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# Disruption of the odour-mediated mating behaviour of *Plodia interpunctella* using high-frequency sound

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**Key words:** antipredator behaviour, calling behaviour, *Plodia interpunctella*, Lepidoptera, Pyralidae, mating disruption, orientation, pest control, sex pheromone, ultrasound

## Abstract

Indian meal moths, *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae), have ears which are sensitive to high-frequency calls produced by echolocating, insectivorous bats. The influence of artificially generated, high-intensity, ultrasound signals (25 kHz, 106 dB SPL at 1 m distance) on different parameters involved in the odour-mediated mating behaviour of this species and its potential use in population control was investigated. All moths flying towards olfactory cues in flight tunnel experiments reacted strongly to a 1 s ultrasound pulse by cessation of flight and falling out of the odour plume. The source contact proportion of both male moths orienting towards the female-produced sex pheromone and of mated female moths orienting towards an oviposition cue was reduced by 40%, compared to unexposed moths. Calling females responded to the sound by retraction of the ovipositor or by falling to the ground. Long-term exposure to repetitive pulses of ultrasound suppressed female calling by up to 27%. Furthermore, mating in plastic tents was disrupted by up to 58% in ultrasound-treated tents using different sound regimens, compared to control tents. The results are discussed in relation to the potential use of ultrasound technology for the population control of pyralid stored product pests.

## Introduction

Ultrasonic hearing exists in several families of moths for the detection and avoidance of attacking echolocating bats (Scoble, 1992; Fullard & Yack, 1993). As many of the economically important lepidopteran species belong to 'eared' families, manipulation of their acoustic sensory systems may provide new ways of controlling them. Whereas much research has focused on the use of olfactory cues, especially pheromones, for the population control of moths, few studies have been conducted which have evaluated the potential of acoustic signals for the same purpose. So far, attempts to control moth populations by high-frequency sound have been restricted to a few agricultural species with varying degrees of success in the field (Belton & Kempster, 1962; Agee & Webb, 1969). Oviposition in *Trichoplusia ni* Hübner was reduced by up to 66% using high-frequency sound (Payne & Shorey, 1968), whereas no effect was observed for *Heliothis zea*

Boddie under a similar sound regimen (Shorey et al., 1972). The lack of a disruption effect was explained by the sound shadows produced by high-growing plants, high humidity, equipment failure, and habituation of moths to the stimulus. Little information is available on the use of high-frequency sound to control indoor pests. This habitat, with smaller areas to be treated, more stable meteorological conditions, and a lower rate of immigration of moths compared to agricultural conditions, may create an optimal environment for control by sound.

Many species of the family Pyralidae, including Indian meal moths, *Plodia interpunctella* Hübner, are serious pests of stored products. As their populations have developed resistance to insecticide treatments, there is a need for alternative methods of controlling this species (Arthur et al., 1988). The sex pheromone has been identified and used in both field trapping and small-scale mating disruption experiments (Kuwahara et al., 1971; Zhu et al., 1999; Ryne et al., 2001). Both sexes of *P. interpunctella* have a pair of tympanic hearing organs located on the first abdominal segment (Mullen & Tsao, 1971), which are thought to function both for the detection of bat cries and for intraspecific communication (Trematerra & Pavan, 1995).

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In this study the effect of high-intensity ultrasound signals on different behaviours involved in the odour-mediated mating system of *P. interpunctella* were investigated: (i) female calling behaviour, (ii) male flight response to sex pheromones, and (iii) female flight response to odour cues involved in oviposition. In addition, experiments were also done to evaluate the potential for suppressing mating using high-frequency sound.

## Materials and methods

### Insects

Males and females of *P. interpunctella* were obtained from a laboratory culture maintained at the Department of Ecology, Lund University, Sweden, for ca. 40 generations and originally established from a laboratory culture at the Danish Pest Infestation Laboratory at Lyngby, Denmark. Moths from the Central Science Laboratory, UK, were incorporated into the culture after ca. 20 generations. Larvae were reared on an artificial diet described by Zhu et al. (1999) at 24 °C and 60% r.h., and pupae were separated according to sex. Moths used in orientation and calling behaviour experiments were placed in a reversed L17:D7 photoperiod at 22 °C and 60% r.h., and moths used in mating suppression experiments were placed in a natural L17:D7 photoperiod at 24 °C and 60% r.h. Two- to five-day-old moths were used in all experiments.

Odour-mediated flight behaviour was investigated for both female and male moths in wind tunnel experiments. To obtain mated females, male–female pairs were placed in 500 ml plastic jars. Mated females were tested 1 day after mating.

### Odour stimuli

Female-produced sex pheromone was used as an odour cue for males in flight tunnel experiments. The pheromone consisted of four components: (*Z,E*)-9,12-tetradecadienyl acetate (Z9,E12-14:OAc), (*Z,E*)-9,12-tetradecadienal (Z9,E12-14:Ald), (*Z,E*)-9,12-tetradecadienol (Z9,E12-14:OH), and (*Z*)-9-tetradecenyl acetate (Z9-14:OAc) in the ratios 100 : 11 : 18 : 12 (Kuwahara et al., 1971; Zhu et al., 1999). Synthetic Z9,E12-14:OAc, Z9,E12-14:OH, and Z9-14:OAc were purchased from DLO Plant Research International PRI, Wageningen, The Netherlands, and Bedoukian Research Inc., Danbury, USA, whereas Z9,E12-14:Ald was synthesised from Z9,E12-14:OH at the Department of Chemistry, Royal Institute of Technology, Stockholm, Sweden. A rubber septum (red sleeve, 16 × 9 mm, catalogue no. 1780J07, Thomas Scientific, Swedesboro, USA) was used as a dispenser. A synthetic four-component blend diluted in redistilled hexane was applied on the dispenser at 5 µg of the main component Z9,E12-14:OAc and placed in a

fume hood for 3 h to allow the hexane to evaporate. The septum was stored in a freezer (−18 °C) between flight tunnel experiments.

An artificial diet with larvae was used as the odour stimulus for females in the flight tunnel experiments. Food sources infested by conspecific larvae have been shown to significantly increase the upwind flight of females compared to food alone (Phillips & Strand, 1994). Five 5th instar larvae were placed in 10 g of the artificial diet described by Zhu et al. (1999). The diet was placed in a 30 ml plastic jar and used in flight experiments the following day.

### Sound stimuli

The acoustic cues were presented from modified electronic sound emitters, i.e., dog whistles (Pet Agree, K-II Enterprises, Camillus, NY, USA). The sound emitted had a peak frequency of 25 kHz and an intensity of 106 dB SPL at 1 m (sound levels are given as dB pe (peak equivalent) SPL (sound pressure levels) relative to 20 µPa r.m.s.). Although the peak frequency was outside the range of best hearing of *P. interpunctella* (40–70 kHz), the sound pressure level was still far above the threshold necessary for flight cessation (G.P. Svensson, C. Löfstedt, and N. Skals, unpubl.).

### Female calling behaviour

Both the short- and long-term effects on calling behaviour (extrusion of the ovipositor) of females exposed to ultrasound were investigated. The short-term effect of the ultrasound pulse on calling behaviour was studied by placing individual females in 250 ml glass cylinders prior to the scotophase. One to two hours into the scotophase, the stimulus (the same as in flight tunnel experiments) was presented manually at a distance of 20 cm to a calling female and its behaviour was recorded.

The long-term effect on calling behaviour was analysed by introducing the females to a paper box (30 × 30 × 30 cm) with two opposite walls covered with cloth, which attenuated the sound by less than 2 dB. Repetitive pulses (1 s) of ultrasound were presented from a sound emitter controlled by a custom-built pulse generator (interpulse length: 10 s) at 150 cm distance. Conditions during the trials were: temperature 21–22 °C, 25–50% r.h., and light intensity 5 Lux. Each day, ten females were introduced into the box 1.5 h before the onset of the scotophase and observed every hour, starting 1 h before the end of the photophase and ending 5 h into the scotophase. The experiments were repeated eight times, i.e., a total of 80 females were used. The difference in proportions of calling females was compared between groups using repeated measurement ANOVA on arcsin  $\sqrt{x}$  transformed data.

### Flight tunnel experiments

The effect of a single pulse of high-frequency sound on the odour-mediated upwind flight behaviour of *P. interpunctella* was tested in a  $0.9 \times 0.9 \times 3$  m Plexiglas flight tunnel as described by Valeur & Löfstedt (1996). The floor was covered by a white paper with scattered dark spots ( $\varnothing$  10 cm). Moths were tested at: temperature 21–22 °C, 25–35% r.h., a wind speed of  $0.3 \text{ ms}^{-1}$ , and a light intensity of 5 Lux. The odour source was placed at 40 cm height and 2.5 m upwind from the release point. A sound emitter was placed at 40 cm height and 100 cm downwind from the odour source. The opposite wall of the flight tunnel was covered with a  $90 \text{ cm} \times 30 \text{ cm}$  cloth to attenuate reflections of the ultrasound stimulus.

Moths were introduced to the flight tunnel room before the onset of the scotophase. One to three hours into the scotophase, moths were individually transferred to small glass cylinders ( $8 \text{ cm} \times \varnothing 2.5 \text{ cm}$ ) with one end covered with a net and exposed to the odour plume for at least 5 s. After they had left the cylinder their behaviour was observed for 3 min. An ultrasound pulse (1 s) was delivered manually from the sound emitter when a moth was ca. 30 cm from the whistle. On each day, 5–12 individuals per group (exposed or unexposed) were tested. The percentages of moths reaching the odour source were compared between groups using  $\chi^2$  analysis. In addition, the source location times were compared between groups using unpaired t-tests.

### Mating frequency

Mating disruption experiments were conducted in a greenhouse at Lund University, Sweden. Transparent plastic tents ( $2.5 \times 2.5 \times 2.5$  m) purchased from the Central Science Laboratory, Slough, UK, were used. Ultrasound pulses (1 s long) were delivered automatically from two sound emitters placed at 2.4 m height on opposite walls in each tent. The sound emitters were controlled by a custom-built pulse generator and the stimulus was delivered asynchronously from the two sources. In the first trial, an interpulse length of 5 s was used, i.e., each apparatus emitted a pulse every 10th second. Conditions during the experiments were: temperature: 20–21 °C, 60–70% r.h., and ambient light, thus matching the light conditions in the rearing room. In the second trial, the interpulse length was decreased to 2.5 s, i.e., each apparatus emitted a pulse every 5th second. Conditions during the experiments were: temperature: 18–21 °C, 60–70% r.h., and ambient light.

Moths (ten of each sex) were introduced to the tents and recaptured the following day. Females were frozen and subsequently dissected and checked for the presence of spermatophores indicating a successful mating. The proportions of mated females were compared between groups using unpaired t-tests on  $\arcsin \sqrt{x}$  transformed data.

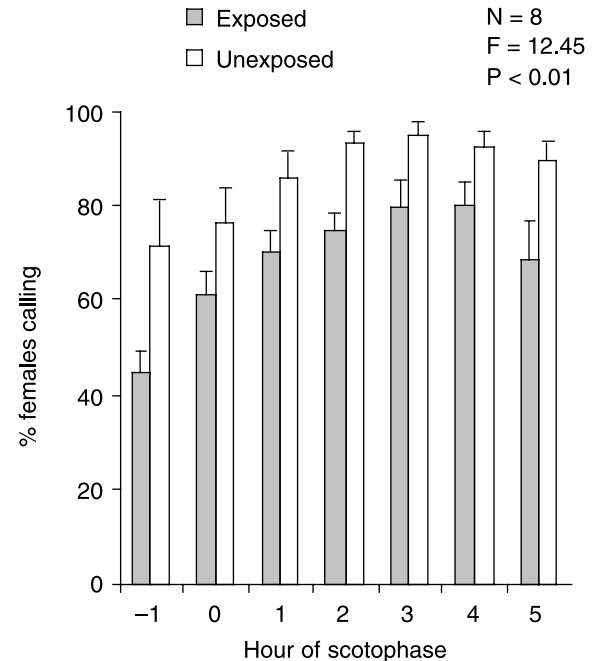
### Sound regimen analyses

During each experimental day, a microphone (Ultrasound Detector D 240x, Pettersson Elektronik AB, Sweden), transforming high-frequency stimuli to audible sound, was used to check sound emissions from the sound emitters. Sound intensities from different positions in the flight tunnel and in the plastic tents were measured by a 0.25-inch microphone Type 40 BF (frequency response:  $\pm 1$  dB at 10 Hz–40 kHz) without grid, a preamplifier Type 26 AC, and a measuring amplifier Type 12 AK (frequency response:  $\pm 1$  dB at 2 Hz–200 kHz) (G.R.A.S. Sound and Vibration, Vedbæk, Denmark). In order to calculate the intensity of the sounds, the microphone was calibrated against a sound calibrator Type 42 AB (G.R.A.S. Sound and Vibration, Vedbæk, Denmark).

## Results

### Female calling behaviour

All 15 calling females responded to a single pulse of ultrasound. Seven retracted their ovipositor and eight fell to the ground. Repetitive stimulation of ultrasound significantly suppressed female calling ( $F = 12.45$ ,  $P < 0.01$ , Figure 1) throughout 5 h of the scotophase. The reduction in calling frequency ranged from 13% to 27%.



**Figure 1** Effect of exposure to repetitive 1 s pulses of ultrasound (25 kHz, 106 dB, at 1 m distance, interpulse length: 10 s) on the calling behaviour of female *P. interpunctella*. Percentages (mean  $\pm$  SE) of calling females at different times before and during the scotophase. Ten females were used in each of the eight replicates.

### General flight response to ultrasound

In the flight tunnel, all individuals of both sexes responded in a stereotypic way to the acoustic stimulus by cessation of flight, i.e., diving to the flight tunnel floor. A similar response was observed for moths soon after their introduction to the greenhouse tents.

### Male flight response

Although all the males interrupted their upwind flight when exposed to the ultrasound, they resumed flight within a few seconds. However, not all moths reached the odour source within the time limit of 3 min. Source contact was reduced by 40% for ultrasound-exposed males compared to the unexposed ones (sound-exposed: 53%, unexposed: 88%,  $\chi^2 = 14.2$ , d.f. = 1,  $P < 0.001$ , Figure 2A). Moreover, the source location time was significantly increased for sound-exposed males compared to unexposed (sound-exposed:  $49 \pm 6$  s, unexposed:  $30 \pm 5$  s,  $T = 3.33$ , d.f. = 58,  $P < 0.01$ , Figure 2B).

### Female flight response

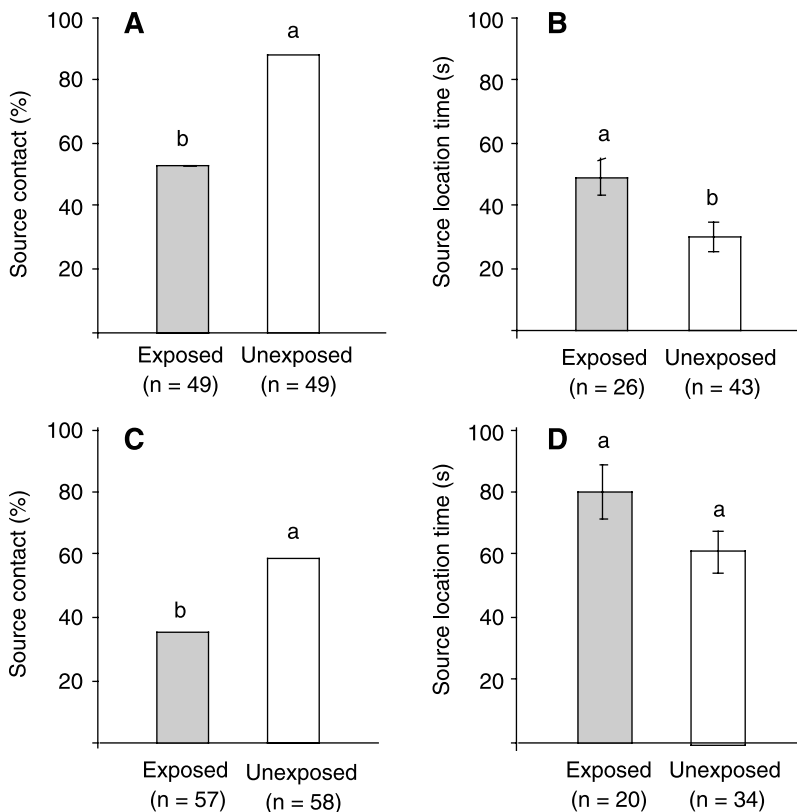
In preliminary trials, the effect of mating status on female response to the odour stimulus was investigated. Unmated females which initiated upwind flight did not differ in source location time compared to mated females (mated:

$61 \pm 6$  s, unmated:  $71 \pm 8$  s,  $T = 1.00$ , d.f. = 80,  $P > 0.05$ ). However, the percentage of females reaching the odour source was significantly higher for mated compared to unmated (mated: 62%, unmated: 40%,  $\chi^2 = 7.92$ , d.f. = 1,  $P < 0.01$ ). This difference was caused by a large proportion of unmated females (> 25%) initiating calling behaviour instead of heading upwind towards the odour source. Based on these results, only mated females were used in flight experiments using ultrasound.

Significantly fewer mated females reached the odour source when stimulated with ultrasound compared to unexposed mated females (sound-exposed: 35%, unexposed: 59%,  $\chi^2 = 6.4$ , d.f. = 1,  $P < 0.05$ , Figure 2C), which corresponded to a 40% reduction in source contact. No significant difference was observed in source location time between treatments (sound-exposed:  $84 \pm 9$  s, unexposed:  $64 \pm 7$  s,  $T = 1.77$ , d.f. = 52,  $P > 0.05$ , Figure 2D). Many female moths were sitting motionless on the floor for several minutes after exposure to high-frequency sound.

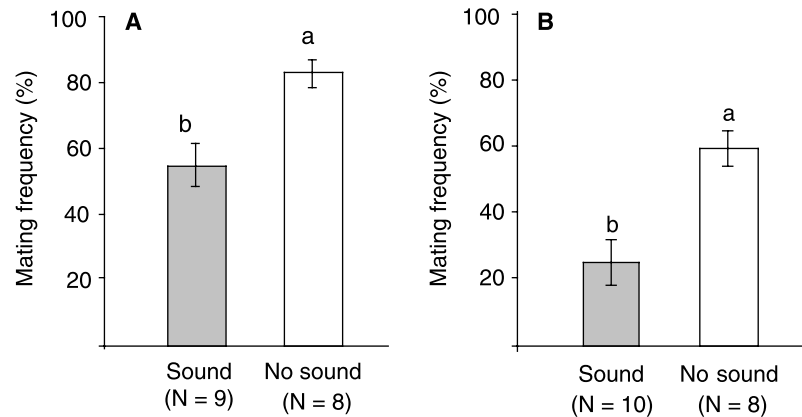
### Mating frequency

Repetitive pulses of high-frequency sound had a significant effect on mating ability of the moths in the tents. In the first trial, using an interpulse length of 5 s, the mating frequency of ultrasound-exposed moths was  $55 \pm 6\%$  compared to



**Figure 2** Effect of exposure to a 1 s pulse of ultrasound (25 kHz, 106 dB, at 1 m distance) on the odour-mediated flight behaviour of *P. interpunctella*. (A) Source contact and (B) source location time (mean  $\pm$  SE) of males flying towards a sex pheromone cue. (C) Source contact, and (D) source location time (mean  $\pm$  SE) of females flying towards an oviposition cue. Bars with different letters are statistically different.

**Figure 3** Effect of exposure to repetitive 1 s pulses of ultrasound (25 kHz, 106 dB, at 1 m distance) on the mating ability of *P. interpunctella* under semifield conditions. Percentages (mean  $\pm$  SE) of mated females when sound was emitted with an interpulse length of (A) 5 s and (B) 2.5 s. Bars with different letters are statistically different.



83  $\pm$  4% for the unexposed moths ( $T = 3.39$ , d.f. = 15,  $P < 0.01$ , Figure 3A), which corresponded to a mating suppression of 34%. In the second trial, using an interpulse length of 2.5 s, the mating frequency of ultrasound-exposed moths was 25  $\pm$  7% compared to 59  $\pm$  6% for unexposed moths ( $T = 3.19$ , d.f. = 16,  $P < 0.01$ , Figure 3B), which corresponded to a mating suppression of 58%.

## Discussion

This study has shown that the different odour-mediated behaviours of *P. interpunctella* can be manipulated by a general ultrasound stimulus emitted at high intensity from dog whistles. In flight tunnel experiments, both males and females responded in a stereotypic way to an acoustic stimulus by the cessation of flight. Our results show that *P. interpunctella* have retained ultrasonic hearing and associated defence behaviours, in spite of the lack of predation pressure from bats in indoor facilities. However, the shift from the outdoor habitat is recent and individuals disperse during warmer periods of the year, thus exposing them to the threat of echolocating bats. Other pyralid moths, e.g., *Galleria mellonella* L. (Skals & Surlykke, 2000; N. Skals, P.G. Valeur, and C. Löfstedt, unpubl.), *Cadra cautella* Walker and *Ephestia kuehniella* Zeller (G.P. Svensson, N. Skals, and C. Löfstedt, unpubl.) show a similar behaviour when exposed to high-frequency sound.

The source location time was significantly increased after ultrasound exposure in males but not in mated females, which was unexpected according to the difference in post-exposure behaviour mentioned above. A large variation was observed in ability to relocate and orient in the odour plume after exposure to the acoustic cue. Moths that elicited normal orientation flight before ultrasound exposure showed arrested flight for several minutes after relocating the odour plume, indicating a long-term effect of the ultrasound exposure on the odour-mediated flight

response. The strong behavioural response observed to ultrasound in females was not surprising. Although calling females may not be as vulnerable to aerially hawking bats as mate searching males are, females also spend long periods in flight, e.g., when searching for oviposition sites.

Few studies have investigated the trade-off between pheromone-mediated flight behaviour and predator avoidance in male moths. Baker & Cardé (1978) observed that male gypsy moths, *Lymantria dispar* L., deviate from a sex pheromone plume when exposed to high-frequency sound from jingling keys. Acharya & McNeil (1998) found a correlation between the level of predation risk and the number of aborted flights of male *Ostrinia nubilalis* Hübner and *Pseudaletia unipuncta* Haworth, when exposed to high-frequency sound cues. N. Skals, P.G. Valeur, and C. Löfstedt (unpubl.) studied the evasive behaviour of male *Agrotis segetum* Denis and Schiffermüller exposed to high-frequency sound when orienting in a sex pheromone plume. Males exposed to ultrasound left the odour plume less often when close to the pheromone source, as compared to males, which were further away from the source.

The majority of *P. interpunctella* females retracted their ovipositors when exposed to a single pulse of ultrasound, but started to call again after a few seconds. Acharya & McNeil (1998) obtained similar results on female *O. nubilalis*. A high proportion (95%) stopped calling as a short-term effect of ultrasound exposure. Our study showed a suppression of female calling in *P. interpunctella* by 27% in response to repetitive pulses of ultrasound, suggesting a limited long-term effect of the treatment on calling behaviour.

A significant suppression of mating was achieved by the use of ultrasound in tent experiments. The effect seemed to be stronger when using a 2.5 s interpulse length regimen compared to a 5 s interpulse length regimen. However, it is hard to compare the experiments as the overall mating activity differed between them, probably due to a few degrees difference in average temperature in the tents. In

an earlier study (Ryne et al., 2001) where sex pheromone was applied as disruptant using the same type of tents and moth density, mating frequencies were reduced by up to 93%, indicating a stronger disruption potential when using olfactory cues compared to acoustic ones. However, the use of ultrasound to reduce pest damage should be investigated further, since other sound regimens may be more effective. Other pyralid moths infesting stored products, e.g., *E. kuehniella* and *C. cautella*, show similar antipredator behaviour as *P. interpunctella*. In addition, exposing these species to high-frequency sound may interfere with their short-range ultrasound sexual communication. By fine-tuning the system using stimuli mimicking the attack calls by echolocating bats, auditory-based technology alone or in combination with pheromones could make an important contribution to integrated pest management.

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