

Reproductive Isolation of *Ips nitidus* and *I. shangrila* in Mountain Forests of Western China: Responses to Chiral and Achiral Candidate Pheromone Components

Fredrik Schlyter¹ · Rastislav Jakuš^{2,3} · Fu-Zhong Han⁴ · Jian-Hai Ma⁵ · Blanka Kalinová^{3,6} · Pavel Mezei² · Jiang-Hua Sun⁷ · Liana Ujhelyiová⁸ · Qing-He Zhang⁹

Received: 23 December 2014 / Revised: 5 May 2015 / Accepted: 25 May 2015 / Published online: 26 June 2015
© Springer Science+Business Media New York 2015

Abstract Eastern Palearctic conifers are subject to frequent bark beetle outbreaks. However, neither the species responsible nor the semiochemicals guiding these attacks are well understood. Two high-mountain *Ips* species on Qinghai spruce, *Picea crassifolia*, *I. shangrila* and *I. nitidus*, are typical in this regard. Six synthetic candidate pheromone components that we earlier identified from hindguts of unmated males of these two *Ips* species were tested for field activity in Qinghai province, P. R. China. For *I. nitidus*, racemic ipsdienol ((±)-Id) could replace the naturally-produced blend of enantiomers containing 74 % (–)-(S)-Id (74:26 S:R), in attractive ternary or binary blends. In contrast, sympatric *I. shangrila* were attracted mainly to blends including Id of opposite chirality, 97 %-(+)-(R)-Id. Of the verbenols, (–)-*trans*-verbenol was inactive for *I. nitidus* or inhibitory for *I. shangrila*, but (–)-*cis*-verbenol (cV) was a key component of the pheromone in both species. Two fully factorial

experiments demonstrated that (±)-Id, cV, and 2-methyl-3-buten-2-ol (MB) are components of the aggregation pheromone of *I. nitidus*, whereas only (+)-Id and cV are essential components of the aggregation pheromone of *I. shangrila*. While MB is not necessary for attraction of *I. shangrila*, it is an active antagonist and likely functions in species isolation. A review of the pheromone production and responses in Palearctic *Ips* and *Pseudoips* showed that cV is more common than methylbutenols, and both elicit qualitatively variable responses. Ipsdienol is the most common component with variable chirality, and is a necessary, but often not sufficient, factor for determining pheromone specificity.

Keywords Species separation · Pheromone component · Enantiomer · Attractant · Trap · Full factorial design · Coleoptera · Curculionidae · Scolytinae · *Picea crassifolia*

Fredrik Schlyter and Rastislav Jakuš contributed equally to this work.

✉ Fredrik Schlyter
fredrik.schlyter@slu.se

Rastislav Jakuš
jakuš@savzv.sk

¹ Chemical Ecology, Department of Plant Protection Biology, Swedish University of Agricultural Sciences, SE-230 53 Alnarp, Sweden

² Institute of Forest Ecology, Slovak Academy of Sciences, Štúrova 2, 960 53 Zvolen, Slovakia

³ Faculty of Forestry and Wood Sciences, Czech University of Life Sciences, Kamýcká 129, 165 21 Praha 6 – Suchbátka, Czech Republic

⁴ Maixiu Forest Park of Qinghai Province, Huangnan 811300, People's Republic of China

⁵ Qinghai Provincial Centre of Wetland Protection and Management, Xining 810000, People's Republic of China

⁶ Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, Prague, Czech Republic

⁷ State Key Laboratory of Integrated Management of Pest Insect & Rodents, Institute of Zoology, Chinese Academy of Sciences, DaTun Road, Chaoyang District, Beijing 100101, People's Republic of China

⁸ Fytofarm Ltd, Dúbravská cesta 21, 845 08 Bratislava 45, Slovak Republic

⁹ Sterling International, Inc., 3808 N. Sullivan Rd, Bldg 16, Spokane, WA 99216, USA

Introduction

Forests face a crisis globally in the wake of climate change and global warming (Allen et al. 2010). Somewhat surprisingly, this is reflected in bark beetle outbreaks in cold, high altitude forests of inner Eastern Palearctic mountains (Jakuš et al. 2011). New and improved strategies to prevent and control bark beetle outbreaks are required for forest protection under these changing conditions. In China, emerging knowledge is enabling better differentiation of taxa, including a taxonomic division of Palearctic species (Stauffer et al. 2001), recognition of the taxa that are present, and description of new taxa (Cognato and Sun 2007). Further study of Eastern Palearctic taxa is generating evidence for pheromone dialects (Chen et al. 2015; Song et al. 2011), and pheromones of new taxa (Zhang et al. 2009b).

The importance of pheromone blend specificity for species separation and mate recognition has long been recognised for both moths (Cardé and Baker 1984) and bark beetles (Wood 1982). The significance of pheromone blend specificity has been highlighted by examples of evolutionary patterns of pheromone specificity among competing, closely related, sibling, or incipient species (e.g., Mitchell et al. 2015; Saveer et al. 2014; Schlyter et al. 1992; Seybold et al. 1995; Song et al. 2011).

Ips nitidus Eggers and a newly described sympatric bark beetle, *Ips shangrila* Cognato and Sun, have caused significant tree mortality in plantations and natural stands of Qinghai spruce, *Picea crassifolia* Komarov, in China (Yang et al. 2014) over the past 10 years (Cognato and Sun 2007; Jakuš et al. 2011; Liu et al. 2008). Both species normally infest weakened, wind-thrown, or burned trees, but at high population densities they may also attack apparently healthy spruce trees. *Ips nitidus* occurs naturally in North-western China. It has two generations a year within 2800–3000 m elevation, but only one complete generation above 3000 m. Adults from overwintering broods begin to attack host trees in early to mid-May. The new callow adults start to emerge in late June through mid-July. The second generation is much shorter than the first one. The young adults of the second generation appear from early until late August. The larger species, *I. nitidus*, prefers to attack trunks of mature trees situated in sunny exposures, especially in the middle to lower part of trunks 15–25 cm diam (Liu et al. 2008). *Ips shangrila* often is present in association with *I. nitidus*, and also completes two generations per year at low elevations. The onset of its spring flight and tree colonization occurs about half a month later than that of *I. nitidus*. The smaller species, *I. shangrila*, always infests from the top of the trunk, on branches larger than 3 cm diam in the crown, and causes tree mortality together with *I. nitidus*, which primarily infests the base of the trunk. Sometimes *I. shangrila* alone colonizes entire young trees and quickly kills the tree (Liu et al. 2007).

Potential male-produced aggregation pheromone components of these two sympatric and partially competitive species were recently identified (Hoskovec et al. 2012; Zhang et al. 2009a, b). The 74 %-(*S*)-ipsdienol (2-methyl-6-methylene-2,7-octadien-4-ol; Id), i.e., 74:26 *S*:*R* or 48 % enantiomeric excess (ee), (*S*)-(*S*)-*cis*-verbenol (cV), and 2-methyl-3-buten-2-ol (MB) were considered as pheromone components for *I. nitidus* (Zhang et al. 2009a). Racemic Id, and a possible importance of (*S*)-*trans*-verbenol (tV), also have been described by Hoskovec et al. (2012). For *I. shangrila*, cV, MB, and the opposite enantiomer of Id, 99 %-(*R*)-ipsdienol (1:99 *S*:*R* or 98 % ee), were reported to be pheromone components (Zhang et al. 2009b).

The disparity in enantiomeric compositions of the ipsdienol produced by the sympatric species, *I. nitidus* and *I. shangrila*, might be responsible for their reproductive isolation even though the pheromones have otherwise similar chemical compositions (Zhang et al. 2012). Such separation was earlier well described from the Western Palearctic (Bakke 1978) and the Nearctic (Seybold et al. 1995; Wood 1982). The reported field attraction of the two species to at least four of these identified pheromone candidates in partial combinations with subtractive approach seemed to support their status as pheromone components (Zhang et al. 2009a, b). However, more detailed field trapping bioassays, especially those with a full factorial designs were needed to evaluate the function of individual pheromone candidates and their possible synergistic or antagonistic interactions. For beetle management in these high altitude areas, it also was desirable to determine the potential for practical applications using the less expensive racemic Id, but still maintain a species-specific trapping system.

Our hypotheses to be tested were: 1) Not all oxygenated terpenoids found in male hindguts are components of an attractive pheromone blend; 2) Differences in chirality of Id are necessary, but not sufficient, for species separation; 3) There will be both synergistic and antagonistic effects in the blends reported; and 4) Racemic (\pm)-Id in synthetic blends allows sufficient attraction for practical applications.

To this end, we performed four field-trapping experiments in SE Qinghai province at the northeast margin of the Tibetan plateau. The first two experiments in 2009 compared 74 %-(*S*)-Id, 97 %-(*R*)-Id, and the less expensive (\pm)-Id in combinations with three other synthetic pheromone candidates of *I. nitidus* (cV, tV, and MB). The second two experiments in 2011 were full factorial experiments, separately for *I. nitidus* and *I. shangrila*, with all possible combinations of the three major components (cV, MB, and Id), for each species using its own specific Id chirality. We also reviewed present knowledge of pheromone components in Palearctic Ipini taxa in respect of their function in species separation.

Methods and Materials

Insect Identification During the outbreaks in 2001–2005, *I. nitidus* was misidentified as *Ips typographus* (L.) by Chinese entomologists (Xue et al. 2003), but in 2006 it was correctly identified (pers. comm., Miloš Knížek, Forestry and Game Management Research Institute, Czech Republic). In the past in China, *I. shangrila* also was incorrectly recognized as *I. mannsfeldi* (Wachtl. 1879) (Yin et al. 1984) even though its host range (*Picea* spp.) in China was different from those recorded for its European populations (*Pinus* spp.) (Stauffer et al. 1997). The status of the latter species in the Qinghai province was first challenged by M. Knížek in 2006, and was later recognized, together with specimens from Yunnan and Sichuan, as a new species based on a cladistic analysis of both DNA and diagnostic morphological characters (Cognato and Sun 2007). The two target species are separated easily by the naked eye, both by size (*I. nitidus* is much larger) and by shape (*I. shangrila* is more elongated). However, due to the recent problems and progress of taxonomy, we checked trap catches under a stereomicroscope in the laboratory. Only very small numbers of other *Ips* or *Ipini* specimens were occasionally observed in trap catches.

Field Experiments

Four field trapping experiments were carried out at the Maixiu Forest Park, Qinghai, P. R. China (35°08′–35°30′ N; 101°33′–102°03′ E; ca. 2900–3000 m elevation) during early summers of 2009 and 2011.

Effect of Enantiomeric Composition of Ipsdienol on Trapping *Ips nitidus*, 2009 Two field trapping experiments were carried out from 30 April to 14 May 2009 to test the behavioral activity of various partial combinations of three major candidate components of the male-produced pheromone (MB, cV, and Id), plus a minor component, (–)-*trans*-verbenol (tV), for *I. nitidus* (Zhang et al. 2009a). Subtractive pheromone testing design (Byers 1992) was followed in this testing, but was incomplete in that one compound (MB) was not subtracted.

Experiment 1 tested the effect of 74 %-(–)-(*S*)-Id, the enantiomeric blend produced by *I. nitidus* males, against the inexpensive racemic (±)-Id in binary, ternary, and quaternary blends (Table 1, Fig. 1a). Experiment 2 tested the effect of 97 %-(+)-(*R*)-Id, approximately the enantiomeric ratio produced by *I. shangrila* males, against the inexpensive (±)-Id in binary, ternary, and quaternary blends (Fig. 1b).

Four sets of cross-barrier traps (50×30 cm, Pherobio Technology Co., Ltd., Beijing, China) were deployed for each experiment along the edge of a *P. crassifolia* forest stand on a northern slope next to a creek at Douheyuan, with > 50 m

between the two experiments, >30 m between trap sets (within each experiment), ca. 10 m between traps within each set, and >10 m from the nearest trees. For each experiment, eight traps within each set were baited with different blends of the three major male-produced volatile compounds, plus a minor component, tV; a ninth trap was left unbaited as a negative control (Fig. 1a and b). Each tested compound was released from a separate polyethylene bag (with or without inserted substrate felt; and with different sizes and thickness). The dispenser types, purities, loadings, and release rates of the tested semiochemicals are described in Table 1. For each experiment, the positions of traps together with dispensers within each set were initially randomized, and the dispensers' positions were re-randomized after each replicate when >20 beetles were caught in the best trap(s), to minimize the positional effects (Byers 1993; Fettig et al. 2006).

Full Factorial Trapping Experiments, 2011 Two more field trapping experiments were conducted to test the behavioral activity of the three major potential aggregation pheromone components (three factors) and all their possible combinations for *Ips nitidus* (Experiment 3) and *I. shangrila* (Experiment 4) in two full factorial experimental designs. The minor component, tV, was not included in these two full factorial tests since it did not show any behavioral effect on *I. nitidus* in earlier research (Zhang et al. 2009a, b) and, based on results of our Experiments 1 and 2, was actually inhibitory against *I. shangrila* (Zhang et al. 2009b). The trapping experiments were carried out from 30 April to 10 May 2011. Two sets of cross-barrier traps for Experiment 3 and one set for Experiment 4 were set up along the edge of a 15-yr-old *P. crassifolia* forest plantation near the Maixiu Forest Nursery, with > 15 m between trap sets and ca. 10 m between traps within each set, and >10 m from the nearest trees. For each experimental set, seven traps within each set were baited with three individual pheromone components and all their possible binary/ternary combinations; an eighth trap was left unbaited as a negative control. Each compound was released from separate dispensers supplied by commercial sources. In the 2011 experiments, the same dispenser type was used for each compound tested in both experiments. The dispenser types, purities, and release rates of the tested semiochemicals are described in Table 1. For each experiment, the positions of traps with dispensers within each set were initially randomized, and the positions of the dispensers were re-randomized after each replicate (Byers 1993; Fettig et al. 2006) when >10 beetles were caught in the best trap(s), to minimize the positional effect.

Statistics We followed the standards for statistical reporting in experiment design as well as descriptive and analytical statistics that were recently set (Hillebrand and Gurevitch 2013). We present trap catch data as relative catches per

Table 1 Details of synthetic compounds and dispensers used in field experiments

Chemical	Sources ^a	Chemical purity (%)	Enantiomeric purity (%) ^b	Release rate ^c (mg/day)	Dispensers ^c	Experiment No.
2-methyl-3-buten-2-ol	Acros, NJ, USA	97		60	3 ml in a PE-bag (6.0×5.0 cm; 0.04 mm thick; with a 5.0×4.5 cm felt)	1
	Acros, NJ, USA	97		25	1 ml in a PE-bag (3.5×5.0 cm; 0.05 mm thick; with a 2.5×4.5 cm felt)	2
	Fytofarm Ltd., Slovakia	98		50 ^d	4.5 ml in an ampule tube made of PE (45 mm height, 22 mm Ø, 1.5 mm thick)	3, 4
(–)- <i>cis</i> -verbenol	Bedoukian	98		0.5	60 mg in a PE-bag (3.0×5.0 cm, 0.1 mm thick, without felt)	1
	Bedoukian	98		0.4	50 mg in a PE-bag (3.0×5.0 cm, 0.15 mm thick, without felt)	2
	Fytofarm Ltd., Slovakia	98		2 ^d	100 mg in a PE-bag (5.0×6.0 cm, 0.1 mm thick without felt)	3, 4
(±)- <i>ipsdienol</i>	Bedoukian	95		1.5	40 mg in a PE-bag (2.5×5.0 cm; 0.31 mm thick; with a 1.5×4.5 cm felt)	1, 2
	Contech	95		0.2 ^d	40 mg in a Bubble cap	3, 4
74 %-(–)-(<i>S</i>)- <i>ipsdienol</i>	Contech	95	74 (–)	1.5	40 mg in a PE-bag (2.5×5.0 cm; 0.31 mm thick; with a 1.5×4.5 cm felt)	1
97 %-(+)-(<i>R</i>)- <i>ipsdienol</i>	Contech	95	97 (+)	1.5	40 mg in a PE-bag (2.5×5.0 cm; 0.31 mm thick; with a 1.5×4.5 cm felt)	2
	Contech	95	97 (+)	0.2 ^d	40 mg in a Bubble cap	3, 4
(–)- <i>trans</i> -verbenol	Contech	>95		0.3	20 mg a PE-bag (2.0×5.0 cm; 0.31 mm thick; with a 1.0×4.5 cm felt)	1
	Contech	>95		0.2	20 mg a PE-bag (1.8×5.0 cm; 0.31 mm thick; with a 1.0×4.5 cm felt)	2

^a Contech: Contech Enterprises, Inc., Victoria BC, Canada; Bedoukian: Bedoukian Research Inc., Danbury CT, USA; PE) polyethylene; thick) Refers to wall thickness of PE bag and tube, respectively

^b % of major enantiomer, i.e. 74 % = 74:26 *S*:*R*, corresponding to 48 % enantiomeric excess

^c Dispensers prepared in our laboratory; release rate measured by weight loss in a fume hood at 20–21 °C for two weeks

^d Compound in device from supplier of compound, release rate given by supplier

^e PE: low density polyethylene (Uline, Pleasant Prairie, WI 53158, USA)

replicate (P). In addition to allowing comparisons of data from different experiments on a similar scale, such a relative measure helps to reduce the variation inherent in absolute catches due to influences of uncontrolled factors, such as weather and beetle population levels, on flight activity. We used the conventional arcsin \sqrt{P} transformation to achieve normal distributions and homogeneous variances (checked by Levene's test) and kept α at 0.05 for hypothesis testing. Software IBM SPSS 19.0.0 and 21 (for Windows) was used for descriptive and analytical statistics. Routines ONEWAY and UNIANOVA were used for mean separation and factorial analysis, respectively. We used factorial ANOVA to determine the importance of each component in a blend for activity alone as well as possible synergisms or antagonisms (Schlyter et al. 1987; Zhang and Schlyter 2003); this analytical approach is superior to relying only on less informative *post-hoc* range pairwise tests (Jones 1984; Slinker 1998). Separations of means were

carried out by REGW multiple Q test after ANOVA on the arcsin \sqrt{P} transformed data.

Results

Ipsdienol Enantiomeric Combinations for *Ips nitidus* In Experiment 1, a total of 1981 *I. nitidus* were captured in seven replicates (Fig. 1a). The numbers of *I. nitidus* in traps baited with the binary blends containing MB were not different from those in the unbaited traps. The four-component blend containing MB, 74 %-(–)-(*S*)-Id, cV, and the minor component (–)-tV, caught the highest numbers of *I. nitidus*, but it was not different from the quaternary blend that contained (±)-Id (Fig. 1a). The ternary blends containing 74 %-(–)-(*S*)-Id or (±)-Id without (–)-tV also caught significantly more beetles than did the unbaited control and the binary blends, but they were not significantly different from the two attractive quaternary blends (Fig. 1a). Thus, subtraction of (–)-tV from either

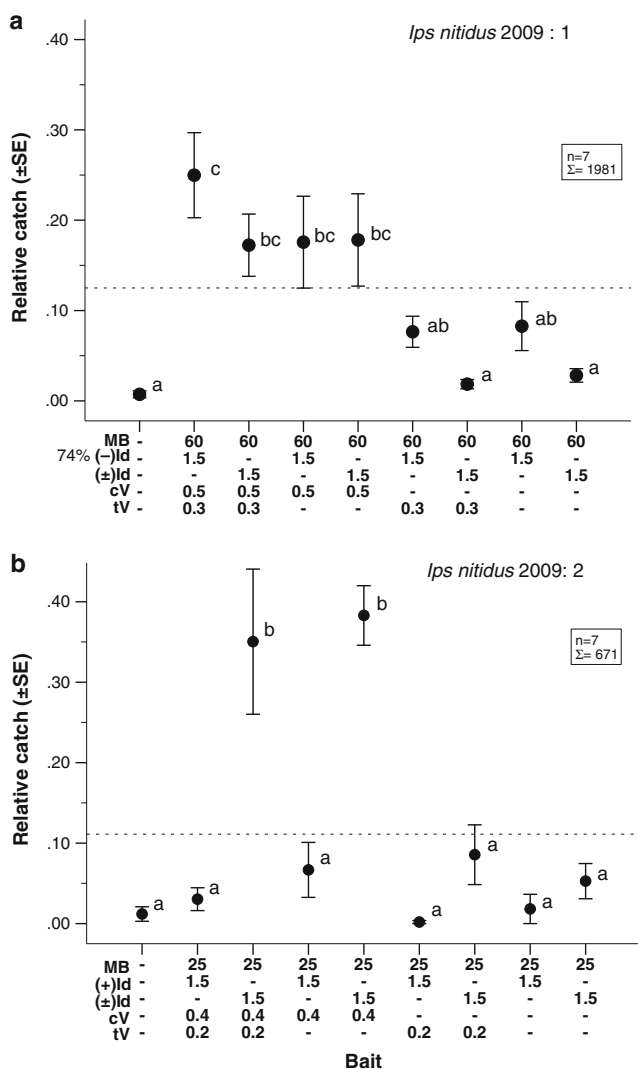


Fig. 1 **a** Mean proportions of total catch of *Ips nitidus* in cross-barrier traps baited with different combinations of male-produced volatile compounds in their naturally-produced ratios, or racemic forms; **b** Mean proportions of total catch of *Ips nitidus* in cross-barrier traps baited with different combinations of *Ips shangrila* male-produced volatile compounds in their naturally-produced ratios, or racemic forms (April 30 to May 14, 2009, Maixiu Forest Park, Qinghai, China; $N=7$; mean proportions with the same letter are not significantly different ($P>0.05$) by REGW multiple Q test after one-way ANOVA on the arcsin square root transformed data of the proportion of captured beetles within each replicate; dashed line is the catch proportion expected by chance alone for nine treatments (11.1 %); compounds and dispensers as in Table 1)

quaternary blend did not reduce the trap catches; and replacing 74 %-(*S*)-Id with the racemate (\pm)-Id in the blends did not result in any trap catch reduction. However, subtraction of cV from the quaternary and ternary blends resulted in significant reductions in trap catch (Fig. 1a), to a level not different from those in unbaited control traps. Both MB and Id were present in all the treatment traps, and so the effects of their absence were not determined in this

test. Only a few individuals of *I. shangrila* were captured in Experiment 1 without showing a significant pattern of attraction to any particular treatment.

In Experiment 2, which was originally designed for *I. shangrila*, only 66 *I. shangrila* were captured due to very low populations of this species in the test area. Detailed trap catch data on *I. shangrila* were previously published in Zhang et al. (2009b). In contrast to the low catches of *I. shangrila*, a total of 671 *I. nitidus* were captured in seven replicates. The unbaited traps and traps baited with any blends (binary/ternary/quaternary) having 97 %-(+)-(*R*)-Id as one of the major components, caught zero or close to zero *I. nitidus* beetles (Fig. 1b). However, the two quaternary and ternary blends that contained the racemic (\pm)-Id caught significantly more *I. nitidus* than did the unbaited control and all other blends (Fig. 1b). Subtraction of the minor component, (-)-tV from the attractive quaternary blend did not reduce the trap catches. Interestingly, removal of cV from the active ternary and quaternary blends totally shut off the attraction to *I. nitidus* (Fig. 1b). As in Experiment 1, MB and Id also were present in all the treatment traps in Experiment 2, and so the effects of their absence from various combinations was not determined. In both Experiment 1 and Experiment 2, and the published data (Zhang et al. 2009a, b), no individual components were tested; and thus their behavioral activity as independent factors cannot be judged. To this end, we devised factorial tests for both species with all combinations of the relevant candidates tested in all possible combinations, using the relevant Id enantiomers from both Experiment 1 and Experiment 2.

Full Factorial Trapping Experiment for *Ips nitidus*

Favorable weather conditions allowed us to complete 8 replications from each of the two sets of traps in Experiment 3, resulting in a total capture of 810 *I. nitidus* in 16 replicates. The factorial experiment clearly showed a specific positive response to treatments including (\pm)-Id (Fig. 2), similar to that of the 2009 experiments (Fig. 1) and reported data (Zhang et al. 2009a).

Factorial ANOVA analysis on the effects of the three major components, MB, (\pm)-Id and cV, and all their interactions (synergism or antagonism) showed an overall highly significant effect ($F_{7,120}=97.6$, $P<0.001$) (Fig. 2). First, there was the expected strong impact of (\pm)-Id as a factor (effect size as partial $\eta^2>0.8$, $F_{1,120}=557$). Second, the MB ($F_{1,120}=13.7$) or cV ($F_{1,120}=76.1$) alone and their two-way interaction (Id \times cV, $F_{1,120}=31.8$) also showed strong significance levels (Table 2). Third, the two-way interaction MB \times cV was hardly significant ($F_{1,120}=3.1$), while other interactions were insignificant (Table 2). This corresponds to the

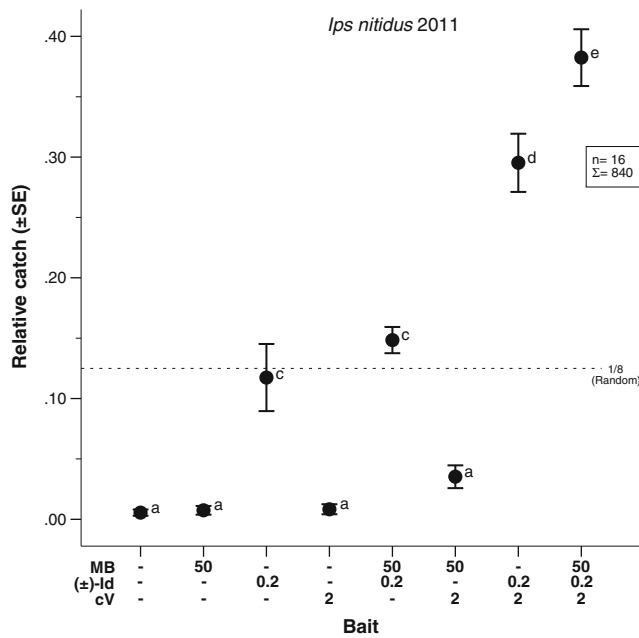


Fig. 2 Mean proportions of total catch of *Ips nitidus* in cross-barrier traps baited with three potential aggregation pheromone components in a full factorial design (April 30 to May 10, 2011, Maixiu Forest Park, Qinghai, China; $N=16$; means with the same letter are not significantly different ($P>0.05$) by REGW multiple Q test after one-way ANOVA on the arcsin square root transformed data of the proportion of captured beetles within each replicate; dashed line is the catch proportion expected by chance alone for eight treatments (12.5 %); compounds and dispensers as in Table 1)

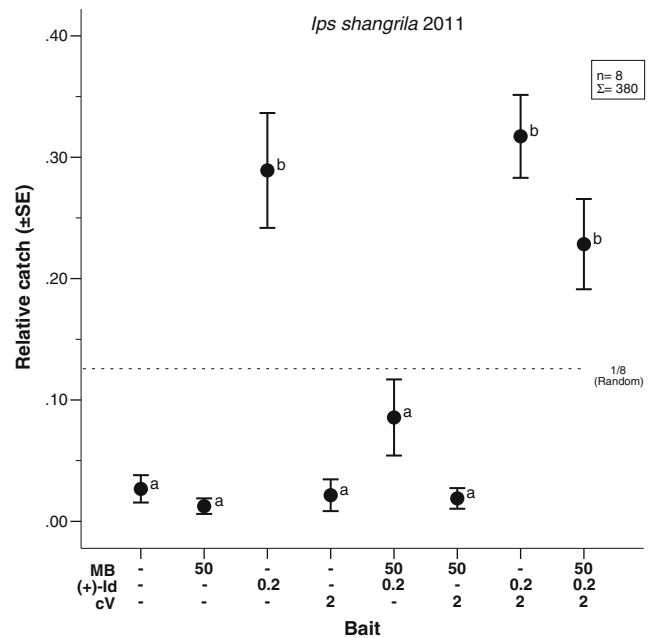


Fig. 3 Mean proportions of total catch of *Ips shangrila* in cross-barrier traps baited with three potential aggregation pheromone components in a full factorial design (April 30 to May 10, 2011, Maixiu Forest Park, Qinghai, China; $N=8$; means with the same letter are not significantly different ($P>0.05$) by REGW multiple Q test after one-way ANOVA on the arcsin square root transformed data of the proportion of captured beetles within each replicate; dashed line is the catch proportion expected by chance alone for eight treatments (12.5 %); compounds and dispensers as in Table 1)

synergistic effect of (±)-Id and cV, while MB seems to have little activity when combined with cV or Id (Fig. 2).

Means separations (Fig. 2) showed all treatments involving (±)-Id to be above or at the random expectation of relative catch of 0.125. In contrast, both MB or (–)-cV alone, and their combination, and the blank, had close to zero catches.

Interestingly, the highest trap catch was with the ternary blend including MB, significantly higher than the synergistic binary blend of (±)-Id + (–)-cV. Thus, a ternary blend of all three major components, MB, (–)-cV, and (±)-Id or 74 %-(–)-(S)-Id is supported as the aggregation pheromone system for *I. nitidus* by the one-way ANOVA *post-hoc* analysis. Very few *I. shangrila* and *Thanasimus* spp. clerid predators were

Table 2 Results for tests of between-subject effects by factorial ANOVA on relative catches of *Ips nitidus* in Experiment 3, 2011 (dependent variable is catch for a treatment as a proportion of total catch in a replicate, transformed to arcsin square root; . Levene’s test of

equality of error variances $F_{7,120}=2.0, P=0.06$ (NS); values for SS and *d.f.* for overall estimates as in corrected model, intercept, error etc. are not given here to improve clarity; error *d.f.*=120, total 128)

Source of variation	Type III SS	<i>d.f.</i>	<i>F</i>	<i>P</i>	Partial η^2
Factors					
2-Methyl-3-buten-2-ol (MB)	0.14	1	13.7	<0.001	0.10
(±)-Ipsdienol (Id)	5.7	1	556.9	<0.001	0.82
(S)-(–)- <i>cis</i> -Verbenol (cV)	0.78	1	76.1	<0.001	0.39
Interaction of factors					
MB×Id	0.01	1	0.6	0.45 NS	0.01
MB×cV	0.31	1	3.1	0.08 NS	0.02
Id×cV	0.32	1	31.8	<0.001	0.21
MB×Id×cV	0.01	1	0.8	0.39 NS	0.01

caught in treatment traps and none were caught in unbaited control traps.

Full Factorial Trapping Experiment for *Ips shangrila* In Experiment 4, *I. shangrila* was again in lower abundance than *I. nitidus*, but eight replicates were achieved ($n=8$) with a total of 358 beetles captured. As with *I. nitidus* in Experiment 3, there was an apparent overall strong influence of Id, but here of the opposite (+)-Id enantiomer (Fig. 3).

A factorial ANOVA of the three major components, MB, cV, and (+)-Id, and their interactions showed an overall highly significant effect ($F_{7, 56}=25.8$, $P<0.001$) with quite homogeneous variances (Table 3). There was an expectedly strong impact of (+)-Id alone as a factor (with effect size as partial $\eta^2>0.7$; $F_{1,56}=145$), and also of the other compounds: MB ($F_{1,56}=14.2$), but less strong for cV ($F_{1, 56}=4.2$), with several interactions at significant levels (Table 3). The interaction MB \times Id was highly significant ($F_{1,56}=7.3$, $P<0.009$), while MB \times cV and Id \times cV were less so, and non-significant for the third level interaction MB \times cV \times Id. Thus (+)-Id and (–)-cV act synergistically to increase attraction, while MB has strong activity when combined with Id. Unexpectedly, the MB-related interactions here were all antagonistic, due to a decrease in trap catches compared to treatments containing (+)-Id without MB. In contrast, cV increased attraction (Fig. 3).

All treatments involving (+)-Id, except the one combined with only MB, had means far above a relative catch of 0.125, and were significantly different from other treatments (Fig. 3). The inhibitory MB, and the less attractive cV alone or their combination, did not differ from the blank with catches near zero. Thus, only (+)-Id and (–)-cV combined are supported as active and synergistic aggregation pheromone components for *I. shangrila*; (+)-Id is the most critical component in its pheromone system, whereas MB does not seem to be a part of the aggregation pheromone blend. In contrast, any combinations

with MB caught less than their corresponding combinations without MB, a highly significant effect according to ANOVA (Table 3). As in Experiment 3, only a few Ipinids and *Thanasimus* spp. were caught.

Discussion

The present paper clearly illustrates an example of pheromone response diversity as a basis for mate recognition in two species of Eastern Palearctic *Ips*. For these two syntopic species, we find that both the compositions of the major components, chirality of the major compound Id, and some minor components contribute to signal specificity. The response specificity is a function not only of attractive properties of synergistic pheromone components, but also of antagonistic effects of some other components.

Our first hypothesis, that not all chemically identified constituents from unmated males are attractive, is borne out by the fact that tV is inactive in *I. nitidus* or inhibitory in *I. shangrila*, and that MB is an antagonist for *I. shangrila*. Our current results for *I. shangrila* are different from the three-component pheromone system of MB, (+)-Id, and (–)-cV reported by Zhang et al. (2009b). The difference in respect of MB is presumably due to the fact MB was included in all the treatment traps in the earlier study. Production of MB and tV in the hindguts of male *I. shangrila* reached the highest level (760 ng/male and 180 ng/male, respectively) after the males mated with four females (Zhang et al. 2009b), providing circumstantial evidence for the antagonistic effects of these two components in this species. After mating, the key pheromone component, (+)-Id, was at the lowest level (<100 ng/male) (Zhang et al. 2009b).

Table 3 Results for tests of between-subject effects by factorial ANOVA on relative catches of *Ips shangrila* in Experiment 4, 2011 (dependent variable is catch for a treatment as a proportion of total catch in a replicate, transformed to arcsin square root; . Levene's test of

equality of error variances $F_{7,56}=0.65$, $P=0.71$ (NS); values for SS and *d.f.* for overall estimates as in corrected model, intercept, error etc. are not given here to improve clarity; error *d.f.*=56, total 64)

Source of variation	Type III SS	<i>d.f.</i>	<i>F</i>	<i>P</i>	Partial η^2
Factors					
2-Methyl-3-buten-2-ol (MB)	0.22	1	14.2	<0.001	0.21
97 %-(+)-(R)-Ipsdienol (Id)	2.2	1	144.8	<0.001	0.72
(S)-(–)- <i>cis</i> -verbenol (cV)	0.07	1	4.2	0.044	0.07
Interaction of factors					
MB \times Id	0.12	1	7.3	0.009	0.12
MB \times cV	0.07	1	4.3	0.044	0.07
Id \times cV	0.08	1	4.9	0.030	0.08
MB \times Id \times cV	0.02	1	1.0	0.31 NS	0.02

(–)-Ipsenol was detected from male hindguts of both species as a minor/trace component (Hoskovec et al. 2012), but its behavioral function has not yet been tested. Other compounds frequently found in many *Ips*, but never confirmed as pheromone components, such as myrtenol and 2-phenylethanol, also were detected as minor components of species studied here (Zhang et al. 2009a, b).

The second hypothesis, that variation in Id chirality was a necessary, but not sufficient, factor for species separation, is partly supported by the two first experiments reported here and by earlier studies (Zhang et al. 2009a, b). Results for (±)-Id based blends tested here indicate that racemic, or close to racemic Id, is the critical component for attraction of *I. nitidus*. In contrast, the sympatric *I. shangrila* is attracted to blends containing 97 %-(+)-(R)-Id, which is close to the naturally produced enantiomeric ratio of 99:1 R :S; (Zhang et al. 2009b). Such strong disparity in the enantiomeric composition of the critical shared pheromone component Id is evidently a necessary factor for maintaining the reproductive isolation among these two syntopic and sympatric species. In addition to the key factor of Id chirality, there was a strong effect of cV for increasing *I. nitidus* attraction. On the other hand, MB improves attraction of *I. nitidus*, but is an antagonist for *I. shangrila*. Thus, at least two identified terpenic alcohols (cV and MB) other than Id in each species contribute to full species isolation by pheromone.

The third hypothesis, stating the existence of both antagonistic and synergistic effects of hindgut compounds, is strongly supported for both species. Our full factorial experiments in 2011 demonstrated that 74 %-(–)-(S)-Id or (±)-Id, (–)-cV, and MB, are the three synergistic components of the male-produced aggregation pheromone of *I. nitidus*, thus supporting the finding of Zhang et al. (2009a); whereas 99 %-(+)-(R)-Id and (–)-cV are the two synergistic components of the aggregation pheromone of *I. shangrila*. In contrast, MB, one of major male-produced hindgut volatiles, has antagonistic effects on the attraction of *I. shangrila* (current study). Furthermore, the minor component, tV, was inactive in *I. nitidus* (current study and Zhang et al. 2009a), but showed strong inhibitory effect on *I. shangrila* (Zhang et al. 2009b).

Pheromone Production and Responses in Palearctic *Ips* and *Pseudoips* Among the 14 major Eurasian Ipini bark beetle examples listed in Table 4, there are eight common pheromone components: both enantiomers of Id, (–)-Ie, cV, *E*-myrcenol (EM), amitinol (AM), and two isomers of methylbutenol (2-methyl-3-buten-2-ol and 3-methyl-3-buten-1-ol) that, in theory, could create 255 different blends.

Typically, only two or three components are noted for 13 of 15 blends reported. The *Larix* infesting taxa of *Ips* shows an interesting variety of blend components; in Europe four components have been reported for *I. cembrae* (Kohnle et al. 1988b), while the East Palearctic *Larix* beetle (*I. subelongatus*) reportedly has two components in Jilin, NE China (Song et al. 2011), but only one component in Inner Mongolia, NE China (Zhang et al. 2000, 2007a). Id is the most common pheromone component, used in 11 of the 14 species of these Eurasian Ipini bark beetles; (+)-Id in five species, (–)-Id in three species, and both enantiomers in three species. In *I. nitidus*, neither pure enantiomer is attractive, which is rather unusual among insect pheromones and obviously a critical factor in species specificity. However, in our set of species we found this in three of 14 cases (*I. nitidus*, *I. duplicatus*, and *I. sexdentatus*), but in each case one [*I. sexdentatus*; or possibly more, see Etxebeste et al. (2012)] or two additional synergistic components are known. Ie is the most common secondary *Ips* pheromone component found in eight species, followed by cV present in six species (Table 4); followed by AM in five species and 232-MB in four species. Two components, *E*-myrcenol (EM) and 331-MB are reportedly pheromone components only for *I. duplicatus* and *I. cembrae*, respectively. The cV seems to be a conservative motif in all four *Ips* and *Pseudoips* taxa that are uniquely distributed in the high altitude regions of Himalayas and Tibetan-Qinghai Plateau. No common aggregation pheromone blend is shared by any of the Eurasian Ipini species listed in Table 4, not even those phylogenetically closely related sister species pairs, such as *I. typographus* and *I. nitidus*; *I. shangrila* and *I. amitinus* (Cognato and Sun 2007); or *I. cembrae* and *I. subelongatus* (Stauffer et al. 2001). A possible exception is the pair *I. schmutzenhoferi* and *Ps. orientalis*, which have been reported to use Ie and cV. However, for *I. schmutzenhoferi*, neither chirality of Ie nor response to full-blend candidates is known (Eidmann and Birgersson 1988; Kohnle et al. 1988a).

Strong geographical variation in pheromone response, and to a lesser extent in production, occurs between European and Asian populations of *I. duplicatus*; (Lakatos et al. 2007; Schlyter et al. 1992, 2001a) or even among Chinese populations of *I. subelongatus* (Chen et al. 2015; Song et al. 2011; Zhang et al. 2007a). Similar geographical variations also might occur for other Eurasian Ipini species, of which pheromone identifications are mostly from European populations that were investigated >30 years ago prior to the advent of GC-EAD. Thus, re-examination of the aggregation pheromones of “Pan” Eurasian species using GC-EAD/MS technology, and field trapping bioassays in both Europe and Asia with a full factorial experimental design approach, could uncover any potential regional or continental variations in pheromone production and/or responses. Updating the knowledge of bark beetle

Table 4 Aggregation pheromone systems reported for major Eurasian Ipsini bark beetles

Species	Distribution	Major host genera	Attractive pheromone components ^{a,b}						Inhibitors	References
			(+)-Id	(-)-Id	(+)-Id	(-)-Id	(+)-Id	(-)-Id		
<i>Ips nitidus</i> Eggers	Western China	<i>Picea</i>	26 %	74 %	✓			✓	Zhang <i>et al.</i> (2009a)	
			50 %	50 %	✓			-	Hoskovec <i>et al.</i> (2012)	
			50 %	50 %	✓			✓	Current paper	
<i>Ips shangrila</i> Cognato & Sun	Western China	<i>Picea</i>	99 %	1 %	✓				Zhang <i>et al.</i> (2009b)	
			99 %	1 %	✓				Current paper	
<i>Ips typographus</i> L.	Eurasia	<i>Picea</i>		?	?			✓	Hoskovec <i>et al.</i> (2012)	
<i>Ips duplicatus</i> (Sahlb.)	Europe	<i>Picea</i>	50 %	50 %			✓		±Ie	Schlyter <i>et al.</i> (1987)
							✓			(-)-Vn ^d
<i>Ips amitinus</i> (Eichhoff)	Europe	<i>Picea</i>	50 %	50 %				✓	(-)-Vn	Byers <i>et al.</i> (1990); Schlyter <i>et al.</i> (1992)
			50 %	50 %			✓	✓ ^e		(-)-Vn
<i>Ips sexdentatus</i> Boerner	Central Europe	<i>Picea</i>	5 %	95 %	✓			✓		Francke <i>et al.</i> (1980)
			50 %	50 %			✓			(±)-Ie
<i>Ips cembrae</i> (Heer)	Europe	<i>Larix (Picea)</i>	95 %	5 %	5 %	95 %	✓	✓		Kohlme <i>et al.</i> (1988b)
							✓			(-)-cV; (-)-Vn
<i>Ips subelongatus</i> Motschulsky	Asia, Inner Mongolia	<i>Larix</i>	96 %	4 %	✓					Zhang <i>et al.</i> (2007a)
							✓			(-)-cV; (-)-Vn
<i>Ips acuminatus</i> Gyllenhal	Eurasia	<i>Pinus</i>	95 %	5 %	5 %	95 %	✓			Bakke (1978); Francke <i>et al.</i> (1986); Kohlme <i>et al.</i> (1988b)
							✓			(-)-Id, AM
<i>Ips schmutzenhoferi</i> Holzschuh	Himalayas	<i>Picea, Pinus</i>		?	?		✓			Kohlme <i>et al.</i> (1993)
							✓			AM; (-)-cV; (-)-Vn
<i>Orthotomicus mansfeldti</i> (Wachtl.)	Central Europe	<i>Pinus</i>	✓				✓			Kohlme <i>et al.</i> (1993)
							✓			(+)-Id
<i>Orthotomicus erosus</i> (Wollaston)	Eurasia	<i>Pinus</i>		✓				✓		G. Birgersson, SLU, Alnarp (unpubl.)
										(+)-Id
<i>Orthotomicus tridentatus</i> Eggers	(introduced to U.S.A.)	<i>Cedrus</i>		✓						
										(±)-Id
<i>Pseudops orientalis</i> (Wood and Ym)	Western China	<i>Picea</i>	5 %	95 %	✓			✓		
										(±)-Id

^a Id: ipsdienol; Ie: ipsenol; cV: *cis*-verbenol; tV: *trans*-verbenol; AM: amitinol; EM: *E*-myrcenol; 232-MB: 2-methyl-3-buten-2-ol; 331-MB: 3-methyl-3-buten-1-ol; Vn: verbenone;

^b ✓: confirmed or reported pheromone component; ? : enantiomeric composition not determined; if determined, % of (+) or (-) enantiomer is indicated

^c Value of 5 % correspond to experimental uncertainty, the (+)-enantiomer has not been proven to exist in *Ips* (Birgersson, personal communication)

^d In contrast to *Dendroctonus* spp., in *Ips* mostly not produced by the insect but by associated yeasts in gallery walls, thus more of a microhabitat cue than a pheromone signal (Schlyter and Birgersson 1999)

^e A higher proportion of EM (60 % relative to Id) is the best blend

chemical ecology will likely allow an improvement of semiochemical-based beetle control of economically important Eurasian Ipsini bark beetles.

Ips nitidus, *I. shangrila*, and *Ps. orientalis* are not only sympatric, but also share common or similar spatial and temporal niches in *P. crassifolia* forests (Liu et al. 2007). This may result in strong interspecific competition and evolutionary pressure for reproductive isolation (Wood 1982). *Ps. orientalis* produces an aggregation pheromone blend of 95 %-(–)-Id and (–)-cV that is quite different from those of *I. nitidus* and *I. shangrila* (Table 4). Such a strong disparity in pheromone systems among the sympatric bark beetle species would amplify the potential semiochemical interactions such as mutual or unidirectional inhibition (Wood 1982). In turn, these interactions play a role in maintaining partial niche separation and reproductive isolation, and may regulate both spatial and temporal competition (Byers 1989; Schlyter et al. 1992, 2001a; Zhang et al. 2009a, b). Whether in general there is an effect on the evolution of their active pheromone components due to the sympatry or syntopy of bark beetle species on the same host tree species has not yet been formally tested.

The fourth hypothesis that racemic Id could be used instead of pure enantiomers of Id holds true only for *I. nitidus*. For *I. shangrila*, a two-component pheromone blend of (+)-Id and (–)-cV, is required as a selective monitoring-bait, while mass-trapping *I. shangrila* is less likely due to the current high costs of synthetic (+)-Id. In contrast, for *I. nitidus* where less expensive racemic Id can replace the naturally produced enantiomers, the future for pheromone monitoring or mass-trapping is much brighter. In fact, the racemic (±)-Id was recently reported from guts of both male and female *I. nitidus*, and from their frass, via chiral GC×GC-TOFMS analysis (Hoskovec et al. 2012). The importance of a synthetic pheromone blend that is competitive to natural sources is illustrated by the successful mass-trapping of *Ips duplicatus* in Inner Mongolia (Schlyter et al. 2001b).

Acknowledgments The authors thank the staff of the Maixiu Mountain Forest Park of Qinghai Province for cooperation in data collection. We thank Drs Y. Wang and J. R. Aldrich, at SLU and USDA, respectively, for constructive comments on an earlier draft. The study was funded by the APVV project “Study of semiochemicals-based technology to control aggressive spruce bark beetles in province Qinghai, China” (SK-CN-0025-09) and project VEGA (2/0194/12) “Interactions between forest trees and bark beetles (Col. Scolytinae)”.

References

- Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowell N, Vennetier M, Kitzberger T, Rigling A, Breshears DD, Hogg E (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *For Ecol Manag* 259:660–684
- Bakke A (1978) Aggregation pheromone components of the bark beetle *Ips acuminatus*. *Oikos* 31:184–188
- Byers JA (1989) Chemical ecology of bark beetles. *Experientia* 45:271–283
- Byers JA (1992) Optimal fractionation and bioassay plans for isolation of synergistic chemicals: the subtractive-combination method. *J Chem Ecol* 18:1603–1621
- Byers JA (1993) Randomization algorithms in BASIC for experimental design. *Comput Biol Med* 23:167–176
- Byers JA, Schlyter F, Birgersson G, Francke W (1990) E-myrcenol in *Ips duplicatus*: an aggregation pheromone component new for bark beetles. *Experientia* 46:1209–1211
- Cardé RT, Baker TC (1984) Sexual communication with pheromones. In: Cardé RT, Baker TC (eds) *Chemical ecology of insects*. Chapman and Hall Ltd., p 355–383
- Chen DF, Li YJ, Zhang Q-H, Zhang SF, Wang HB, Zhang Z, Zhao LL, Kong XB (2015) Population divergence of aggregation pheromone responses in *Ips subelongatus* in Northeastern China. *Insect Sci*. doi: 10.1111/1744-7917.12221
- Cognato AI, Sun JH (2007) DNA based cladograms augment the discovery of a new *Ips* species from China (Coleoptera: Curculionidae: Scolytinae). *Cladistics* 23:539–551
- Eidmann HH, Birgersson G (1988) Semiochemicals in the East Himalaya spruce bark beetle. *Anz Schadlingskd Pfl* 61:147–148
- Etxebeste I, Álvarez G, Pérez G, Pajares J (2012) Field response of the six-toothed pine bark beetle, *Ips sexdentatus* (Col.: Curculionidae, Scolytinae), to pheromonal blend candidates. *J Appl Entomol* 136: 431–444
- Fettig CJ, Dabney CP, McKelvey SR, Borys RR (2006) An assessment of re-randomization methods in bark beetle (Scolytidae) trapping bioassays. *Agric For Entomol* 8:267–271
- Francke W, Sauerwein P, Vité JP, Klimetzek D (1980) The pheromone bouquet of *Ips amitinus*. *Naturwissenschaften* 67:147–148
- Francke W, Pan ML, Bartels J, König W, Vité J, Krawielitzki S, Kohnle U (1986) The odour bouquet of three pine engraver beetles (*Ips* spp.). *J Appl Entomol* 101:453–461
- Giesen H, Kohnle U, Vité J, Pan ML, Francke W (1984) Das aggregationspheromon des mediterranen Kiefernborckenkäfers *Ips (Orthotomicus) erosus*. *Z Angew Entomol* 98:95–97
- Hillebrand H, Gurevitch J (2013) EDITORIAL reporting standards in experimental studies. http://www.wiley-docs.com/Checklist_for_reporting_experimental_details.pdf
- Hoskovec M, Kalinová B, Knižek M (2012) Chiral and Nonchiral GC×GC/TOFMS analysis of natural compounds: the case of possible aggregation pheromones of Chinese bark beetles *Ips shangrila* and *Ips nitidus*. In: Salih B, Çelikbiçak Ö (eds) *Gas chromatography in plant science, wine technology, toxicology and some specific applications*. InTech Europe, Rijeka, pp 325–346. doi:10.5772/33934
- Jakuš R, Kalinová B, Hoskovec M, Knižek M, Schlyter F, Sun J-H, Zhang Q-H (2011) Outbreak of *Ips nitidus* and *Ips shangrila* in northeastern margin of the Tibetan Plateau. In: 10th IUFRO Workshop of WP 7.03.10 “Methodology of forest insect and disease survey in central Europe”: Biotic risks and climate change in forests, September 20–23, 2010, Freiburg, Germany, 2011. *Berichte Freiburger Forstliche Forschung*, p 111–117
- Jones D (1984) Use, misuse, and role of multiple-comparison procedures in ecological and agricultural entomology. *Environ Entomol* 13: 635–649
- Kohnle U, Schmutzenhofer H, Bartels J, Francke W (1988a) Oxygenated terpenes in the chemical communication system of the bark beetle, *Ips schmutzenhoferi* (Col., Scolytidae), a species recently described for the Southeastern Himalaya. *J Appl Entomol* 106:46–51
- Kohnle U, Vité J, Erbacher C, Bartels J, Francke W (1988b) Aggregation response of European engraver beetles of the genus *Ips* mediated by terpenoid pheromones. *Entomol Exp Appl* 49:43–53

- Kohnle U, Pajares J, Bartels J, Meyer H, Francke W (1993) Chemical communication in the European pine engraver, *Ips mansfeldi* (Wachtl)(Col., Scolytidae). *J Appl Entomol* 115:1–7
- Lakatos F, Grodzki W, Zhang Q-H, Stauffer C (2007) Genetic comparison of *Ips duplicatus* (Sahlberg, 1836)(Coleoptera: Curculionidae, Scolytinae) populations from Europe and Asia. *J For Res* 12:345–349
- Liu L, Yan W, Luo Y-Q, Wu J, Li Z-Y, Ma J-H (2007) Spatial niches of bark beetle population in *Picea crassifolia* natural forests (In Chinese; Abstract in English). *J Beijing For Univ* 29:165–169
- Liu L, Wu J, Luo Y-Q, Li Z-Y, Wang G-C, Han F-Z (2008) Morphological and biological investigation of two pioneer *Ips* bark beetles in natural spruce forests in Qinghai Province, northwest China. *For Stud China* 10:19–22
- Mitchell RF, Reagel PF, Wong JC, Meier LR, Silva WD, Mongold-Diers J, Millar JG, Hanks LM (2015) Cerambycid beetle species with similar pheromones are segregated by phenology and minor pheromone components. *J Chem Ecol*. doi:10.1007/s10886-015-0571-0
- Saveer AM, Becher PG, Birgersson G, Hansson BS, Witzgall P, Bengtsson M (2014) Mate recognition and reproductive isolation in the sibling species *Spodoptera littoralis* and *Spodoptera litura*. *Front Ecol Evol Chem Ecol* 2:18
- Schlyter F, Birgersson G (1999) Forest beetles. In: Hardie J, Minks AK (eds) Pheromones in non-Lepidopteran insects associated with agricultural plants. CAB International, Oxford, pp 113–148
- Schlyter F, Byers J, Löfqvist J (1987) Attraction to pheromone sources of different quantity, quality, and spacing: density-regulation mechanisms in bark beetle *Ips typographus*. *J Chem Ecol* 13:1503–1523
- Schlyter F, Birgersson G, Byers JA, Bakke A (1992) The aggregation pheromone of *Ips duplicatus* and its role in competitive interactions with *I. typographus* (Coleoptera: Scolytidae). *Chemoecology* 3: 103–112
- Schlyter F, Svensson M, Zhang Q-H, Knizek M, Krokene P, Ivarsson P, Birgersson G (2001a) A model for peak and width of signaling windows: *Ips duplicatus* and *Chilo partellus* pheromone component proportions—does response have a wider window than production? *J Chem Ecol* 27:1481–1512
- Schlyter F, Zhang Q-H, Liu GT, Ji LZ (2001b) A successful case of pheromone mass-trapping of the bark beetle *Ips duplicatus* in a forest island, analysed by 20-year time-series data. *Integr Pest Manag Rev* 6:185–196
- Seybold SJ, Ohtsuka T, Wood DL, Kubo I (1995) Enantiomeric composition of ipsdienol: a chemotaxonomic character for north American populations of *Ips* spp. in the *pini* subgeneric group (Coleoptera: Scolytidae). *J Chem Ecol* 21:995–1016
- Seybold SJ, Huber DPW, Lee JC, Graves AD, Bohlmann J (2006) Pine monoterpenes and pine bark beetles: a marriage of convenience for defense and chemical communication. *Phytochemistry Reviews* 5: 143–178
- Slinker BK (1998) The statistics of synergism. *J Mol Cell Cardiol* 30: 723–731
- Song LW, Zhang QH, Chen YQ, Zuo TT, Ren BZ (2011) Field responses of the Asian larch bark beetle, *Ips subelongatus*, to potential aggregation pheromone components: disparity between two populations in northeastern China. *Insect Sci* 18:311–319
- Stauffer C, Lakatos F, Hewitt GM (1997) The phylogenetic relationships of seven European *Ips* (Scolytidae, Ipinae) species. *Insect Mol Biol* 6:233–240
- Stauffer C, Kirisits T, Nussbaumer C, Pavlin R, Wingfield MJ (2001) Phylogenetic relationships between the European and Asian eight spined larch bark beetle populations (Coleoptera: Scolytidae) inferred from DNA sequences and fungal associates. *Eur J Entomol* 98:99–106
- Wood DL (1982) The role of pheromones, kairomones, and allomones in the host selection and colonization behavior of bark beetles. *Annu Rev Entomol* 27:411–446
- Xue Y-G, Ma Y-S, Wang X-P (2003) Occurrence and damages of *Ips typographus* in Huangnan State, Qinghai Province. *Sci Technol Qinghai Agric For* 3:18–19 (In Chinese; Abstract in English)
- Yang B, Qin C, Wang J, He M, Melvin TM, Osborn TJ, Briffa KR (2014) A 3,500-year tree-ring record of annual precipitation on the northeastern Tibetan Plateau. *Proc Natl Acad Sci U S A* 111:2903–2908
- Yin H-F, Huang F-S, Li Z-L (1984) *Ips mansfeldi* Wachtl. In: Economic insect fauna of China (Coleoptera: Scolytidae), vol Fasc. 29. p 129
- Zhang Q-H, Schlyter F (2003) Redundancy, synergism, and active inhibitory range of non-host volatiles in reducing pheromone attraction in European spruce bark beetle *Ips typographus*. *Oikos* 101:299–310
- Zhang Q-H, Birgersson G, Schlyter F, Chen G-F (2000) Pheromone components in the larch bark beetle, *Ips cembrae*, from China: quantitative variation among attack phases and individuals. *J Chem Ecol* 26: 841–858
- Zhang Q-H, Schlyter F, Chen G, Wang Y (2007a) Electrophysiological and behavioral responses of *Ips subelongatus* to semiochemicals from its hosts, non-hosts, and conspecifics in China. *J Chem Ecol* 33:391–404
- Zhang Q-H, Schlyter F, Liu G-T, Sheng M-L, Birgersson G (2007b) Electrophysiological and behavioral responses of *Ips duplicatus* to aggregation pheromone in Inner Mongolia, China: amitinol as a potential pheromone component. *J Chem Ecol* 33:1303–1315
- Zhang Q-H, Ma J-H, Zhao F-Y, Song L-W, Sun J-H (2009a) Aggregation pheromone of the Qinghai spruce bark beetle, *Ips nitidus* Eggers. *J Chem Ecol* 35:610–617
- Zhang Q-H, Song L-W, Ma J-H, Han F-Z, Sun J-H (2009b) Aggregation pheromone of a newly described spruce bark beetle, *Ips shangrila* Cognato and Sun, from China. *Chemoecology* 19:203–210
- Zhang Q-H, Ma J-H, Zhao F-Y, Song L-W, Sun J-H, Cognato AI (2011) Aggregation pheromone of the Oriental spruce engraver *Pseudips orientalis*. *Agric For Entomol* 13:67–75
- Zhang Q-H, Ma J-H, Zhao F-Y, Shi Q, Wang G (2012) Research progress on aggregation pheromone systems of major *Ips* / *Pseudoips* bark beetles attacking the thickleaf spruce: a mini-review. *Sci Silvae Sin* 48:118–126 (In Chinese with English Abstract)