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Ultrasound affects spermatophore transfer, larval numbers, and larval weight of *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae)☆

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Abstract

Effects of ultrasound from a commercial device on reproduction in the Indianmeal moth, *Plodia interpunctella* (Hübner), were investigated in paired Plexiglas enclosures, one with ultrasound and one without (control treatment). In each of the five paired trials, 10 newly emerged male and 10 female moths were introduced into each enclosure. The commercial device produced peak frequencies at 21, 25, and 35 kHz, and a 94 dB sound pressure level at a distance of 50 cm. In enclosures with ultrasound, female moths had 27% fewer spermatophores and produced 48% fewer larvae than those not exposed to ultrasound. Furthermore, ultrasound reduced total and individual larval weights by 66% and 35%, respectively, when compared with the control treatment. About 17% more moths were found on the enclosure floor in the presence of ultrasound when compared with those not exposed to ultrasound. This is the first paper documenting the effects of ultrasound on reproductive performance of *P. interpunctella*. These laboratory data suggest that the use of ultrasound against *P. interpunctella* may be an appealing and effective behavioral management strategy.

Keywords: Stored products; Indianmeal moth; Ultrasound; Non-chemical management

1. Introduction

The Indian meal moth, *Plodia interpunctella* (Hübner), is an important pest of stored raw grains and processed cereals worldwide (Sinha and Watters, 1985). Male and female adult moths possess

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tympanic membranes on the lateral sides of the first abdominal segment (Mullen and Tsao, 1971) that respond to ultrasounds similar to those produced by insectivorous bats (Spangler, 1988). Several moths belonging to the families Pyralidae and Noctuidae that are not associated with stored products also have tympanic membranes. The tympanic membranes have evolved over time to allow moths to avoid predation by insectivorous bats (Spangler, 1988; Conner, 1999) that emit ultrasonic pulses in the range 20–200 kHz (Fullard, 1998). Moths in flight show evasive maneuvers when exposed to ultrasounds in that frequency range (Treat, 1955; Roeder, 1962; Fullard and Barclay, 1980). These maneuvers include flying away from the sound or suddenly dropping to the ground and remaining motionless.

Mating behaviors and reproduction of moths associated with field crops were affected by ultrasound (Payne and Shorey, 1968; Baker and Cardé, 1978). Acharya and McNeil (1998) have shown that males of the European corn borer, *Ostrinia nubilalis* (Hübner) and the true army worm, *Pseudaletia unipuncta* (Haworth), flying upwind in response to female sex pheromone in a wind tunnel aborted the upwind flight when exposed to simulated aerially hawking bat sounds (50 kHz, 50–100 pulses/s, and 90 dB sound pressure level (dB SPL)). Females of both the species stopped calling when exposed to hawking bat sounds (Acharya and McNeil, 1998). Therefore, ultrasounds that simulate those produced by hawking bats could alter reproductive activities of moths.

P. interpunctella courtship and mating behaviors were described by Trematerra and Pavan (1995). The females curve their abdomen upward and release a pheromone that attracts males. Males fly upwind to the pheromone source, locate the females, come to rest face to face and then turn around to copulate. During courtship behaviors, *P. interpunctella* males produce ultrasound in the range 50–70 kHz by wing fanning. Wing fanning ceases when males are exposed to ultrasound frequencies of 40–50 kHz (Trematerra and Pavan, 1995).

The responses of several non-stored product moths to ultrasound, and limited evidence on *P. interpunctella* male responses to ultrasound (Trematerra and Pavan, 1995), suggest that the use of this technology may be a promising non-chemical pest management method for two reasons. First, *P. interpunctella* males and females do not feed as adults and live for 1–2 weeks after emergence (Sinha and Watters, 1985); their sole purpose is to mate and lay eggs. Second, during the short adult life, if moths are exposed to ultrasound they may invest more energy into evasive maneuvers and less into finding mates and in courtship behaviors. By reducing the chance of successful mating, populations may be reduced to a level where they do not cause economic damage to stored products. In our laboratory, detailed studies are underway to characterize the effects of ultrasound on courtship and mating behaviors of male and female *P. interpunctella*. In this paper, we determined the effect of ultrasound produced from a commercial device on spermatophore transfer by male to female *P. interpunctella* and larval production and weight.

2. Materials and methods

2.1. Insects

Cultures of *P. interpunctella* were reared on a poultry-mash diet (Subramanyam and Cutkomp, 1987) at 28°C, 65% r.h., and 14 h:10 h light:dark cycle. The diet (200 g) in 0.95-l glass jars was seeded with approximately 200 eggs. Corrugated paper spools, placed above the diet in each jar, served as pupation sites for wandering larvae. Pupae collected from spools were sexed using characters described by Butt and Cantu (1962). Male and female pupae were placed in separate 0.95-l jars. Jars were checked twice daily, and moths (0–12-h-old) that emerged were used in the tests.

2.2. Test enclosures

Enclosures measuring $1.2\,m \times 1.2\,m \times 1.2\,m$ were built using Plexiglas (Fig. 1). A pair of enclosures was used for each test. Each of the six sides of each enclosure was divided into 16, 0.09-m^2 quadrats. A commercial ultrasonic unit (CIX 0600, Weitech, Inc., Sisters, OR, USA) was mounted on the top left-hand corner of one enclosure. In the other enclosure, the unit was mounted on the top right-hand corner. On the floor of each enclosure, one Petri dish (9 cm diam $\times$ 1.8 cm high), containing 20 g of *P. interpunctella* diet, was placed in the center of each $0.09\,m^2$ quadrat (Fig. 1).

2.3. Measurement of sound output

Sound measurements were made at a distance of 50 cm from the ultrasonic unit's transducer, with the unit running in "Quiet and Mode B" setting. Measurements were made using a Bruel and Kjaer (B&K) type 4939 condenser microphone, B&K type 2670 preamplifier, and B&K NEXUS conditioning amplifier. Sound measurements included peak frequencies, sound cycles, and sound pressure levels. Data were collected using a Tektronix 544A digitizing oscilloscope. Measurements were calibrated using a B&K type 4231 sound level calibrator.

SPLs at the bottom (floor), middle, or top levels within an enclosure were also determined by placing the microphone in the center of each of the 16 quadrats. Contour plots of SPL distributions at the three levels were generated using Surfer® software (Keckler, 1995).

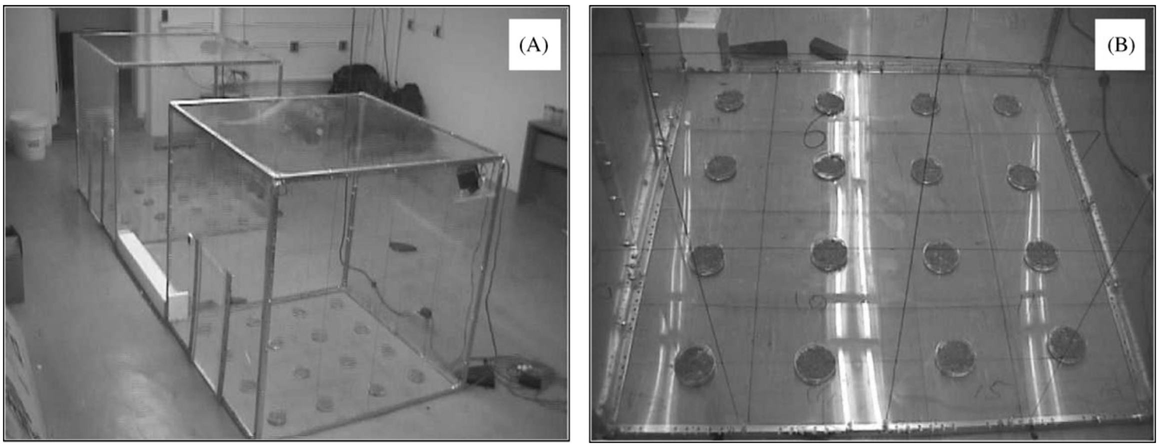

Fig. 1. Paired Plexiglas enclosures used to evaluate effects of ultrasound on *P. interpunctella* reproduction (A). The diet (20 g) was placed in the center of 16, $0.09\,m^2$ quadrats on the enclosure floor (B).

2.4. Test procedures

Five paired tests or replications were conducted to evaluate *P. interpunctella* responses to ultrasound. For each test, a pair of enclosures was used. Ten pairs of newly emerged *P. interpunctella* adults were released into each enclosure. The ultrasonic unit in one of the paired enclosures was turned and kept "on" after moth introduction until the termination of the test, while the unit in the other enclosure was kept in the "off" position. The total number of live *P. interpunctella* moths alighting on the sidewalls, tops, and bottoms of the enclosures were recorded daily once (8:30 am, tests 3–5) or twice (8:00 am and 5:00 pm, tests 1 and 2). Tests were terminated after 18–30 d. At this time all the moths were dead, and the larvae in diet were easy to recover. All dead female moths were preserved in vials containing absolute ethanol for dissection under a stereomicroscope to count spermatophores in the bursa copulatrix (Lum, 1979). Live larvae in diet from each of the 16 locations were separated, counted, and weighed.

Temperature and humidity levels inside each enclosure during all the tests were electronically measured with HOBO units (Onset Computer Corporation, Pocasset, MA, USA). The environmental conditions varied among the trials. During the first test, temperatures ranged from 22.5°C to 24°C and r.h. ranged from 59% to 96%. Temperature and r.h. ranges were 20–24°C and 25–37%, respectively, for the second and third tests, and 20–22.5°C and 27–51%, respectively, for the fourth and fifth tests. All tests were conducted at 13 h:11 h light:dark cycle.

2.5. Data analysis

Differences in the number of spermatophores per female, number of larvae, total larval weight, weight per larva, and percentage of moths on the enclosure floor in the presence and absence of ultrasound were determined using paired *t*-tests (SAS Institute, 1990). For each trial, the relationship between weight per larva and number of larvae among the 16, 0.09 m^2 quadrats, in the presence or absence of ultrasound, was determined using the PROC REG procedure of SAS (SAS Institute, 1990). The percentage of moths on the enclosure floor was calculated by dividing the cumulative number of live moths observed on the floor by the cumulative number of live moths within the enclosure. These percentages were transformed to angular values before the analysis to normalize heteroscedastic data.

Data on the number of larvae in each of the 16 locations in the presence or absence of ultrasound across all five tests were pooled, and expressed as a percentage by dividing the total number of larvae at a particular location by the total number of larvae in that enclosure. Contour maps based on these percentages were drawn using Surfer® software (Keckler, 1995), to determine the impact of ultrasound on larval distributions within enclosures.

3. Results

3.1. Sound measurements

The SPL in the enclosure without ultrasound when the other enclosure had an active ultrasonic unit was undetectable (below the level of 0.01 Pa). The ultrasonic device used in all five tests generated peak frequencies at 21, 25, and 35 kHz (Fig. 2A). The units produced a 94 dB SPL at a distance of 50 cm from the source ($0\,dB = 20\log_{10}(20\,\mu Pa/20\,\mu Pa)$). The waveform plot (Fig. 2B) showed the sound cycle duration to be 0.123 s. In each sound cycle, there were two groups of pulses with eight pulses per group. The first group of weaker pulses was followed by a group of stronger pulses. The interval between the two groups of pulses was 0.038 s. SPL distributions within an enclosure were slightly different among the three levels (Fig. 3). SPLs ranged from 76 to 78, 76 to 84, and 76 to 87 dB at the bottom, middle, and top levels, respectively. SPLs recorded just above or near the units at the top level were higher than those recorded from other areas.

3.2. Effect of ultrasound on spermatophore transfer, larval numbers, and larval weight

Females from enclosures without ultrasound had 2–3 spermatophores, whereas in enclosures with ultrasound, females had 1–2 spermatophores. Across all five tests, each female, on an average, had 2.1 spermatophores in the absence of ultrasound and 1.5 spermatophores in the presence of ultrasound (Table 1). This difference was significant ($t = 3.15$; d.f. = 4, $P = 0.035$).

Significantly fewer larvae were found in enclosures with ultrasound when compared with those found in enclosures without ultrasound ($t = 6.07$, d.f. = 4, $P = 0.0037$; Table 1). Also, the total weight of larvae (biomass) in enclosures with ultrasound was significantly less than the weight of

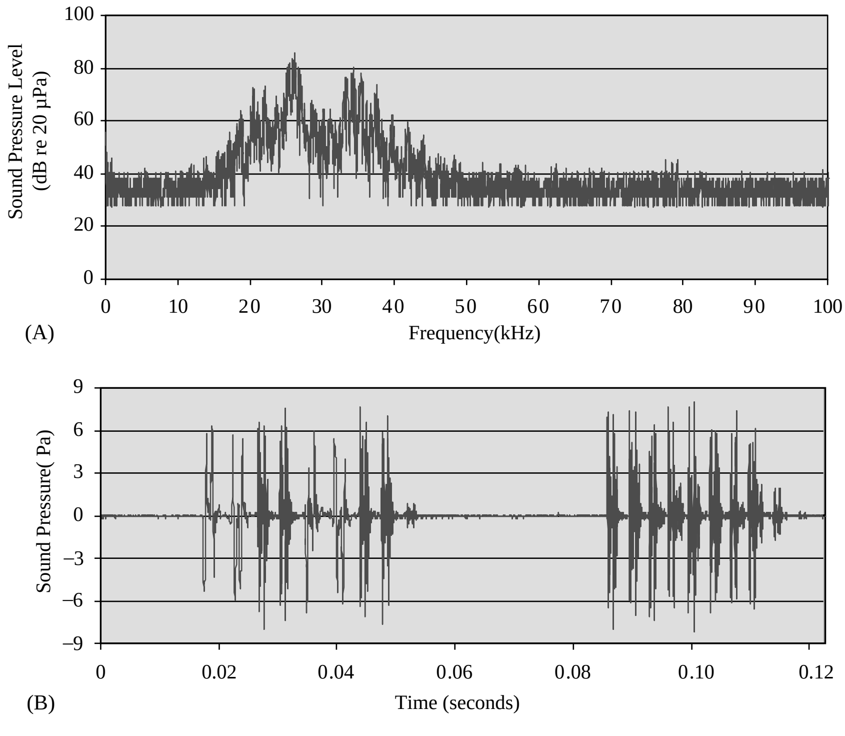

Fig. 2. Sound frequency spectra (A) and waveform plot (B) for the commercial ultrasonic device measured at a distance of 50 cm from the unit.

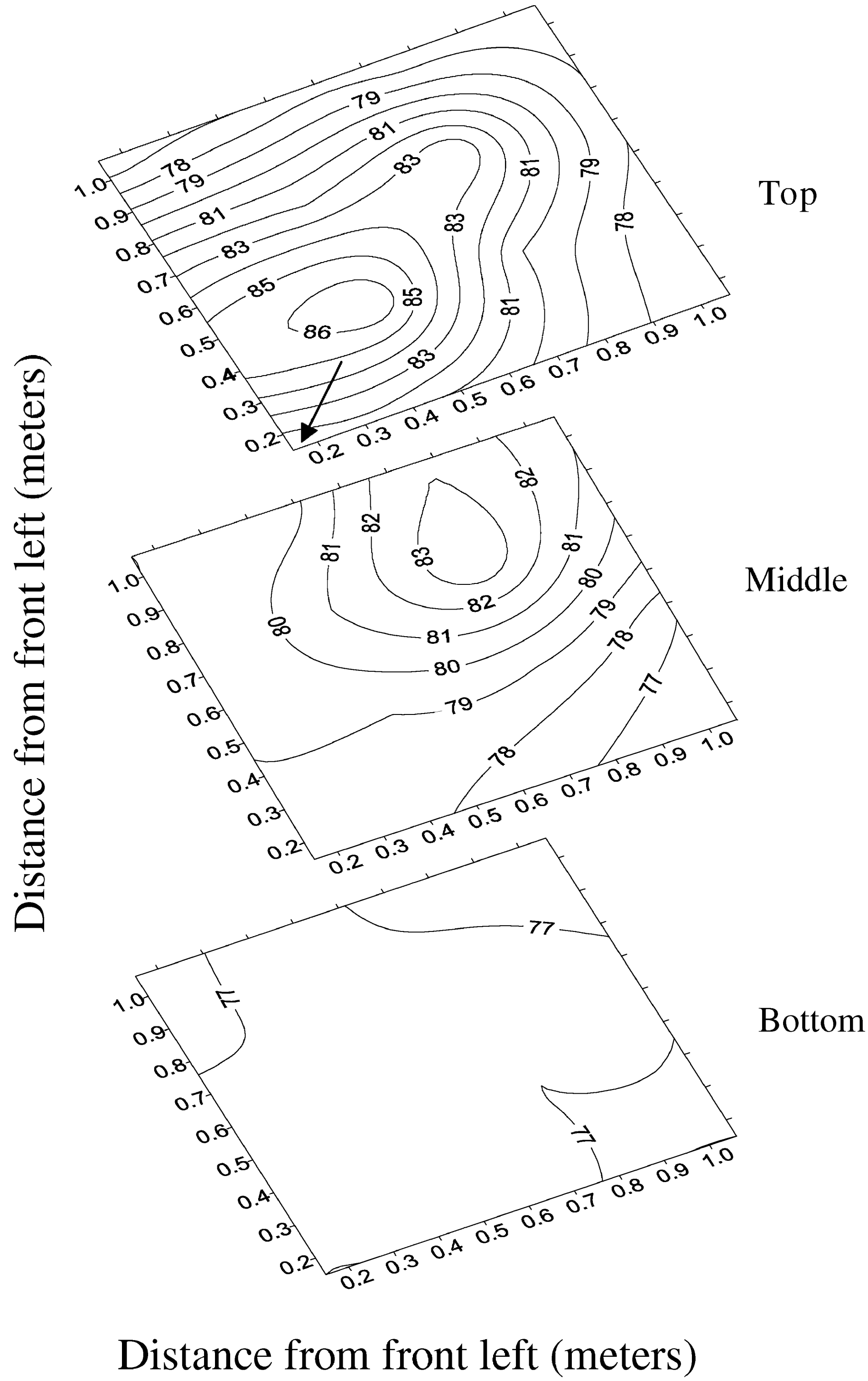

Fig. 3. Contour maps showing distribution of SPLs (dB, $0\,dB = 20\log_{10}(20\,\mu Pa/20\,\mu Pa)$) produced by the commercial ultrasonic unit in the bottom, middle, and top levels within an enclosure. The device was positioned at (0,0; arrow) coordinates near the top level within the enclosure.

larvae in enclosures without ultrasound ($t = 3.24$, d.f. = 4, $P = 0.0315$). When larval weight was corrected for the number of larvae or expressed as weight per larva, differences were present at slightly higher than the 5% significance level ($t = 2.7$, d.f. = 4, $P = 0.0538$). There was no linear relationship ($P > 0.2$) between larval numbers and weight per larva within and among the five trials, in the presence or absence of ultrasound. This indicated that larval weight was not affected by larval crowding, because there was adequate food for all developing larvae.

Table 1
Reproductive performance of *P. interpunctella* in the presence and absence of ultrasound produced by a commercial device

Ultrasound status	Mean ± SE[a]			
	No. spermatophores/female[b]	No. larvae/enclosure[b]	Total larval biomass (mg)[b]	Weight/larva (mg)[c]
Off	2.1 ± 0.3	1464.8 ± 230.8	4106.7 ± 1438.1	2.61 ± 0.68
On	1.5 ± 0.1	765.0 ± 217.5	1412.8 ± 720.7	1.69 ± 0.45

[a] Each mean is based on $n = 5$ replications.
[b] For each response variable, difference between off and on status of ultrasound was significant ($P < 0.05$; paired t-test).
[c] Significant ($P < 0.06$; paired t-test).

Table 1

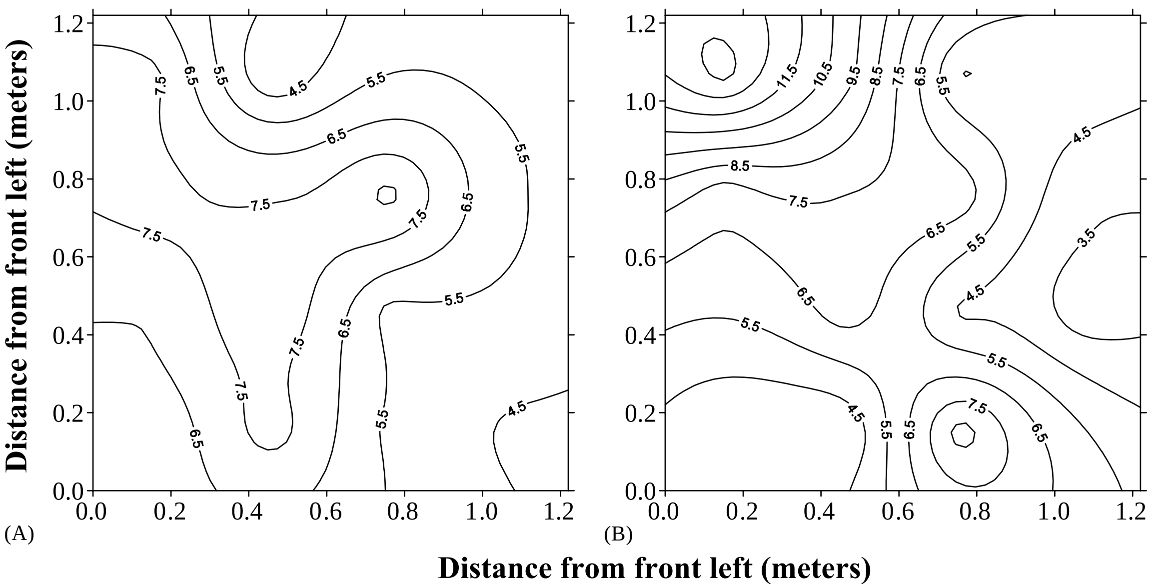

Fig. 4. Contour maps showing distribution of larvae, expressed as percentage of total, within enclosures in the absence (A) and presence (B) of ultrasound produced by a commercial device. The device was positioned at (0,0) coordinates near the top level within the enclosure.

3.3. Effect of ultrasound on adult distribution

In the presence of ultrasound, a greater percentage of live moths (mean $\pm$ SE, 41.6 $\pm$ 2.5%) was found on the enclosure floor than was found in the absence of ultrasound (34.4 $\pm$ 4.8%). However, this difference was not significant at the 5% level ($t = 2.21$, d.f. = 4, $P = 0.0918$).

3.4. Effect of ultrasound on larval distribution

There were differences in larval distribution within enclosures in the presence and absence of ultrasound. In the absence of ultrasound, larvae were distributed more uniformly (Fig. 4A). In the presence of ultrasound, a greater percentage of larvae were found near the enclosure walls on either side of the ultrasonic unit (0,0 coordinates) (Fig. 4B).

4. Discussion

The number of spermatophores per female, larval numbers, and larval weight of *P. interpunctella* decreased significantly when the insects were exposed to ultrasound from a commercial device. In *P. interpunctella*, a single successful mating is indicated by the presence of a spermatophore in the female (Brower, 1975). The reduced number of spermatophores in females in the presence of ultrasound could be due to a decrease in the number of successful matings or delayed mating. Several researchers demonstrated that ultrasound could disrupt courtship and mating behaviors of tympanate moths. For example, *P. unipuncta* and *O. nubilalis* moths significantly reduced their mate-seeking behavior when exposed to ultrasound simulating a hawking bat (Acharya and McNeil, 1998). Males of the day-flying gypsy moth, *Lymantria dispar* (L.), while flying upwind toward a pheromone source, respond to >15 kHz sounds by deviating sharply from the established flight course or by flying away rapidly (Baker and Cardé, 1978). Free-flying bollworm, *Helicoverpa zea* (Boddie), and 10 other noctuid species of tympanate moths made evasive maneuvers when they were exposed to pulsed ultrasound (Agee, 1969).

Huang and Subramanyam (2002) reported a decrease in the number of spermatophores in female *P. interpunctella* with an increase in the age of males or females at mating. Delayed mating also reduced the number of eggs laid by female *P. interpunctella* (Huang and Subramanyam, 2003). Belton and Kempster (1962) reported that sound resembling that produced by a single bat decreased *O. nubilalis* oviposition by $\geqslant 50\%$ in sweet corn fields. Payne and Shorey (1968) found that oviposition of the cabbage looper, *Trichoplusia ni* (Hübner), was reduced by 41%, 23%, and 30% when exposed to 20, 30 and 40 kHz sound, respectively, in field plots of lettuce and broccoli. Kirkpatrick and Harein (1965) reported that *P. interpunctella* adults exposed for 4 d to amplified low-frequency sounds (120–2000 Hz) produced 75% fewer larvae than those not exposed to the sound.

The significant reduction in total larval biomass in enclosures with ultrasound is partially due to the reduced number of larvae recovered when compared with those recovered from enclosures without ultrasound. However, even after correcting larval weight for the number of larvae in enclosures, differences were still present in average weight per larva. One plausible reason is delayed mating, and consequently, delayed egg laying by moths exposed to ultrasound relative to those not exposed to ultrasound. When separating larvae from the diet, we noticed that larvae in enclosures with the active ultrasonic unit were smaller in size than those from enclosures without ultrasound. Another reason may be that ultrasound retarded larval development. There is no evidence of ultrasound retarding larval development, but there is evidence of infrasound (10–25 Hz, 124 dB SPL) accelerating *P. interpunctella* larval development (Mullen, 1973).

Distribution of *P. interpunctella* adults inside the enclosures was affected by exposure to ultrasound, with more moths being found on the enclosure floor. Lollis (1971) reported that free flying *P. interpunctella* in laboratory tests showed vigorous evasive flying and diving maneuvers in response to ultrasound (20 kHz). Rydell et al. (1997) also found that ultrasonic pulses (27 kHz, 110 dB SPL) sent the flying males of the dotted border moth, *Agriopis marginaria* F., spiraling or diving toward the ground. The effect of ultrasound on adult distribution is also reflected by changes in the distribution of larvae in Petri dishes within enclosures. This suggested that *P. interpunctella* oviposition patterns may be affected by ultrasound, with moths selectively laying more eggs in certain Petri dishes than others.

5. Conclusions

Ultrasound produced from a commercial device had a significant impact on *P. interpunctella* adult distribution and reproduction. We are presently studying the effect of ultrasound on *P. interpunctella* courtship, mating, and oviposition behaviors. The positive findings reported in this paper suggest that ultrasound technology could be exploited for managing *P. interpunctella* reproductive behaviors. The short, non-feeding adult life of these tympanate moths makes exploring such a technology feasible.

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