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Publication date:
2004

Permanent link:
https://doi.org/10.3929/ethz-b-000422591

Originally published in:
Chemoecology 14(3-4), https://doi.org/10.1007/s00049-004-0281-5

Chemoecology14:217–223 (2004)
0937–7409/04/040217–7

DOI 10.1007/s00049-004-0281-5

CHEMOECOLOGY

Identification, synthesis and activity of sex pheromone gland components of the autumn gum moth (Lepidoptera: Geometridae), a defoliator of *Eucalyptus*

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Summary. The autumn gum moth, *Mnesampela privata* (Guenée) (Lepidoptera: Geometridae), is native to Australia and can be a pest of plantation eucalypts. Field-collected and laboratory-reared female autumn gum moths were dissected to remove glands likely to contain components of the sex pheromone. Using gas chromatography (GC) and combined gas chromatography–mass spectrometry (GC-MS), three compounds were identified from female extracts, namely (3*Z*,6*Z*,9*Z*)-3,6,9-nonadecatriene, 1-hexadecanol and 1-octadecanol (confirmed by comparison with synthetic samples). Nonadecatriene elicited an antennal response in male autumn gum moth during gas chromatographic analyses combined with electroantennographic detection (GC-EAD). In electroantennogram (EAG) recording male *M. privata* antennae responded to the nonadecatriene. Nonadecatriene was synthesised via Kolbe electrolysis, starting with (9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoic acid (linolenic acid) and propanoic acid or via an alternative four-step method also starting from linolenic acid. In field trials (3*Z*,6*Z*,9*Z*)-3,6,9-nonadecatriene proved attractive to male moths. Thus, we conclude that (3*Z*,6*Z*,9*Z*)-3,6,9-nonadecatriene is a sex pheromone component of autumn gum moth. This component has been identified in extracts from other geometrids in the same subfamily, Ennominae. However, to our knowledge this is the first example where (3*Z*,6*Z*,9*Z*)-3,6,9-nonadecatriene has been found in females and also proved attractive to male moths when presented on its own. Our results are discussed in relation to other geometrid pheromones.

Key words. (3*Z*,6*Z*,9*Z*)-3,6,9-nonadecatriene – gas chromatography-mass spectrometry – GC-EAD – EAG – synthesis

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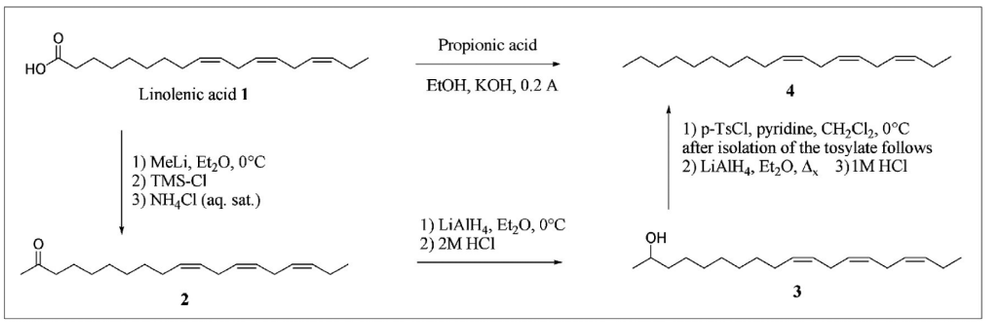
Introduction

Mnesampela privata is an endemic Australian moth whose larvae feed upon species of *Eucalyptus* (McQuillan 1985). The peak season of moth activity is typically from late February until mid April, and the larvae appear shortly after adult emergence (Elliott & Bashford 1978; Steinbauer *et al*. 2001; Steinbauer 2003).

Among the most preferred eucalypt hosts of *M. privata* are the species *E. globulus* and *E. nitens* (both are commonly referred to as bluegums). Coincidentally, these two species are among the most important species used in commercial plantations in Australia. There has been rapid, large-scale and widespread planting of these two eucalypts in south-eastern and south-western Australia over the last decade. The autumn gum moth represents a threat to this industry and has become one of the most significant outbreak insects of plantation eucalypts in south-eastern Australia (Abbott 1993; Bashford 1993; Neumann 1993; Phillips 1993; Stone 1993; Elliott *et al*. 2000). The leaves of these eucalypts are only attractive to adults while they are still juvenile (Steinbauer 2002); after 3-4 years (depending on the species and site) the foliage changes to adult leaves. Consequently, plantation bluegums only need to be protected from *M. privata* larvae for the duration of this stage of their growth.

Autumn gum moth larvae are usually controlled using aerial applications of alpha-cypermethrin (Neumann & Collett 1997). The use of a sex pheromone for monitoring moth populations could be part of a more environmentally sustainable management strategy for this insect. Presently, populations of the autumn gum moth are usually monitored using more labour-intensive methods, either by counts of "hot spots" (i.e. leaves skeletonised by feeding of first instar larvae) or by defoliation estimates of whole trees (J. Bulinski & J. Elek pers. comm.).



Schema 1 Two different synthetic routes to (3*Z*,6*Z*,9*Z*)-3,6,9-nonadecatriene ("**4**"), both starting from 9*Z*,12*Z*,15*Z*)-octadeca-5,8,11-trienoic acid (linolenic acid "**1**")

The purpose of this study was to identify the sex pheromone of autumn gum moth. This entailed chemical analyses of female glands, recording of male antennal response, and field screening with synthetic replica compounds. The need for replica compounds offered the opportunity to investigate novel methods of synthesis.

Materials and methods

Insects

In 1999 139 female pupae were collected from a plantation of *E. grandis* near Mildura, Victoria (34°17' S 142°05' E). Pupae were also reared from eggs collected at Cornelian Bay, Tasmania (42°51' S 147°18' E) in 2000 and Ginninderra Experiment Station, Australian Capital Territory (35°09' S, 149°02' E) in 1999 to 2003. Larvae were reared according to protocols in Steinbauer and Matsuki (2004).

Sexed pupae were kept at 18 °C, 24 h scotophase and ambient humidity until ommatidia and wing pads had darkened. Near eclosion pupae were placed in individual containers in a controlled temperature cabinet set to a 12 h:12 h light cycle. The temperature in this cabinet stayed within 16-19 °C.

Chemical analyses

Extract preparation: Results of analyses of pupae from different geographic locations are not reported separately. One- to two-day old virgin female moths were placed in a freezer for 4 to 5 min. The ovipositor, with as little other tissue as possible, was removed and placed in a small tube with 20-30 µL of HPLC-grade hexane. The tube was capped with aluminium foil and stored in a freezer. The extracts were refined by a mild thermal desorption technique (Whittle *et al*. 1991). The hexane extract was injected onto glass beads tightly packed in a tube, and the hexane was evaporated in a stream of nitrogen, 20 mL/min, at room temperature. The tube was placed in a small oven arranged so that a stream of helium passed through the tube at 12 mL/min. The oven was maintained at around 135 °C. The effluent from the tube was trapped in a capillary tube (150 mm x 1.6 mm) cooled with solid CO^2. Desorption was conducted for 8 to 15 min. The condensate was removed from the capillary walls in 2 µL solvent (hexane or dichloromethane) prior to analysis.

Gas chromatography of pheromone extracts: GC analyses were conducted using a Carlo Erba Model 5300 instrument. Two columns were used: BP1 (dimethylsiloxane), 25 m × 0.32 mm id phase thickness 0.25 µm; and BP10 (14 % cyanopropylphenyl polysiloxane) 25 m × 0.32 mm id, phase thickness 0.25 µm; both supplied by SGE, Melbourne. The injection was on-column. The sample was injected over approximately 1 min at 60 °C. After 6 min the temperature was raised as quickly as possible (nominally at 49.9 °C per min) to 200 °C and maintained at that temperature for the duration of the run. Retention data were collected on an integrator (HP 3392A). Retention indices were calculated from runs in which hydrocarbon standards (for BP1 17, 18, 20 and 21 and for BP10 18, 20 and 22) were co-injected and calculations done using the program INDEX.

Gas chromatography-mass spectrometry: GC-MS analyses were undertaken using a VG Trio 2000 mass spectrometer interfaced directly to a Hewlett Packard 5890 Plus gas chromatograph and PC-based MassLynx software. Electron ionisation (EI) mass spectra were obtained at an ionisation voltage of 70 eV and a source temperature of 180 °C. The GC column used was a BPX35 (bonded-phase 35 % phenyl polysilphenylene-siloxane, SGE, Australia), 30 m × 0.32 mm id, phase thickness 0.25 µm. The column was preceded by a retention gap (length 3 m), and samples were introduced using cool on-column injection. After 2 min at 40 °C, the column was programmed at 20 °C/min to 150 °C then at 10 °C/min to 300 °C, followed by an isothermal period of 10 min. Pheromone extracts and standards were dissolved in HPLC grade dichloromethane.

Syntheses

The (3*Z*,6*Z*,9*Z*)-3,6,9-nonadecatriene ("4" in Schema 1) has earlier been synthesised using different strategies (reviewed in Millar 2000). Initially, we applied Kolbe electrolysis (see Schema 1) starting from pure linolenic acid and propionic acid using MeOH as solvent (Bestmann *et al*. 1987). This method is known to result in low yields (Millar 2000; references therein) and we obtained a complex mixture of products from which it was difficult to isolate a pure product, but after altering the voltage and changing to EtOH as solvent, we obtained a more easily purified product mixture. This gave us a small amount of pure (> 98 % by GC) nonadecatriene "4" that was needed at that time. Analytical and spectroscopic data were identical with the product obtained below.

When more nonadecatriene was needed for biological purposes we developed an alternative method (as described below and in Schema 1), again starting from linolenic acid which was reacted with an excess of methyl lithium (Rubottom & Kim 1983) to produce the corresponding ketone "2" at 61 % yield. After reduction with $LiAlH_4$, the secondary alcohol "3" was obtained in near quantitative yield. The alcohol was then converted to a tosylate which, when followed by $LiAlH_4$ reduction (Kabalka *et al*. 1986), produced nonadecatriene at > 98 % purity. Specific details about each stage of the syntheses are presented below.

(10Z,13Z,16Z)-nonadeca-10,13,16-trien-2-one ("2"): The alkylation follows that described by Rubottom & Kim (1983) for other types of acids. (9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoic acid (2.83 g, 10.2 mmol) was dissolved in dry Et_2O (75 mL) and cooled to 0 ºC. Methyl lithium (1.6 M in Et_2O, 31.7 mL, 50.8 mmol) was added to this solution, and stirring was continued at 0 ºC. After 2.3 h TMS-Cl (6.45 mL, 50.8 mmol) was added to the reaction mixture, and the mixture was allowed to reach room temperature, then aqueous saturated NH_4Cl (50 mL) was added and stirring continued for another 0.5 h. The organic phase was separated, and the aqueous phase was extracted with Et_2O (3 × 50 mL). The combined ether

extracts were washed with aqueous saturated $NaHCO_3$ (50 mL), H_2O (50 mL) and dried ($MgSO_4$). Evaporation of the solvent gave 3.08 g of the product along with some tertiary alcohol (ketone:tertiary alcohol, ~3:1). Flash chromatography (EtOAc/cyclo-hexane) gave the ketone "2", a yellow oil, in 61 % yield and 99 % purity by GC (1.71 g, 6.19 mmol). ^{1}H NMR 250 Mhz (CDC_{l3}): δ 0.98 (3H, t, *J* = 7.5 Hz), 1.23-1.37 (8H, m), 1.51-1.63 (2H, m), 2.02-2.11 (4H, m), 2.14 (3H, s), 2.42 (2H, t, *J* = 7.4 Hz), 2.78-2.83 (4H, m), 5.26-5.46 (6H, m). ^{13}C NMR 62.9 MHz (CDC_{l3}): δ 14.3, 20.6, 23.8, 25.5, 25.6, 27.2, 29.1 (2C), 29.3, 29.6, 29.9, 43.8, 127.1, 127.7, 128.3 (2C), 130.3, 132.0, 209.4. MS (CI) *m/z* (relative intensity): 277 (MH^+, 46 %), 276 (M^+, 6), 189 (42), 177 (100), 163 (93), 149 (58), 135 (63), 123 (90), 109 (92), 95 (83), 81 (63). *Anal. Calcd. For $C_{19}H_{32}O$: C, 82.6; H, 11.4. Found: C, 82.4; H, 11.5.*

(10Z,13Z,16Z)-nonadeca-10,13,16-trien-2-ol ("3"): The ketone "2" (1.64 g, 5.94 mmol) was dissolved in dry Et_2O (25 mL) and cooled to 0 ºC, followed by addition of $LiAlH_4$ (225 mg, 5.94 mmol). After 1.5 h the reaction was quenched with aqueous 2 M HCl. The organic phase was separated and the aqueous phase was extracted with Et_2O (5 × 25 mL). The combined ether extracts were washed with brine (50 mL), H_2O (50 mL) and dried ($MgSO_4$). Evaporation of solvent gave 1.58 g of the title compound, which after flash chromatography (Et_2O/pentane) gave the alcohol "3", a yellow oil, in 93 % yield and 99.5 % purity by GC (1.53 g, 5.50 mmol). ^{1}H NMR 250 MHz ($CDCl_3$): δ 0.98 (3H, t, *J* = 7.5 Hz), 1.19 (3H, d, *J* = 6.2 Hz), 1.23-1.42 (13H, m), 2.02-2.14 (4H, m), 2.75-2.87 (4H, m), 3.73-3.85 (1H, m), 5.26-5.46 (6H, m). ^{13}C NMR 62.9 MHz ($CDCl_3$): δ 14.3, 20.6, 23.5, 25.5, 25.6, 25.8, 27.2, 29.2, 29.5, 29.6 (2C), 39.4, 68.2, 127.1, 127.7, 128.3 (2C), 130.3, 132.0. MS (CI) *m/z* (relative intensity): 279 (MH^+, 21 %), 278 (M^+, 4), 261 (MH^+–H_2O, 55), 191 (42), 177 (50), 163 (60), 149 (66), 135 (63), 123 (75), 109 (100), 95 (75). *Anal. Calcd. For $C_{19}H_{34}O$: C, 82.0; H, 12.3. Found: C, 81.6; H, 11.9.*

(3Z,6Z,9Z)-nonadeca-3,6,9-triene("4"): The tosylation followed that described by Kabalka *et al*. (1986) for other alcohols. Alcohol "3" (1.45 g, 5.20 mmol) was dissolved in dry CH_2Cl_2 (10 mL) and cooled to 0 ºC, followed by addition of dry pyridine (1.26 mL, 15.6 mmol) and p-toluenesulphonyl chloride (1.98 g, 10.4 mmol). After stirring at 0 ºC for 44 h, Et_2O (40 mL) and H_2O (10 mL) was added to the reaction mixture and the aqueous phase was removed. The organic phase was washed with aqueous 2 M HCl (10 mL), aqueous saturated $NaHCO_3$ (10 mL), H_2O (10 mL) and dried ($MgSO_4$). After evaporation of the solvent the crude product was purified by flash chromatography (EtOAc/cyclohexane), which yielded 1.83 g (approx. 88 % purity by GC) of the tosylate.

The crude tosylate (1.83 g) was dissolved in dry Et_2O and $LiAlH_4$ (262 mg, 6.87 mmol) was added. After refluxing for 8 h, the reaction mixture was cooled to 0 ºC and quenched with aqueous 1 M HCl. The organic phase was separated and the aqueous phase was extracted with Et_2O (3 × 40 mL). The combined ether extracts were washed with aqueous saturated $NaHCO_3$ (75 mL), brine (75 mL), H_2O (75 ml) and dried ($MgSO_4$). Evaporation of the solvent gave a yellow oil, which was purified by flash chromatography using ~10 wt. % $AgNO_3$ on silica gel (Et_2O/pentane). The triene was obtained as an oil in 32 % yield from the alcohol and with 98 % purity by GC (435 mg, 1.66 mmol). ^{1}H NMR data were similar to those in Bestmann *et al*. (1987). ^{13}C NMR 62.9 MHz (CDC_{l3}): δ 14.1, 14.3, 20.5, 22.7, 25.5, 25.6, 27.2, 29.3 (2C), 29.5, 29.6, 29.7, 31.9, 127.1, 127.6, 128.2, 128.3, 130.4, 131.9. MS (EI) data were identical to those in Millar (2000).

GC-EAD and EAG studies

Combined pheromone gland extracts (3 females, 6 µL) were injected on-column at 40 °C (1 min) into a gas chromatograph (Varian 3400) followed by programming to 300 °C at a rate of 10 °C per min. The GC was equipped with an EC-1 column (dimethylpolysiloxane), 30 m × 0.32 mm id, phase thickness 0.25 µm. Helium was used as a carrier gas. A GC effluent splitter (SGE, Australia; split ratio 1:1) was used, and the outlet was added to a purified and humidified air stream, directed over the excised antenna of a male moth. The tip of the antenna was cut off and the antenna mounted on an antennal holder using electrode gel. The electrode holding the base of the antenna was grounded. The distal end of the antenna was connected via an interface box (Syntech, Hilversum, the Netherlands) to a PC. EAD signals and flame ionization detector (FID) responses were simultaneously recorded. Another two GC-EAD runs were conducted each with 2 µL of sample (1 female equivalent) extracted from individual females.

A GC-EAD active compound, namely (3*Z*,6*Z*,9*Z*)-3,6,9-nonadecatriene (following identification from the GC and GC-MS data), was validated by co-injection of the synthetic analogue.

For EAG recordings male antennae were prepared as described above for the GC-EAD recordings, and the same type of interface was used. The cut antennae (including the tip) were placed between two electrodes that were made of thin glass capillaries filled with Ringer solution. The antennae were continuously exposed to charcoal-filtered and moistened air with a flow of 0.5 m/s. Stimuli were injected into the air stream through an opening in the glass tube 20 cm upstream from the antennae, with the outlet 1 cm from the antennae. Stimuli were administered by a device (Murphy Developments, Hilversum, the Netherlands) that delivered a 0.5 s puff of air at a flow rate of 5 mL/s. Our "standard stimulus" was prepared by dissolving hexane only onto a piece of filter paper placed in a Pasteur pipette and delivering air from this using the above mentioned device to the antennae. Prior to the experiments the solvent was allowed to evaporate. Synthetic samples, dissolved in hexane, were administered in the same way. An EAG recording comprised the sequential delivery of a standard stimulus, two synthetic samples and a standard stimulus. EAG recordings were analysed using 'EAG version 2.2a' (Syntech, The Netherlands 1993).

Field trials

The (3*Z*,6*Z*,9*Z*)-3,6,9-nonadecatriene used in field trials during 2002 and 2003 was synthesised by AN, FA and EH at Mid Sweden University, Sweden. The 1-hexadecanol (97 % purity) and 1 octadecanol (98 % purity) were recrystallised commercial samples.

From early February to mid April 2002, two field sites within the ACT were used. Lyneham Ridge (35°14' S 149°07' E) is a 0.27 ha *E. globulus* plantation established in 1994. The trees were felled in 1999 to induce coppice. Ginninderra is a 0.69 ha plantation established in 1998 that comprises *E. globulus* and *E. nitens* as well as other species of *Eucalyptus.* All the *E. globulus* and *E. nitens* in these two plantations were in juvenile foliage at the time of the field tests.

Unitraps (Phero Bank, the Netherlands) filled with soapy water were hung from purpose-built poles approximately 1.4 m above the ground and spaced at least 10-15 m from one another. As dispensers, 4 cm lengths of medical rubber tubing of approximately 4 mm id (Graham Rubber, Granville, USA) were used. The synthetic triene and alcohols were dissolved in hexane, the solution allowed to soak into the rubber tubing and the solvent allowed to evaporate. Virgin females used in the field tests were placed in the same lure holder (a small basket with a cap) as the rubber septa. On some occasions, instead of emerged females, single pupae (with the moth close to emergence) were placed in the trap.

In 2003, aluminium foil was wrapped around the rubber septa to protect the pheromone components from degradation through exposure to the sun. Elsewise, dispensers were prepared as in 2002. In 2003 we also used blank (unbaited) traps on some occasions. Unitraps hung from poles of the same height were either filled with soapy water, 40 % ethanol or the lower half of the trap was sprayed with an insecticide (Baygon, Bayer, Pymble NSW). All traps were placed at least 25-30 m from each other and their position was shifted when > 10 moths had been caught in the best trap within each set-up.

Apart from the two plantations in the ACT, we also used a plantation of *E. grandis* in Victoria where an outbreak of *M. privata* was in progress during 2003. This gave us a chance to test the lures at a high population density site. This 1.4 ha plantation was established in 2001 and approximately 30 % of the trees' foliage were juvenile. The baits were prepared as in 2002, and the Unitraps were hung from poles at the same height. Catches were analysed with Kruskal-Wallis ranking tests, followed by Mann-Whitney tests for pair-wise comparisons.

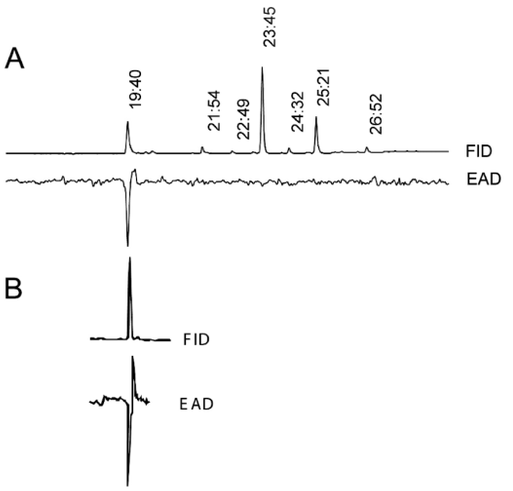


Fig. 1 Gas chromatographic analyses (FID) of (**A**) a female *M. privata* pheromone gland extract and (**B**) synthetic (3*Z*,6*Z*,9*Z*)-3,6,9-nonadecatriene with electroantennographic detection (EAD) using male antennae (note, **B** is aligned to time interval given for corresponding peak in **A**). One electrophysiologically-active component of the female extract eluted, in this instance, at a retention time of 19.40 min. Another 6 compounds (mainly straight hydrocarbons, C_{21}-C_{27}) in the extract shown in **A** did not elicit an electrophysiological response in the male antenna

Results

Identification of electrophysiologically-active pheromone components

Using GC-EAD we observed a strong response of male *M. privata* antennae to the peak eluting from the GC column at a retention time of 19.40 min (Fig. 1A). None of the other peaks in the chromatogram were found to elicit an EAD reaction. The identities of the principal components of the extracts from *M. privata* females were established because they coincided precisely with the synthetic analogues of (3*Z*,6*Z*,9*Z*)-3,6,9-nonadecatriene, 1-hexadecanol and 1-octadecanol, both in their EI mass spectra and their GC retention times on columns of low and high polarities. The other components in significant abundance were identified as a homologous series of saturated hydrocarbons. The symmetrical GC peak shape for the natural triene was maintained throughout the range of phase polarities, indicating that other stereo- or regio-isomers of the (3*Z*,6*Z*,9*Z*)-triene were not present in any significant amounts. In addition, there was no detectable amount evident of an analogous heptadecatriene. The antennae of male moths responded both to the natural component and to synthetic (3*Z*,6*Z*,9*Z*)-3,6,9-nonadecatriene in GC-EAD runs (Fig. 1B).

Selected monitoring of diagnostic ions in their EI mass spectra (*m/z* 252.2, 224.2, 196.2, 125.1, 111.1, 97.1, 83.1) demonstrated that the relative proportion of (3*Z*,6*Z*,9*Z*)-3,6,9-nonadecatriene, 1-hexadecanol and 1-octadecanol fluctuated markedly between individual female extracts. That is, the ratios of (3*Z*,6*Z*,9*Z*)-3,6,9-nonadecatriene, 1-hexadecanol and 1-octadecanol fluctuated markedly between individuals (This variation in composition may be due to the age of the moths, whether or not they had begun release of pheromone

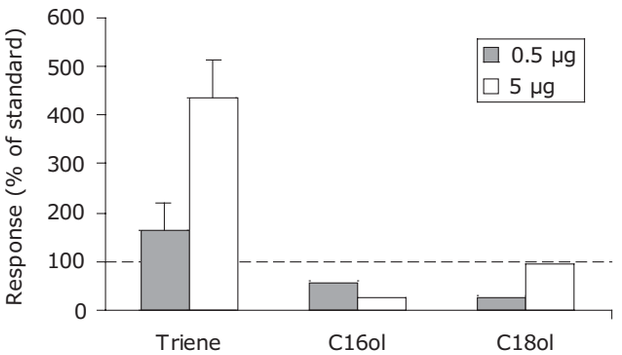


Fig. 2 Results of EAG recordings of male *M. privata* antennae to different compounds found in female extracts. Response (± standard deviation) is expressed as a % of the "standard stimulus" (see text). "triene" is short for (3*Z*,6*Z*,9*Z*)-3,6,9-nonadecatriene and "C_{16}ol" and "C_{18}ol" are short for 1-hexadecanol and 1-octadecanol, respectively. In all cases $n = 2$ except for "C_{16}ol" and "C_{18}ol" where $n = 1$

and/or their geographic origins). Typically, the triene was found by GC and GC-MS in all female extracts whereas either or both of the alcohols were not always detected in the extracts. The amount of triene in individual females varied up to approximately 110 ng ($n = 19$) (as judged by the size of the peaks on the chromatograms).

The alcohols and the triene were also examined for activity in male antennae during electroantennogram recordings (EAG). The triene again elicited a response, whereas neither of the alcohols elicited an antennal response different from that of a blank stimulus (Fig. 2).

Field trials

In the low-density areas (i.e. Lyneham Ridge and Ginninderra, ACT), the traps baited with virgin females caught some males, although the catch of individual females varied considerably (Table 1). The majority of virgin females (8 of 12) caught none or only one male during their time in the trap. With the exception of one female, which attracted 48 males over three consecutive nights, virgin females did not catch more males than did the traps baited with the synthetic (3*Z*,6*Z*,9*Z*)-3,6,9-nonadecatriene. However, a female only lived for 3 to 4 days in a Unitrap whereas the synthetic baits caught males for 7 or 8 weeks. Although the traps with synthetic triene caught more autumn gum moth males than unbaited traps (up to a total of 4 males compared with 0 in the blank traps), the catches in the low-density areas were too small to allow for any statistical comparisons.

In the high population density area the synthetic (3*Z*,6*Z*,9*Z*)-3,6,9-nonadecatriene proved attractive to autumn gum moth males. The traps baited with the triene caught significantly more moths than unbaited traps (Table 2). We only tested virgin females in the high-density area during one night (data not shown), and the only female that emerged caught one male during the first night. The next morning she was dead.

There was no indication in these field trials that either of the alcohols worked synergistically with the triene. That is, the traps with the triene and either of the alcohols did not catch more males than did the triene by itself. Late in the

Table 1 Catches of male *Mnesampela privata* attracted by individual virgin females caged in Unitraps in the ACT from February until April 2002. Catches are totals for the 3 to 4 days that the females remained alive in Unitraps

Females	Number of males caught
Female 1	48
Female 2	4
Female 3	3
Female 4	2
Females 5, 6 & 7	1
Females 8, 9, 10, 11 & 12	0

season of 2003 we also tested other ratios of the alcohols + triene, but again there was no indication that any of the alcohols worked synergistically.

The (3*Z*,6*Z*,9*Z*)-3,6,9-nonadecatriene also attracted individuals of three closely related species (all belonging to the same subfamily). In the ACT we caught approximately 9 *Dolabrossa amblopa* Guest and 1 *Mnesampela heliochrysa* (Lower) and in Victoria we caught 3 *M. arida* McQuillan.

Discussion

Through GC and GC-MS analyses, together with GC-EAD and EAG recordings and field screening, we conclude that (3*Z*,6*Z*,9*Z*)-3,6,9-nonadecatriene is a sex pheromone component of *M. privata*. Female pheromone gland extracts also contained two alcohols, namely 1-hexadecanol and 1-octadecanol, but these substances never proved attractive to the males. Both alcohols have previously been recorded in other female moths. 1-hexadecanol has been recorded in females from six different families of Lepidoptera, whereas 1-octadecanol has been found in three families (El-Sayed 2004; Witzgall *et al*. 2004). To our knowledge this is the first time that they have been recorded in the sex pheromone gland of a geometrid.

(3*Z*,6*Z*,9*Z*)-3,6,9-nonadecatriene has been recorded as a sex pheromone component in other geometrids (and in other moths, e.g. noctuids) (Millar 2000; El-Sayed 2004; Witzgall *et al*. 2004). To our knowledge this is the first time that it has been identified in female moths and *also* proved attractive to males in field when tested on its own. Earlier records have found (3*Z*,6*Z*,9*Z*)-3,6,9-nonadecatriene to work synergistically or to be a pheromone precursor. Trap catches of both *Alsophila pometaria* Harris in Canada (Wong *et al*. 1984) and *Peribatodes rhomboidaria* Denis & Schiffermüller in Hungary and Switzerland (Tóth *et al*. 1987) increased significantly when the triene was added to two tetraenes and one ketone (the main pheromone components), respectively. Similarly, in field trapping in Hungary for three other geometrids (*Colotois pennaria* Linnaeus, *Erannis defoliaria* Clerck and *Agriopis marginaria* Fabricius), adding the triene to the main component produced a more attractive lure than the main component alone (Szöcs *et al*. 1993). Field catches of *Biston robustum* Butler in Japan increased when triene was added to a three component blend of diene + two epoxides (Yamamoto *et al*. 2000), but the catches were low and no statistical analyses were performed.

Table 2 Catches of male *Mnesampela privata* in traps baited with synthetic (3*Z*,6*Z*,9*Z*)-3,6,9-nonadecatriene ("triene") in Mildura from late January until June, 2003. Totals in the same column with different letters differ significantly ($P < 0.05$) from each other following Kruskal-Wallis tests (followed by Mann-Whitney U-tests)

Lure composition	28 January to 30 March ($n = 7$)	31 March to 11 June ($n = 7$)	31 March to 11 June ($n = 7$)
Unbaited Unitrap (blank)	–*	7^{A}	7^{A}
500 µg triene	11^{A}	92^{B}	–
1000 µg triene	13^{A}	94^{B}	59^{B}

*no blank was used during this period

In closely related geometrids, namely *Boarmia (Ascotis) selenaria* Schiffermüller and *Ascotis selenaria cretacea* Butler, (3*Z*,6*Z*,9*Z*)-3,6,9-nonadecatriene has been identified from females but no effect has been found in males in field (Ando *et al*. 1997) or wind tunnel + field studies (Cossé *et al*. 1992). It has instead been suggested that the triene acts as a biochemical precursor to the pheromone epoxynonadecadiene (Cossé *et al*. 1992; Ando *et al*. 1997). In yet other species of geometrids and noctuids, (3*Z*,6*Z*,9*Z*)-3,6,9-nonadecatriene has proved attractive to males in field screening, but no analyses of female extracts were done (e.g. Szöcs *et al*. 1984; Subchev *et al*. 1986; Millar *et al*. 1990; 1992; Witjaksano *et al*. 1999). Whether the triene is also a component of the sex pheromones of *M. arida*, *M. heliochrysa* and *D. amblopa,* which we caught in traps containing (3*Z*,6*Z*,9*Z*)-3,6,9-nonadecatriene, also remains to be determined. When employing the screening method for pheromone "identification" some bias is bound to arise based on the choice of compounds tested. Proof of a sex attractant need not mean that the particular compound is used as a sex pheromone (Millar 2000).

It is tempting to speculate that the autumn gum moth probably uses more compounds in its sex pheromone, even though only one EAD-active component was detected. Our field catches were quite poor. Even in the outbreak population, where a light trap regularly caught at least the same number of moths as did our best pheromone trap, pheromone trap catches were low. The findings from some 35 species of geometrid suggest that the triene acts either as a synergist or as the main component (El-Sayed 2004; Witzgall *et al*. 2004). Consequently, co-occurring moth species must require additional pheromone components to be able to find individuals of the opposite sex. The autumn gum moth is the only native Australian geometrid that has so far been shown to use the triene as a component of its sex pheromone. In fact, (3*Z*,6*Z*,9*Z*)-3,6,9-nonadecatriene is probably the first identified sex pheromone component of a native Australian geometrid.

There are 3 possible reasons why additional EAD-active components were not detected in our analyses. Firstly, the peak at retention time 19.40 min (Fig. 1A) may not be homogeneous and a second isomer of (3*Z*,6*Z*,9*Z*)-3,6,9-nonadecatriene may be superimposed. However, such an isomer, coinciding precisely with the (3*Z*,6*Z*,9*Z*)-standard

over a range of phase polarities, has not been recorded in the literature. Secondly, the additional component may be too fragile to survive the refinement process or the GC conditions. However, such a chemical would not be typical of other lepidopteran pheromone structures. Thirdly, the additional component (or components) may be formed enzymatically from the triene or the alcohols on the surface of the gland before release as effluvium and therefore would be barely represented in solvent extracts. We consider this possibility to be the most likely alternative and that the trapped effluvium could reveal its identity by GC-MS and GC-EAD. However, *M. privata* females were loathe to display calling behaviour under laboratory conditions and therefore it was not possible to entrap the effluvium, e.g. within capillary tubes (Lacey & Saunders 1992).

As for using pheromone traps to monitor populations of autumn gum moth, this study has brought us much closer to an alternative to light trapping. However, additional studies are needed to improve our knowledge of the sex pheromone of this moth and its efficacy in mate location. We have only begun the process of developing a sex pheromone-based monitoring system for *M. privata*. We are continuing our collaborative research to address outstanding issues.

Acknowledgments

We thank Testu Ando, Ken Dickson, Peter Ebner, Ted Edwards, William Foley, Wittko Francke, Peter McQuillan, Michelle Michie, Rod Peakall, Luke Rapley and Rob Rhind for their advice and assistance. FÖ and MJS would particularly like to thank the CF Lundströms Stiftelse and Hellmuth Hertz Foundation (financial support) as well as the Swedish and Australian Embassies because without their combined assistance FÖ would not have been able to come to Canberra to collaborate with MJS and his Australian colleagues on this project. August 2002 to August 2003 was a year of good research, great company and enjoyable cultural exchanges.

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Received 19 March 2004; accepted 9 June 2004.