J Chem Ecol (2007) 33:555–565
DOI 10.1007/s10886-006-9246-1

Sex Pheromone of *Lonomia obliqua*: Daily Rhythm of Production, Identification, and Synthesis

Paulo H. G. Zarbin · Lisete M. Lorini · Bianca G. Ambrogi · Diogo M. Vidal · Eraldo R. Lima

Received: 11 September 2006 / Accepted: 18 December 2006 /
Published online: 25 January 2007

Abstract The sex pheromone of *Lonomia obliqua* Walker (Lepidoptera: Saturniidae) was studied in the laboratory. All female calling occurred during the scotophase. Most females (70.6%) called first within 24 hr of eclosion. Calling varied with age of female, with older (5- to 6-day-old) females calling earlier in the scotophase and for longer durations than younger (0- to 1-day-old) females. The sex pheromone gland of 1- to 3-day-old virgin females was extracted during the calling peak. A Y-olfactometer bioassay showed significant attraction of males to a filter paper containing the female gland extract. Gas chromatographic-electroantennogram detection (GC-EAD) analysis of the extract indicated the presence of at least two possible pheromone components. Gas chromatographic-mass spectrometric analysis of the major GC-EAD-active peak indicated a hexadecenyl acetate; chemical derivatization indicated $\Delta 11$ unsaturation. Synthetic samples of (*E*)- and (*Z*)-11-hexadecenyl acetate were obtained by coupling 10-bromo-1-decanol and 1-hexyne, utilizing lithium chemistry. The comparison of the retention time of dimethyl disulfide derivatives of the natural compound, to those of synthetic chemicals, confirmed the natural compound as (*E*)-11-hexadecenyl acetate. The minor component was identified as the related alcohol, (*E*)-11-hexadecenol. The ratio of the two components in female extract was 100:35. Preliminary tests of males in a Y-olfactometer showed that their response to a mixture of the two compounds was not significantly different from that to gland extract.

Keywords Hemorrhagic caterpillar · Sex pheromone · (*E*)-11-Hexadecenyl acetate · (*E*)-11-Hexadecenol · Calling behavior

P. H. G. Zarbin (✉) · L. M. Lorini · B. G. Ambrogi · D. M. Vidal
Departamento de Química, Laboratório de Semioquímicos, Universidade Federal do Paraná,
C. Postal 19081, 81531-990 Curitiba-PR, Brazil
e-mail: pzarbin@quimica.ufpr.br

E. R. Lima
Laboratório de Semioquímicos e Comportamento de Insetos, Departamento de Biologia Animal,
Universidade Federal de Viçosa, 36570-000 Viçosa-MG, Brazil

L. M. Lorini
Departamento de Biologia, Universidade de Passo Fundo,
C. Postal 611, 99001-70 Passo Fundo-RS, Brazil

Introduction

Lonomia obliqua Walker (Lepidoptera: Saturniidae) is one of the most important urban insects in Brazil due to the urticating spines on the larva. Contact with the toxin present in the spines can cause burns, bleeding, and, in worst cases, acute renal failure, hemorrhagic disorder, and death (Duarte et al., 1990). In Brazil, the distribution of the insect is concentrated in the southern states. Aspects of the biology and morphology of *L. obliqua* have been reported (Lorini, 1999, 2004; Lorini and Corseuil, 2001).

Compared to some families of Lepidoptera, there are relatively few studies on the identification of sex pheromones of Saturniidae. The sex pheromones of *Antheraea polyphemus* (Kochansky et al., 1975), *Nudaurelia cytherea* (Henderson et al., 1973), *Antheraea pernyi* and *Samia cynthia ricini* (Bestmann et al., 1989), and some species in the genera *Hemileuca* and *Saturnia* (McElfresh and Millar, 1999a,b,c; McElfresh et al., 2001a, b) are known.

The aim of the present study was to determine the effect of age on the calling behavior of *L. obliqua* females and to identify the sex pheromone of the species. Additionally, the behavioral responses of males to gland extract and to a blend of synthetic chemicals were also evaluated.

Methods and Materials

Insect Rearing Adults used in the experiments were obtained from caterpillars collected in the field in the northern region of the state of Rio Grande do Sul, Brazil. Insects were maintained in a controlled-environment room, with a mean temperature of 25±0.5°C, RH 65±15%, and a 13:11 (L to D) photoperiod. Larvae were allowed to feed on the leaves of *Platanus acerifolia* until pupation. Pupae were transferred into plastic containers (8×3 cm) lined with filter paper and maintained in an environmental chamber under the same conditions as for the larvae. The filter papers in the pupal containers were moistened daily. At the beginning of each scotophase, adults were collected and sexed according to the method of Lorini (1999).

Calling Behavior of Females After emergence, virgin females were transferred to a room at the same conditions as used for pupae. Females were placed in individual cages made of plastic tubes (PVC), measuring 15×10 cm, sealed on one side with transparent glass and on the other with nylon net. They were observed during the scotophase of each day until death. A tungsten lamp, covered with three layers of red cellophane paper and positioned approximately 80 cm from the cage, was used during observations of behavior. To determine the effect of age on calling, 65 virgin females were observed from the first to the sixth day after emergence, at 10-min intervals during each scotophase. From these observations, we obtained information about the time of onset of calling, the time spent calling, and the number of calling bouts.

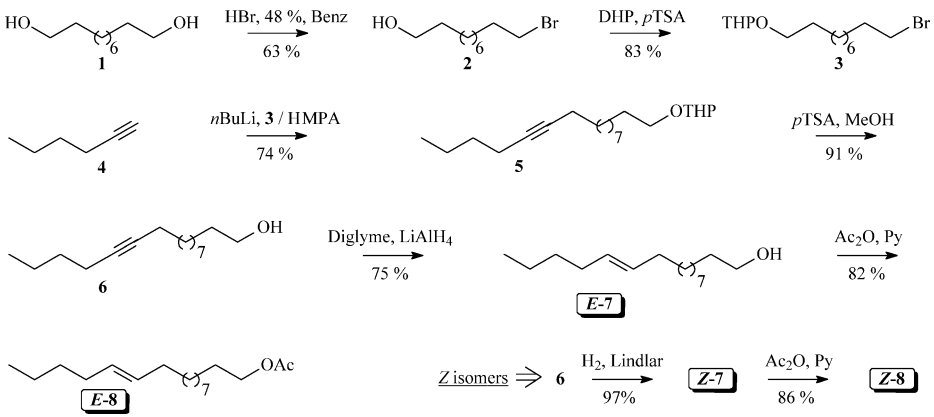
Extraction of Sex Pheromone from Glands Light pressure was applied to the terminal part of the abdomen of a female to expose the last abdominal segments on which the sex pheromone gland is located. The gland was dissected and extracted for 30 min in a 2-ml glass vial containing 0.5 ml of hexane. The extract was transferred to a clean vial, concentrated under a flow of argon, and stored at −20°C until use.

Analytical Procedures Coupled gas chromatographic-electroantenographic detector (GC-EAD) analysis was performed on male antennae with a system based on that of Leal et al. (1992, 1994). A Varian 3800 gas chromatograph, equipped with a DB-5 column (30 m× 0.25 mm×0.25 μm; Agilent Technologies, Santa Clara, CA, USA) and a splitless injector, was used in these analyses. The oven was programmed from 70°C (1 min hold) to 250°C at 7°C/min and kept at this temperature for 5 min. Gas chromatography-mass spectrometry (GC-MS) analyses were performed with a Varian 3800–Saturn 2000 GC-MS-MS ion trap detector, fitted with a DB-5 capillary column, under the same conditions as described above.

Synthesis High-grade reagents and solvents were used in the syntheses. Chromatographic purifications were carried out on silica gel 60, Merck, 230–400 mesh. Dimethyl disulfide (DMDS) derivatization was carried out according to the method of Vincenti et al. (1987). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-400 (400 and 100 MHz, respectively).

The starting material, 1,10-decanediol (**1**), was subjected to monobromination to yield 10-bromodecan-1-ol (**2**), which was subsequently protected with DHP to give ether **3**. The anion generated with *n*-BuLi from 1-hexyne (**4**) was alkylated with bromide **3**, affording compound **5**, which was directly deprotected to yield alcohol **6**, the direct precursor of the pheromones, because it possesses a triple bond at C-11 and functionality at C-1. The isomer (*E*)-11-hexadecenol (**7**) was obtained by reacting alcohol **6** with $LiAlH_4$, under reflux of diglyme, while the isomer (*Z*)-11-hexadecenol (**7**) was prepared by catalytic hydrogenation of the same compound **6**. The ^{1}H NMR analysis of the vinyl protons of the resulting compounds indicated an isomeric purity >97%. The isomers (*E*)- and (*Z*)-11-hexadecenyl acetate (**8**) were obtained by acetic anhydride acetylation of the corresponding alcohols (*E*)-**7** and (*Z*)-**7**, respectively (Scheme 1).

10-Bromodecan-1-ol (2) To a stirred solution of 1,10-decanediol (**1**) (5.2 g; 30.4 mmol) in benzene (100 ml), 48% aqueous HBr (5.0 ml) was added at room temperature. The reaction was refluxed for 24 hr, trapping water with a Dean-Stark apparatus. The mixture was successively washed with 1 M NaOH, 10% HCl, and brine. The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The crude oil obtained



Scheme 1 Synthesis of the components of the sex pheromone of *L. obliqua* and their (*Z*)-isomers

was distilled to give compound **2** in 63% yield (4.4 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.28–1.46 (m, 12H); 1.56 (quint, J=6.8 Hz, 2H); 1.65 (bs, 1H); 1.85 (quint, J=6.8 Hz, 2H); 3.41 (t, J=6.8 Hz, 2H); 3.63 (t, J=6.8 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 25.72, 28.15, 28.74, 29.37 (2C), 29.47, 32.75, 32.81, 34.00, 62.98. GC-MS *m/z* (%): 220/218 (M-18, 0.14), 190/192 (7.7), 162/164 (10.8), 148/150 (54), 97 (39.8), 83 (53.4), 69 (93.4), 55 (96.6), 43 (43.7), 41 (100).

1-(Tetrahydro-2'-pyranyloxy)-10-bromodecane (3) To a stirred solution of **2** (3.5 g; 15.25 mmol) in dry THF (15 ml), some crystals of *p*TSA and dihydropyran (1.65 ml; 18 mmol) were added. The reaction was stirred at room temperature for 4 hr and then washed with brine. The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. Crude product **3** (83%, 3.9 g) was employed in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.28–1.46 (m, 12H); 1.50–1.62 (m, 6H); 1.68–1.75 (m, 1H); 1.81–1.89 (m, 3H); 3.35–3.42 (m, 1H); 3.43 (t, J=6.8 Hz, 2H); 3.48–3.53 (m, 1H); 3.73 (dt, J=6.8 and 9.6 Hz, 1H); 3.84–3.90 (m, 1H); 4.56-4.58 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 19.72, 25.52, 26.22, 28.17, 28.75, 29.37, 29.43 (2C), 29.75, 30.80, 32.83, 34.02, 62.35, 67.66, 98.85. GC-MS *m/z* (%): 320/322 (M, 0.38), 109 (0.9), 101 (8.61), 85 (100), 69 (8.40), 55 (16.5), 41 (23.1).

1-(Tetrahydro-2'-pyranyloxy)-11-hexadecyne (5) To a stirred solution of 1-hexyne (**4**) (2.34 ml; 20.2 mmol) in dry THF (10 ml), under argon, *n*-BuLi (6.0 ml, 2.0 M in hexane) was added dropwise at −78°C. The solution was slowly warmed to 0°C and stirred at this temperature for 40 min. The reaction was cooled again to −78°C, and compound **3** (3.0 g, 10 mmol) in dry HMPA (2.0 ml) was added and left for 1 hr. The mixture was warmed to 0°C and stirred for 50 min. The reaction was extracted with ethyl ether and washed with brine. The organic layer was separated, dried (Na_2SO_4), and concentrated *in vacuo*. The crude product was purified by column chromatography (silica gel, hexane to ethyl acetate 9:1), affording **4** in 74% yield (2.2 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.91 (t, J=6.8 Hz, 3H); 1.45–1.52 (m, 24H); 1.53–1.58 (m, 2H); 2.11–2.16 (m, 4H); 3.35–3.41 (m, 1H); 3.47–3.53 (m, 1H); 3.73 (dt, J=6.8 and 9.6 Hz, 1H); 3.84–3.90 (m, 1H); 4.56–4.58 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.65, 18.46, 18.77, 19.72, 21.95, 25.54, 26.27, 28.87, 29.19 (2C), 29.51 (2C), 29.57, 29.78, 30.81, 31.30, 62.32, 67.69, 80.19 (2C), 98.83. GC-MS *m/z* (%): 322 (M, 0.44), 280 (0.33), 265 (1.26), 207 (1.1), 165 (1.84), 121 (4.07), 101 (27.9), 85 (100), 81 (35.4), 67 (41.2), 55 (48.7), 41 (71.1).

11-Hexadecyn-1-ol (6) To a stirred solution of **5** (2.0 g, 6.2 mmol) in MeOH (10 ml), some crystals of *p*TSA were added. The mixture was stirred at room temperature for 4 hr. Methanol was removed, and the residue was diluted in ethyl ether and washed with saturated solutions of $NaHCO_3$ and brine. The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. Crude product **6** (91%, 1.34 g) was employed in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.90 (t, J=6.8 Hz, 3H); 1.25–1.51 (m, 21H); 2.12–2.16 (m, 4H); 3.63 (t, J=6.8, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.64, 18.46, 18.77, 21.94, 25.76, 28.86, 29.18 (2C), 29.44, 29.48, 29.57, 31.29, 32.82, 63.05, 80.19 (2C). GC-MS *m/z* (%): 164 (1.48), 149 (2.06), 135 (4.23), 121 (10.1), 110 (19.4), 96 (88.6), 81 (100), 67 (76.8), 55 (69.7), 41 (99).

(E)-11-Hexadecen-1-ol (7) A solution of compound **6** (1.5 g, 6.30 mmol) in diglyme (10 ml) was added dropwise to a suspension of $LiAlH_4$ (0.23 g, 6.30 mmol) in diglyme (10 ml) at room temperature. The resulting mixture was stirred at 130°C, under argon, for

5 hr. The reaction was quenched by the addition of aqueous NaOH, 0.1 mol/l (10 ml), followed by the addition of ether (50 ml). The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The crude product was purified by column chromatography (silica gel, hexane to ethyl acetate 9:1), affording (*E*)-**7** in 75% yield (1.1 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.88 (t, J=6.8 Hz, 3H); 1.25–1.40 (m, 19H); 1.53–1.61 (m, 2H); 1.94–1.99 (m, 4H); 3.65 (t, J=6.8, 2H); 5.37–5.40 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.97, 22.20, 25.75, 29.16, 29.44, 29.50, 29.56 (2C), 29.60, 29.66, 31.85, 32.29, 32.61, 32.83, 63.11, 130.32 (2C). GC-MS *m/z* (%): 240 (M, 0.09), 222 (5.23), 194 (1.87), 180 (0.92), 152 (3.0), 138 (7.23), 123 (14.0), 109 (24.4), 96 (64.5), 82 (92.3), 67 (55.4), 55 (100), 41 (75.0).

(Z)-11-Hexadecen-1-ol (7) To a stirred solution of **6** (0.5 g, 2.1 mmol) in MeOH (5 ml), 5% $Pd/BaSO_4$ (20 mg) and quinoline (two drops) were added. The mixture was stirred under hydrogen atmosphere (25 psi) in a Parr® apparatus for 4 hr and filtered on Celite®. The solvent was evaporated *in vacuo*, and the residue was extracted with ether and washed with 10% HCl, water, and brine. The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The product was purified by column chromatography (silica gel, hexane to ethyl acetate 9:1), affording (*Z*)-**7** in 97% yield (0.48 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.90 (t, J=6.8 Hz, 3H); 1.24–1.39 (m, 19H); 1.53–1.60 (m, 2H); 1.97–2.04 (m, 4H); 3.64 (t, J=6.8, 2H); 5.33–5.36 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.97, 22.20, 25.75, 29.16, 29.44, 29.50,29.56 (2C), 29.60, 29.66, 31.85, 32.29, 32.61, 32.83, 63.11, 130.32 (2C). The MS data are the same as described for the (*E*)-isomer **7**. The retention times of compounds (*Z*)- and (*E*)-**7** were identical to that of the minor component of the natural pheromone.

(E)-11-Hexadecenyl acetate (8) To a stirred solution of pyridine (0.5 ml) and compound (*E*)-**7** (0.5 g, 2.1 mmol), acetic anhydride (0.4 ml, 4.0 mmol) was added, and the resulting mixture was stirred overnight at room temperature. The reaction was diluted in ethyl ether (20 ml) and washed with saturated $NaHCO_3$ solution, 10% HCl, and brine. The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The product was purified by column chromatography (silica gel, hexane to ethyl acetate 9:1), affording (*E*)-**8** in 82% yield (0.47 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.89 (t, J=6.8 Hz, 3H); 1.22–1.40 (m, 18H); 1.55–1.65 (m, 2H); 1.96–2.02 (m, 4H); 2.05 (s, 3H); 4.05 (t, J=6.8, 2H); 5.37–5.40 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 21.02, 22.20, 25.93, 28.62, 29.15, 29.27, 29.50 (2C), 29.52 (2C), 29.66, 31.85, 32.29, 32.61, 64.67, 130.33 (2C), 171.22. GC-MS *m/z* (%): 282 (M, 0.09), 222 (15.2), 194 (2.90), 180 (1.82), 166 (5.30), 152 (5.44), 138 (13.45), 124 (23.8), 110 (33.7), 96 (82.9), 82 (100), 67 (56.4), 55 (78.8), 43 (81.8), 41 (61.2).

(Z)-11-Hexadecenyl acetate (8) In the same manner as for the (*E*)-isomer, the alcohol (*Z*)-**7** (0.2 g, 0.83 mmol) afforded the acetate (*Z*)-**8** in 86% yield (0.2 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.90 (t, J=6.8 Hz, 3H); 1.25–1.38 (m, 18H); 1.58–1.65 (m, 2H); 1.98–2.03 (m, 4H); 2.05 (s, 3H); 4.05 (t, J=6.8, 2H); 5.33–5.36 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.00, 21.02, 22.36, 25.92, 26.93, 27.20, 28.61, 29.28 (2C), 29.52 (3C), 29.77, 31.98, 64.67, 129.87 (2C), 171.24. The MS data are the same as described for (*E*)-**8**. The retention times of compounds (*Z*)- and (*E*)-**8** were identical to that of the major component of the natural pheromone.

Olfactometer Tests The behavior of males in the presence of the natural female pheromone was observed in a Y-olfactometer. In the first test, a glass olfactometer, with arms 10×

40 cm, was used. The bioassay was conducted during the scotophase at 25±1.0°C and 65±15% RH. The airflow, produced by a vacuum pump, was 2.5 l/min. A piece of filter paper, 2 cm^2, loaded with 5 μl (one female equivalent) of female gland extract, was placed at the extremity of one arm, and a filter paper with solvent applied (and left to evaporate) was placed in the other arm as a control. A virgin male was placed in the olfactometer, and which of the two arms he moved into was recorded; if he did not move into either arm, he was recorded as a nonresponder. Twelve replicates were performed. For the second test, 0.1 μg of a 1:0.35 mixture (on filter paper) of synthetic (*E*)-11-hexadecenyl acetate:(*E*)-11-hexadecenol was tested against a solvent blank. In the third test, the same synthetic blend as in the second test was tested against one female equivalent of extract. Ten replicates (each a single male) were performed for the last two tests.

Statistical Analyses Comparisons of the calling behavior on different days were made by ANOVA, followed by a Tukey highest significant difference test (Statistica and Stat Soft, Inc., 1999). The olfactometer responses were analyzed by using the R Statistical System (R Development Core Team, 2005). The choice experiment (gland extract vs. hexane) was tested with a GLM for Quasibinomial data using the logit link function (Crawley, 2005). Proportions were calculated by dividing the numbers of insects that chose a particular arm by the total insects tested. The analysis was carried out as follows. A full model was fitted, and significance was assessed by testing the change in deviance after the removal of a term from the model. After fitting the full model, the data were examined for overdispersion by dividing the residual deviance by the residual degrees of freedom to give the dispersion parameter; relatively large values of residual deviance suggest overdispersion and risk potential overestimation of the significance level (Crawley, 2002). To account for this, the residual deviance was rescaled by the dispersion parameter, and an *F* test was used to evaluate whether the removal of a term caused a significant increase in deviance. In addition, model fit was checked by examining the distribution of residuals. The validity of each analysis was verified with residuals analysis (Crawley, 2005). All significant differences are reported at $P<0.05$.

Results and Discussion

Virgin *L. obliqua* females exhibited calling behavior only during the scotophase. The behavior consisted of a female positioning with the wings totally opened, showing the end part of the abdomen, and exposing the ovipositor and pheromone gland. This compares to the work by Fornés and Hernández (2000) on *Hylesia metabus* (Lepidoptera: Saturniidae), who showed that females are active during crepuscular–nocturnal hours but not active during the photophase.

The mean age of females calling for the first time was 13.9±12.6 hr after emergence, with the range being 1.2–51.5 hr. The percentage of females that first called on the first day after emergence was 70.6%, on the second day, 27.8%, and on the third day, 1.6%. Among the females, 92.3% were observed to call once a day, 6.2% twice a day, and only 1.6% three times during the same day. Because not all female callings happened at the same chronological age, comparisons were made by "calling age" (Turgeon and McNeil, 1982).

Age-related data for onset and duration of calling are reported in Table 1. In general, females began calling earlier in the scotophase as they aged, with the mean onset of calling being significantly different between 1-day-old and 5- and 6-day-old females. Furthermore, older females tended to call for a longer duration than younger females. These age-related

Table 1 Mean onset time (hours into the scotophase) and mean time spent calling during the scotophase for different-age virgin females of *L. obliqua* (±SEM), (*N*=65)

Days	Mean Onset Time of Calling[1]	Time Spent Calling (hr)
1	6.0±0.2 a	3.20±0.21
2	5.35±0.2 ab	3.81±0.27
3	5.2±0.2 ab	4.26±0.30
4	5.25±0.2 ab	4.00±0.35
5	4.85±0.4 b	4.17±0.50
6	4.84±0.4 b	4.37±0.51

[1] Means in the column followed by the same letter are not significantly different (Tukey HSD test, $P<0.05$).

effects on calling probably ensure that older, unmated females will mate by attracting males earlier in the scotophase than younger females are capable of doing (Swier et al., 1977).

Sex pheromone glands from virgin females, 1- to 3-day-old, were extracted at peak calling (sixth–seventh hour into scotophase), and the extracts were tested in the olfactometer. A significantly greater percentage ($F_{1,10}=5.82$, $P=0.016$) of males were attracted to the arm containing the pheromone extract than to the control arm (Fig. 1). Responses of males in the olfactometer were similar to those observed for *Phyllocnistis citrella* (Gracillariidae) (Parra et al., 2002; Parra-Pedrazzoli et al., 2006) and suggested that the gland extracts elicit sexual attraction of males.

GC-EAD analysis of extract by using male antennae showed antennal responses to two components present in the gland extract (Fig. 2). The major EAD-active peak was at 25.31 min, whereas the minor one was at 23.51 min. The flame ionization detector showed the two compounds to be present in the extract at a ratio of 100:35. GC-MS analysis of the major EAD-active peak gave identifying features for a monounsaturated C16-acetate: a strong peak at *m/z* 43 and a peak at *m/z* 222, resulting from loss of acetic acid from the molecular ion *m/z* 282 (confirmed by chemical ionization measurement). The minor constituent was presumed to be the related alcohol because the mass spectrum showed a *m/z* of 222, suggesting loss of water from the molecular ion. The molecular weight was confirmed as 240 by chemical ionization.

Double-bond positions in the two components of the *L. obliqua* pheromone extract were determined by reaction with DMDS. GC-MS analysis of the resulting mixture showed the

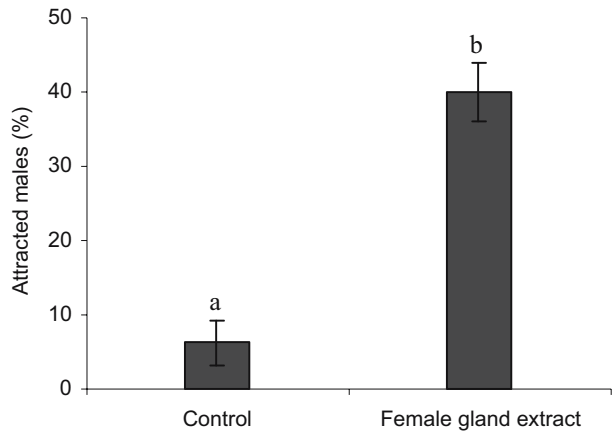


Fig. 1 Y-olfactometer test of *L. obliqua* to virgin female pheromone gland extract and a control. Different letters atop bars indicate significantly different means ($P<0.05$)

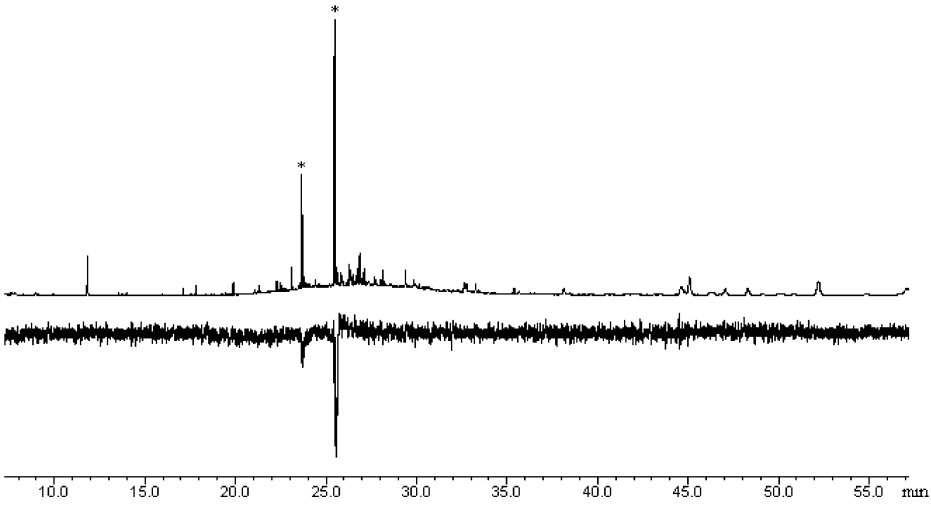


Fig. 2 Coupled gas chromatograph-electroantennogram detection (GC-EAD) analysis of female *L. obliqua* pheromone gland extract; the flame ionization detector response is above the EAD response. (*) = EAD-active peak

presence of the DMDS adduct of a Δ11-hexadecenyl acetate [*m/z* 376 (M^+) and major fragments at *m/z* 259 and 117] and of a Δ11-hexadecenol [*m/z* 334 (M+), *m/z* 217, and *m/z* 117] (Fig. 3).

To determine the geometry of the double bond of the compounds and to provide samples for laboratory and future field bioassays, we carried out a six-step synthesis of (*E*)- and (*Z*)-

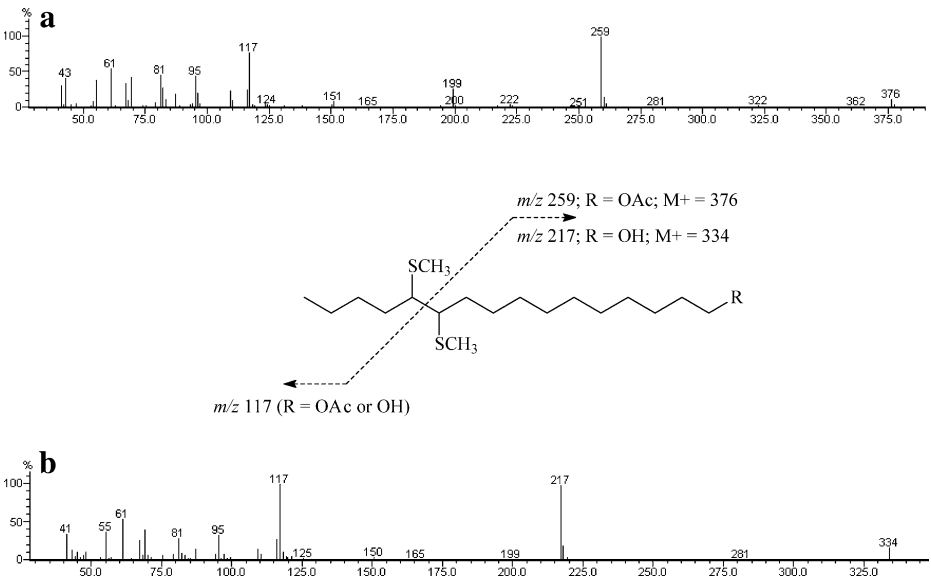


Fig. 3 Mass spectra of derivatives obtained by DMDS reaction with the two components of the pheromone gland extract of *L. obliqua*. The major product (A) and the minor (B) gave a fragmentation pattern that confirmed the location of the double bond at position 11

isomers of 11-hexadecenyl acetate (**8**) and 11-hexadecen-1-ol (**7**) based on the work of Kang and Park (1988) (see Scheme 1).

The mass spectra of synthetic alcohols **7** and acetates **8** were indistinguishable from those of the minor and major components of the natural pheromone, respectively. However, retention time data alone did not establish the geometry of the double bond because the isomers were poorly resolved on the DB-5 column. Therefore, all the synthetic isomers of **7** and **8** were converted to DMDS adducts, and the retention times of the synthetic adducts compared with those of the DMDS adducts of compounds in the pheromone extract. The addition of DMDS to double bonds for monounsaturated compounds is a stereospecific process, with the (*Z*)-isomer affording the *threo*-adduct and the (*E*)-isomer giving the *erythro*-adduct (Caserio et al., 1985). Even though adducts of regio-isomers show nearly identical EI-mass spectra, they typically have different retention times on capillary gas chromatography (Attygalle, 1998).

Almost baseline separation of (*Z*)- and (*E*)-11-hexadecenyl acetate–DMDS derivatives (34.7 and 35.1 min) was achieved by using the DB-5 column. The corresponding DMDS derivative in the pheromone extract had the same retention time as the (*E*)-isomer. DMDS adducts of (*Z*)- and (*E*)-hexadecenol were also easily resolved (32.4 and 32.8 min, respectively). The corresponding adduct in the pheromone extract had the same retention time as that of the (*E*)-isomer (Fig. 4). Based on these results, the two compounds were identified as (*E*)-11-hexadecenyl acetate (major component) and (*E*)-11-hexadecenol (minor component).

In the preliminary olfactometer trial that tested the synthetic blend of the two compounds versus a solvent blank, 60% of the males moved into the arm with the synthetic blend, whereas only 10% moved into the solvent blank arm (the remainder were nonresponders). A further preliminary olfactometer trial that tested the synthetic blend against female extract showed that 40% of the males moved into the arm with the synthetic blend and 30% into the arm with the extract. Although preliminary in nature, these experiments suggest that the two-component synthetic blend does attract male *L. obliqua*. Together, our data indicate that a mixture of (*E*)-11-hexadecenyl acetate and (*E*)-11-hexadecenol forms the sex pheromone of *L. obliqua*. This is the first report of these compounds as sex pheromone components of a saturniid moth.

In summary, we have described the periodicity of the calling behavior of *L. obliqua* females and identified their sex pheromone. Field experiments are now in progress, which aim to try to develop a program to monitor populations and prevent problems caused by this pest.

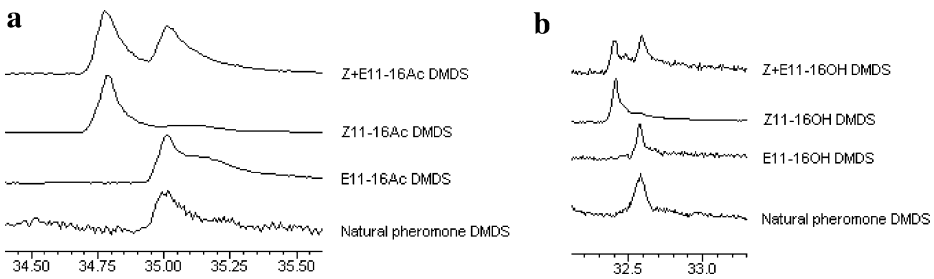


Fig. 4 Determination of the double-bond geometry of (A) 11-hexadecenyl acetate (Z+E11-16Ac) and (B) 11-hexadecenol (Z+E11-16OH) of the two sex pheromone components of *L. obliqua* using DMDS derivatization

Acknowledgments We thank the International Foundation for Science—Sweden, the Organization for the Prohibition of Chemicals Weapons—The Netherlands, CNPq, Fundação Araucária, and the Embrapa/Prodetab for the financial support.

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