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Aggregation pheromone of a newly described spruce bark beetle, *Ips shangrila* Cognato and Sun, from China

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Abstract Volatiles from male hindgut extracts of a newly described spruce bark beetle, *Ips shangrila* Cognato and Sun, from different attack phases were analyzed by GC–MS/FID with both polar and enantioselective columns. The GC–MS/FID analyses showed that unmated males (Phase 1) or males mated with <3 females (Phases 2–4) produced 2-methyl-3-buten-2-ol and 99%-(+)-ipsdienol as major components, and (−)-*cis*-verbenol, (−)-*trans*-verbenol, myrtenol and 2-phenyl ethanol as minor or trace components. The release of these male-produced compounds was confirmed by the analysis on aeration sample of an *I. shangrila* infested wind-thrown spruce trunk. The quantities of 2-methyl-3-buten-2-ol, *cis*-verbenol and *trans*-verbenol from male hindgut extracts were almost unchanged or even slightly increased during gallery development, while ipsdienol decreased dramatically after mating with three females. No obvious *Ips*-related aggregation pheromone components were detected in the female hindgut extract. A field trapping bioassay in Qinghai, China, showed that the ternary blends containing 2-methyl-3-buten-2-ol, (−)-*cis*-verbenol and 97%-(+)-ipsdienol or (±)-ipsdienol, caught significantly more *I. shangrila* (♂:♀ = 1:2.14) than did the unbaited control. Replacing 97%-(+)-ipsdienol (close to the naturally produced enantiomeric ratio) with (±)-ipsdienol in the ternary blend seemed to reduce trap catches by 50%, but the difference was not statistically significant. Surprisingly, addition of (−)-*trans*-verbenol (at 0.2 mg/day) to the active ternary blends significantly reduced traps catches to the level not different from the unbaited control. Our results suggest that the two major components, 2-methyl-3-buten-2-ol and 99%-(+)-ipsdienol, plus a minor component, (−)-*cis*-verbenol, produced by fed males, are likely the aggregation pheromone components of *I. shangrila.*

Keywords Enantiomeric composition · *Ips shangrila* · Ipsdienol · 2-Methyl-3-buten-2-ol · *Picea* · Scolytidae · Semiochemical · *cis*-Verbenol

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Introduction

Ips shangrila Cognato and Sun (Coleoptera: Scolytidae) is a newly described bark beetle species based on specimens collected from several species of spruce (*Picea* spp.) in Sichuan, Yunnan and Qinghai provinces, China (Cognato and Sun 2007). This new species was misidentified as *Ips mannsfeldi* (Wachtl. 1879) in the past by the Chinese entomologists (Yin et al. 1984) even though its host range

(*Picea* spp.) in China was significantly different from those recorded for its European populations (*Pinus* spp.) (Stauffer et al. 1997). Its species status in the Qinghai province was first challenged by Milŏs Knízek (Forestry and Game Management Research Institute, Czech Republic) during a field visit in July 2006, and was later recognized together with the specimen from Yunnan and Sichuan by Anthony I. Cognato (Michigan State Univ.) as a new species based on a cladistic analysis of both DNA and diagnostic morphological character data (Cognato and Sun 2007).

This bark beetle normally infests weakened, wind-thrown, or burned trees, but at high population densities it can also attack healthy spruce trees. In fact, *I. shangrila* together with *Ips nitidus* Eggers and *Pseudips orientalis* (Wood and Yin) (Cognato 2000), were recently recognized as the most destructive forest beetles in the Maixiu Forest Park of Qinghai Province, China; causing significant tree mortality in both plantations and natural stands of the Qinghai spruce [*Picea crassifolia* (Kom.)] since 2001 (Xue et al. 2003; Liu et al. 2007). The basic biology and host colonization sequence (temporal and spatial) in relation to other sympatric bark beetle species were reported in two lately published papers by You-Qing Luo and coworkers (Liu et al. 2007, 2008). However, nothing is known about the aggregation pheromone system of this new species.

As part of our research effort on the chemical communication systems of the three key spruce bark beetles native solely to western China, *Ips nitidus*, *I. shangrila* and *Ps. orientalis*, we recently identified three aggregation pheromone components for *I. nitidus* (Zhang et al. 2009). Our objectives of the present study were to (1) identify the aggregation pheromone of *I. shangrila*, (2) analyze the quantitative variation of pheromone components from different attack phases, (3) determine the enantiomeric compositions of major chiral pheromone components, and (4) test the behavioral activity of the key male-specific compounds in the field in Qinghai, China.

Materials and methods

Collection and preparation

Field collections of live *I. shangrila* adult beetles of different attack phases [Phase 1, unmated males finishing nuptial chambers; Phases 2–5, mated males with 1–4 females in the galleries] were taken from naturally attacked wind-thrown spruce trees (*Picea crassiflia*) at Maixiu Forest Farm (35°08′–35°30′ N; 101°33′–102°03′ E; ca. 2,900–3,000 m elevation), Huangnan Tibetan District, Qinghai province, China (from 22–25 May 2008). Beetles from the same family were placed in a 2-ml polyethylene centrifuge tube (Fisher Scientific) and immediately put into an outdoor cooler (ca. 4°C). The centrifuge tubes were separated into categories of attack phases on the same day of collection in the laboratory, and the hindguts were dissected, and the sex was determined based on their elytral spine differences (Cognato and Sun 2007) and if necessary on the presence of the aedeagus or eggs. The guts of the same sex from the same attack phase were immediately transferred to a glass vial with 1 ml redistilled pentane (with 2 μg of heptyl acetate as internal standard).

Headspace volatiles from the trunk of a wind-thrown spruce tree, newly attacked by *I. shangrila* for <5 days, were sampled in situ by a battery-operated pump and a high-density polyacetate film (cut from a 48.2 × 59.6 cm Reynolds® Oven Bag; Richmond, VA, USA) enclosure with two activated charcoal filter tubes in the air inlet (Zhang et al. 2000b), on 22nd May 2008. The plastic film around the trunk (at mid-section of the tree trunk) was joined by folding/taping on the side and tightened to the trunk with tape at both open ends. The distance between the film and bark surface was ca. 1 cm. The volatiles in the enclosure (with a sampling bark area of ca. 2,000 cm^2; diameter, 13 cm; length, 50 cm) were trapped on two Porapak Q tubes (50/80 mesh; 30 mg in Teflon tube, 3 × 35 mm) for 2 h (airflow 350 ml/min) and extracted with 1 ml redistilled pentane (with 2 μg of heptyl acetate as internal standard). After the aeration, the aerated trunk portion was debarked and the number and status of *I. shangrila* attacks within the sampled area were determined as 21 attacks from mixed Phases 1–4. The same aeration procedure was also used to collect volatiles from a spruce log (diameter, 15 cm length, 30 cm; in a Reynolds® Oven Bag) freshly cut from an un-attacked healthy spruce tree.

Both hindgut (4–15 guts/sample for males and 15 guts/sample for females) and aeration extracts were shipped to the USA by express mail and kept at −20°C until they were analyzed chemically.

Chemical analysis

All hindgut and aeration samples were analyzed on a combined Agilent 6890N gas chromatograph (GC) and an Agilent 5973N mass selective detector (MSD) equipped with a polar column (INNOWax; 60 m × 0.25 mm × 0.5 μm film thickness; Agilent Technologies, Wilmington, DE, USA). The GC condition and temperature program were the same as described in Zhang et al. (2009) for *I. nitidus* samples. Compounds were identified by comparison of retention times and mass spectra with those of authentic standards (see "Chemical standards" below).

GC-FID analysis

The hindgut and aeration samples were also injected into a Varian CP-3800 GC equipped with a polar column (INNOWax; 30 m × 0.53 mm × 1.0 μm film thickness; Agilent Technologies, Wilmington, DE, USA) and FID for compound quantification based on the internal standard (IS: 2 μg of heptyl acetate in each sample; assuming similar or identical response factors between the analytes and the IS). The GC condition and temperature program were as described for *I. nitidus* samples (Zhang et al. 2009).

Enantioselective GC-FID

The enantiomeric analyses of male hindgut extracts (from Phases 1–2), and a synthetic mixture of several compounds known as key components of *Ips* pheromones including (±)-ipsenol, (±)-ipsdienol, (1*S*,2*S*)-(−)-*cis*-verbenol, (1*S*,2*R*)-(−)-*trans*-verbenol, amitinol, (1*S*)-(−)-verbenone, and *E*-myrcenol (50 ng/μl each in hexane) were conducted by injecting the samples splitless on a Varian CP-3800 GC equipped with an Rt-bDEXm^{TM} column (30 m × 0.25 mm × 0.25 μm film thickness; Restek, Bellefonte, PA, USA). Helium was used as the carrier gas, and the injector/detector temperatures were both 230°C. Column temperature was 80°C for 1 min and programmed to 200°C at 2°C/min. Elution orders of the (−)- or (+)-enantiomers of ipsenol and ipsdienol [(−)-eluted before (+)-for both compounds] were determined by injecting SPME (CAR/PDMS, 75 μm; Supelco, Bellefonte, PA, USA) samples of synthetic 97%-(+)-ipsdienol and 97%-(−)-ipsenol onto the same column. Since no pure (+)-enantiomers of *cis*- or *trans*-verbenol, and verbenone were available for comparison, our suggestions on their enantiomeric assignments were based entirely on the retention time matches to the synthetic (−)-enantiomers. Therefore, these assignments should be considered tentatively.

Chemical standards

Synthetic compounds were obtained from various commercial and noncommercial sources: (±)-ipsenol (95%, chemical purity: cp), (±)-ipsdienol (95% cp), (−)-*cis*-verbenol (98% cp, unknown enantiomeric purity-ep), and (−)-verbenone (99% cp, unknown ep) (Bedoukian Research Inc., Danbury, CT, USA); amitinol (98% cp, W. Francke, Universität Hamburg, Hamburg, Germany); *E*-myrcenol (95.2% cp, SciTech, Prague, Czech Republic); 2-methyl-3-buten-2-ol (97% cp, Acros, Morris Plains, NJ, USA); (−)-ipsenol (95% cp; 97% ep), (+)-ipsdienol (95% cp; 97% ep), (−)-ipsdienol (95% cp; 97% ep) and (−)-*trans*-verbenol (>95% cp, unknown ep) [Pherotech (now Contech) International, Inc., Delta, BC, Canada]; and heptyl acetate (>98% cp, food grade) (Sigma–Aldrich, St. Louis, MO, USA).

Field trapping

To test for the behavioral activity of the potential semiochemicals, a field trapping experiment was carried out from 30 April to 14 May 2009 at the Maixiu Forest Park, Qinghai, China. Four sets of cross-barrier type traps (Pherobio Technology Co., Ltd., Beijing, China) were set up along the edge of a *P. crassifolia* forest stand on a northern slope next to a creek at Douheyan, with >30 m between trap sets and ca. 10 m between traps within each set, and >10 m from the nearest trees. Within each set, eight traps were baited with different blends (full or partial blends; racemic or enantiomerically pure) of the key male-produced volatile compounds; a ninth trap was left unbaited as a negative control (Fig. 4). Each tested compound was released separately from a polyethylene bag (with or without substrate felt; and with different sizes and thickness). The dispenser types, loading and release rates of the tested semiochemicals are described in Fig. 4. The positions of traps together with dispensers within each set were assigned randomly.

Statistical analyses

Trap catch data were converted to proportion (P) of total captured beetles within each replicate. Data were then transformed by $\arcsin\sqrt{P}$ to meet the assumptions of normality and homogeneity of variances for ANOVA. Means were compared by ANOVA followed by the Ryan–Einot–Gabriel–Welsh (REGW) multiple Q test (SPSS 16.0 for Windows) at $\alpha = 0.05$ (Day and Quinn 1989).

Results

Chemical analysis

Over ten volatile compounds from the extracts of male hindguts were identified by GC–MS and quantified by GC-FID. The major components from the male extracts included 2-methyl-3-buten-2-ol and ipsdienol, followed by *cis*-verbenol and *trans*-verbenol, while ipsenol and verbenone were almost absent (Fig. 1; Table 1). Other volatiles found from *I. shangrila* were myrtenol and 2-phenyl ethanol, 3-hydroxy-2-butanone, 1-heptanol and isobutyric acid.

GC–MS/FID analysis of aeration samples of *I. shangrila* infested spruce trunk showed the release of male-produced compounds, 2-methyl-3-buten-2-ol, ipsdienol, *cis*-verbenol and *trans*-verbenol (Fig. 2). These compounds were not

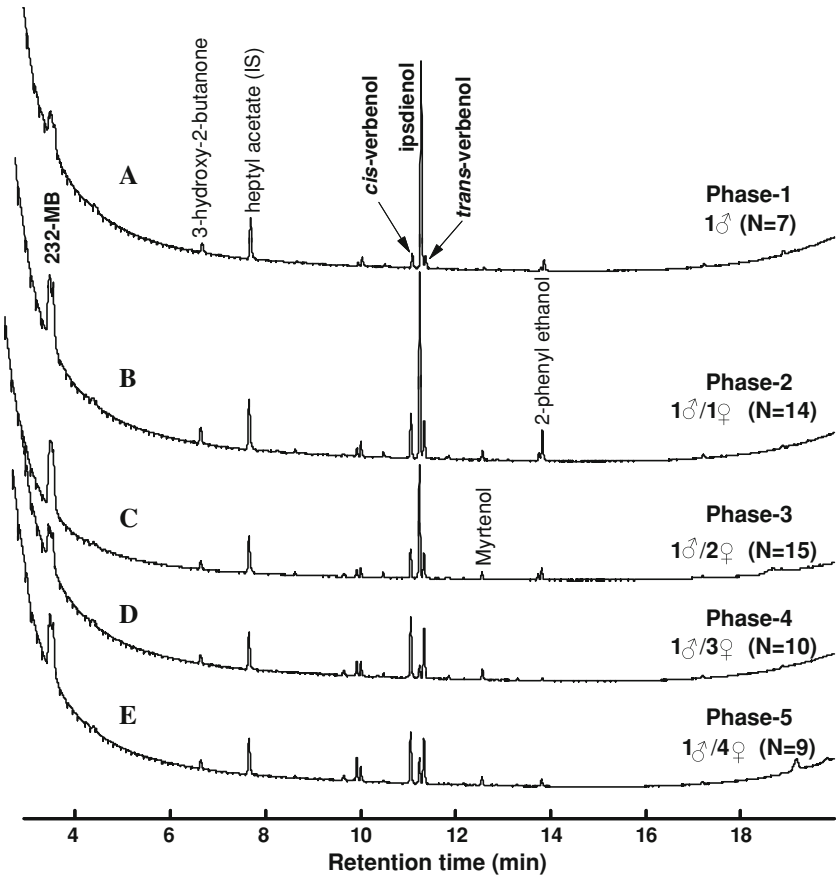

Fig. 1 Representative gas chromatograms (polar column with FID detection) of compounds in hindgut extracts of male *Ips shangrila* from different attack phases (A–E). Heptyl acetate (2 μg/sample) was added as an internal standard to the hindgut extracts. 232-MB: 2-methyl-3-buten-2-ol

Table 1 Quantities of potential semiochemicals (in ng) identified from hindgut extracts of *Ips shangrila* males/females of different attack phases, Qinghai Province, China, 22–25 May 2008

Retention time (min)	Chemical	Male hindguts from different attack phases										Mated ♀ hindguts (ng/♀) ($N = 1$; $n = 15$)
		1♂ ($N = 2$; $n = 7$–10)*		1♂1♀ ($N = 2$; $n = 14$)		1♂2♀ ($N = 2$; $n = 8$–15)		1♂3♀ ($N = 2$; $n = 4$–5)		1♂4♀ ($N = 1$; $n = 9$)		
		ng/♂	%	ng/♂	%	ng/♂	%	ng/♂	%	ng/♂	%	
3:28	2-Methy-3-buten-2-ol	503.3	31.0	477.8	23.7	538.6	27.4	412.9	36.6	764.6	40.2	Trace
6:39	3-Hydroxy-2-butanone	57.4	3.5	73.3	3.6	65.9	3.3	83.2	7.4	69.9	3.7	70.0
8:39	1-Heptanol	0.0	0.0	9.1	0.5	0.0	0.0	34.2	3.0	42.2	2.2	30.0
9:41	(−)-Ipsenol	0.0	0.0	0.0	0.0	6.5	0.3	0.0	0.0	0.0	0.0	
10:02	Isobutyric acid	81.1	5.0	97.9	4.9	103.9	5.3	41.4	3.7	168.4	8.9	80.0
11:05	(*S*)-*cis*-verbenol	136.6	8.4	120.1	6.0	139.5	7.1	200.7	17.8	257.5	13.6	
11:16	(+)-Ipsdienol (99%)	661.6	40.8	1021.2	50.7	904.9	46.0	37.3	3.3	99.5	5.2	Trace
11:22	(−)-*trans*-verbenol	72.5	4.5	100.8	5.0	112.1	5.7	172.7	15.3	175.5	9.2	Trace
11:53	(−)-verbenone	0.0	0.0	0.0	0.0	0.0	0.0	57.1	5.1	0.0	0.0	
12:35	Myrtenol	29.5	1.8	39.1	1.9	42.5	2.2	58.2	5.2	43.8	2.3	
13:51	2-Phenyl ethanol	80.5	5.0	73.4	3.6	54.6	2.8	29.3	2.6	56.6	3.0	

* N number of hindgut extracts per attack phase, n number of hindguts per extract

detected from the aeration sample of the freshly cut (un-attacked) spruce log, from which several monoterpenes (including two major components, α-pinene and β-pinene, accounting for >70% total volatiles), sesquiterpenes and other conifer-related compounds were identified (data not shown).

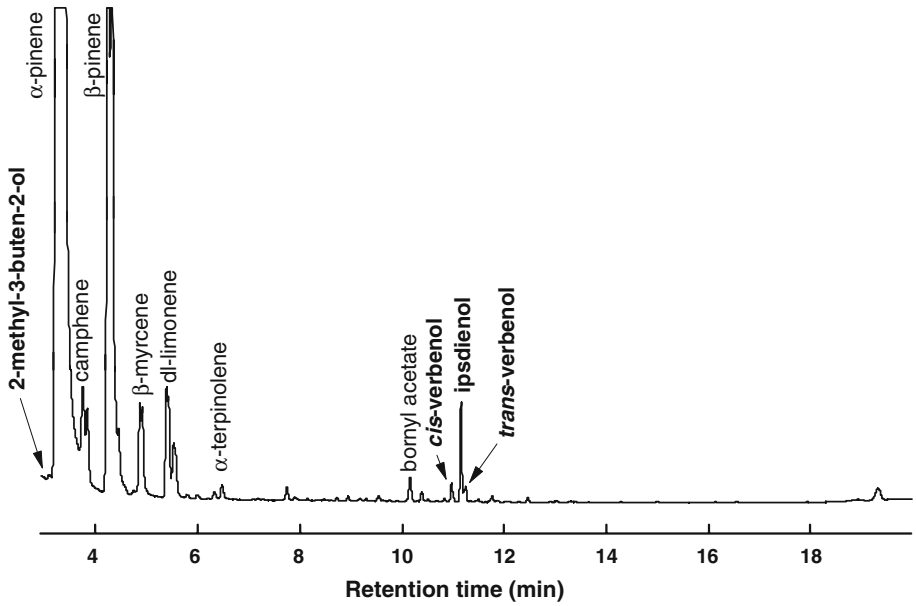

Fig. 2 Representative gas chromatogram (polar column with FID detection) of the aeration extract of an *Ips shangrila* infested spruce trunk. Beetle-produced volatiles are *highlighted in bold*

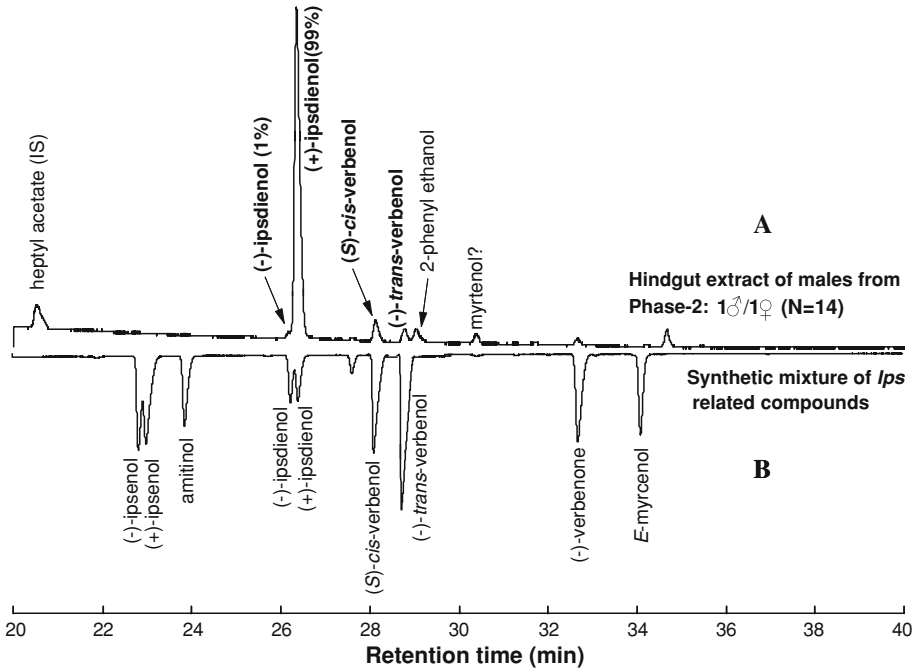

Fig. 3 Enantioselective GC-FID analyses (Rt-bDEXm^{TM} column) of (**A**) compounds in a hindgut extract of unpaired male *Ips shangrila* from attack Phase 2 and (**B**) a synthetic mixture of *Ips*-related compounds, including (±)-ipsenol (Ie), (±)-ipsdienol (Id), amitinol (At), (−)-*cis*-verbenol (*c*V), (−)-*trans*-verbenol (*t*V), (−)-verbenone (Vn), and (*E*)-myrcenol (EM) (ca. 50 ng/each compound). Amitinol and *E*-myrcenol are achiral. Heptyl acetate (2 µg/sample) was added as an internal standard to the hindgut extract

GC-FID analysis with an enantioselective stationary phase of the hindgut extracts of the *I. shangrila* males from Phases 1–2 and a synthetic mixture of *Ips* pheromone compounds showed that *I. shangrila* males produced nearly pure (+)-ipsdienol (∼99%) (Fig. 3). The predominant enantiomers of other chiral compounds were tentatively determined as (−)-*cis*- and (−)-*trans*-verbenol (Fig. 3).

Mean amounts ($N = 2$) of 2-methyl-3-buten-2-ol, ipsdienol, *cis*-verbenol and *trans*-verbenol from Phase 1 were about 500, 660, 140 and 70 ng/male, respectively (Table 1). Although no statistical analysis was performed because of the limited data set, the amounts of 2-methyl-3-buten-2-ol, *cis*-verbenol and *trans*-verbenol were almost unchanged or even increased during gallery development (Phases 2–5, i.e. one male mated with 1–4 females), while ipsdienol decreased dramatically after males mated with more than three females (Fig. 1; Table 1).

No obvious *Ips*-related aggregation pheromone components were detected in the female hindgut extracts (data not shown). However, 3-hydroxy-2-butanone, 1-heptanol and isobutyric acid were detected in the female extracts at about the same level as found in the male extracts.

Field trapping experiment

Due to extremely low populations of *I. shangrila* and continued rains during the short spring flight, only three physical replicates were achieved in the field trapping experiment (end of April to mid-May 2009) with a total catch of 66 beetles. The unbaited traps and traps baited with binary or four-component blends caught zero or close to zero beetles (Fig. 4). However, ternary blends containing 2-methyl-3-buten-2-ol and *cis*-verbenol plus 97%-(+)-ipsdienol or (±)-ipsdienol caught significantly more beetles than did the unbaited control and several other blends (Fig. 4). The ternary blend with 97%-(+)-ipsdienol (close to naturally produced enantiomeric composition) seemed to catch more *I. shangrila* beetles than did the ternary blend with (±)-ipsdienol, but data were not statistically different. Addition of *trans*-verbenol (at 0.2 mg/day release) to the active ternary blends significantly reduced the trap catches (Fig. 4). The sex ratio of captured beetles was estimated as 1:2.14(♂/♀), based on the pooled sample.

Discussion

This is the first report on chemical and behavioral analysis of aggregation pheromone system of the newly described scolytid species, *Ips shangria*. Our GC–MS/FID analyses clearly showed that unmated males (after finishing the nuptial chambers) or males mated with <3 females produced 2-methyl-3-buten-2-ol and ipsdienol as major components, and *cis*-verbenol, *trans*-verbenol, myrtenol and 2-phenyl ethanol as minor or trace components (Fig. 1; Table 1). The release of these male-produced compounds was confirmed by the aeration sample of an *I. shangria* infested wind-thrown spruce trunk (Fig. 2). It should be pointed out that the release ratio of 2-methyl-3-buten-2-ol in the aeration sample was smaller than the production ratio in the hindgut extracts. Such difference is simply due to its significant breakthrough volume of the extremely volatile compound from the Porapak Q tubes during the aeration (Birgersson and Bergström 1989). Similar to *I. nitidus*, 3-hydroxy-2-butanone, 1-heptanol, and isobutyric acid, commonly related to insect fat tissues (Zhang et al. 2006),

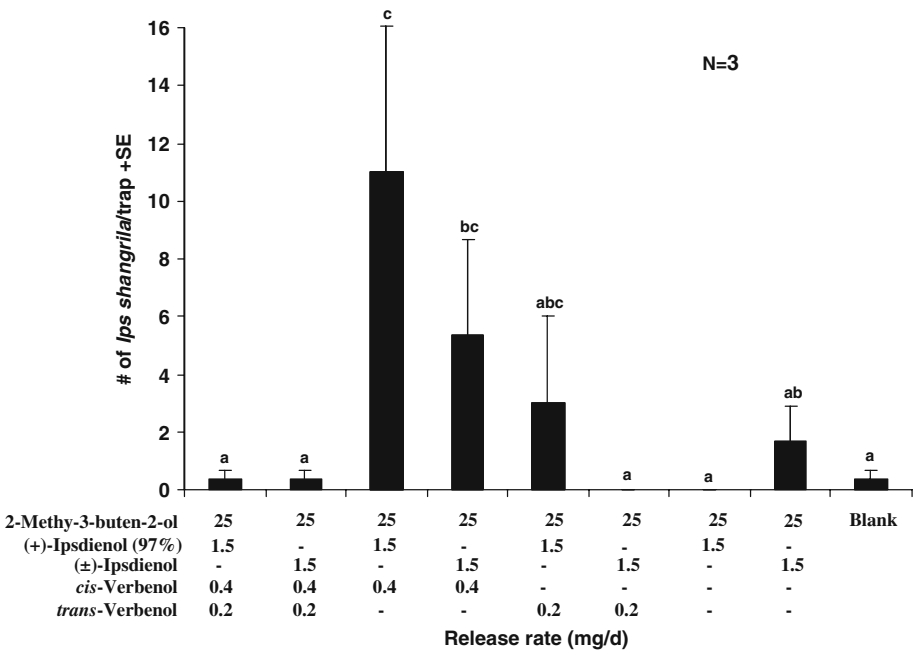

Fig. 4 Mean captures ($N = 3$) of *Ips shangrila* in cross-barrier traps baited with different combinations of the key male-produced volatile compounds in their natural production ratios, 30 April to 14 May 2009, Maixiu Forest Park, Qinghai, China. An unbaited trap served as the negative control. *Bars with the same letter* are not significantly different ($P > 0.05$) by REGW multiple Q test after ANOVA on the arcsin$\sqrt{P}$ transformed data of the relative catches, i.e., proportion (P) of total captured beetles within each replicate. Each tested compound was released separately from a polyethylene (PE) bag dispenser. 2-Methyl-3-buten-2-ol: 1 ml in a 2 mil (~0.051 mm thickness) PE-bag (3.5 × 5.0 cm; with a 2.5 × 4.5 cm felt); 97%-(+)- or (±)-ipsdienol: 40 mg in a 12 mil (~0.305 mm) PE-bag (2.5 × 5.0 cm; with a 1.5 × 4.5 cm felt); (−)-*cis*-verbenol: 50 mg in 6 mil (~0.152 mm) PE-bag (3.0 × 5.0 cm, without felt); (−)-*trans*-verbenol: 20 mg a 12 mil (~0.305 mm) PE-bag (1.8 × 5.0 cm; with a 1.0 × 4.5 cm felt). The release rates were measured by suspending dispensers in a laboratory hood at 21–22°C and measuring weight loss over 7–10 days

were also present in all male and female hindgut extract samples of *I. shangrila*, and are presumably not part of the male-produced aggregation pheromone system (Zhang et al. 2009). Chemical analysis of the hindgut extracts of mated females showed little or trace amounts of these semiochemicals (Table 1), in agreement with the preponderance of studies that indicate that female *Ips* do not produce behaviorally relevant amounts of aggregation pheromones during attacks (Wood 1982; Byers 1989b).

Unlike in many other *Ips* spp. (e.g. *Ips nitidus, I. typographus* and *I. subelongatus*), mating shows little or weak effect on the production of the key *I. shangria* male-produced volatiles, especially when the harem size is <3 (Fig. 1; Table 1). This might be due to the fact that each *I. shangria* male is normally joined by >5 females in a typical family gallery system, while the above-mentioned other *Ips* species only have 2–3 females per family (Birgersson et al. 1984; Zhang et al. 1992, 2000a, 2009).

The chemical composition of *I. shangria* male-produced volatiles seems to be quite similar to a sympatric and partially competitive species, *I. nitidus* (Zhang et al. 2009). However, the enantiomeric composition of the major component ipsdienol in *I. shangrila* [~99%-(+)] (Fig. 3) is totally different from that of the *I. nitidus* [74%-(−)] (Zhang et al. 2009). Such strong disparity in the enantiomeric composition should be one of the key factors for maintaining the reproductive isolation among these two closely related and sympatric (or syntopic) species. Production of >90%-(+)-ipsdienol as a part of aggregation pheromone systems have been reported in several *Ips* species, such as *I. acuminatus* (Kohnle et al. 1988), *I. cembrae* (Francke and Vité 1983), *I. latidens* (Savoie et al. 1998), *I. mannsfeldi* (Kohnle et al. 1993), *I. paraconfusus* (Silverstein et al. 1966), *I. plastographus* (Warren et al. 1996) and *I. subelongatus* (Zhang et al. 2000a). Two other chiral compounds were tentatively assigned to be (−)-*cis*- and (−)-*trans*-verbenol based on comparison of retention times of natural products with synthetic standards (Fig. 3), which are similar to the reported absolute configurations for many *Ips* bark beetles (Kohnle et al. 1988) including the sympatric *I. nitidus* (Zhang et al. 2009).

Our field trapping data showed that the ternary blends containing the two major components, 2-methyl-3-buten-2-ol, 97%-(+)- or (±)-ipsdienol, plus a minor component, (−)-*cis*-verbenol, caught significantly more *I. shangrila* beetles than did the unbaited control (Fig. 4). Replacing 97%-(+)-ipsdienol (close to the naturally produced enantiomeric ratio) with (±)-ipsdienol in the ternary blend seemed to reduce trap catches by 50%, but their difference was not statistically significant. Such insignificant difference in trap catches might be due to the large variations among the treatments, likely resulted from the extremely low population level and limited physical replicates obtained. More field trapping experiments are needed to determine whether the natural ratio of ipsdienol enantiomers will prove superior to racemic ipsdienol. Surprisingly, addition of (−)-*trans*-verbenol to the active ternary blends significantly reduced traps catches to the level not different from the unbaited control. This compound was also detected in several other Eurasian *Ips* species as a minor component, such as *I. nitidus* (Zhang et al. 2009), *I. typographus* (Birgersson et al. 1984), and *I. duplicatus* (Byers et al. 1990; Schlyter et al. 1992; Zhang et al. 2007), but was not considered to be part of their aggregation pheromone systems (Schlyter et al. 1987).

Ips shangrila is recently considered as a monophyletic sister species with *I. amitinus* based on the cladistic analysis of nucleotide data by Cognato and Sun (2007). However, our proposed aggregation pheromone for *I. shangrila* contains three components: 2-methyl-3-buten-2-ol, (+)-ipsdienol, and (−)-*cis*-verbenol, which is quite different from the aggregation pheromone system reported for *I. amitinus* [(−)-ipsdienol, (+)-ipsenol and amitinol] (Kohnle et al. 1988). *I. shangrila* also shows a strong difference in the pheromone systems from a morphologically similar species (Cognato and Sun 2007), *I. duplicatus* [(±)-ipsdienol and *E*-myrcenol] (Byers et al. 1990; Schlyter et al. 1992; Zhang et al. 2007) and a previously misidentified species, *I. mannsfeldi* (now recognized as *Orthotomicus mannsfeldi*; (Cognato and Vogler 2001) [(+)-ipsdienol, (−)-ipsenol and amintinol] (Kohnle et al. 1993). *I. shangrila* and two other sympatric bark beetles, *I. nitidus* and *Ps. orientalis,* share a common or similar spatial and temporal niche(s) in *Picea crassifolia* (Liu et al. 2007). This may result in strong interspecific competition and reproductive isolation pressures (Wood 1982). The latest effort and progress on identification of the aggregation pheromone system of *Ps. orientalis* indicates that this species produced an aggregation pheromone blend (Zhang et al., in prep.) different from both *I. shangrila* (this paper) and *I. nitidus* (Zhang et al. 2009). Such a disparity in pheromone systems (discrimination among their pheromone blends) among the sympatric (competitive or cooperative) bark beetle species and their potential semiochemical interactions may play an important role in maintaining their mass-attack sequences (e.g., partial niche separation) and reproductive isolation, and regulating spatial and temporal competition (Wood 1982; Byers 1989a, b; Schlyter et al. 1992; Zhang et al. 2008, 2009). Whether or not the sympatry or syntopy of different bark beetle species on the same host tree species has any effect on the evolution of their pheromone systems is still questionable (Symonds and Elgar 2004).

Our results suggest that the two major components, 2-methyl-3-buten-2-ol and (+)-ipsdienol, plus a minor component, (−)-*cis*-verbenol, produced by fed males, are

likely the aggregation pheromone components of *I. shangrila*. More field trapping experiments on optimal component ratios (including the enantiomeric ratios of ipsdienol), release rates, and dispenser technology are underway. Traps baited with synthetic aggregation pheromone lures will have great potential as a monitoring and mass-trapping tool in integrated pest management directed against this serious forest pest (Schlyter et al. 2001).

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