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Attraction of Male Pine Sawflies, *Diprion jingyuanensis*, to Synthetic Pheromone Candidates: Synergism between Two Stereoisomers

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Abstract: The pine sawfly *Diprion jingyuanensis* Xiao and Zhang (Hymenoptera: Diprionidae) is a serious pest of *Pinus tabulaeformis* Carr. in the Shanxi, Gansu, and Inner Mongolia provinces in P. R. China. The sex pheromone of *D. jingyuanensis* was shown to be the propionate ester of 3,7-dimethyl-2-tridecanol. Virgin females contained an approximate 1:3 blend of the pheromone precursors *erythro*-(2*S*,3*S*,7*R*/*S* and 2*R*,3*R*,7*R*/*S*)-3,7-dimethyl-2-tridecanol and *threo*-(2*S*,3*R*,7*R*/*S* and 2*R*,3*S*,7*R*/*S*)-3,7-dimethyl-2-tridecanol, but the exact stereoisomers were not determined. Males responded the strongest to the propionate ester of the two *threo*-isomers, (2*S*,3*R*,7*R*) and (2*S*,3*R*,7*S*), in electroantennogram (EAG) recordings, followed by a significant EAG response to the (2*S*,3*R*,7*R*) propionate of diprionol (pheromone component of *D. similis*), whereas the remaining two isomers (2*S*,3*S*,7*S* and 2*S*,3*S*,7*R*) of the propionate ester of 3,7-dimethyl-2-tridecanol and the acetate of the (2*S*,3*R*,7*R*) isomer (one of the two pheromone components of *D. pini*) did not elicit any significant increase in antennal response. In the field, the strongly EAG-active (2*S*,3*R*,7*R*)-isomer alone was only weakly (but significantly) attractive to *D. jingyuanensis* males at 100 µg, while the equally EAG-active (2*S*,3*R*,7*S*)-isomer alone at the same loading was 8–14 times more attractive than was the (2*S*,3*R*,7*R*)-isomer alone. Traps baited with the same amounts of the two *threo*-isomers ((2*S*,3*R*,7*R*) and (2*S*,3*R*,7*S*), 100 µg + 100 µg) caught significantly more males than did traps baited with other isomers, either of the two isomers alone or other proportions of the two isomers. Thus, the (2*S*,3*R*,7*S*)-isomer is considered as a strong and essential sex-attractant component for *D. jingyuanensis* males, whereas the (2*S*,3*R*,7*R*)-isomer is a weak but synergistic sex-attractant. This is one of the few examples of a pine sawfly responding significantly stronger to a binary blend of stereoisomers in a synergistic fashion than to a single stereoisomer alone.

Keywords: Hymenoptera; Diprionidae; sex pheromone; gas chromatography-mass spectrometry; electroantennography; 3,7-dimethyl-2-tridecanol; attractant

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1. Introduction

Sawflies of the family Diprionidae (Hymenoptera, Symphyta) belong to the most important defoliators of pines, *Pinus* spp., over a large part of the northern hemisphere [1].

In many countries, notably in Central Europe and China, outbreaks have regularly been controlled by chemical insecticides. Attempts to develop environmentally more friendly control methods have included investigations of diprionid sex pheromones. Moreover, from basic science point of view, the chemical communication in this insect group has received some attention. Diprionid females attract males by pheromones that consist of a unique series of methyl-branched long chain esters, with two to four stereogenic centers [2,3]. Although many stereoisomers of the pheromone can exist, it appears that several species use the same stereoisomers as attractant [2,4]. This led to investigations about species recognition and reproductive isolation, especially among the North American diprionids [5,6].

Until recently only few diprionid species from the Far East were known, but during the last three decades, several new taxa have been described from China [7] and Korea [8]. Many of these are considered severe pests and the target of the present study, *Diprion jingyuanensis* Xiao and Zhang, is no exception [9]. Large areas planted with *Pinus tabulaeformis* Carr. in the provinces of Shanxi, Gansu, and Inner Mongolia, P. R. China, have been defoliated by this sawfly since the 1990s, around 10,000 to 200,000 ha per year [10,11] (Biological Disaster Prevention and Control Center, National Forestry and Grassland Administration, P.R. China, unpublished). It has one generation per year and overwinters as prepupae in cocoons in the litter or topsoil beneath its host trees. Adults emerge from late May to early August, with at least one flight peak occurring during mid to late June [12,13]. Calling of virgin females and male responses to traps baited with virgin females occur during the daytime, with a major peak at 9:00–13:00 and a minor peak around 17:00–19:00 [14].

In a first attempt to find an attractant for *D. jingyuanensis*, several earlier described diprionid pheromone substances were used in a field screening test [14]. It was found that the propionate, but not the acetate, of (2*S*,3*R*,7*R*)-3,7-dimethyl-2-tridecanol attracted males. The acetate and propionate of diprionol (3,7-dimethyl-2-pentadecanol), both as eight-isomer mixtures and as pure (2*S*,3*S*,7*S*)-isomer, were found to be inactive, as was the acetate of (2*S*,3*R*,7*R*)-3,7-dimethyl-pentadecanol. Thus, it was concluded that the propionate of (2*S*,3*R*,7*R*)-3,7-dimethyl-2-tridecanol might be a component of the *D. jingyuanensis* sex pheromone. The same compound was earlier identified from *Diprion pini* [15,16]. Traps baited with a four-*threo*-isomer mixture of 3,7-dimethyl-2-tridecanol propionate (1 to 2 mg/trap) were also attractive to *D. jingyuanensis* males and have been used to monitor the sawfly adult flight activity and its population dynamics since the late 1990s [12]. We herein report further work to identify the pheromone of *D. jingyuanensis*, including chemical analysis, electrophysiology, and field tests. Carbon numbers are from here on omitted before the chiral identity.

2. Materials and Methods

2.1. Insects, Extraction, and Liquid Chromatography

Insects were collected as cocoons in the litter in plantations of *Pinus tabulaeformis* situated in Qingyuan County, Shanxi Province, P.R. China, and stored at room temperature until they started to emerge. When emergence started, male cocoons were put in a refrigerator to delay development and were taken out when needed for electrophysiological studies. Whole bodies of females were extracted in ethyl acetate for 72 h at room temperature—43 females for identification and 118 females for chiral analyses. Initially, the extract was purified by liquid chromatography (LC) on Chromabond (SiOH) columns [16].

2.2. Chemicals

The blends of isomers and samples of highly pure single stereoisomers were synthesized at Mid Sweden University as described by Bergström et al. [16]. The chemical and stereochemical purity of each compound used in the present study is listed in Table 1, and their structural formulas presented in Figure 1.

Table 1. Chemical and stereoisomeric purity of the synthetic compounds used for electroantennography and in field tests.

Compound	Chemical Purity (%)	Stereochemical Purity (%)	Other Isomers Present (%)
Propionate of *threo*-3,7-dimethyl-2-tridecanol	>99	>99.9	*3rythron* (<0.05)
Propionate of *SRR*-3,7-dimethyl-2-tridecanol	99.5	>99.3	*SRS* (0.2), *RSR*, (<0.4) *RRR* (<0.03), *SSR* (<0.03)
Propionate of *SRS*-3,7-dimethyl-2-tridecanol	>96	>98.9	*SRR* (0.6), *RSS* (<0.4), *SSS* (<0.03), *RRS* (<0.03)
Propionate of *RSS*-3,7-dimethyl-2-tridecanol	>96	>99.2	*RSR* (<0.6), *SSS* (<0.05), *RRS* (<0.05), *SRS* (<0.05)
Propionate of *RSR*-3,7-dimethyl-2-tridecanol	>98	>99.6	*RSS* (0.2), *RRR* (<0.05), *SRR* (<0.05), *SSR* (<0.05)
Propionate of *SSS*-3,7-dimethyl-2-tridecanol	>96	>99.2	*SSR* (0.6), *RSS* (<0.05), *RRS* (<0.05), *RSR* (<0.05)
Propionate of *SSR*-3,7-dimethyl-2-tridecanol	>99	>99.6	*SSS* (0.2), *RRR* (<0.05), *SRR* (<0.05), *RSR* (<0.05)
Acetate of *SRR*-3,7-dimethyl-2-tridecanol	>98	>99.3	*RSR* (<0.4), *SRS* (0.2), *RRR* (<0.03), *SSR* (<0.03)
Propionate of *SRR*-3,7-dimethyl-2-pentadecanol	98.5	87	*SRS* (13), *SSR* (0.5), *RSR* (0.5)

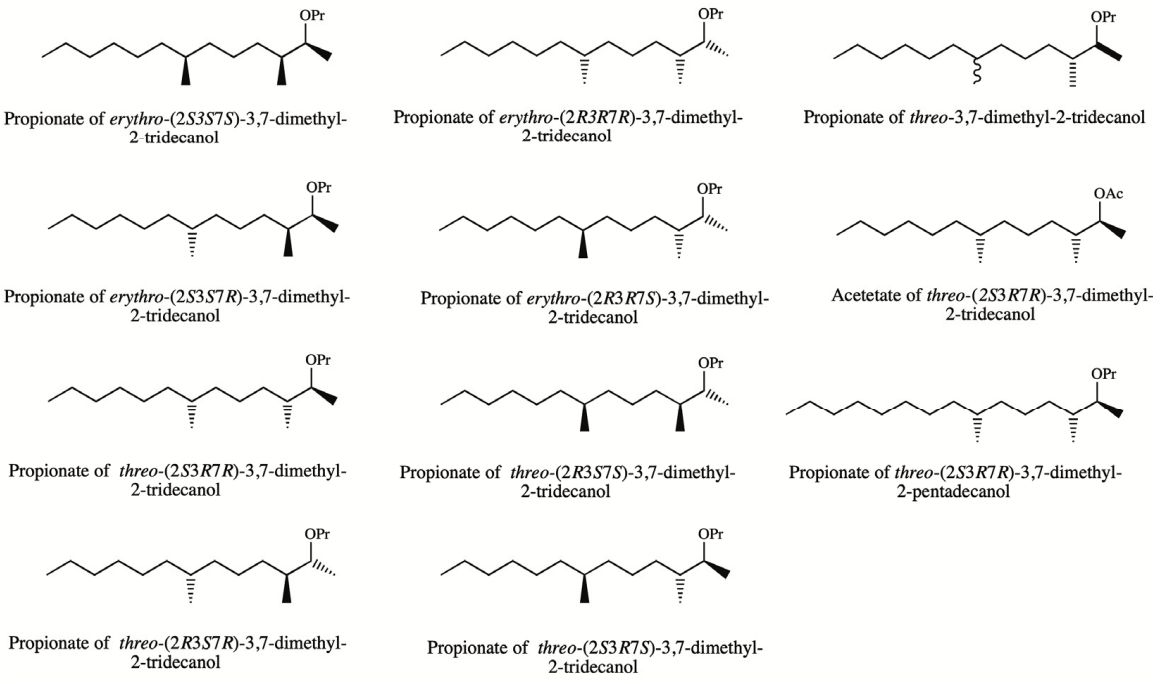

Figure 1. Structural formulas of all stereoisomers of the propionate esters of 3,7-dimethyl-2-tridecanol, of which six stereoisomers were tested in this work, and also formulas of three other pheromone candidates that also were tested in this work.

2.3. Gas Chromatography with Mass Spectrometry Detection (GC-MS)

Analyses of the isolated fractions 7 and 8 from liquid chromatography were performed by combined gas chromatography and mass spectrometry (GC-MS) on a Hewlett-Packard GC-5890 gas chromatograph coupled with a Finnigan TSQ -700 quadrupole mass spectrometer in electron impact (EI) mode. The gas chromatograph was fitted with an analytical fused silica capillary column (30 m × 0.25 mm I.D.) coated with a 0.20 μm layer of CP-WAX 58 (OV-351) as a stationary phase. Helium was used as carrier gas at a flow of 25 cm/s. After sample injection, the temperature was held constant at 50 °C for 5 min, then raised

10 °C/min to 220 °C, and then isothermal 20 min. Injector and transfer line temperatures were 220 °C and 240 °C, respectively.

2.4. Gas Chromatography with Electron Capture Detection (GC-ECD)

Gas chromatographic separations of the natural stereoisomers as well as synthetic 3,7-dimethyl-2-tridecanol as pentafluorobenzoate derivatives were carried out on a HP Agilent 6890 gas chromatograph equipped with an electron capture detector (ECD). A fused silica capillary column (50 m × 0.25 mm I.D.) was coated with the stationary phase CP-Sil-88, d_f = 0.20 μm [17].

2.5. Electrophysiology

In the electroantennographic (EAG) recordings, antennae were cut off from heads of live pine sawfly males, and each was placed between two silver wire-glass capillary electrodes filled with Beadle-Ephrussi Ringer solution. The antennal base was in connection with the ground electrode, whereas the antennal tip was inserted into the recording electrode that was connected to a high-impedance amplifier. The antennal signals were acquired and stored on a PC equipped with a serial IDAC interface box (Syntech, Hilversum, the Netherlands). The antennal preparation was held 1 cm in front of a humidified and charcoal filtered air stream flowing at 0.5 m/s. The stimulus was injected into the air stream through a hole in the glass tube 20 cm upstream of the antenna. A stimulus was created by a stimulus controller (CS-05; Syntech, Hilversum, the Netherlands), which delivered the stimulus in a 0.5 s puff at a flow rate of 5 mL/s. The stimulus was made of a Pasteur pipette containing a piece of filter paper. Different dosages of the candidate synthetic compounds were diluted in cyclohexane, and then each was added to the filter paper and the solvent was allowed to evaporate. A blank stimulus was prepared using only the solvent. During the recordings, stimulation with a standard stimulus (0.01 μg of the propionate ester of (2*S*,3*R*,7*R*)-3,7-dimethyl-2-tridecanol) was done every third time, including the first and the last one. The EAG data were analyzed using the 'EAG version 2.2a' Software (Syntech). Since no pine sawfly species has been shown to use a sex pheromone having a '2R' configuration [2], the EAG recordings were concentrated to the four non-'2R' stereoisomers: propionate esters of *SRR*-, *SRS*-, *SSS*-, and *SSR*-3,7-dimethyl-2-tridecanol, plus the acetate of the *SRR*-isomer (a pheromone component of *D. pini*) and the propionate of *SRR*-3,7-dimethyl-2-pentadecanol (diprionol) (a pheromone component of *D. similis*).

2.6. Field Experiments

Field tests were made in the same pine plantations as the cocoon sampling took place from 1996 to 1998. The plantations were about 35 years old, and the pine trees were approximately 5 m high with a diameter at breast height of 12 cm. The area is situated 1000–1200 m above sea level. Lund-I cardboard sticky traps [18] were used. Traps within each test set were hung in a line from pine trees (about 1.5 m above ground and at least 30 m apart) with their initial positions randomized. In order to minimize any positional effects, the trap positions within the same set were re-randomized when the traps were checked.

Synthetic pheromone candidates (individuals or blends) were diluted with a known amount of hexane or cyclohexane, and then applied to 1 × 4 cm dental cotton rolls (Celluron® No. 2, Paul Hartmann AG, Heidenheim, Germany) as dispensers. The release rates of the tested compounds decrease exponentially with this type of dispenser [19], but the proportion among stereoisomers should remain constant. Tethered live virgin females, 1–3 d old and one per trap, were also included in the 1996 tests as active controls.

2.7. Statistical Analysis

The EAG data were normalized relative to the average EAG response to the standard stimulus (0.01 μg of the propionate ester of *SRR*-3,7-dimethyl-2-tridecanol) (% of STD; Figure 2). The normalized EAG data were then analyzed by one-way ANOVA followed by Tukey's test at α = 0.05. Trap catch data were transformed by log(catch + 1) to improve

normality and homogeneity of variances for ANOVA. Means were compared by one-way ANOVA followed by Tukey's HSD procedure at $\alpha = 0.05$. Analysis of trap catches from tests performed in 1998 was done by the G-test, due to few replicates ($n = 3$).

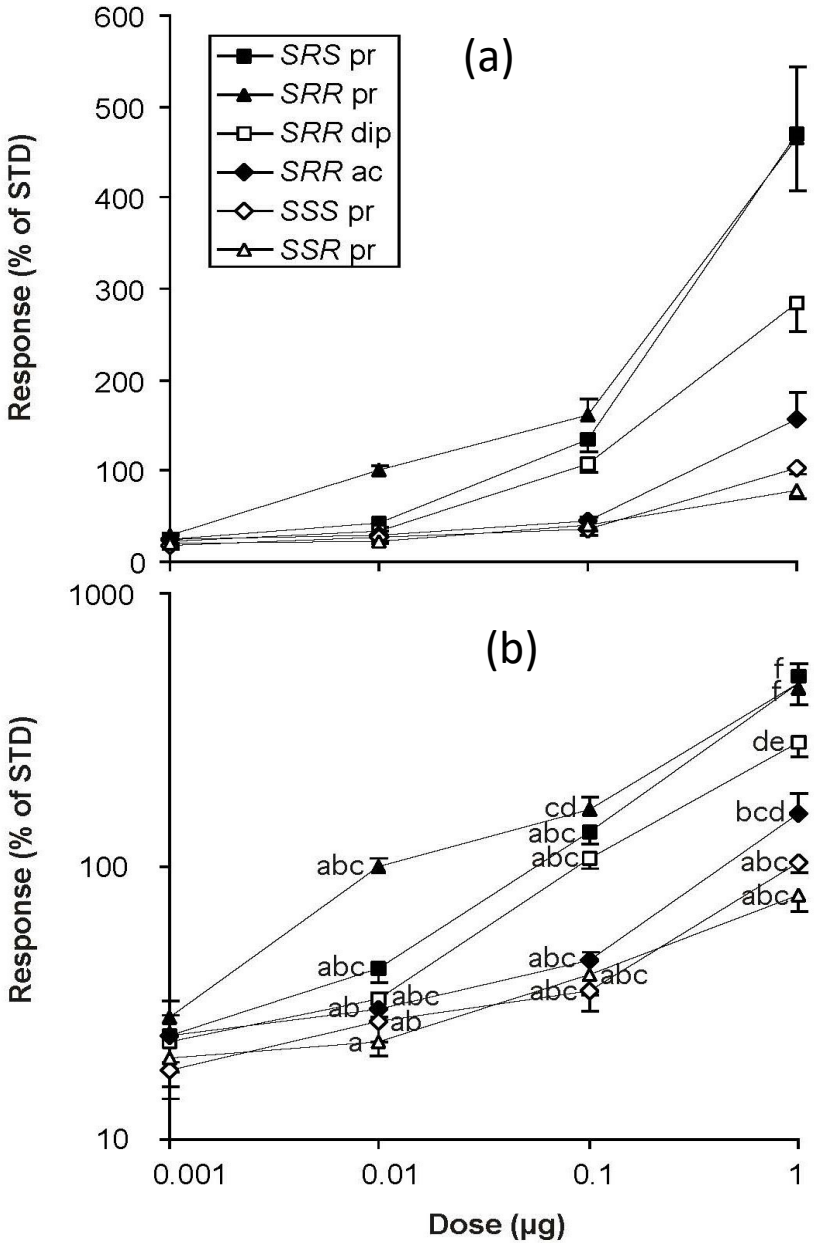

Figure 2. (**a**) Mean (±SE) normalized electroantennographic dose–response curves of male *D. jingyuanensis* antennae (n =10) stimulated with the propionate ester of different 3,7-dimethyl-2-tridecanol isomers and of *SRR*-diprionol and with the acetate of *SRR*-3,7-dimethyl-2-tridecanol. The response to a blank was 22 ± 13%, and 0.01 µg of the propionate ester of *SRR* 3,7-dimethyl-2-tridecanol was used as standard stimulus. (**b**) The same data as in (**a**) but in order to improve separation the *Y*-axis was fitted with a logarithmic scale. Responses with the same letters are not significantly different, ANOVA followed by Tukey's test ($p < 0.05$).

3. Results

3.1. Chemical Analysis

In the female extract, both *erythro*- and *threo*-3,7-dimethyl-2-tridecanol were identified using GC retention time and mass spectra for the compounds of interest. For the identifica-

tion, we used two synthetic reference-mixtures, one consisting of the four stereoisomers of *erythro*-3,7-dimethyl-2-tridecanol and the second of the four stereoisomers of *threo*-3,7-dimethyl-2-tridecanol. The ratio of *erythro/threo* isomers was approximately 1:3 when analyzing the female extract. The two well-resolved peaks in the GC-MS analysis showed that the first eluting peak represents one or more of the erythro-stereoisomers (*SSS, RRR, SSR, RRS*) and the later eluting peak represents one or more of the *threo*-stereoisomers (*SRR, RSS, SRS, RSR*). Although we did not use a tandem system in our analysis, the eluting order of *erythro*- and *threo*-stereoisomers was suggested to be the same as for the GC-separation of *erythro*- and *threo*-3,7-dimethyl-2-pentadecanol in Högberg et al. [20]. In the GC-analysis, with ECD detector, the two well-resolved peaks had a 1:2 ratio when analyzing the female extract. The first eluting peak now probably represents one or more of the *erythro*- and *threo*-isomers: *SSS, RRR, SSR, RRS, SRS, RSR*. Thus, the second peak now probably represents one or two of the *threo*-isomers, *SRR* or *RSS*. Moreover, in this analysis, the eluting order of *erythro*- and *threo*-stereoisomers was suggested to be the same as for the GC-separation of *erythro*- and *threo*-3,7-dimethyl-2-pentadecanol in [20]. Our suggestion of which stereoisomers that would be represented by the first and second peaks may, however, not fully explain why the ratio of *erythro/threo* isomers was 1:3 in the first analysis (GC-MS) and 1:2 in the second (GC-ECD). However, either or both of the two strongly EAG-active threo-isomers, *SRR* and *SRS* (see below for details), likely existed in the female extracts.

3.2. Electrophysiology

Two of the propionate threo-isomers of 3,7-dimethyl-2-tridecanol, namely, *SRS* and *SRR*, induced the strongest responses from male antennae during the EAG recordings (Figure 2). At the highest tested dose (1 µg), the *SRR*- and the *SRS*-isomers elicited significantly higher responses in the male antennae than did the remaining compounds. The *SRR*-propionate of diprionol (a pheromone component of *D. similis*) exhibited the third highest antennal activity, whereas the remaining two isomers (propionate esters of *SSS*- and *SSR*-3,7-dimethyl-2-tridecanol) plus the acetate of the *SRR*-isomer (one of the two pheromone components of *D. pini*) did not elicit any significant increase in antennal response (Figure 2).

3.3. Field Experiments

In the field test in 1996, the propionate *threo*-blend of 3,7-dimethyl-2-tridecanol was compared with the *SRR*-isomer alone. The per-isomer amount was kept constant. The *SRR*-isomer alone at low dosages was inactive and was weakly (but significantly) attractive at the highest dose (1000 µg loading) in at least one set-up (along the stand margin). The *threo*-blend caught significantly more (>5 times more) males than did the *SRR*-isomer alone at the highest dose in both set-ups and at the second highest dose in one set-up (Table 2). The trap baited with a virgin female caught approximately as much as the strongest *threo*-lure, but significantly more males than the single *SRR*-isomer (Table 2). The dose–response relationship showed that approximately 400 µg of the *threo*-blend was needed to attract males in 1996 (Table 2) but only around 100 µg in 1997 (Table 3).

The two isomers showing the highest EAG activity, *SRS* and *SRR*, were tested alone and combined and with the *threo*-blend. The *SRR*-isomer alone was only weakly attractive at 100 µg, whereas the *SRS*-isomer alone at the same loading was 8–14 times more attractive to *D. jingyuanensis* males than was the *SRR*-isomer alone (Table 4). In both set-ups, the combination of the two strongly EAG-active isomers caught significantly more males than the *threo*-blend or the *SRR*-isomer alone. This binary blend (*SRS* and *SRR*) was also significantly more attractive to the males than was the *SRS*-isomer alone in Set-Up 1. Thus, this test indicated, first, that the optimal attractant might be a mixture of the *SRR*- and *SRS*-isomers, and second, that one or both of the remaining two *threo*-isomers (*RSR* and *RSS*) might have an antagonistic effect. These assumptions were tested in a number of tests during 1998. In three of them (Tests 1–3), different ratios between the *SRR*- and *SRS*-isomers were tested, and the results revealed that both isomers are necessary to attract and catch

large numbers of males (Table 5). The ratio between the two isomers did not seem to be very critical, and a 1:1 blend caught large numbers in all tests (Figure 3). In Test 4, the effect of adding the remaining *threo*-isomers (*RSR* and *RSS*: not tested in EAG recordings) to the attractive binary (*SRR* and *SRS*) blend was investigated. No antagonistic activity of these two isomers was found (Table 5).

Table 2. Catch of male *D. jingyuanensis* in traps baited with various amounts of the propionate ester of *threo*-3,7-dimethyl-2-tridecanol or *SRR*-3,7-dimethyl-2-tridecanol, Shanxi Province, P. R. China, 27 June–4 July, 1996. Means followed by the same letter are not significantly different according to one-way ANOVA of log(catch + 1) transformed data followed by Tukey's HSD procedure ($p < 0.05$).

Bait		**Average Catch (±SD)**	
Compound	**Amount (µg)**	**Set-Up 1 Stand Margin (*n* = 12)**	**Set-Up 2 Inside Stand (*n* = 16)**
Blank	-	0.0 ± 0.0 a	0.0 ± 0.0 a
SRR	1	0.0 ± 0.0 a	0.0 ± 0.0 a
SRR	10	0.0 ± 0.0 a	0.0 ± 0.0 a
SRR	100	0.0 ± 0.0 a	0.0 ± 0.0 a
SRR	1000	16.3 ± 16.4 b	0.4 ± 0.7 ab
threo	4	0.0 ± 0.0 a	0.0 ± 0.0 a
threo	40	0.0 ± 0.0 a	0.0 ± 0.0 a
threo	400	3.6 ± 5.4 a	1.9 ± 1.7 b
threo	4000	88.8 ± 20.4 c	93.6 ± 13.3 d
Virgin female	-	65.7 ± 34.6 c	62.2 ± 47.3 c

Table 3. Catch of male *D. jingyuanensis* in traps baited with different amounts of the propionate ester of *threo*-3,7-dimethyl-2-tridecanol, Shanxi Province, P. R. China, 13–23 June 1997. Means followed by the same letter are not significantly different according to one-way ANOVA of log(catch + 1) transformed data followed by Tukey's HSD procedure ($p < 0.05$).

Amount (µg)	**Average Catch (±SD) (*n* = 6)**
Blank	0.0 ± 0.0 a
0.1	0.2 ± 0.4 a
1	0.0 ± 0.0 a
10	0.2 ± 0.4 a
100	8.3 ± 5.5 b
1000	102.3 ± 14.1 c

Table 4. Catch of male *D. jingyuanensis* in traps baited with different isomers or combinations of isomers of propionate ester of 3,7-dimethyl-2-tridecanol, Shanxi Province, P. R. China, 13–18 June 1997. Means followed by the same letter are not significantly different according to one-way ANOVA of log(catch + 1) transformed data followed by Tukey's HSD procedure ($p < 0.05$).

Bait		**Average Catch (±SD)**	
Compound	**Amount (µg)**	**Set-Up 1 Stand Margin (*n* = 8)**	**Set-Up 2 Inside Stand (*n* = 10)**
Blank	-	0.0 ± 0.0 a	0.0 ± 0.0 a
threo	400	35.6 ± 24.8 c	23.5 ± 27.1 b
SRR	100	5.1 ± 4.5 b	4.8 ± 9.4 a
SRS	100	40.5 ± 23.4 c	66.0 ± 39.4 bc
SRR + *SRS*	100 + 100	112.9 ± 17.5 d	99.8 ± 36.4 c

Table 5. Catch of male *D. jingyuanensis* in traps baited with different combinations of isomers of the propionate ester of 3,7-dimethyl-2-tridecanol, Shanxi Province, P. R. China, 13–21 June 1998. Catch figures within each test having the same letters are not significantly different ($p < 0.05$). Total catches compared with G-test including Sidák's compensation for mass significance. Each test is based on three replicates.

	Bait: Isomer, Amount (µg)				Total Catch
	SRR	***SRS***	***RSR***	***RSS***	
Test 1			Blank		0 a
	100	0			13 a
	90	10			10 a
	50	50			236 c
	10	90			238 c
	0	100			170 b
Test 2			Blank		0 a
	100	0			1 a
	100	1			3 a
	100	11			3 a
	100	100			546 b
Test 3			Blank		0 a
	0	100			18 a
	1	100			29 a
	11	100			317 b
	100	100			693 c
Test 4			Blank		0 a
	50	50			181 b
	50	50	50		232 c
	50	50		50	280 d

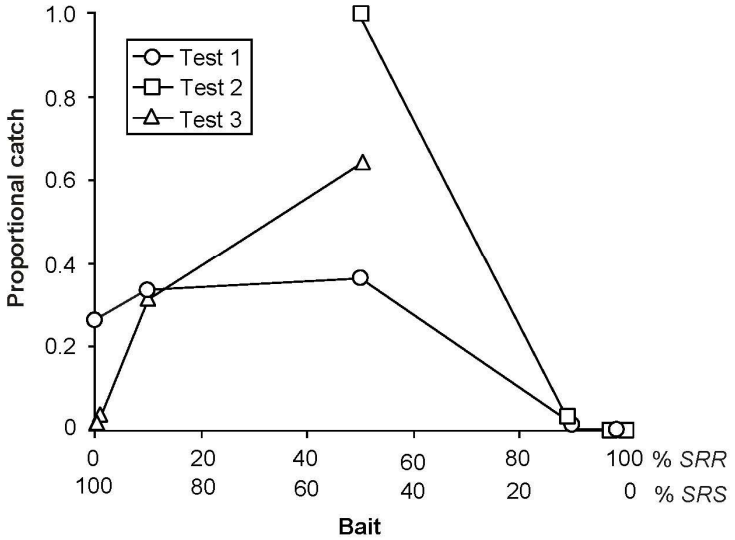

Figure 3. Relative catch of male *D. jingyuanensis*, expressed as the proportion of the total (within each test), for different ratios of the *SRR*- and *SRS*-isomers of the propionate ester of 3,7-dimethyl-2-tridecanol. Data shown separately for Tests 1–3 in 1998 (see also Table 5).

4. Discussion

We have shown that the propionate ester of the *SRR*-isomer of 3,7-dimethyl-2-tridecanol was weakly but significantly attractive to *D. jingyuanensis* males; thus, it might be part of the pine sawfly sex pheromone system as a synergistic component, whereas the *SRS*-isomer seemed to be a key/essential pheromone component since it alone was strongly attractive to the males. The blend of all four *threo*-isomers (2*S*,3*R*,7*R*/*S* and 2*R*,3*S*,7*R*/*S*)

was also significantly attractive in the current study and seemed to be a good and effective sex-attractant, confirming the results of earlier field screening [14] and monitoring [12].

Chemical analyses of the female whole-body extracts indicated that either or both of the two *threo*-isomers (*SRR/SRS*) likely presented in the second peak in the GC-MS analysis, whereas the *SRS*-isomer might be a part the first peak and *SRR*-isomer possibly presented in the second peak in the GC-ECD analysis. However, as the amount of insect material was only sufficient for mass spectrometric identification and one analysis of pentafluorobenzoate by electron capture detector, the extensive stereochemical analysis of the precursors for determining the complete isomer composition was not completed.

Together with the *SRS*-isomer, the *SRR*-isomer evoked the strongest antennal response in *D. jingyuanensis* males. These two isomers elicited significantly stronger EAG responses than the other tested compounds. In field experiments, the combinations of these two strongly EAG-active isomers (*SRR* and *SRS*; their alcohol precursors likely present in the female whole-body extract) were significantly more attractive to males than either isomer alone, indicating a significant synergism. Females also contained the *erythro*-isomer(s) *SSS/SSR* or *RRR/RRS* (in the first peak in the GC-MS analysis of the female extract), but neither *SSS* nor *SSR* were active in the EAG recordings. Since no pine sawflies have been shown to use a sex pheromone having a '2*R*' configuration [2], *RRR/RRS* or *RSR/RSS* isomers will unlikely be a part of the *D. jingyuanensis* sex pheromone system. However, it is premature to suggest that the sex pheromone of *D. jingyuanensis* consists of both the *SRR*- and the *SRS*-isomers as the exact stereoisomer composition of the female content is still unknown.

Synergism between different steroisomers has earlier been reported in a few species of pine sawflies. In the widely distributed *Neodiprion sertifer*, Anderbrant et al. [21] examined the attractivity of males to different proportions of either *SRR*- or *SRS*-isomers added to the sex pheromone, the acetate ester of *SSS*-3,7-dimethyl-2-pentadecanol (diprionol), in eight places on the Northern hemisphere from Japan in the East to Canada in the West, via sites in Asia and Europe. Significantly more *N. sertifer* males were captured only in Siberia when *SRR*- or *SRS*- were added to the *SSS*-isomer at 0.01%–100% of that of the *SSS* bait.

Males of *Neodiprion pratti banksianae* were significantly more attracted to *SSS* + *SRR* of diprionyl acetate than to the single *SSS*-stereoisomer, although the catches were quite low [22]. Similarly, males of both *Neodiprion rugifrons* and *Neodiprion dubiosus* were more attracted to *SRR* + *SRS* diprionyl propionate than to any of the isomers alone [23]. However, again, the catches were low, and these experiments need to be repeated. In both *N. rugifrons* and *N. dubiosus*, the male responses in EAG recordings were stronger to *SRS* than to *SRR*, although the doses used were relatively high [23].

In all the cases where males of different species were more attracted to combinations of steroisomers rather than to the individual stereoisomers, little information about female content and released amount exists. As no gland responsible for pheromone production has been detected in diprionid females, whole-body extracts are often used, making analysis more difficult. It would be desirable to analyze what the female actually releases instead of what she contains. The first case where synergism in male response between different isomers occurs simultaneously with a female release of these compounds still remains to be identified.

5. Conclusions

The few *Diprion* species investigated so far all seem to make use of the *SRR* configuration of their pheromone molecules as the sex attractant. Males of *D. similis and D. nipponica* were attracted to propionate esters of *SRR*-3,7-dimethyl-2-pentadecanol and *SRR*-3,7-dimethyl-2-undecanol, respectively ([2] and references therein). The attraction of *D. similis* to the *SRR*-isomer was reported to be synergized by three other isomers, viz., *SSS, SSR,* and *SRS* [6], but this could not be confirmed in a later study [24]. In both *D. pini* and *D. jingyuanensis,* males responded to the propionate ester of *SRR*-3,7-dimethyl-2-tridecanol. Males of *D. pini* also responded to the acetate of this molecule [15,16]. Sur-

prisingly, *D. jingyuanensis* males in the current study were not only strongly attracted to the propionate ester of *SRS*-3,7-dimethyl-2-tridecanol (as a key/essential attractant), but also weakly and synergistically to the *SRR*-isomer (as a weak but synergistic attractant). For maximum trapping of *D. jingyuanensis* males, a synergistic mixture of *SRR*- and *SRS*-isomers (at 1:1 ratio) is recommended. However, due to the difficulty (expensiveness) of the chiral synthesis, the inexpensive propionate *threo*-blend of 3,7-dimethyl-2-tridecanol seems to be adequate for development of an effective pheromone-based monitoring and/or mass-trapping program against this economically important pine sawfly.

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