Effects of Ultrasound on Reproduction of Indianmeal Moth, *Plodia interpunctella* (Hubner) (Lepidoptera: Pyalidae)

Bhadriraju Subramanyam, Fangneng Huang, and Russell Taylor*

Department of Grain Science and Industry, Kansas State University, Manhattan, KS 66506

*Electronics Design Laboratory, Kansas State University, Manhattan, KS 66506

E-mails: bhs@wheat.ksu.edu; fhuang@oznet.ksu.edu; rdtaylor@ksu.edu

Introduction

Responses of several non-stored product moths to ultrasound, and limited evidence on *Plodia interpunctella* responses to ultrasound, suggest that the use of ultrasonic technology may be a promising nonchemical method for two reasons: (1) *P. interpunctella* males and females do not feed as adults and live for 1-2 weeks after emergence; their sole purpose is to mate and lay eggs. (2) 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, we may be able to reduce populations to a level where they do not cause economic damage to stored products.

Materials and Methods

Insects: *P. interpunctella* were reared on a poultry-mash diet at 28ºC, 65% RH, and 14 h light:10 h dark cycle. Male and female pupae were placed in separate 0.95-liter jars. Newly-emerged moths (0 to 12-h-old) were used in tests.

Measurements of sound output: Sound measurements were made at a distance of 50 cm from the ultrasonic unit. 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 recorded included peak frequencies, sound cycles, and sound pressure levels (SPLs). Data were collected using a Tektronix 544A digitizing oscilloscope. Measurements were calibrated using a B&K type 4231 sound level calibrator.

Test procedures: Five paired tests were conducted to evaluate *P. interpunctella* responses to ultrasound produced from a commercial ultrasonic device. For each test, a pair of enclosures (Figure 1) was used. The floor of each enclosure was divided 16 (0.09m^2) quadrats. In each quadrat, 20 g of *P. interpunctella* diet was placed in a 9 cm petri dish. Ten pairs of newly emerged 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. Live *P. interpunctella* moths alighting on the sidewalls, tops, and bottoms of the enclosures were recorded daily once or twice. Tests were terminated after 18-30 d. All dead female moths were preserved in 100% ethanol for dissection under a stereomicroscope to count spermatophores in bura copulatrix. Live larvae in diet from each of the 16 locations were separated, counted, and weighed.

The environmental conditions varied among the trails. During the first test, the temperature was 22.5-24ºC and RH was 59–96%. Temperature and RH. were 20-24ºC and 25–37%, respectively, for the second and third tests, and 20-22.5ºC and 27–51%, respectively, for the fourth and fifth tests. All tests were conducted at 13 h:11 h light:dark cycle.

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. 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. Percentages were transformed as arcsine before analysis to normalize the data.

Across all five tests, the number of larvae in each of the 16 locations in the presence or absence of ultrasound was 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, to determine the impact of ultrasound on larval distributions.

Figure 1. Plexiglas enclosures used to evaluate effects of ultrasound on *P. interpunctella* reproduction.

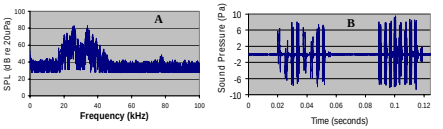

Figure 2. Sound frequency spectrum (A) and waveform graph (B) produced by a commercial ultrasonic device. Measurements were made 50 cm from the transducer.

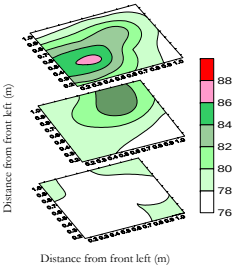

Figure 3. Contour maps showing distribution of SPLs (dB) within an enclosure at the bottom, middle, and top levels. The ultrasonic device position was at the (0,0) coordinates.

Table 1. Reproductive performance of *P. interpunctella* in the presence and absence of ultrasound.

Ultrasound Status	Mean ± SE[a]			
	No. spermatophores/ female[b]	No. larvae/ enclosure[b]	Total larva biomass (mg)[b]	Wt/larva (mg)[c]
Off	2.1±0.3	1465±231	4107±1438	2.6±0.7
On	1.5±0.1	765±218	1413±721	1.7±0.5

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

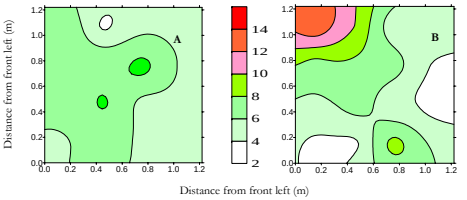

Figure 4. Contour maps showing distribution of larvae, expressed as percentage of total, within enclosures in the absence (A) and presence (B) of ultrasound. The device was positioned at (0,0) coordinates.

Results

Sound output: SPLs within the enclosure 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, 35, and 41 kHz (Figure 2A). The units produced a 94 dB SPL at a distance of 50 cm from the source (0 dB=20 log10(20 uPa/20 uPa). The waveform plot (Figure 2B) showed the sound cycle duration to be 0.123 second. In each sound cycle, there were two groups of pulses with 8 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 second. SPL distributions within an enclosure were slightly different among the three levels (Figure 3). SPLs ranged from 76–78, 76–84, and 76–87 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.

Effect of ultrasound on spermatophore transfer: 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 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.

Effect of ultrasound on larval numbers and larval weight: Significantly fewer larvae were found in enclosures with ultrasound when compared with those found in enclosures without ultrasound (Table 1). Also, the total weight of larvae (biomass) in enclosures with ultrasound was significantly less than the weight of larvae in enclosures without ultrasound. However, when larval weight was corrected for the number of larvae. or expressed as weight per larva, differences were still present, but at a slightly higher than the 5% significance level ($P = 0.0538$).

Effect of ultrasound on adult distribution: In the presence of ultrasound, a greater percentage of live moths (mean ± SE, 41.6 ± 2.5%) was found on the enclosure floor when compared with those found in the absence of ultrasound (34.4 ± 4.8%). This difference was significant at the 10% level ($t = 2.21$, df = 4, $P = 0.092$).

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 (Figure 4). 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).

Conclusions

The number of spermatophores transferred and number of larvae produced by *P. interpunctella* decreased significantly when moths were exposed to the ultrasound from a commercial device. Ultrasound affected adult as well as larval distribution. This is the first report documenting the effects of ultrasound on the reproductive performance of *P. interpunctella.* These laboratory data suggest that use of ultrasound might be an appealing and effective behavioral management strategy.

Acknowledgment

This research was supported by Weitech, Inc., Sisters, OR.