

Disruption of sexual communication in the mirid bug *Lygocoris pabulinus* by hexyl butanoate

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- Abstract**
- 1 The metathoracic scent gland in *Lygocoris pabulinus* contains mostly hexyl butanoate. As secretions of this gland in Heteroptera may serve as an alarm pheromone, we determined whether hexyl butanoate is released by disturbed bugs, and whether this compound disrupts sexual attraction of *L. pabulinus* males towards females.
 - 2 Undisturbed males and females, and disturbed males released less than 100 ng/h hexyl butanoate, whereas disturbed females released a highly variable amount, ranging from 25 ng/h to more than 1 µg/h.
 - 3 In the field, traps with virgin females and rubber septa containing 20 mg hexyl butanoate, caught a total of one male in a month. In control traps without hexyl butanoate, 36 males were caught in the same period.
 - 4 In Y-track olfactometer tests, males were not attracted to virgin females when a dispenser with 20 mg hexyl butanoate was placed in the bottle with females. Males were attracted to females when the dispenser was placed downwind from the females, but upwind from the point of male release.
 - 5 These results suggest that males are not repelled by hexyl butanoate, but that this compound inhibits sex pheromone release in females. Application possibilities for pest management are discussed.

Keywords Alarm pheromone, dispersal behaviour, Heteroptera, metathoracic scent gland, Miridae, pest management, sex pheromone inhibition.

Introduction

True bugs are notorious for their defensive secretions when they are attacked or disturbed (Remold, 1963; Carayon, 1971; Staddon, 1986; Aldrich, 1988; Aldrich *et al.*, 1997). Intraspecifically, these secretions may function as an alarm pheromone, causing dispersal and escape from confrontations with predators (Blum, 1985; Leal & Kadosawa, 1992; Leal *et al.*, 1994). As dispersal will reduce the chance of sexual encounters, alarm pheromones may disturb sexual communication.

In adult heteropterans, the metathoracic scent gland is generally described as the gland where defensive secretions are stored or synthesized (Remold, 1963; Carayon, 1971; Games & Staddon, 1973; Staddon, 1986; Aldrich, 1988). The content of this gland has been chemically analysed in many species (Levinson & Bar Ilan, 1971; Games & Staddon, 1973; Aldrich &

Yonke, 1975; Oetting & Yonke, 1978; Staddon & Daroogheh, 1981; Aldrich *et al.*, 1984; Knight *et al.*, 1984; Lockwood & Story, 1987; Kou *et al.*, 1989; Aldrich *et al.*, 1996, 1997; Blatt *et al.*, 1998). Actual toxicity of compounds from this gland for predators was demonstrated only by Remold (1963) and Aryeetey & Kumar (1973). A few other studies have determined that these compounds cause intraspecific dispersal behaviour (Levinson & Bar Ilan, 1971; Lockwood & Story, 1987; Kou *et al.*, 1989; Blatt *et al.*, 1998). When chemical identification of compounds is not succeeded by bioassays, the function of such compounds remain subject to speculation.

In mirids, the content of the metathoracic scent gland is similar among species. For example, the major components in *Lygus lineolaris*, *L. elisus* and *L. hesperus* are hexyl butanoate and (*E*)-2-hexenyl butanoate (Gueldner & Parrott, 1978; Aldrich *et al.*, 1988), in *Pilophorus perplexus* they are butyl butanoate and hexyl butanoate (Knight *et al.*, 1984), and in *Blepharidopterus angulatus* the major components are hexyl hexanoate and (*E*)-2-hexenyl hexanoate (Knight *et al.*, 1984). In

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this family, females attract males by means of a sex pheromone (reviewed by McBrien & Millar, 1999). To our knowledge, no study has determined which, if any, of these compounds may disrupt sexual communication in mirid bugs, and how such disruption would affect sexual attraction of males towards females.

The metathoracic scent gland of *Lygocoris pabulinus* (L.) (Heteroptera: Miridae) contains up to 90% hexyl butanoate (F. P. Drijfhout, unpublished data). In this study, we determined: (A) whether this compound is released by males and females in undisturbed and disturbed conditions, (B) whether hexyl butanoate disrupts sexual attraction of males towards females, and (C) which sex is actually affected by hexyl butanoate, i.e. are the males repelled by this compound, or do females stop attracting males?

Materials and methods

Insects

Lygocoris pabulinus was reared on potted potato plants, cultivar Bintje, in wooden cages in greenhouses ($22 \pm 2^\circ\text{C}$, r.h. = $65 \pm 5\%$, LD 18:6 h), following Blommers *et al.* (1997). Every 2–3 days newly emerged adults were collected from the rearing cages, after which the sexes were isolated in separate rearing cages. In this way, virgin females and males of known age were continuously available (Groot *et al.*, 1998).

Release rates of hexyl butanoate in undisturbed and disturbed bugs

To determine amounts of hexyl butanoate released by *L. pabulinus*, five 5–8-days-old virgin females or males were placed in a 250 mL glass bottle (height 14 cm, diameter 6.5 cm) together with wet oasis (i.e. artificial soil for flowers, used by florists) and pollen. The inlet of these glass bottles were connected to the air flow of the Y-track olfactometer setup, as described below (see also Fig. 1). One hour prior to sampling, the bottles were placed in the set-up to avoid possible release of hexyl butanoate as a result of disturbance before the experiment. After one hour the outlet of the bottle was connected to a glass tube filled with 200 mg Tenax®, on which the headspace was collected during 1 h. To collect headspace from undisturbed bugs, females and males were left untreated for one hour. Headspace from disturbed bugs was sampled by vigorously shaking the bottles for 1 min, 3–4 times during the sampling. After collection, the headspace was thermally desorbed at 250°C (5 min) and analysed by GC (Drijfhout *et al.*, 2000). The release rate of hexyl butanoate was determined by comparing the peak area of hexyl butanoate with that of an external standard.

Disruption of sexual communication

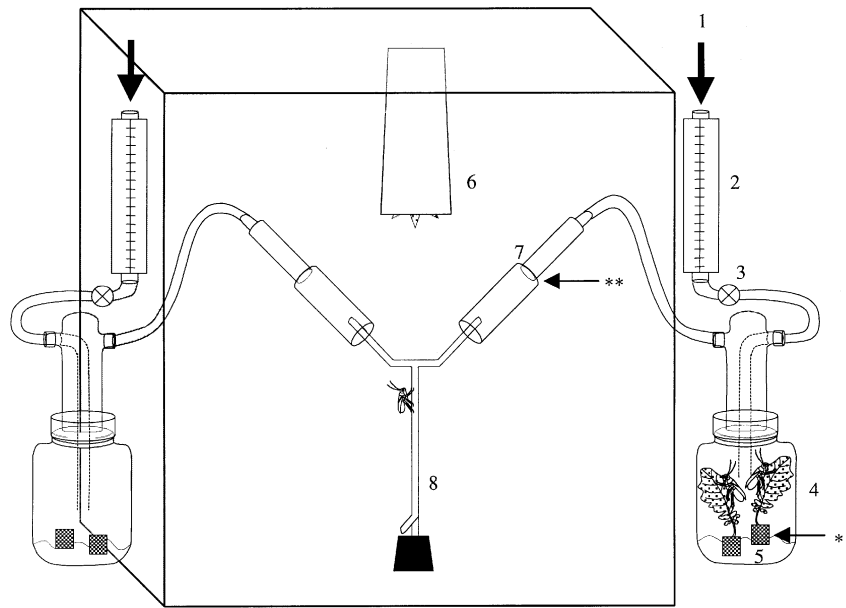
To determine whether hexyl butanoate disturbs sexual communication, a field test was conducted from 6 August to 2 September 1999 at the experimental orchard 'Schuilenburg', Kesteren, The Netherlands. Two kinds of traps with virgin females were tested in order to compare the number of males caught: (a) six traps with rubber septa containing hexyl butanoate, and (b) six traps

with rubber septa without hexyl butanoate. All traps contained three virgin, 5–8-day-old females, caged in a PVC cage (diameter 7 cm, length 7.5 cm, closed at both sides with gauze lids), with pollen and a small potato sprout, of which the roots were wrapped in wet cotton and aluminium foil to delay desiccation. Around each cage with females, two rubber septa were placed, both in front of the gauze lids. These septa were filled with 100 μL dichloromethane. In this solvent 4 mg 2,6-di-*t*-butyl-*p*-cresol ($\approx 20\%$ of the total amount) and 2 mg 2-hydroxy-4-methoxybenzophenone ($\approx 10\%$ of the total amount) were dissolved as antioxidants. Additionally, 20 mg hexyl butanoate was dissolved in the 100 μL dichloromethane when the septa for traps (a) were filled. The septa for traps (b) only contained the antioxidants. All cages were placed in delta traps (30 (length) \times 20 (width) \times 20 (height) cm), after which the 12 traps were arranged alternately in six 0.4 ha plots in apple trees at ± 1.50 m height, with at least 10 m or three rows of apple trees between adjacent traps. Numbers of males caught were counted every 3–4 days. Every week the cages were cleaned and refilled with pollen, new potato sprouts and fresh 5–8-day-old females, and all dispensers were renewed. Twice, the release rate of a rubber septum with 20 mg hexyl butanoate was measured by weighing an empty septum, then weighing the septum filled with the antioxidant and 20 mg hexyl butanoate. Subsequently, the dispenser was placed outside in a delta trap and weighed again after 24, 48 and 62 h.

Are males and/or females affected by hexyl butanoate?

To determine which sex is affected by hexyl butanoate, Y-track olfactometer assays were conducted as described by Visser & Piron (1998), with specific adjustments (Fig. 1). The Y-track was placed in a black box under a halogen lamp (4–12 V DC, 10 VA). To suppress flight intention of males, this lamp was placed in a black socket sealed with a red filter, so that the light intensity at the base of the Y-track was 6.3–6.5 lux only. One hour before each experiment five virgin *L. pabulinus* 5–8-day-old females were placed in a 250 mL glass bottle (height 14 cm, diameter 6.5 cm), together with two small potato sprouts in wet oasis, and pollen. The control glass bottles contained only wet oasis and pollen. Both bottles were placed outside the black box, one on each side, under a desk-lamp with a 25 W light bulb. Three situations were tested: (I) a similar rubber septum as used in the field test, containing 20 mg hexyl butanoate, was placed in the bottle together with the females, (II) the rubber septum with 20 mg hexyl butanoate was placed downwind from the females, in the glass tube slipping over the Y-track, behind gauze (indicated by the arrow in Fig. 1), (III) a rubber septum was filled with only 0.2 mg hexyl butanoate (i.e. 10 times less than used above, arbitrarily chosen to determine if reactions would be similar at lower concentration), and placed in the bottle with the females. Hence, in situations (I) and (III) females were in the odour of hexyl butanoate, or in situation (II) they were not. For males, situations (I) and (II) did not differ in the sense that they were offered 20 mg hexyl butanoate together with the odour of females in both situations. In situation (III) males were offered a lower dose of hexyl butanoate. All experiments were repeated three to five times. One test consisted of 20 males, tested individually, that were offered an odour source vs. control. After

Figure 1 Schematic drawing of open Y-track olfactometer positioned in black box, with its open front covered by a black cloth during experiments. (1) Incoming clean air, (2) air flow control, (3) open/close valve, (4) glass bottle under a desk lamp (25 W), (5) square of wet oasis on a bottom of wet cotton, (6) lamp in black cylinder with red filter, (7) glass tube with gauze (arrow), (8) brass rod (diameter 4 mm, length to junction 13 cm) with extension at base for placing a male. Arrows indicate position of rubber septum in experiments I and III (*) and experiment II (**).



testing five males, the bottles, glass tubes and connecting tubes were exchanged among the olfactory arms to correct for possible positional effects. The release rate of hexyl butanoate from the septa in the Y-track olfactometer was determined by placing one dispenser with hexyl butanoate in a similar glass bottle as used in the tests. The inlet of this glass bottle was connected to the air flow of the olfactometer set-up, the outlet to a glass tube filled with Tenax®. Headspace of a septum with 20 mg hexyl butanoate was collected for 15 min, headspace of a septum with 0.2 mg hexyl butanoate was collected for 30 min. The release rates were determined by comparing the peak area of hexyl butanoate with that of an external standard (as described above).

Results

Release rates of hexyl butanoate in undisturbed and disturbed bugs

Undisturbed and disturbed males released similar amounts of hexyl butanoate, about 10 ng/h (Fig. 2), which was the smallest amount that could be measured. Once, disturbed males released 45 ng/h. Undisturbed females released 10–92 ng/h hexyl butanoate (mean 47 ng/h). Upon disturbance the released amount was highly variable: the lowest amount measured was 25 ng/h, the highest was 1081 ng/h.

Disruption of sexual communication

In traps with septa containing hexyl butanoate, only one male was caught over one month. In traps with septa containing only the antioxidants, 36 males were caught in the same period (Fig. 3). The release rate of a septum with 20 mg hexyl butanoate in the field was 3.7–3.9 mg/day, which is 154–163 µg/h.

Are males and/or females affected by hexyl butanoate?

In the Y-track olfactometer, when a dispenser with 20 mg hexyl butanoate was placed in the odour source bottle with females, males did not preferentially walk towards the females (Fig. 4I). Overall, significantly more males walked towards the control instead, suggesting that males are repelled by the odour source. When a similar rubber septum with hexyl butanoate was placed in the glass tube downwind from the females, significantly more males walked towards the females than towards the control (Fig. 4II), which resembles the reaction towards virgin females in this bioassay (Groot & Visser, 2000). As attraction was restored in the latter set-up, experiments with 0.2 mg hexyl butanoate dispensers were only conducted with a dispenser placed in the bottle with females. Females with these dispensers were also attractive to males (Fig. 4III). The release rate of a septum with 20 mg hexyl butanoate was about 92 µg/h, whereas the release rate of a septum with 0.2 mg hexyl butanoate was about 1 µg/h.

Discussion

Hexyl butanoate is present in similar amounts in both sexes of *L. pabulinus*, one adult may contain up to 100 µg (F. P. Drijfhout, unpublished data). Males do not seem to emit the scent upon disturbance, such as when the glass bottles containing them were vigorously shaken. Females did release additional hexyl butanoate upon disturbance, although a maximum of 1 µg released by five females in one hour is only a fraction of the amounts present in these females. Possibly, upon sudden attack much larger amounts can be released. If it would be possible for females to release their total gland content at once, a maximum of 300 µg would be released by the three caged females in the field, which is similar to the amount released by the two rubber

septa in one hour. However, males in the field are not constantly exposed to odour released from a trap. This only happens when they are in the vicinity of the trap. A male, flying in the surroundings of a trap with two dispensers, each with 20 mg hexyl butanoate, would experience about 5–5.5 µg hexyl butanoate in 1 min. In the Y-track olfactometer, in 1 min each male would be exposed to about 1.5 µg hexyl butanoate from the 20 mg dispenser. Although males preferentially walked towards the control instead of towards females with this amount of hexyl butanoate (experiment C I), males were attracted to the odour source when the dispenser was placed downwind from the females (experiment C II). Hence, hexyl butanoate was not directly responsible for the repellent effect in *L. pabulinus* males. When in the vicinity of attractive females, males seem to disregard the large concentration of hexyl butanoate.

Females were no longer attractive to males when exposed to a high concentration of hexyl butanoate. This suggests that large amounts of hexyl butanoate inhibit sex pheromone release in *L. pabulinus* females. Females were unable to disperse away from the scent in the field cages or in the Y-track experiments. In the field traps, females were constantly exposed to the scent for a whole week. Under such circumstances, habituation or sensory

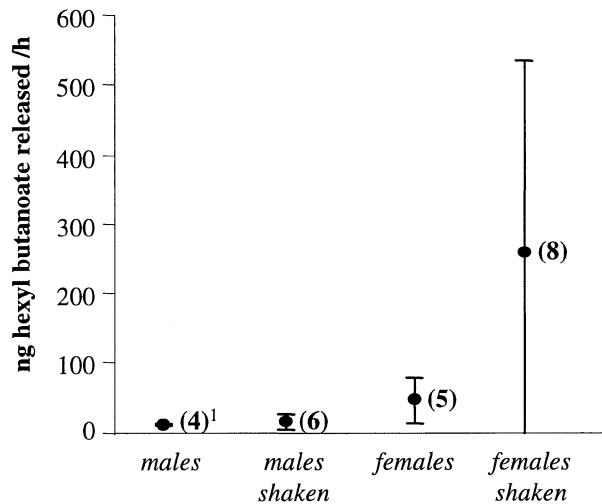


Figure 2 Amount (\pm c.i.) of hexyl butanoate, released from five males or females in 1 h, in glass bottles that have been shaken or not. ¹Number of replicates. See text for further explanation.

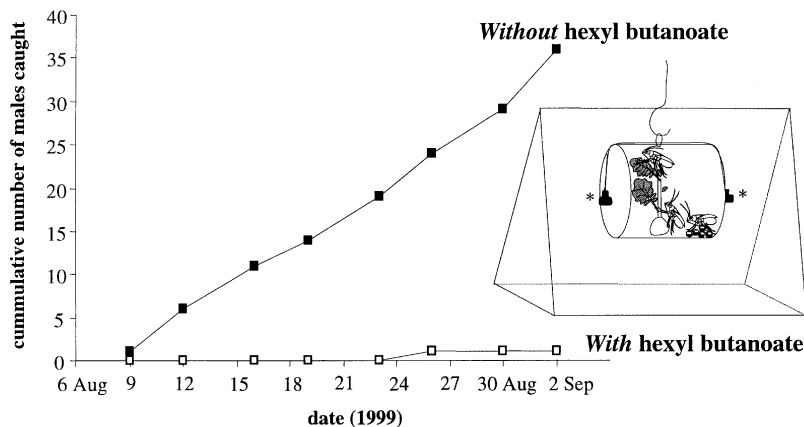


Figure 3 Trap catches of virgin female traps with two rubber septa (*) loaded either with 20 mg hexyl butanoate each, or with antioxidants only. See text for further explanation.

adaptation to hexyl butanoate is likely to occur (Calam & Youdeowei, 1968; Oetting & Yonke, 1978; Blum, 1985; Lockwood & Story, 1987; El-Agamy & Haynes, 1992; Blatt *et al.*, 1998). If habituation or sensory adaptation would have occurred, males would have been caught in the traps with hexyl butanoate some days after females were placed in the traps. As in one month only one male in total was attracted, such adaptation to the scent did not seem to occur in females.

Because of the persistent effect of hexyl butanoate in the field, this compound may be used to inhibit matings in fruit orchards, where *L. pabulinus* is a pest. Its pest status is caused by the nymphs, emerging in spring from overwintering eggs laid in fruit trees. These nymphs feed on plant ovaries and young fruitlets, which cause russeted malformations on the fruits (Blommers, 1994). *Lygocoris pabulinus* oviposits its overwintering eggs in autumn, after migration from herbaceous plants to woody hosts (Petherbridge & Thorpe, 1928; Küllenberg, 1946; Southwood & Leston, 1959). When adults of the summer generation could be prevented to mate so that fertile eggs would not be deposited, no nymphs would emerge the following spring to cause damage. Population densities of *L. pabulinus* during remigration to woody hosts seem to be low. Bus *et al.* (1985) caught a maximum of 316 green capsid bugs in one 0.4 ha plot in 2 months. Blommers *et al.* (1988) caught on average about five males per week per virgin female-baited trap, and in 1998 we caught an average of four males per week in similar traps (A. T. Groot, unpublished data). In such low densities, it may be feasible to effectively disrupt mating. Before and during migration to woody hosts, large amounts of hexyl butanoate dispensers should be introduced in fruit orchards. Also, dispensers should be placed at potential summer sites, such as potato fields, because mating may occur before migration. When alternative oviposition sites for winter eggs are offered in the periphery of the orchard, for example black currant plants (Wightman, 1969), the density of damaging nymphs the following spring in orchards may be minimized.

Hexyl butanoate may not only be effective in disrupting sexual attraction in *L. pabulinus*, but also in other mirids. During the same period in which we caught 36 *L. pabulinus* males in virgin female traps without hexyl butanoate, we also caught 28 other male bugs (*Phytocoris longipennis* Flor, *P. dimidiatus* (Kirschbaum) and *P. varipes* Boheman, all identified by B.

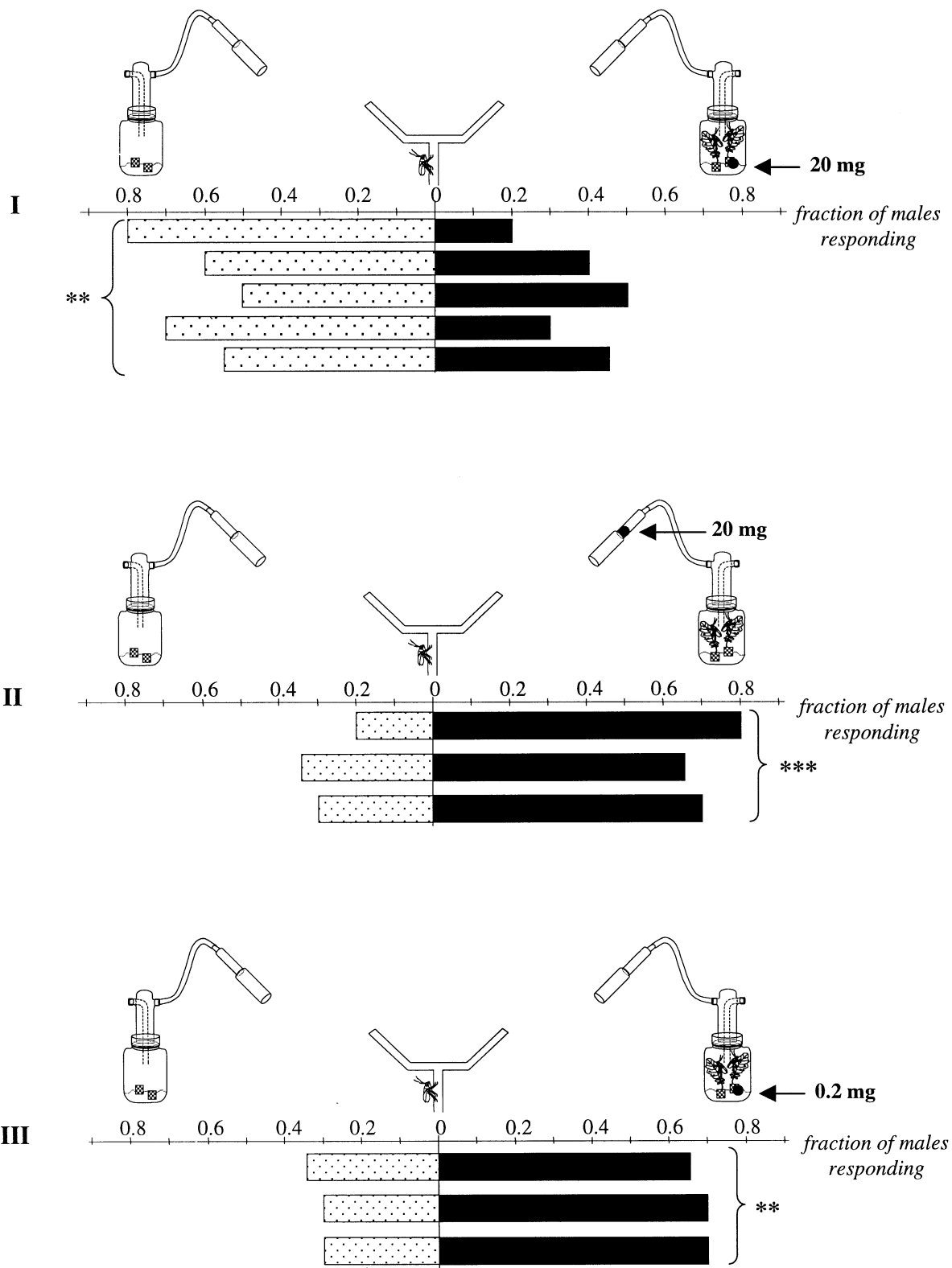


Figure 4 Y-track olfactometer tests. Odour sources tested: five females and a rubber septum, filled with 20 mg hexyl butanoate, (I) placed in the bottle with females, (II) placed downwind from the females, (III) filled with 0.2 mg hexyl butanoate, placed in the bottle with females (arrows indicate position of septa). Each bar represents one test with 20 males choosing. Preferences for odour sources were determined by using the two-sided binomial test for the mean results (after summing the replicates); ***0.01 < *P* < 0.001.

Aukema, Wageningen, The Netherlands), whereas the traps with hexyl butanoate were devoid of these bug species.

In conclusion, hexyl butanoate appears to be repellent for *L. pabulinus* males in the absence of sex pheromone, but not in the presence of pheromone. Hexyl butanoate inhibits sex pheromone release in females. As sex pheromone inhibition will reduce sexual encounters, and potentially the amount of eggs oviposited, this compound has potential for pest management. To optimize such a management strategy, the time and place of matings in the field before ovipositing overwintering eggs should be studied in detail.

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