Improved Semiochemical-Based Trapping Method for Old-House Borer, *Hylotrupes bajulus* (Coleoptera: Cerambycidae)

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ABSTRACT The old-house borer, *Hylotrupes bajulus* (Linnaeus) (Coleoptera: Cerambycidae), has been a very serious pest of structural timber in many parts of the world. The available semiochemical-based trapping method typically results in low capture rates. In this study, efforts were made to improve the semiochemical-based trapping method using screen cage assays conducted in the greenhouse. Ground traps baited with two vials of (3*R*)-ketol + 1-butanol (pheromone baited) caught >21.0% adults than the alternative trap and were seen to be superior to ramp traps baited with identical compounds. Based on adult catches with pheromone-baited traps with different colors, attraction of *H. bajulus* adults to black was significantly greater than other colors, followed by brown, gray and yellow, red, white, green, and blue. No differences were found in adult catches to pheromone-baited traps with respect to different trap sizes. There were no statistical differences between the different semiochemicals tested. Therefore, black ground traps baited with two vials of (3*R*)-ketol + 1-butanol plus two vials of ethyl acetate can be effective in the monitoring and potential control of *H. bajulus*.

KEY WORDS pheromone, *Hylotrupes bajulus*, ground trap, ramp trap, insect behavior

The old-house borer, *Hylotrupes bajulus* L. (Coleoptera: Cerambycidae), is a very destructive pest of structural timber worldwide (Becker 1979). The larvae of *H. bajulus* can bore and damage the sapwood of pine, spruce, and fir (White 1954) and also infest a number of hardwood species (Duffy 1963). However, the Dunarobba fossil forest wood is very resistant to attack by *H. bajulus* (Palanti et al. 2004) and South African yellow-wood (*Podocarpus* sp.) (Dürr 1954), in which the larvae did not survive. Insecticides must have good penetration characteristics to control this wood borer because larvae of *H. bajulus* feed below the surface of wood (Dodson and Robinson 1989). However, the application of insecticides is detrimental and frequently undesirable, particularly in the human dwellings. In this context, the development of an efficient semiochemical-based trapping method could provide an alternative control measure, as well as providing a mechanism for monitoring populations of this insect.

The pheromone components (−)-verbenone and p-cymen-8-ol produced in the frass of the wood-boring larvae of *H bajulus* mediate the oviposition behavior of adults (Higgs and Evans 1978). A sex pheromone produced by adult males is attractive to female beetles, and the major component of this pheromone has been identified as (3*R*)-3-hydroxy-2-hexanone (3*R*)-ketol) (Schröder et al. 1994). The sex pheromone is produced by exocrine glands in the prothorax of the male beetle (Noldt et al. 1995). Chemical analysis of hexane extracts obtained by surface extraction of dissected prothoracic glands and headspace samples revealed the male-specific compounds: (3*R*)-ketol, 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, and 1-butanol (Fettköther et al. 1995). The authors reported that unmated females were attracted to pheromone blends (headspace extracts of males and synthetic blends of other major glandular compounds) and also to the compound (3*R*)-ketol in wind tunnel experiments (Fettköther et al. 1995). The presence of males on pine wood increased the attractiveness of the wood to virgin females responding to the pheromone compounds (Plarre and Hertel 2000).

In a previous study, a ground trap baited with synthetic sex pheromone (3*R*)-ketol + 1-butanol was the most efficient trap in capturing *H. bajulus* in both greenhouse and field tests (Reddy et al. 2005a). Fettköther et al. (2000) reported that female *H. bajulus* were also attracted to a blend of monoterpenes (−)-verbenone, (+)-α-pinene, (+)-terpinen-4-ol, and (−)-*trans*-pinocarveol, whereas male beetles were attracted to a mixture of monoterpenes (+)-terpinen-4-ol, (+)-α-pinene, and (−)-*trans*-pinocarveol in wind tunnel experiments. However, a blend of the synthetic sex pheromone (3*R*)-ketol + 1-butanol or (±)-3-ketol +

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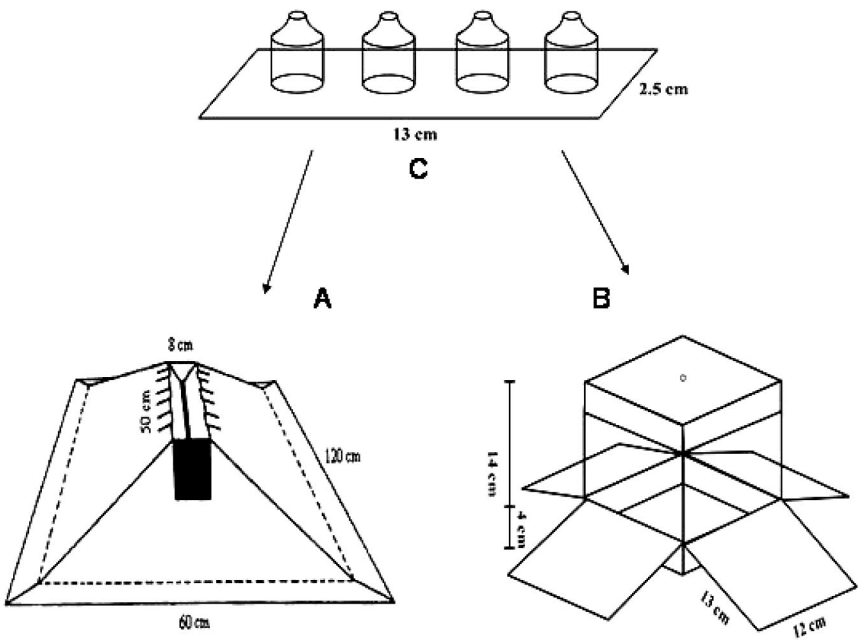

Fig. 1. Schematic illustration of traps evaluated in this study: (A) ground trap, (B) ramp trap, and (C) test chemicals in glass vials with rubber septa secured on cardboard with a sealing wax and placed in the traps.

1-butanol combined with monoterpenes resulted in the capture of significantly more females than either the synthetic sex pheromone or the monoterpene mixture alone (Reddy et al. 2005b). However, capture rates remained relatively low when ground traps baited with synthetic sex pheromone were used.

In this study, I carried out investigations in the green house into lure and trap characteristics to develop a more effective monitoring and mass trapping technique for *H. bajulus* adults. Second, experiments were conducted on the effects of visual cues as previous experiments had highlighted the importance of visual cues in this species. Here, I studied the color response of *H. bajulus* to traps baited with synthetic pheromones.

Materials and Methods

Experimental Procedures

Rearing of *H. bajulus*. *Hylotrupes 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 held in separate rooms at 20°C, 12 L:12 D photoperiod until needed (Reddy et al. 2005a). Food was not provided, because adults do not feed (Becker 1944). Beetles used in any given experiment were of the same age; however, age ranged from 5 to 15 d across all experiments (Fettköther et al. 1995).

Traps. Two ground-based pheromone trap types were evaluated including a ground trap and ramp trap. The ground trap (Fig. 1A) was constructed from a 5-mm-thick brown piece of cardboard, measured 120 by 60 cm with a baffle 50 by 8-cm fitted in the top that served to prevent beetles that fell through the slit from escaping (Reddy et al. 2005a). Traps were coated on the inner side of the collection container with Hostaflon TF 5035 (polytetrafluoroethylene [PTFE]; Werk Gendorf, Burgkirchen, Germany) to prevent trapped beetles from escaping. The ramp trap (Fig. 1B) was based on the design of Vernon (2004). The ramp trap closely resembles the commercial version manufactured by ChemTica Internacional (Heredia, Costa Rica). The ramp trap is made locally of durable polyvinyl chloride (PVC) and consists of two box-shaped components, with inside dimensions of 14 cm wide by 4 cm high. The other component is a slope section, four of which are attached into the ends of one of the open boxes. To assist beetles in climbing, the slope section was 4 cm high by 13 cm long by 12 cm wide and slid into slots in the floor of the box component. The other box has four corner ridges (6 cm high) that extend downward into the bottom box. Water with detergent (1–3%) was used in the container bottom to retain adults that walked into traps.

Dispensers. Dispensers were made from glass screw top vials (N 8–1 brown, 23 by 5.5 by 1.0 mm, capacity 1 ml; Macherey-Nagel, Duren, Germany) with a straight end capped with a rubber septum (15 by 8 mm; Thomas Scientific, Swedesboro, NJ). Each vial was filled with 1 ml of the test compound diluted in hexane. Each treatment was replicated four times.

Test Chemicals. The synthetic sex pheromone blend + hexane, at the ratio of 1:1:100 (by vol) (3*R*)-ketol + 1-butanol (purity ≥98.5%, with a release rate of 3 mg/d; hereafter referred to as sex pheromone) was obtained from the Department of Chemistry, Cornell University, Ithaca, NY. The selected monoterpenes (+)-α-pinene, (−)-verbenone, (−)-*trans*-pinocarveol, and (+)-terpinen-4-ol (purity ≥99%) were obtained from Fluka Chemika (Steinheim, Germany) and mixed in a 1:1:1:1 ratio diluted in 100 ml of hexane (hereafter referred to as the monoterpene blend). Ethyl acetate (purity ≥95%) and hexane (high-performance liquid chromatography grade) were obtained from Merck (Darmstadt, Germany). Ethyl acetate (release rate = 250 mg/d) was used without dilution (hereafter referred to as ethyl acetate).

Greenhouse Conditions. Experiments were conducted between 1200 and 1730 hours, a period when most beetles produce and respond to pheromones (Fettköther et al. 2000). All experiments were conducted in a greenhouse using a screen cage (450 cm long by 230 cm wide by 100 cm high) with six openings to insert or remove both traps and beetles. Contaminated air (i.e., air containing sex pheromone and monoterpene/ethyl acetate volatiles) was exchanged by an exhaust fan located at the top of the greenhouse during the experiment. The average temperature, relative humidity and wind speed were measured with a Meteo Digit III (Lambrecht, Klimatologische Messtechnik, Göttingen, Germany), whereas light intensity was recorded using a Lux-Meter (Testo, Lenzkirch, Germany).

Trapping Method. All trap studies used 30 recently emerged unmated female beetles (5–15 d old) that were released in the screen cage but outside of the traps on the downwind side at a distance of 3 m. One ramp or ground trap, when baited with either the pheromone or monoterpenoid blends or ethyl acetate, was placed on the ground in the greenhouse screen cage with 2 h before the release of the beetles, which allowed the pheromone or monoterpenoid blend or ethyl acetate vapors to spread throughout the cage. A running fan was installed behind the trap with several tightened gauze layers over its downwind opening to produce a uniform moderate airflow through the screen cage. Contaminated air from the screen cage was removed by a downwind exhaust system. Test chemicals in glass vials with rubber septa were fixed with sealing wax on a 5-mm-thick brown cardboard (12 cm long by 3 cm width; Fig. 1C). The card with test chemicals was subsequently placed within the ground trap. In the case of the ramp trap, the baited vials on the cardboard were suspended 8 cm from its top using a wire.

Statistical Analysis. Data were analyzed using Kruskal--Wallis nonparametric tests to compare the evaluation of trap designs, frequency of response to different colors, effect of trap size, and effect of traps baited with different semiochemicals in capturing *H. bajulus.* Mann–Whitney *U* tests were used to test for significant differences between the treatments. All statistical procedures were conducted using SPSS 13.0 for Windows software.

Evaluation of Two Different Trap Designs for Capture Efficiency of *H. bajulus.* Ground or ramp traps baited with two vials of the sex pheromone were tested consequently in the screen cage to determine the capture efficiency of female *H bajulus.* One cage was used for all trap testing. Traps without lure were used for control treatments. Counts were made of the number of beetles that were caught by the traps during a 3-h (1230–1530 hours) experimental period. Captured beetles were immediately removed from the trap. For each treatment, a total of four replicates were conducted.

Effect of Trap Color on the Capture Efficiency of Female *H. bajulus*

Trap Color Measurements. Color characteristics were measured using a Konica Minolta CR-410 Chromameter (Minolta Instrument Systems, Ramsey, NJ). The measurements were obtained from the average of three readings for each trap color. The hue angle (h°) and chroma (C*) were calculated from a* and b* color coordinates. Hue angle and chroma are calculated as arc tan (b*/a*) and $(a^{*2} + b^{*2})^{0.5}$, respectively. Chroma provides a measure of color intensity, whereas hue angle indicates the sample color (Wrolstad et al. 2005). Hue angle is expressed on a 360° grid where 0° = red, 90° = yellow, 180° = green, and 270° = blue.

This experiment was conducted in the screen cage with ground traps of different colors, baited with two vials of the sex pheromone. The ground trap was selected because it has a 21.4% higher *H. bajulus* capture rate than the ramp trap. Brown, black, gray, yellow, red, white, green, and blue trap colors were tested independently with four replicates per color. These experiments were conducted between 1230 and 1530 hours. Data collected included the number of females caught by the trap. Trapped beetles were removed during the course of the experiment.

Effect of Trap Size on Capture Efficiency of Female *H. bajulus.* The efficiency of seven different sizes (140 by 70, 120 by 60, 100 by 50, 80 by 40, 60 by 30, 40 by 20, and 30 by 15 cm) of black-colored ground traps was studied. Thirty unmated females (5–15 d old) were released 3 m downwind from a ground trap baited with two vials of the sex pheromone blend in a screen cage. The number of beetles trapped over the 3-h interval was recorded. Trapped beetles were removed from the trap after capture. A total of four replicates were conducted for each trap size treatment.

Effect of Traps Baited with Pheromone, Monoterpenoid Blend, Ethyl Acetate, or Their Combination on Capture Efficiency of Female *H. bajulus.* Black-colored ground traps received one of the following treatments: two vials of pheromone blend, two vials of monoterpene blend, two vials of ethyl acetate, two vials of pheromone plus two vials of monoterpene blend, or two vials of pheromone blend plus two vials of ethyl acetate. The experiment was conducted between 1230 and 1530 hours. The number of beetles trapped over the 3-h interval was recorded. Beetles were removed from the experiment as trapped. A total of four replicates were conducted for each treatment.

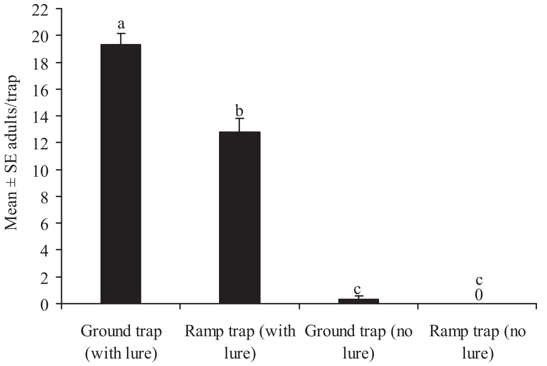

Fig. 2. Mean (±SE) catches of *H. bajulus* in ground and ramp traps baited with and without (3*R*)-ketol + butanol. Different lowercase letters indicate significant differences between treatments (Kruskal–Wallis ANOVA and Mann–Whitney *U* test, $P < 0.05$). Means were generated from four tests each using 30 insects.

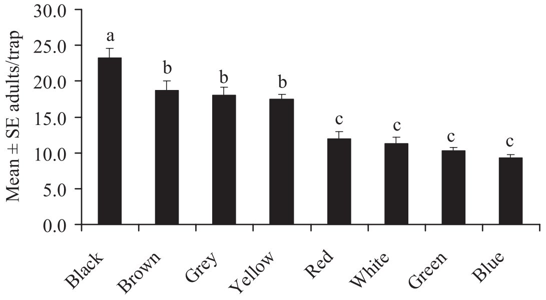

Fig. 3. Mean (±SE) catches of *H. bajulus* in pheromone-baited ground traps with different colors. Different lowercase letters indicate significant difference between treatments (Kruskal–Wallis ANOVA and Mann–Whitney *U* test, $P < 0.05$). Means were generated from four tests each using 30 insects.

Results

The average temperature, relative humidity, light intensity, and wind speed that prevailed during the experimental period were 31.2°C, 33.0%, 79 lux, and 2.5 m/s.

Evaluation of Two Different Trap Designs for Capture Efficiency of *H. bajulus*

In the screen cage arena, the ground trap captured significantly more females than ramp trap baited with pheromone mixture ($P < 0.001$; Fig. 2). Nonbaited ground and ramp traps, used as controls, captured no beetles.

Effect of Trap Color on the Capture Efficiency of Female *H. bajulus*

Trap color measurement values (L*, a*, b*, chroma, and hue angle) are given in Table 1. Adult catches in ground traps were affected by trap color ($P < 0.001$; Fig. 3). Black was the most preferred by *H. bajulus* adults. Red, white, green, and blue trap colors were significantly less effective at trapping *H. bajulus* than black or other trap colors that were intermediate in effectiveness like brown, gray, and yellow (Fig. 3).

Effect of Trap Size on Capture Efficiency of Female *H. bajulus*

No differences were detected between trap size treatments ($P > 0.05$). Average *H. bajulus* trap collection was 18.8, 20.0, 19.7, 18.7, 18.7, and 18.2 on trap sizes of 140 by 70, 120 by 60, 100 by 50, 80 by 40, 60 by 30, 40 by 20, and 30 by 15 cm, respectively.

Effect of Traps Baited with Pheromone, Monoterpenoid Blend, Ethyl Acetate, or Their Combination on Capture Efficiency of Female *H. bajulus*

Ground traps baited with two vials of sex pheromone plus two vials of monoterpene blend caught 86% of released adults, whereas traps baited with two vials of sex pheromone plus two vials of ethyl acetate caught 79% of total *H. bajulus* and proved to be more attractive ($P < 0.001$; Fig. 4) than the other lures tested. No statistical difference was observed between the catches by traps baited with sex pheromone plus monoterpenoid and sex pheromone plus ethyl acetate ($P < 0.05$). Traps baited with two vials of sex pheromone caught 66% of the beetles, a value significantly higher than the sex pheromone/monoterpenoid blends or ethyl acetate ($P < 0.05$). Collections of *H. bajulus* in the monoterpenoid blend or ethyl acetate baited traps were statistically equivalent to the control.

Table 1. Color measurements of traps used in this study

Trap color	L*	a*	b*	Chroma (C)	Hue angle (h°)
Black	33.8 ± 0.1	7.6 ± 0.2	−5.4 ± 0.1	9.28 ± 0.1	328.3 ± 0.7
Brown	62.3 ± 0.4	5.6 ± 0.3	19.23 ± 0.3	20.0 ± 0.3	52.1 ± 0.3
Grey	56.7 ± 0.0	−1.8 ± 0.1	−23.7 ± 0.0	23.7 ± 0.0	213.7 ± 0.0
Yellow	96.0 ± 0.3	−8.8 ± 0.1	24.5 ± 0.3	26.7 ± 0.4	129.2 ± 0.1
Red	48.7 ± 0.1	53.9 ± 0.1	23.1 ± 0.1	22.1 ± 0.1	58.6 ± 0.1
White	94.4 ± 0.1	−0.8 ± 0.1	4.86 ± 0.1	4.9 ± 0.0	125.5 ± 0.1
Green	56.0 ± 0.3	−35.2 ± 1.4	6.5 ± 2.3	35.8 ± 1.8	169.8 ± 3.3
Blue	48.7 ± 0.0	16.4 ± 0.1	−36.7 ± 0.1	40.1 ± 0.1	311.0 ± 0.1

Means were generated from three observations.

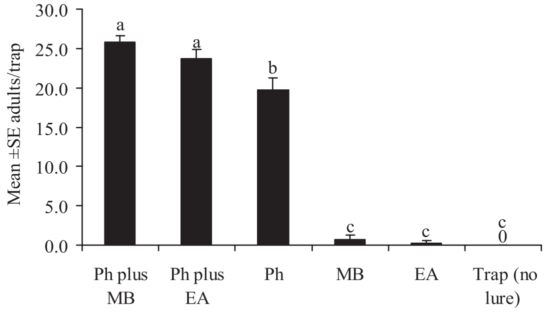

Fig. 4. Mean (±SE) catches of *H. bajulus* in ground traps baited with different attractant blends. Ph, pheromone [(3*R*)-ketol + 1-butanol]; MB, monoterpenoid blend [(+)-α-pinene, (−)-verbenone, (−)-*trans*-pinocarveol, (+)-terpinen-4-ol]; EA, ethyl acetate. Different lowercase letters indicate significant differences between treatments (Kruskal–Wallis ANOVA and Mann–Whitney *U* test, $P < 0.05$). Means were generated from four tests each using 30 insects.

Discussion

In previous studies, ground traps baited with sex pheromone compounds were statistically more effective than no-baited traps, but adult catches were still relatively low and inadequate for survey and mass trapping of *H. bajulus* (Reddy et al. 2005a). It was hypothesized that low captures rates were caused by the absence of visual trap cues that were omitted from the experimental design (Reddy et al. 2005b). Therefore, this study undertook a comparative evaluation with other ground-based pheromone traps such as ramp traps. Results from this study indicate ground traps caught higher numbers of adults than the ramp traps. The ramp trap used in this study is an efficient design for capturing other beetles like *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) (G.V.P.R., unpublished data) and also *Agriotes lineatus* L. and *Agriotes obscurus* L. (Coleoptera: Elateridae) (Vernon 2004). It is possible that ground traps could perform better in the field conditions rather than the controlled and artificial environment of the glasshouse. In comparison to the ramp trap, the ground trap is less expensive to make and easier to use.

Experiments with different colored ground traps baited with sex pheromone indicate black-colored traps were the most attractive. The trap color effect indicates the importance of visual factors, in addition to olfactory cues, for mate location in *H. bajulus* traps. Previous studies used colored brown traps, which caught 15.4% fewer insects than the black traps used in this study. However, it is not known why *H. bajulus* should be attracted to colored black traps in preference to the other trap colors tested. Trap characteristics such as color are important for orientation of some insects (Suckling et al. 2005). Mate location and recognition are the first steps in mating behavior, followed by courtship and copulation (Fukaya et al. 2005). Sex pheromone is thought to be the most important cue for mate recognition in cerambycid beetles (Hanks 1999, Allison et al. 2004). Many borers are known to orient to dark silhouettes for reasons that may not be mate related (de Groot and Nott 2001). It may be a perceived host or reproductive site and not direct orientation to mates. However, shape, size, colors, and movement of adult beetles are also potential visual cues for mate location/recognition (Engelmann 1970, Thornhill and Alcock 1983, Wang 2002).

In this study, the trap size did not influence capture efficiency for *H. bajulus.* Although this study was conducted under greenhouse conditions, further experiments are required to support these results at the field level. It is expected that greenhouse studies may represent *H. bajulus* behavior in other structures like domestic dwellings. However, in the Cerambycidae, mate body size is reported to influence mate location and mating success (Hanks et al. 1996, Fukaya 2004, Fukaya et al. 2005) but such visual cues had been considered to be much less important than olfactory cues (Fukaya et al. 2005). Trap design and size were inconsistent in capturing other Cerambycid species like *Tetropium fuscum* (Fabr.) (Coleoptera: Cermabycidae) (Sweeney et al. 2006). *H. bajulus* seems to have a similar response to trap size and shape as other cerambycids like *T. fuscum.*

Traps baited with sex pheromone plus monoterpenoid blends caught males equivalent to traps baited with sex pheromone plus ethyl acetate. In a previous study, the use of traps baited with sex pheromone plus the monoterpenoid blend was recommended for monitoring and control of *H. bajulus* (Reddy et al. 2005b). However, monoterpenoid blends consists of four different compounds and may be expensive and difficult to obtain. In this study, it was found that traps baited with the pheromone blend and ethyl acetate could attract as many beetles as traps baited with the monoterpenoid blend. Therefore, the use of ethyl acetate with the pheromone blend can serve as an equivalent substitute for the monoterpenoid blend. Host plant volatiles are reported to have synergistic effect on trap catches when combined with pheromone compounds (Reddy and Guerrero 2004). In coleopteran insects, ethyl acetate can have a synergistic effect like this study when added with pheromone compounds (Giblin-Davis et al. 1996, Reddy et al. 2005). The presence of males of *H. bajulus* on pine wood (*Pinus sylvestris* L., Pinaceae) increased its attractiveness for virgin females (Plarre and Hertel 2000).

The results indicate a ground trap baited with a pheromone/ethyl acetate blend can efficiently trap adult *H. bajulus.* Importantly, trap color was a significant factor in trap efficacy. Ground traps used in this study could be effectively deployed for the monitoring of *H. bajulus* and may facilitate a measure of population control. A reduction in *H. bajulus* population may reduce the need for insecticide treatment of human dwellings infested with or at risk of infestation by *H. bajlus.*

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