(2*S*,12*Z*)-2-Acetoxy-12-heptadecene: Major Sex Pheromone Component of Pistachio Twig Borer, *Kermania pistaciella*

**Regine Gries · Grigori Khaskin · Hassan Daroogheh ·
Cafer Mart · Serpil Karadag · M. Kubilay Er ·
Robert Britton · Gerhard Gries**

Abstract The sex pheromone of the pistachio twig borer, *Kermania pistaciella* (Lepidoptera: Oinophilidae), one of the most important insect pests of pistachio, *Pistacia vera*, in Turkey and Iran, was identified. In gas chromatographic-electroantennographic detection (GC-EAD) and GC-mass spectrometric analyses of pheromone gland extracts of female *K. pistaciella* from Turkey, (2*S*,12*Z*)-2-acetoxy-12-heptadecene was identified as the major candidate pheromone component. In field experiments in Turkey, lures containing synthetic (2*S*,12*Z*)-2-acetoxy-12-heptadecene attracted large numbers of male moths. Its attractiveness was significantly reduced by the presence of the *R*-enantiomer or of either enantiomer of the corresponding alcohol. (2*S*,12*Z*)-2-Acetoxy-12-heptadecene is the first pheromone component identified in the Oinophilidae and the first secondary acetate pheromone component identified in the Lepidoptera.

Keywords Pistachio twig borer · *Kermania pistaciella* · (2*S*,12*Z*)-12-Heptadecen-2-ol · (2*R*,12*Z*)-12-Heptadecen-2-ol · (2*S*,12*Z*)-2-Acetoxy-12-heptadecene · (2*R*,12*Z*)-2-Acetoxy-12-heptadecene · Sex pheromone · Secondary acetate

R. Gries · G. Khaskin · G. Gries (✉)
Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia,
Canada, V5A 1S6
e-mail: gries@sfu.ca

C. Mart · M. K. Er
Department of Crop Protection, Faculty of Agriculture, Kahramanmaras Sutcu Imam University,
Kahramanmaras, Turkey

S. Karadag
Pistachio Research Institute, Gaziantep, Turkey

R. Britton
Department of Chemistry, Simon Fraser University, Burnaby, British Columbia, Canada, V5A 1S6

Present Address:
H. Daroogheh
Nelson Ave, West Vancouver, British Columbia, Canada, V7V 2R6

Introduction

The pistachio twig borer, *Kermania pistaciella* (Lepidoptera: Oinophilidae), is one of the most important insect pests in plantations of pistachio, *Pistacia vera*, in Turkey and Iran. Females lay eggs close to shoot tips and fruit clusters. Larvae bore into and feed on terminal buds and in twigs and shoots formed the previous year. Larval feeding causes abscission of fruit buds and die-back of twigs (Küçükarslan 1966) and may directly damage fruit clusters (Mart et al., 1995). Pupation takes place in cocoons attached to the branch surface near larval exit holes. After 20–25 days, adults eclose, and females lay up to 60 eggs. The 1-month flight period of first-generation adults occurs in April–May (Küçükarslan 1966).

Many pistachio plantations in Turkey and Iran are treated annually with insecticides to reduce damage caused by *K. pistaciella* larvae. For example, in southeast Turkey in 2005, 548,600 trees were sprayed with insecticides to suppress *K. pistaciella* populations (Anonymous 2005). Identification of the *K. pistaciella* sex pheromone might be a first step toward pheromone-based control tactics (e.g., attract and kill) of *K. pistaciella* populations. We report the identification, synthesis, and field testing of (2S,12Z)-2-acetoxy-12-heptadecene as the major component of the sex pheromone of *K. pistaciella*.

Methods and Materials

Experimental Insects In early March 2001, in several locations of the Nizip province of Gaziantep (Turkey), sections of pistachio tree branches bearing pupal cocoons were cut off and sent under import permit from Agriculture and Agri-Food Canada (Food Production and Inspection Branch) to Simon Fraser University. Insects were kept at 24°C in mesh cages under a photoperiod of 13-hr light/11-hr dark. Eclosed males were transferred to and kept individually in filter paper-lined Petri dishes. The abdominal tip with pheromone gland of 1- to 2-d-old females was removed and extracted with hexane for 15 min.

Analyses of Extracts Aliquots of 1 female equivalent (FE) of such extracts were analyzed by coupled gas chromatographic-electroantennographic detection (GC-EAD; Arn et al., 1975; Gries et al., 2002), employing a Hewlett-Packard (HP) 5890 gas chromatograph fitted with a column (30 m×0.25 or 0.32 mm ID) coated with DB-5, DB-23, or DB-210 (J&W Scientific, Folsom, CA, USA). Helium was used as carrier gas (35 cm/sec), with temperature programs described in the captions of Figs. 2 and 4. GC-mass spectrometry (MS) of compounds eliciting responses from antennae and of synthetic standards employed a Saturn 2000 Ion trap GC-MS (Varian Instruments) fitted with the DB-5 column referred to above.

Instrumentation Nuclear magnetic resonance (NMR) spectra of synthetic compounds were taken on a Varian AS500 spectrometer at 499.77 MHz for ^{1}H and 125.68 MHz for ^{13}C spectra with chemical shifts reported in parts per million relative to tetramethylsilane (^{1}H, δ 0.00) and CDCl$_3$ (^{13}C, δ 77.00). Elemental analyses were performed using a Carlo-Erba model 1106 elemental analyzer. Optical rotations were measured with a Perkin-Elmer 341 polarimeter.

The major candidate pheromone component in gland extracts was isolated by high-performance liquid chromatography (HPLC), employing a Waters LC 625 HPLC equipped with a Waters 486 variable wavelength UV–visible detector set at 210 nm, HP Chemstation

software (Rev.A.07.01), and a reverse phase Nova Pak® C_{18} (3.9×300 mm) column (Waters) eluted with acetonitrile (1 ml/min).

The absolute configuration of the HPLC-isolated natural pheromone component **B** and the enantiomeric excess of synthetic standards were determined by analyses of samples on a custom-made chiral GC column coated with a 1:1 mixture of heptakis-(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin and OV-1701 (König et al., 1992; Pietruszka et al., 1992).
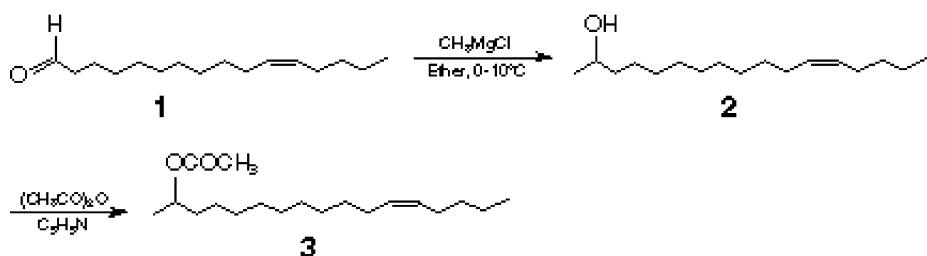
Syntheses

(*Z*)-12-Heptadecen-2-ol (compound **2** in scheme 1, Fig. 1). Racemic (*Z*)-12-heptadecen-2-ol was synthesized starting with (*Z*)-11-hexadecenal (**1**, scheme 1, Fig. 1; Bedoukian Research, Danbury, CT, USA). Compound **1** (225 g, 0.915 mol) in 400 ml of ether was added dropwise under argon at 0–10°C in 3 hr to a stirred solution of methylmagnesium chloride (3 M in ether; 333 ml, 1 mol). The mixture was allowed to warm to room temperature, quenched with 200 ml of saturated aqueous NH_4Cl, and extracted with ether (3×200 ml). Extracts were combined, washed with brine, dried (anhydrous $MgSO_4$), filtered, and evaporated *in vacuo*, affording 233 g of (*Z*)-12-heptadecen-2-ol (**2**, 99% pure by GC, 0.906 mol, 99% yield) as a slightly yellowish oil. ^{1}H NMR (CDCl₃) δ: 0.89 (t, 3H, *J*=7.1 Hz), 1.18 (d, 3H, *J*=6.3 Hz), 1.21–1.49 (m, 24H), 2.05 (m, 1H), 3.78 (m, 1H), 5.34 (m, 2H). ^{13}C NMR (CDCl₃) δ: 129.84, 129.82, 68.15, 39.34, 31.94, 29.74, 29.63, 29.59, 29.55, 29.51, 29.27, 27.16, 26.88, 25.76, 23.45, 22.32, 13.98. Anal. calcd. for $C_{17}H_{34}O$ (%): C, 80.24; H, 13.47; found C, 79.96; H, 13.24.
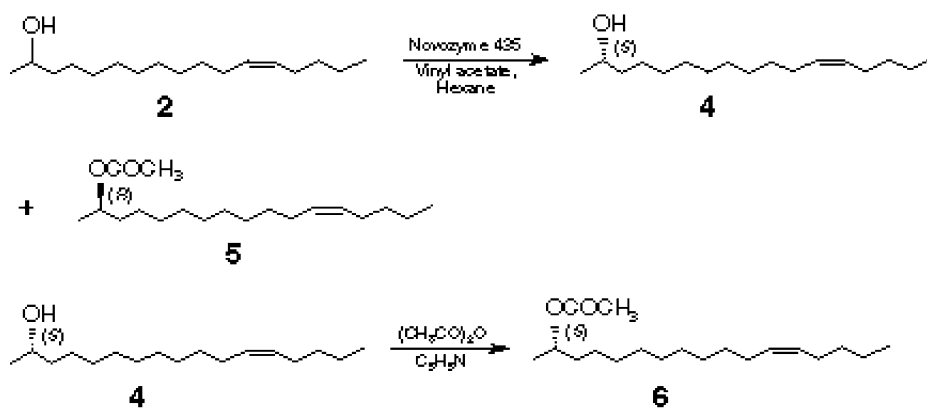
(*Z*)-2-Acetoxy-12-heptadecene (compound **3** in scheme 1, Fig. 1). Racemic (*Z*)-2-acetoxy-12-heptadecene was synthesized by adding 94 ml (0.99 mol) of acetic anhydride dropwise at room temperature to a stirred solution of 233 g (0.906 mol) of alcohol **2** in 100 ml (1.23 mol) of pyridine. After stirring for 6 hr, the reaction was quenched with water, and the product was extracted with ether/hexane (1:1) (3×250 ml). Combined extracts were washed successively with saturated aqueous $NaHCO_3$, HCl (10%), water, and brine, then dried (anhydrous $MgSO_4$). Filtration and removal of solvents afforded 270 g of (*Z*)-2-acetoxy-12-heptadecene (**3**, 99.5% pure by GC, quantitative yield). ^{1}H NMR (CDCl₃) δ: 0.87 (t, 3 H, *J*=7.0 Hz), 1.18 (d, 3 H, *J*=6.3 Hz), 1.21–1.35 (m, 22 H), 1.50 (m, 2 H), 2.00 (s, 3 H), 4.86 (m, 1 H), 5.33 (m, 2 H). ^{13}C NMR (CDCl₃) δ: 170.68, 129.79, 129.77, 70.99, 35.88, 31.92, 29.72, 29.50, 29.49, 29.47, 29.42, 29.24, 27.14, 26.87, 25.37, 22.30, 21.31, 19.90, 13.94. Anal. calcd. for $C_{19}H_{36}O_2$ (%): C, 80.24; H, 13.47; found C, 79.96; H, 13.24.

(2*S*,12*Z*)-12-Heptadecen-2-ol, (2*R*,12*Z*)- and (2*S*,12*Z*)-2-acetoxy-12-heptadecene (compounds **4**, **5**, and **6** in scheme 2, Fig. 1). Enantioselective syntheses of these compounds were initiated by adding 70 mg of immobilized lipase Novozym 435 (10,000 units per gram, Sigma Chemical, St. Louis, MO, USA; Xiao and Kitazume, 1997) to a stirred solution of 0.600 g of racemic alcohol **2** (2.36 mmol; scheme 2, Fig. 1) and 0.53 ml of vinyl acetate (5.75 mmol, Aldrich Chemical, Milwaukee, WI, USA) in 3 ml of hexane. After stirring for 4 hr at 40°C, the Novozym-containing resin was filtered off, solvents were evaporated *in vacuo*, and alcohol **4** (0.280 g) and acetate **5** (0.340 g) were separated by flash chromatography [10 g of SiO_2, ether/hexane (1:20) as eluent]. Subsequent acetylation of alcohol **4** with acetic anhydride in pyridine and usual work-up (see above) afforded 0.320 g (1.08 mmol) of (2*S*,12*Z*)-2-acetoxy-12-heptadecene [**6**, 99.5% pure, 45.8% yield (theory: 50%)], $[\alpha]_D^{20} = +0.83°$ (c 4.5; CHCl₃). The NMR spectrum of acetate **6** was consistent with that of racemic acetate **3**. The enantiomeric excesses (ee) of **4**, **5**, and **6** were
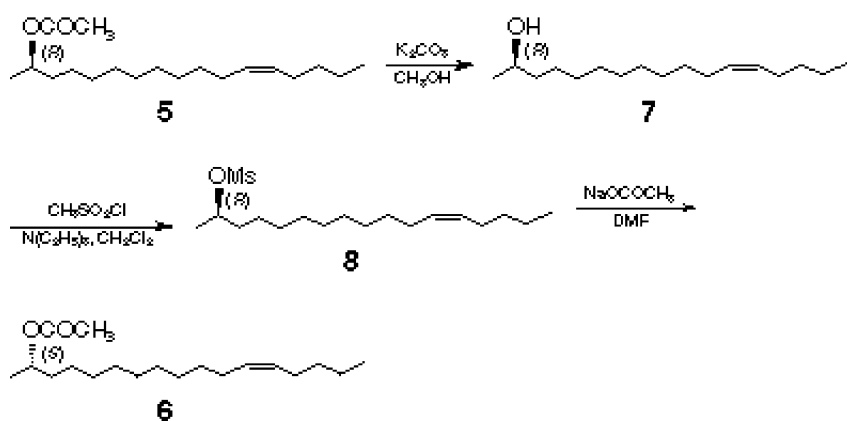
SCHEME 1

1 → (CH₃MgCl / Ether, 0–10°C) → **2**

(CH₃CO)₂O / C₅H₅N → **3**

SCHEME 2

2 → (Novozyme 435 / Vinyl acetate, Hexane) → **4** (2S,12Z)

+

5 (2R)

4 (S) → (CH₃CO)₂O / C₅H₅N → **6** (S)

SCHEME 3

5 (R) → (K₂CO₃ / CH₃OH) → **7** (R)

CH₃SO₂Cl / N(C₂H₅)₃, CH₂Cl₂ → **8** (R) OMs → (NaOCOCH₃ / DMF)

6 (S)

Fig. 1 Synthesis of racemic (Z)-12-heptadecen-2-ol (**2**) and (Z)-2-acetoxy-12-heptadecene (**3**) (scheme 1); enantioselective syntheses of (2S,12Z)-heptadecen-2-ol (**4**), (2R,12Z)-2-acetoxy-12-heptadecene (**5**) and (2S,12Z)-2-acetoxy-12-heptadecene (**6**) (scheme 2); and synthesis of (2S,12Z)-2-acetoxy-12-heptadecene (**6**) from (2R,12Z)-2-acetoxy-12-heptadecene (**5**) (scheme 3)

99, 95, and 99%, respectively, as determined by gas chromatography that separated enantiomers with baseline resolution (Fig. 2).

Additional quantities of **6** were obtained by a three-step synthesis (scheme 3, Fig. 1), based on inversion of the absolute configuration of **5**. A mixture of **5** (0.280 g) and K_2CO_3 (1.0 g) in methanol (10 ml) was stirred for 18 hr at room temperature. After removal of 9 ml of methanol *in vacuo*, ether (20 ml) and water (5 ml) were added to the reaction mixture. The organic layer was washed with water and brine, dried (anhydrous $MgSO_4$) and solvents were removed *in vacuo* to afford (2R,12Z)-12-heptadecen-2-ol (**7**, 95% pure by GC). Without further purification, alcohol **7** was mesylated at 0°C with methanesulfonyl chloride (1.5 ml) in the presence of triethylamine (2 ml) in dichloromethane (10 ml). After 1 hr, the reaction was quenched with aq. $NaHCO_3$. Extraction with ether (50 ml), washing of the extract with 10% HCl, water, and brine, and drying and evaporation of solvent afforded the (*R*)-mesylate **8**. Without further purification, mesylate **8** was stirred for 96 hr at 60–70°C in dry dimethylformamide (10 ml) with 3 g of sodium acetate. Usual work-up of the reaction mixture and purification of the product by flash chromatography yielded 0.180 g of pure **6** with 90% ee. Overall yield based on **5** was 64%.

Assignment of the absolute configuration of **6** was confirmed by an alternative synthesis, which comprised converting (*Z*)-1-bromo-9-tetradecene to the Grignard reagent and then to the monocuprate, adding (*S*)-propylene oxide and acetylating the resulting alcohol **4**. This
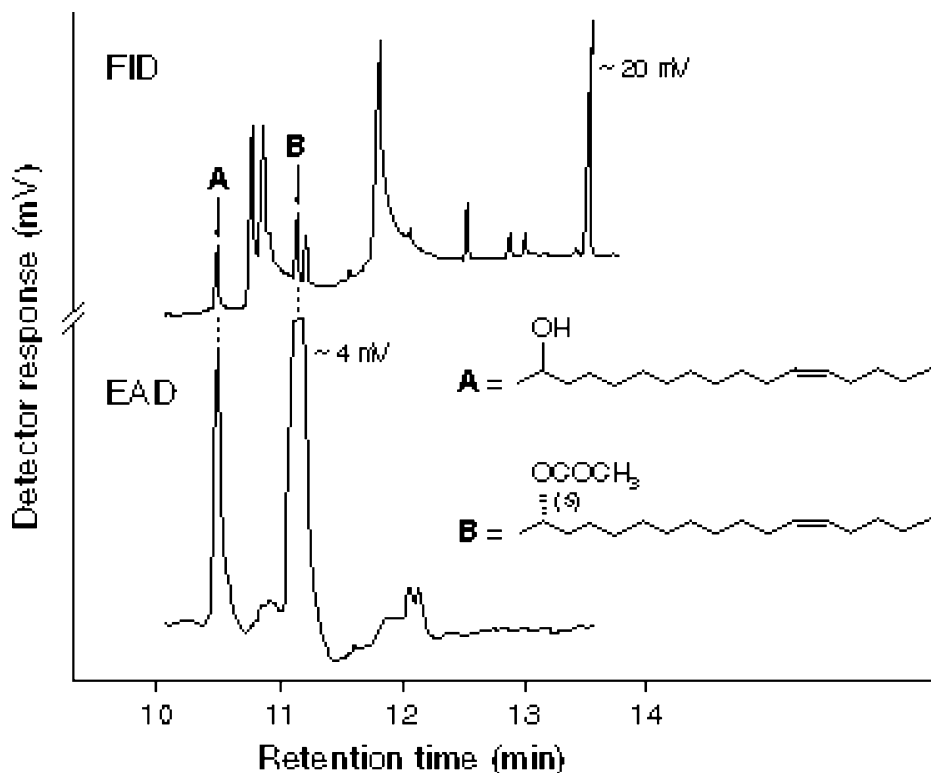

Fig. 2 Representative recording of flame ionization detector (FID) and electroantennographic detector (EAD: male *Kermania pistaciella* antenna) responses to one female equivalent of female *K. pistaciella* pheromone extract. Chromatography: DB-5 column; splitless injection, temperature of injection port and FID: 240°C; temperature program: 1 min at 50°C, then 20°C/min to 280°C

approach also proved conducive to large-scale synthesis of **6** (Britton and Khaskin, unpublished data).

Acetoxyheptadecanes (Table 1) were produced from the corresponding alcohols synthesized by Grignard reactions of suitable aldehydes with *n*-alkylmagnesium bromides. 2-Acetoxyheptadecenes with various (*E*)- and (*Z*)-double bond positions (Table 1) were synthesized from the corresponding hexadecenals according to scheme 1.

Field Experiments Field experiments were conducted in a pistachio orchard of the Gaziantep Pistachio Research Institute in Gaziantep, Turkey. Experiments employed a randomized complete block design with 8–10 replicates each. Delta-like traps were made from 2-L milk carton (Gray et al., 1984), coated with Tanglefoot (The Tanglefoot, Grand Rapids, MI, USA) and suspended from trees ~1.5 m above ground with ~16-m spacing. Traps were baited with a gray sleeve stopper (West Pharmaceutical Services, Lionville, PA, USA) impregnated with candidate pheromone components in HPLC-grade hexane.

Experiment 1 tested whether racemic (*Z*)-2-acetoxy-12-heptadecene was effective in attracting male *K. pistaciella*. Experiment 2 tested whether the corresponding alcohol, (*Z*)-12-heptadecen-2-ol, enhanced attractiveness of the acetate or was attractive by itself. Experiment 3 explored which enantiomer of (*Z*)-2-acetoxy-12-heptadecene was attractive and whether there were synergistic or inhibitory interactions between enantiomers. Considering the inhibitory nature of the *R*-enantiomer in experiment 3, experiment 4 retested the *S*-enantiomer by itself and in combination with either one or both enantiomer(s) of (*Z*)-12-heptadecen-2-ol. Doses used in field experiments are shown in Fig. 5.

Trap catch data were subjected to nonparametric analysis of variance by ranks (Friedman's test) followed by comparison of means (Scheffé test; Zar 1984; SAS/STAT 1988).

Table 1 Retention indices (RI) of synthetic standards and of components **A** and **B** in pheromone gland extracts of female *Kermania pistaciella*

Compounds	RI on GC column		
	DB-23	DB-5	DB-210
A in Fig. 2	2,376	1,890	2,093
B in Fig. 2	2,355	2,011	2,290
B1 (= hydrogenated **B**)	2,309	2,021	2,286
(*Z*)-12-Heptadecen-2-ol	2,376	1,890	2,093
(*Z*)-2-Acetoxy-12-heptadecene	2,355	2,011	2,290
(*Z*)-2-Acetoxy-10-heptadecene	2,342	2,002	2,281
(*Z*)-2-Acetoxy-11-heptadecene	2,348	2,007	2,284
(*E*)-2-Acetoxy-13-heptadecene	2,342	2,014	2,276
(*Z*)-2-Acetoxy-13-heptadecene	2,363	2,019	2,297
Heptadec-1-yl acetate	2,450	2,105	2,386
2-Acetoxyheptadecane (= **B1**)	2309	2021	2286
3-Acetoxyheptadecane[a]	2284	2001	2262
4-Acetoxyheptadecane[a]	2254	1981	2237
5-Acetoxyheptadecane[a]	2240	1970	2226
6-Acetoxyheptadecane[a]	2231	1965	2217
7-Acetoxyheptadecane[a]	2227	1962	2215

[a] Mass spectra of 2-, 3-, 4-, 5-, 6-, and 7-acetoxyheptadecane did not have fragment ions diagnostic of the acetoxy position.

Results and Discussion

GC-EAD analyses of pheromone gland extracts from female *K. pistaciella* revealed two components (**A** and **B**) that elicited strong responses from male *K. pistaciella* antennae (Fig. 2). The mass spectrum of compound **B** (Fig. 3) with diagnostic fragment ion *m/z* 61 indicated an acetate functionality. Moreover, fragment ion *m/z* 236 [MW of $C_{19}H_{36}O_2$ (296)− 60], instead of *m/z* 238 [MW (298)−60] expected for a saturated compound (Table 1), suggested that compound **B** had one double bond.

Hydrogenation (Millar and Haynes, 1998 and references cited therein) of pheromone gland extract, followed by repeated GC-EAD and GC-MS analyses, revealed a new EAD-active compound (**B1**) with different retention characteristics (Table 1). **B1** did not co-chromatograph with synthetic heptadec-1-yl acetate, suggesting that it might be a saturated secondary or tertiary acetate. It was determined to be 2-acetoxyheptadecane by GC-MS analyses and comparison of its GC retention indices (Table 1) with those of seven synthetic acetoxyheptadecanes (acetoxy group at C1, C2, C3, C4, C5, C6, and C7, respectively).

To determine the position of the double bond in 2-acetoxyheptadecene (**B**), HPLC-isolated **B** was treated with dimethyl disulfide (DMDS; Dunkelblum et al., 1985). The mass spectrum of the DMDS-derivative (MW=390) had adduct fragment ions [*m/z* 117 (26%) $(CH_3-S=CH-(CH_2)_3-CH_3)^+$; *m/z* 213 (100%) $(273-HOAc)^+$; *m/z* 273 (15%) $(CH_3-CHOAc-(CH_2)_9-CH=S-CH_3)^+$; and *m/z* 390 (M⁺, 16%)] that revealed a double bond at C12. Synthetic (*Z*)-2-acetoxy-12-heptadecene, but not the (*E*)-isomer, and none of (*Z*)-2-acetoxy-10-heptadecene, (*Z*)-2-acetoxy-11-heptadecene, and (*Z*)- or (*E*)-2-acetoxy-13-heptadecene co-chromatographed with female-produced **B** (Table 1).

Comparative GC analyses of synthetic racemic (*Z*)-2-acetoxy-12-heptadecene, the synthetic *S*-enantiomer, and insect-produced **B** revealed that female *K. pistaciella* produces the *S*-enantiomer of **B** (Fig. 4). Component **A** (Fig. 2) had retention indices (Table 1)
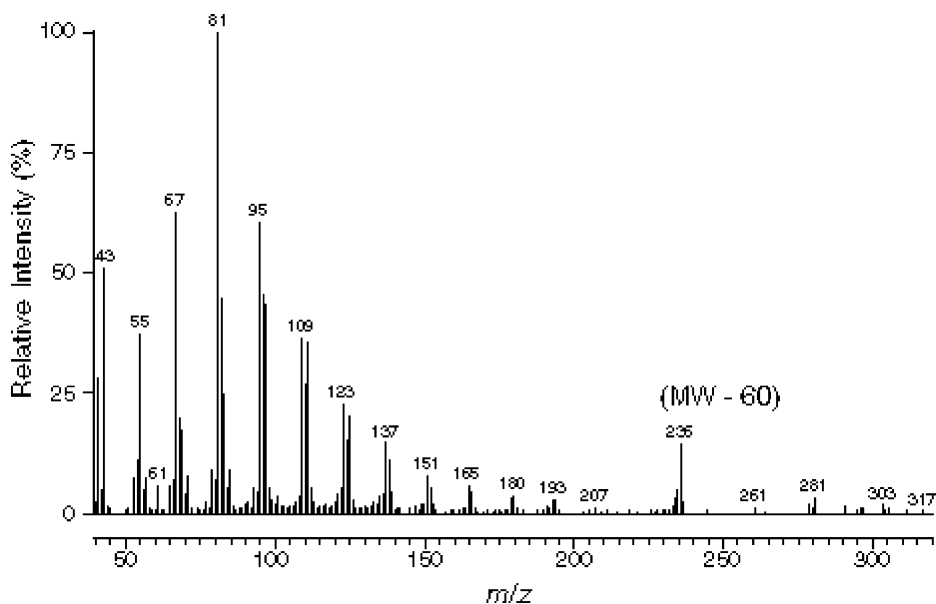

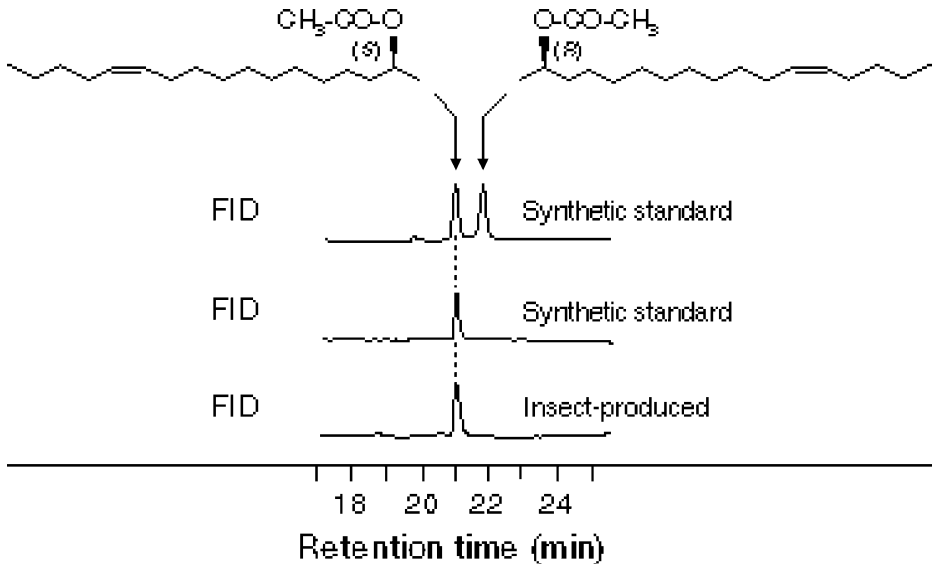**Fig. 3** Ion trap mass spectrum of compound **B** in Fig. 2 (compound **6** in Fig. 1)

Fig. 4 Flame ionization detector (FID) chromatograms of racemic (Z)-2-acetoxy-12-heptadecene (**3** in scheme 1, Fig. 1), (2S,12Z)-2-acetoxy-12-heptadecene (**6** in schemes 2, 3; Fig. 1); and HPLC-isolated, insect-produced **B** (Fig. 2); chromatography: custom-made GC column coated with a 1:1 mixture of heptakis-(2,6-di-O-methyl-3-O-pentyl)-β-cyclodextrin and OV-1701 (König et al., 1992; Pietruszka et al., 1992); splitless injection; temperature of injection port and FID, respectively: 240 and 250°C; temperature program: 1 min at 100°C, then 10°C/min to 180°C (20 min)

indicative of an alcohol functionality. It was shown to be (Z)-12-heptadecen-2-ol by comparison of its GC and GC-MS data with those of authentic **2**.

In field experiment 1, traps baited with racemic (Z)-2-acetoxy-12-heptadecene (**3**) captured large numbers of male *K. pistaciella* (Fig. 5). The corresponding alcohol, (Z)-12-heptadecen-2-ol (**2**), was not attractive, and a 1:1 (w/w) mixture of **2** and **3** (as found in pheromone gland extracts) was significantly less attractive than **3** alone (Fig. 5, experiment 2). However, the addition of different ratios of alcohol **2** to the acetate **3** may have enhanced, instead of reduced, the attractiveness of the lure. This remains to be determined by further field tests. (2S,12Z)-2-Acetoxy-12-heptadecene attracted many males, whereas the R-enantiomer was not attractive and, when added to the S-enantiomer, significantly reduced its attractiveness (Fig. 5, experiment 3). Attractiveness of the S-acetate was reduced by either or both enantiomers of the corresponding alcohol (Fig. 5, experiment 4).

(2S,12Z)-2-Acetoxy-12-heptadecene is the first secondary acetate reported as a sex pheromone component in the Lepidoptera. In contrast, secondary alcohols have been identified in several species of the Lepidoptera, including *Stigmella malella* [(S)-(E)-6,8-nonadien-2-ol and (S)-(Z)-6,8-nonadien-2-ol (Tóth et al., 1995)], *Eriocrania cicatricella* [(2R)-heptan-2-ol (Zhu et al., 1995)], *Eriocrania sangii* [(2S,6Z)-nonen-2-ol (Kozlov et al., 1996)], *Eriocrania semipurpurella* [(2S,6Z)-nonen-2-ol and (2R,6Z)-nonen-2-ol (Kozlov et al., 1996)], and *Orgyia detrita* [(11S,6Z,9Z)- and (11R,6Z,9Z,)-6,9-heneicosadien-11-ol (Gries et al., 2003)].

Straight-chain secondary acetate sex pheromones also commonly occur in cecidomyiid midges, including the Hessian fly *Mayetiola destructor* [(2S,10E)-2-acetoxy-10-tridecene; Foster et al., 1991; Harris and Foster, 1991; Millar et al., 1991], pea midge *Contarinia pisi* [(S,S)-2,11-diacetoxytridecane, (S,S)-2,12-diacetoxytridecane and 2-acetoxytridecane;
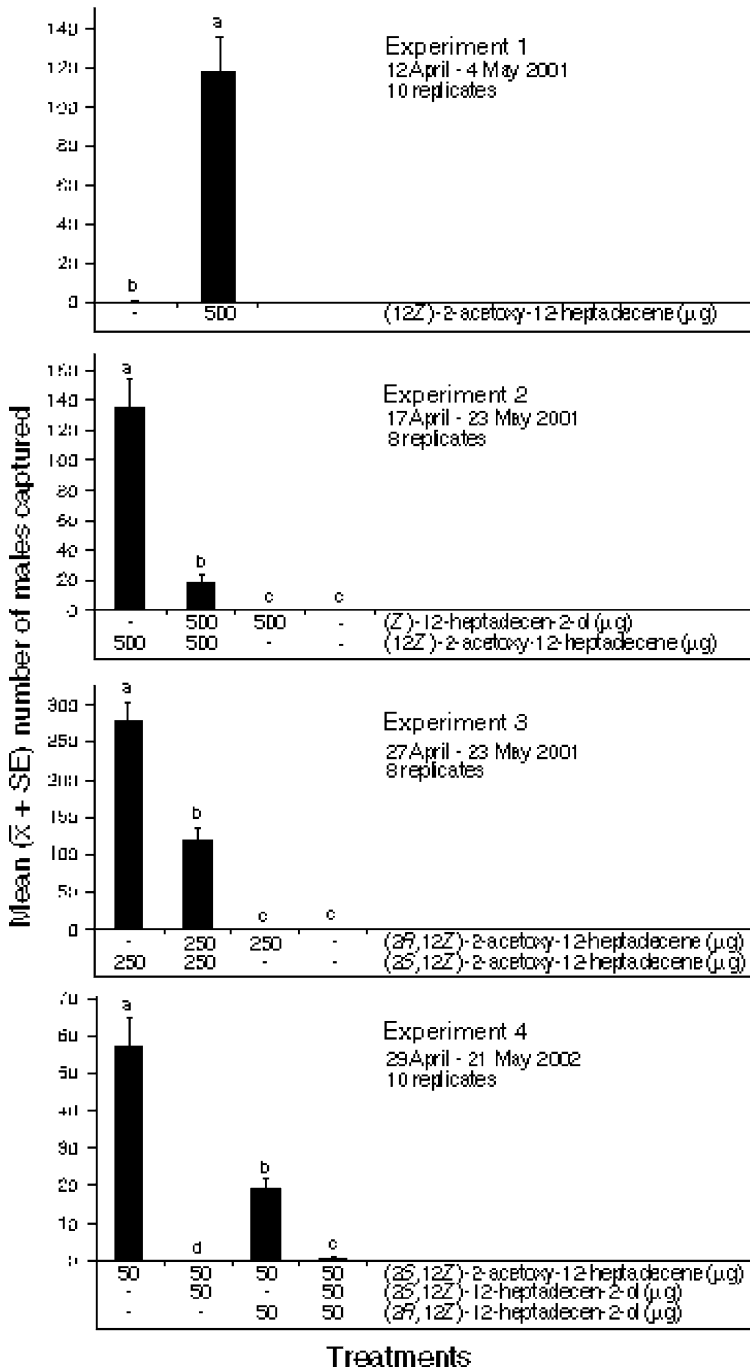
Fig. 5 Mean numbers (+SE) of male *Kermania pistaciella* captured per experimental period in experiments 1–4 in sticky traps baited with various candidate pheromone components, placed in pistachio orchards of the Gaziantep Pistachio Research Institute in Gaziantep, Turkey. In each experiment, bars with different letter superscripts are significantly different; nonparametric analysis of variance by ranks (Friedman's test) followed by comparison of means (Scheffé test), $P<0.05$ (Zar 1984; SAS/STAT 1988)

Hillbur et al., 1999, 2000, 2001], Douglas fir cone gall midge *Contarinia oregonensis* [(2*S*,4*Z*,7*Z*)-2-acetoxy-4,7-tridecadiene; Gries et al., 2002], *Aphidoletes aphidimyza* [(*R*,*S*)-2,7-diacetoxytridecane; Choi et al., 2004], swede midge *Contarinia nasturdii* [(*S*,*S*)-2,9-diacetoxyundecane, (*S*,*S*)-2,10-diacetoxyundecane, (*S*)-2-acetoxyundecane; Hilbur et al., 2005], and western red cedar cone midge *Mayetiola thujae* [(*S*,*S*)-2,12-diacetoxyheptadecane, (*S*,*S*)-2,13-diacetoxyheptadecane, (*S*,*S*)-2,14-diacetoxyheptadecane; Gries et al., 2005]. Cecidomyiid midges and *K. pistaciella* belong to discrete taxonomic orders (Diptera and Lepidoptera, respectively) of the Insecta. However, these two orders are related, which might explain the similarity of some of their sex pheromone structures.

With the *K. pistaciella* pheromone identified and shown to attract large numbers of male moths, it may be possible to develop pheromone-based tactics for control of *K. pistaciella*. In a 75-ha experiment in commercial pistachio orchards in Iran in 2005, plots treated with a proprietary attract-and-kill formulation containing chiral pheromone had significantly fewer larvae-infested fruit bunches (>120,000 assessed) than insecticide-treated plots or control plots (unpublished data; two PCT patent applications filed in 2006).

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