Sex pheromone of the blue-striped nettle grub moth *Parasa lepida* (Cramer) (Lepidoptera: Limacodidae): Identification and field attraction

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Abstract

An EAG-active compound in the extract of virgin females of the limacodid, *Parasa lepida* (Cramer) was identified as (Z)-7,9-decadien-1-ol (Z7,9-10:OH). This compound was observed to attract conspecific males in the field. When 1 mg of Z7,9-10:OH was applied to a rubber septum and used as bait in sticky traps, its attraction was equivalent to that of two 2- to 5-d-old virgin females. GC-EAD analyses suggested weak EAG responses, but the most likely candidate compounds, (Z)-7-decen-1-ol and 9-decen-1-ol, exhibited neither synergistic nor inhibitory effects when either or both were blended with Z7,9-10:OH. Z7,9-10:OH was thus considered as a sex pheromone component of this species. However, the attractiveness of the synthetic sex pheromone declined rapidly over a 12-d exposure period in the field. This decline was likely attributed to a loss of the Z7,9-10:OH which has a low molecular weight (154) and is highly volatile.

Key words: (Z)-7,9-decadien-1-ol; sex attractant pheromone; GC-EAD

INTRODUCTION

The caterpillars of limacodid moths carry stinging spines on their dorsal thorax and abdomen. When the insect is disturbed, the spines sting anything that accidentally brushes against the tree leaf on which it is perched. Contact with these specially-equipped caterpillars can produce severe irritation and inflammation of the skin. The blue-striped nettle grub moth *Parasa lepida* (Cramer), which is one of those species, is found in India, Southeast Asia including the Sunda Islands and the Philippines, China, and Okinawa, Kyushu, Shikoku and Honshu of Japan (Hirashima, 1989). This insect is regarded as exotic in Japan; Distribution has gradually extended toward the east and north along the urban areas of Honshu.

Larvae of *P. lepida* feed on a variety of plant leaves mainly in gardens and parks and along streets. Hosts include rose, magnolia, cherry and plane trees, Chinese tallow trees, Japanese pho-
Parasa lepida were collected from cherry (Plunus yedoensis) trees on the campus of the University of Shiga Prefecture at Hikone, Shiga, Japan, on 29 July and 3 August 2005. The larvae were reared on cherry leaves until they formed cocoons. Prepupae and cocoons were kept at room temperature and natural light conditions. Cocoons were dissected to obtain 19 female and 6 male pupae; 15 female and 2 male moths emerged. The females were kept at room temperature. The males were provided with honey solution and kept at 15°C until use.

**Extraction.** Extract was obtained from 3-d-old virgin females 1 h before the end of the dark period when the virgin females most frequently assume a calling posture (Oda, 1981). Gentle pressure was exerted on the abdomens of the females to extrude the ovipositor, and the abdominal tips were then removed with fine forceps. The abdominal tips containing pheromone glands were extracted with ca. 100 μl of hexane for 15 min, and the residue was rinsed twice with the same volume of the solvent. The extracts accumulated for 15 females were stored at -10°C until use.

**Florisil column chromatography.** The crude extract was poured onto 200 mg of Florisil in a Pasteur pipette. The compounds were eluted with 1 ml each of pure hexane, 5%, 15% and 50% ether in hexane, and pure ether. Each fraction was concentrated to ca. 30 μl at below 30°C under reduced pressure.

**Gas chromatography (GC) and electroantennographic detection (GC-EAD).** GC analyses were conducted on a Hewlett-Packard (HP) 5890 series II gas chromatograph equipped with a split/splitless injector and a flame ionization detector (FID). An apolar HP-1 or polar INNOWAX fused silica column (15 m or 30 m×0.25 mm I.D.×0.25 μm film thickness; Agilent) was used at a column head pressure of 55 kPa or 110 kPa of helium as carrier gas, respectively. Injection was made in splitless mode at 220°C for 1 min. The temperature program for the column oven was 50°C for 1 min, 50 to 100°C at 25°C/min, 100 to 250°C at 5°C/min. The final temperature was then held for 5 min.

An electroantennographic detector was used according to Struble and Arn (1984). For certain analyses, n-alkanes with an even number of carbon atoms (between 12 and 28) were added as internal standards for calculation of the Kováts retention index (KI; Kováts, 1965).

**Gas chromatography–mass spectrometry (GC-MS).** GC-MS analyses were conducted with an HP6890 gas chromatograph interfaced to a JEOL JMS SX-102A mass spectrometer (EI mode, 70 eV). An INNOWAX column was operated under the same conditions as the GC-EAD system but He was used at a constant flow rate of 1.6 ml/min.

**Diimide reduction.** In accordance with the method described by Yamaoka et al. (1976), a solution of polyenyl compound was concentrated to ca. 5 μl in a taper-bottomed vial and then combined with 5 μl each of hydrazine and hydrogen peroxide solution in ethanol (N₂H₂: 0.3 ml of hydrazine hydrate in 10 ml of ethanol, H₂O₂: 0.04 ml of 31% H₂O₂ in 10 ml of ethanol). The mixture was maintained at 55°C for 15 min, and then immediately cooled to −20°C. The mixture of 1.5 μl was directly injected into the GC-MS after blending with the internal standards in order to calculate KI values.

**Chemicals.** All possible positional and geometric isomers of decen-1-ol were synthesized from terminal alkyne and bromide compounds via acetylene coupling reaction and subsequent hydrogenation. (Z)-7,9-Decadien-1-ol (Z7,9-10:OH) was synthesized from 7-hydroxyheptyl(triphenyl)phosphonium bromide and propenal via Wittig reaction according to the method described by Horiike et al. (1980). The synthetic Z7,9-10:OH was more than 99.5% pure with respect to positional and geometric isomerism.

**Traps.** Sticky traps (SE-trap, Sankei Chem. Co., Kagoshima), with a triangular section and dimensions of 29 cm×32 cm×8 cm height, and a 24 cm×30 cm sticky plates were used. Synthetic chemicals were impregnated into rubber septa (gray, 8 mm O.D., 19 mm ht., West Co., Singapore) by applying 300 μl of the hexane solution into the depression. Each septum was placed in a draft chamber for ca. 12 h at room temperature to allow the solvent to evaporate. The septa were stored below −20°C until use. Each septum was suspended on a wire about 3 cm above the sticky plate in the trap.

A net cage (6 cm diam.) containing two 2- to 5-d-old virgin females was placed beneath the roof of a sticky trap. As a control, a rubber septum containing no pheromone chemical but treated with the same amount of solvent was suspended in a
Field test. Field tests were conducted on campuses of the University of Shiga Prefecture, Hikone, Shiga and the Agricultural, Food and Environmental Sciences Research Center of Osaka Prefecture, Habikino, Osaka, and in private gardens around the Research Center. Traps were placed 1.2–1.7 m above the ground at about 5–10 m intervals. The traps were checked for catches during the daytime and trap locations were rotated on the site to cancel any possible effects of trap location. Experiments were repeated on each of the three sites.

Data (X) were transformed to \((X+0.5)^{1/2}\) and submitted to a 2-way-layout analysis of variance and subsequently to Tukey’s ranking when ANOVA was significant. The values shown in the figures are the backtransformed means and SE, and the means designated by the same letter are not significantly different at \(p=0.05\).

RESULTS

When one female equivalent (1 FE) of the crude extract of abdominal tips of virgin females was injected into the GC-EAD equipped with a polar INNOWAX column, several EAG responses were observed, while only one response was reproducible at \(t_R=24.450\) min \((KI 1954)\) in the 50%-ether-in-hexane fraction (50% fraction) after chromatography on Florisil (Fig. 1). This response was observed at \(t_R=12.980\) min \((KI 1276)\) on an apolar HP-1 column. The amount of this compound was estimated at ca. 20 ng/FE from the area of the corresponding FID peak (Fig. 1).

GC-MS analysis of the EAG-active compound in the 50% fraction gave a mass spectrum (Fig. 2): Molecular ion was observed at \(m/z 154\) (relative intensity: 8.1%) and the M–18 ion at \(m/z 136\) (23%) led to an estimation that this compound was a diene alcohol. The other major fragment ions were \(m/z 121\) (13%), 107 (21%), 95 (24%), 93 (31%), 82 (31%), 81 (51%), 80 (89%), 79 (74%), 68 (50%), 67 (base), 55 (23%), 54 (72%) and 41 (53%). These fragment ions suggested a terminal 1,3-diene system (Ando et al., 1988). The large difference of \(KI\) values between the polar and apolar columns (1954–1276=678) supported this estimation.

Diimide reduction of the 50% fraction yielded two products at \(t_R=19.04\) min \((KI 1768)\) and \(t_R=20.23\) min \((KI 1824)\) in GC-MS analyses using the polar column. Mass spectrum and \(KI\) value for the compound at \(t_R=19.04\) min were identical to those for authentic decan-1-ol, which confirmed that the original compound had a straight carbon chain and primary alcohol group. Mass spectrum of the second compound indicated that it was an isomer of decen-1-ol. Comparative analyses with all the possible positional and geometric isomers of decen-1-ol showed that the mass spectrum and the
KI value were identical to those for (Z)-7-decen-1-ol (Z7-10:OH). However, the other possible monoeneol product was not detected. This indicates that the second double bond might be terminal since the terminal olefins is reduced 8 to 10 times faster with diimide (Aisinger et al., 1964) while the position and geometry of the double bond have a rather small effect on the reduction rate (Scholfield et al., 1969; House, 1972). Thus, the original compound should be (Z)-7,9-decadien-1-ol (Z7,9-10:OH). This estimation was confirmed by a comparative GC-MS analysis of the natural component and the synthetic Z7,9-10:OH, which gave identical mass spectra and KI values on both the polar and apolar columns. The amount of this compound was estimated to be ca. 20 ng/FE based on a comparison of the FID peak areas of the crude virgin female (Fig. 1) and the known amount of authentic decan-1-ol.

In the GC-EAD analyses, in addition to the single major response, weak and uncertain EAG responses were observed at around $KI \sim 1820$ (1815–1825) on the polar INNOWAX column (Fig. 1). Their KI values corresponded to those for decen-1-ol isomers. From the similarity of chemical structure, Z7:10-10:OH and/or 9-decen-1-0l (9-10:OH) appeared to be the most likely components. The amounts of possible minor component should be less than 1% of the major compound, if it occur at all, since no corresponding FID peak was observed (Fig. 1). Although these weak responses are only experimental noise at this point, further examination of this activity is worthwhile.

When the rubber septa impregnated with 0.1 to 1 mg of synthetic Z7,9-10:OH were used as a lure in the field traps, the trap catch increased depending on the amount of Z7,9-10:OH (Fig. 3). The trap catch neither increased nor diminished when Z7-10:OH and/or 9-decen-1-ol (9-10:OH) were blended to Z7,9-10:OH (Fig. 4). Trap catches with the septa impregnated with 1.0 mg of Z7,9-10:OH were approximately half of those obtained with virgin females (Fig. 5), but the difference was not significant. The trap catches drastically diminished after the 12-d exposure in the field (Fig. 6).

Throughout the field experiments, males of congeneric species $P. \text{s}inica$ Moore were captured with Z7,9-10:OH and Z7,9-10:OH plus 1% amount of 9-10:OH: one male with 0.1 mg/septum, four with 0.3 mg/septum, and five with 1 mg/septum of single use of Z7,9-10:OH, and one male with 1 mg/septum of Z7,9-10:OH plus 1% of 9-10:OH.

**DISCUSSION**

A single compound Z7,9-10:OH was identified as a major EAG-active compound in the hexane extract. This compound showed potent attractiveness to $P. \text{l}epida$ males in the field (Fig. 3). Weak EAG responses suggested minor component but the most
likely candidate compounds, Z7-10:OH and 9-10:OH showed neither synergistic nor inhibitory effects in the field test when blended with Z7,9-10:OH (Fig. 4). Attractiveness of Z7,9-10:OH loaded on a rubber septum (1.0 mg/septum) was comparable to that of two 2-d-old virgin females (Fig. 5). Thus Z7,9-10:OH was considered to be a component of the sex attractant pheromone of *P. lepida*.

In Limacodidae, sex pheromone has been identified in several species, which are sympatric and coseasonal, feed on a wide range of host plants and have become economically important as a defoliator of oil palms in Southeast Asia: *Darna bradleyi* Holloway, *D. trima* Moore (Sasaerila et al., 2000c), *Setothosea asigna* van Eecke (Sasaerila et al., 1997) and *Setosa nitens* Walker (Sasaerila et al., 2000b). In the former two species, (E)-7,9-decadienoic acid esters of species-specific alcohols act as the sex pheromone: isobutyl and methyl esters in *D. bradleyi* and (E)-2-hexenyl and (S)-2-methylbutyl esters in *D. trima*, while the unsaturated C_{12}-aldehydes: (E)-9-dodecenal and (E)-9,11-dodecadienal in *S. asigna* and (Z)-9-dodecenal and (Z)-9,11-dodecadienal in *S. nitens* act as the pheromone in the latter two species. The former two diurnal and the latter two nocturnal species discriminate their own mates by olfaction (Sasaerila et al., 2000a). In North America, a 10:1 blend of (Z)-9,11- and (E)-9,11-dodecadienals was observed to attract males of *Tortricidia testacea* (Packard) (Chisholm et al., 1985), but the constituents of this blend have not been confirmed to be sex pheromone components.

In our field experiments, males of congeneric *P. sinica* were captured with Z7,9-10:OH, which suggests that this species also may share this compound as a sex pheromone component with *P. lepida*. *P. sinica* is distributed in temperate East Asia: southeast Siberia, China, Korean Peninsula, and Japan (with exception of the Ryukyu Islands) (Hirashima, 1989). Larvae of domestic *P. sinica* and exotic *P. lepida* feed on common plants in temperate Japan. It is, therefore, interesting to examine the sex pheromone component(s) of *P. sinica*, since this might reveal interspecific interaction of pheromonal communication between domestic and exotic species.

To our knowledge, Z7,9-10:OH is new as a component of the sex pheromone, whereas the sex pheromone components of the other limacodid moths have a terminal 1,3-diene structure in the straight C_{10}- or C_{12}-carbon chain (Chisholm and Reed, 1985; Sasaerila et al., 1997, 2000a, b). Among the sex pheromone compounds released by female Lepidoptera, Z7,9-10:OH (M.W. 154) is one of the compounds with the lowest molecular weight (see Ando, 2006). When this compound was loaded onto a rubber septum, the attractiveness declined rapidly within 12 d (Fig. 6). This decline is likely due to the rapid decrease of Z7,9-10:OH by evaporation. When a blend of (Z)-5- and (Z)-7-decenyl acetates (M.W. 196) was loaded onto a rubber septum formulation, the released amount decreased to 1/10 of the original level within 6 d in
the laboratory (Kozai and Wakamura, 1982). This suggests that reduction rate of Z7,9-10:OH would be considerably higher than these compounds since its molecular weight (154) is lower than that of the decenyl acetates (198). A controlled release formulation is needed to extend the useful life of this highly volatile compound to achieve stable male attraction in the field.

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