Behavioral and reproductive effects of ultrasound on the Indian meal moth, *Plodia interpunctella*

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Abstract

The daily activity patterns of adult movement, female calling, and mating of the Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), were examined both in the absence and presence of ultrasound. Moths were exposed to ultrasound from a commercial ultrasonic device (Cix 0600) that produces constant sound patterns, and from a device developed at Kansas State University (KSU device) that produces random sound patterns. Daily activity patterns of adult movement, female calling, and mating followed a similar trend in the absence or presence of ultrasound. Female calling and mating, both in the absence and presence of ultrasound, primarily occurred during scotophase (21.00–07.00 hours). Ultrasound from the two devices significantly reduced the frequency of female calling and mating relative to unexposed moths. Consequently, the number of spermatophores transferred by males to females and egg production were lower in females exposed to ultrasound compared with unexposed females. In the absence of ultrasound, female *P. interpunctella* mated 2.9 times, resulting in 2.8 spermatophores/female. In the presence of ultrasound from the Cix 0600 device, a female mated 2.1 times and had 1.7 spermatophores. Corresponding values for the KSU device were 1.9 and 1.4, respectively. In the absence of ultrasound, 78% of the matings lasted 30–90 min, whereas in the presence of ultrasound 45–58% of the matings lasted either less than 30 min or more than 90 min. Moths exposed to ultrasound laid 96–130 eggs female–1 compared with 229 eggs female–1 for unexposed moths. Ultrasound did not affect the pre-oviposition period and adult longevity of *P. interpunctella*.

Introduction

Aerial hawking bats capture flying insects using echolocation signals (Fullard, 1998). The sound frequencies used by the echolocating bats generally range from 20 to 100 kHz (Miller & Surlykke, 2001). Many lepidopterous moths have tympanic organs for detecting the ultrasound produced by insectivorous bats (Roeder & Treat, 1957; Roeder, 1967; Mullen & Tsao, 1971; Fullard, 1998; Miller & Surlykke, 2001). Moths use ultrasound for intraspecific communication such as calling, courtship, territorial proclamation, and for predator avoidance (Spangler, 1988; Trematerra & Pavan, 1995; Acharya & McNeil, 1998; Fullard, 1998; Conner, 1999; Fullard et al., 2003; Skals et al., 2003). In flight, many moths perform evasive maneuvers when exposed to ultrasounds (Baker & Cardé, 1978; Acharya & McNeil, 1998).

The males and females of the Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), have tympanic organs on the lateral sides of their first abdominal segment (Mullen & Tsao, 1971). These moths also use ultrasound during pheromone-mediated courtship behavior (Trematerra & Pavan, 1995). In enclosure tests in the laboratory, ultrasound produced from a commercial device, Cix 0600 (Huang et al., 2003), and an experimental device developed at Kansas State University (KSU device) (B. Subramanyam, F. Huang & R. Taylor, unpubl.), significantly reduced the spermatophore transfer and progeny production of *P. interpunctella*. Svensson et al. (2003) also reported that ultrasound signals modified from a dog whistle (25 kHz) adversely affected the female calling behavior and mating success of *P. interpunctella*. The present study was...
designed to quantitatively analyze the effects of ultrasound produced by the Cix 0600 and KSU devices on adult movement, courtship and mating behaviors, and the reproduction of *P. interpunctella*, an economically important insect pest of stored raw grains and processed foods worldwide (Sinha & Watters, 1985).

**Materials and methods**

**Insects**

Cultures of *P. interpunctella* were reared on a chicken mash diet (Subramanyam & Cutkomp, 1987) in growth chambers (Percival Scientific, Perry, Iowa, USA) at 28 °C, 65% r.h., and a L14:D10 cycle. Corrugated paper spools, placed above the diet in culture jars, served as pupation sites for the wandering larvae (Huang & Subramanyam, 2003). Pupae were collected from the paper spools and sexed using characters described by Butt & Cantu (1962). Male and female pupae were placed in separate 0.95-l glass jars and newly eclosed virgin males and females (0–6 h old) were used in all tests.

**Ultrasonic devices and sound measurements**

Two ultrasonic devices were evaluated, Cix 0600, a commercial device manufactured by Weitech, Inc., Sisters, Oregon, USA (Huang et al., 2003), and the KSU device that was developed in the Electronics Design Laboratory, Kansas State University, Manhattan, Kansas, USA (Bh. Subramanyam, F. Huang & R. Taylor, unpubl.).

Ultrasonic properties were measured using a Bruel and Kjaer type 4939 condenser microphone (Bruel and Kjaer North America, Norcross, Georgia, USA), a B&K type 2670 preamplifier and a B&K Nexus conditioning amplifier. Data were captured using a laptop computer equipped with a DAQCARD-AI-16E-4 acquisition card (National Instruments, Austin, Texas, USA). Data were collected at 200 000 samples/s and had a 12-bit resolution. Sound pressure level (SPL) measurements were based on 10 readings at any given position. SPL measurements were expressed in decibels with respect to 20 micropascals [µP] (0 dB = 20 µP).

Huang et al. (2003) reported the ultrasound frequencies and SPLs produced by the Cix 0600 device. The device generated peak frequencies at 21, 25, and 35 kHz with a mean ± SE SPL of 98.4 ± 0.4 dB at a distance of 50 cm from the sound source. The waveform plot generated by this device had a 0.123 second sound cycle.

All sound parameters generated by the KSU device are fully programmable via a computer. The KSU device produces both the ultrasound frequencies between 20 and 100 kHz and sound durations at random. In addition, a quiet period, when none of the frequencies is on, was also programmed as a random event. In the present study, the KSU device was programmed to generate frequencies between 20 and 80 kHz. The KSU device uses a computer to generate waveform pulses via a DAQCard-6715 analog output channel (National Instruments, Austin, Texas). The analog output was amplified to dual electrostatic transducers. The amplitude of the sine waves was 2.25 V at the peaks. A LABview (National Instruments, Austin, TX, USA) random number generator (0–1) (Wichmann & Hill, 1982) was used to produce the random sound durations and frequencies. The mean ± SE SPL produced was 115.1 ± 0.6 dB at a distance of 50 cm.

**Female calling behavior**

Virgin *P. interpunctella* females were placed individually in glass vials (6.5 cm high × 3 cm in diameter). The open ends of the vials were tightly covered with wire mesh screens (1.58 mm2 openings) that were glued to the vials. Two Cix 0600 or two KSU units were placed 43 cm above the wire mesh covering the vials in growth chambers. The SPL just above the wire mesh was 99.7 ± 0.1 dB for the Cix 0600 device and 118.4 ± 0.8 dB for the KSU. Vials with females, in separate growth chambers, unexposed to ultrasound, served as the control treatment.

Five paired tests were done with each device. In each paired test, 15 vials with females were exposed to ultrasound in a growth chamber and 15 vials were placed in another growth chamber without ultrasound. All tests started at 19.00 hours and ended at 19.00 hours the next day. Lights were turned off at 21.00 hours and turned on at 07.00 hours. Visual observations were taken hourly to count the number of calling females (abdomen curved upward with extrusion of the genitalia). A red light modified from a Fiber-lite illuminator (Model MI-150 BY, Dolan-Jenner, Lawrence, MD, USA), with a light intensity of 2.0 lux near the vials, facilitated counting during the scotophase. To reduce noise levels, fans in the growth chambers were turned off during the tests. All tests were conducted at 28 °C and 65% r.h.

**Adult movement, mating success, and reproduction**

Glass cylinders (4 cm high by 5.5 cm diam) were covered with wire mesh (1.58 mm2 holes) on one end, and the mesh was held in place using glue. A pair of virgin *P. interpunctella* male and female adults was introduced into each cylinder. The open end of the glass cylinder was covered with a wire mesh and glued to the cylinder. The cylinders were placed over 6-cm glass Petri dishes to collect any eggs that dropped through the mesh. Two Cix 0600 or two KSU units were placed 43 cm above wire meshes covering the cylinders.

A total of 15 tests was conducted, five with the Cix 0600 device, five with the KSU device, and five without ultrasound, following a completely random design. In each test,
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six adult pairs, each confined to a separate cylinder, were kept with or without ultrasound in a chamber at 28 °C and 65% r.h. The six glass cylinders in each test were laid out in two rows with three cylinders in a row on a 12 × 18 cm paper. The SPLs produced by the Cix 0600 device just above the cylinders were 99.9 ± 0.2 dB at the center position and 99.0 ± 0.2, 99.3 ± 0.3, 99.2 ± 0.1, and 99.2 ± 0.2 dB at the four corner positions. SPL values for the KSU device were 119.2 ± 1.1 dB at the center position and 111.6 ± 1.8, 110.7 ± 1.7, 111.5 ± 1.3, and 110.4 ± 1.8 dB at the four corner positions. Sound frequencies and waveform plots for the Cix 0600 and the KSU devices measured at the center positions are shown in Figure 1. Each test started at 15.00 hours. Lights in the growth chamber were turned off at 21.00 hours and turned on at 07.00 hours. Insect activity was recorded continuously by digital video camera (Canon ZR 10, Canon USA Inc., Lake Success, NY, USA). The camera was placed 50 cm above the cylinders, so that moths in all cylinders could be recorded. The camera was connected to a monitor (GE 13TVR62, Thomson Consumer Electr onics, Indianapolis, IN, USA), placed outside the chamber. Recordings were made for 24 h a day until all moths were dead. A red light (1.5 lux of photo intensity over the cylinders) in the chamber, that turned on automatically when the lights were off, facilitated video recording during the scotophase. The number of adults in cylinders that were moving, mating, or dead was counted by reviewing the videotapes. Adult mortality data were used to determine the longevity of males and females in the absence and presence of ultrasound. Adult movements were based on counting moths that were active during the first 5 min of a 30 min interval throughout the test. Eggs collected daily were counted at 17.00 hours. Eggs adhering to the mesh were gently removed with a hairbrush. All eggs were placed in glass Petri dishes (1 cm high × 2.5 cm diam.) to determine egg viability by using the methods described in Huang & Subramanyam (2003). Petri dishes were examined under a stereomicroscope to determine egg viability, by counting the number of eggs that failed to hatch. At the end of each test, all females were dissected to determine the number of spermatophores transferred by males to females in the bursa copulatrix (Lum, 1979).

**Data analysis**

Female calling and mating occurred during the scotophase. Thus, only behavioral data recorded during scotophase were analyzed. Data on the number of calling females in the absence or presence of ultrasound at each observation time were first analyzed as a split-plot design (SAS Institute, 1999), with treatment (with or without ultrasound laid out in a randomized complete block) serving as the whole plot and time (repeated measurements over time) as the subplot factor. Differences in the number of calling females at each observation time in the absence or presence of
ultrasound were further analyzed using a paired t-test (SAS Institute, 1999).

The number of adults that moved during each observation period and the number of mating pairs observed in 30-min intervals over the first 5 days were analyzed as a split-plot design (SAS Institute, 1999), with the treatment (with and without ultrasound laid out in a complete random arrangement) as the whole plot treatment and the repeated observations over time as the subplot factor. Data collected during the first 5 days were analyzed, because adult movement and mating were not apparent after 5 days. Differences among treatments (Cix 0600 device, KSU device, and control) at each observation time were determined using one-way analysis of variance (ANOVA), followed by Fisher’s Protected Least Significant Difference (LSD) test (SAS Institute, 1999). The number of times each pair mated during its lifetime, number of spermatophores in females, number of eggs laid by females, egg viability, preoviposition period, and adult longevity were subjected to one-way ANOVA, and treatment means were separated using Fisher’s Protected LSD test (SAS Institute, 1999).

Data on the number of matings, and the number of spermatophores were pooled over the five replicates. The pooled data were subjected to χ² analysis (SAS Institute, 1999). The percentage of matings lasting up to 30, 60, 90, 120, and >120 min were subjected to χ² analysis (SAS Institute, 1999).

Adult activity, mating, spermatophore, and egg laying data were transformed to log(x + 1). Egg viability data were transformed to angular values (Zar, 1984). All treatment differences were considered significant at the α<0.05 level.

Results

Female calling behavior

Females were calling only during scotophase, both in the absence and presence of ultrasound. Females started calling immediately after the lights were turned off (21.00 hours), and ceased calling after the lights were turned on (07.00 hours) (Figure 2A,B). Diel rhythms of calling behavior were similar in the absence or presence of ultrasound.

Fewer females were observed calling when exposed to ultrasound from the Cix 0600 device compared with unexposed females (F₁,₁₄ = 12.31, P = 0.025), and these differences were also significant over time (F₅,₆₄ = 2.64, P = 0.04). The treatment by time interaction was not significant (F₅,₆₄ = 1.77, P = 0.099), because the diel calling pattern was similar between the treatments during the observation period (Figure 2A). In the absence of ultrasound, a mean ± SE of 67.1 ± 2.4% of females were observed calling across the observation times, whereas in the presence of ultrasound, 58.5 ± 2.1% of females were observed calling.

In tests with the KSU device, a mean ± SE of 65.7 ± 3.9% of females were observed calling in the absence of ultrasound, whereas 50.3 ± 3.8% were observed calling in the presence of ultrasound (Figure 2B). The main treatment effect (with or without ultrasound) on female calling was not significant (F₁,₁₄ = 2.8, P = 0.170) but the interaction between treatment and time was significant (F₅,₆₄ = 2.17, P = 0.043), because fewer females (paired t-test, P<0.05) called at 22.00, 23.00, and 24.00 hours in the presence of ultrasound.

Adult movement

The adult movement of P. interpunctella was not affected by ultrasound produced from the two ultrasonic devices (F₁,₁₂ = 0.08, P = 0.925 for main treatment; and F₇₉,₁₀₆₈ = 0.85, P = 0.913 for the interaction of treatment and time), while the main effect of time was significant (F₉₅,₁₀₆₇ = 15.37, P<0.0001). The movement of P. interpunctella adults was limited during the first day, except when the moths were exhibiting courtship and mating behaviors. After the first
day, adults were only active during the scotophase. The most active period was from 21.00 hours to 24.00 hours.

**Mating**

The diel pattern of *P. interpunctella* mating was similar in the absence or presence of ultrasound. More mating pairs were observed during the first night than any other night during the 5-day observation period. The peak of mating occurred soon after the lights were turned off (21.00 hours) and lasted until 23.00 hours.

The main effect of treatment on the number of adult pairs observed mating was not significant ($F_{2,12} = 2.42$, $P = 0.131$), but the main effect of time, and the interaction between treatment and time were significant ($F_{89,178} = 6.18$, $P < 0.0001$ for time and $F_{178,1068} = 1.43$, $P = 0.0005$ for the interaction), because under ultrasound exposure, fewer moths ($P < 0.05$) mated during 21.00–22.30 hours compared with unexposed moths. There were no significant differences ($P > 0.05$) between the treatments for other observation intervals.

A pair of *P. interpunctella* adults, on average, mated 2.9 times during their lifetime in the absence of ultrasound. The number of pairs mating decreased to 2.1 and 1.9 times in the presence of ultrasound produced by the Cix 0600 and KSU devices, respectively. However, these differences among treatments were not significant at the 5% level ($F_{2,12} = 3.72$, $P = 0.055$).

In the absence of ultrasound, most pairs (90%) mated more than twice (Figure 3), whereas in the presence of ultrasound, a few (8%) pairs failed to mate and more than 30% of the pairs mated only once. There were significant differences among treatments in the mating frequency distribution ($\chi^2 = 26.50$, d.f. = 12, $P = 0.009$).

In the absence of ultrasound, most of the matings (78%) lasted 30–90 min, while in the presence of ultrasound, many matings (52%) lasted either less than 30 min or longer than 90 min (Figure 4). The distribution of mating durations was significantly different among the treatments ($\chi^2 = 37.73$, d.f. = 8, $P < 0.0001$).

**Spermatophore transfer**

The frequency distribution of spermatophores found per female was significantly different among the treatments ($\chi^2 = 34.64$, d.f. = 12, $P = 0.0001$). In the absence of ultrasound, all females mated successfully and most females (90%) contained two or more spermatophores (Figure 5), while in the presence of ultrasound, 37% of females had one or zero spermatophores.

The average number of spermatophores transferred was different between the treatments ($F_{2,12} = 14.66$, $P = 0.0006$). In general, fewer spermatophores were found in females exposed to the Cix 0600 (1.7 ± 0.1 female$^{-1}$) and KSU (1.4 ± 0.1 female$^{-1}$) device compared to unexposed females (2.8 ± 0.3 female$^{-1}$).

**Pre-oviposition period**

The preoviposition period of females was similar in the absence or presence of ultrasound ($F_{2,12} = 1.54$, $P = 0.254$).

**Oviposition pattern and egg viability**

The number of eggs laid by females in the control treatment increased gradually with adult age up to 6 days, and then decreased gradually over the next 5 days (Figure 6). Moths exposed to ultrasound from the Cix 0600 device laid most of their eggs between the second and fifth day. Moths exposed to ultrasound from the KSU device laid most of their eggs between the fifth and seventh day.

Ultrasound from both devices significantly reduced the number of eggs laid by *P. interpunctella* ($F_{2,12} = 31.44$, $P < 0.0001$). The number of eggs laid by females was reduced by 44 and 58% when exposed to ultrasound from the Cix 0600 and KSU devices, respectively, compared to the control treatment. Moth exposed to ultrasound from the KSU device laid fewer eggs ($P < 0.05$) than those exposed to the Cix 0600 device.
Viability of *P. interpunctella* eggs was significantly different between the treatments ($F_{2,12} = 8.32, P = 0.005$). Viability of eggs laid by females in the control treatment was 98%, but those exposed to ultrasound from the KSU device was 82%, and this difference was significant ($P < 0.05$). Egg viability in the Cix 0600 treatment (95%) did not differ significantly ($P > 0.05$) from the control treatment.

### Adult longevity

The longevity of male or female *P. interpunctella* was not significantly different between the treatments ($F_{2,12} = 1.35, P = 0.295$ for males; $F_{2,12} = 1.47, P = 0.269$ for females).

### Discussion

The reduction in the number of pairs mating under ultrasound exposure could be due to a disruption of the calling behavior of *P. interpunctella*, because significantly fewer females exposed to ultrasound were calling compared with unexposed females. Our findings are consistent with those reported by Svensson et al. (2003) and Acharya & McNeil (1998). Svensson et al. (2003) reported that calling female *P. interpunctella* responded to ultrasound signals by retracting the ovipositor. Under continuous exposure to ultrasound, female calling was reduced by 27% relative to unexposed females. In addition, when moths were exposed to ultrasound with an interpulse length of 2.5 s in plastic tents, the mating frequency was reduced by 58% compared with unexposed moths. Female moths of the European corn borer, *Ostrinia nubilalis* (Hübner), and the armyworm, *Pseudaletia unipuncta* (Haworth), ceased their calling behavior in the presence of ultrasound that simulated a hawking bat (Acharya & McNeil, 1998). Similar results were observed in several other moth species (Baker & Cardé, 1978; Fullard et al., 2003; Skals et al., 2003). Ultrasound may have reduced mating by affecting the behavior of males responding to the calling female. Trematerra & Pavan (1995) reported that during courtship behavior, male *P. interpunctella* produce ultrasounds in the range of 50–70 kHz by wing fanning. Wing fanning stopped when the males were exposed to ultrasound frequencies of 40–50 kHz. Disruption of this male courtship behavior by ultrasound may also have resulted in fewer males mating with females.

The presence of a spermatophore in the bursa copulatrix of female *P. interpunctella* indicates a successful mating (Brower, 1975). A greater reduction in spermatophore
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...transfer (39–50%) compared to the reduction in the number of matings (28–34%) suggests that ultrasound adversely affected successful mating. Under ultrasound exposure, the duration of mating was often either unusually long or unusually short.

All *P. interpunctella* females tested laid eggs, but exposed females laid fewer eggs than unexposed females. Several other studies have found that ultrasound can significantly affect the reproduction of moth pests of field crops. For example, ultrasound significantly reduced the oviposition of *O. nubilalis* in corn fields (Belton & Kempster, 1962), and that of the cabbage looper, *Trichoplusia ni* (Hübner), in lettuce and broccoli plots (Payne & Shorey, 1968).

Females of *P. interpunctella* without a spermatophore are essentially unmated. The retention of mature oocytes in unmated *P. interpunctella* females or females without spermatophores (Mbata, 1985) may have resulted in fewer eggs being laid. Mbata (1985) reported that unmated females laid about 24 eggs. A reduction in number of eggs laid under ultrasound exposure was evident, even after the data on the females that did not mate successfully were excluded. For example, a successfully mated female *P. interpunctella* that was not exposed to ultrasound laid about 229 eggs, whereas those exposed to ultrasound from the Cix 0600 and KSU devices laid about 132 and 112 eggs, respectively. The physiological mechanisms for the decreased egg laying by ultrasound-stressed female *P. interpunctella* warrants further study.

In summary, ultrasound from a commercial and KSU device adversely affected the mating and reproduction of *P. interpunctella*. The detailed observations made in this study provide a valid basis for explaining the reproductive effects of ultrasound observed on *P. interpunctella* in our enclosure tests (Huang et al., 2003; Bh. Subramanyam, F. Huang & R. Taylor, unpubl.). The reduction in mating and egg laying observed under ultrasound exposure is not adequate to recommend it as a stand-alone pest management strategy. We suggest exploring this technology in conjunction with pheromone-based behavioral management strategies to further reduce the mating and reproduction of *P. interpunctella*.

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