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Sex pheromone of the larch caterpillar moth, *Dendrolimus superans*, from northeastern China

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Abstract

The larch caterpillar moth, *Dendrolimus superans* Butler (Lepidoptera: Lasiocampidae), is a serious pest in the northeastern part of China. In order to develop an effective method for monitoring *D. superans*, we began a project to identify the sex pheromone of *D. superans* and preliminary results were published in a note in 2001. Here we report complete laboratory and field results to support the identification. (*Z*,*E*)-5,7-Dodecadienal, (*Z*,*E*)-5,7-dodecadienol, (*Z*)-5-dodecenol, and (*Z*)-5-dodecenal (100:95:75:71) were identified by gas chromatography (GC) and coupled GC-mass spectrometry in extracts of pheromone glands of female *D. superans*. However, only (*Z*,*E*)-5,7-dodecadienal elicited strong responses from conspecific male antennae in coupled GC-electroantennography studies. Field tests with synthetic compounds indicated that baits containing (*Z*,*E*)-5,7-dodecadienal as a single component attracted male *D. superans* moths, whereas addition of one, two, or all three of the possible minor components did not increase the attractiveness of lures. (*Z*,*E*)-5,7-Dodecadienyl acetate, one of the pheromone components in sympatric *Dendrolimus* species, was antagonistic, but the analog (*Z*,*E*)-5,7-dodecadienyl propionate, another pheromone component in sympatric *Dendrolimus* species, was not. Since 2005, pheromone traps baited with (*Z*,*E*)-5,7-dodecadienal have been used effectively for monitoring populations of *D. superans* in various areas of northeastern China.

Introduction

The larch caterpillar moth, *Dendrolimus superans* Butler (Lepidoptera: Lasiocampidae), is a serious pest, which is widely distributed in northeastern China, Siberia, Korea, and Japan. The preferred host is *Larix gmelinii*, but larvae also feed on *Pinus* species, such as *Pinus koraiensis*, *Pinus tabulaeformis*, *Pinus sylvestris*, and *Picea koraiensis* (Hou, 1987). For the past few decades, integrated methods of controlling *D. superans* have been investigated in efforts to minimize forest loss with ecologically based pest-management systems. However, there are difficulties in forecasting population outbreaks so that integrated control methods can be timed for maximum efficacy. Thus, synthetic sex pheromones for use as trap baits would be valuable for population monitoring.

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To date, sex pheromones have been identified in three species in the genus *Dendrolimus*. The sex pheromone of *Dendrolimus punctatus* consists of (*Z*,*E*)-5,7-dodecadienol (*Z*5,*E*7-12:OH), (*Z*,*E*)-5,7-dodecadienyl acetate (*Z*5,*E*7-12:OAc), (*Z*,*E*)-5,7-dodecadienyl propionate (*Z*5,*E*7-12:OPr), (*Z*)-5-dodecenol (*Z*5-12:OH), and (*Z*)-5-dodecenyl acetate (*Z*5-12:OAc) in a ratio of 7.9:6.5:2.4:2.6:1.0 (Zhao et al., 1993), whereas the sex pheromones of *Dendrolimus spectabilis* and *Dendrolimus pini* consist of *Z*5,*E*7-12:OH (Ando et al., 1982) and (*Z*,*E*)-5,7-dodecadienal (*Z*5,*E*7-12:Ald), respectively (Priesner et al., 1984). Recently, *Z*5,*E*7-12:OH has been identified as another pheromone component in *D. pini* (Kovalev et al., 1993), and *Z*5,*E*7-12:OAc and *Z*5,*E*7-12:OPr have been identified as minor pheromone components in *D. spectabilis* (Kong et al., 2003). Because of its importance to Chinese forestry, as early as the late 1970s, an effort to elucidate the sex pheromone of *D. superans* was started, but little progress was made partly due to the very small amount of sex pheromone in the pheromone glands (C-H Zhao, unpubl.). A sex attractant for *Dendrolimus superans*

sibiricus was found by a joint American-and-Russian effort (Klun et al., 2000). The sex attractant is a mixture of aldehydes [64% *Z*5,*E*7-12:Ald, 10% (*Z*)-5-dodecenal (*Z*5-12:Ald), 18% (*E*)-7-dodecenal, and 8% (*E*)-6-dodecenal] and alcohols [64% *Z*5,*E*7-12:OH, 10% (*Z*)-5-dodecenol (*Z*5-12:OH), 18% (*E*)-7-dodecenol, and 8% (*E*)-6-dodecenol]. In a preliminary note, we tentatively identified the sex pheromone of *D. superans* as a mixture of *Z*5,*E*7-12:Ald and *Z*5,*E*7-12:OH (Kong et al., 2001). In the present article, complete laboratory and field data are given to support the identification of the sex pheromone of *D. superans*. In particular, our most recent result indicated that the sex pheromone of *D. superans* in northeastern China consists only of *Z*5,*E*7-12:Ald.

Materials and methods

Insects

Cocoons of *D. superans* were collected from the host trees, *L. gmelinii*, in Xifeng County, Pulandian city of Liaoning Province and Weichang County of Hebei Province, China. Cocoons were maintained in a rearing cabinet with a reversed photoperiod of L16:D8, 24–26 °C, and 60–80% r.h. Emerging moths were immediately sexed and were maintained under the same conditions. Males were used for electroantennographic studies and females as the source of pheromone.

Extraction of sex pheromone glands

Zero- to 2-day-old calling females (calling behavior occurs 6–8 h into the scotophase) were used for pheromone extraction. The pheromone gland was extruded by applying gentle pressure to the abdomen, and the gland was dissected. Single glands were immersed in 10 µl distilled hexane containing 1.4 ng of (*Z*)-12-tetradecenyl acetate as internal standard for ca. 20 min. Extracts were used immediately for gas chromatography (GC) analysis or were stored at –20 °C for other studies.

Preparation and purification of chemicals

*Z*5,*E*7-12:OAc, *Z*5,*E*7-12:OH, and (*E,Z*)-5,7-dodecadienol (*E*5,*Z*7-12:OH) were purchased from Chemtech B.V. (Amsterdam, The Netherlands). Other isomers of 5,7-dodecadienol were from our laboratory collections. All C_{12} monoene alcohols used in the present study were prepared from the corresponding acetates that were in our laboratory collection, by hydrolysis in 0.5 M KOH in methanol. All C_{12} monoene and diene aldehydes were prepared by oxidation of the corresponding alcohols with pyridinium chlorochromate (Corey & Suggs, 1975). 4-Methyl-1,2,4-triazoline-3,5-dione (MTAD) was obtained from J. Millar as a gift.

Synthetic chemicals used for GC-electroantennographic detection (GC-EAD) analyses and field trials were purified by preparative GC as described in a previous study (Kong et al., 2001). The compounds used for GC-EAD analyses were ≥99% chemically and isomerically pure and the compounds used for field trials were ≥99% in chemical purity and ≥96% in isomeric purity.

Gas chromatography

Extracts and reference compounds were analyzed using a Hewlett Packard 5890 Series II GC (Santa Clara, CA, USA) with a flame ionization detector (FID). The GC was used in splitless mode with nitrogen as carrier gas. Oven temperature programs were as follows: BP-20 column [50 m × 0.25 mm ID, 0.25-µm film; Scientific Glass Engineering Pty. Ltd. (SGE), Melbourne, Australia], 80 °C for 1 min, 10 °C per min to 200 °C, hold for 30 min; BP-1 column (50 m × 0.25 mm ID; SGE) 80 °C for 1 min, 10 °C per min to 230 °C, hold for 40 min.

Coupled gas chromatography-electroantennography

A Hewlett Packard 5890 Series II GC equipped with a BP-20 capillary column (25 m × 0.25 mm ID, 0.25-µm film, SGE) programed from 80 °C for 1 min, then to 200 °C at a rate of 4 °C per min and hold for 30 min was used. An effluent splitter (SGE, part no. OSS-2) was used to allow simultaneous flame ionization and EAD of the separated compounds. Nitrogen was used as carrier gas and the effluent split ratio was approximately 1:1. Make-up gas was added to the effluent splitter (10 ml min^{-1}). The EAD effluent was directed through a transfer line (250 °C) to an L-shaped glass tube (7 mm id), where it was mixed with purified humidified air (600 ml min^{-1}), and then directed over the antennal preparation. The male antennae were mounted between two glass capillary electrodes filled with saline (7.5 g NaCl, 0.35 g KCl, and 0.21 g $CaCl_2$ in 1 l distilled water). A silver wire down the center of each electrode provided electrical contact.

Electroantennographic detection responses were amplified (10 ×), recorded, and processed with an EAD amplifier and software from Syntech (Hilversum, The Netherlands).

Coupled gas chromatography-mass spectrometry

Gland extracts and MTAD derivatives were analyzed with a Finnigan Trace 2000-Voyager GC-MS running WindowsNT/Xcalibur software (EI, 70 eV). Twelve female equivalent (FE) extracts were analyzed with a 30 m × 0.32 mm HP-5 capillary column programed at 80 °C for 1 min, then 10 °C per min to 270 °C and held for 20 min. Helium was used as the carrier gas (0.6 ml min^{-1}). Compounds in the gland extract were identified by comparison of retention times and mass spectra with those of reference compounds.

MTAD was used to determine the double bond positions of conjugated dienes in the gland extracts as described previously (Young et al., 1990; McElfresh & Millar, 1999).

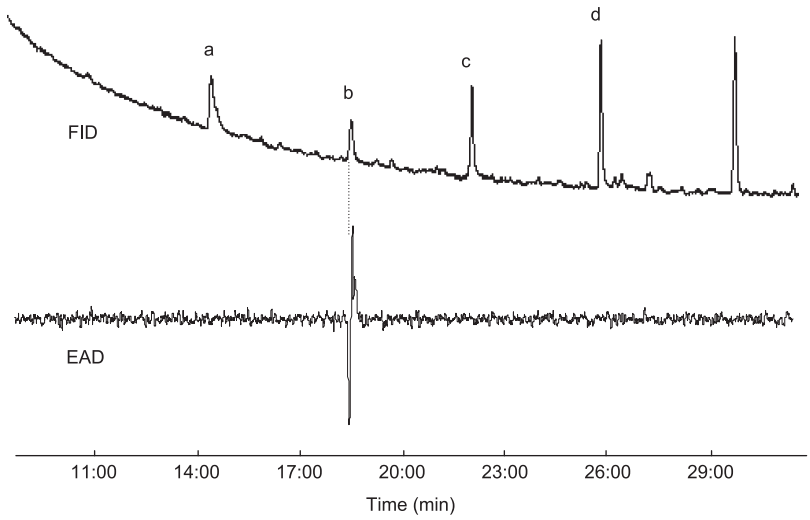

Figure 1 Gas chromatography–electroantennographic detection (GC-EAD) response of antennae of male *Dendrolimus superans* to 19 female equivalents (FE) of pheromone gland extracts. A BP-20 column was used (GC conditions are described in Materials and methods). Identifications of peaks: (a) *Z*5-12:Ald, (b) *Z*5,*E*7-12:Ald, (c) *Z*5-12:OH, and (d) *Z*5,*E*7-12:OH.

Combined gland extracts (40 FE) were concentrated to <2 µl, and then treated with 5 µl of MTAD solution (1 mg ml^{-1} in dichloromethane). The resulting faint pink solution was kept 100 min, then the concentrated sample was analyzed by GC-MS with a 50 m × 0.2 mm HP-1 capillary column, using a temperature program of 80 °C for 0 min, 15 °C per min to 280 °C, held for 20 min, with the injector temperature at 300 °C. Identifications were confirmed by analyses of adducts prepared in identical fashion from reference compounds.

Field trials

Synthetic pheromone candidates with 2,6-di-tert-butyl-p-cresol as antioxidant (ratio of pheromone candidates and antioxidant was ca. 10:1) were formulated as hexane solutions. Pheromone blends in hexane containing the appropriate ratios of components were loaded onto gray rubber septa (The West Company, Phoenixville, PA, USA). Once the solvent had evaporated, two 100-µl aliquots of dichloromethane were added to the septum to help transport any residual pheromone into the septa. The field trial was conducted from 8 to 11 July 2001; from 8 to 12 July 2002; from 21 to 25 July 2002 in Weichang County, Hebei Province, China. Sticky wing traps constructed from two pieces of cardboard (42 × 28 cm) were used in all trails. Traps were hung on the branches of larch trees ca. 2 m above ground, with at least 15 m between traps in a complete block design. Moth catches were checked daily and trap positions were randomized every 2 days within a replicate to minimize the effects of habitat heterogeneities.

Statistical analysis

For the statistical analysis of field trials, day effect was eliminated by pooling the daily trap counts for each treatment in a given block. These sums (x) were regarded as replicates and statistically analyzed after log (x + 1) transformation and then subjected to a one-way analysis of variance (ANOVA). If the F-value was significant, differences between treatment means were tested for significance by Student–Newman–Keuls ($\alpha = 0.05$) tests (SAS/STAT User's Guide, 1988, release 6.03 edition, SAS Institute, Cary, NC, USA).

Results

GC-EAD analysis of pheromone gland components

Gas chromatography–electroantennographic detection analyses of pheromone gland extracts revealed only one peak that consistently elicited strong EAD signals from antennae of male *D. superans* (Figure 1, peak 'b'). The retention time of this peak corresponded to that of synthetic *Z*5,*E*7-12:Ald. The three other peaks whose retention times corresponded respectively to *Z*5-12:Ald (a), *Z*5-12:OH (c), and *Z*5,*E*7-12:OH (d) did not elicit antennal responses. Gas chromatography–electroantennographic detection analysis of a mixture of seven known pheromone components from various *Dendrolimus* species, demonstrated that only *Z*5,*E*7-12:Ald consistently elicited strong responses from male *D. superans* antennae (n = 6; Figure 2). *Z*5-12:Ald elicited a small EAD response in two of six GC-EAD runs and no responses were detected from any other compound (Figure 2).

GC and GC-MS analysis of pheromone gland extracts

BP-20 capillary column GC analyses of pheromone gland extracts showed that the average amounts (± SD) of *Z*5,*E*7-12:Ald, *Z*5,*E*7-12:OH, Z5-12:Ald, and Z5-12:OH in a single pheromone gland were 0.40 ± 0.30, 0.38 ± 0.25, 0.29 ± 0.27, and 0.30 ± 0.18 ng, respectively, with a ratio of 100:95:71:75

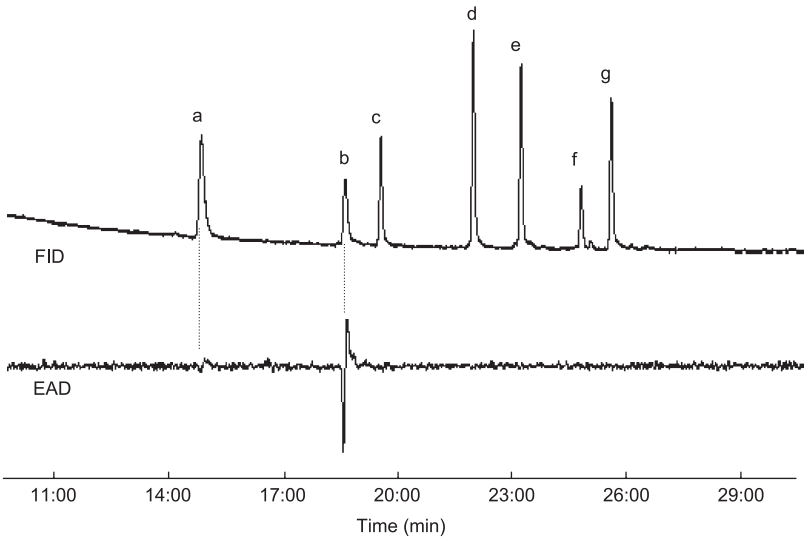

Figure 2 Gas chromatography–electroantennographic detection (GC-EAD) response of antennae of male *Dendrolimus superans* to a mixture of seven synthetic compounds including (a) *Z*5-12:Ald, (b)*Z*5,*E*7-12:Ald, (c) *Z*5-12:OAc, (d) *Z*5-12:OH, (e) *Z*5,*E*7-12:OAc, (f) *Z*5,*E*7-12:OPr, and (g) *Z*5,*E*7-12:OH. A BP-20 column was used and the GC conditions were described in Materials and methods.

(Table 1). Further GC analysis using a BP-1 capillary column confirmed the presence of the four components in gland extracts.

Analytical results obtained by GC and GC-EAD were confirmed by GC-MS analysis of pheromone gland extracts and MTAD derivatives of the extracts. Diagnostic ions of each identified component in the extracts were all found as follows: m/z 164 (8%, M^+-H_2O), 138 (42%), and 67 (100%) for *Z*5-12:Ald; m/z 166 (18%, M^+-H_2O), 138 (20%), and 82 (100%) for *Z*5-12:OH; m/z 180 (7%, M^+), 136 (15%), and 79 (100%) for *Z*5,*E*7-12:Ald; and m/z 182 (7%, M^+), 164 (12%), and 79 (100%) for *Z*5,*E*7-12:OH. These diagnostic ions were also found in the corresponding reference compounds. The mass spectrum of the MTAD derivative of *Z*5,*E*7-12:OH in the gland extracts gave a molecular ion at m/z 295 (8%), and diagnostic fragments at m/z 222 (100%) and m/z 238 (29%), locating the double bonds of the C_{12} dienic alcohol in the 5,7 positions. Although the molecular ion of the MTAD derivative of the C_{12} dienic aldehyde was not seen, the diagnostic fragments locating the double bonds of the dienic aldehyde at the 5,7 positions, [m/z 222 (100%) and 236 (17%)] were present.

The double bond position and geometry of the tentatively identified *Z*5-12:Ald (peak 'a') and *Z*5-12:OH (peak 'c') in the gland extracts were further determined by comparing their retention times with those of a series of synthetic C_{12} monounsaturated alcohols and aldehydes on BP-20 capillary column (Table 2). The retention times of peaks 'a' and 'c' matched that of both *Z*5-12:Ald and *Z*5-12:OH, respectively. Thus the GC analyses provided further evidence that peaks 'a' and 'c' are probably *Z*5-12:Ald and *Z*5-12:OH, respectively. The configurations of the 5,7-dodecadienal (peak 'b') and 5,7-dodecadienol (peak 'd') in the gland extracts were determined by comparison of their retention times with

Table 1 Components, methods of identification, and absolute and relative amounts of *Z*5,*E*7-12:Ald in pheromone gland extracts of *Dendrolimus superans*

	Identification methods used[1]				Mean amount (ng per gland ± SD)[2]	
Compound	GC	GC-MS	MTAD	GC-EAD	Absolute	Relative
*Z*5-12:Ald	X	X			0.29 ± 0.27	71 ± 66.8
*Z*5,*E*7-12:Ald	X	X	X	X	0.40 ± 0.30	100
*Z*5-12:OH	X	X			0.30 ± 0.18	75 ± 45.8
*Z*5,*E*7-12:OH	X	X	X		0.38 ± 0.25	95 ± 62.2

[1]GC, retention time on two columns matches with reference compound; GC-MS, mass spectrum matches with reference compound; MTAD, all diagnostic fragments of the mass spectrum from the MTAD derivative of dienic components in gland extracts were found; GC-EAD, male antennal response at retention time of reference compound.

[2]Means of absolute amounts and relative amounts to *Z*5,*E*7-12:Ald were calculated from GC analyses with single gland extracts (n = 23). All four gland components were detectable in each replicate.

Table 2 Retention times (min) of isomers of synthetic alcohols and aldehydes of C_{12} monoene, and diene and pheromone gland components of *Dendrolimus superans*[1]

	Synthetic compound		Component in the gland extracts	
Isomer of C_{12} monoene	Ald	OH	Peak 'a'	Peak 'c'
*E*4		22.954		
*Z*4		23.018		
*E*5	16.217	23.181		
*Z*5	16.227	23.308	16.235	23.310
*E*6	16.432	23.129		
*E*7	16.430	23.198		
*Z*7	16.832	23.393		
*E*8		23.223		
*Z*8		23.576		
Isomer of C_{12} diene	Ald	OH	Peak 'b'	Peak 'd'
*Z*5,*E*7	20.116	27.080	20.116	27.087
*E*5,*Z*7	20.536	27.258		
*Z*5,*Z*7	20.819	27.628		
*E*5,*E*7	21.172	27.956		

[1]Column used was BP-20 (50 m × 0.25 mm id, 0.25 μm film; program used was 80 °C for 1 min, then to 200 °C at a rate of 4 °C per min, held for 30 min.

those of synthetic reference compounds. All four geometrical isomers of 5,7-dodecadienal and 5,7-dodecadienol were well separated by BP-20 capillary column with different retention times (Table 2), and both dienes were determined to have the *Z*5,*E*7 configuration.

Field trials

Based on analyses of the pheromone gland extracts, especially the determination of EAD active peak, traps containing *Z*5,*E*7-12:Ald with or without one, two, or three other gland components (ratios were similar to that in the pheromone glands), were tested (Table 3). All baits containing *Z*5,*E*7-12:Ald with or without other gland components trapped more male *D. superans* than controls. No significant differences in male captures were found between baits containing *Z*5,*E*7-12:Ald alone and *Z*5,*E*7-12:Ald plus any one, two, or three other gland components, indicating that the sex pheromone of *D. superans* appears to consist of only *Z*5,*E*7-12:Ald (Tables 3 and 4).

In *D. pini, E*5,*Z*7-12:Ald is a powerful antagonist to its sex pheromone, *Z*5,*E*7-12:Ald (Priesner et al., 1984). However, in *D. superans*, addition of *E*5,*Z*7-12:Ald (20% of *Z*5,*E*7-12:Ald) to the baits containing *Z*5,*E*7-12:Ald had no significant effect on male catches (Table 4).

Table 3 Captures of male *Dendrolimus superans* in traps baited with different combinations of components found in female pheromone gland

Composition of baits (μg)				
*Z*5,*E*7-12:Ald	*Z*5,*E*7-12:OH	*Z*5-12:Ald	*Z*5-12:OH	Catch per trap (mean ± SE)[1]
500				17.5 ± 3.4a
500	480			19.3 ± 1.9a
500		360		21.3 ± 1.0a
500	480	360		15 ± 1.3a
500	480	360	380	21 ± 1.6a
500	480		380	20 ± 2.0a
500		360	380	22 ± 1.5a
500			380	20 ± 3.1a
Blank				2.3 ± 0.5b

[1]Numbers followed by the same letter are not significantly different at the 5% confidence level by Student–Newman–Keuls tests ($F_{7,24} = 5.46$, $P << 0.0008$; $n = 4$).

Table 4 Field trapping of male *Dendrolimus superans* in traps baited with *Z*5,*E*7-12:Ald and analogs

Bait composition (μg)					
*Z*5,*E*7-12:Ald	*Z*5,*E*7-12:OH	*Z*5-12:Ald	*Z*5-12:OH	*E*5,*Z*7-12:Ald	Catch per trap (mean ± SE)[1]
600					14.3 ± 3.4a
600	570	426	450		21.3 ± 2.9a
600				120	20.3 ± 2.2a

[1]Numbers of males captured were not significantly different at the 5% confidence level by Student–Newman–Keuls tests ($F_{2,6} = 2.07$, $P<0.2$; n = 3).

Because *Z*5,*E*7-12:OAc and *Z*5,*E*7-12:OPr also are known pheromone components in several sibling species (Zhao et al., 1993; Kong et al., 2003), different doses of these components were tested to see if these components had any antagonistic or synergist effect on the sex pheromone. Baits containing all four gland components (*Z*5,*E*7-12:Ald, *Z*5,*E*7-12:OH, *Z*5-12:Ald, and *Z*5-12:OH) were used as controls. Addition of 10 μg *Z*5,*E*7-12:OAc to the above baits had no significant effect, compared to the control group. As the dose of *Z*5,*E*7-12:OAc was increased to 100 μg, male captures were significantly decreased, indicating an increasing antagonistic effect on the sex pheromone (Table 5). In contrast, *Z*5,*E*7-12:OPr had no antagonistic effect even at a dose of 100 μg.

Discussion

Because of its importance as a pest in Chinese and Russian forests, efforts to identify the sex pheromone of *D. superans* have spanned more than two decades (C-H Zhao, pers. obs.; Klun et al., 2000). One of the difficulties in clarifying the sex pheromone in *D. superans* was that only very small amount of the sex pheromone were obtained from virgin females. Gas chromatography analysis showed that only 0.40 ± 0.30 ng *Z*5,*E*7-12:Ald per gland (Table 1) could be recovered from gland extracts of calling females, while only 0.15 ± 0.14 ng per gland (n = 14) was extractable from non-calling females (data not presented). The pheromone titer in *D. superans* is the smallest among pheromone titers that have been reported in *Dendrolimus* species (Ando et al., 1982; Priesner et al., 1984; Zhao et al., 1993, 2001; Kong et al., 2001).

In combination with field tests, analyses of pheromone gland extracts by GC, GC-EAD, and GC-MS including double-bond location indicated that the sex pheromone of *D. superans* probably consists only of *Z*5,*E*7-12:Ald. In an early study, EAG analyses with eight pheromone-like compounds, including compounds found in the pheromone gland extracts of *D. superans*, indicated that the male antennal EAG response to *Z*5,*E*7-12:Ald was much higher than to any other compound (Kong et al., 2001). Our results from GC-EAD analyses using the extracts and pheromone-like compounds (Figures 1 and 2) were in agreement with those from the previous EAG analyses, in which antennae of males responded strongly to *Z*5,*E*7-12:Ald, only weakly to *Z*5,*E*7-12:OH, *Z*5,*E*7-12:OAc, and several related compounds (Kong et al., 2001). Also, much smaller doses were used in the EAD tests reported here (ca. 5–10 ng) than in previous EAG tests (10 μg).

In a preliminary field test conducted in 2000, *Z*5,*E*7-12:Ald was a necessary component in traps for male *D. superans* whereas the role of *Z*5,*E*7-12:OH was unclear (Kong

Table 5 Captures of male *Dendrolimus superans* in traps baited with four gland components, adding different amounts of *Z*5,*E*7-12:OAc or *Z*5,*E*7-12:OPr

Bait composition (μg)						
*Z*5,*E*7-12:Ald	*Z*5,*E*7-12:OH	*Z*5-12:Ald	*Z*5-12:OH	*Z*5,*E*7-12:OAc	*Z*5,*E*7-12:OPr	Catch per trap (mean ± SE)[1]
100	90	70	80			12.7 ± 2.3a
100	90	70	80	10		5.3 ± 2.9ab
100	90	70	80	100		2.6 ± 0.3b
100	90	70	80		10	13 ± 4.1a
100	90	70	80		100	8.3 ± 0.3ab
100	90	70	80	50	50	6.0 ± 1.5ab

[1]For one-way analysis of variance (ANOVA), data transformed to log (x + 1), numbers followed by the same letter are not significantly different at 5% confidence level by Student–Newman–Keuls tests ($F_{5,12} = 3.75$, $P = 0.028$; n = 3).

et al., 2001). Field tests conducted in 2001 and 2002 suggested that the other three gland components are not pheromone components, and that *Z*5,*E*7-12:Ald is the sole component of the sex pheromone. This suggestion was strongly supported by the GC-EAD analyses using both the gland extracts and synthetic compounds (Figures 1 and 2). The fact that *Z*5,*E*7-12:OH in *D. superans* female gland extracts did not elicit responses from antennae of male moths, coupled with the observations from field tests, suggests that this compound may be a precursor to the aldehyde pheromone component (e.g., Teal & Tumlinson, 1986). However, the possibility that *D. superans* may have one or more minor pheromone component(s) cannot be ruled out, because the amounts of these pheromone components may have been below the detection levels of our GC or GC-MS system.

In a dose–response trial, trap captures with 3000 μg dosages were significantly higher than those for doses of 120 and 24 μg, but not for a 600-μg dose (one-way ANOVA, $P \leq 0.05$; $n = 3$). We also found that the numbers of males caught in traps containing *Z*5,*E*7-12:Ald were significantly lower 3–4 days after the field tests began. The gray rubber septa used in the present study were also used in *D. punctatus* pheromone traps containing *Z*5,*E*7-12:OAc, *Z*5,*E*7-12:OH, and *Z*5,*E*7-12:OPr, and these traps lasted for at least 20 days in the field without losing activity (Gao et al., 2001). The reduced activity of the pheromone traps reported here may be due to higher volatility or greater instability of *Z*5,*E*7-12:Ald than that of the analogs, alcohol, acetate, or propionate. Furthermore, the trapping efficiency of the Siberian moth pheromone lures loaded with 2 mg of a 1:1 blend of *Z*5,*E*7-12:Ald, and *Z*5,*E*7-12:OH was reported to decrease significantly after aging in the laboratory for 2 weeks (Khrimian et al., 2002), and those authors suggested that lures should be replaced at intervals of less than 2 weeks. Consequently, we suggest that lures loaded with 1 mg of *Z*5,*E*7-12:Ald be replaced at intervals of 10 days until more long lasting formulations can be developed.

Male *D. superans sibricus* in the Shira Region, Republic of Khakassiya, Siberia, Russia, were attracted by a 1:1 mixture of *Z*5,*E*7-12:Ald and *Z*5,*E*7-12:OH, but a mixture of aldehydes containing *Z*5,*E*7-12:Ald without any alcohol was not attractive (Klun et al., 2000). However, our field tests in northern Hebei Province showed no difference in male catches between traps containing a 1:1 blend of *Z*5,*E*7-12:Ald and *Z*5,*E*7-12:OH and traps containing *Z*5,*E*7-12:Ald alone (Table 3). This difference could be caused by a geographic variation in the sex pheromone blend of *D. superans*, as has been observed in other species (Tóth et al., 1992; Picimbon et al., 1997; McElfresh & Millar, 1999). Furthermore, in 2005 and 2006, pheromone lures containing 1 mg of *Z*5,*E*7-12:Ald were successfully used to monitor *D. superans* in northeastern China, including Inner Mongolia Autonomous Region, Heilongjiang, and Jillin Province (C-H Zhao, unpubl.).

The major sex pheromone component of a sibling species, *D. pini*, also consists of *Z*5,*E*7-12:Ald (Priesner et al., 1984). In addition to *Z*5,*E*7-12:Ald, other gland components of *D. superans*, including *Z*5,*E*7-12:OH, *Z*5-12:OH, and *Z*5-12:Ald were not detected in a German strain of *D. pini*, and *Z*5,*E*7-12:OH and *E*5,*Z*7-12:OH had no effect on trap captures of *D. pini*. However, Russian scientists reported that *Z*5,*E*7-12:OH was detected in extracts of the abdominal tips of *D. pini*, and field tests in Khashuri leskhoz, Georgia showed that attraction of males to pheromone lures containing *Z*5,*E*7-12:Ald and *Z*5,*E*7-12:OH was substantially increased, compared to *Z*5,*E*7-12:Ald as a single component (Kovalev et al., 1993). Thus, the difference in the sex pheromone compositions between Germany and Georgia also could be due to geographic variation in the sex pheromone. Furthermore, in *D. pini*, a 1% addition of *Z*5,*E*7-12:OAc to lures containing *Z*5,*E*7-12:Ald abolished trap captures (Priesner et al., 1984). In contrast, in *D. superans,* *Z*5,*E*7-12:OAc had a weaker antagonistic effect on trap captures in the present study (Table 5). In some areas of northeastern China, *D. superans* is sympatric with *D. spectabilis* or with *Dendrolimus tabulaeformis* (Hou, 1987). One of the pheromone components in *D. spectabilis* and *D. tabulaeformis* is *Z*5,*E*7-12:OAc, and *Z*5,*E*7-12:Ald has an antagonistic effect on the sex pheromones of *D. spectabilis* and *D. tabulaeformis* (Kong et al., 2001, 2003; C-H Zhao, unpubl.). This suggests that the pheromone components *Z*5,*E*7-12:OAc and *Z*5,*E*7-12:Ald play an important role in the reproductive isolation between *D. superans* and *D. spectabilis* or *D. tabulaeformis*.

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