Female Sex Pheromone of the Cone Moth, *Dioryctria mendacella*: Investigation of Synergism between Type I and Type II Pheromone Components

David R Hall • Dudley Farman
Natural Resources Institute, University of Greenwich, Chatham Maritime, Kent ME4 4TB UK

Juan C. Domínguez
Centro de Sanidad Forestal, Junta de Castilla y León, Polígono industrial de Villamuriel de Cerrato s/n, 34190 Palencia, Spain

Juan A Pajares •
Sustainable Forest Management Research Institute, University of Valladolid CIFOR INIA, Av. Madrid 44, 34004 Palencia, Spain

Author for correspondence: David R Hall, d.r.hall@gre.ac.uk

Abstract Polyunsaturated hydrocarbons (Type II pheromone components) have been reported to be synergists for unsaturated acetates, alcohols or aldehydes (Type I components) in the sex pheromones of several species of Lepidoptera. However, there is some debate over whether the active components are the hydrocarbons themselves or more volatile degradation products. Extracts of pheromone glands of adult females of the cone moth, *Dioryctria mendacella* (Lepidoptera: Pyralidae), contain (*Z,E*)-9,11-tetradecadienyl acetate (ZE9,11-14:Ac) and at least ten times as much (*Z,Z,Z,Z,Z*)-3,6,9,12,15-pentacosapentaene (ZZZZZ3,6,9,12,15-25:H). The former elicits a strong electroantennogram response from males while no response could be recorded to the latter. In field trapping tests, both compounds were individually unattractive to male *D. mendacella* moths, but blends of the two compounds containing at least a 10:1 ratio of ZZZZZ3,6,9,12,15-25:H : ZE9,11-14:Ac were highly attractive. The relatively involatile hydrocarbon was shown to be released from the dispensers used and no significant degradation could be detected. Furthermore, blends of ZE9,11-14:Ac and analogs of ZZZZZ3,6,9,12,15-25:H with fewer carbons and/or double bonds that might be expected to produce similar degradation products to ZZZZZ3,6,9,12,15-25:H were unattractive. This indicated a specific response to the

hydrocarbon itself, further substantiated by the observation that related hydrocarbons did not interfere with the activity of ZZZZZ3,6,9,12,15-25:H. Thus a three-step conversion of fish oil was used to produce a blend of unsaturated hydrocarbons containing ZZZZZ3,6,9,12,15-25:H as the major component, albeit only 30% of the total, and a blend of this material with ZE9,11-14:Ac was as attractive to male *D. mendacella* moths as blends with an equivalent amount of the purified material. This mixture of unsaturated hydrocarbons is much cheaper to produce than the pure pentaene, and may be useful in lures for other species using these compounds. *Dioryctria mendacella* is a major constraint to production of edible pine kernels throughout the Mediterranean region. Pheromone traps will provide a means to improve monitoring of seasonal flight patterns and changes in population abundance of this pest.

Key Words: Lepidoptera, Pyralidae, trapping, (*Z,E*)-9,11-tetradecadienyl acetate, (*Z,Z,Z,Z,Z*)-3,6,9,12,15-pentacosapentaene, *Pinus pinea*

Introduction

The cone moth, *Dioryctria mendacella* (Staudinger, 1859) (Lepidoptera: Pyralidae: Phycitinae), attacks the cones of several pine species, such as *Pinus pinea*, *P. halepensis*, *P. brutia* and *P. pinaster*, around the Mediterranean region (Karsholt and van Nieukerken 2013; Knölke 2007). In particular, this pest is a major constraint on production of pine nuts, the edible kernel of the Mediterranean stone pine, *P. pinea*. Larvae bore galleries into the cones of all ages, reducing cone production and yield, e. g. up to 80 % loss of marketable nuts cones in Italy (Innocenti and Tiberi 2002) or between 20% and 56% in Spain (Gordo et al. 1997; Mutke et al. 2013). The pest may also reduce tree reproductive success, impacting on the quality of seed supply for regeneration and reforestation, and affecting abundance, distribution and dynamics of tree populations (Boivin and Auger-Rozenberg 2016).

The cryptic feeding behavior of this species makes study of its biology and ecology difficult, and little can be done to protect pine cones from this pest at present. The level of infestation varies markedly from year to year and from site to site (e.g. Bracalini et al. 2013) and the reasons for this, beyond the spatial and temporal variability of fruiting structures, are not well

understood. Pheromone traps are efficient monitoring tools that could greatly help improve knowledge of the biology and population dynamics of this pest, necessary for a sound integrated pest management that includes silvicultural, mechanical and biological methods

Female-produced sex pheromones have been identified for ten *Dioryctria* species (El-Sayed 2016). These comprise unsaturated alcohols, acetates and aldehydes, typical Type I pheromone components (Ando et al. 2004). However, in three species the attractiveness of the Type I component is strongly synergized by (*Z,Z,Z,Z,Z*)-3,6,9,12,15-pentacosapentaene, a Type II pheromone component (Ando et al. 2004). These are the European species *D. abietella* Denis and Schiffermüller (Löfstedt et al. 2012) and the American species *D. abietivorella* Grote (Millar et al. 2005; Strong et al. 2008) and *D. amatella* Hulst (Miller et al. 2010).

Here we describe identification of a similar blend of Type I and Type II components in the female-produced sex pheromone of *D. mendacella,* and further investigation of the role of the Type II component. We also report use of a cheaper substitute for the pure Type II component that may be useful in other species using such blends.

Methods and Materials

Insect Material *Dioryctria mendacella* were collected as late-instar larvae from stone pine cones in the province of Valladolid (Castile and León, Spain) and allowed to pupate within a plastic box (40 cm x 30 cm x 30 cm) containing a 4 cm deep layer of sand. Pupae were separated by sex according to the presence or absence of a genital slot characteristic of the female, and sent to UK. There they were maintained in individual plastic pots (30 mm high x 40 mm diameter; Talon Direct, London, UK) on a reversed light/dark cycle (12:12 h L:D) with temperatures at 25 °C and 20 °C respectively. The sex of eclosed adults was confirmed by examining the abdominal tip for brushes in the male and a genital slot in the female.

Pheromone Collection Pheromone was extracted from batches of 1-3 virgin female moths aged 2-4 d after emergence and at 2 h (N = 2), 3 h (N = 2), 4 h (N = 6) or 6 h (N = 2) into the dark cycle. Moths were lightly anaesthetized with carbon dioxide and the abdomen squeezed gently to extrude the ovipositor which was excised with dissection scissors directly into hexane (10 µl/female; Distol-Pesticide Residue Grade; Fisher Scientific, Loughborough, Leicestershire, UK) in a conical

vial (1.1 ml; Chromacol, Welwyn Garden City, Herts., UK). After 15 min the hexane was transferred with a microsyringe to a clean vial and the abdominal tips were extracted with another aliquot of hexane (10 µl) which was also transferred to the second vial. Extracts were stored at -20°C until analysis.

For collection of volatiles, virgin female moths (1 d old) were housed individually in silanized glass vessels (12 cm x 4 cm) with a glass frit at the upwind end. Air (2 l/min) was drawn into the vessel through an activated charcoal filter (20 cm x 2 cm, 10-18 mesh; Fisher Scientific) and out through a collection filter consisting of a Pasteur pipette (4 mm i.d.) containing Porapak Q (200 mg, 50/80 mesh; Supelco, Gillingham, Dorset, UK) held between plugs of silanized glass wool. The Porapak Q was extracted with chloroform for 8 h in a Soxhlet apparatus and washed with dichloromethane (Distol Pesticide Residue Grade, Fisher Scientific) immediately before use. Volatiles were collected during the dark period and then desorbed from the Porapak with dichloromethane (1 ml) and stored at -20 °C until analysis (N = 4).

Analyses by Gas Chromatography coupled to Mass Spectrometry (GC-MS) Analyses were performed on a CP-3800 GC coupled directly to a Saturn 2200 MS (Varian, now Agilent, Cheadle, UK) using fused silica capillary GC columns (30 m x 0.25 mm i.d. x 0.25 µ film thickness) coated with non-polar VF5 (Varian) or polar DBWax (Supelco). Carrier gas was helium (1 ml/min) and the oven temperature was held at 40 ºC for 2 min then programmed at 10 ºC/min to 250 ºC and held for 5 min. The NIST/NIH/EPA Mass Spectral Library v2.0d (2005) supplied and a custom-built library were used for initial identifications.

Analyses by Gas Chromatography with Flame Ionization Detection (GC-FID) Analyses were carried out on a HP6850 GC (Agilent) with GC columns (30 m x 0.32 mm i.d. x 0.25 µ film) coated with non-polar HP5 (Agilent) or polar DB Wax (Supelco) with helium carrier gas (2.4 ml/min), splitless injection (220°C), and flame ionization detection (FID) (250°C). The oven temperature was held at 50 °C for 2 min, then programmed at 10°C/min to 250°C and held for 5 min.

Later analyses of synthetic compounds were carried out on the HP5 column with oven temperature held at 60 °C for 2 min then programmed at 10 °C/min to 300 °C as this gave more reproducible quantification of the unsaturated hydrocarbons.

Analyses by Gas Chromatography coupled to Electroantennographic (GC-EAG) Recording

For GC-EAG analyses, a HP6890 instrument (Agilent) was fitted with fused silica capillary columns (30 m x 0.32 mm i.d. x 0.25 µ film) coated with non-polar SPB1 (Supelco) and polar DBWax (Supelco). The ends of the two columns were connected to a short piece of deactivated fused silica tubing with a glass, push-fit Y-piece (Supelco). The effluent from this was then split by means of a similar Y-piece with half going to the flame ionization detector and half to a silanized, glass T-piece (arms 5 cm, i.d. 4 mm), using similar lengths of deactivated fused silica tubing. One arm of the T-piece was connected to a device delivering air (200 ml/min) in a 3-sec pulse at 17-sec intervals. The third arm of the T-piece passed through the GC oven wall to the insect EAG preparation (Cork et al. 1990). In this way, the GC column effluent was accumulated in the glass T-piece during 17 sec before being blown over the EAG preparation in a single pulse.

EAG recording was carried out with a portable device (INR-02; Syntech, Hilversum, The Netherlands, now Kirchzarten, Germany) consisting of integrated electrode holders, micromanipulators, and amplifier. Electrodes were silver wires fitted into glass electrodes pulled to a fine point with an electrode puller and containing saline solution (0.1 M potassium chloride with 1% polyvinylpyrrolidone to reduce evaporation). A male *D. mendacella* moth (0-2 d old) was lightly anesthetized with carbon dioxide and one antenna was excised at the base and suspended between the glass electrodes, which were cut so that they just accommodated the ends of the antenna. The signal was amplified x 10 and the amplifier was connected to the GC as a detector device. Data were processed with EZChrom Elite v3.0 (Agilent).

Synthesis

(*Z*,*E*)-9,11-Tetradecadienyl acetate (ZE9,11-14:Ac) This compound was synthesized as described by Hall et al. (1975). The resulting mixture of *Z*,*E* and *E*,*E* isomers (90:10) was treated with tetracyanoethylene in dichloromethane for 24 h at room temperature to react selectively with the *E*,*E* isomer. The reaction mixture was chromatographed on silica gel with 2% diethyl ether in petroleum spirit. The product was distilled in a kugelrohr oven at 150 °C and 0.02 mm Hg and had an isomeric composition by GC analysis on a polar column of ZE : EZ : ZZ : EE 98.4 : 0.3 : 0.6 : 0.7.

(*Z*,*Z*,*Z*,*Z*,*Z*)-3,6,9,12,15-Pentacosapentaene (ZZZZZ3,6,9,12,15-25:H) Syntheses of unsaturated hydrocarbons are summarized in Fig. 1 and described in detail in the Supplementary Material.

In initial work (Fig. 1), fish liver oil (Super EPA Fish Oil Concentrate, Holland and Barrett, Nuneaton, Warwickshire, UK) was dissolved in methanol with a catalytic amount of boron trifluoride etherate in ether and stirred for 6 d at room temperature. GC-MS analysis of the resulting mixture of methyl esters indicated that the single most abundant component (approx 34% of total) was methyl (*Z*,*Z*,*Z*,*Z*,*Z*)-5,8,11,14,17-eicosapentaenoate, with the second most abundant component methyl (*Z*,*Z*,*Z*,*Z*,*Z*,*Z*)-4,7,10,13,16,19-docosahexaenoate (20%). Other components were mainly saturated and unsaturated 18-, 20- and 22-carbon esters.

The mixture of methyl esters was chromatographed on silica gel impregnated with 10% silver nitrate (230 mesh; prepared in our laboratory or from SigmaAldrich, Gillingham, Dorset, UK) eluted with a gradient of increasing concentrations of diethyl ether in petroleum spirit (b.p. 40-60 °C) to give methyl (*Z*,*Z*,*Z*,*Z*,*Z*)-5,8,11,14,17-eicosapentaenoate in 87% purity with 5% and 8% respectively of the 21- and 22-carbon homologues.

The purified methyl (*Z*,*Z*,*Z*,*Z*,*Z*)-5,8,11,14,17-eicosapentaenoate was reduced to the corresponding alcohol with lithium aluminum hydride in diethyl ether. The alcohol was dissolved in dichloromethane containing pyridine and reacted with trifluromethanesulfonic anhydride at -30 °C. After removal of solvents, the residue was dissolved in tetrahydrofuran with a catalytic amount of lithium tetrachlorocuprate and reacted with pentylmagnesium bromide at -60 °C as described by Wang and Zhang (2007). After aqueous work-up, the reaction product was chromatographed on silica gel to give (*Z*,*Z*,*Z*,*Z*,*Z*)-3,6,9,12,15-25:H in 50% overall yield from the methyl ester with similar 87% purity (Fig. 2). The main component had GC retention times on polar and non-polar columns and a mass spectrum identical to those of a sample provided previously by Prof Jocelyn Millar (UC Riverside, CA). Spectral data are given in the Supplementary Material.

Subsequently it was shown that the fish oil could be reduced with lithium aluminum hydride in ether to give the mixture of alcohols directly. This could be chromatographed on silica gel impregnated with silver nitrate to isolate (*Z*,*Z*,*Z*,*Z*,*Z*)-5,8,11,14,17-eicosapentaen-1-ol which could be processed as above.

As an alternative route to the mixture of unsaturated hydrocarbons more suited to larger scale production, the mixture of alcohols was reacted with *p*-toluenesulfonyl chloride in diethyl ether in the presence of powdered sodium hydroxide to give a mixture of the corresponding tosylates. This was dissolved in tetrahydrofuran containing a catalytic amount of lithium tetrachlorocuprate and reacted with pentylmagnesium bromide in ether at -60°C. The mixture of hydrocarbons was obtained in 90% overall yield from the alcohols and the (*Z,Z,Z,Z,Z*)-3,6,9,12,15-25:H was the major component at approximately 34% of the total (Fig. 2).

(*Z,Z,Z,Z,Z*)-3,6,9,12,15-Tricosapentaene (ZZZZZ3,6,9,12,15-23:H) This compound was prepared from purified (*Z,Z,Z,Z,Z*)-5,8,11,14,17-eicosapentaen-1-ol via the tosylate and reaction with propyl magnesium bromide in the presence of lithium tetrachlorocuprate catalyst. Details and spectral data are given in the Supplementary Material.

(*Z,Z,Z*)-3,6,9-Pentacosatriene (ZZZ3,6,9-25:H) This compound was prepared from methyl linolenate (methyl (*Z,Z,Z*)-9,12,15-octadecatrienoate; SigmaAldrich) by reduction with lithium aluminium hydride in ether and reaction of the crude product with trifluoromethanesulfonic anhydride and pyridine followed by heptyl magnesium bromide in the presence of lithium tetrachorocuprate as described above (Wang and Zhang 2007) in 50% overall yield. Details and spectral data are given in the Supplementary Material.

(Z,Z,Z)-3,6,9-tricosatriene (ZZZ3,6,9-23:H) This compound was provided by Dr. Bhanu (Biocontrol Research Laboratories, Bangalore, India) and was >95% pure by GC analysis. Spectral data are given in the Supplementary Material.

Pheromone Dispensers and Measurement of Release Rates

Pheromone Dispensers Dispensers were white rubber septa (2 cm long x 1 cm dia cup; International Pheromone Systems Ltd., Wirral, UK) or low-density polyethylene vials (22 mm x 8 mm x 1 mm thick; Just Plastics, London, UK). The pheromone blend, containing 10% butylated hydroxytoluene (BHT) as anti-oxidant, was applied in hexane (0.1 ml) and the solvent allowed to evaporate in a fume hood.

Measurement of Release Rates by Extraction For laboratory studies, dispensers were loaded with a blend of ZE9,11-14:Ac (0.1 mg) and ZZZZZ3,6,9,12,15-25:H (1 mg) and maintained in a laboratory wind tunnel (120 cm x 40 cm x 40 cm; 27 °C; 8 km/h wind speed) illuminated by domestic fluorescent lights. Septa and vials from a field experiment had been exposed for 30 d (3 September – 3 October 2013) in traps under field conditions and contained the same two-component blend.

Lures were extracted individually in hexane (5 ml) containing tetradecyl acetate (14:Ac; 1 mg) as an internal standard overnight at room temperature before analysis by GC-FID on the non-polar HP5 column. Results are means of analyses of two dispensers.

Measurement of Release Rates by Collection of Volatiles For volatile collections, dispensers were maintained in a laboratory wind tunnel as above and collections were made in the same controlled-temperature room (27 °C). Individual dispensers were held in a glass vessel (8 cm x 3 cm) and air drawn in at 2 l/min through a charcoal filter (20 cm x 2 cm; 10-18 mesh) and out through a collection filter (4 mm i.d.) containing Porapak Q (200 mg, 50-80 mesh) for 2-3 h. Volatiles were eluted with dichloromethane (Pesticide Residue Grade, 1ml). Dodecyl acetate (12:Ac 5 µg) was added as an internal standard and the solutions were analyzed by GC-MS and GC-FID after concentration approximately ten-fold under a gentle stream of nitrogen. Amounts of pheromone components were quantified by comparison of peak areas with that of the internal standard and results are the means of measurements on two dispensers.

Field Trapping Tests Field trapping tests were carried out in natural stands of Mediterranean stone pine near Nava del Rey (Valladolid, Castile and León, Spain), between 41º 27' 1.61" N 5º 3' 40.50" W and 41º 26' 34.85" N 5º 2' 38.24" W, at 700 m altitude. The experimental stand consisted of mature pines over 80-100 years old with an understory of young regenerated pines. Pheromone dispensers were polyethylene vials or rubber septa as above and traps were sticky delta traps (21 cm x 20 cm x 11 cm high; ECONEX S.L., Murcia, Spain). The dispensers were positioned in the roof of the trap to minimize exposure to direct sunlight. Traps were hung at ca. 2.5 m above ground from the ends of 60 cm long wire supports extending from the trunks of the pines. A replicate of each treatment was positioned within each of seven experimental blocks in a

247 randomized complete block design. Traps were at least 80 m apart and nearest blocks were 300 m
248 apart. Trap catches were recorded every week.
249 In Experiment 1, traps were baited with ZE9,11-14:Ac (100 μg), ZZZZZ3,6,9,12,15-25:H
250 (1000 μg), or a combination of the two (100 μg + 1000 μg) dispensed from rubber septa, or were
251 unbaited. The experiment was run from 11 July – 3 September 2013 without renewing the lures.
252 In Experiment 2, traps were baited with the binary blend of ZE9,11-14:Ac and
253 ZZZZZ3,6,9,12,15-25:H (100 μg + 1000 μg respectively) dispensed from both rubber septa and
254 polyethylene vials or were unbaited. The experiment was run from 3 September – 3 October 2013
255 without renewing the lures.
256 In Experiment 3, traps were baited with ZE9,11-14:Ac (100 μg) alone or in three binary
257 blends with ZZZZZ3,6,9,12,15-25:H (100 μg + 100 μg, 100 μg + 300 μg, 100 μg + 1000 μg). The
258 blends were tested in both rubber septa and polyethylene vials as dispensers, and the experiment
259 ran from 14 May – 2 July 2014 without renewing the lures.
260 In Experiment 4, the effects of increasing the proportion of ZZZZZ3,6,9,12,15-25:H
261 further and increasing the overall loading were investigated by comparing catches in traps baited
262 with blends of 100 μg + 1000 μg, 100 μg + 3000 μg and 300 μg + 3000 μg ZE9,11-14:Ac and
263 ZZZZZ3,6,9,12,15-25:H respectively. Further treatments were included to determine the
264 possibility of replacing the purified ZZZZZ3,6,9,12,15-25:H in the 100 μg + 1000 μg blend with
265 material derived directly from fish oil containing an equivalent amount of ZZZZZ3,6,9,12,15-
266 25:H, or with the 23-carbon homolog ZZZZZ3,6,9,12,15-23:H or analog ZZZ3,6,9-23:H. The
267 blends were dispensed from rubber septa and the experiment ran from 4 July – 10 September 2014
268 with lures renewed every four weeks.
269 Finally in Experiment 5, catches were compared in traps baited with the binary blend of
270 ZE9,11-14:Ac and ZZZZZ3,6,9,12,15-25:H (100 μg + 1000 μg) with the latter in purified or
271 unpurified form, a blend of ZE9,11-14:Ac and the 25-carbon, tri-unsaturated hydrocarbon
272 ZZZ3,6,9-25:H, and an unbaited trap. The blends were dispensed from both rubber septa and
273 polyethylene vials and the experiment ran from 10 September – 8 October 2014 without renewing
274 the lures.
275 Although catches were recorded and discarded weekly during the experiments, the data
276 were analysed using the total number of insects caught during each of the experimental periods as
277 the response variable. Mean total trap catches were fitted against treatment and block factors and

to a Poisson error distribution in a generalized linear model (GLM) with a loglink function. If significant treatment effects ($P < 0.05$) were detected, Tukey's honestly significant difference test to the value of $\alpha = 0.05$ was used for comparisons of means. All statistical computing was carried out using the R software package (The R Development Core Team, 2011).

Results

Analyses of Pheromone Collections In GC-EAG analyses of pheromone extracts from virgin female *D. mendacella* with a male moth EAG preparation, a single strong EAG response was observed on both polar and non-polar GC columns (Fig. 3). The retention time of the EAG response corresponded to a very small peak in the GC-FID trace (<< 1 ng/female). This was identified by GC-MS analyses and comparison of retention times with those of synthetic standards as ZE9,11-14:Ac (GC Retention Indices (RI) relative to retention times of *n*-alkanes for GC-EAG 1821 on SPB1, 2283 on DBWax; GC-MS 1835 on VF5, 2272 on DBWax). Synthetic ZE9,11-14:Ac elicited a strong EAG response from the antenna of a male *D. mendacella* moth, as did (*Z*)-9-tetradecenyl acetate and (*Z,E*)-9,12-tetradecadienyl acetate (Supplementary Material Fig. S5).

With reference to the components of the pheromones of other *Dioryctria* species reported, re-examination of the GC-EAG and GC-MS analyses of pheromone extracts from female *D. mendacella* showed the presence of ZZZZZ3,6,9,12,15-25:H (RI for GC-EAG 2426 on SPB1, 2683 on DBWax; GC-MS 2441 on VF5, 2673 on DBWax), although no EAG response was recorded to this compound (Fig. 3). A clean peak for the pentaene was observed in GC-MS analyses of all the gland extracts ($N = 12$). The relative amount of ZE9,11-14:Ac was difficult to measure because of the very small amount present and the presence of impurities at that level, but this was 1 : 9.5 ZE9,11-14:Ac : ZZZZZ3,6,9,12,15-25:H in the cleanest extract made 4 h into the dark period. No other polyunsaturated hydrocarbons could be detected in GC-MS analyses by single ion scanning at *m/z* 79, characteristic of polyunsaturated hydrocarbons with at least three double bonds in the 3-, 6- and 9-positions, such as ZZZZZ3,6,9,12,15-23:H, ZZZ3,6,9-25:H or ZZZ3,6,9-23:H.

In GC-EAG analyses of volatiles collected from virgin female *D. mendacella* on the polar GC column, a response was observed corresponding to the retention time of ZE9,11-14:Ac. However, amounts present were too low for reliable detection in GC-MS analyses, and ZZZZZ3,6,9,12,15-25:H could not be detected.

Release of Pheromone from Dispensers Analyses of collection of volatiles from dispensers maintained in the laboratory windtunnel showed that ZZZZZ3,6,9,12,15-25:H was released at measurable rates from both rubber septa and polyethylene vial dispensers, as verified by GC retention times on both polar and non-polar GC columns and by GC-MS analyses.

For the rubber septa, release of ZE9,11-14:Ac was relatively constant at approx. 0.6 µg/d over the period of measurement of 37 d at 27 °C. The release rate of ZZZZZ3,6,9,12,15-25:H increased from 0.01 to 0.05 µg/d (Fig. 4). Thus the ratio of ZZZZZ3,6,9,12,15-25:H : ZE9,11-14:Ac increased from 0.03 to 0.08. Given the ratio of material loaded in the septum was 10:1, this indicates the release rate of ZZZZZ3,6,9,12,15-25H was approx. 0.003 that of ZE9,11-14:Ac.

For the polyethylene vials, release of ZE9,11-14:Ac was faster than from the septa and declined from approx. 2.5 µg/d to 0.9 µg/d over the period of measurement of 37 d at 27 °C. That of the ZZZZZ3,6,9,12,15-25H was also faster and increased from 0.03 µg/d to 0.23 µg/d (Fig. 4). The ratio of ZZZZZ3,6,9,12,15-25H : ZE9,11-14:Ac increased from 0.014 to 0.26. Given the ratio of material loaded in the septum was 10:1, this indicates the release rate of ZZZZZ3,6,9,12,15-25H was approx. 0.001 that of ZE9,11-14:Ac.

Analysis of the pheromone remaining in the lures exposed in the laboratory showed the percentage of ZE9,11-14:Ac remaining in the septa was higher than that in the vials, as expected from the lower release rate from septa than vials (Table 1). However, the percentage of ZZZZZ3,6,9,12,15-25H remaining in the septa was lower than that in the vials which was unexpected, given the lower release rate from the septa. This indicated more degradation of the pentaene may have been occurring in the septa than in the vials. Analyses of the pheromone remaining in lures exposed in the field for 30 d were consistent with these results with more ZE9,11-14:Ac in the septa than the vials but less ZZZZZ3,6,9,12,15-25H (Table 1).

Field Tests In Experiment 1, traps baited with ZE9,11-14:Ac or ZZZZZ3,6,9,12,15-25:H alone caught no more male *D. mendacella* moths than unbaited traps. However, a blend of the two compounds in a 1:10 ratio respectively was highly attractive (Fig. 5a; $F = 62.24$, df = 3,27, $P < 0.001$).

Catches with rubber septa and polyethylene vials as dispensers for the 1:10 blend of ZE9,11-14:Ac and ZZZZZ3,6,9,12,15-25:H in Experiment 2 were not significantly different from

each other over the 30-d period tested, and were both significantly higher than those in unbaited traps (mean catches 46.7 ± 25.5 (SEM) with rubber septa, 51.5 ± 26.0 with polyethylene vials and 0.0 ± 0.0 in unbaited traps; $F = 1.75$, df = 2,20, $P < 0.001$).

Results in Experiment 3 were similar with rubber septa or polyethylene vials as dispensers ($F = 1.22$, df = 1,13, $P = 0.29$) and these were combined for analysis. Catches in traps baited with a 1:1 or 1:3 blend of ZE9,11-14:Ac and ZZZZZ3,6,9,12,15-25:H were not significantly greater than those in unbaited traps and significantly greater catches were only obtained with the 1:10 blend (Fig. 5b; $F = 15.26$, df = 3,27, $P < 0.001$).

Increasing the proportion of ZZZZZ3,6,9,12,15-25:H relative to that of ZE9,11-14:Ac to 1:30 ZE9,11-14:Ac and ZZZZZ3,6,9,12,15-25:H or increasing the amount of the 1:10 blend three-fold in Experiment 4 did not increase catches (Fig. 6a; $F = 14.26$, df = 6,48, $P < 0.001$). Replacing the purified ZZZZZ3,6,9,12,15-25:H in the blend with an equivalent amount of the unpurified material obtained directly from fish oil gave at least as high catches, but blends in which the 25-carbon pentaene was replaced with the 23-carbon homologue ZZZZZ3,6,9,12-23:H or the 23-carbon triene ZZZ3,6,9-23:H were unattractive (Fig. 6a).

In the final Experiment 5, results with rubber septa and polyethylene vials as dispensers were similar and these were combined for analysis ($F = 1.53$, df = 1,13, $P = 0.239$). It was confirmed that the purified ZZZZZ3,6,9,12-25:H could be replaced with the unpurified material in the 1:10 blend of ZE9,11-14:Ac and ZZZZZ3,6,9,12,15:H without loss of attractiveness, but replacing this with the 25-carbon triunsaturated analog ZZZ3,6,9-25:H gave an unattractive blend (Fig. 6b; $F = 24.31$, df = 3,27, $P < 0.001$).

Discussion

In this study, ZE9,11-14:Ac and ZZZZZ3,6,9,12,15-25:H were identified as components of the sex pheromone of female *D. mendacella* moths. The former elicited a strong EAG response from male moths, but the latter did not. Neither was attractive to male moths in field trapping tests when used alone, but a 1:10 blend was highly attractive, showing quite remarkable synergy. The relative amount of the pentaene could not be reduced without reducing attractiveness, but an equivalent amount of the crude hydrocarbon mixture obtained directly from fish oil (approx. 30% ZZZZZ3,6,9,12,15-25:H) could be used without loss of attractiveness. This greatly reduces the cost of the lure.

A similar blend has been reported for the sex pheromones of female spruce moth, *D. abietella* Denis and Schiffermüller (Löfstedt et al. 2012) and *D. abietivorella* Grote (Millar et al. 2005; Strong et al. 2008). The former is widely distributed across Eurasia, from UK to Japan (UK CAB International, 1991) and may overlap that of *D. mendacella* in some Mediterranean areas such as France, Croatia and Italy. Both species have similar feeding habits on cones but use different hosts: *D. abietella* thrives on *Picea*, *Abies*, *Cedrus*, *Larix* and also on some pine species in China, whereas *D. mendacella* is restricted to Mediterranean pines (Knölke 2007). *Dioryctria abietivorella* is a native of the USA. Attraction of *D. amatella* Hulst male moths to (*Z*)-11-hexadecenyl acetate was greatly increased by addition of ZZZZZ3,6,9,12,15-25:H (Miller et al. 2010).

There have been an increasing number of reports of Lepidopteran sex pheromones containing both Type I components (such as ZE9,11-14:Ac) and Type II components (such as ZZZZZ3,6,9,12,15-25:H) (Ando et al. 2004). In addition to the species of *Dioryctria* mentioned above, (*E*)-11-hexadecenol and ZZZ3,6,9-23:H are pheromone components of *Neoleucinodes elegantalis* Guenée (Lepidoptera: Crambidae) (Cabrera et al. 2001; Jaffe et al. 2007), (*Z,Z*)-11,13-hexadecadienal, ZZZZZ3,6,9,12,15-23:H and ZZZZZ3,6,9,12,15-25H are essential pheromone components of *Amyelois transitella* Walker and *Pyralis farinalis* L. (Lepidoptera: Pyralidae) (Leal et al. 2005), (*Z*)-11-hexadecenal and ZZZ3,6,9-23:H are pheromone components of *Deanolis sublimbalis* Snellen (Lepidoptera: Crambidae) (Gibb et al. 2007), (*E*)-10-hexadecenal, (*E,E*)-10,12-hexadecadienal and ZZZ3,6,9-23:H are pheromone components of *Conogethes pluto* Butler (Lepidoptera: Crambidae) (El-Sayed et al. 2013), (*E*)- and (*Z*)-10-hexadecenal and ZZZ3,6,9-23:H are pheromone components of *Conogethes punctiferalis* Guenée (Lepidoptera: Crambidae) (Xiao et al. 2012), (*E,E*)-10,14-hexadecadienal and ZZZ3,6,9-23:H are pheromone components of *Omphisa plagialis* Wileman (Lepidoptera: Crambidae) (Yan et al. 2014), while (*E,Z*)-10,12-hexadecenal, the corresponding acetate and ZZZ3,6,9-23:H are pheromone components of *Rehimena surusalis* Walker (Lepidoptera: Crambidae) (Honda et al. 2015).

Where EAG studies have been carried out, the Type II hydrocarbon components have generally been found to elicit very weak responses from the male moths in contrast to the Type I components (e.g. Leal et al. 2005). In the work described here, no convincing EAG response was recorded from the antennae of male moths of *D. mendacella* to ZZZZZ3,6,9,12,15-25:H in GC-EAG analyses of extracts of the pheromone glands of female moths although a strong response

was recorded to ZE9,11-14:Ac, in spite of the fact that the amount of the former was at least ten times that of the latter. Furthermore, the GC-EAG system used here accumulated the column effluent in a reservoir in the GC oven before delivering it in a pulse of air to the EAG preparation (Cork et al. 1990). This would be anticipated to be much more effective at delivering relatively involatile compounds, such as ZZZZZ3,6,9,12,15-25:H, than the alternative approach of passing the column effluent into a relatively slow flow of air at room temperature used in other studies above.

Even though ZZZZZ3,6,9,12,15-25:H is present at over ten times the amount of ZE9,11-14:Ac in the pheromone gland extracts, the amount released will be very much lower than the amount of the latter, as indicated by the release rate studies here. Given the low electrophysiological activity of the pentaene, there has been some debate over whether the active pheromone component is actually the relatively involatile, long-chain, unsaturated hydrocarbon or some more volatile product of oxidative degradation, as has been reported for species of sawfly such as *Pikonema alaskensis* Rohwer (Hymenoptera: Tenthredinae) (Bartelt and Jones 1983). In this study we showed that ZZZZZ3,6,9,12,15-25:H is released in detectable amounts from both polyethylene vial and rubber septa dispensers. Furthermore, the fact that analogs of ZZZZZ3,6,9,12,15-25:H cannot replace this compound in the blend without loss of attractiveness even though they could produce similar degradation products also suggests that the pentaene is indeed the active pheromone component. The latter result is in contrast to those obtained with *O. plagialis* by Yan et al. (2014) where the pheromone component ZZZ3,6,9-23:H could be replaced by the 21-carbon or 22-carbon analogous trienes or by ZZZZZ3,6,9,12,15-23:H without loss of attractiveness.

Rubber septa and polyethylene vial dispensers gave similar results with the various blends tested here, despite rather different release characteristics. It would seem there is a certain threshold for the blend composition released to be attractive to male *D. mendacella* moths, perhaps somewhere in the region of 0.02 ZZZZZ3,6,9,12,15-25:H : ZE9,11-14:Ac that is achieved or exceeded with the 10:1 blend in the dispenser. Blends with a lower proportion of ZZZZZ3,6,9,12,15-25:H in the dispenser and hence in the blend released were unattractive, and increasing the proportion of ZZZZZ3,6,9,12,15-25:H in the blend above this threshold did not increase attractiveness.

The crude blend of unsaturated hydrocarbons derived from fish oil contained ZZZZZ3,6,9,12,15-25:H as the most abundant component, albeit at only approximately 30% of the mixture. An equivalent amount of this mixture was just as effective as the purified ZZZZZ3,6,9,12,15-25:H at synergizing the attractiveness of ZE9,11-14:Ac to male *D. mendacella* moths, also suggesting that the male moths are responding very specifically to the 25-carbon pentaene. This observation also makes it possible to decrease the cost of the lure substantially. (*Z,Z,Z,Z,Z*)-5,8,11,14,17-Eicosapentaenoic acid costs $750 for 100 mg from SigmaAldrich whereas 100 g of the fish oil is available for $15. It will be interesting to see if this crude blend can be used in lures for other species using ZZZZZ3,6,9,12,15-25:H or the 23-carbon analogue as pheromone components.

Thus lures containing ZE9,11-14:Ac (100 µg) and ZZZZZ3,6,9,12,15-25:H (1000 µg) in purified or unpurified form, dispensed from either rubber septa or polyethylene vials can be used to bait traps for *D. mendacella*. There was a suggestion that some degradation of the ZZZZZ3,6,9,12,15-25:H may occur in the rubber septa and we favor the latter in our work. Both types of dispenser remain effective for at least 30 d in the field in Spain and probably for at least two months. Further work is in progress using the pheromone traps to monitor populations of *D. mendacella* and gain a better understanding of its life cycle and population dynamics. Mediterranean forests are nowadays subjected to climate change which is expected to result in changes in the physiology, phenology and distribution of forest pests. Furthermore, tree species can also suffer changes in their phenology and vigor, becoming more susceptible to native and introduced organisms. At the same time, there is an increasing demand for ecosystem services and products. Development of management tools such as pheromone trapping will help forest managers to face these challenges.

Acknowledgements

We would like to acknowledge the field assistance and support provided A. Ponce, F.J. Gordo, the forest officers at the Castilla y León Forest Service in Valladolid and the crew at Castilla y León Forest Health Center (Calabazanos, Palencia). G. Álvarez-Baz assisted with the statistical analyses. This work has been financed by grants by the Diputación de Valladolid (PROPINEA project) and by the Castilla y León Government.

References

Ando T, Inomate SI, Yamamoto M (2004) Lepidopteran sex pheromones. In: The chemistry of pheromones and other semiochemicals (ed. Schulz, S.) 51–96. Springer, Berlin, Heidelberg, New York

Bartelt RJ, Jones RL (1983) (*Z*)-10-Nonadecenal: a pheromonally active air oxidation product of (*Z*,*Z*)-9,19-dienes in the yellow-headed spruce sawfly. J Chem Ecol 9:1333-1342

Boivin T, Auger-Rozenberg M-A (2016) Native fruit, cone and seed insects in the Mediterranean Basin. In: Insects and diseases in Mediterranean Forest Systems. eds T.D. Paine and F. Lieutier. Springer, The Netherlands. pp. 47-88

Bracalini M, Benedetelli S, Croci F, Terreni P, Tiberi R, Panzavolta T (2013). Cone and seed pests of *Pinus pinea*: assessment and characterization of damage. J. Econ. Entomol. 106: 229 - 234

Cabrera A, Eiras A, Gries G, Gries R, Urdaneta N, Miras B, Badji C, Jaffe K (2001) Sex pheromone of tomato fruit borer, *Neoleucinodes elegantalis*. J Chem Ecol 27:2097-2107

Cork A, Beevor PS, Gough AJE, Hall DR (1990). Gas chromatography linked to electroantennography: a versatile technique for identifying insect semiochemicals. In: Chromatography and Isolation of Insect Hormones and Pheromones. eds. A. R. McCaffery and I. D. Wilson. Plenum Press, New York and London. pp. 271-279

El-Sayed AM (2016). The Pherobase: Database of Pheromones and Semiochemicals. http://www.pherobase.com

El-Sayed AM, Gibb AR, Mitchell VJ, Manning L-A M, Revell J, Thistleton B, Suckling DM (2013) Identification of the sex pheromone of *Conogethes pluto*: a pest of Alpinia. Chemoecology 23:93-101

Gibb AR, Pinese B, Tenakanai D, Kawi AP, Bunn B, Ramankutty P, Suckling DM (2007) (*Z*)-11-Hexadecenal and (3*Z*,6*Z*,9*Z*)-tricosatriene: sex pheromone components of the red banded mango caterpillar *Deanolis sublimbalis*. J Chem Ecol 33:579-589

Gordo J, Mutke S, Gil L (1997). Variabilidad en la producción de fruto de *Pinus pinea* L. en la provincia de Valladolid. I Congreso Forestal Hispano-Luso/II Congreso Forestal Español. Pamplona June 1997. Abstracts, 327-332.

492 Hall DR, Beevor PS, Lester R, Poppi RG, Nesbitt BF (1975). Synthesis of the major sex
493 pheromone of the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.). Chem Ind
494 216-217
495 Honda H, Yamasaki R, Sumiuchi Y, Uehara T, Matsuyama S, Ando T, Naka H (2015) Hybrid
496 sex pheromones of the hibiscus flower-bud borer, *Rehimena surusalis*. J Chem Ecol
497 41:1043-1049
498 Innocenti M, Tiberi R (2002) The cone and seed pests of *Pinus pinea* L. in Central Italy. Redia
499 85: 21-28
500 Jaffe K, Miras B, Cabrera A (2007) Mate selection in the moth *Neoleucinodes elegantalis:*
501 evidence for a supernormal chemical stimulus in sexual attraction. Anim Behav 73:727-
502 734
503 Karsholt O, van Nieukerken EJ, Erik J, (2014). Fauna Europea: *Dioryctria mendacella*. In: Fauna
504 Europaea - all European animal species on the web. eds Y. de Jong *et al.* . Biodiversity
505 Data Journal 2. e4034.
506 Knölke S. (2007). A revision of the European representatives of the microlepidopteran genus
507 *Dioryctria* Zeller, 1846 (Insecta: Lepidoptera: Pyralidae: Phycitinae). Ludwig
508 Maximilians-Universität München, München, Germany.
509 Leal WS, Parra-Pedrazzoli AL, Kaissling K-E, Morgan TI, Zalom FG, Pesak DJ, Dundulis EA,
510 Burks CS, Higbee BS (2005) Unusual pheromone chemistry in the navel orangeworm:
511 novel sex attractants and a behavioral antagonist. Naturwissenschaften 92:139-146
512 Löfstedt C, Svensson GP, Jirle EV, Rosenberg O, Roques A, Millar JG (2012)
513 (3Z,6Z,9Z,12Z,15Z)-Pentacosapentaene and (9Z,11E)-tetradecadienyl acetate: sex
514 pheromone of the spruce coneworm *Dioryctria abietella* (Lepidoptera: Pyralidae). J Appl
515 Entomol 136:70-78
516 Millar JG, Grant GG, McElfresh JS, Strong W, Rudolph C, Stein JD, Moreira JA (2005)
517 (3Z,6Z,9Z,12Z,15Z)-Pentacosapentaene, a key pheromone component of the fir coneworm
518 moth, *Dioryctria abietivorella*. J Chem Ecol 31:1229-1234
519 Miller DR, Millar JG, Mangini A, Crowe CM, Grant GG (2010) (3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-
520 Pentacosapentaene and (*Z*)-11-hexadecenyl acetate: sex attractant blend for *Dioryctria*
521 *amatella* (Lepidoptera: Pyralidae). J Econ Entomol 103:1216-1221

Mutke S, Piqué M, Calama R (2013) AGROPINE 2011 Meeting conclusions. In : Mediterranean stone pine for agroforestry. Zaragoza Eds. Mutke S., Piqué M., & Calama R.. Options Méditerranéennes Série A. Séminaires Méditerranéens; n. 105 CIHEAM/FAO/INIA/IRTA/CESEFOR/CTFC. p 111-112

Strong WB, Millar JG, Grant GG, Moreira JA, Chong JM, Rudolph C (2008) Optimization of pheromone lure and trap design for monitoring the fir coneworm, *Dioryctria abietivorella*. Entomol Exp Appl 126:67-77

The R Development Core Team (2011) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN: 3-900051- 07-0

UK CAB International (1991) Distribution Maps of Plant Pests. CAB International Wallingford UK, Map 520

Wang S, Zhang A (2007). Facile and efficient syntheses of (3Z,6Z,9Z)-3,6,9-nonadecatriene and homologues: pheromone and attractant components of Lepidoptera. J Agric Food Chem 55:6929-6932

Xiao W, Matsuyama S, Ando T, Millar JG, Honda H (2012) Unsaturated cuticular hydrocarbons synergize responses to sex attractant pheromone in the yellow peach moth, *Conogethes punctiferalis*. J Chem Ecol 38:1143-1150

Yan Q, Vang LV, Khanh CNQ, Naka H, Ando T (2014) Reexamination of the female sex pheromone of the sweet potato vine borer moth: identification and field evaluation of a tricosatriene. J Chem Ecol 40:590-598

Table 1. Percentages of original loadings of pheromone components remaining in dispensers maintained in laboratory (27°C) and field experiment in Spain (N = 2).

	Exposure (d)	% Remaining	
		ZE9,11-14:Ac	ZZZZZ3,6,9,12,15-25:H
Laboratory vial	37	24	86
Laboratory septum	37	45	58
Field vial	30	41	84
Field septum	30	66	77

Fig. 1 Syntheses of (*Z,Z,Z,Z,Z*)-3,6,9,12,15-pentacosapentaene: (i) MeOH/BF_3 etherate; (ii) chromatography on silica gel impregnated with 10% silver nitrate; (iii) $LiAlH_4$/ether; (iv) *p*-toluenesulfonyl chloride/NaOH/ether; (v) triflic anhydride/pyridine/dichloromethane; (vi) $C_5H_{11}MgBr/Li_2CuCl_4$/THF

Fig. 2. GC-FID analyses of (*Z,Z,Z,Z,Z*)-3,6,9,12,15-pentacosapentaene crude direct from fish oil (upper) and from the methyl ester purified by chromatography on silica gel impregnated with silver nitrate (lower) on non-polar HP5 GC column.

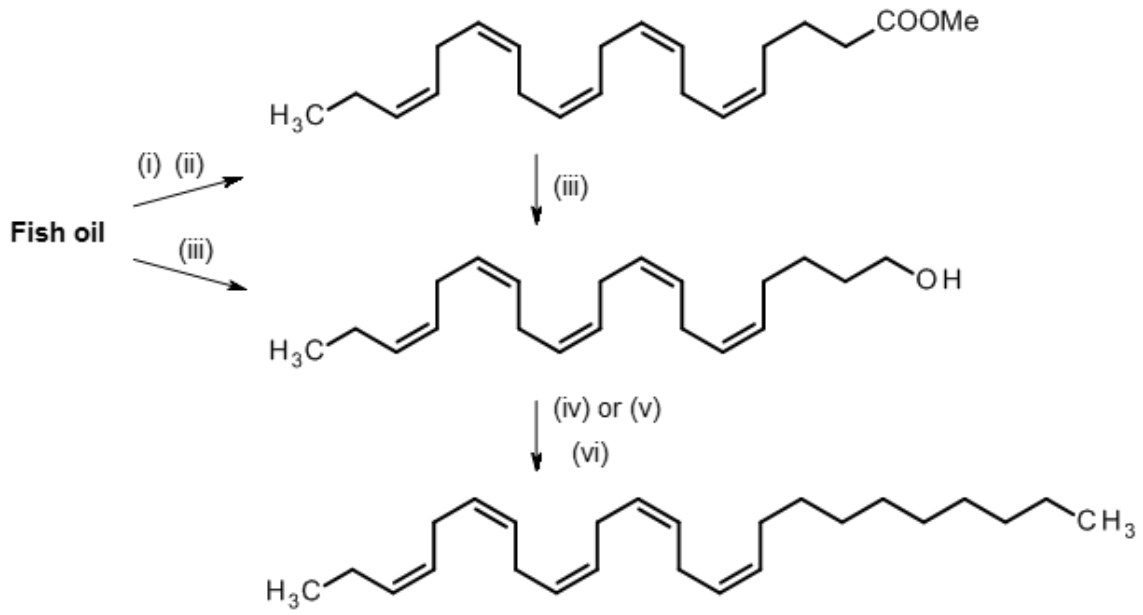
Fig. 3 GC-EAG analysis of pheromone extracts from virgin female *Dioryctria mendacella* with a male moth EAG preparation on non-polar GC column (lower panel is expansion of upper; in each lower trace is GC-FID, upper EAG responses to intermittent delivery of accumulated column effluent; (1) ZE9,11-14:Ac at 17.16 min, (2) ZZZZZ3,6,9,12,15-25:H at 22.50 min)).

Fig. 4 Release rates (µg/d) of pheromone components from rubber septum and polyethylene vial dispensers measured at 27°C with lures maintained at 27°C and 8 km/h windspeed between measurements (mean of two replicates, bars show spread).

Fig. 5 Means of total catches (+ standard error) of male *Dioryctria mendacella* moths in traps baited with (a) ZE9,11-14:Ac, ZZZZZ3,6,9,12,15-25:H, a blend of the two or unbaited (Experiment 1; 11 July – 3 September 2013; rubber septa as dispensers; $N = 7$); (b) blends of ZE9,11-14:Ac and ZZZZZ3,6,9,12,15-25:H (Experiment 3; 14 May – 2 July 2014; results with rubber septa and polyethylene vials as dispensers combined, $N = 14$); means with different letters are significantly different at $P < 0.05$)

Fig. 6 Means of total catches (+ standard error) of male *Dioryctria mendacella* moths in traps baited with (a) blends of ZE9,11-14:Ac with ZZZZZ3,6,9,12,15-25:H in pure or crude (c) form, ZZZ3,6,9-23:H or ZZZZZ3,6,9,12,15-23:H (Experiment 4; 4 July – 10 September 2014; rubber septa as dispensers; $N = 7$); (b) blends of ZE9,11-14:Ac with ZZZZZ3,6,9,12,15-25:H in pure or crude (c) form, or ZZZ3,6,9-25:H (Experiment 5; 10 September – 8 October 2014; results with

579 rubber septa and polyethylene vials as dispensers combined, $N = 14$); means with different letters
580 are significantly different at $P < 0.05$)
581
582



583

584 **Fig 1.** Syntheses of (*Z*,*Z*,*Z*,*Z*,*Z*)-3,6,9,12,15-pentacosapentaene: (i) MeOH/BF_3 etherate; (ii)
585 chromatography on silica gel impregnated with 10% silver nitrate; (iii) $LiAlH_4$/ether; (iv) *p*-
586 toluenesulfonyl chloride/NaOH/ether; (v) triflic anhydride/pyridine/dichloromethane; (vi)
587 $C_5H_{11}MgBr/Li_2CuCl_4$/THF

588

589

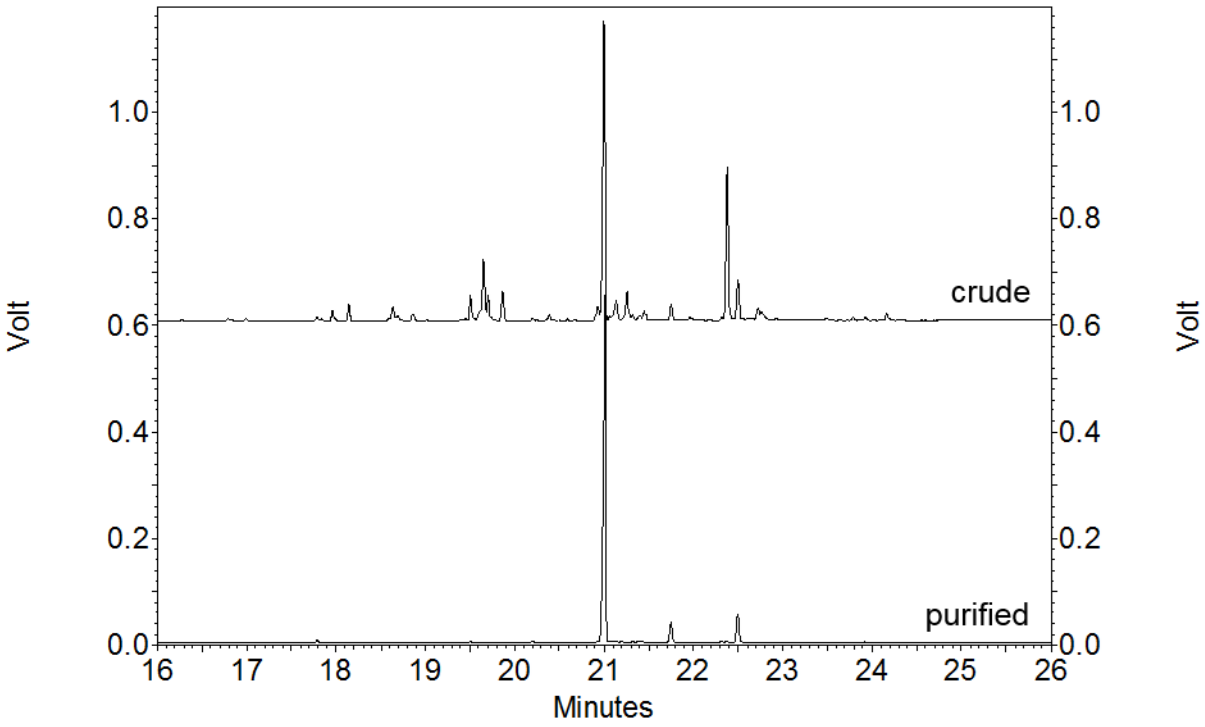
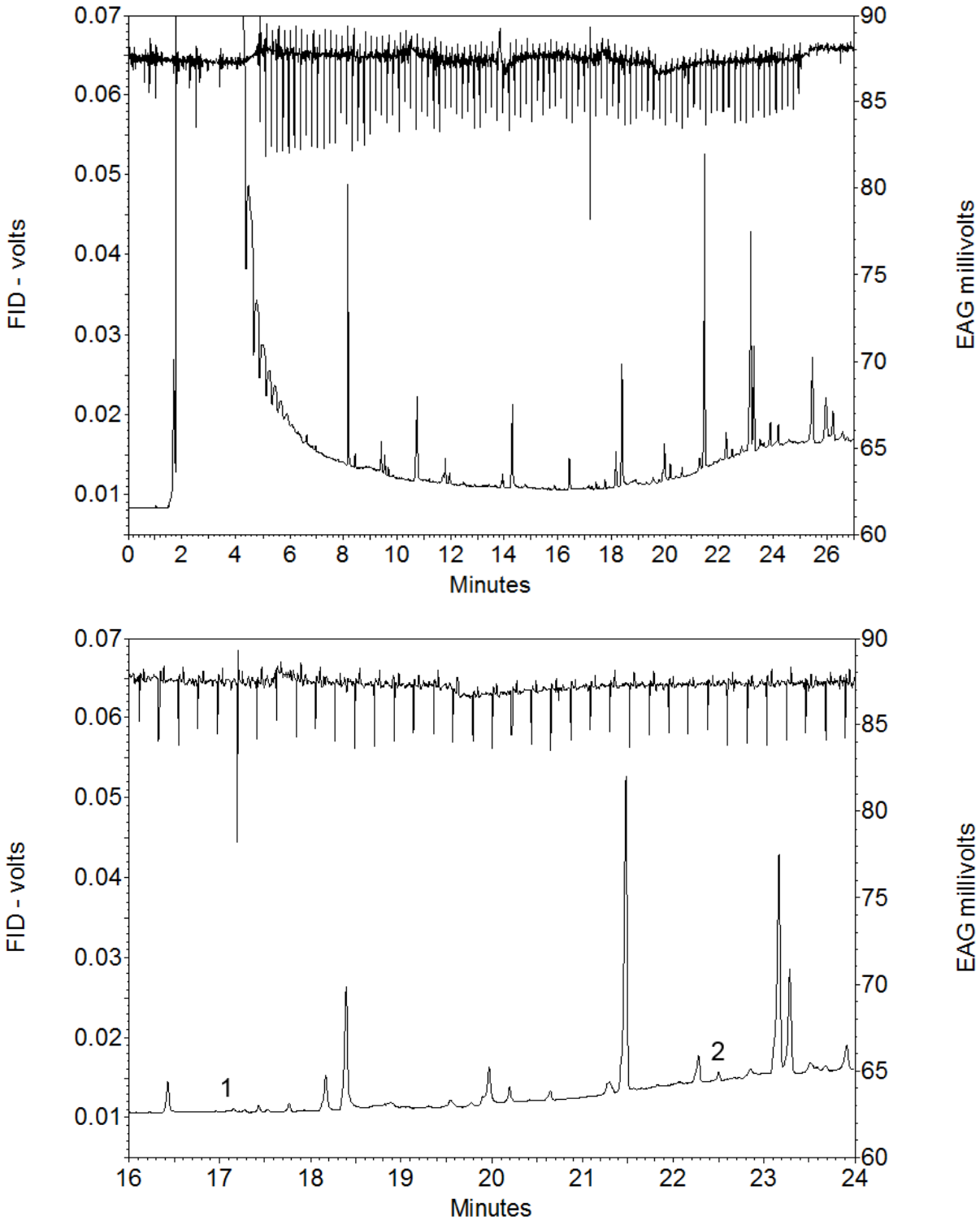


Fig. 2. GC-FID analyses of (*Z*,*Z*,*Z*,*Z*,*Z*)-3,6,9,12,15-pentacosapentaene crude direct from fish oil (upper) and from the methyl ester purified by chromatography on silica gel impregnated with silver nitrate (lower) on non-polar HP5 GC column.



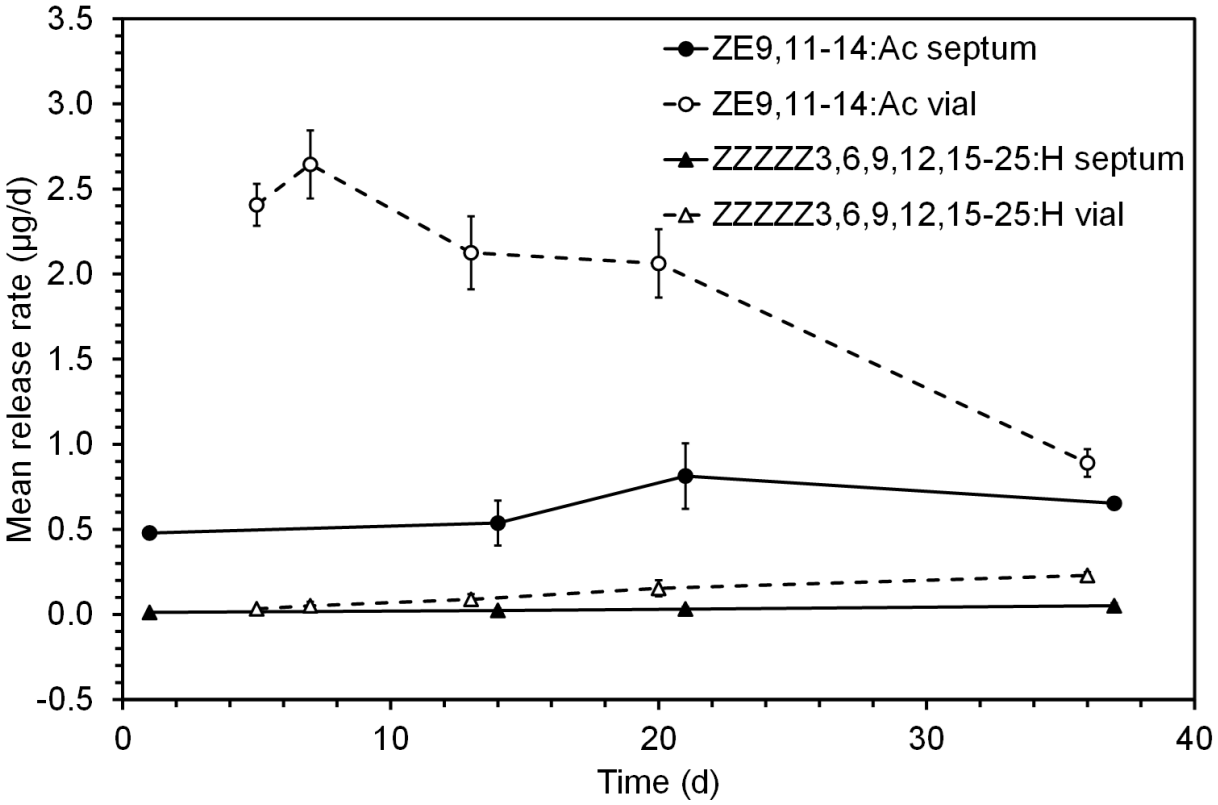
596

597

598 **Fig. 3.** GC-EAG analysis of pheromone extracts from virgin female *Dioryctria mendacella* with

599 a male moth EAG preparation on non-polar GC column (lower panel is expansion of upper; in

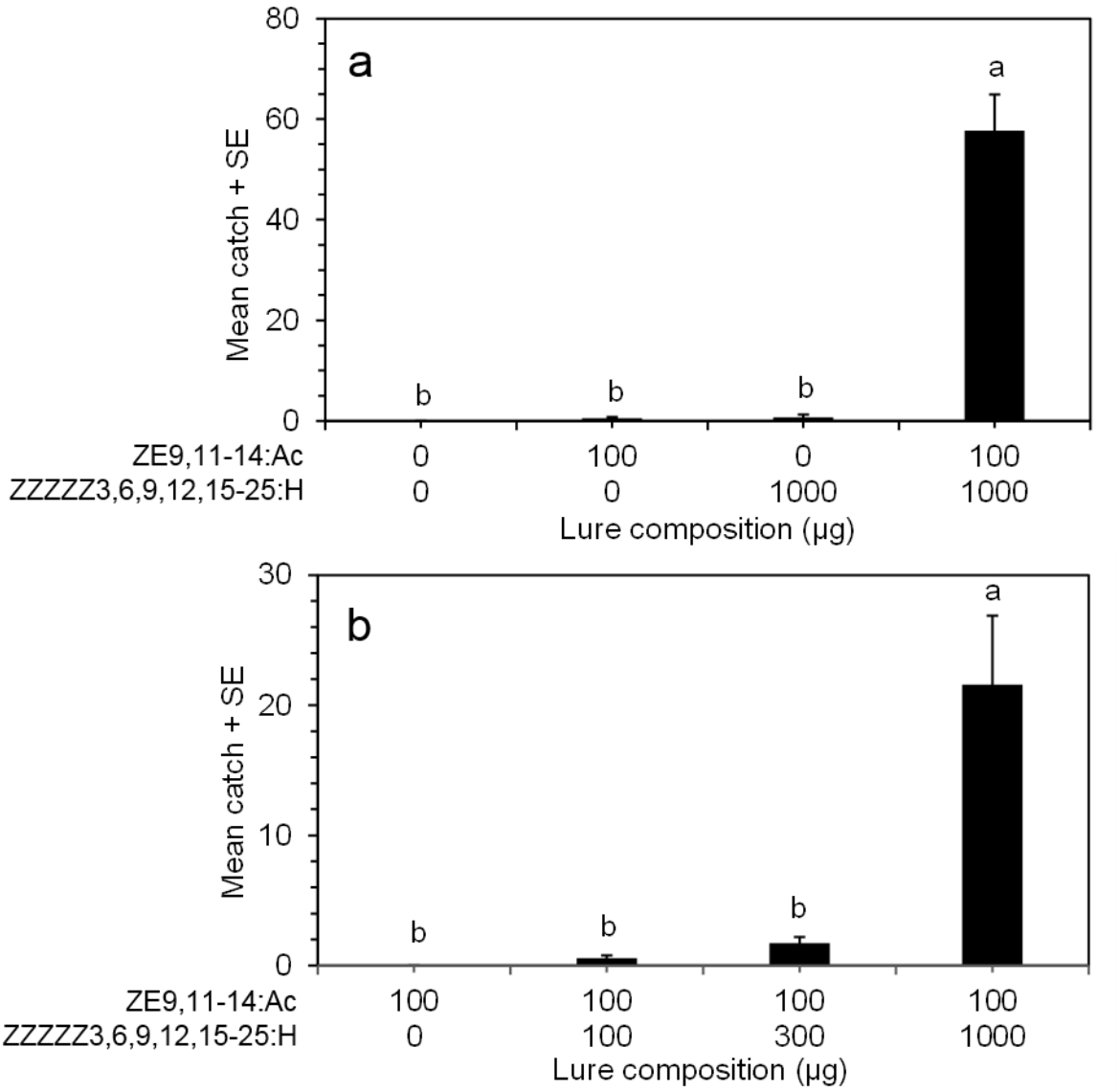
each lower trace is GC-FID, upper EAG responses to intermittent delivery of accumulated column effluent; (1) ZE9,11-14:Ac at 17.16 min, (2) ZZZZZ3,6,9,12,15-25:H at 22.50 min)).



603

604 **Fig. 4** Release rates (µg/d) of pheromone components from rubber septum and polyethylene vial
605 dispensers measured at 27 °C with lures maintained at 27 °C and 8 km/h windspeed between
606 measurements (mean of two replicates; bars show spread).

607

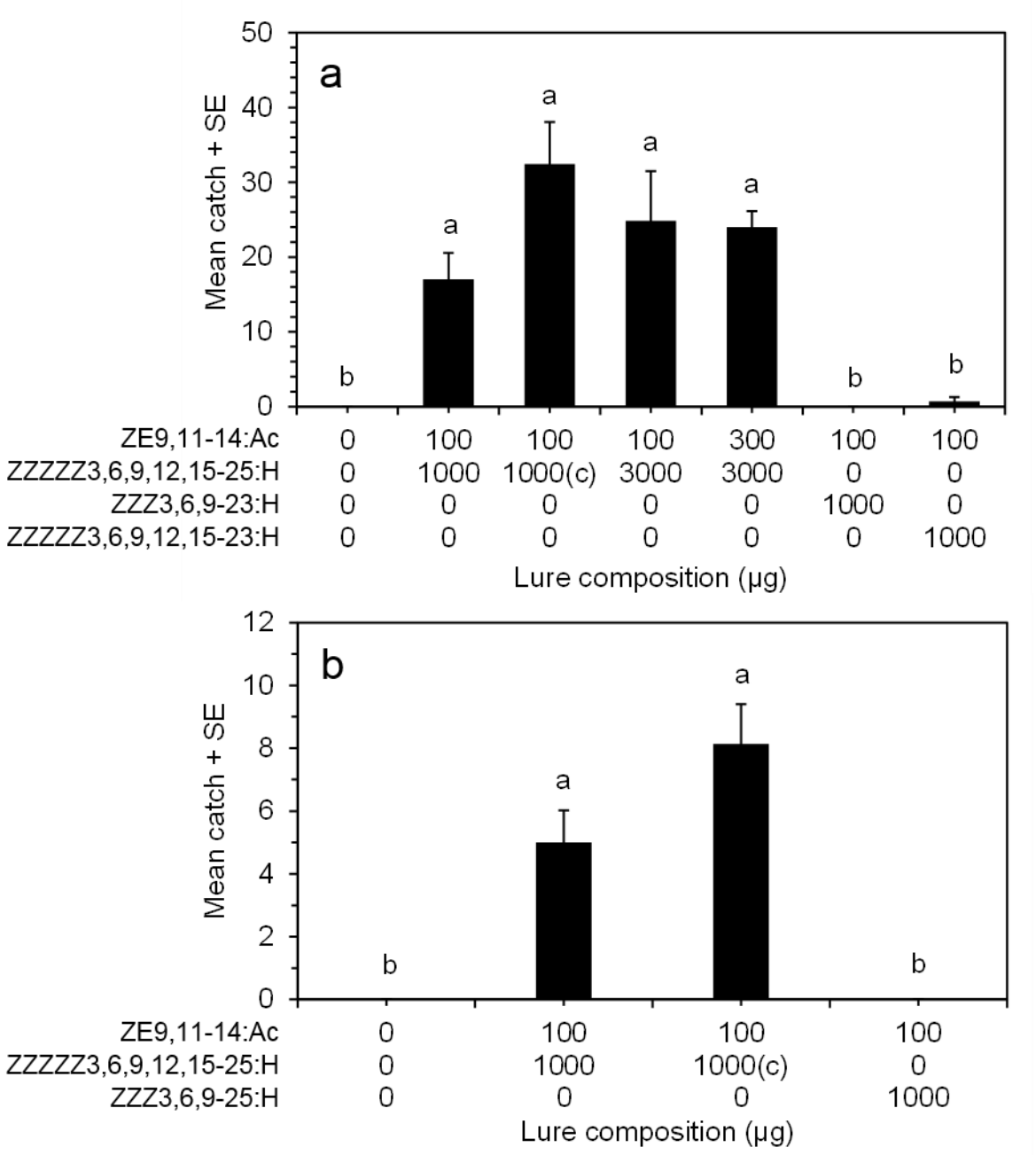


608

609

610 **Fig. 5** Means of total catches (+ standard error) of male *Dioryctria mendacella* moths in traps
611 baited with (a) ZE9,11-14:Ac, ZZZZZ3,6,9,12,15-25:H, a blend of the two or unbaited
612 (Experiment 1; 11 July – 3 September 2013; rubber septa as dispensers; $N = 7$); (b) blends of
613 ZE9,11-14:Ac and ZZZZZ3,6,9,12,15-25:H (Experiment 3; 14 May – 2 July 2014; results with
614 rubber septa and polyethylene vials as dispensers combined, $N = 14$); means with different letters
615 are significantly different at $P < 0.05$)

616



617

618 **Fig. 6** Means of total catches (+ standard error) of male *Dioryctria mendacella* moths in traps
619 baited with (a) blends of ZE9,11-14:Ac with ZZZZZ3,6,9,12,15-25:H in pure or crude (c) form,
620 ZZZ3,6,9-23:H or ZZZZZ3,6,9,12,15-23:H (Experiment 4; 4 July – 10 September 2014; rubber
621 septa as dispensers; $N = 7$); (b) blends of ZE9,11-14:Ac with ZZZZZ3,6,9,12,15-25:H in pure or
622 crude (c) form, or ZZZ3,6,9-25:H (Experiment 5; 10 September – 8 October 2014; results with
623 rubber septa and polyethylene vials as dispensers combined, $N = 14$); means with different letters
624 are significantly different at $P < 0.05$)