slowly approaching the source, 4) landing near the source and wing fanning while walking (mating dance), and 5) contact with the source. Figure 2 shows that a 3-component mixture containing Z-9-HDAL added to the primary components, Z-11-HDAL and Z-13-HDAL, elicited the complete mating behavior pattern as did the crude extract and the 6-component mixture. On the other hand, the primary components alone in most cases elicited only up to response step (2). Neither the mixture of saturated aldehydes, HDAL and ODAL, nor the alcohol, Z-11-HDOL, had any appreciable effect on male behavior when mixed with the primary components in ratios similar to those in the crude extract. These results suggested that only Z-9-HDAL was behaviorally active among the 4 components newly detected and played an important role in eliciting the close-range orientation behavior such as landing and wing fanning in the male C. suppressalis. Presumably only Z-9-HDAL acted as a synergist to the primary components attracting the male in the field test. Also, the few male catches by the primary components could be the result of the absence of their close-range orientation behavior. On the contrary, an appreciable number was caught with the primary components in the previous field tests (Tatsuki et al., 1977; Tatsuki et al., 1979). This discrepancy might be due mainly to differences in ecological and environmental factors between the flight seasons, since the previous work was done with overwintering generation moths in the first flight season (May-June). Further field tests are planned for the coming flight season.

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REFERENCES