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Effect of host and larval frass volatiles on behavioural response of the old house borer, *Hylotrupes bajulus* (L.) (Coleoptera: Cerambycidae), in a wind tunnel bioassay

Regina Fettköther[1], **Gadi V. P. Reddy**[1], **Uwe Noldt**[2] **and Konrad Dettner**[1]

[1]Lehrstuhl für Tierökologie II, Universität Bayreuth, Postfach 101251, D-95440 Bayreuth, Germany, e-mail: k.dettner@uni-bayreuth.de
[2]Bundesforschungsanstalt für Forst- und Holzwirtschaft, Institut für Holzbiologie und Holzschutz, D-21027 Hamburg, Germany

Summary. In a wind tunnel bioassay the effect of three concentrations of natural extracts of (1) Scots pine wood, *Pinus sylvestris*, and (2) larval frass on the behavioural response of unmated females and males of the old house borer, *Hylotrupes bajulus*, was tested and compared to the behavioural effects of the male-produced sex pheromone (3*R*)-3-hydroxy-2-hexanone. The influence on the behaviour of both sexes was found to be equally significant for the two higher concentrated hexane extracts of wood and larval frass. Therefore several synthetic monoterpenes present in the extracts and ethanol were tested at the two higher concentrations (1:100, 1:1000 vol/vol). Among the higher concentrated monoterpenoid hydrocarbons [(+)-α-pinene, (+)-β-pinene, (+)-limonene], only α-pinene increased the activity, orientation towards scent source and interest towards conspecifics. The tests with higher concentrated ethanol and the oxygenated monoterpenes [(−)-verbenone, (−)-*trans*-pinocarveol, (+)-terpinen-4-ol, (+)-α-terpineol, (−)-myrtenol] revealed that verbenone is the most effective stimulant for the females, followed by *trans*-pinocarveol, terpinen-4-ol and α-terpineol. For males, terpinen-4-ol was the only mediator significantly inducing attraction and orientation towards the scent source combined with an interest in conspecifics apparent by fighting or courtship behaviour. Males did not respond to verbenone which is a main compound of larval frass. Myrtenol and ethanol were ineffective in both sexes. In fact behavioural observations suggest that the beetles were repelled by the high dose of myrtenol. Using the ten-fold lower dose of the synthetic monoterpenes (1:1000 vol/vol), all semiochemicals except myrtenol lost activity. Myrtenol, however, induced behavioural responses, like increased activity and orientation towards scent source, only at the low concentration. Based on the results, primary attraction of unmated old house borer is probably mediated by monoterpenes of coniferous wood, while secondary attraction to infested wood would occur in response to volatiles of larval frass.

Key words. *Hylotrupes bajulus* – Coleoptera – Cerambycidae – primary and secondary attraction – pheromone – host volatiles – monoterpenes – dose-response dependency

Introduction

The larvae of *Hylotrupes bajulus* (L.) (old house borer, house longhorn beetle, European house borer) are structural insect pests of world-wide importance, as this species has been introduced from Europe to all major continents (Becker 1979). The larvae infest and damage most of the common seasoned coniferous timbers used in buildings (White 1954), and their tunnelling often results in loss of structural integrity of infested wood and financial losses incurred by the treatment and replacement of damaged wood (Cannon & Robinson 1982). The development of a biological control method would be a useful support to the conventional controlling of the old house borer.

Pheromone- or host kairomone-baited traps play an important role in the integrated control of other wood- and bark-boring pests since the host tree selection of many scolytids and curculionids is well studied (Borden 1985). Volatiles released by host plants are also known to be attractive to many cerambycids including some important pest species. Some flower-visiting longicorn species are attracted to traps baited with chemicals resembling floral fragrances, e. g. benzyl acetate, methyl benzoate, methyl phenylacetate, eugenol or linalool (Nakashima *et al.* 1994, Maetô *et al.* 1995; Shibata *et al.* 1996). The attraction of female *Anaglyptus subfasciatus* Pic was enhanced when methyl phenylacetate was combined with the male sex pheromones (Nakamuta *et al.* 1997). Many wood-boring cerambycid species were captured in traps baited with turpentine, logs, logs treated with ethanol (Fatzinger 1985, Phillips *et al.* 1988, Dunn & Potter 1991, Brattli *et al.* 1998) or traps baited with synthetic monoterpenes and ethanol (Ikeda *et al.* 1980, Montgomery & Wargo 1983, Atkinson *et al.* 1988, Chénier & Philogène 1989, Miller & Borden 1990, Schroeder & Weslien 1994, Shibata *et al.* 1996).

Correspondence to: K. Dettner

H. bajulus develops only in coniferous softwood. It seems reasonable to assume that chemical stimuli that are characteristic of these host plants might be involved in primary attraction of the adults. Using a sample choice test, Becker (1944) studied the stimulation of egg-laying females by turpentine and 36 terpenoid substances, most of them identified in essential oils of *Pinus spp*. The author reported that the bicyclic hydrocarbons, α- and β-pinene, attracted mated females and stimulated oviposition. Furthermore, Higgs & Evans (1978) found that oviposition behaviour of females is mediated by two oxygenated monoterpenes, (−)-verbenone and *p*-cymen-8-ol, detected in larval frass of the old house borer. The potential attractiveness of monoterpenoid volatiles of coniferous softwood or larval frass pellets to unmated adults of *H. bajulus* has not been investigated previously.

Schröder *et al.* (1994) identified the pheromone compounds produced in the prothoracic exocrine glands of male *H. bajulus* (Noldt *et al.* 1995). In wind tunnel bioassays unmated female beetles were activated and attracted to a scent source baited with pheromone blends (headspace extracts of males and synthetic pheromone blends) as well as to single pheromone compounds like (3*R*)-3-hydroxy-2-hexanone (Fettköther *et al.* 1995).

Our objective was to identify possible chemical mediators in dead or infested Scots pine wood, *Pinus sylvestris* L., involved in host finding of unmated *H. bajulus* that might be combined with synthetic pheromones to develop baited traps for the control of this wood-boring pest.

Materials and methods

Insects

Hylotrupes bajulus was reared in wooden blocks of *P. sylvestris* at the Institute for Wood Biology and Wood Protection, Hamburg, Germany (Noldt *et al.* 1995). Freshly-emerged beetles were held in Bellaplast™ boxes lined with moistened filter paper until used (Fettköther *et al.* 1995). Virgin males and females were kept in separate chambers at 20°C and a 12:12 L:D photoregime, and were tested within 8–21 days after emergence.

Preparation and GC/MS-analysis of extracts of pine wood and larval frass pellets

Frass (fecal) pellets produced by tunnelling larvae and sawdust of comparable grain size were obtained from the culture blocks. A 23 g portion of each material was Soxhlet-extracted for 8 h with hexane and ether, respectively. After evaporation of the solvent the resulting yellow oil was resuspended in the original solvent. For behavioural experiments the crude extracts were tested at concentrations of 1:100, 1:1000 and 1:10,000 (vol/vol), representing 8.6 µg, 0.86 µg, 0.086 µg wood extract or 9.5 µg, 0.95 µg, 0.095 µg frass extract per µl solvent, respectively.

The extracts were analysed in a GC 6000 Vega Series 2 (Carlo Erba) coupled with a Finnigan Iontrap ITD 800 (carrier gas Helium; 230°C injector temperature; 25-m fused silica capillary column SGE HT8; 0.25 mm ID; temperature program with 50°C for 3 min, heating rate 7°C/min, final temperature 270°C). Electron impact (70 mV) mass spectra were obtained in total ion chromatograms. Compounds were identified by comparison with mass spectra and retention times of authentic monoterpenes. Our search focused on monoterpenoid substances known from turpentines (Becker 1944), larval frass (Evans & Higgs 1975) and head space analysis of *P. sylvestris* trees (Sadof & Grant 1997). Hexane and ether extracts of *P. sylvestris* wood were almost identical concerning quality and quantity of the following monoterpenes. Percentages in brackets are given if the peak area amounts for more than 1% of the total peak area amount in the chromatogram: α-pinene (45%), β-pinene, 3-carene (15%), camphene, limonene, sabinene, myrcene, terpinolene, *m*- and *p*-cymene, α- and γ-terpinene, α-thujene, terpinen-4-ol, α-terpineol and verbenone (7%). The three latter chemicals are oxygenated monoterpenes which may be a product of autoxidation of hydrocarbons during extraction procedure. β-Phellandrene also occurs in *P. sylvestris* (Sadof & Grant 1997), but was not available as a standard. The extracts of larval frass pellets contained fatty acids in high amounts. Verbenone, an oxidized product of α-pinene, was the main terpenoid compound in the frass, which also contained α-pinene, 3-carene, β-pinene, camphene, *p*-cymene, γ-terpinene in trace amounts. *p*-Cymene-8-ol, the second major component of larval frass according to Evans & Higgs (1975) could not be identified in our samples.

Selection of test chemicals

We tested the responses of unmated adult *H. bajulus* to hexane and ether extracts of wood and larval frass, synthetic monoterpenoids and ethanol, and compared it to the effectiveness of the main pheromone component (3*R*)-3-hydroxy-2-hexanone. The synthetic pheromone compound (ee ≥ 98.5%) was obtained from the Institute for Organic Chemistry, University of Hamburg, Germany. The selected monoterpenes were (+)-α-pinene, (+)-β-pinene, (+)-limonene, (−)-verbenone, (−)-*trans*-pinocarveol, (+)-terpinen-4-ol, (+)-α-terpineol, (−)-myrtenol (chemical purity ≥ 99%, Fluka, Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany) representing a selection of monoterpenoid hydrocarbons of pine wood logs (Ikeda *et al.* 1980), and mono-oxygenated monoterpenes found in extracts of larval frass of *H. bajulus* boring in pine wood (Higgs & Evans 1978). Hexane, ether and ethanol (purity > 99.5%) were purchased from Merck KGaA, Darmstadt, Germany. Ethanol was selected for testing as this compound is known to be produced in dead or stressed pine tree tissues (Ikeda *et al.* 1980, Sjödin *et al.* 1989). We found that ether was unsuitable as solvent treatment, because ether alone showed repelling effects on the beetles and ether extracts elicited no behavioural response. Because there were no significant differences of beetles responding to hexane control and blank, all synthetic chemicals were diluted with hexane yielding concentrations of 1:100 and 1:1000 (vol/vol), respectively.

Wind tunnel bioassay

Methods and wind tunnel design were identical to those used to test the response of *H. bajulus* females to pheromone components (Fettköther *et al.* 1995). Micropipettes (Blaubrand, intra MARK) containing the chemical samples (5 µl) served as scent sources and guaranteed a continuous and complete evaporation within 15 min. The dispenser was positioned horizontally in a gauze box attached to a box wire screen. The Plexiglas tube of the wind tunnel was sectioned into four zones (I–IV) each 25 cm long. Thirty beetles were tested to each stimulus. In every test run, two unmated individuals of the same sex were released on the start wire screen 100 cm downwind of the chemical source. Testing of two conspecifics per run allowed observation of interaction between them. The behaviour of each beetle was recorded for 15 min.

Definition of behavioural responses

Ten behavioural responses (italics) were recorded (Tables 1–4) and grouped under three general classes of behavioural modifications:

(a) activation
  - *starting to move*
  - *initiating flight* ('*flying*' in tables)
  - *running time* (duration of running)

Table 1 Behavioural response of **unmated female** *H. bajulus* to blank and solvent controls, stimuli: pheromone compound (3*R*)-3-hydroxy-2-hexanone, extracts of *P. sylvestris* wood and larval frass, synthetic monoterpenes and ethanol dissolved at the ratio of **1 : 100** in hexane

scent source	starting to move	arrivals (%) in/at						hovering	extension of ovipositor	courtship behaviour	flying
	(%)	zone I	zone II	zone III	zone IV	box wire screen	gauze box	(%)	(%)	(%)	(%)
controls:											
blank	67	63	60	50	20	10	3	0	0	0	0
hexane	70	70	67	57	37	17	3	0	3	0	0
(3*R*)-3-hydroxy-2-hexanone	100***	100***	100***	97***	83***	63***	47***	23**	47***	57***	37***
hexane extract of wood	100***	100***	100***	93**	87***	57**	53***	23**	47***	43***	33***
hexant extract of larval frass	100***	100***	100***	100***	93***	67***	53***	20*	53***	53***	27**
monoterpenoid hydrocarbons:											
(+)-α-pinene	100***	100***	93*	87*	83***	60***	33**	13	30**	33***	27**
(+)-β-pinene	97**	83	77	63	53	27	13	10	17	17*	13
(+)-limonene	93*	87	77	67	53	33	17	13	20*	23**	17*
mono-oxygenated monoterpenes:											
(−)-verbenone	100***	100***	97**	93**	87***	60***	47***	20*	63***	33***	27**
(−)-*trans*-pinocarveol	100***	97**	93*	87*	80***	60***	37**	17*	47***	30***	20*
(+)-terpinen-4-ol	100***	97**	93*	87*	80***	60***	37**	13	33**	27**	20*
(+)-α-terpineol	100***	93*	90*	87*	77**	57**	33**	13	27*	30***	17*
(−)-myrtenol	80	67	43	30	20	7	0	0	7	0	0
ethanol	70	57	50	40	27	7	3	3	3	10	7

Thirty females were tested per treatment. Asterisks indicate significant differences (Fisher's exact test) from hexane control: *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. All results without asterisks were not significant different.

Table 2 Behavioural response of **unmated female** *H. bajulus* to blank and solvent controls, stimuli: pheromone compound (3*R*)-3-hydroxy-2-hexanone, extracts of *P. sylvestris* wood and larval frass, synthetic monoterpenes and ethanol dissolved at the ratio of **1 : 1000** in hexane. Extracts were also tested at the concentration **1 : 10,000**

scent source	starting to move	arrivals (%) in/at						hovering	extension of ovipositor	courtship behaviour	flying
	(%)	zone I	zone II	zone III	zone IV	box wire screen	gauze box	(%)	(%)	(%)	(%)
controls:											
blank	67	63	60	53	27	10	0	0	0	0	0
hexane	73	73	67	60	40	20	3	0	3	0	3
(3*R*)-3-hydroxy-2-hexanone	100**	97*	90*	87*	67*	23	17	17*	30**	27**	20*
hexane extract of wood	100**	100**	97**	93**	90***	60**	50***	20*	47***	37***	30**
hexane extract of larval frass	100**	100**	100***	97***	87***	70***	50***	17*	57***	53***	27*
hexane extract of wood (**1 : 10,000**)	93*	80	67	53	47	23	10	10	27*	20*	10
hexane extract of larval frass (**1 : 10,000**)	97*	83	70	60	53	27	13	13	33**	23**	17
monoterpenoid hydrocarbons:											
(+)-α-pinene	97*	87	73	63	47	27	13	7	23*	17*	7
(+)-β-pinene	93*	87	77	67	53	20	17	10	7	17*	3
(+)-limonene	87	77	67	53	37	17	13	7	10	13	10
mono-oxygenated monoterpenes:											
(−)-verbenone	97*	87	73	63	47	30	17	7	17	20*	10
(−)-*trans*-pinocarveol	93*	83	73	57	43	27	7	7	13	17*	10
(+)-terpinen-4-ol	90	87	77	63	57	33	17	10	17	20*	13
(+)-α-terpineol	93*	83	73	60	50	27	13	10	7	17*	3
(−)-myrtenol	100**	100**	97**	93**	73**	53**	27*	23**	37**	13	27*
ethanol	83	73	57	50	37	27	17	13	13	17*	17

Thirty females were tested per treatment. Asterisks indicate significant differences (Fisher's exact test) from hexane control: $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$. All results without asterisks were not significant different.

(b) attraction
  - *arrival in zones I–IV* (in the wind tunnel upwind zone I is furthest from the scent source; zone IV is closest)
  - *arrival at scent source (*either the box wire screen or the gauze box containing the dispenser)
  - *hovering* (beetle remains close to the scent source, spins around its own axis or moves away and returns in zigzag path.
  - *extension of ovipositor* (female partially extrudes its ovipositor sweeping over the Plexiglas surface or wire screens of the wind tunnel)

(c) increase of interest in conspecifics
  - *fighting* (antennal fencing, butting or biting by males sometimes resulting in the loss of antennal or leg segments)
  - *courtship behaviour* (one test beetle chases the other and licks the prothoracic dorsum and elytra with the maxillary and labial palpi)
  - *homosexual mounting* (one test female succeeds in mounting the other after performing courtship behaviour, aligns her body axis in the same direction as the mounted individual, which in turn starts walking without parting)

The results of the running time were plotted (box and whisker plots). For descriptive statistics, the number of responding beetles (percentages in Tables 1–4) were analysed for significant differences between hexane control and test stimuli (pheromone compound, extracts and synthetic monoterpenes) or blank capillaries using Fisher's exact test, $\alpha = 0.05$. We assumed the control as independent and did not apply Bonferroni correction in our tests, since the two hexane controls carried out for the different concentrations of the test stimuli yielded nearly identical results.

Results

Response of unmated females

In control bioassays with hexane or without solvent, 63–70% of the test females started to move, only 20–37% reached zone IV and no female hovered or showed any interest in conspecifics (Table 1, 2). The medians of running time did not exceed 50% of the experiments' duration (Fig. 1A, B). Exposed to the high dose of the pheromone compound (3*R*)-3-hydroxy-2-hexanone (1:100), test females ran almost the entire experimental duration (Fig. 1A: 863 s median running time), 83% reached zone IV and many reacted with searching behaviour and increased interest in conspecifics (Table 1), including homosexual mounting (53%),

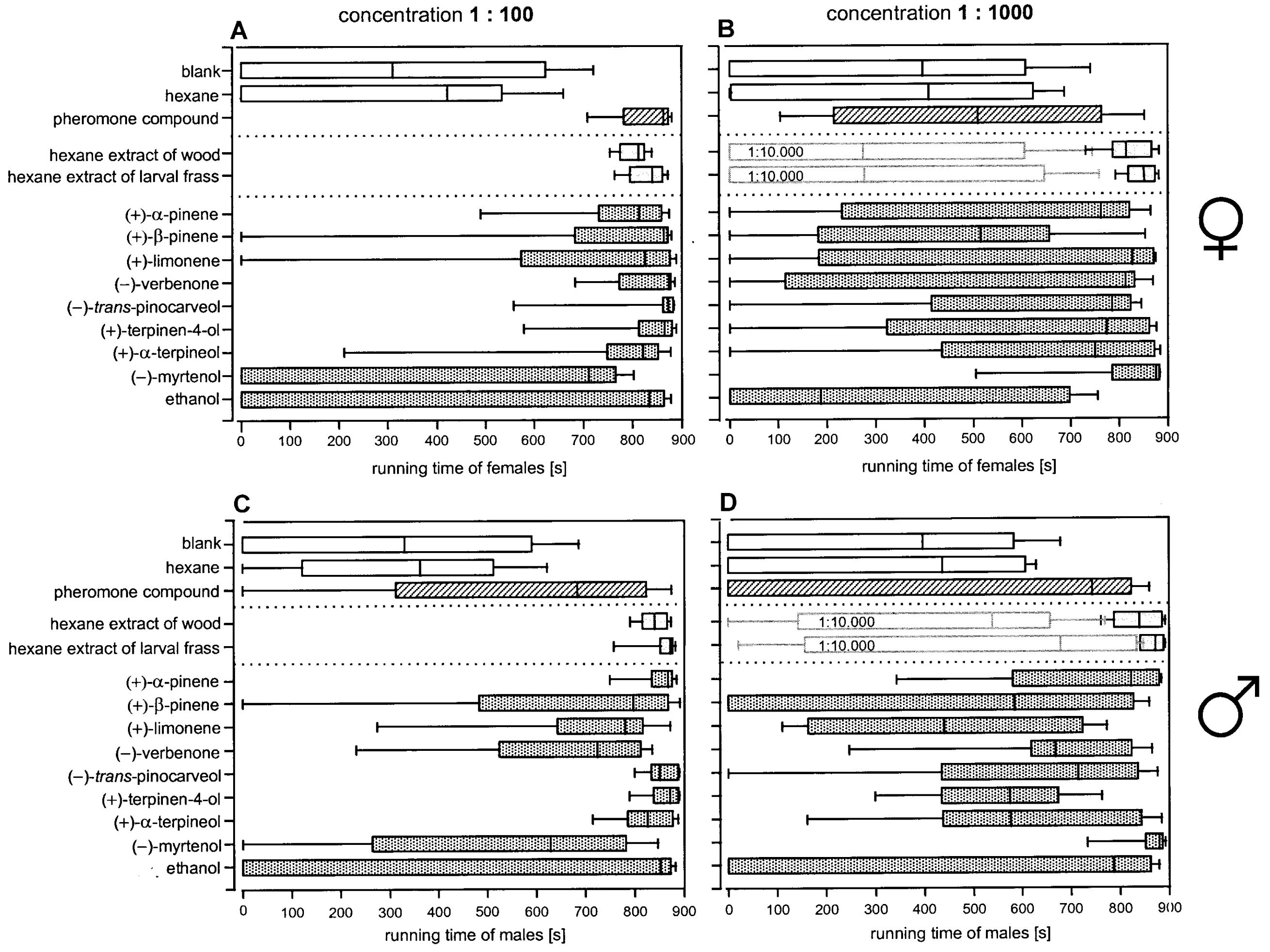

Fig. 1 Median (vertical lines within boxes), 25th and 75th percentile (boxes) and 10th and 90th percentile (bars) of the running time of **unmated females (A,B) and males (C,D)** of *H. bajulus* (N = 30) in the wind tunnel during an experimental duration of 900 s. All stimuli including the pheromone compound (3*R*)-3-hydroxy-2-hexanone, extracts of *P. sylvestris* wood and larval frass, synthetic monoterpenes and ethanol were dissolved in hexane and offered in concentrations (vol/vol) 1:100 **(A,C)** and 1:1000 **(B,D)**. Extracts were additionally tested at a dilution 1:10,000 (**B,D**: grey-lined box and whisker plots)

Table 3 Behavioural response of **unmated male** *H. bajulus* to blank and solvent controls, stimuli: pheromone compound (3*R*)-3-hydroxy-2-hexanone, extracts of *P. sylvestris* wood and larval frass, synthetic monoterpenes and ethanol dissolved at the ratio of **1 : 100** in hexane

scent source	starting to move	arrivals (%) in/at						hovering	fighting	courtship behaviour	flying
	(%)	zone I	zone II	zone III	zone IV	box wire screen	gauze box	(%)	(%)	(%)	(%)
controls:											
blank	83	73	67	57	10	3	0	0	0	0	0
hexane	87	77	67	53	17	7	3	0	0	0	0
(3*R*)-3-hydroxy-2-hexanone	93	87	73	53	37	13	7	0	3	0	3
hexane extract of wood	100	100**	100***	100***	87***	67***	67***	30***	37***	23**	27**
hexane extract of larval frass	100	100**	100***	97***	90***	77***	73***	33***	43***	23**	30***
monoterpenoid hydrocarbons:											
(+)-α-pinene	100	100**	100***	97***	77***	57***	53***	17*	23**	13	17*
(+)-β-pinene	90	87	63	47	27	10	7	0	3	0	3
(+)-limonene	97	97*	83	67	47*	27*	23*	7	7	3	3
mono-oxygenated monoterpenes:											
(−)-verbenone	93	93	77	57	37	23	13	3	3	0	3
(−)-*trans*-pinocarveol	100	100**	97**	77	57**	37**	30**	7	7	7	3
(+)-terpinen-4-ol	100	100**	100***	100***	83***	70***	67***	27**	27**	20*	20*
(+)-α-terpineol	97	97*	87	63	43*	37**	33**	7	13	7	10
(−)-myrtenol	97	80	57	37	13	3	0	0	3	0	0
ethanol	77	73	67	63	27	7	0	0	3	0	0

Thirty males were tested per treatment. Asterisks indicate significant differences (Fisher's exact test) from hexane control: *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. All results without asterisks were not significant different.

Table 4 Behavioural response of **unmated male** *H. bajulus* to blank and solvent controls, stimuli: pheromone compound (3*R*)-3-hydroxy-2-hexanone, extracts of *P. sylvestris* wood and larval frass, synthetic monoterpenes and ethanol dissolved at the ratio of **1 : 1000** in hexane. Extracts were also tested at the concentration **1 : 10,000**

scent source	starting to move	arrivals (%) in/at						hovering	fighting	courtship behaviour	flying
	(%)	zone I	zone II	zone III	zone IV	box wire screen	gauze box	(%)	(%)	(%)	(%)
controls:											
blank	87	77	57	50	20	7	0	0	3	0	0
hexane	87	80	70	63	43	23	0	0	3	0	0
(3*R*)-3-hydroxy-2-hexanone	90	83	67	47	33	13	3	0	3	0	0
hexane extract of wood	100	100**	100***	97**	83**	70***	67***	27**	33**	23**	23**
hexane extract of larval frass	100	100**	100***	100***	87***	77***	73***	27**	37**	23**	33***
hexane extract of wood (**1 : 10,000**)	100	93	77	63	53	33	20*	0	3	3	0
hexane extract of larval frass (**1 : 10,000**)	100	93	77	63	43	27	17*	7	7	0	3
monoterpenoid hydrocarbons:											
(+)-α-pinene	100	100**	83	63	43	33	23**	3	7	3	7
(+)-β-pinene	83	73	53	40	13	17	3	0	0	0	3
(+)-limonene	93	93	67	53	47	27	17*	3	7	3	3
mono-oxygenated monoterpenes:											
(−)-verbenone	97	97	83	73	43	33	17*	10	13	7	10
(−)-*trans*-pinocarveol	93	87	57	43	30	20	17*	7	10	3	7
(+)-terpinen-4-ol	100	100**	100***	80	67	43	27**	7	3	3	0
(+)-α-terpineol	97	90	53	33	20	17	13	3	3	3	0
(−)-myrtenol	100	100**	100***	87*	77**	57**	33***	20*	30**	13	23**
ethanol	73	63	57	53	23	7	3	0	3	0	0

Thirty males were tested per treatment. Asterisks indicate significant differences (Fisher's exact test) from hexane control: *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. All results without asterisks were not significant different.

which was only elicited when the pheromone component was introduced as scent source.

In the presence of highly concentrated hexane extracts of wood and larval frass virgin females showed similar behavioural modifications as induced by the sex pheromone compound (Table 1, Fig. 1A). In high doses, (+)-α-pinene was the most potent monoterpenoid hydrocarbon significantly eliciting activation, attraction and courtship behaviour. Females were also activated by (+)-β-pinene and (+)-limonene, but did not orient towards the scent source. Among the mono-oxygenated compounds, (−)-verbenone attracted most females and also induced ovipositor extension and display of courtship behaviour (Table 1). (−)-*Trans*-pinocarveol, (+)-terpinen-4-ol and (+)-α-terpineol were almost as effective as verbenone. The behaviour of test females was not modified by highly concentrated ethanol or (−)-myrtenol.

Lowering the concentration of hexane extracts of wood or larval frass (1:1000) did not affect the beetles' response (Table 2, Fig. 1B). In contrast, only (−)-myrtenol was capable to induce behaviour at this dose whereas the other single substances lost their activating and attracting effect. Only a slight increase in courtship behaviour was noticed. At the ratio of 1:1000, (−)-myrtenol turned out to be the most potent activating agent and attractant among the synthetic stimuli. At a dilution of 1:10,000, the activating and attracting effects of wood and frass extracts were lost, as were hovering and flying (Fig. 1B, Table 2).

Response of unmated males

In the control assays without host or larval frass volatiles the test males behaved in the same way as virgin females, but unlike the females, they did not respond to the sex pheromone (3*R*)-3-hydroxy-2-hexanone (Fig. 1C, D; Table 3, 4).

At a dose of 1:100, hexane extracts of wood and larval frass elicited strong behavioural responses in terms of activation, directed orientation, courtship behaviour and fighting (Fig. 1C, Table 3). As for females, (+)-α-pinene at the high dose was the most effective behavioural elicitor among the monoterpenoid hydrocarbons, while (+)-β-pinene and (+)-limonene did not attract males nor did they evoke fighting or courtship behaviour. Males were unresponsive to (−)-verbenone, which appears to be a female-specific mediator. (+)-Terpinen-4-ol was the strongest agent among the oxygenated monoterpenes, followed by (−)-*trans*-pinocarveol and (+)-α-terpineol. Ethanol and (−)-myrtenol were inactive.

As for females, males responded to the two higher doses of hexane extracts of wood and larval frass, but not to the lowest (1:10,000) dose (Fig. 1 C, D; Table 3, 4). Again as for females, synthetic compounds diluted 1:1000 already lost behavioural activity, but (−)-myrtenol elicited a high level of activity (Fig. 1D, Table 4).

Discussion

Mares *et al.* (1986) proposed that *H. bajulus* is guided in host selection by light and odours of bicyclic monoterpenes ($C_{10}H_{16}$). In our wind tunnel experiments however, activation elicited by most terpenoid stimuli was not necessarily followed by attraction or further behavioural responses. Only certain dosages of natural blends of pine wood and larval frass as well as α-pinene and several mono-oxygenated monoterpenes induced orientation of unmated beetles towards scent sources. Some stimuli triggered an intense searching by females with the ovipositor extended, possibly in preparation for copulation, because the cascade of responses was often completed by courtship behaviour as third behavioural modification. These responses were also observed in experiments with unmated females exposed to male-produced sex pheromones (Fettköther *et al.* 1995), but more females extended the ovipositor in response to (3*R*)-3-hydroxy-2-hexanone in the present control series. These females were tested during a later period of their life and may have had a higher ovipositing pressure than those tested by Fettköther *et al.* (1995). Female mounting behaviour, as seen in this study, may reflect an adaptive mechanism displayed when mate finding is controlled by a male sex pheromone, e. g. in the mating system of the cerambycid *Xylotrechus pyrrhoderus* Bates (Iwabuchi 1987). Female mounting of inactive *X. pyrrhoderus* males resulted in successful copulation. Therefore, this mechanism may have its biological significance in maximizing reproductive success. The lack of attraction of unmated *H. bajulus* males to their own pheromone compound supports its characterization as a sex pheromone.

The increased interest of males and females in their same-sex test partners in the presence of host- and larvae-produced volatiles suggests that mating in nature occurs readily in an atmosphere enriched with such odours. The stronger response by females and males to hexane extracts of pine wood and larval frass than to the synthetic components tested separately suggests either that the volatile blends contain bioactive compounds that were not among those tested separately, or that synergism of compounds occurs in the volatile blends.

α-Pinene is known to be one of the dominant monoterpenes in Scots pine and other conifers. In addition, large quantities of α-pinene vaporize during induced defence from resin exuding from freshly sawn logs of conifers (Ikeda *et al.* 1980, Strömvall & Petersson 1991). Our extraction of seasoned Scots pine wood also revealed α-pinene and additionally 3-carene as main residual monoterpenes. Therefore, it is not surprising that among the terpenoid hydrocarbons tested in our study (+)-α-pinene was the most potent agent for unmated females and males. Becker (1944) found that among 36 terpenes, α-pinene was the strongest attractant and oviposition stimulus for mated *female H. bajulus*. β-Pinene also attracted ovipositing females, whereas other bicyclic monoterpenes like carene and sabinene were less effective. The (+)-enantiomer of β-pinene, which we tested, did not elicit attraction of unmated beetles. However, we did not examine chiral specificity of *H. bajulus* to monoterpenes. Such specificity of chemoreceptors to host odours or kairomones is not generally known among insect herbivores, though many host monoterpenes are optically active (Hobson *et al.* 1993). These authors could show the importance of chirality of host monoterpenes in the host finding of the bark beetle *Dendroctonus valens* LeConte. A differentiation of chiral host terpenes is questionable for a conifer generalist like the old house borer, that is able to infest sapwood of most coniferous species which produce different combinations of monoterpenes in different enantiomeric ratios. In addition, large variations in the relative amounts of the monoterpene hydrocarbons and their enantiomeric composition were found between the different varieties of conifers as well as between and within individual trees (Borg-Karlson *et al.* 1993, Persson *et al.* 1993, 1996, Sjödin *et al.* 1996, Ochocka *et al.* 1997, Sadof & Grant 1997).

Besides the monoterpenoid hydrocarbons, the fermentation product ethanol is utilized by many wood-boring species searching for suitable host material. Several cerambycids, especially saprophagous species, were attracted to ethanol alone or in combination with monoterpenes (Ikeda *et al.* 1980, Montgomery & Wargo 1983, Fatzinger 1985, Dunn *et al.* 1986, Atkinson *et al.* 1988, Phillips *et al.* 1988, Chénier & Philogène 1989, Dunn & Potter 1991, Schroeder & Weslien 1994). The lack of response by *H. bajulus* beetles to ethanol in our wind tunnel study is consistent with its preference for dry and seasoned host material.

Evans & Higgs (1975) identified eight monoterpenes in the frass pellets of tunnelling *H. bajulus* larvae, and Higgs & Evans (1978) reported that oviposition by mated females is mediated mainly by (−)-verbenone and *p*-cymene-8-ol. This finding is verified by our results, in which verbenone was the main monoterpenoid compound in larval frass from Scots pine wood and *H. bajulus* females showed a strong behavioural response to synthetic (−)-verbenone.

Additionally, Higgs and Evans (1978) found that myrtenol in combination with the two terpenes mentioned before enhanced oviposition response by mated females but that it was ineffective when tested alone. However, we could show that the response of the old house borer depended upon the dosage of myrtenol tested in the wind tunnel. Although the wind tunnel design is not optimal to observe repellent effects, a number of beetles tested against the high dose of (−)-myrtenol entered zone II-IV moving toward the scent source, stopped and returned to the starting zone I for the rest of the observation time. We hypothesize that a high amount of larval frass pheromones, especially of minor products like myrtenol, may signal an overpopulated infestation, hence the repellency of (−)-myrtenol at a high dose, and an attraction and stimulatory effect at a ten-fold lower dose.

A dose-dependent response of *H. bajulus* to monoterpenes was already evident in earlier experiments studying the oviposition preference of mated females. Becker (1944) used high doses of oxygenated monoterpenes and found that they were either inactive or repelling. Higgs & Evans (1978) applied almost the same bioassay as Becker (1944), but found that oviposition by females was mediated by (−)-verbenone and *p*-cymene-8-ol at lower doses than Becker (1944) had used. As highest dosage, we offered a release rate of about 5 µl/day of essential oils or single compounds because we intended to mimic evaporation rates that would occur in nature. For example, monoterpenes (72–86% α-pinene) vaporized at 9.5 and 13.7 µl/kg per day on the first day after cutting of Japanese red pine, *Pinus densiflora* Sieb. et Zucc., but decreased to 5–10% of that rate within two weeks (Ikeda *et al.* 1980). The old house borer prefers to attack dead and seasoned wood of conifers, and we assume that the beetles are attracted to lower doses of monoterpenes than species attracted to stressed or decaying wood.

Our results suggest that primary attraction of unmated *H. bajulus* to its coniferous hosts is mediated mainly by certain monoterpenoid hydrocarbons and that secondary attraction to infested hosts is mediated mainly by chemicals excreted with larval frass pellets. The old house borer is adapted to reinfest previously damaged wood, making use of breeding material for several generations. If there is a small amount of frass in infested wood and therefore a low concentration of larval frass pheromones, *H. bajulus* females are stimulated to lay eggs on that wood (Mares & Robinson 1985). Obviously, this pest species shows a very sedentary behaviour using already successful breeding sites. However, pioneering *H. bajulus* males are responsible for the colonization of new and uninfested host locations in order to avoid densely populated habitats which are characterized by high amounts of larval frass pheromones. Therefore, males should respond in nature more sensitively to monoterpenoid hydrocarbons, indicating undamaged host material, than to larvae-produced pheromones like verbenone. The male-released sex pheromone might be used to attract females to new hosts.

In general, the natural system and response mechanisms have to be fully understood if the practical use of semiochemicals in trap lures is scrutinized (Borden 1985). The results found in our wind tunnel study are only a first step to understand the role of semiochemicals in the chemical ecology of *H. bajulus*.

Combinations of host- and larvae-produced monoterpenes might be useful as a bait for trapping *H. bajulus*. Becker (1944) concluded that lures containing monoterpenes would not be effective for capturing *H. bajulus* in its natural habitat. As a main argument for his prediction he referred to his observation that the mated females already laid several egg patches during their orientation towards the terpene source. However, the effective monoterpenes found in our study might have an additive or synergistic influence on the attraction of unmated *H. bajulus* females if they are combined with the male-produced sex pheromone components. We are currently investigating this hypothesis.

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