

## CAPTURE OF FEMALE *Hylotrupes bajulus* AS INFLUENCED BY TRAP TYPE AND PHEROMONE BLEND

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**Abstract**—Three different types of traps were evaluated for a semiochemical-based trapping method for *Hylotrupes bajulus* (L.) (Cerambycidae). One, designated the ground trap, was the most efficient under both laboratory and natural conditions and had an active space of about 3.5 m. Significantly more beetles were captured in traps baited with a mixture of synthetic pheromones, (3*R*)-3-hydroxy-2-hexanone [(3*R*)-ketol] + 1-butanol, than in those with either single compound or with racemic mixtures. Furthermore, the synthetic lure captured more females than two virgin males in a laboratory bioassay. In addition, doubling the concentration of the synthetic pheromone significantly increased trap catches. The present findings have potential value of the management of this pest.

**Key Words**—*Hylotrupes bajulus*, Coleoptera, Cerambycidae, trap designs, effective distance, pheromone lure, pheromone mixture.

### INTRODUCTION

Long-horned beetles have received attention in the recent past because of their pest potential (Hanks, 1999; Allison et al., 2004; Nerg et al., 2004, and references therein). The old-house borer, *Hylotrupes bajulus* (L.) (Coleoptera: Cerambycidae), is a cosmopolitan insect of high economic importance due to its

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great potential to damage wood in houses. The beetle normally only feeds on sapwood, but in severe infestations may also attack the heartwood (White, 1954). Attacks often begin in the roof where larvae bore through rafters made of pine, spruce, and fir (Cannon and Robinson, 1982). Infestations spread when beetles fly from house to house in hot, sunny weather (Mares and Robinson, 1985), so an effective semiochemical-based trapping method would help to monitor and control *H. bajulus* populations.

Schröder et al., (1994) demonstrated that *H. bajulus* males produce a sex pheromone and identified (3*R*)-3-hydroxy-2-hexanone [(3*R*)-ketol] as a major component. Subsequently, chemical analysis of hexane extracts of dissected prothoracic glands, the source of the sex pheromone (Noldt et al., 1995), and headspace samples of both sexes revealed several male-specific compounds that were attractive to virgin females: (3*R*)-ketol, 2-hydroxy-3-hexanone, the diastereomeric alcohols [(2*R*, 3*R*)-2,3-hexanediol and (2*S*, 3*R*)-2,3-hexanediol, 2,3-hexanedione], and 1-butanol (Fettköther et al., 1995).

The objectives of this study were to determine the optimal pheromone dose, develop dispensers baited with pheromone mixtures, define the effective distance of the pheromone lure, and determine the efficiency of pheromone mixtures for use in trapping strategies.

#### METHODS AND MATERIALS

*H. bajulus* larvae are reared in darkness at 25°C and 75% RH at the Institute of Wood Biology and Wood Protection, Hamburg (Germany). The original culture was established in 1965, with adults collected at various roof sites in Schleswig-Holstein and Hamburg, and regularly restocked with specimens from other locations (Noldt et al., 1995). Newly emerged males and females were placed in individual Bellaplast boxes (Bellaplast AG, Altstätten, Switzerland) lined with moistened filter paper and then held in separate rooms at 20°C, 12 L:12 D photoperiod until needed. Food was not provided, since adult beetles do not feed under natural conditions (Becker, 1944). Beetles used in any given experiment were of the same age; however, age ranged from 5 to 15 days across all experiments.

Experiments were carried out in a greenhouse from April 15 to June 20, 1999, between 1200 and 1730 hours when insects are likely to be producing and responding to pheromones. Traps were evaluated in a gauze cage (450 cm long × 230 cm wide × 100 cm high) with six openings to insert or remove both traps and beetles. A ventilator was installed to facilitate a constant airflow of 2.5 m/sec and the contaminated air was removed during the experiment with an exhaust fan located on top of the cage.

Three trap types were evaluated, named ground, hanging, or wall traps, according to where they are placed. The ground trap (Figure 1A) measured 120 by 65 cm and had a slitted 50 by 8-cm baffle. The hanging trap (Figure 1B), suspended 75 cm from the floor, measured 50 by 12 cm and had a baffled slit on the upper surface. Wall traps (Figure 1C) were box shaped (40 cm high and 11 × 11 cm in cross section) with two 22-cm-long opening slits on the sides adjacent to the wall. All were constructed from 5-mm-thick brown cardboard, with a baffle near the openings to ensure that beetles caught did not escape.

Pheromone dispensers were made from 1-ml brown glass screw-top vials (N 8-1, Macherey-Nagel, Duren, Germany) capped with a 15 × 8-mm rubber septum (Thomas Scientific, Swedesboro, NJ, USA). Each vial was filled with 1,000 ml of pheromone mixture (purity ≥98.5%) obtained from the Department of Chemistry, Cornell University (USA) and the Institute of Organic Chemistry, University of Hamburg (Germany). Test chemicals were (3*R*)-ketol, 1-butanol (3 mg/vial/d), or racemic 3-ketol [(±)-3-ketol] (2.8 mg/vial/d), either alone at 1:100 or combined at 1:1:100 (by volume in hexane), respectively. Two

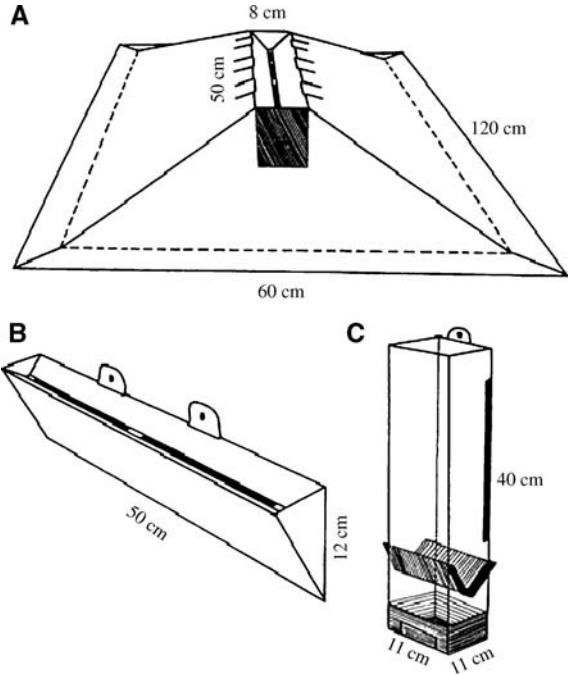


FIG. 1. Schematic diagram of three different pheromone traps tested for monitoring *H. bajulus*: (A) Ground trap, (B) hanging trap, and (C) wall trap.

dispenser vials were used per trap, unless otherwise stated. Dispensers were prepared the day before the experiment to allow volatile compounds to penetrate the rubber septa.

*General Trapping Method.* A trap baited with the test mixture was placed inside the greenhouse cage 2 hr before the release of the beetles so that pheromone could spread throughout the cage. Ten virgin female *H. bajulus* were released downwind of the trap at 1230 hr, and the number captured was counted after 3 hr. Temperature, relative humidity, and wind speed were measured with Meteo Digit III (Lambrecht, Klimatologische Messtechnik, Göttingen, Germany), whereas light intensity was recorded using a Lux-Meter (Testo GmbH & Co., Lenzkirch, Germany). All laboratory experiments were replicated eight times.

*Radius of Maximum Attraction of Synthetic Pheromone Mixture.* To determine the radius of maximum attraction of the synthetic pheromone, the ground trap was used, since it proved the most efficient in our previous experiment. Ten unmated females were released 1, 2, 3, 3.5, or 4 m downwind of a trap baited with (3*R*)-ketol + 1-butanol.

*Effect of Pheromone Lure Composition on Capture of Unmated Females.* Each trap was baited with (3*R*)-ketol (1.4 mg/vial/d), 1-butanol (1.6 mg/vial/d), (±)-3-ketol (1.2 mg/vial/d), or either two or four vials of (3*R*)-ketol + 1-butanol (standard; 3.0 mg/vial/d) or (±)-3-ketol + 1-butanol (2.6 mg/vial/d). In each replicate, ten unmated females were released 3 m downwind of the trap.

*Trapping Efficiency of Pheromone and Racemic Mixtures in the Presence of Males.* Ten unmated females (5–15 d-old) were released 3 m downwind from two competing pheromone sources that were 50 cm apart. One was a ground trap baited the pheromone mixture (3*R*)-ketol + 1-butanol or (±)-3-ketol + 1-butanol and the other, two virgin males (5–15 d-old). The males, placed in a cavity within two pieces (13 × 13 × 13 cm) of pine wood held together with a rubber band were enclosed in a 30 × 20 × 20 cm nylon screen cage to prevent them from escaping. The beetles were replaced every 2 d. Data on the number of females approaching and entering the trap or coming into contact with the nylon cage containing the males were recorded. Females remaining on the nylon cage for 5 min were considered as trapped. Trapped beetles were removed from the experimental arena.

*Efficiency of Traps under Natural Infested Conditions in the Field.* The three trap designs were evaluated from July to October, 1999, in five naturally infested sites around the town of Bayreuth, Germany. Four ground, 30 wall, and 21 hanging traps were deployed in each of the five sites. The intertrap distance was 10 m, and their positions were re-randomized between replicates once every week. Traps were coated on the inner side with Hostaflo TF 5035 (polytetrafluoro ethylene) (Werk Gendorf, Burgkirchen, Germany) to prevent

trapped beetles from escaping. Four unbaited control traps of each type were used in the experiments. The number of beetles captured was recorded every morning.

*Effect of Different Pheromone Blends in Recapturing H. bajulus in the Field.* Ground traps baited with either the standard pheromone mixture (3*R*)-ketol + 1-butanol (3.0 mg/vial/d), an 86:14 blend of (3*R*)-ketol, and (3*S*)-ketol + 1-butanol (2.8 mg/vial/d), a 1:1 mixture of (±)-3-ketol + 1-butanol (2.6 mg/vial/d), as well as (3*R*)-ketol (1.4 mg/vial/d) or 1-butanol (1.6 mg/vial/d) as single components. Experiments were set up in barns at four different locations near Bayreuth, Germany, during July to October, 1999, with one trap in each location. The intertrap distance was 10 m in each location, whereas interlocation distance was approximately 2 km. Ten unmated females marked with color were released 2 m from the trap. Releases were made in the afternoon and the number of trapped beetles was recorded the following morning. The untrapped beetles were collected at the end of the experiment.

*Statistical Analysis.* Data were analyzed by Kruskal–Wallis nonparametric tests to compare the efficiency of traps, effective distance of pheromone mixture, or effect of pheromone compositions in recapturing *H. bajulus*. Mann–Whitney *U* tests (Sokal and Rohlf, 1995) were used to test for significant differences between the treatments.

## RESULTS

*Evaluation of Traps.* In the greenhouse arena, the ground trap captured significantly more females than other traps baited with identical pheromone mixture ( $P \leq 0.05$ , Table 1). A similar trend was seen when tests were conducted in the infested roofs, trusses, and barns ( $P = 0.001$ , Table 1). Traps without pheromone mixtures, used as control, captured no beetles so they were not included in the analyses.

*Radius of Maximum Attraction of Synthetic Pheromone Mixture.* The baited ground trap was equally effective at distances up to 3.5 m, distances greater than previously reported for this species (Fettköther et al., 1995, 2000). However, there is a significant decline in captures when females are released 4m from the trap ( $P \leq 0.05$ ; Table 2). The average temperature, relative humidity and light intensity prevailing during the study were 29.5°C, 30.5% and 1297.0 lx.

*Effect of Different Pheromone Lures on Capture of Unmated Females.* Ground traps baited with pheromone mixtures, except (±)-3-ketol + 1-butanol, were generally more attractive than those baited with any single compound ( $P \leq 0.05$ ; Table 3). Increasing the dose increased trap catches with both blends,

TABLE 1. THE EFFICACY OF DIFFERENT TRAP DESIGNS, BAITED WITH (3R)-KETOL AND 1-BUTANOL, IN CAPTURING FEMALE *H. bajulus*

Trap design	Median captures of ten released females in a screen cage <sup>a</sup>	Capture of females in infested roof trusses and barns	
	Median $\pm$ MAD/trial ( $N = 8$ )	No. of traps used	Mean number of females caught $\pm$ SE
Ground trap	7.5 $\pm$ 0.5a	4	2.8 $\pm$ 0.2a
Wall trap	6.0 $\pm$ 0.5b	30	1.2 $\pm$ 1.0b
Hanging trap	3.5 $\pm$ 0.5c	21	0.1 $\pm$ 0.5c

<sup>a</sup>Captures in both greenhouse assays (Kruskal–Wallis ANOVA,  $P = 0.001$ ) and naturally infested sites (Kruskal–Wallis ANOVA,  $P = 0.001$ ) were significantly different. Median/Means followed by the same letter are not significantly different (Mann–Whitney  $U$  test,  $P \leq 0.05$ ). MAD = median absolute deviation.

but for any given concentration the (3R)-ketol + 1-butanol blend caught significantly more beetles than the corresponding racemic mixture (Table 3). The average temperature, relative humidity, and light intensity prevailing during this experimental period were 31°C, 25%, and 971 lx, respectively.

*Trapping Efficiency of Two Pheromone Blends in the Presence of Males.* Ground traps baited with the pheromone mixture captured significantly more females (median of six beetles captured) than two live males (median of four beetles captured) when tested concurrently in the greenhouse ( $F = 36.22$ ,  $P \leq 0.05$ ; data not shown). In contrast, traps baited with ( $\pm$ )-3-ketol + 1-butanol captured significantly ( $F = 28.14$ ,  $P \leq 0.05$ ) fewer females than the males (medians of 3.0 and 5.0, respectively). The temperature, relative humidity, and

TABLE 2. EFFECT OF DISTANCE ON THE NUMBER OF *H. bajulus* FEMALES CAPTURED IN GROUND TRAPS BAITED WITH THE STANDARD PHEROMONE MIXTURE IN A GREENHOUSE TRIAL

Distance (m)	Median captures of ten released females in screen cage trial <sup>a</sup>
	Median $\pm$ MAD/trial ( $N = 8$ )
1	9.0 $\pm$ 0.5a
2	8.0 $\pm$ 1.0a
3	7.0 $\pm$ 1.0a
3.5	6.5 $\pm$ 1.5a
4	3.5 $\pm$ 0.5b

<sup>a</sup>Captures were significantly different (Kruskal–Wallis ANOVA;  $P = 0.011$ ). Medians followed by the same letters are not significantly different (Mann–Whitney  $U$  test;  $P \leq 0.05$ ). MAD = median absolute deviation.

TABLE 3. EFFECT OF DIFFERENT PHEROMONE LURES ON THE CAPTURE OF UNMATED *H. bajulus* FEMALES IN GROUND TRAPS

Attractants	Quantity (no. of vials)	No. of females captured <sup>a</sup>
		Median ± MAD/trial
<i>Single pheromone</i>		
1-butanol	2	2.0 ± 1.5c
(3 <i>R</i> )-ketol	2	2.0 ± 0.5c
(±)-3-ketol	2	1.0 ± 0.5d
<i>Blend of pheromone</i>		
(3 <i>R</i> )-ketol + 1-butanol (standard)	2	6.0 ± 1.0b
(3 <i>R</i> )-ketol + 1-butanol	4	8.0 ± 0.5a
(±)-3-ketol + 1-butanol	2	3.0 ± 0.5c
(±)-3-ketol + 1-butanol	4	5.0 ± 1.0b

<sup>a</sup> Captures were significantly different (Kruskal–Wallis ANOVA,  $P = 0.012$ ). Medians followed by the same letters are not significantly different (Mann–Whitney  $U$  test;  $P \leq 0.05$ ). MAD = median absolute deviation.

light intensity prevailing during this experimental period were 31.5°C, 21.5%, and 983.0 lx, respectively.

*Efficiency of Different Pheromone Mixtures in the Field.* As with the greenhouse experiments, traps baited with pheromone mixtures were more efficient than those baited with either single pheromone component ( $P \leq 0.05$ ; Table 4). Furthermore, the standard blend was more effective than any of the racemic mixtures (Table 4).

TABLE 4. PERCENTAGE OF FEMALE *H. bajulus*, RELEASED 2 M FROM GROUND TRAPS BAITED WITH DIFFERENT PHEROMONE MIXTURES, WHICH WERE RECAPTURED IN BARNs AT FOUR DIFFERENT LOCATIONS

Attractants	Percent females captured				Average ± SE <sup>a</sup>
	Barn 1a	Barn 1b	Barn 1c	Barn 2	
(3 <i>R</i> )-ketol + 1-butanol (standard)	70	60	52	73	63.7 ± 8.3a
Mixture of 86% (3 <i>R</i> )-ketol +14% (3 <i>S</i> )-ketol + 1-butanol	50	45	45	56	49.0 ± 4.5b
(±)-3-ketol + 1-butanol	45	35	28	53	40.2 ± 9.5c
Mixture of (±)-3-ketol + (±)-2-ketol + 1-butanol	27	24	40	50	35.2 ± 10.4c
(3 <i>R</i> )-ketol	20	30	10	24	21.0 ± 7.2d
1-butanol	15	40	25	33	28.2 ± 9.3d

<sup>a</sup> Catches were significantly different (Kruskal–Wallis ANOVA,  $P = 0.001$ ). Means followed by the same letters are not significantly different (Mann–Whitney  $U$  test;  $P \leq 0.05$ ).

## DISCUSSION

The ground trap proved to be the most effective design tested partly because although females initially fly upwind in the pheromone plume they generally walk the final distance (about 50 cm) to the source, whereas the relative response of different beetles to racemic and pure pheromone blends varies with species (Iwabuchi et al., 1986; Hallett et al., 1995; Giblin-Davis et al., 1996; Miller et al., 1997). *H. bajulus* females are clearly less attracted to racemic blends. Furthermore, the racemic blend proved less effective than virgin males. Thus, we believe that ground traps baited with (3*R*)-ketol + 1-butanol offer a viable approach for the monitoring and control of *H. bajulus*.

However, additional work is required to further refine the system. For example, research examining the possible effects of physical characteristics such as color on the efficacy of the ground traps merits attention (Suckling et al., 2005). With respect to the lure, we saw an increase in trap catch when the concentration of the pheromone was doubled (Table 3). Thus, we need to determine if doses higher than those tested in this study increase the numbers of beetles caught. This merits attention because different dose-dependent responses were reported on other beetle species (Fadamiro, 1996; Fukaya and Honda, 1996). In addition, if trap catches do increase with increasing concentrations of pheromone, one needs to examine if this is related to having a larger active space. Furthermore, our previous studies (Fettköther et al., 2000) showed that the addition of  $\alpha$ -pinene to the pheromone increased attraction and orientation of both sexes toward the source. Therefore, trials need to be carried out to determine how this and other compounds such as (–)-verbenone, *trans*-pinocarveol, and terpinen-4-ol (Fettköther et al., 2000) might be used with the pheromone to increase trap efficacy.

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## REFERENCES

- ALLISON, J. D., BORDEN, J. H., and SEYBOLD, S. J. 2004. A review of the chemical ecology of the Cerambycidae (Coleoptera). *Chemoecology* 14:123–150.
- BECKER, G. 1944. Sinnesphysiologische Untersuchungen über die Eiablage des ausbockkäfers. *Z. Vgl. Physiol.* 30:253–299.

- CANNON, K. F. and ROBINSON, W. H. 1982. Notes on the biology and distribution of *Hylotrupes bajulus* (L.) (Coleoptera: Cerambycidae) in Virginia. *Entomol. News* 93:173–176.
- FADAMIRO, H. Y. 1996. Influence of stimulus dose and wind speed on the orientation behaviour of *Prostephanus truncatus* (Coleoptera: Bostrichidae) to pheromone. *Bull. Entomol. Res.* 86:659–665.
- FETTKÖTHER, R., DETTNER, K., SCHRÖDER, F., MEYER, H., FRANCKE, W., and NOLDT, U. 1995. The male pheromone of the old house borer *Hylotrupes bajulus* (L.) (Coleoptera: Cerambycidae): identification and female response. *Experientia* 51:270–277.
- FETTKÖTHER, R., REDDY, G. V. P., NOLDT, U., and DETTNER, K. 2000. Effect of host and larvalfrass volatiles on behavioural response of the old house borer *Hylotrupes bajulus* (L.) (Coleoptera: Cerambycidae), in a wind tunnel bioassay. *Chemoecology* 10:1–10.
- FUKAYA, M. and HONDA, H. 1996. Reproductive biology of the yellow-spotted longicorn beetle, *Psacotha hilaris* (Pascoe) (Coleoptera: Cerambycidae): V. Male mating responses to male extract. *Appl. Entomol. Zool.* 31:95–98.
- GIBLIN-DAVIS, R. M., PENA, J. E., OEHLISCHLAGER, A. C., and PEREZ, A. L. 1996. Optimization of semiochemical-based trapping of *Metamasius hemipterus sericeus* (Oliver) (Coleoptera: Curculionidae). *J. Chem. Ecol.* 22:1389–1410.
- HALLETT, R. H., PEREZ, A. L., GRIES, G., GRIES, R., PIERCE, H. D. JR., YUE, J., OEHLISCHLAGER, A. C., GONZALEZ, L. M., and BORDEN, J. H. 1995. Aggregation pheromone of coconut rhinoceros beetle, *Oryctes rhinoceros* (L.) (Coleoptera: Scarabaeidae). *J. Chem. Ecol.* 21:1549–1570.
- HANKS, L. M. 1999. Influence of the larval host plant on reproductive strategies of cerambycid beetles. *Annu. Rev. Entomol.* 44:483–505.
- IWABUCHI, K., TAKAHASHI, J., NAKAGAWA, Y., and SAKAI, T. 1986. Behavioral responses of female grape borer *Xylotrechus pyrrhoderus* BATES (Coleoptera: Cerambycidae) to synthetic male sex pheromone components. *Appl. Entomol. Zool.* 21:21–27.
- MARES, J. T. and ROBINSON, W. H. 1985. The old house borer and wood damage go hand-in hand. *Pest Control* 53:31–32.
- MILLER, D. R., GIBSON, K. E., RAFFA, K. F., SEYBOLD, S. J., TEALE, S. A., and WOOD, D. L. 1997. Geographic variation in response of pine engraver, *Ips pini*, and associated species to pheromone, lanierone. *J. Chem. Ecol.* 23:2013–2031.
- NERG, A.-M., HEIJARI, J., NOLDT, U., VIITANEN, H., VUORINEN, M., KAINULAINEN, P., and HOLOPAINEN, J. K. 2004. Significance of wood terpenoids in the resistance of Scots pine provenances against the old house borer, *Hylotrupes bajulus*, and brown-rot fungus, *Coniophora puteana*. *J. Chem. Ecol.* 30:125–141.
- NOLDT, U., FETTKÖTHER, R., and DETTNER, K. 1995. Structure of the sex pheromone-producing prothoracic glands of the male old house borer, *Hylotrupes bajulus* (L.) (Coleoptera: Cerambycidae). *Int. J. Insect Morphol. Embryol.* 24:223–234.
- SCHRÖDER, F., FETTKÖTHER, R., NOLDT, U., DETTNER, K., KÖNIG, W. A., and FRANCKE, W. 1994. Synthesis of (3R)-3-hydroxy-2-hexanone, (2R, 3R)-2, 3-hexanediol and (2S, 3R)-2, 3-hexanediol, the male sex pheromone of *Hylotrupes bajulus* and *Pyrrhidium sanguineum* (Cerambycidae). *Liebigs Ann. Chem.* 12:1211–1218.
- SOKAL, R. R. and ROHLF, F. J. 1995. *Biometry: The Principles and Practices of Statistics in Biological Research*. Freeman and Company, New York.
- SUCKLING, D. M., GIBB, A. R., BURNIP, G. M., SNELLING, C., DE RUITER, J., LANGFORD, G., and EL-SAYED, A. M. 2005. Optimization of pheromone lure and trap characteristics for currant clearwing, *Synanthedon tipuliformis*. *J. Chem. Ecol.* 31:393–406.
- WHITE, M. G. 1954. The house longhorn beetle *Hylotrupes bajulus* L. (Col. Cerambycidae) in Great Britain. *Forestry* 27:31–40.