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Aggregation Pheromone of the Qinghai Spruce Bark Beetle, *Ips nitidus* Eggers

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Abstract Volatiles from hindgut extracts of males of the Qinghai spruce bark beetle, *Ips nitidus*, from different attack phases (phase 1: unpaired males and phases 2–4: males joined with one to three females) and hindgut extracts of mated females were analyzed by gas chromatography–mass spectrometry (GC–MS)/flame ionization detection (FID) with both polar and enantioselective columns. The GC–MS/FID analyses demonstrated that unpaired males from attack phase 1 (nuptial chamber constructed) produced 2-methyl-3-buten-2-ol, approx. 74%-(−)-ipsdienol, and (−)-*cis*-verbenol as major hindgut components, and (−)-*trans*-verbenol, (−)-ipsenol, (−)-verbenone, myrtenol, and 2-phenylethanol as minor or trace components. The quantities of 2-methyl-3-buten-2-ol and especially ipsdienol decreased after mating during phases 2–4, whereas the quantities of (−)-*cis*- and (−)-*trans*-verbenol did not change. In contrast, the quantity of (−)-ipsenol seemed to increase as mating activity progressed. After mating with three females (harem size=3; phase 4), only trace to small amounts of male-specific compounds were detected from *I. nitidus* male hindguts. Chemical analysis of the hindgut extracts of mated females showed only trace amounts of semiochemicals. A field-trapping bioassay in Qinghai, China showed that the four-component "full blend" containing the three major components, 2-methyl-3-buten-2-ol, (±)-ipsdienol, and (−)-*cis*-verbenol, plus a minor component, (−)-*trans*-verbenol, caught significantly more *I. nitidus* (♂/♀=1:2.2) than did the unbaited control and two binary blends. The replacement of (±)-ipsdienol with nearly enantiomerically pure (−)-ipsdienol in the "full blend" significantly reduced trap catches, which suggests that both enantiomers are needed for attraction. On the other hand, removal of (−)-*trans*-verbenol from the active "full blend" had no significant effect on trap catches. Our results suggest that the three major components, 2-methyl-3-buten-2-ol, 74%-(−)-ipsdienol, and (−)-*cis*-verbenol (at 7:2:1), produced by unpaired fed males, are likely the aggregation pheromone components of *I. nitidus*, thus representing the first characterization of an aggregation pheromone system of a bark beetle native solely to China.

Keywords Attractant · Coleoptera · Enantiomeric composition · GC–MS · *Ips nitidus* · Ipsdienol · 2-Methyl-3-buten-2-ol · *Picea* · Scolytidae · Semiochemical · Trap · *cis*- and *trans*-Verbenol

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Introduction

The Qinghai spruce bark beetle, *Ips nitidus* Eggers (Coleoptera: Scolytidae), was first described in 1933 from specimens collected by Eggers from Muke Tatslenlu (Kangding), Sichuan Province, China. Fu (1983) recorded

I. nitidus from several species of spruce (*Picea*) in five western provinces of China (Gansu, Qinghai, Sichuan, Xinjiang, and Yunnan). In the 1970s, *I. nitidus* was recognized as one of the dominant bark beetle species in Qinghai spruce forests [*Picea crassifolia* (Kom.)] of the Qilian Mountain, Gansu Province (Fu 1983). This bark beetle infests weakened, wind-thrown, or burned trees, and at high population densities, it attacks healthy spruce trees. Recently, *I. nitidus* together with two other newly described sympatric bark beetles, *Ips shangrila* Cognato and Sun (Cognato and Sun 2007) and *Pseudips orientalis* (Wood & Yin) (Cognato 2000), were identified as the most destructive forest pest insects in the Maixiu Forest Park of Qinghai Province, China; where, since 2001, their outbreaks (independent or mixed) have caused significant tree mortality both in plantations and natural stands of *P. crassifolia* (Xue et al. 2003; Liu et al. 2007, 2008).

During the outbreaks in 2001–2005, the species was mis-identified as *Ips typographus* (L.) by Chinese entomologists (Xue et al. 2003), but in 2006, it was correctly identified by Milos Knížek (Forestry and Game Management Research Institute, Czech Republic) as *I. nitidus*. The basic biology and host colonization behavior of this species in natural spruce forests were recently described (Liu et al. 2007, 2008). First attempts to catch adult beetles with traps baited with *I. typographus* pheromone lures during their dispersal flights were made in the mid 1980s by Zhou et al. (1995) in Gansu Province with some positive results (assuming the captured beetle species was correctly identified). Additional bouts of field screening of potential attractants (for future monitoring or mass-trapping operations) with the known *Ips* aggregation pheromone components and their various combinations have been undertaken by local forest departments in recent years, but these efforts have yielded little success.

In contrast to the previous focus on screening semiochemicals by evaluating flight responses, our objectives were to (1) identify the aggregation pheromone of *I. nitidus*, (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 as flight attractants in the field in Qinghai, China.

Methods and Materials

Collection and Preparation of Samples Live adult *I. nitidus* of different subcortical attack phases [phase 1: unpaired male in nuptial chamber; phase 2: one mated male with one female; phase 3: one mated male with two females; and phase 4: one mated male with three females in the galleries] were collected between 22 and 25 May 2008 from two naturally attacked wind-thrown Qinghai spruce trees [*P. crassifolia*)] at Maixiu Forest Park (35°08′–35°30′ N; 101° 33′–102°03′ E; ca. 2,900–3,000 m elevation), Huangnan Tibetan District, Qinghai Province, China. Beetles from the same family gallery system were placed in a 2-ml polyethylene centrifuge tube (Fisher Scientific, Pittsburgh, PA, USA) 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 quickly. Sexes were distinguished based on their elytral spine differences (Third elytral spine of males is much larger and more strongly capitate than the other three spines, whereas in females there are no obvious differences among the second, third, and fourth spines; Song et al. unpublished data) and presence of the aedeagus (male) or eggs (female) in cases where the spines were damaged. Male hindguts from the same attack phase and the same sample tree were extracted immediately with 1 ml redistilled pentane (with 2 μg of heptyl acetate as internal standard) in a 2-ml amber glass vial. Female hindguts from various attack phases and both sample trees were pooled and extracted in the same fashion as the male hindguts. Hindgut extracts (four to 27 guts/sample for males from different attack phases and 19 guts/sample for mated females) were shipped to the USA by express mail and kept at −20°C until analyzed chemically [gas chromatography with mass spectrometric detection (GC–MS) and gas chromatography with flame ionization detection (GC–FID)].

GC–MS Analysis All hindgut samples were analyzed on a combined Agilent 6890N gas chromatograph (GC) and an Agilent 5973N mass selective detector equipped with a polar column (INNOWax; 60 m×0.25 mm×0.5 μm film thickness; Agilent Technologies, Wilmington, DE, USA). The GC oven was programmed at 50°C for 1 min, with a rise to 230°C at a rate of 10°C/min, and held at 230°C for 25 min. Injector and transfer line temperatures were both 250°C. Helium was used as carrier gas at a constant flow of 26 cm/s. Compounds were identified by comparison of retention times and mass spectra with those of authentic standards (see Chemical Standards below). All of the analytes are previously known bark beetle semiochemicals.

GC–FID Analysis Hindgut samples also were 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). Helium was used as the carrier gas at a constant flow of

26 cm/s, and the injector and detector temperatures were 220°C and 300°C, respectively. Column temperature was 50°C for 1 min, rising to 240°C at 10°C/min, and then held for 10 min.

Enantioselective GC–FID The enantiomeric analyses of male hindgut extracts (from phases 1 to 2), and a synthetic mixture of several key *Ips* pheromone compounds 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™ column (30 m×0.25 mm×0.25 μm film thickness; Restek, Bellefonte, PA, USA). Helium was used as carrier gas, and the injector/detector temperatures were both 230°C. Column temperature was 80°C for 1 min and rose 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, which confirmed the previous reports with a similar stationary phase (Seybold et al. 1995b; Macías-Sámano et al. 1997; Savoie et al. 1998). 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 provisionary.

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 (97.3% cp; 97.5% ep), (+)-ipsdienol (96% cp; 98.2% ep), (−)-ipsdienol (95% cp; 97% ep) and (−)-*trans*-verbenol (>95% cp, unknown ep) [Pherotech (now Contech) International, Inc., Delta, BC, Canada]; heptyl acetate (>98% cp, food grade) and (±)-1,3-butanediol (99% cp) (Sigma-Aldrich, St. Louis, MO, USA).

Field Trapping To assay for behavioral activity of the potential semiochemicals, a field-trapping experiment was carried out from 30 July to 24 August 2008 at the Maixiu Forest Park, which is the same park from where the *I. nitidus* hindgut samples were collected. Three sets of cross-barrier type traps (Sino-Czech Trading Co. Ltd. Beijing, China; see photo insert in Fig. 3) 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 in their natural production ratios; a ninth trap was left unbaited as a negative control (Table 1). Release rates of the potential semiochemicals varied from <0.3 to 30 mg/day (Table 1). The positions of traps together with dispensers within each set were assigned randomly, and to minimize any positional effects, the dispensers' positions were re-

Table 1 Semiochemical treatments and dispensers for field-trapping experiment with the Qinghai spruce bark beetle, *Ips nitidus*, Qinghai Province, China, 30 July–24 August, 2008

Chemical	Treatments and loading (mg/dispenser)[a]								
	A	B	C	D	E	F	G	H	I
2-Methy-3-buten-2-ol[b]	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	0
(−)-*cis*-Verbenol[c]	15	15	15	15	0	0	0	0	0
(−)-Ipsdienol (97%) [c]	30	0	30	0	30	0	30	0	0
(±)-Ipsdienol [c]	0	30	0	30	0	30	0	30	0
(−)-*trans*-Verbenol [c]	7	7	0	0	7	7	0	0	0
1,3-Butanediol (inert solvent)	750	750	750	750	750	750	750	750	750

[a] Loading ratios were based on the natural ratios of these chemicals produced by unpaired males from phase 1 (Table 2)

[b] 2-Methyl-3-buten-2-ol alone was released from a 2-mil polyethylene bag (7×5 cm) with felt. Release rate was 30 mg/day measured at 22°C in a laboratory fumehood

[c] These semiochemicals (individuals: G/H or in combinations: A/B/C/D/E/F) were dissolved in 750 ml of 1,3-butanediol (as an inert solvent) and loaded in a 2-mil polyethylene bag (3×5 cm) with felt. The exact release rates of these chemicals were not determined with normal gravimetric methods due to the hygroscopic nature of the inert solvent. Estimated release rates by solid phase microextraction (SPME) followed by GC analysis indicated a low overall release rate (<0.3 mg/day) for individual components or mixtures of ipsdienol, and *cis*- and *trans*-verbenol

randomized after each replicate (Byers 1991) when >10 beetles were caught in the traps with the most attractive bait.

Statistical Analyses Trap catch data were converted to proportion (P) of total captured beetles within each replicate. Data then were 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 α=0.05 (Day and Quinn 1989). Due to the zero responses to the unbaited control and the two binary blends, these three treatments were not included in the ANOVA and range tests. Trap catch data (untransformed) were also analyzed with the nonparametric Kruskal–Wallis ANOVA on ranks followed by the Student–Newman–Keuls test to separate means of the treatments with and without zero catches (Zar 1984).

Results

Chemical Analysis Eleven volatile compounds from the extracts of male hindguts were identified by GC–MS and quantified by GC–FID. These compounds included 2-methyl-3-buten-2-ol, ipsdienol, and *cis*-verbenol (at 7:2:1) as major components, and *trans*-verbenol, ipsenol, and verbenone as minor or trace components (Fig. 1, Table 2). Myrtenol and 2-phenylethanol, found in several *Ips* beetles (Zhang et al. 2000), but not confirmed as pheromone components, also were detected as minor components (Fig. 1, Table 2). 3-Hydroxy-2-butanone, 1-heptanol, and isobutyric acid were present in all samples.

GC–FID analysis with an enantioselective stationary phase of the hindgut extracts of the *I. nitidus* males from phases 1 to 2 and a synthetic mixture of *Ips* pheromone compounds showed that *I. nitidus* males produced 74±3%-(−)-ipsdienol (mean±SE; N=4; Fig. 2). The predominant enantiomers of other chiral compounds were tentatively determined as (−)-*cis*- and (−)-*trans*-verbenol, (−)-verbenone, and likely (−)-ipsenol (Fig. 2).

Mean amounts (N=2) of the three major components (2-methyl-3-buten-2-ol, ipsdienol, and *cis*-verbenol) from phase 1 were 2,800, 760, and 330 ng/male (ca. 7:2:1 ratio), respectively, which are 2–100 times higher than the three minor components (*trans*-verbenol, ipsenol, and verbenone; Fig. 1, Table 2). Although no statistical analysis was performed because of the limited data set, the quantities of 2-methyl-3-buten-2-ol and especially ipsdienol decreased after mating (i.e., during phases 2–4), whereas the quantities of *cis*- and *trans*-verbenol, myrtenol, and 2-phenylethanol showed no obvious changes (Fig. 1, Table 2).

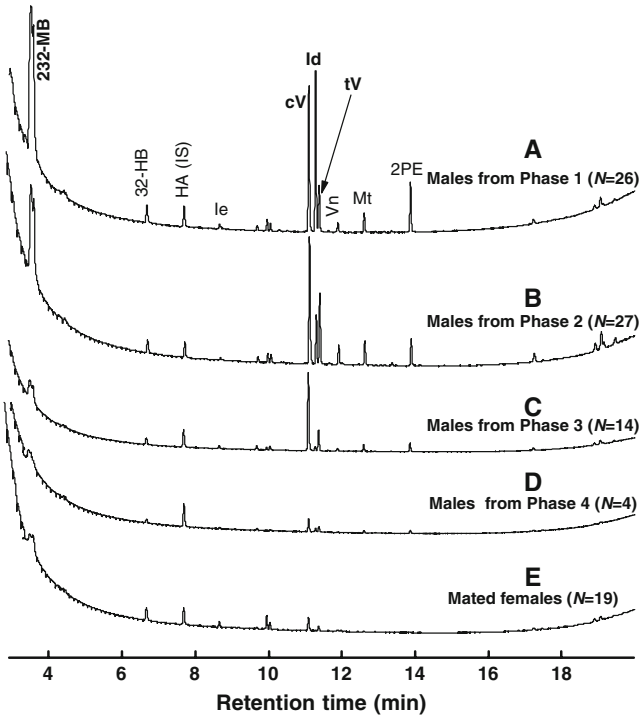

Fig. 1 Representative gas chromatograms (polar column with FID detection) of compounds in hindgut extracts of male *Ips nitidus* from different attack phases (*A–D*) and mated females from mixed phases (*E*). Heptyl acetate (HA; 2 µg/sample) was added as an internal standard to the hindgut extracts. Analytes included 2-methyl-3-buten-2-ol (*232-MB*), *cis*-verbenol (*cV*), ipsdienol (*Id*), ipsenol (*Ie*), *trans*-verbenol (*tV*), (−)-verbenone (*Vn*), 3-hydroxy-2-butanone (*32-HB*), myrtenol (*Mt*), and 2-phenylethanol (*2PE*)

Interestingly, the quantity of ipsenol seemed to increase as the mating activity progressed (Table 2).

In the hindgut extracts of mated females (taken from mixed phases), only trace amounts of male *Ips*-related semiochemicals were detected by GC–MS or GC–FID (Fig. 1 E). These traces may have been due to contamination in the gallery from semiochemical-producing males. In female extracts, we detected quantities of 3-hydroxy-2-butanone, 1-heptanol, and isobutyric acid that were similar to those in male extracts (Table 2).

Field-Trapping Experiment Due to extremely low populations of *I. nitidus* sister broods and heavy winds and rains during the period of our field study (late July to mid August), only six replicates were obtained from two sets of traps (sets 2–3). Traps and dispensers in set 1 were damaged by a flood or partially missing, thus giving no valid data. The unbaited control traps and traps baited with the binary blends (Table 1, treatments G and H): 2-methyl-3-buten-2-ol plus (±)-ipsdienol or (−)-ipsdienol, did not catch any beetles (Fig. 3). Ternary blends with 97%-(−)-ipsdienol caught only a few beetles and seemed to be less attractive than ternary or quaternary blends with (±)-

Table 2 Quantities of potential semiochemicals (in nanogram) identified from hindgut extracts of *Ips nitidus* males/females of different attack phases, Qinghai Province, China, May 22–25, 2008

Retention time (min)	Chemical	Male hindguts from different attack phases								Mated ♀ hindguts (*N*=1; *n*=19) ng/♀
		Phase 1		Phase 2		Phase 3		Phase 4		
		1♂ (*N*=2; *n*=16–19)		1♂1♀ (*N*=1; *n*=27)		1♂2♀ (*N*=2; *n*=8–14)		1♂3♀ (*N*=2; *n*=4–5)		
		ng/♂	%	ng/♂	%	ng/♂	%	ng/♂	%	
3:28	2-Methy-3-buten-2-ol	2816.5	61.9	1060.6	42.5	844.5	41.3	924.3	47.1	trace
6:39	3-Hydroxy-2-butanone	82.9	1.8	69.5	2.8	88.6	4.3	104.2	5.3	107.0
8:39	1-Heptanol	39.4	0.9	19.3	0.8	38.0	1.9	61.6	3.1	45.0
9:41	(−)-Ipsenol	9.5	0.2	37.2	1.5	60.9	3.0	112.1	5.7	
10:02	Isobutyric acid	49.2	1.1	59.8	2.4	72.9	3.6	140.9	7.2	89.0
11:05	(−)-*cis*-Verbenol	332.5	7.3	507.2	20.3	549.0	26.9	219.4	11.2	
11:16	74%-(−)-Ipsdienol	760.7	16.7	183.0	7.3	47.0	2.3	71.9	3.7	trace
11:22	(−)-*trans*-Verbenol	163.8	3.6	265.8	10.7	164.2	8.0	137.7	7.0	trace
11:53	(−)-Verbenone	83.5	1.8	76.7	3.1	34.0	1.7	20.2	1.0	
12:35	Myrtenol	73.6	1.6	93.9	3.8	66.3	3.2	76.6	3.9	
13:51	2-Phenylethanol	142.1	3.1	119.8	4.8	77.5	3.8	91.9	4.7	

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

ipsdienol (Fig. 3). The four-compoment "full blend" containing 2-methyl-3-buten-2-ol, (±)-ipsdienol, (−)-*cis*-verbenol, and (−)-*trans*-verbenol, caught the most beetles. This response was significantly higher than that to the quaternary blend that contained 97%-(−)-ipsdienol (Table 1, treatment A, Fig. 3). Subtraction of (−)-*trans*-verbenol from the most active "full blend" [containing (±)-ipsdienol] slightly reduced trap catches, but this response was not significantly different from that to the quaternary blend (Fig. 3). The sex ratio of captured beetles was estimated as 1:2.2 (♂/♀), based on the pooled sub-samples.

Discussion

This is the first chemical and behavioral analysis of the aggregation pheromone system of the Qinghai spruce bark beetle, *I. nitidus*. GC–MS and GC–FID results demonstrate that males from attack phase 1 (nuptial chamber constructed, unpaired) produced 2-methyl-3-buten-2-ol, ipsdienol, and *cis*-verbenol as major hindgut components, and *trans*-verbenol, ipsenol, and verbenone as minor or trace components (Fig. 1 A, Table 2). Other compounds frequently found in many *Ips*, but not confirmed as pheromone components,

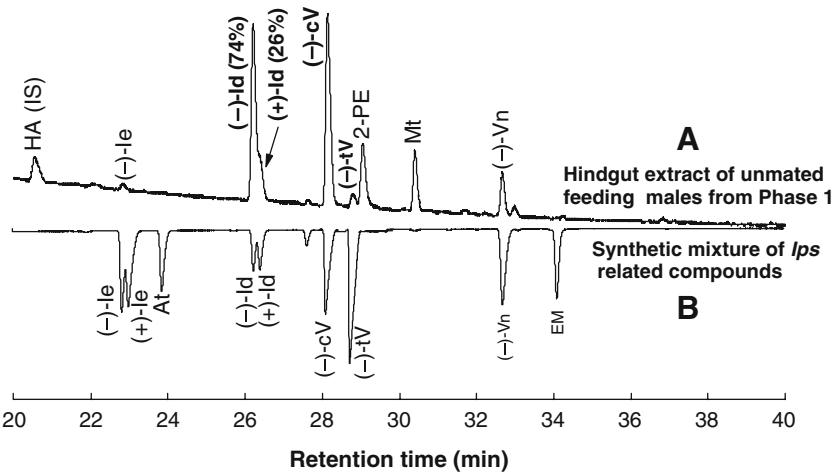

Fig. 2 Enantioselective GC–FID analyses (Rt-bDEXm™ column) of *A* compounds in a hindgut extract of unpaired male *Ips nitidus* from attack phase 1 and *B* a synthetic mixture of *Ips*-related compounds, including (±)-ipsenol (*Ie*), (±)-ipsdienol (*Id*), amitinol (*At*), (−)-*cis*-verbenol (*cV*), (−)-*trans*-verbenol (*tV*), (−)-verbenone (*Vn*), and (*E*)-myrcenol (*EM*; ca. 50 ng/each compound). Amitinol and *E*-myrcenol are achiral. Heptyl acetate (HA; 2 μg/sample) was added as an internal standard to the hindgut extract

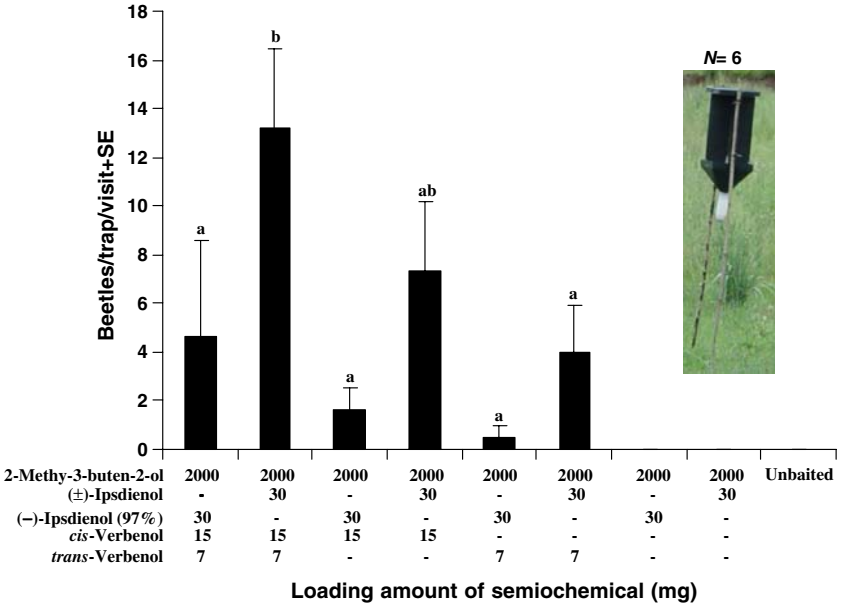

Fig. 3 Mean captures (N=6) of *Ips nitidus* in cross-barrier traps (*photo insert*) baited with different combinations (Table 1) of the key male-produced volatile compounds in their natural production ratios, July 30 to August 24, 2008, Douheyan, Maixiu Forest Park, Qinghai, China. An unbaited trap served 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. Due to the zero responses to the unbaited trap and the two binary blends, these three treatments were not included in the ANOVA and range tests, but were further compared by using the nonparametric Kruskal–Wallis ANOVA on ranks followed by Student–Newman–Keuls test to separate means of the treatments with and without zero catches

such as myrtenol and 2-phenylethanol, also were detected as minor components (Birgersson et al. 1984; Zhang et al. 2000, 2007; Fig. 1, Table 2). 3-Hydroxy-2-butanone, 1-heptanol, and isobutyric acid, commonly related to insect fat tissues (Zhang et al. 2006), were present in all samples, and presumably are not part of the male-produced aggregation pheromone system. Chemical analysis of the hindgut extracts of mated females showed trace quantities of these semiochemicals (Fig. 1 E, Table 2), 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).

GC–FID analyses indicated a large variation in the quantities of male-produced volatiles during different attack phases. The maximum production of the major components occurred in phases 1–2, when the nuptial chamber was finished, or only one female was accepted (Fig. 1 A, B). Mating reduced production of the male-specific major hindgut volatiles, especially for 2-methyl-3-buten-2-ol and ipsdienol, whereas the quantities of ipsenol appeared to increase as colonization progressed (Table 2). This might indicate a potential repellent effect of ipsenol during the development of attacks, as reported for *I. typographus* (Schlyter et al. 1992). After mating with three females (harem size=3; phase 4), only trace to tiny amounts of male-specific compounds were detected in *I. nitidus* male hindgut extracts, suggesting that males in this phase may not be capable of attracting females or other males (Fig. 1 D).

Enantioselective GC analysis indicated that *I. nitidus* males produced approx. 74%-(−)-ipsdienol (Fig. 2). This enantiomeric composition is different from those reported for other Eurasian *Ips* spp. (Kohnle et al. 1988, 1991; Zhang et al. 2007), including its sibling species *I. typographus* [95%-(−)] (Kohnle et al. 1991) and the sympatric *I. shangrila* [99%-(+)] and *P. orientalis* [67%-(+)] (Zhang et al., unpublished). However, this enantiomeric ratio is similar to that of eastern populations of North American *Ips pini* (Seybold et al. 1995a). Other chiral compounds were tentatively determined as follows: (−)-*cis*- and (−)-*trans*-verbenol, (−)-verbenone, and likely (−)-ipsenol, based on the retention time comparison with the synthetic standards (Fig. 2), which are similar to the reported absolute configurations for many *Ips* bark beetles (Kohnle et al. 1988).

Our field-trapping data showed that the four-component "full blend" containing the three major components, 2-methyl-3-buten-2-ol, (±)-ipsdienol, and (−)-*cis*-verbenol, plus a minor component, (−)-*trans*-verbenol, caught significant numbers of *I. nitidus* (both females and males; Fig. 3). Replacing (±)-ipsdienol with 97%-(−)-ipsdienol in the "full blend" significantly reduced trap catches, indicating that both enantiomers of ipsdienol might be needed for attraction. This response preference to both enantiomers is

supported by a naturally occurring blend of 74%-(−)-ipsdienol extracted from male hindgut tissue (from phases 1 to 2; Fig. 2). It remains unknown if the natural ratio of ipsdienol enantiomers will prove superior to racemic ipsdienol. Subtraction of (−)-*trans*-verbenol from the active "full blend" (Byers 1992) had no significant effect on trap catches. In fact, this compound also was detected in other Eurasian *Ips* species as a minor component, such as *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).

I. nitidus and *I. typographus* form a monophyletic group and are sibling species (Cognato and Sun 2007). The proposed aggregation pheromone for *I. nitidus* contains three components: 2-methyl-3-buten-2-ol, ipsdienol, and *cis*-verbenol, whereas the aggregation pheromone system for *I. typographus* contains two components: 2-methyl-3-buten-2-ol and *cis*-verbenol (Kohnle et al. 1988). Ipsdienol is produced as a minor component by mated male *I. typographus* during late attack phases (Birgersson et al. 1984), but has not been considered as part of its aggregation pheromone system (Schlyter et al. 1987; Kohnle et al. 1988). The enantiomeric compositions of ipsdienol isolated from the two species differ [95%-(−) for *I. typographus* (Kohnle et al. 1991) and 74%-(−) for *I. nitidus* (Fig. 2)]. Any differences in the enantiomeric compositions of *cis*-verbenol from these two species have not been reported. Thus, there appears to be some level of disparity in the aggregation pheromone attractants for *I. nitidus* and *I. typographus* that may allow these species to maintain their reproductive isolation within their zone of sympatry (e.g., native spruce forests in Qinghai and Gansu Provinces, China). These differences in the pheromone systems may not be as pronounced as predicted by the saltational evolution hypothesis proposed by Symonds and Elgar (2004), i.e., within certain phylogenetic constraints, pheromone evolution in bark beetles is characterized by large saltational shifts, resulting in substantial phenotypic (i.e., pheromonal composition) differences between sibling species. In fact, this hypothesis is not supported either by experimental data on cross-attraction in the field between two sympatric sibling *Ips* species, *Ips hoppingi* and *Ips confusus*, in the southwestern USA (Cane et al. 1990) or by data on laboratory responses to pheromones by 17 species of *Ips* (Lanier and Wood 1975). *I. nitidus* and two other sympatric bark beetles, *I. shangrila* and *P. orientalis*, share a common or similar spatial and temporal niche(s) in *P. crassifolia* (Liu et al. 2007). This may result in strong interspecific competition and reproductive isolation pressures (Lanier and Wood 1975; Wood 1982). The latest effort and progress on identification of the aggregation pheromone systems of *I. shangrila* and *P. orientalis* indicates that these two newly described species produced different aggregation pheromone blends from each other and from *I. nitidus* (Zhang et al., in preparation). 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 (Birch and Wood 1975; Lanier and Wood 1975; Byers and Wood 1980; Cane et al. 1990; Švihra et al. 1980; Paine et al. 1981; Wood 1982; Byers 1989a, b; Schlyter et al. 1992; Zhang et al. 2008).

Our results suggest that the three major components, 2-methyl-3-buten-2-ol, 74%-(−)-ipsdienol, and (−)-*cis*-verbenol (at 7:2:1), produced by unpaired fed males, are likely the aggregation pheromone components of *I. nitidus*. Another semiochemical found in male hindgut tissue, (−)-*trans*-verbenol, may not be part of the aggregation pheromone system, but it merits further field bioassays to determine its potential functionality. This is the first characterization of an aggregation pheromone system of a bark beetle that is native solely to China. 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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