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SYNERGISTIC SEX PHEROMONE COMPONENTS OF WHITE-SPOTTED TUSSOCK MOTH, *Orgyia thyellina*

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Abstract—In 1996, the exotic white-spotted tussock moth (WSTM), *Orgyia thyellina* (Lepidoptera: Lymantriidae), was discovered in Auckland, New Zealand. Because establishment of WSTM would threaten New Zealand's orchard industry and international trade, eradication of WSTM with microbial insecticide was initiated. To monitor and complement eradication of WSTM by capture of male moths in pheromone-baited traps, pheromone components of female WSTM needed to be identified. Coupled gas chromatographic–electroantennographic detection analysis of pheromone gland extract revealed several compounds that elicited responses from male moth antennae. Mass spectra of the two most EAD-active compounds suggested, and comparative GC-MS of authentic standards confirmed, that they were (*Z*)-6-heneicosen-11-one (*Z*6–11-one) and (*Z*)-6-heneicosen-9-one, the latter termed here "thyellinone." In field experiments in Japan, *Z*6–11-one plus thyellinone at a 100:5 ratio attracted WSTM males, whereas either ketone alone failed to attract a single male moth. Addition of further candidate pheromone components did not enhance attractiveness of the binary blend. Through the 1997–1998 summer, 45,000 commercial trap lures baited with 2000 μg of *Z*6–11-one and 100 μg of thyellinone were deployed in Auckland towards eradication of the residual WSTM population.

Key Words—Lepidoptera, Lymantriidae, white-spotted tussock moth, *Orgyia thyellina*, (*Z*)-6-heneicosen-11-one, (*Z*)-6-heneicosen-9-one, (*Z*)-6,(*E*)-8-

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[4]Dedicated to my dear mother in honor of her 75th birthday.

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heneicosadien-11-one, sex pheromone, synergism, quarantine insect, international trade, eradication, *Bacillus thuringiensis*, microbial insecticide.

INTRODUCTION

In April 1996, white-spotted tussock moths (WSTM), *Orgyia thyellina* (Lepidoptera: Lymantriidae), were discovered in suburbs of Auckland City, New Zealand. WSTM is native to eastern Asia, including Japan (Inoue, 1956), Korea (Kim et al., 1982), the Russian Far East (Kozhanchikov, 1950), eastern China (Zhao, 1982), and Taiwan (Inoue, 1956, but see Kishida, 1992). Caterpillars feed on a variety of host plants in the rose (Rosaceae), willow (Salicaceae), birch (Betulaceae), legume (Fabaceae), oak (Fagaceae), mulberry (Moraceae), ebony (Ebenacae), elm (Ulmacae), and maple (Aceraceae) families (Kozhanchikov, 1950; Kim et al., 1982). In Japan and northern populations, there are two or three generations per year in which female WSTM show wing dimorphism. They are fully winged and flight capable in the spring or summer generation(s), but wingless and mainly sedentary in the fall generation (Inoue, 1956; Kimura and Masaki, 1977). With this considerable potential to disperse and the wide range of host plants, WSTM's spread and establishment throughout New Zealand was very likely. WSTM could become New Zealand's most serious defoliator of pip, stone, and berry fruits and may also become a serious pest of urban amenity plantings. Because female WSTM oviposit also on wooden crates, pallets, logs, containers, and other export material, and because egg masses are resilient, establishment of WSTM would likely affect New Zealand's international trade. A direct risk from WSTM to human health stems from the urticating hairs produced by caterpillars. Scientists working with the moth developed respiratory problems and rashes. Once sensitized, people exposed to the hairs may develop symptoms at increasingly low exposure. Furthermore, establishment of WSTM in urban environments would lead to increased and widespread use of chemical insecticides by both local authorities and home owners (Hosking, 1997).

In October 1996, New Zealand's Ministry of Forestry launched "Operation Ever Green" aimed at eradicating WSTM. This eradication program used a combination of aerial and ground spraying of the microbial insecticide *Bacillus thuringiensis* var. *kurstaki* (*Btk*). In the first phase of the operation (October–December 1996), 40 km^2 of Auckland were treated nine times from the air with a DC-6 aircraft and ~300 properties were sprayed from the ground using mist blowers. In the second phase (January–April 1997), 3 km^2 were treated 14 times with a helicopter, and ~200 properties were sprayed from the ground (Hosking, 1997).

Capture of male moths in traps baited with WSTM females were employed to assess progress towards eradication of WSTM. However, supply of female

moths for trapping of males was interrupted when a virus infection caused collapse of the WSTM colony reared in a quarantine facility. If sex pheromone components of female WSTM were known, synthetic pheromone baits—instead of female moths—could be emloyed as trap baits. Large numbers of pheromone-baited traps would provide a continuous measure of the residual WSTM population and would complement other eradication efforts, such as *Btk* sprays. We report identification and field testing of WSTM sex pheromone components.

METHODS AND MATERIALS

Insect Culture and Pheromone Extraction

WSTM caterpillars were reared on apple foliage (photoperiod: 14L : 10D; temperature: 24°C; relative humidity: 55–60%) at the Forestry Research Institute Rotorua, Auckland, New Zealand. Abdominal tips with pheromone glands of calling, 1- to 2-day-old virgin female moths were removed and placed in redistilled hexane. After 30–60 min of extraction, the supernatant was withdrawn, syringed into ampoules, and shipped (together with male pupae) to Simon Fraser University.

Laboratory Analyses, Instruments, and General Procedures

Aliquots of 1 female equivalent (FE) of pheromone gland extract were analyzed by coupled gas chromatographic–electroantennographic detection (GC-EAD) (Arn et al., 1975), using a Hewlett Packard (HP) 5890A gas chromatograph fitted with an on-column injector and equipped with a fused silica column (30 m × 0.25 or 0.32 mm ID) coated with either DB-210, DB-5, or DB-23 (J&W Scientific, Folsom, CA 95630). GC–mass spectrometry (MS) of synthetic or antennally active WSTM-produced compounds in full scan electron impact mode employed a Varian Saturn II Ion Trap GC-MS fitted with the DB-210 column referred to above. Synthetic (*Z*)-6,(*E*)-8-heneicosadien-11-one (Gries et al., 1997) was purified by high-performance liquid chromatography (HPLC) with a Waters LC 626 high-performance liquid chromatograph equipped with a Waters 486 variable wavelength UV visible detector set to 220 nm, an HP integrator, and a Nova Pak C_{18} (3.9 × 300 mm) column with 1 ml/min of acetonitrile flow. Nuclear magnetic resonance (NMR) spectroscopy of synthetic compounds was conducted on a Bruker AMX-400 spectrometer at 400.13 MHz for ^{1}H NMR spectra. ^{1}H chemical shifts are reported as parts per million (ppm; δ) relative to TMS (0.00 ppm). Elemental analyses were performed using a Carlo Erba model 1106 elemental analyzer. Chemicals obtained from commercial sources were used without further purification unless otherwise indicated. All moisture- and air-sensitive reactions were conducted under argon. Column chromatogra-

phy refers to flash chromatography on silica gel 60 (230–400 mesh, E. Merck, Darmstadt, Germany) (Still et al., 1978).

Syntheses

6-Heneicosyn-9-ol (4) (Figure 1, Scheme 1). 1-Tetradecane (**2**) obtained from myristyl bromide (**1**) with lithium diisopropylamide (LDA) at 0°C was converted with *m*-Cl-peroxybenzoic acid to 1,2-epoxy-tetradecene (**3**) (87% yield). Epoxide **3** (4.50 g, 21.2 mmol) was added under argon to a prepared reaction mixture of heptyne (5.25 ml, 40 mmol), BuLi (40 mmol), and hexamethylphospho rous triamide (HMPA) (30 ml) in 200 ml of dry tetrahydrofuran (THF) at −30°C.

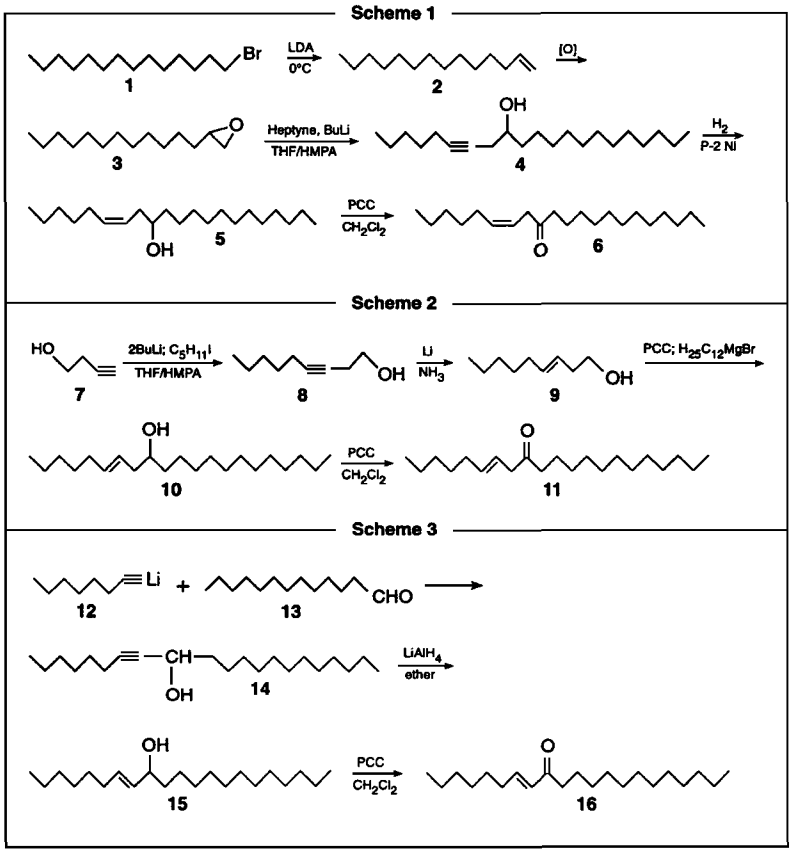

FIG. 1. Schemes for the syntheses of (*Z*)-6-heneicosen-9-one (Scheme 1), (*E*)-6-heneicosen-9-one (Scheme 2), and (*E*)-7-heneicosen-9-one (Scheme 3).

The mixture was gradually warmed to 50°C and kept 4 hr under argon with stirring. It was then cooled to room temperature and quenched with NH_4Cl. It was extracted (3 × 100 ml) with hexanes–ether (1 : 1). The organic layer was washed with saturated NaCl, dried, and purified by column chromatography [hexanes–ether (98 : 2)], and gave **4** (6.35 g, 92% yield; mp: 31°C). Anal. calcd. for $C_{21}H_{40}O$: C, 81.73; H, 13.07; found: C, 81.72; H, 13.02; ^{1}H NMR: 3.68 (m, 1H); 2.40, 2.26 (ab quartet, 2H, split further into double triplets due to long range coupling); 2.17 (tt, 2H); 1.50 (m, 2H); 1.22–1.40 (m, 26H), 0.87 (t, 3H), 0.89 (t, 3H).

(Z)-*6-Heneicosen-9-ol (5) (Figure 1, Scheme 1).* Alcohol **4** (6.30 g, 20 mmol) was hydrogenated with P-2 Ni, and the reaction was monitored by GC. The resulting olefinic alcohol (**5**) was column chromatographed [hexanes–ether (95 : 5)], yielding 5.80 g of **5** with 93.5% chemical purity. Anal. calcd. for $C_{21}H_{42}O$: C, 81.20; H, 13.63; found: C, 80.89; H, 13.97. ^{1}H NMR: 5.56 (m, 1H); 5.40 (m, 1H); 3.61 (m, 1H); 2.21 (dd, 2H); 2.05 (dt, 2H); 1.23–1.48 (m, 28H), 0.88 (m, 6H).

(Z)-*6-Heneicosen-9-one (6) (Figure 1, Scheme 1).* Alcohol **5** (6.0 g, 19.3 mmol) was stirred 90 min in 20 ml of CH_2Cl_2 with pyridinium chlorochromate (PCC) (6.25 g, 28.5 mmol). Hexane (30 ml) was added and the mixture column chromatographed [hexanes–ether (99 : 1)]. Ketone-containing fractions, monitored by GC, were combined yielding 5.1 g of the ketone **6** (85.5% yield). Anal. calcd. for $C_{21}H_{40}O$: C, 81.73; H, 13.07; found: C, 81.69; H, 13.22. ^{1}H NMR: 5.56 (m, 2H); 3.15 (d, 2H); 2.42 (t, 2H); 2.02 (m, 2H); 1.55 (m, 4H); 1.20–1.42 (m, 22H); 0.88 (m, 6H).

(E)-*6-Heneicosen-9-ol (10) (Figure 1, Scheme 2).* Butyl lithium-induced coupling of 3-butyn-1-ol (**7**) with iodopentane in the presence of HMPA, and subsequent reduction of the resulting 3-nonyn-1-ol (**8**) with lithium in liquid ammonia afforded (*E*)-3-nonen-1-ol (**9**) (Katritzky et al., 1996) at 36% yield (based on **7**). It was purified (98%) on a silica column containing 20% silver with hexane–ether–benzene (3 : 1 : 2) as eluents. To alcohol **9** (1.2 g, 8.45 mmol) in CH_2Cl_2 (10 ml) at room temperature, PCC (12.7 mmol) was added. After 1 hr of stirring, the reaction mixture was quenched with hexane (30 ml) and flash chromatographed with hexane–ether (10 : 1). Aldehyde-containing fractions, as monitored by GC, were combined, concentrated to 10 ml, placed into a dropping funnel, and added to a Grignard reagent freshly prepared from dodecyl bromide (10 mmol) and magnesium (12 mmol) in ether. After 15 min of stirring, the reaction mixture was gently quenched with aqueous ammonium chloride. Extraction (3 × 100 ml of ether), separation, and washing of organic layers with saturated NaCl, drying, and column chromatography [hexane–ether (98 : 2)] yielded alcohol **10** (1.50 g, 57% yield) as a solid compound (mp 32–33°C); anal. calcd. for $C_{21}H_{42}O$: C, 81.20; H, 13.63; found: C, 80.70; H, 13.85; ^{1}H NMR: 5.54 (m, 1H); 5.40 (m, 1H); 3.57 (m, 1H); 2.23 (m, 1H); 1.98–2.09 (m, 3H); 1.21–1.46 (m, 28H); 0.87 (two t, 6H).

(E)-*6-Heneicosen-9-one (11) (Figure 1, Scheme 2).* Ketone **11** was obtained

(85% yield) from alcohol **10** following the procedure applied for conversion of compound **5** to **6.** Anal. calcd. for $C_{21}H_{40}O$: C, 81.73; H, 13.07: found; C, 81.39; H, 13.03; ^{1}H NMR: 5.51 (m, 2H); 3.08 (d, 2H); 2.41 (t, 2H); 2.02 (m, 2H); 1.5–1.6 (m, 4H); 1.18–1.38 (m, 22H); 0.88 (m, 6H).

(E)-*7-Heneicosen-9-one (16) (Figure 1, Scheme 3).* GC analysis of synthetic ketone **6** resulted in the formation of rearrangement products [4–10% of **6** depending on the GC injector (split/splitless or on-column)]. Heat exposure of ketone **6** (5 mg) (180°C for 5 min under argon) and subsequent two-dimensional ^{1}H NMR spectroscopy suggested, and synthesis confirmed, that the major rearrangement product was (*E*)-7-heneicosen-9-one (**16**). For synthesis of **16** (Figure 1, Scheme 3), lithium octynide (**12**) was coupled with tridecanal (**13**), affording 7-heneicosyn-9-ol (**14**), which was reduced overnight with $LiAlH_4$ in ether to (*E*)-7-heneicosen-9-ol (**15**) and oxidized with PCC to give **16** (62% overall yield). ^{1}H NMR: 6.82 (two t, 1H); 6.08 (td, 1H); 2.52 (t, 2H); 2.20 (q, 2H); 1.59 (m, 2H); 1.45 (m, 2H); 1.21–1.35 (m, 22H), 0.86 (m, 6H).

The following compounds were produced to confirm that their GC retention times differed from that of Z6–9-one (Figure 1, Scheme 1). Because these compounds are not synthetic intermediates for pheromones and do not have pheromonal activity, their syntheses are reported without spectroscopic data or schemes, as follows:

(Z)-*4-Heneicosen-9-one.* 5-Hexyn-1-ol was added to 2.5 equivalents of butyl lithium in THF. Addition of bromopropane in HMPA resulted in the formation of 5-nonyn-1-ol. Hydrogenation over P-2 Ni followed by oxidation with PCC gave (*Z*)-5-nonenal, which was added to a solution of dodecyl magnesium bromide, resulting in (*Z*)-4-heneicosen-9-ol. Oxidation with PCC yielded (*Z*)-4-heneicosen-9-one.

(Z)-*5-Heneicosen-9-one.* 4-Pentyn-1-ol was added to 2.5 equivalents of butyl lithium in THF, followed by bromobutane in HMPA. Oxidation of the resulting 4-nonynol with PCC provided the aldehyde, which was added to dodecylmagnesium bromide, resulting in 5-heneicosyn-9-ol. Oxidation with PCC and hydrogenation over P-2 Ni yielded (*Z*)-5-heneicosen-9-one.

(E)-*5-Heneicosen-9-one.* 4-Nonyn-1-ol was treated with lithium in liquid ammonia to form (*E*)-4-nonen-1-ol, which was oxidized with PCC to (*E*)-4-nonenal. Reaction with dodecyl magnesium bromide and oxidation as above provided (*E*)-5-heneicosen-9-one.

(*Z*)-6,(*E*)-8-Heneicosadien-11-one (*Z*6,*E*8–11-one) and (*Z*)-6-heneicosen-11-one (Z6–11-one) were available from previous research (Gries et al., 1997). (*Z*)-6,(*Z*)-9-Heneicosadiene (*Z*6,*Z*9–21-Hy) was generously provided by the National Research Council of Canada, Plant Biotechnology Institute, Saskatoon, Saskatchewan.

All synthetic chemicals used for GC-EAD, wind-tunnel, and field bioassays were >95% chemically pure.

Wind-Tunnel, Field-Cage, and Field Experiments

Wind-Tunnel Bioassays. In the bioassays we employed a wind tunnel and protocol as developed by Miller and Roelofs (1978). The airspeed was 30 cm/sec and the temperature was 20°C (±2°C). Both bioassay experiments tested gray sleeve stopper (The West Company, Lionville, Pennsylvania 19341) lures impregnated with test chemicals in HPLC grade hexane. Six criteria (Table 1) were used to assess the attractiveness of lures. The first bioassay experiment tested Z6–11-one alone and in combination with either Z6–9-one or E6–9-one. The second bioassay experiment tested Z6–11-one alone and in combination with either Z6–9-one, Z6,E8–11-one, or both.

Field-Cage Bioassays. Field-cage bioassays were conducted in Aukland, New Zealand, employing a cage (10 × 4 × 2.4 m high) with a steel tubing frame and heavy mesh cover. Experimental replicates (*N*) were conducted from 10:00 AM to 1:00 PM on January 13–15 (*N:* 1–3) and January 27–29 (*N:* 4–6). Each replicate employed 10 green delta traps (IPM Technologies, Inc., Portland, Oregon 97217) suspended 2 m above ground at 2-m spacings, with rerandomized positions between replicates. Five traps were baited with a commercial formula-

TABLE 1. WIND-TUNNEL BIOASSAYS WITH MALES OF WHITE-SPOTTED TUSSOCK MOTH, *Orgyia thyellina*, RESPONDING TO GRAY SLEEVE STOPPERS IMPREGNATED WITH CANDIDATE PHEROMONE COMPONENTS

Exp.[a]	Treatment[b]	Percent males responding to assessment criteria[c]					
		I	O	H	A	L	T
1	Z6–11-one (100)	0	0	0	0	0	0
	Z6–11-one (100) + E6–9-one (1)	0	0	0	0	0	0
	Z6–11-one (100) + E6–9-one (10)	0	0	0	0	0	0
	Z6–11-one (100) + Z6–9-one (1)	100	100	75	75	0	25
	Z6–11-one (100) + Z6–9-one (10)	50	50	50	50	0	0
2	Z6–11-one (100)	17	0	0	0	0	0
	Z6–11-one (100) + Z6,E8-11-one (2)	12	0	0	0	0	0
	Z6–11-one (100) + Z6–9-one (2)	68	39	12	12	12	10
	Z6–11-one (100) + Z6–9-one (2) + Z6,E8–11-one (2)	80	73	54	54	54	46

[a]Bioassay experiments 1 and 2 tested 8 and 41 male moths, respectively, for each of the treatments.
[b]Z6–11-one = (Z)-6-heneicosen-11-one; Z6–9-one = (Z)-6-heneicosen-9-one; Z6,E8–11-one = (Z)-6,(E)-8-heneicosadien-11-one; Z6,Z9–21-Hy = (Z)-6,(Z)-9-heneicosadiene. Numbers in parentheses are micrograms.
[c]I = initiation of flight; O = orientation towards pheromone lure, H = halfway flight towards lure, A = approaching lure, L = landing on platform that carries lure, T = touching and attempting to mate with lure.

tion (IPM Technologies) of *Z*6–11-one (2000 μg) plus thyellinone (100 μg), and five traps were baited with a virgin female WSTM. Each female was retained in a stainless steel wire mesh cylinder with plywood discs at the top and bottom. Because females lack functional mouthparts, no food or water was provided. The same females were used for replicates 1–3 and 4–6, respectively. Numbers of active males per replicate ranged between 17 and 44.

Field Experiments. Field experiments were conducted in and around Morioka, Japan. Green belts and forest margins stocked with decidious trees and various shrubs and herbs served as experimental sites. Sticky 2-liter Delta milk carton traps (Gray et al., 1984) were suspended from trees 1.5 m above ground in randomized complete blocks. Traps within blocks (= replicates) were spaced at intervals of 15–20 m, whereas replicates (in response to low WSTM populations) were set 0.1–4 km apart from another. Traps were baited with gray sleeve stoppers impregnated with synthetic candidate pheromone components in HPLC grade hexane. The first experiment tested *Z*6–11-one in binary, ternary, and quaternary combinations with *Z*6–9-one, *Z*6,*E*8–11-one, and *Z*6,*Z*9–21-Hy. Experiment 2 tested *Z*6–11-one alone and in combination with *Z*6–9-one at ratios of 100 : 10 and 100 : 1. Experiments 3–5 tested *Z*6–11-one plus *Z*6–9-one alone and in combination with either *Z*6,*Z*9–21-Hy (experiment 3) or *Z*6,*E*8–11-one (experiments 4 and 5) at different ratios. Experiment 6 tested *Z*6–11-one and *Z*6–9-one singly and in combination. The final experiment 7 tested increasing doses of *Z*6–11-one, *Z*6–9-one and *Z*6,*E*8–11-one at a blend ratio of 100 : 5 : 1.

RESULTS AND DISCUSSION

GC-EAD analyses of pheromone gland extract of female WSTM revealed five compounds that consistently elicited responses from male moth antennae (Figure 2). Retention indices on three columns and mass spectra of EAD-active compounds A and C suggested, and GC-MS of authentic standards confirmed, that they were *Z*6,*Z*9–21-Hy (A) and *Z*6–11-one (C). Quantities of compound E were below the detection threshold of both the flame ionization detector (FID) and the mass spectrometer. However, its retention indices on DB-5, DB-210, and DB-23 coated columns were identical to those of previously reported *Z*6,*E*8–11-one (Gries et al., 1997). Corresponding retention times and comparable antennal activity of synthetic *Z*6,*E*8–11-one and WSTM-produced E supports structural assignment of *Z*6,*E*8–11-one for the component *E*. Retention indices (DB-5: 2261; DB-210: 2641; DB-23: 2753) of the second strongest EAD-active compound (D in Figure 2) were indicative of a C_{21} ketone with one or two (nonconjugated) double bonds. GC-MS analyses of a concentrated (> 50 female equivalents) pheromone extract revealed a weak but diagnostic mass spectrum for compound D (Figure 3). The molecular ion m/z 309 (M+1) suggested a hene-

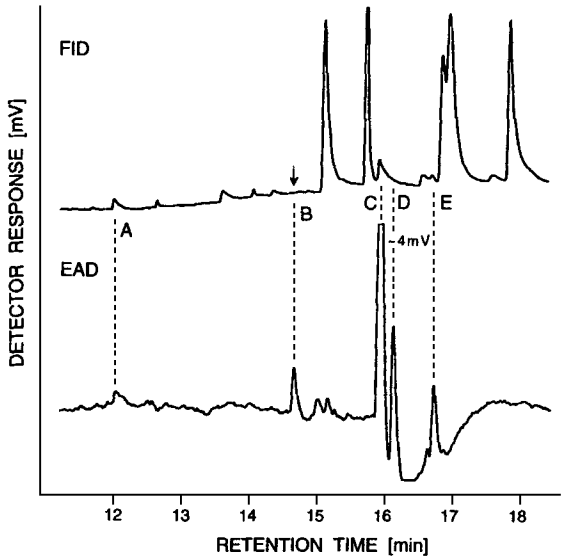

FIG. 2. Flame ionization detector (FID) and electroantennographic detector (EAD: male *O. thyellina* antenna) responses to one female equivalent of pheromone gland extract. Hewlett Packard 5890A gas chromatograph fitted with on-column injection device and equipped with a fused silica column (30 m × 0.25 mm ID) coated with DB-210; temperature program: 50°C for 1 min, 25°/min to 100°C, then 10°/min to 220°C, (hold for 10 min).

icosene ketone, and fragmentation ion *m/z* 197 indicated a keto group in C-9 (Figure 3). Allylic (*E*)-7- or (*Z*)-7 double bonds would have resulted in a distinctively higher retention index, and thus could be excluded as double bond positions. Our hypothesis of a (*Z*)-6 double bond in WSTM-produced D was based on findings that all known keto pheromones in *Orgyia* spp. contain a *Z* double bond in C-6. Synthetic (*Z*)-6-heneicosen-9-one had a mass spectrum consistent with that of WSTM-produced D, retention times corresponding with D on all three columns (DB-5, DB-210, and DB-23), and EAD activity comparable to that of D. Synthetic (*E*)-6-heneicosen-9-one, cochromatographing with Z6–9-one, was only weakly EAD active and had no pheromonal activity in wind-tunnel bioassays (Table 1). Synthetic (*E*)-5- and (*Z*)-5- as well as (*E*)-4- and (*Z*)-4-heneicosen-9-one had different retention times than WSTM-produced D, and thus could be eliminated as candidate pheromone components. EAD-active compound B in pheromone gland extracts (Figure 2) is still unknown.

In field experiments in Japan, pheromone blends with Z6–11-one plus Z6–9-one—the latter ketone termed here "thyellinone"—synergistically attracted

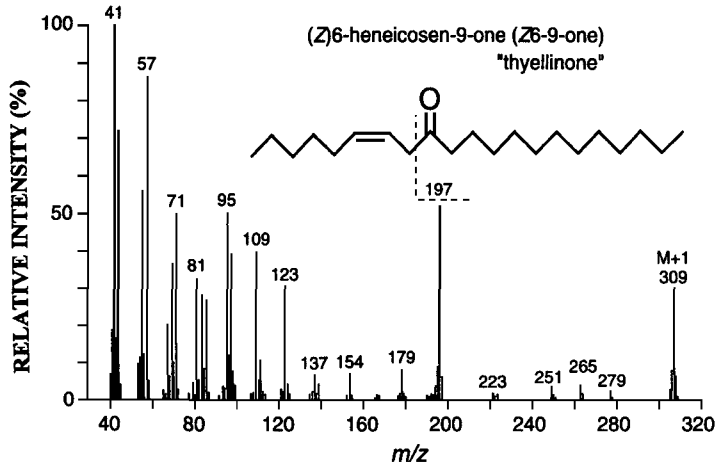

FIG. 3. Full-scan electron impact mass spectrum of (Z)-6-heneicosen-9-one; obtained with a Varian Saturn II Ion Trap GC-MS.

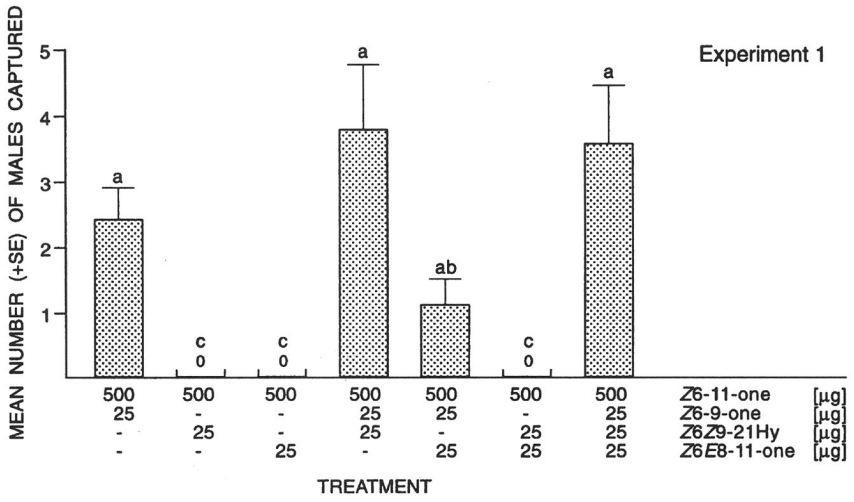

FIG. 4. Mean number of male *O. thyellina* captured in sticky 2-liter Delta milk carton traps baited with (Z)-6-heneicosen-11-one (Z6–11-one) in combinations with (Z)-6-heneicosen-9-one (Z6–9-one), (Z)-6,(E)-8-heneicosadien-11-one (Z6,E8–11-one) and (Z)-6,(Z)-9-heneicosadiene (Z6,Z9–21-Hy); 10 replicates; July 29–August 8, 1997; Morioka, Japan. Bars with the same letter superscript are not significantly different; nonparametric analysis of variance by ranks (Friedman's test) followed by comparison of means [Bonferroni (Dunn) t test, $P < 0.05$] (SAS/STAT, 1988).

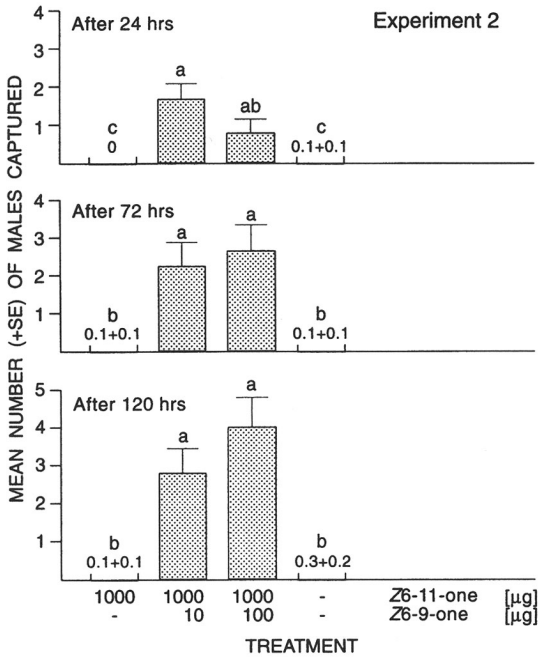

FIG. 5. Mean number of male *O. thyellina* captured in sticky 2-liter Delta milk carton traps baited with (*Z*)-6-heneicosen-11-one (*Z*6–11-one) alone and in combination with (*Z*)-6-heneicosen-9-one (*Z*6–9-one); 10 replicates; July 31–August 5, 1997; Morioka, Japan. Bars with the same letter superscript are not significantly different; nonparametric analysis of variance by ranks (Friedman's test) followed by comparison of means [Bonferroni (Dunn) *t* test, $P < 0.05$] (SAS/STAT, 1988).

WSTM males (Figure 4, experiment 1; Figure 5, experiment 2; Figure 7, experiment 6). Either ketone alone (Figure 7, experiment 6) or volatile blends without thyellinone (Figure 4, experiment 1; Figure 5, experiment 2) failed to attract any male moths. Collectively the data suggest that both *Z*6–11-one and thyellinone are essential pheromone components in the WSTM. This result contrasts with findings in other *Orgyia* spp. for which *Z*6–11-one by itself constitutes an attractive but not species-specific sex pheromone component or sex attractant (Arn et al., 1992). Although admixture of *Z*6,*E*8–11-one to the blend of *Z*6–11-one plus thyellinone did not enhance attractiveness of the pheromone lure in field experiments in Japan (Figure 4, experiment 1; Figure 6, experiments 4 and 5), *Z*6,*E*8–11-one did play a role in wind-tunnel bioassays in New Zealand. In these bioassays, blends with *Z*6,*E*8–11-one as a third volatile component caused significantly more WSTM males to contact the pheromone lure (Table 1). This

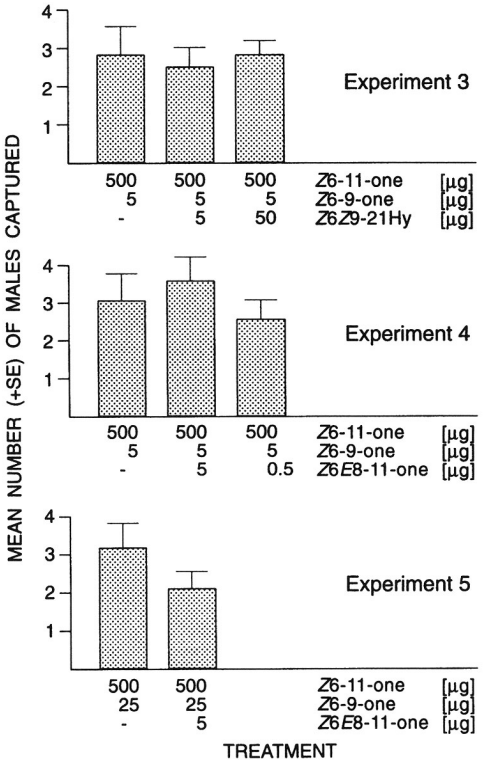

FIG. 6. Mean number of male *O. thyellina* captured in sticky 2-liter Delta milk carton traps baited with (*Z*)-6-heneicosen-11-one (Z6–11-one) plus (*Z*)-6-heneicosen-9-one (Z6–9-one) alone and in combination with either (*Z*)-6,(*Z*)-9-heneicosadiene (Z6,Z9–21-Hy) (experiment 3), or (*Z*)-6,(*E*)-8-heneicosadien-11-one (Z6,E8–11-one) (experiments 4 and 5); 10 replicates for each experiment; August 1–7, 1997 (experiment 3), August 3–8, 1997 (experiment 4), and August 4–8, 1997 (experiment 5); Morioka, Japan; treatments in experiments 3–5 were not significantly different; analysis of variance, followed by comparison of means [Bonferroni (Dunn) *t* test, $P > 0.05$] (SAS/STAT, 1988).

effect may not have been expressed in field experiments, as male moths are captured on tanglefoot already when they enter the milk carton trap or attempt to approach the lure therein. Alternatively, WSTMs that were introduced, reared, and bioassayed in a wind tunnel in New Zealand did not originate from Japan and may indeed use a three-component instead of a two-component pheromone blend.

(*Z*)-6-Heneicosen-9-one (thyellinone) is reported here for the first time as a sex pheromone component in the Lepidoptera and is only one of six pheromone components identified thus far in the genus *Orgyia*. Z6–11-one (Smith et al.,

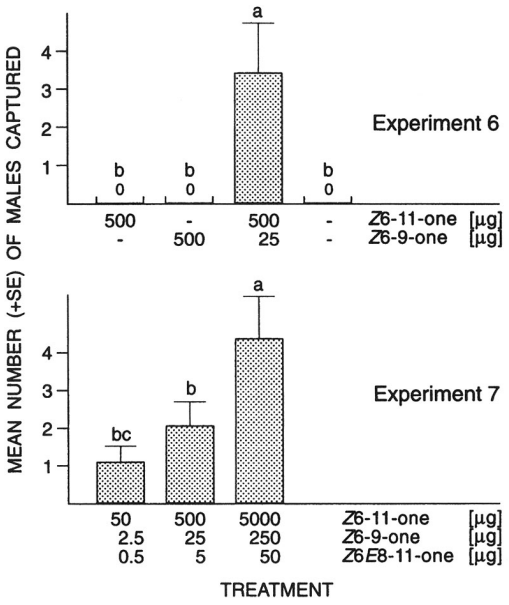

FIG. 7. Mean number of male of *O. thyellina* captured in 2-liter Delta milk carton traps baited with (*Z*)-6-heneicosen-11-one (*Z*6–11-one) and (*Z*)-6-heneicosen-9-one (*Z*6–9-one) singly and in combination (experiment 6; 5 replicates) or baited with a three-component blend [*Z*6–11-one; *Z*6–9-one and (*Z*)-6,(*E*)-8-heneicosavien-11-one (*Z*6,*E*8–11-one)] at increasing doses (experiment 7; 6 replicates); August 4–9, 1997 (experiment 6) and August 6–9, 1997 (experiment 7); Morioka, Japan. Bars with the same letter superscript in experiments 6 or 7 are not significantly different; nonparametric analysis of variance by ranks (Friedman's test) followed by comparison of means [Bonferroni (Dunn) *t* test, $P < 0.05$] (SAS/STAT, 1988).

1975) and *Z*6,*E*8–11-one (Gries et al., 1997) are sex pheromone components of the Douglas-fir tussock moth, *O. pseudotsugata* (McDunnough), and the hydrocarbons *n*-tricosane, *n*-tetracosane, *n*-pentacosane, and *n*-heptacosane in body scales of female white-marked tussock moth (WMTM), *O. leucostigma* (J. E. Smith), release copulatory behavior in conspecific male moths (Grant et al., 1987).

In field-cage, binary choice experiments, traps baited with the commercial lure formulation *Z*6–11-one plus thyellinone captured 8.8 times more male WSTM than those baited with a caged virgin female WSTM [1.4 (mean) + 0.29 (SE) versus 0.16 (mean) + 0.07 (SE) males per trap]. Through the 1998 summer, 45,000 of these synthetic lures were employed in defined areas of Auckland to attempt eradication of a residual WSTM population.

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