0014-4754/95/030270-08$1.50 + 0.20

The male pheromone of the old house borer *Hylotrupes bajulus* (L.) (Coleoptera: Cerambycidae): identification and female response

R. Fettköther, K. Dettner*, F. Schröder[a], H. Meyer[a], W. Francke[a] and U. Noldt[b]

Lehrstuhl für Tierökologie II, Universität Bayreuth, Postfach 101251, NWI, D-95440 Bayreuth, [a]Institut für Organische Chemie und Biochemie der Universität Hamburg, D-20146 Hamburg, and [b]Bundesforschungsanstalt für Forst- und Holzwirtschaft, Institut für Holzbiologie und Holzschutz, D-20127 Hamburg (Germany)
Received 26 July 1994; accepted 7 October 1994

Abstract. We report here the identification of the long-range, male-produced sex pheromone of the Old house borer *Hylotrupes bajulus*. Chemical analysis of hexane extracts obtained by surface extraction from dissected prothoracic glands and from headspace samples of the two sexes, revealed male-specific compounds: (3*R*)-3-hydroxy-2-hexanone, 2-hydroxy-3-hexanone, the diastereomeric diols (2*R*, 3*R*)-2,3-hexanediol and (2*S*, 3*R*)-2,3-hexanediol, 2,3-hexanedione, as well as 1-butanol.

In wind tunnel bioassays we tested the influence of these male-specific compounds from the prothoracal glands on the behaviour of unmated and mated females. Specific behavioural sequences of the tested females (activity, running behaviour, searching, cleaning, flying, extension of ovipositor) were recorded. Unmated females were attracted by male beetles, headspace extracts of males, synthetic blends of the major pheromone compounds as well as by the components (3*R*)-3-hydroxy-2-hexanone, and the diastereomeric diols. Hexane, female beetles and 2,3-hexanedione did not attract unmated females. The reactions of mated females to male beetles and headspace samples did not differ significantly from those of the controls.

The results of the bioassays show that the two-stage premating behaviour is initiated by emission of a long-range sex pheromone from the male prothoracal glands, which functions as an activator, attractant, and possibly aphrodisiac for unmated females.

Key words. *Hylotrupes bajulus*; Coleoptera; Cerambycidae; male sex pheromone; (3*R*)-3-hydroxy-3-hexanone; wind tunnel; female response; premating behaviour.

The woodboring larvae of the longhorn beetle *Hylotrupes bajulus* (L.) (old house borer, House Longhorn beetle) are a common pest of coniferous woods in Central Europe and North America. This beetle has been spread worldwide by import of infested woods[1]. It is able to infest and to damage most of the common coniferous timbers used in buildings, irrespective of age or nutritional content of the wood.

In order to develop biological control methods it would be useful to know more about the chemical mediators of mating and oviposition behaviour of *H. bajulus*. Female *H. bajulus* are known to prefer oviposition sites which are infested by conspecifics[2]. Moreover, the extracted monoterpenes from the larval frass stimulate oviposition.

There are several reports indicating that sex pheromones might be involved in the mating behaviour of cerambycids: male volatiles may attract females from a distance, and female contact pheromones are thought to stimulate mating behaviour in various species[3–6]. As far as we know, the only reported chemical structure for a male sex pheromone is from *Xylotrechus* species[7,8].

Previous studies on its biology[9,10], aggression, stridulation behaviour[11], and copulatory behaviour[12] in *H. bajulus* suggest there is no long-range female sex pheromone. In another study the existence of a close-range female sex pheromone which elicits the orientation of males was assumed[13], however, these results could not be confirmed[14] (Noldt, pers. commun.). On the other hand, reports on the ecology of the old house borer cannot absolutely exclude the existence of male-produced pheromones[9,11].

Evidence exists indicating that females are attracted to males by chemical mediators. Exocrine glands were found in the macerated prothoraces of male *H. bajulus* (fig. 1)[15]. Numerous glandular units form an internal glandular matrix in the entire prothorax. Each glandular unit is supplied with a cuticular receiving and conducting canal. Clusters of glandular units are arranged below externally visible, shallow pore pits with their openings concentrated in pore fields (fig. 1B).

In the present paper we provide evidence for the existence of a male sex pheromone in *H. bajulus* and report on the identification of its chemical structure. We also present the first results of wind tunnel bioassays.

* To whom correspondence should be addressed.

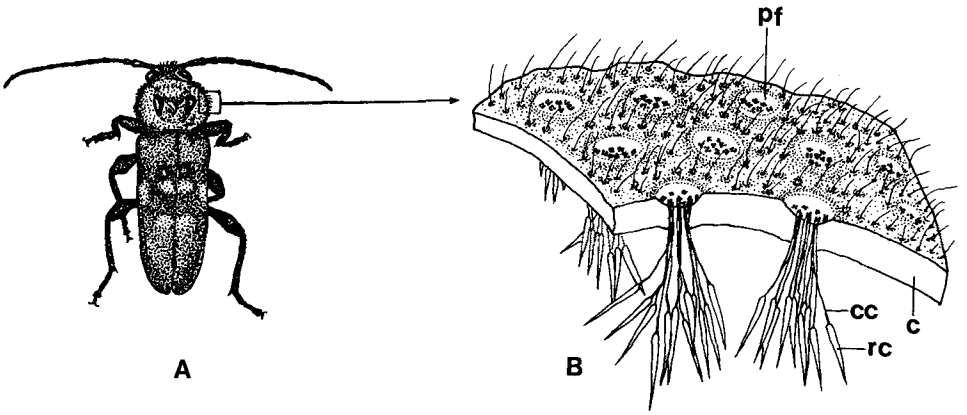

Figure 1. *A* Dorsal view of the male longhorn beetle *Hylotrupes bajulus* and *B* magnification of a KOH-macerated piece of its pronotal integument.
c: cuticle, cc: conducting canal of a gland cell unit; pf: pore field; rc: receiving canal of a gland cell.

Materials and methods

Insects. Experimental insects were reared in a laboratory colony of the old house borer at the Institute for Wood Biology and Wood Protection, Hamburg (Germany)[15]. Prior to their use in wind tunnel bioassays, newly emerged beetles were stored, separately, for 8 days in plastic boxes lined with moistened filter paper. Food was not provided, since adult beetles do not feed under natural conditions[9]. Males and females were maintained in separate chambers (20 °C and 12:12 L:D photoperiod). Over a period of 7 days beetles of the same age were used in the various test series of wind tunnel bioassays. Thus, the age of the beetles used in tests was 9–15 days after emergence from the rearing wood blocks. Females of *H. bajulus* have been observed to copulate from the 3rd to the 19th day after emergence[9].

After completion of wind tunnel tests, unmated females were allowed to copulate once and were then separated. Mated females were tested in the wind tunnel 24 h after copulation.

Chemical investigations: A. Analysis. Samples were obtained by surface washings of whole males or females, by solvent extraction of dissected male prothoraces and by headspace sampling using closed loop stripping techniques (CLSA)[16]. Pentane or methanol (Merck Uvasol) were used as solvents. Crude extracts were concentrated in microvials[17] and directly submitted to gas chromatography (GC) or to a combination of gas chromatography and mass spectrometry (GC/MS). Volatile compounds were separated using free fatty acid phase (FFAP) (50 m, 0.25 mm i. d. fused silica column, purchased from Macherey & Nagel (Düren, Germany) as a stationary phase, programmed from 60 to 220 °C at a rate of 2 °C/min. Mass spectrometric investigations were performed on a double focusing VG 70-250SE instrument using EI (70 eV)-mode, CI with ammonia as reactant gas, and high resolution GC/MS (HR-GC/MS).

Gas chromatographic separation of enantiomers was carried out on a chiral stationary phase: a 30 m, 0.25 mm i. d. fused silica column coated with heptakis-(2,6-di-O-methyl-3-O-pentyl)-β-cyclodextrin was used at 70 °C, hydrogen was used as the carrier gas.

For CLSA-extracts used in the wind tunnel bioassays, the pheromone emitted by 8 unmated male beetles was collected by CLSA for 24 h on a 1.5 mg charcoal filter under constant fluorescent light. The filter was extracted with 15 µl hexane[18]. To check for the presence of the target compounds, the extracts were examined by GC/MS-analysis (Finnigan Iontrap ITD 800).

B. Synthesis. Synthesis of racemic reference compounds followed figure 2. In a Corey-Seebach sequence, n-butanal (**5**) was coupled to 2-methyl-1,3-dithiane (**6**) to yield 1-(2-methyl-1,3-dithian-2-yl)butan-1-ol (**7**)[19] which was transformed to 3-hydroxy-2-hexanone (**1**)[20]. A complementary reaction using 2-propyl-1,3-dithiane (**8**) and acetaldehyde (**9**) produced 1-(2-propyl-1,3-dithan-2-yl)ethan-1-ol (**10**), which was transformed to 2-hydroxy-3-hexanone (**2**). Reduction of (**2**) yielded a 1:1 mixture of the diols (**3**) while oxidation produced the diketone (**4**)[21]. Reactions were monitored by thin layer chromatography; final products were purified by distillation and column chromatography (silica gel Merck 60; hexane/ethyl acetate). The synthesis of optically active compounds will be published separately[22].

Wind tunnel experiments. Wind tunnel experiments were conducted in an environmental chamber at a temperature of 30 °C and relative humidity of 20–30%, illuminated by 8 neon tubes directly above the setup (8000 lux inside the wind tunnel). The wind tunnel which was placed on a metal guide, was made of transparent acryl plexiglass tube (length 100 cm, internal diameter 19 cm) and was sectioned into 4 marked zones (I–IV) each 25 cm long (fig. 3). The tube openings were closed by a start wire screen and a box wire screen. Beetles were released at the start wire screen in zone I (starting zone), while the test stimuli were introduced in

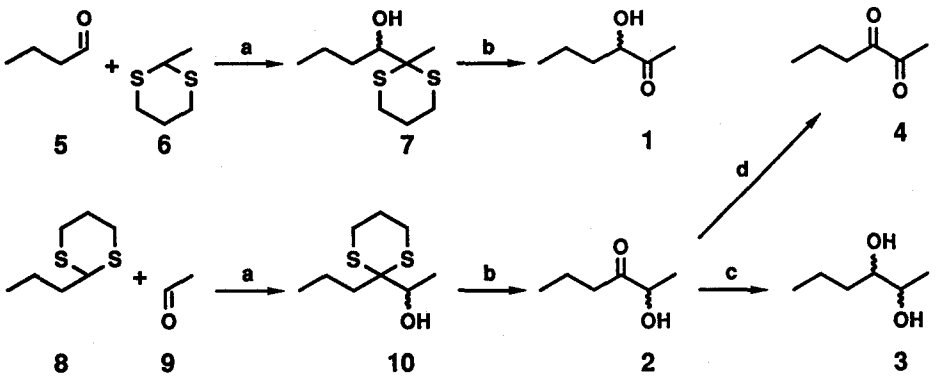

Figure 2. Syntheses of compounds (**1**)–(**4**).

a gauze box (a 14 cm petri dish with gauzes in the bottom and in the lid) attached to the box wire screen in zone IV (source zone). A ventilator was installed behind the box wire screen, with several tightened gauze layers over its downwind opening, in order to produce a uniform and reduced laminar airflow across the tunnel zones. Contaminated air from the tunnel was removed by a downwind exhaust system. The following wind velocities were measured in the tunnel: 0.65 m/s at the start wire screen, 0.71 m/s between zone II and III, and 2.3 m/s in front of the box wire screen beside the gauze box containing the stimulus source. Due to the 2 extra gauze layers, the wind speed in front of the gauze box was decreased to 0.02 m/s. Living beetles or capillaries filled with hexane solutions (Merck Uvasol) of the males' CLSA filtrate or of synthetic compounds, were placed in the gauze box. To test the attractiveness of live insects, 4 unmated females or 8 unmated males were placed in the petri dish which was divided into separate compartments by pieces of cardboard. The beetles were conditioned to the climatic chamber 2 hours before the tests were run. Stimuli, as synthetics, single compounds or blends, were tested in hexane solutions of 5 µl. For testing single compounds 50 µg of (3*R*)-3-hydroxy-2-hexanone (**1**) (enantiomeric excess [ee] > 99%), of a mixture of the diastereomeric diols (**3**) ((2*R*, 3*R*)-2,3-hexanediol of 31% ee and (2*S*, 3*R*)-2,3-hexanediol of 34% ee at a ratio of 1:3) or of 2,3-hexanedione (**4**) dissolved in 5 µl hexane was applied. A synthetic blend was prepared by adding 50 mg of (3*R*)-3-hydroxy-2-hexanone (**1**), 15 mg of the mixture of the diastereomeric diols (**3**) and 1.5 mg of 2,3-hexandione (**4**) to 5 ml of hexane. The filled 5 µl disposable-micro-pipettes (Blaubrand, intraMARK, Cat. No. 708707) were positioned horizontally in the gauze box. In addition, the synthetic blend was tested at a higher dosage. In these case 50 µl of the synthetic blend was administered in two 100 µl micro-pipettes (Cat. No. 708745)

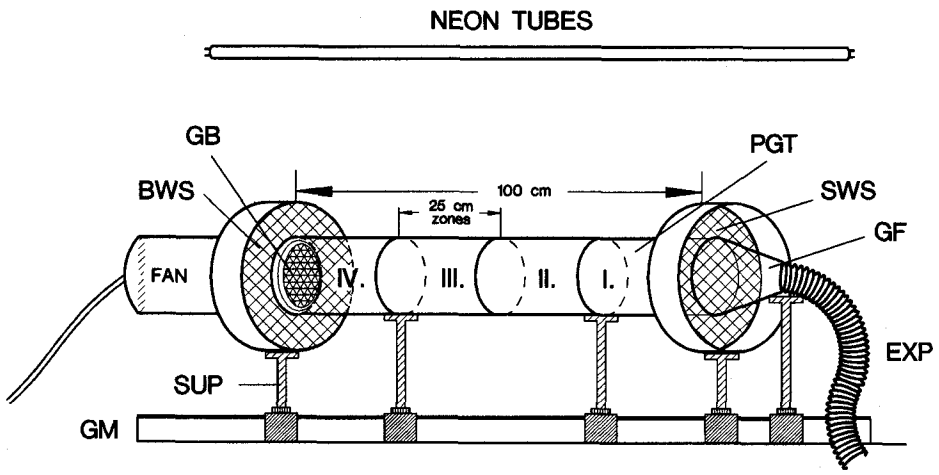

Figure 3. The wind tunnel bioassay design. BWS: box wire screen; EXP: exhaust pipe; GB: gauze box; GF: glass funnel; GM: guide metal; PGT: plexiglass tube; SUP: support metal; SWS: start wire screen; I–IV: zones I–IV.

(each filled with 25 µl solution). The capillary sizes used guaranteed complete vaporization of the solvent in the 15 min of experimental time.

Bioassay procedure. Bioassays were conducted during the daytime from 12.00–17.00. All test insects were conditioned in the test chamber 2 hours prior to tests. In every test run, 2 females were released on the start wire screen and observed for 15 min. The following behavioural responses were recorded: females starting to move; females entering zones I to IV; searching behaviour (i.e., moving in circles and specific antennal movements); stopping (i.e., at the gauze box); cleaning behaviour; flying activity; extension of the ovipositor. The resting time in zone IV (including the box screen wire) and the total time of running activity were also recorded. These positive responses are summarized as activation and attraction (see 'Discussion'). Activation includes percentage of starting females, percentage of arrivals in zone I, and the total time occupied by running activity. Attraction includes percentage of individuals reaching zones II to IV, percentage of females searching and/or stopping, and time spent in zone IV. The number of responding females (percentages in tables 1 and 2) was statistically analyzed using the X^2 test. Average times measured were analyzed using a Kruskal Wallis ANOVA. If significant differences were found, a Mann-Whitney U-test was used to indicate where these differences occurred (table 3).

Results

Chemistry. Gas chromatographic comparison of body washings of the two sexes revealed a number of male-specific compounds, found in the prothorax fraction. The males released the compounds in relatively high concentrations as could be shown by headspace analysis. A gas chromatogram of the methanol extract of a CLSA carbon filter is depicted in figure 4. The mass spectra of two early eluting minor components ($M = 114 = C_6H_{10}O_2$ and $M = 74 = C_4H_{10}O$ established by CI and HR-GC/MS) suggested 2,3-hexanedione (**4**) and 1-butanol (**11**)[23]. This was confirmed by comparison with authentic reference compounds. Of two later eluting compounds of medium volatility ($M = 116 = C_6H_{12}O_2$), the major component exhibited a mass spectrum (fig. 5) which showed some similarities to the data published on 3-hydroxy-2-octanone[7], suggesting this compound to be 3-hydroxy-2-hexanone (**1**). The mass spectrum of the earlier eluting minor component suggested it was the complementary 2-hydroxy-3-hexanone (**2**). The structures of the natural compounds could be confirmed by comparison of gas chromatographic and spectroscopic properties with those of synthetic reference compounds (fig. 2). Two late eluting compounds ($M = 118 = C_6H_{14}O_2$) proved to be the diastereomers of 2,3-hexanediol (**3**).

In a detailed study of the enantiomeric composition of the chiral hydroxyketones and the corresponding diols we used chiral gas chromatography. By means of optically pure synthetic reference compounds we found that 2-hydroxy-3-hexanone (**2**) is almost racemic, while 3-hydroxy-2-hexanone (**1**) showed a (*R*)-configuration and extremely high optical purity (enantiomeric excess > 99.5%). The *erythro* and *threo* isomers of the diols (**3**) consist of enantiomeric mixtures, the-diastereomers with (3*R*)-configuration forming the

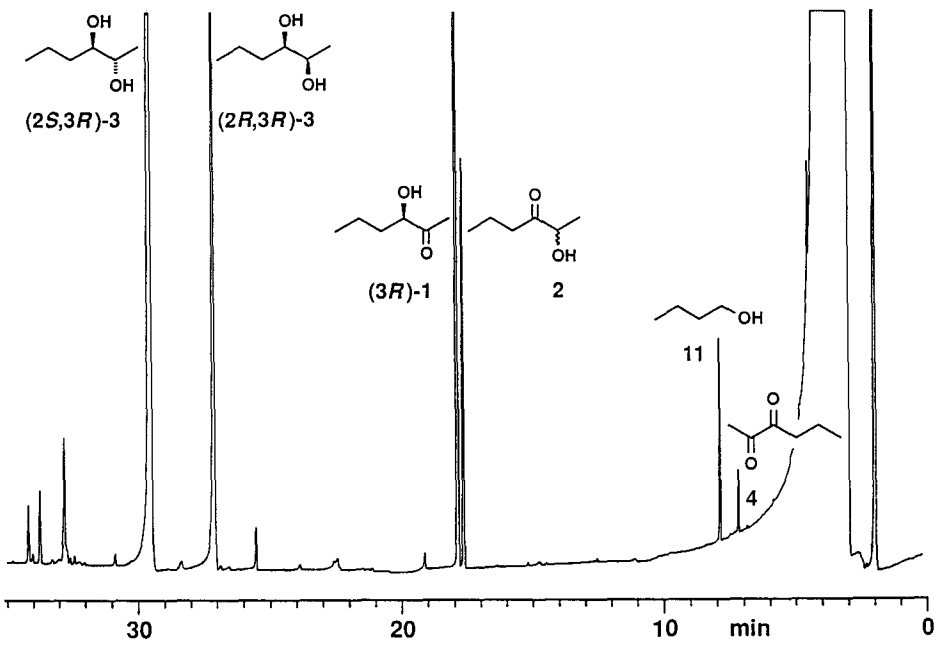

Figure 4. Gas chromatogram of volatile compounds released by males of *Hylotrupes bajulus* 50 m fused silica-free fatty acid phase (FS-FFAP), 3 min at 60 °C, then with 3 °C/min to 220 °C.

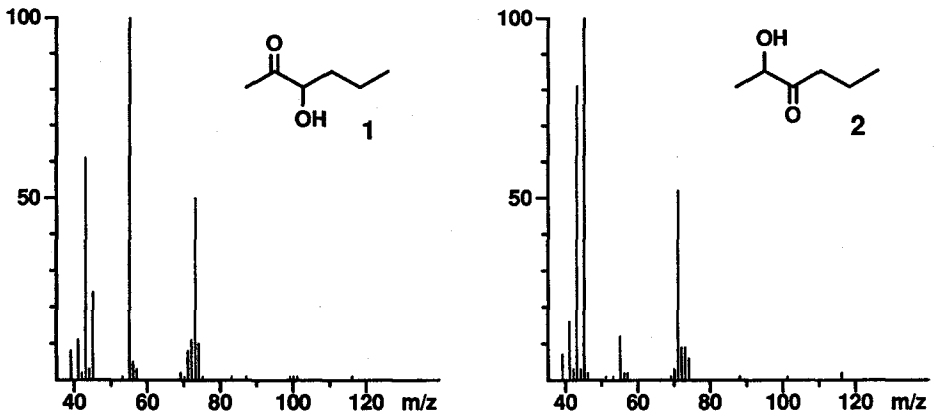

Figure 5. Mass spectra of 3-hydroxy-2-hexanone (**1**) and 2-hydroxy-3-hexanone (**2**).

Table 1. Responses of unmated females to controls, live females or live males, CLSA-male-extract, and synthetic pheromene components.

Scent sources	Tested unmated females (n)	Start (%)	Arrivals in: zone I (%)	zone II (%)	zone III (%)	zone IV (%)	Searching /stopping near scent source (%)	Cleaning (%)
Blank	30	70	67	60	50	37	0	40
Hexane	36	64	64	50	31	6	6	31
4 unmated females	30	97**	93**	80	53	40	10	20
8 unmated males	30	100**	100***	100***	97***	93***	93***	10**
CLSA-extract	27	100**	100***	100***	89**	85***	48***	7**
5 µl synth. blend	30	100**	100***	100***	100***	83***	46***	7**
50 µl synth. blend	32	100***	100***	100***	97***	78***	19*	3***
(3*R*)-3-hydroxy-2-hexanone	32	100***	100***	97***	91***	73*	43***	13*
Mixture of (2*S*, 3*R*)- and (2*R*, 3*R*)-2,3-hexanediol	32	97**	97**	91**	88**	66*	56***	9**
2,3-hexanedione	30	87	83*	83	63	33	7	13*

Asterisks indicate significant differences (χ^2-test) from the blank: *$p \leq 0.05$, **$p \leq 0.01$, ***$p < 0.001$.

Table 2. Responses of mated females to blank, live males, and synthetic pheromone blend.

Scent sources	Tested unmated females (n)	Start (%)	Arrivals in: zone I (%)	zone II (%)	zone III (%)	zone IV (%)	Searching /stopping near scent source (%)	Cleaning (%)
Blank	30	93	83	67	47	43	0	10
8 unmated males	31	97	61	58	48	43	6	0
5 µl synth. blend	30	100	73	63	60	53	3	7

Values are not significantly different (χ^2-test) from the blank.

major products, i.e. (2*R*, 3*R*)-2,3-hexanediol and (2*S*, 3*R*)-2,3-hexanediol showing an enantiomeric excess (ee) of 31% and 34%. Details on the synthesis of optically active reference samples will be published elsewhere[22].

Wind tunnel bioassays: A. Reaction of unmated females. Unmated females were strongly activated by and attracted to unmated males, the volatiles (CLSA-extract) released by living males, synthetic (3*R*)-3-hydroxy-2-hexanone (**1**) (ee > 99%), the synthetic mixture of the diastereomeric diols (**3**), and the synthetic blend of ketol (**1**), diols (**3**) and diketone (**4**) (table 1). Preliminary observations showed that unmated females are less active than males. This was confirmed by results of the controls (blank and hexane): 70% and 64% of the females, respectively, became activated during the experimental period of 15 min. The rest of the females showed cleaning behaviour or stayed motionless on the start wire screen. The percentage of females entering the four zones decreased with increasing distance from the start wire screen. The few entering zone IV did not

Table 3. Time spent in zone IV and time of activity (max. 900 s).

Scent source	Tested females	Unmated females		Mated females	
		Stay in zone IV	Total of running activity in zones I–IV	Stay in zone IV	Total of running activity in zones I–IV
	(n)	(s)	(s)	(s)	(s)
Blank	30	36	467	170	743
Hexane	36	17	413		
4 unmated females	30	46	583		
8 unmated males	30	448***	817***	140	796
CLSA-extract	27	164***	728**		
5 µl synth. blend	30	206***	767***	234	759
50 µl synth. blend	32	87***	767**		
(3*R*)-3-hydroxy-2-hexanone	32	167**	784***		
Mixture of (2*S*, 3*R*)- and (2*R*, 3*R*)-2,3-hexanediol	32	151**	698**		
2,3-hexanedione	30	36**	664*		

Responses of unmated females were significantly different from the blank (Kruskal Wallis ANOVA $p = 0.000$).
Asterisks indicate significant differences (Mann-Whitney U-test) from the blank: *$p \leq 0.05$, **$p \leq 0.001$, ***$p \leq 0.001$.

show searching behaviour, nor did they stop moving (table 1). Caged unmated females as scent source activated significantly more conspecific females than the blank as far as percentages of starting individuals and of those entering zone I are concerned (table 1). However, there were no significant differences in attraction into zones II to IV between the blank and those tests in which unmated females were used as test stimulus.

Significantly higher responses were registered when testing samples of natural or synthetic male volatiles, with all females activated and reaching zone II, and more than 80% entering zone IV. The highest incidence of searching and stopping in zone IV was evoked from unmated females when living males were used as the test stimulus. This response was significantly higher than with the respective CLSA-extract or synthetics. Similarly, females spent more time in zone IV with living males as the test stimulus (table 3). While 33% of the females arrived at the box containing the males, the collected volatiles (CLSA-extract) or synthetics lured a maximum 15% of the introduced females. No female beetles were attracted to the control boxes (blank, with hexane, or with unmated females). The CLSA-extract of living males was less attractive as determined by the arrival in zones III and IV, searching behaviour, and stopping close to the source, compared to tests with males (table 1). This may be due to the concentration of the male pheromone blend in the hexane filtrate. Interestingly, a high dose of the synthetic pheromone blend was less attractive than a low dose of the blend. When the high dose was used, females were obviously less effective in orienting towards the odour source. Activation and attraction of females by only the ketol (**1**) or the mixture of the diols (**3**) was almost as significant as activation and attraction by the low dose of the synthetic blend, whereas 2,3-hexanedione (**4**) did not induce these behavioural responses. This trace component was not significantly different from the blank.

Whereas all natural male pheromone samples increased the total running activity of the unmated females (table 3), cleaning behaviour was significantly reduced, compared to the results with blank, hexane or unmated females as the source (table 1). Hardly any flight activity of unmated females was observed with any of the test stimuli. When two females were placed into the tunnel simultaneously, they showed little interest in each other and usually avoided any contact when tested with the blank, hexane, or unmated females. In contrast, they interacted in diverse ways in the presence of male pheromone stimuli. In a very few cases, the females showed aggressive fighting, characterized by pushing with their heads and antennae. Approaching closely, they turned their body headfirst towards each other and tried to push themselves under the opponent. Several copulatory attempts were noted. The frequency of ovipositor entension, however, was not increased.

B. Reaction of mated females. Mated females behaved totally differently from unmated ones, showing distinctly increased locomotory activity (table 3). Regardless of the test stimuli offered, mated females started to run around immediately after release with the ovipositor extended all of the time. They were presumably searching for an oviposition site. Responses to all test stimuli were similarly low in the mated females (table 2).

Discussion

It has been suggested that short-range chemical stimuli play only a minor part in controlling the mating be-

haviour of *H. bajulus*[12]. However, in the present paper we demonstrate that unmated females are able to perceive and orientate towards pheromones emanating from male beetles. Stimulated by natural and synthetic test stimuli the unmated females respond with significant changes in behaviour compared to their behaviour with a blank, hexane or unmated females as test stimuli. Only 2,3-hexanedione (**4**) did not differ from the controls, indicating that this trace substance is not important for the activation and attraction of the females. Unmated males placed in the gauze box induced a stronger response in the females, i.e., searching behaviour and stopping close to the odour source. This may be due to the non-optimal release rate from artificial sources versus living beetles. The male CLSA-extracts and synthetic pheromone blends might also differ in quantity or in the ratio of the three-component blend from the natural pheromone.

In the present observations no flight initiation was recorded in females, probably because of the unsuitable size of the wind tunnel and the unnatural distribution of the pheromone plume in the tunnel. In their natural habitat beetles prefer running around when they leave their hiding places from about 11.00 to late afternoon, at preferred temperatures of 30–35 °C[9,10].

In the grape borer *Xylotrechus pyrrhoderus* Bates and related species of the cerambycid tribe Clytini, females are attracted to males by a long-range male-produced sex pheromone[8,24]. The chemicals responsible for attraction have been identified as (2*S*, 3*S*)-2,3-octanediol and (2*S*)-2-hydroxy-3-octanone[7]. The blend ratio of the two synthetic male sex pheromone components and the particular stereoisomers present affected the response of the females[25]. In males of *Xylotrechus chinensis* Chevrolat 3-hydroxy-2-octanone has also been reported[26].

The sex pheromone produced by male *H. bajulus* (cerambycid tribe Callidiini) is characterized by bifunctional C_6-components with molecular structures similar to the compounds found in *Xylotrechus* spp. These similarities may indicate a close relationship between the two tribes Callidiini and Clytini of the subfamily Cerambycinae. All compounds identified from *H. bajulus* show close biogenetic relations. Undoubtedly, 3-hydroxy-2-hexanone (**1**) shows the highest optical purity and may be a key compound in the bouquet. The corresponding 2-hydroxy-3-hexanone (**2**) is almost racemic and may well represent a non-enzymatic product of a tautomerization. 2,3-Hexanedione (**4**) and 1-butanol (**11**) may be products of secondary reactions. Interestingly, the two 2,3-hexanediols (**3**) do not show the same stereochemical purity with respect to C-3 as 3-hydroxy-2-hexanone (**1**). This might be due to a reduction step competitive to the above mentioned tautomerization.

In our wind tunnel setup the male sex pheromone of *H. bajulus* initiated a sequence of behavioural steps which began with activation of the resting female. This was followed by an approach to the pheromone source involving searching or stopping near or on the box. Finally, occasional female homosexual mounting and copulatory attempts were observed, a behaviour indicating a high level of female stimulation. Hierarchies of mating behaviour ranging from attraction to copulatory success have been demonstrated for various insect species[27]. Our wind tunnel bioassays showed that in *H. bajulus* the male-produced pheromone acts as an attractant from a distance of at least 1 m; most probably it also functions as an aphrodisiac and arrestant. On the basis of our present observations, we suggest that the premating behaviour of *H. bajulus* can be divided into two main stages: 1) emission of a long-range sex pheromone by males acting as activator, attractant, and aphrodisiac for unmated females, and 2) close-range behaviour between males and females, which is characterized by mechanical stimulation by the males such as licking the female pronotum and dorsal surface of the elytra with the maxillary and labial palps[12]. These mechanical premating stimuli act as releasers of final copulatory behaviour of the female, which is most evident in the extension of the ovipositor. These stimuli are known not only in *H. bajulus*, but also in other cerambycids[28]. It is not known, however, whether long-range male-produced attractants are also present in other cerambycid species besides *H. bajulus* and *Xylotrechus* spp[8]. Many cerambycid species meet their sexual partners on flowers or fresh timber. Besides the physical features of these habitats there may be chemical stimuli emanating from such places, which could lead to the aggregation of individuals, making long-range pheromones superfluous.

The presence of a male-produced sex pheromone in *H. bajulus* fits well into the known bionomics of this species. As early as 1942 it was reported that male *H. bajulus* are barely able to find a female over long distances[9]. On average male beetles from laboratory colonies emerge earlier and in greater numbers than females[9]. This may optimize the reproductive strategy. In the field, we observed that males rested in their hiding places for more than two weeks while waiting for females. Males left their hiding places only when objects similar in size to a conspecific moved nearby and then examined these objects by direct antennal contact.

In studies on male aggressive intra- and intersexual behaviour of *H. bajulus*, males were able to orientate visually to dummies at distances of up to 50 cm and frequently approached them[11]. Females were not tested with respect to perception. In another study on *H. bajulus* males were able to recognize females only at short distances[12]. In addition to the visual stimuli influencing males over short distances and the long-range, male-produced pheromone, the existence of further chemical contact stimuli necessary for the completion of mating cannot be excluded in either sex.

Several studies indicate that female cerambycids release a contact pheromone operating at short distances or as a stimulus to male copulatory behaviour. The presence of a close-range female sex pheromone in *H. bajulus* released from the elytra, which elicits olfactory orientation of the males, was proposed[13]. This finding has not been verified[14]. Based on detailed bioassays, contact sex pheromones released from the female body surface were reported for *Semanotus japonicus* Lacordaire[29] and for *Psacothea hilaris* (Pascoe)[30]. The females of *Megacyllene robiniae* (Forster) deposit sex pheromones on host wood[3]. Males of the Udo longicorn beetle *Acalolepta luxuriosa* Bates attempted to copulate with a dummy treated with female extract, suggesting the presence of a female contact pheromone[31]. A short-range stimulus produced by the female, and affecting male sexual activity was also suggested in *Paraglenea fortunei* Saunders[32]. The mating behaviour of the Japanese pine sawyer beetle *Monochamus alternatus* Hope may be evoked by a male volatile pheromone and a female contact pheromone[4]. Recent field trappings of male *Migdolus fryanus* Westwood indicate that the females attract their mating partners by sex pheromones[5,6]. For all these species the chemistry of the possible female volatiles is not known. The male-produced pheromone of the grape borer *Xylotrechus pyrrhoderus* attracts females over a distance of 1–2 m as determined using a wind tunnel bioassay[24]. The bifunctional C_8 compounds mentioned above represent the only cerambycid male pheromone whose chemical identity is at present known, with the exception of the male pheromone components of *H. bajulus*.

In the present paper the existence and chemical composition of a male-produced long-range sex pheromone in *H. bajulus* have been reported. Further studies are currently being undertaken to determine the optimum synthetic mixture and the quantity of synthetic pheromone blend. Field bioassays will show whether the pheromone can be used as trap bait or in other types of pheromonal control methods.

1 Becker, H., Z. angew. Ent. *61* (1968) 253.
2 Higgs, M. D., and Evans, D. A., Experientia *34* (1978) 46.
3 Galford, J. R., USDA Forest Service Research Note NE-240 1977.
4 Kim. G.-H., Takabayashi, J., Takahashi, S., and Tabata, K., Appl. Ent. Zool. *27* (1992) 489.
5 Bento, J. M. S., Albino, F. E., Della Lucia, T. M. C., and Vilela, E. F., J. chem. Ecol. *18* (1992) 245.
6 Bento, J. M. S., Della Lucia, T. M. C., and Frighetto, R. T. S., J. chem. Ecol. *19* (1993) 2347.
7 Sakai, T., Nakagawa, Y., Takahashi, J., Iwabuchi, K., and Ishii, K., Chem. Lett. *84* (1984) 263.
8 Sakai, T., and Iwabuchi, K., 4th Annl Meet. Int. Soc. chem. Ecol. Hull 1987.
9 Becker, G., Z. hyg. Zool. *34* (1942) 83.
10 Cymorek, S., Z. angew. Ent. *62* (1968) 316.
11 Breidbach. O., Dt. ent. Z., N. F. *37* (1990) 23.
12 Cannon, K. F., and Robinson, W. H., Material and Organismen *16* (1981) 303.
13 Doppelreiter, H., Z. angew. Ent. *88* (1979) 56.
14 Rehrmann, P., Untersuchungen zur intraspezifischen Kommunikation des Hausbockkäfers (*Hylotrupes bajulus* L.) Diploma thesis, Hamburg University 1992.
15 Noldt, U., Fettköther, R., and Dettner, K., Int. J. Insect. Morphol. Embryol. (1995) in press.
16 Boland, W., Ney. P., Jaenicke, L., in: Analysis of Volatiles, 371. Ed. P. Schreier. de Gruyter, Berlin 1984.
17 Klimetzek, D., Köhler, J., Krohn, S., Francke, W., J. appl. Ent. *107* (1989) 304.
18 Habich, A., and Grob, K., J. high Resolution Chromatogr. Chromatogr. Commun. *7* (1984) 492.
19 Seebach, D., Synthesis *69* (1969) 17.
20 Burri, K. F., Cardone, R. A., Chen. W. Y., Rosen, P., J. Am. chem. Soc. *100* (1978) 7069.
21 Corey, E. J., and Schmidt, G., Tetrahedron Lett. *5* (1979) 399.
22 Schröder, F., Fettköther, R., Noldt, U., Dettner, K., König, W. A., and Francke, W., Liebigs Ann. Chem. *12* (1994) 1211.
23 McLafferty, F. W., and Stauffer, D. B., The Wiley/NBS Registry of Mass Spectral Data, John Wiley & Sons, New York 1989.
24 Iwabuchi, K., Appl. Ent. Zool. *17* (1982) 497.
25 Iwabuchi, K., Takahashi, J., Nakagawa, Y., and Sakai, T., Appl. Ent. Zool. *21* (1986) 21.
26 Kuwahara, Y., Matsuyama, S., and Suzuki, T., Appl. Ent. Zool. *22* (1987) 25.
27 Shorey, H. H., A. Rev. Ent. *18* (1973) 349.
28 Michelsen, A., Ent. Meddr *34* (1966) 329.
29 Fauziah, B. A., Tabata, K., Ito, K., Takahashi, S., and Hidaka, T., Appl. Zool. *27* (1992) 19.
30 Fukaya, M., and Honda, H., Appl. Ent. Zool. *27* (1992) 89.
31 Kuboki, M., Atutsu, K., Sakai, A., and Chuman, T., Appl. Ent. Zool. *20* (1985) 88.
32 Wang, Q., Li, J.-S., Zeng, W.-Y., and Yin, X.-M., Annls Ent. Soc. Am. *84* (1991) 107.