Naturwissenschaften (2005) 92:139–146
DOI 10.1007/s00114-004-0598-5

SHORT COMMUNICATION

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Unusual pheromone chemistry in the navel orangeworm: novel sex attractants and a behavioral antagonist

Received: 15 October 2004 / Accepted: 19 November 2004 / Published online: 25 January 2005
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Abstract Using molecular- and sensory physiology-based approaches, three novel natural products, a simple ester, and a behavioral antagonist have been identified from the pheromone gland of the navel orangeworm, *Amyelois transitella* Walker (Lepidoptera: Pyralidae). In addition to the previously identified (*Z*,*Z*)-11,13-hexadecadienal, the pheromone blend is composed of (*Z*,*Z*,*Z*,*Z*,*Z*)-3,6,9,12,15-tricosapentaene, (*Z*,*Z*,*Z*,*Z*,*Z*)-3,6,9,12,15-pentacosapentaene, ethyl palmitate, ethyl-(*Z*,*Z*)-11,13-hexadecadienoate, and (*Z*,*Z*)-11,13-hexadecadien-1-yl acetate. The C_{23} and C_{25} pentaenes are not only novel sex pheromones, but also new natural products. In field tests, catches of *A. transitella* males in traps baited with the full mixture of pheromones were as high as those in traps with virgin females, whereas control and traps baited only with the previously known constituent did not capture any moths at all. The navel orangeworm sex pheromone is also an attractant for the meal moth, *Pyralis farinalis* L. (Pyralidae), but (*Z*,*Z*)-11,13-hexadecadien-1-yl acetate is a behavioral antagonist. The new pheromone blend may be highly effective in mating disruption and monitoring programs.

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Introduction

Female-produced sex pheromones in moths (Lepidoptera) are normally complex mixtures of straight chain acetates, aldehydes, and alcohols, with 10–18 carbon atoms and up to three unsaturations. This group of pheromones, Type I according to Ando's classification (Ando et al. 2004) comprises ca. 75% of the known pheromones. A second major group, Type II (15%) (Ando et al. 2004) consists of polyunsaturated (up to four double bonds) hydrocarbons and epoxy derivatives with long straight chains (C_{17}–C_{23}) (Ando et al. 2004). While Type I pheromones are synthesized de novo (Jurenka 2004; Ando et al. 2004), polyunsaturated hydrocarbons seem to be derived from dietary linoleic and linolenic acid (Jurenka 2004; Ando et al. 2004).

The major constituent of the sex pheromones of two species in the family Pyralidae, the navel orangeworm, *Amyelois transitella* Walker (subfamily: Phycitinae) (Coffelt et al. 1979) and the meal moth, *Pyralis farinalis* Linnaeus (subfamily: Pyralinae) (Landolt and Curtis 1982) have been previously identified as (*Z*,*Z*)-11,13-hexadecadienal belonging to Type I (Ando et al. 2004). It has been suggested that additional pheromone components may be present in the female navel orangeworm moths (Shorey and Gerber 1996), but hitherto conventional approaches have failed to identify the full pheromone system. We have applied molecular- and sensory physiology-based approaches for the characterization of the full pheromone system in the navel orangeworm, a major pest of almond, pistachio, and walnuts in California. Here, we report that the sex pheromone system of *A. transitella* is in fact a hybrid of the two types of pher-

omones, i.e., a combination of aldehyde, acetate, ethyl ester, and novel highly unsaturated hydrocarbons.

Materials and methods

Analytical procedures

Gas chromatography–mass spectrometry (GC–MS) was done with a 5973 Network mass selective detector linked to a 6890 Network GC system (Agilent Technologies, Palo Alto, Calif.) operated either in the electron impact (EI) or chemical ionization (CI) mode. Chromatographic resolution was done on an HP-5MS column (30 m×0.25 mm; 0.25 μm; Agilent) that was operated at 70°C for 1 min, increased to 250°C at a rate of 10°C/min and held at this temperature for 10 min. Vapor phase infrared (IR) spectroscopy was carried out on a Win GC/IR Pro (Varian, formerly Digilab, Randolph, Mass.) with a GC/IR interface and a Scimitar FTS 2000 linked to a 6890 Network GC system (Agilent). Separation was done on a HP-5 column (30 m×0.32 mm; 0.25 μm; Agilent) operated at 100°C for 1 min, increased to 250°C at a rate of 20°C/min and held at this temperature for 5 min; the transfer line and light pipe were operated at 250°C. Gas chromatography with electroantennographic detection (GC–EAD) was done with two different systems: HP 5890 and HP 6890 (Agilent) both having Syntech's GC–EAD transfer lines and temperature control units (Hilversum, The Netherlands). In both systems, the effluent from the capillary column was split into EAD and flame ionization detector (FID) in 3:1 ratios. Male antennae were placed in EAG probes (Syntech) and held in place with Spectra 360 electrode gel (Parker Laboratories, Orange, N.J.). These probes were connected to AM-01 amplifiers (Syntech). The analog signals were fed into A/D 35900E interfaces (Agilent) and acquired simultaneously with a FID signal on an Agilent Chemstation. Chromatographic separations were done either with a HP-5MS column operated as in GC–MS or with a HP-INNOWAX column (30 m×0.32 mm; 0.25 μm; Agilent) operated at 70°C for 1 min, increased to 250°C at a rate of 10°C/min and held at this temperature for 5 min.

Insect rearing, pheromone extraction and fractionation

The navel orangeworm colony started from larvae collected in Bakersfield, California. The larvae were kept on dried and roasted pistachio at 25±2°C, 75±10% relative humidity, and a 16:8 h (light:dark) photoregime. Adults were transferred to aluminum cages (30 cm×30 cm×30 cm) and kept for 48 h to allow copulation. After the first generation, 20% of the emerged adults were used to maintain the colony. The remainder of the pupae was kept individually in culture tubes [17 mm internal diameter (i.d.), 10 cm long]. Upon emergence males were used for EAD and single sensillum recordings (SSR) and females for gland extracts or trap baits. Pheromone glands of 1- to 2-day-old virgin females were extracted 2 h before photophase for 10 min in glass-distilled hexane and kept at −80°C until used. Crude extracts from a pool of 1,500 females were subjected to flash column chromatography on silica gel (60–200 mesh; Fisher Scientific) by successive elution with hexane–ether mixtures in the following order: 100:0 (hexane fraction), 99:1 (1% fraction), 98:2, 97:3, 95:5, 90:10, 50:50, 0:100.

Syntheses

*(11Z,13E)-, (11E,13Z)-, (11E,13E)-, and (11Z,13Z)-11,13-hexadecadienal (**1**)*

The (*Z*,*Z*) isomer was prepared by a previously published method (Sonnet and Heath 1980). The (*Z*,*E*) isomer was prepared by the sequence shown in Scheme 1 (**1**). (*E*)-12-Pentadecen-10-yn-1-ol THP was prepared by palladium- catalyzed cross-coupling of 10-undecyn-1-ol THP (prepared from 10-undecyn-1-ol and dihydropyran) with *E*-1-iodo-1-butene (Zweifel and Whitney 1967; Alami et al. 1993). Addition of dicyclohexyl borane across the triple bond followed by hydrolysis of both the borane and THP protecting group gave the desired (*Z*,*E*) diene stereochemistry (Brown 1975). The alcohol was converted to bromide via the mesylate using conventional methods (Jones 2001). The Grignard reagent of the bromide was then prepared and reacted with triethyl orthoformate to give (*Z*,*E*)-11,13-hexadecadienal diethyl acetal (DeWolfe 1970). Acidic hydrolysis (Greene and Wuts 1999) gave the desired aldehyde. The (*E*,*Z*) isomer was prepared by the sequence shown in Scheme 1 (**2**). (*E*)-10-Pentadecen-12-yn-1-ol THP was prepared from the borane adduct of 10-undecyn-1-ol THP and the lithium salt of 1-butyne (Svirskaya et al. 1984). The rest of the synthesis follows that of the (*Z*,*E*) isomer from the THP stage described above. The (*E*,*E*) isomer was prepared by isomerization of the (*Z*,*Z*) isomer mediated by thiophenol and a radical source (Schwarz et al. 1986) followed by fractional crystallization.

Ethyl (11Z,13Z)-11,13-hexadecadienoate (3)

(*Z*,*Z*)-10,12-Pentadecadien-1-ol was prepared using the appropriate starting materials using a previously reported reaction sequence (Sonnet and Heath 1980). The alcohol was converted to bromide (Scheme 1, **3**). The Grignard reagent of the bromide was prepared and quenched with excess diethylcarbonate (Whitmore and Loder 1943) to give the desired ester **3**.

*(11Z,13Z)-11,13-Hexadecadien-1-yl acetate (**4**)*

Compound **4** was prepared by LAH reduction of the aldehyde (*Z*,*Z*)-**1** followed by acylation of the alcohol with acetyl chloride (Scheme 1, **4**).

*(3Z,6Z,9Z,12Z,15Z)-3,6,9,12,15-Tricosapentaene (**5**) and (3Z,6Z,9Z,12Z,15Z)-3,6,9,12,15-pentacosapentaene (**6**)*

Commercially available methyl (*Z*,*Z*,*Z*,*Z*,*Z*)-5,8,11,14,17-eicosapentaenoate was reduced to the corresponding alcohol with Red-Al (Málek 1988) (Scheme 1, **5**). The alcohol was then converted to bromide, which was coupled to either *n*-propyl or *n*-pentyl Grignard catalyzed by copper (Erdik 1984) to give the **5** and **6** pentaenes, respectively.

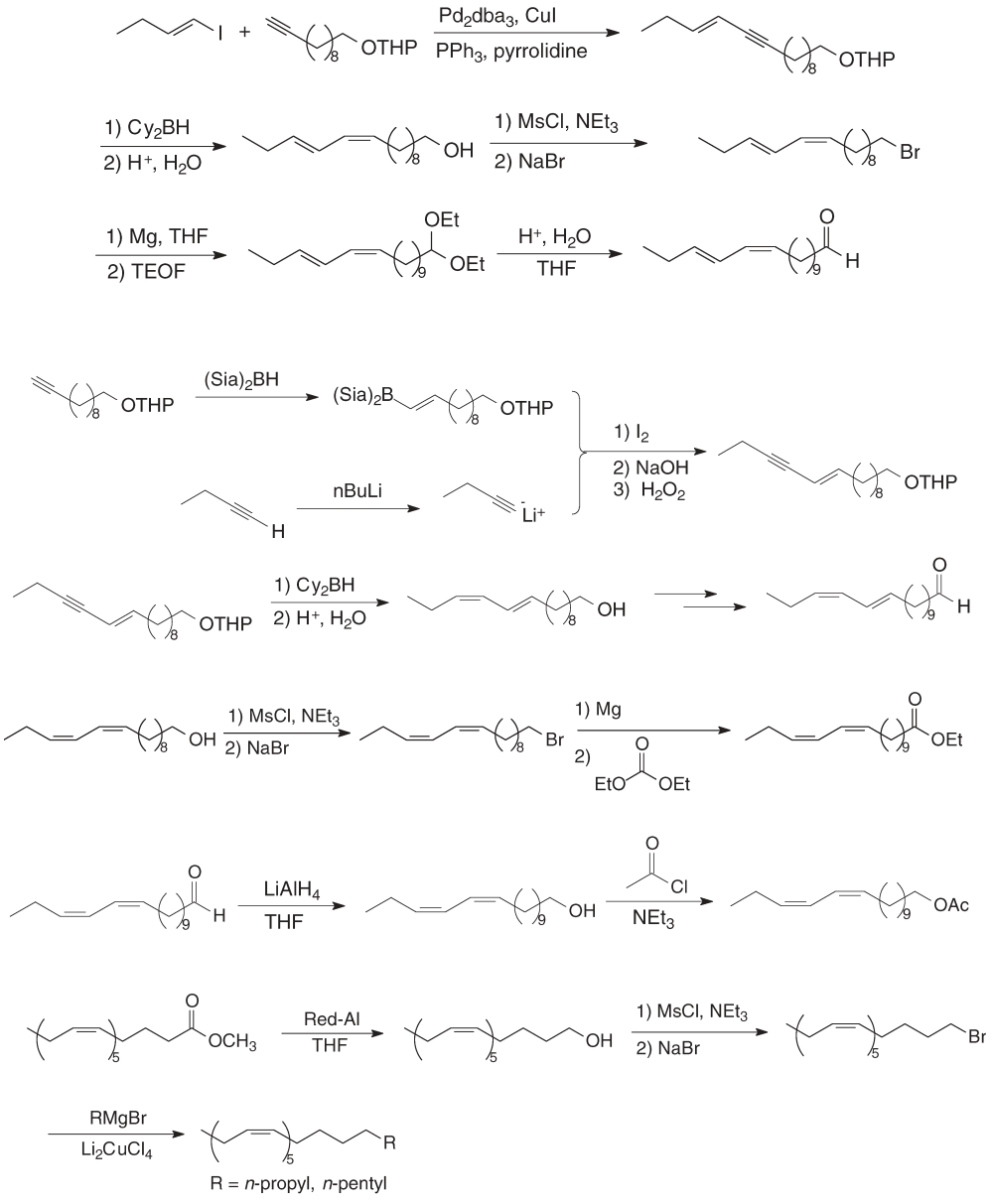
Single sensillum recordings

Male moths were immobilized with dental wax on the recording stage of a single sensillum recording unit (Syntech, INR-02), and the tips of the sensilla were cut (Kaissling 1995) and placed under a stereomicroscope (SZX12; Olympus, Tokyo). The indifferent (ground) electrode was a thin tungsten electrode inserted into the head. The recording glass electrode was slipped over the cut tip of the sensilla with a Piezo Manipulator (PM-10; World Precision Instruments, Sarasota, Fla.) while the signal was monitored with a Tektronix oscilloscope (TDS-2014). The pre-amplified signal was acquired with an acquisition system (IDAC-USB; Syntech) and SSR software (Autospike 2000; Syntech). The antennal preparation was continuously flushed with clean air at 0.5 m/s. Each stimulus was applied to a filter paper, dried for at least 10 min, and placed within a glass cartridge (7 mm i.d., 5 cm long). The cartridge opening was placed 1 cm in front of the antennae. The stimulus air was delivered by a stimulus controller (CD-02/E; Syntech).

Field experiments

Tests were conducted in almond and walnut plot fields in the UC Davis campus. Pheromone samples (0.5 mg) were formulated in rubber septa or in 12-mm-diameter, 3-mm-thick discs (made of ES

Scheme 1 Synthetic routes for compounds **1**, **2**, **3**, **4**, and **5**



fiber; Chisso, Tokyo) and loaded into Pherocon 1C traps (Trece, Salinas, Calif.). Three or five 1- to 3-day-old virgin females were placed in fiberglass screen cages (Curtis and Clark 1984). Baited and control traps were placed at ca. 1.8 m height in randomized blocks with the intertrap distance of ca. 10 m. Capture data were transformed to log (x+0.5) and analyzed by ANOVA (see below).

Results and discussion

We have taken a comprehensive approach to studying chemical communication in the navel orangeworm, *A. transitella*. On the one hand, we have isolated, cloned, and expressed pheromone- and odorant-binding proteins (Y. Ishida et al., manuscript in preparation). Binding assays with recombinant olfactory proteins indicated that the previously identified pheromone, (*Z*,*Z*)-11,13-hexadecadienal (ALD), bound to the major pheromone-binding protein (AtraPBP) with apparent high affinity. As it will be described elsewhere, preliminary screening of potential ligands showed that a related acetate compound, (*Z*,*Z*)-11,13-hexadecadienyl acetate, had similar affinity to AtraPBP. On the other hand, electrophysiological recordings from sensilla trichodea (SSR) in male moth antennae (Fig. 1a) indicated that the navel orangeworm possess multiple olfactory receptors neurons (ORN), which are stimulated by constituents in hexane extracts from pheromone glands (Fig. 1a, right panel). The crude extract was fractionated by flash chromatography with electrophysiological activity being monitored by SSR. Different ORNs were stimulated not only by the ALD-containing fractions (5 and 10% ether), but also by two other fractions: hexane (0% ether) and 3% ether. Based on the spike amplitudes, it was not possible to conclude unambiguously whether different ORNs fired or if the

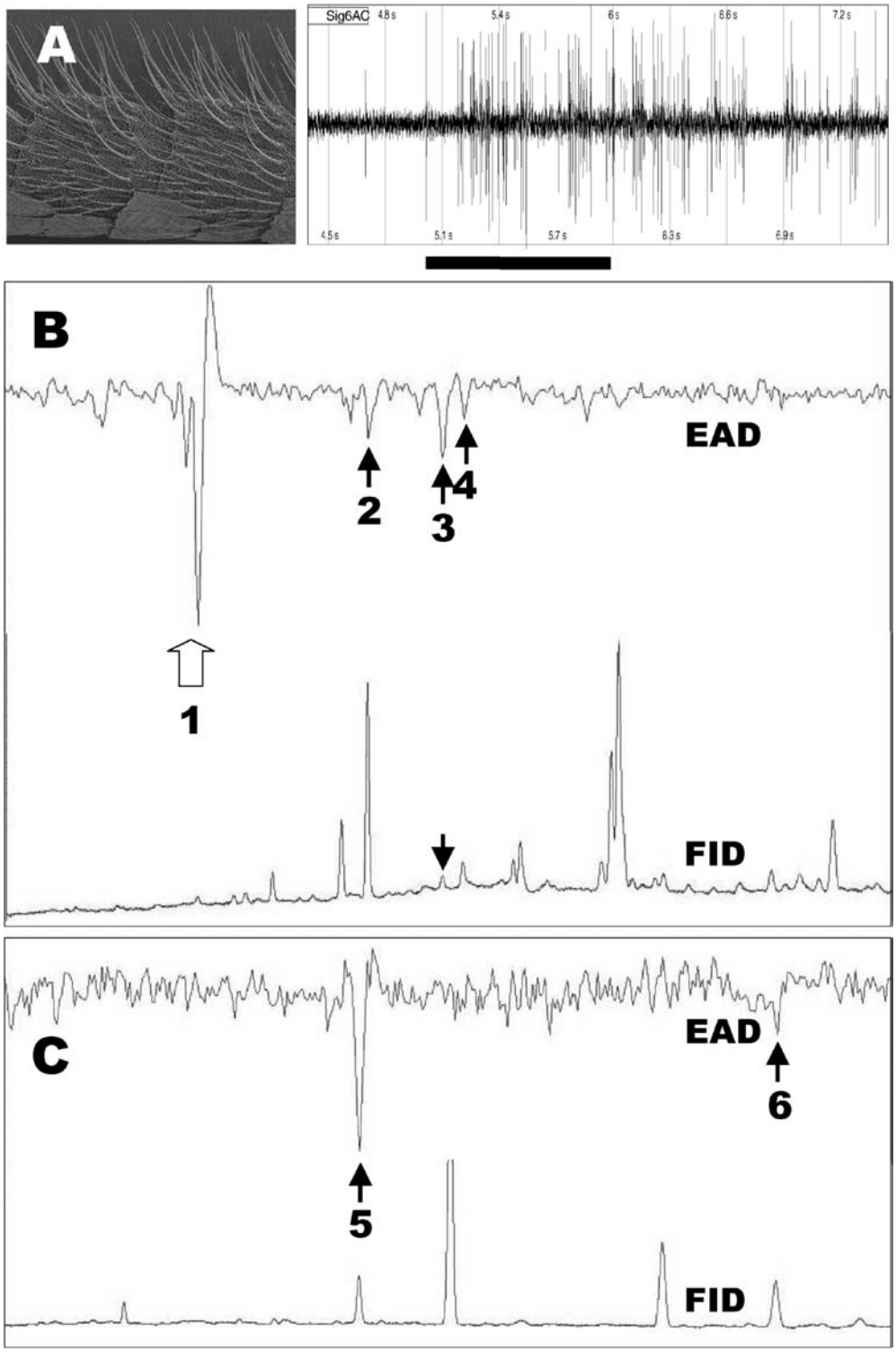


Fig. 1 **a** Scanning electron micrograph (magnification, 300×) of a male antennae of the navel orangeworm (*left*); electrophysiological recording from one of these sensilla trichodea stimulated by five female-equivalents of a gland extract (*right*). The *bar* represents the stimulus duration (1 s). Gas chromatography with electroantennographic detection recordings from the 3% (**b**) and hexane (**c**) fractions after separation of the crude extract by a silica gel column. The peaks highlighted (*arrows*) in the EAD traces were highly reproducible (n=20). Isomers of the known pheromone (ALD) generated a cluster of peaks (*open arrow*)

SSR responses were derived only from minute amounts of ALD, particularly in the 3% fraction. To determine the active constituents in these SSR-active fractions (3% and hexane), we used GC–EAD and used male moth antennae as the sensing element. GC–EAD analyses using a non-polar column (HP-5MS) indicated that in addition to the ALD pheromone (peak **1**), the 3% fraction contained three other EAD-active peaks (**2**, **3** and **4**) (Fig. 1b), whereas the hexane fraction contained two other EAD-active peaks (**5** and **6**) (Fig. 1c). The peaks were numbered in the order of their retention times (t_R) in a non-polar column (**1**, t_R, 17.30 min; **2**, 18.44 min; **3**, 18.96 min; **4**, 19.08 min; **5**, 20.9 min; **6**, 23.8 min). The retention times of these EAD-active peaks in a polar column (HP-INNOWAX) were: 16.59, 17.37, 18.32, and 18.72 min (3% fraction) and 18.52 and 20.22 min (hexane

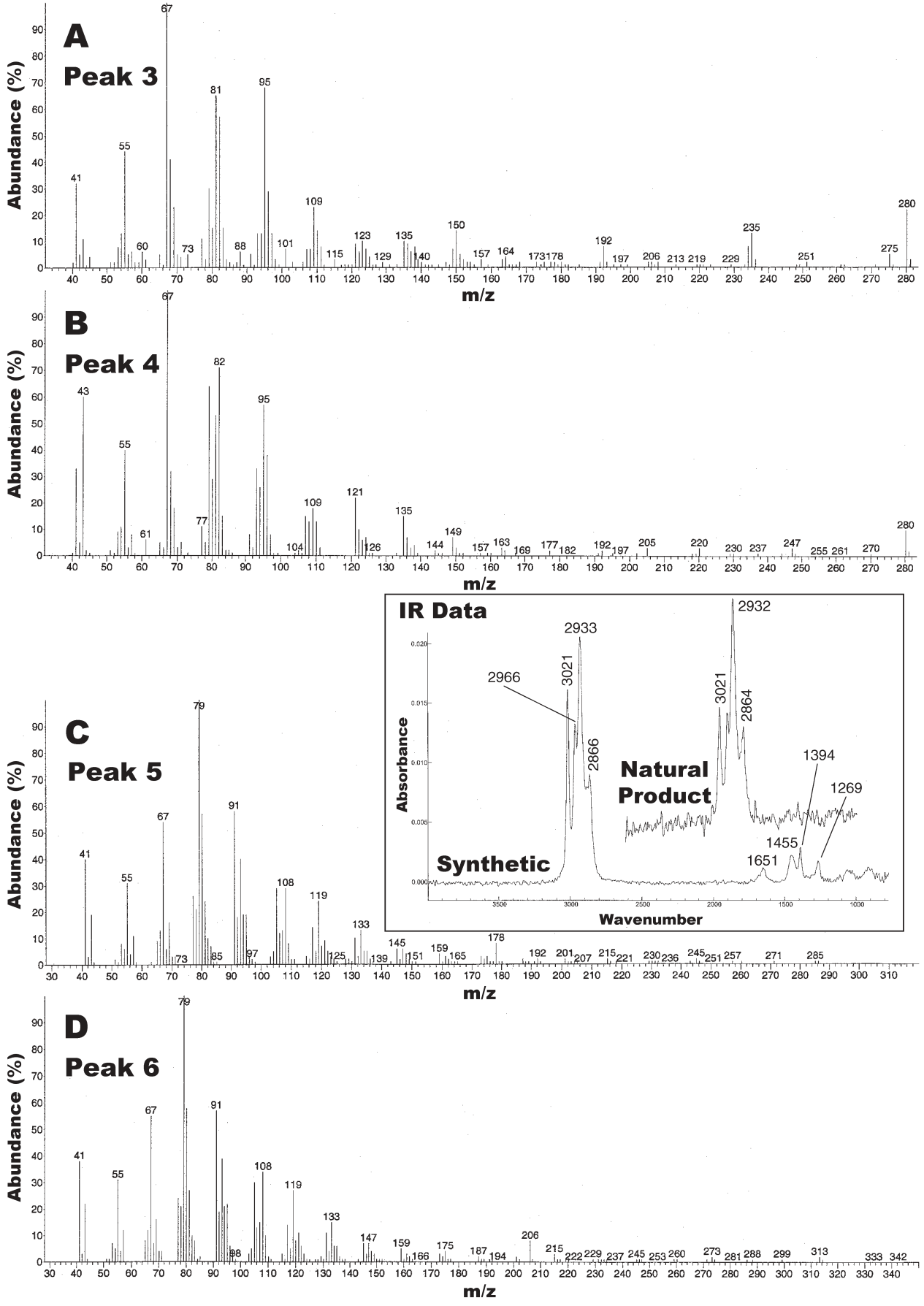
fraction). GC–MS analyses indicated that the cluster of peaks (labeled peak **1** in Fig. 1b) is derived from the isomers of the previously identified pheromone, ALD. Authentic synthetic standards showed the following order of elution by GC–MS: (*Z*,*E*)-, (*E*,*Z*)-, (*Z*,*Z*)-, and (*E*,*E*)-**1** (t_R, 14.77, 14.86, 14.94, and 14.98 min, respectively). The strongest EAD-active peak in the cluster (**1**) corresponds to the (*Z*,*Z*)-isomer, whereas the earlier eluting, small EAD-active peaks are generated by (*Z*,*E*)- and (*E*,*Z*)-isomers. While the occurrence in gland extracts of the major, (*Z*,*Z*)-, and other two minor isomers, i.e., (*Z*,*E*) and (*E*,*Z*), was clearly observed by both GC–EAD and GC–MS, the (*E*,*E*)-isomer was not detectable by these techniques. In SSR experiments, large spike amplitude cells (Fig. 1a) were activated by (*Z*,*Z*)-**1**, whereas synthetic (*E*,*E*)-**1** activated mainly a small spike ORN, with small activation of a large spike cell.

Peak **2** was identified as ethyl palmitate by GC–MS and library (Wiley) search. Co-elution with authentic ethyl palmitate in polar and non-polar columns and EAD activity confirmed the identification. The fragmentation pattern in the MS of peak **3** (Fig. 2a) somewhat resembles that of the ALD constituent. The loss of 45 (molecular ion peak, *m/z* 280 and *m/z* 235) and the peak at *m/z* 88 suggested that **3** was a di-unsaturated ethyl ester. This assignment was also supported by the vapor phase IR spectra with a strong carbonyl stretching band at 1,753 cm^{-1}, as commonly observed in methyl and ethyl esters (Leal 1998). Although it was not possible to assign the location of the double bonds (given the small amounts of the natural product), we suggested on the basis of the MS profile that it might be derived from the same biosynthetic pathway as ALD and, therefore, have the double bonds in positions 11 and 13. Synthetic ethyl (*Z*,*Z*)-11,13-hexadecadienoate was indistinguishable from **3** in the MS and GC-IR profiles, retention times in polar and non-polar columns; synthetic **3** was also EAD active.

Peak **4** gave a MS (Fig. 2b) identical to that of synthetic (*Z*,*Z*)-11,13-hexadecadien-1-yl acetate, utilized in molecular-based approaches for the screening of potential attractants (see above). Synthetic and natural compounds have identical retention times in polar and non-polar columns. Synthetic (*Z*,*Z*)-11,13-hexadecadien-1-yl acetate showed the same electrophysiological activity as the natural product. In summary the 3% fraction contained four EAD-active peaks, which were fully characterized as: **1**, (*Z*,*Z*)-11,13-hexadecadienal; **2**, ethyl palmitate; **3**, ethyl (*Z*,*Z*)-11,13-hexadecadienoate, and **4**, (*Z*,*Z*)-11,13-hexadecadien-1-yl acetate. Whereas mixtures of biosynthetically related aldehydes and acetates are commonly utilized in moth sex pheromones, this is the first identification of a novel ethyl ester likely derived from the same biosynthetic pathway as that of the major pheromone constituent (ALD).

MS data suggested that **5** and **6** were related compounds (Fig. 2c, d). The base peak in the MS of **5** (Fig. 2c) appeared at *m/z* 79; CI (methane) MS indicated that a tiny peak at *m/z* 314 was the molecular peak. CI gave two major peaks at *m/z* 313 ($[M\text{-}H]^+$) and 315 (base peak, $[M+H]^+$). Hydrogenation of the purified compound and MS analyses suggest that **5** is a pentaunsaturated straight chain hydrocarbon. The peak at *m/z* 178 $[Me(CH_2)_6(CH{=}CH)_3H]^+$ suggests the occurrence of six methylenes after the last double bond (Karunen 1974; Youngblood et al. 1971; Lee et al. 1970; Blumer et al. 1970). The occurrence of a double bond in position 3 was inferred by the fragment $[MeCH_2(CH{=}CH)_3H]^+$ at *m/z* 108 (Karunen 1974; Youngblood et al. 1971; Lee et al. 1970; Blumer et al. 1970) and the lack of vinyl CH_2 in vapor phase IR (Leal 1998) at ca. 3,080 cm^{-1} (Fig. 2c, inset). IR and MS suggest that there was no conjugation and the strong IR band at 3,021 cm^{-1} suggests that all double bonds had the *cis* configuration (Leal 1998) (Fig. 2c). MS of **6** showed evidence of eight methylenes after the last double bond: *m/z* 206, $[Me(CH_2)_8(CH{=}CH)_3H]^+$ (Karunen 1974; Youngblood et al. 1971; Lee et al. 1970; Blumer et al. 1970). The molecular peak at *m/z* 342 was confirmed by CI. Like **5**, compound **6** showed no band corresponding to vinyl CH_2 in vapor phase IR (data not shown), no conjugation, and evidence of all-*cis* configuration. Thus, the two compounds were tentatively identified as (*Z*,*Z*,*Z*,*Z*,*Z*)-3,6,9,12,15-tricosapentaene and (*Z*,*Z*,*Z*,*Z*,*Z*)-3,6,9,12,15-pentacosapentaene, respectively. The synthetic polyunsaturated hydrocarbons were indistinguishable from the natural products in their MS, IR, and retention times under GC–EAD and GC–MS separation conditions. Even with a shallow separation method in a polar column (INNOWAX; 70–250°C at 5°C/min), both synthetic and natural products gave the same retention time: (*Z*,*Z*,*Z*,*Z*,*Z*)-3,6,9,12,15-tricosapentaene, 31.33 min; (*Z*,*Z*,*Z*,*Z*,*Z*)-3,6,9,12,15-pentacosapentaene, 34.42 min. The synthetic polyunsaturated hydrocarbons were also EAD-active. Hitherto monoene, diene, triene and tetraene hydrocarbons (C_{17}–C_{23}) have been identified as sex pheromones (Ando et al. 2004), but pentaenes are not known. Both **5** and **6** are novel types of natural products, but a shorter pentaene, (*Z*,*Z*,*Z*,*Z*,*Z*)-3,6,9,12,15-heneicosapentaene has been previously identified from marine benthic algae (Youngblood et al. 1971) and spores of a moss (Karunen 1974). Given the methylene-interrupted pattern of the 3,6,9 moiety, it is conceivable that these novel moth pheromones (**5** and **6**) could be derived from linolenic acid after chain elongation, desaturation and decarboxylation, provided the insect possesses the appropriate enzymes.

The ratio of the six constituents of the sex pheromone system of the navel orangeworm, analyzed by GC with three replicates of gland extracts, was (*Z*,*Z*)-11,13-hexadecadienal 100 (850±97 pg/female); ethyl palmitate, 14±1.3; ethyl-(*Z*,*Z*)-11,13-hexadecadienoate, 4.8±1.4); (*Z*,*Z*)-11,13-hexadecadien-1-yl acetate, 4.9±1.2; (*Z*,*Z*,*Z*,*Z*,*Z*)-3,6,9,12,15-tricosapentaene, 14.9±2.4; and (*Z*,*Z*,*Z*,*Z*,*Z*)-3,6,9,12,15-pentacosapentaene, 17.1±1.4.3. Preliminary field tests in Davis showed that captures in traps baited with the full mixture of the pheromone system (0.5 mg) did not differ significantly from catches in traps baited with 1- to 3-day-old virgin females (Fig. 3a),


A
Peak 3
Abundance (%)
m/z
B
Peak 4
Abundance (%)
m/z
C
Peak 5
Abundance (%)
m/z
IR Data
Absorbance
Wavenumber
2932
2933
3021
2966
3021
2864
2866
Natural
Product
1394
1269
1455
1651
Synthetic
D
Peak 6
Abundance (%)
m/z

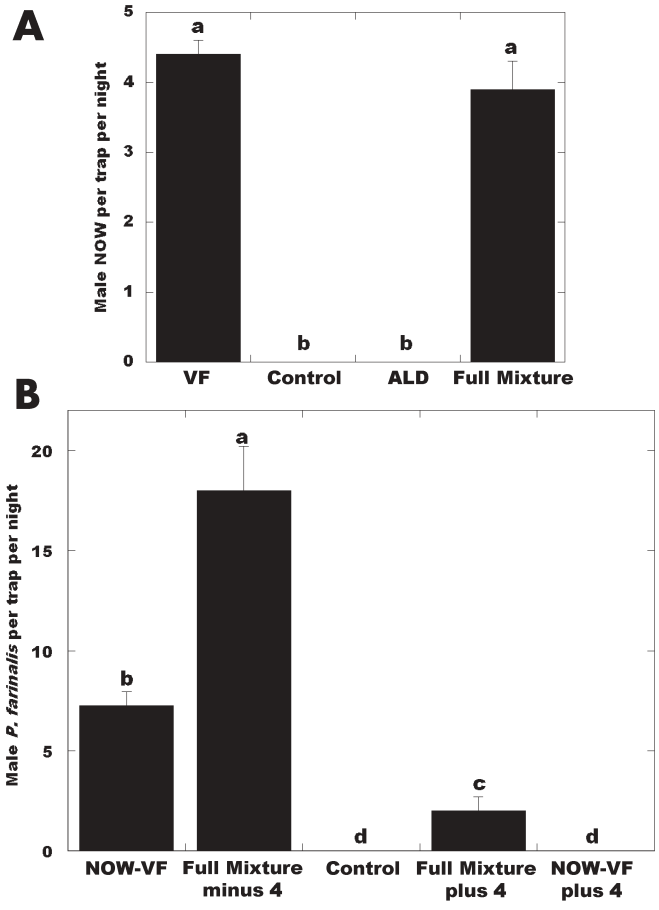


Fig. 3a, b Captures of the navel orangeworm and meal moth in traps baited with virgin females of the navel orangeworm and synthetic pheromone mixtures. **a** Catches of male navel orangeworm in traps baited with the previously identified constituent (ALD), full pheromone mixture and virgin female. **b** Catches of the meal moth in Davis, California in traps baited with virgin females of the navel orangeworm and pheromone mixtures. Note that catches of the meal moth in traps baited with virgin females of the navel orangeworm are completely halted by the addition of **4**, (*Z*,*Z*)-11,13-hexadecadien-1-yl acetate. Captures in traps loaded with the synthetic mixture devoid of **4** were significantly higher than in traps baited with virgin females of the navel orangeworm, indicating that the natural behavioral antagonist fends off the male meal moth to some extent. Treatments with the *same letters* are not significantly different at the 5% level according to the Tukey–Kramer honestly significant difference. Means of captures are untransformed, and *error bars* show 1 SE

whereas traps baited with the single pheromone constituent and control traps captured no moths in a 3-week period of tests. In some locations, traps baited with virgin females of the navel orangeworm captured also males of the meal moth, *P. farinalis*. Interestingly, catches of the meal moth were significantly smaller when traps were baited with a synthetic sample containing the full pheromone system. Tests with partial mixtures showed that removal of (*Z*,*Z*)-11,13-hexadecadien-1-yl acetate increased dramatically captures of male meal moth (Fig. 3b). We, therefore, suggest that this compound is a behavioral antagonist, which may not be strong enough in the natural pheromone to completely repel the meal moth. This assumption is supported by complete lack of captures in traps baited with virgin females and boosted with a synthetic sample (0.5 mg/device) of the acetate. In addition, GC–EAD experiments utilizing antennae of male meal moth captured in the pheromone traps confirmed that *P. farinalis* males do possess detectors tuned to (*Z*,*Z*)-11,13-hexadecadien-1-yl acetate.

Fig. 2a–d Mass spectrometry (MS) and vapor-phase infrared (IR) data of the novel natural products. **a** MS of ethyl (*Z*,*Z*)-11,13-hexadecadienoate. **b** MS of (*Z*,*Z*)-11,13-hexadecadien-1-yl acetate. **c** MS of (*Z*,*Z*,*Z*,*Z*,*Z*)-3,6,9,12,15-tricosapentaene; IR data of the synthetic and natural (*inset*) compound. **d** MS data of (*Z*,*Z*,*Z*,*Z*,*Z*)-3,6,9,12,15-pentacosapentaene

In summary, we have identified, synthesized, and evaluated in the field formulations of the complex pheromone system of *A. transitella.* When one of the constituents is removed, this pheromone system is also attractive to *P. farinalis*. Previously, only one constituent of the pheromone of these species was known. We hope that a more complete pheromone system may improve the efficacy of mating disruption of the navel orangeworm, the potential for which has been previously demonstrated (Landolt et al. 1981; Curtis and Clark 1984; Curtis et al. 1985; Shorey and Gerber 1996). The discovery of novel pheromone constituents belonging to two biosynthetically dichotomous (de novo vs. derived from dietary fatty acids) groups may shed more light on our understanding of pheromone biosynthesis in moths.

Note Added in Proof Thorough analysis of highly concentrated gland extracts (ca. 1000 female-equivalent per injection) led to the identification of trace amounts of three additional EAD-active compounds. They were characterize as (Z)-11-hexadecenal, (Z)-13-hexadecenal, and (Z,Z)-11,13-hexadecadien-1-ol, which appeared in GC-MS at 14.27, 14.39, and 15.57 min, respectively. The addition of trace amounts of these compounds to synthetic lures further improved trap performance.

Acknowledgements This work was supported in part by a research agreement between Bedoukian Research Incorporated and the University of California, the California Pistachio Commission and the Almond Board of California. We thank Dr Yuko Ishida for his assistance in keeping the navel orangeworm colony. Mention of trade names or commercial products in this article are solely for the purpose of providing scientific information and do not imply recommendation or endorsement by the U.S. Department of Agriculture.

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