Effects of ultrasound on Indian meal moth reproduction

Fangneng Huang, Bhadriraju Subramanyam[a,*]

Abstract

Effects of ultrasound emitted from a commercial ultrasonic device (Cix 0600) and a novel unit developed at Kansas State University (KSU) on the reproduction of Indianmeal moth, *Plodia interpunctella* (Hübner), were evaluated in paired Plexiglas enclosures. The commercial ultrasonic device generated peak frequencies at 21kHz, 25kHz and 35kHz, and a 94dB sound pressure level at a distance of 50cm. The KSU unit produced frequency, pulse duration and quiet time at random. Ultrasound emitted from both of the ultrasonic units had a similar and significant impact on the number of spermatophores transferred by males to females, the number of larvae produced, larval weight and adult distribution within enclosures. In the presence of ultrasound, each female, on average, had 1.5 spermatophores. In the absence of ultrasound, each female had 2 spermatophores. Larval numbers were reduced by 48% and 38% when moths were exposed to ultrasound produced by the Cix 0600 device and KSU unit, respectively. Therefore, the total larval biomass in the enclosures with ultrasound was significantly less than that in the enclosures without ultrasound. The distribution of larvae within enclosures was affected by ultrasound emitted by the Cix 0600 device but not by the KSU unit. More *P. interpunctella* moths were found on the enclosure floor in the presence of ultrasound than without ultrasound. These laboratory data suggest that use of ultrasound technology could be exploited for managing *P. interpunctella* reproductive behaviours.

Keywords: Stored products; Indian meal moth; Ultrasound; Nonchemical management

Introduction

Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), is a serious pest of raw and processed cereals worldwide. It is a common insect pest reported from grain storage bins, warehouses, grocery stores, birdseed facilities and food/feed mills (Sinha and Watters, 1985). *P. interpunctella* adults possess tympanic membranes on the sides of their first abdominal segment (Mullen and Tsao, 1971) that respond to ultrasounds produced by insectivorous bats (Spangler, 1988; Conner, 1999).

Tympanic moths in flight show evasive manoeuvres when exposed to ultrasounds. These manoeuvres include flying away from the sound or dropping to the ground and remaining motionless. Mating behaviours and reproduction of several other moths associated with field crops were affected by ultrasound (Payne and Shorey, 1968). In the presence of ultrasound, male moths abort the upwind flight in response to the female sex pheromone and females' calling (Acharya and McNeil, 1998). The responses of several non-stored-product moths to ultrasound and the limited evidence on effects of ultrasound on *P. interpunctella* (Trematerra and Pavan, 1995) prompted us to investigate the effects of ultrasound on *P. interpunctella* reproduction.

Currently, all commercial ultrasonic devices produce constant sound patterns, to which insects may become habituated. Therefore, it is desirable to develop an ultrasonic unit that can produce random sound patterns. Such a unit was recently developed in our laboratory. In this paper, we report laboratory tests designed to determine the effects of ultrasound produced from a commercial ultrasonic device and the KSU unit on the reproductive performance of *P. interpunctella*.

Materials and methods

Insect cultures

A colony of *P. interpunctella* was reared on a poultry-mash diet (Subramanyam and Cutkomp, 1987) at 28°C, 65% r.h. and a 14h:10h light:dark cycle. Samples of 200 grams of diet in 0.95-L glass jars were seeded with approximately 200 eggs each. Corrugated paper spools, placed above the diet in each jar, served as pupation sites. 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 newly emerged moths (≤12h old) were used in the tests (Huang et al., in press).

Test enclosures and ultrasonic units

Enclosure cubes, each measuring 1.2m × 1.2m × 1.2 m, were built using Plexiglas. A pair of enclosures was used for each test. The floor of each enclosure was divided into sixteen 0.09-m^2 quadrats. Two ultrasonic devices were evaluated. One was a commercial ultrasonic device (Cix 0600) manufactured by Weitech, Inc. (Sisters, Oregon, USA), and the other was an experimental unit (KSU unit). The KSU unit used a computer, an arbitrary waveform generator and custom electronics to generate ultrasonic pulses in the 20–100kHz frequency range (Fig. 1a). The computer randomly chooses pulse length, frequency and quiet time between

[a] Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas 66506, USA

* Corresponding author. *E-mail address:* bhs@wheat.ksu.edu. This paper presents research results only. Mention of a proprietary product name does not constitute an endorsement for its use by Kansas State University

pulses across the entire frequency range. The computer then directs the arbitrary waveform generator to construct the pulse. The output of the waveform generator is amplified with custom electronics to the requirements of the electrostatic transducer that ultimately produces the ultrasound desired. One device can drive two ultrasonic emitters simultaneously.

When testing the Cix 0600 device, one unit was mounted on the top left-hand corner of an enclosure. In the other enclosure, a similar unit was mounted on the top right-hand corner. When testing the KSU unit, the two emitters of the random ultrasonic unit were connected to a rotator that was mounted in the top corner within an enclosure (Fig. 1b). The other paired enclosure did not have an ultrasonic unit (control). On the floor of each enclosure, 20g of *P. interpunctella* diet was placed in the centre of each 0.09-m^2 quadrat in a Petri dish (9cm diameter × 1.8cm high) or on a plastic sheet (0.04m^2).

Sound measurements

Sound level 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 as described by Huang et al. (in press). The sound signal was digitised at 500,000 samples per second using a 12 bit PCMCIA data acquisition card. A laptop computer and a custom software interface were used to display, print and record data. The custom software can display the digitised waveform, the frequency spectrum of the waveform and the total sound pressure level (SPL).

For the Cix 0600 device, sound measurements were made at a distance of 50 cm from the ultrasonic unitís transducer. SPLs at the bottom (floor), middle or top levels within an enclosure were also determined by placing the microphone in the centre of each of the sixteen 0.092 quadrats. For the KSU unit, sound measurements were made at the bottom centre of the test enclosures. SPLs were calculated by averaging 10 readings. In the current study, the random ultrasonic unit was tested under the parameters: frequency range 20–80 kHz (in 1000 to 5000 kHz increments); sound duration 50–200 ms (random increments); quiet time 50–500 ms (random increments); and amplitude 2.25 m.

Test procedures

Five paired tests for each ultrasonic unit were conducted to evaluate *P. interpunctella* responses to ultrasound. In each test, 10 pairs of newly-emerged (≤12h old) *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 in the other enclosure, the unit was kept in the "off" position only in tests with the Cix 0600 device. The numbers of live moths alighting on the side walls, tops and bottoms of the enclosures were recorded daily once (8.30a.m.) or twice (8.00a.m. and 5.00p.m.). Tests were terminated after 18–30 days. At this time, all moths were dead and the dead moths were preserved in vials containing absolute ethanol. Moths were dissected under a stereomicroscope to record the number of 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 tests were monitored using HOBO data logging units (Onset Computer Corporation, Pocasset, Massachusetts, USA). The environmental conditions varied among the tests (Huang et al., in press). In evaluation of the Cix 0600 device, temperatures and relative humidities ranged from 22.5–24°C and from 59–96%, respectively, during test 1; 20–24°C and 25–37% during tests 2 and 3; and 20–22.5°C and 27–51% during tests 4 and 5. In testing the KSU unit, temperatures and relative humidities were 21.0–23.2°C and 31–80%, respectively, during test 1; 24.0–27.1 °C and 49–82% during test 2; 25.6–28.3 °C and 51–62% during test 3; 24.0–26.7 °C and 53–63% during test 4; and 23.4–25 °C and 53–79% during test 5. All tests were conducted under a 13h light:11h dark cycle.

Data analysis

Data of 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 analysed using paired *t*-tests (SAS Institute, 1990). For each test, the relationship between weight per larva and number of larvae among the sixteen

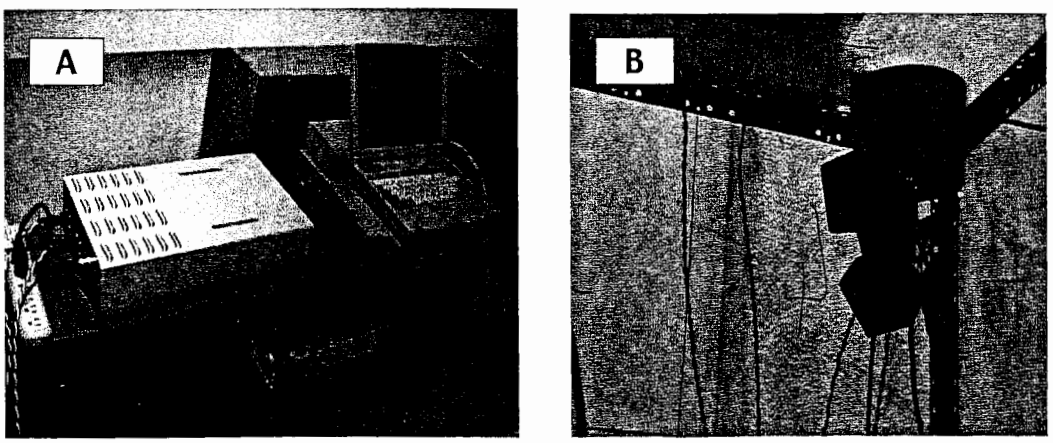

Fig. 1. Random ultrasonic unit developed at Kansas State University: (A) Random ultrasonic generating system with an arbitrary waveform generator (bottom), an ultrasound generator (left) and a computer (right) with electrostatic amplifier; (B) two ultrasonic emitters connected on a rotating head that was mounted on one of the top corners of one enclosure of paired enclosures.

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 with the cumulative number of live moths within the enclosure. Percentages were transformed to angular values to normalise heteroscedastic data before analysis. Data on the number of larvae in each of the 16 locations in the presence or absence of ultrasound were pooled across all five tests and expressed as a percentage, by dividing the total number of larvae at a particular location with the total number of larvae in that enclosure (Huang et al. in press). Contour maps based on these percentages were drawn using Surfer ® software (Keckler, 1995), to determine the impact of ultrasound on larval distributions within enclosures.

Results

Sound outputs

SPLs in the enclosure without ultrasound when the other enclosure had an active ultrasonic unit were undetectable (below the level of 0.01 Pa). The Cix 0600 device 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 (0dB = $20\log_{10}(20\mu Pa/20\mu Pa)$). 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 8 pulses per group. The interval between the two pulse groups of pulses was 0.038 s. 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 somewhat higher than those recorded from other areas.

The KSU random ultrasonic unit produced ultrasonic pulses in the 20–80 kHz range in random patterns over time (Figs. 3b–d). The mean SPL produced was 89.3 dB (0dB = $20\log_{10}(20\mu Pa/20\mu Pa)$) at the centre of the enclosure floor.

Effect of ultrasound on spermatophore transfer

Spermatophores in the bursa copulatrix of female *P. interpunctella* were significantly reduced when moths were exposed to ultrasound, compared with numbers in unexposed moths (Figs. 4a, 5a). In the absence of ultrasound, females had 1–4 spermatophores, whereas in the presence of ultrasound females had 0–3 spermatophores. A female, on average, had 2 spermatophores in the absence of ultrasound and 1.5 in its presence (Figs. 4a, 5a). These differences were significant (for the Cix 0600 device: $t=3.15$; df=4, $P=0.035$; for the KSU unit: $t=5.0$; df=4, $P=0.0075$).

Effect of ultrasound on larval production

Ultrasound emitted from the Cix 0600 and KSU units reduced *P. interpunctella* reproduction (Figs. 4a, 5b). In tests with the Cix 0600 device, only 765 larvae per enclosure were found when moths were exposed to ultrasound, which represents a 48% reduction in larval numbers relative to those found in the enclosures without ultrasound (1465 larvae). In tests with the KSU unit, 2129 larvae were found in the enclosures without ultrasound, whereas 1314 were found in the enclosures with ultrasound—a 38% reduction. Those differences were significant (for the Cix 0600 device: $t=6.07$, df=4, $P=0.0037$; for the KSU unit: $t=8.73$, df=4, $P=0.001$).

Effect of ultrasound on larval weight

The total weight of larvae in the enclosures without ultrasound was higher than in the enclosures with ultrasound (for the Cix 0600 device: $t=3.24$, df=4, $P=0.0315$; for the KSU unit: $t=9.41$, df=4, $P=0.0007$; Figs. 4c, 5c). However, when larval weights were corrected for the number of larvae or expressed as weight per larva, differences were not significant at the 5% level (for the Cix 0600 unit: $t=2.7$, df=4,

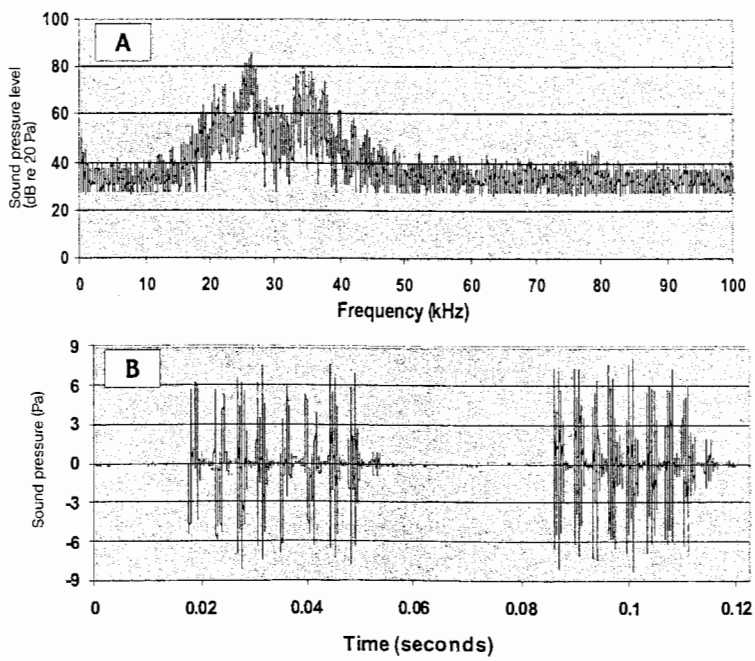

Fig. 2. Sound frequency spectrum (A) and waveform plot (B) produced by the Cix 0600 device measured at a distance of 50 cm from the unit.

P=0.0538; for the KSU unit: t=1.17, df=4, P=0.3053; Figs. 4d, 5d). In the presence or absence of ultrasound, there was no linear relationship (P>0.05) between larval numbers and weight per larva within and among the five tests of each ultrasound unit. This indicated that larval weight was unaffected by larval crowding, because there was adequate food for all developing larvae.

Effect of ultrasound on larval distribution

Difference in larval distribution within enclosures in the presence and absence of ultrasound was observed only with the Cix 0600 device. Larvae were distributed nearly uniformly in the enclosures without ultrasound (Fig. 6a). However, 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. 6b). Ultrasound emitted from the KSU unit did not affect larval distributions within enclosures (Figs. 6c, 6d).

Effect of ultrasound on adult distribution

Exposure to ultrasound from the Cix 0600 unit resulted in 42% of live moths being found on the enclosure floor compared to 34% in the absence of ultrasound (Fig. 7). However, this difference was not significant at the 5% level (t=2.21, df=4, P=0.0918). With the KSU unit, 44% live moths were found on the enclosure floor in the presence of ultrasound compared to only 23% in the absence of ultrasound (Fig. 7). This difference was significant (t=5.14, df=4, P=0.0068).

Discussion

The possibilities of using ultrasound to control insect pests have been explored against a few arthropod species. Belton and Kempster (1962) reported that ultrasound decreased European corn borer, *Ostrinia nubilalis* (Hübner) oviposi-

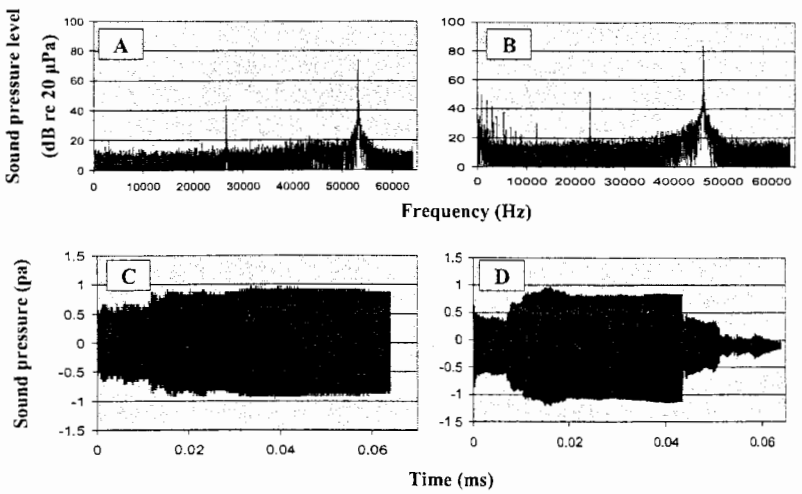

Fig. 3. Two sound outputs from the KSU random ultrasonic unit measured at the centre of the enclosure floor. Figures indicate the changes of the sound frequencies (A and C) and waveform plots (B and D)

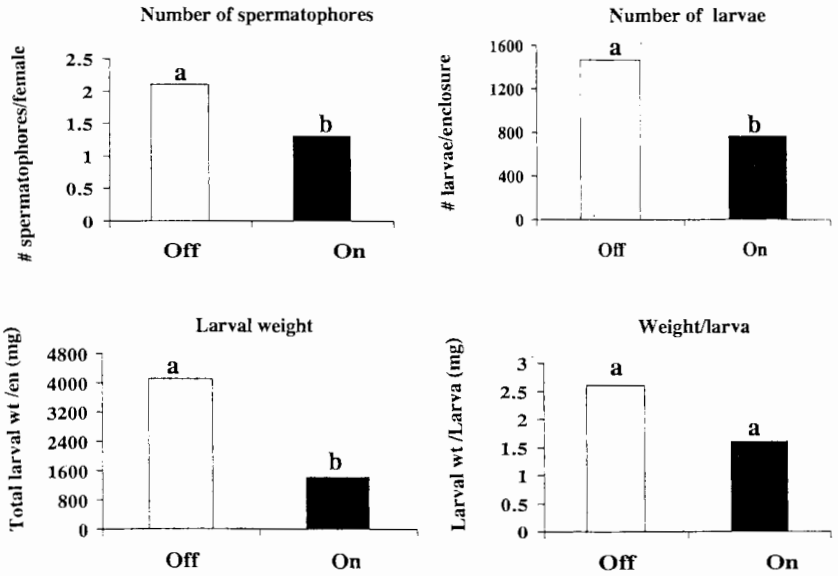

Fig. 4. Number of spermatophores transferred, number of larvae produced, and larval weight of *P. interpunctella* in absence or presence of ultrasound produced by the Cix 0600 device. Within each graph, means with different letters above bars are significantly different (P < 0.05; paired t-test).

tion by ≥50% in sweet-corn fields. Payne and Shorey (1968) also found that oviposition of the cabbage looper, *Trichoplusia ni* (Hübner), was significantly reduced by exposure to ultrasound in field plots of lettuce and broccoli.

Our laboratory tests showed that the number of spermatophores transferred, numbers of larvae produced, and total larval weight of *P. interpunctella* were lower when moths were affected by exposure to ultrasound from either the commercial ultrasonic device or the KSU unit. The reduction in number of spermatophores transferred from males to females in the presence of ultrasound may be due to a decrease in the number of successful matings or to delayed mating. Ultrasound could disrupt courtship and mating behaviours of tympanic moths such as *P.unipuncta* and *O.nubilalis* (Acharya and McNeil, 1998), the gypsy moth, *Lymantria dispar* (L.) (Baker and Cardé, 1978), the bollworm, *Helicoverpa zea* (Boddie), and many other noctuid species (Agee, 1969).

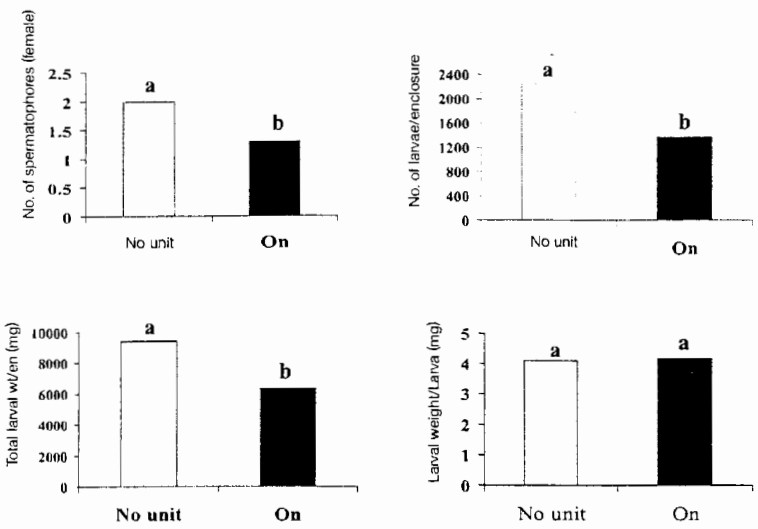

Fig. 5. Number of spermatophores transferred, number of larvae produced, and larval weight of *P. interpunctella*, in absence or presence of ultrasound produced by the KSU unit. Within each graph, means with different letters above bars are significantly different ($P < 0.05$; paired t-test).

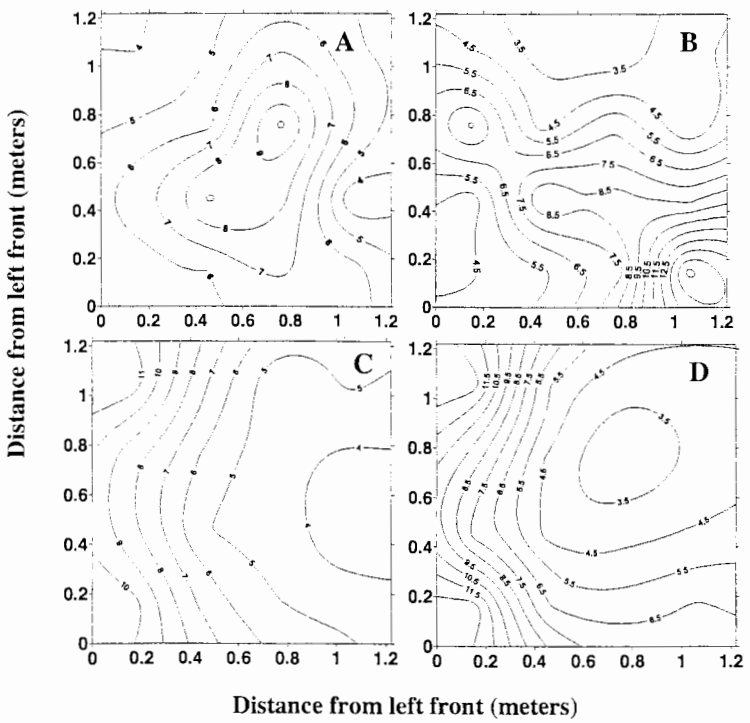

Fig. 6. Contour maps showing distribution of larvae of *P. interpunctella*, expressed as percentage of total, within enclosures in the absence and presence of ultrasound. The device was positioned at (0,0) coordinates near the top level within the enclosure. (A) no ultrasound in test with the Cix 0600 device; (B) ultrasound from Cix 0600 device; (C) no ultrasound in test with the KSU unit; (D) ultrasound from the KSU unit.

Larval distributions inside the enclosures were not affected by ultrasound produced by the KSU unit, which might indicate a uniform coverage of sound inside the enclosures. The two emitters of the KSU unit were connected to a rotator, which resulted in more uniform coverage of sound inside the enclosures.

P. interpunctella adult distributions inside the enclosures were affected by exposure to ultrasound. More moths were located on the enclosure floor when exposed to ultrasound than without ultrasound. Such behavioural responses of *P. interpunctella* to ultrasound have been demonstrated in several other tympanic moth species (Lollis, 1971; Rydell et al., 1997).

Larval production, total larval weight and larval distributions in the test enclosures differed between the tests of the Cix 0600 device and the KSU unit, even in the absence of ultrasound. These differences could be due to differences in test conditions. The tests for the two units were conducted at a different time and in separate rooms. Temperatures and relative humidities varied among the tests. The enclosures used to test the Cix 0600 device were located in a bigger room (5.8m × 11.6m) than the room (2.4m × 4.8m) used for testing the KSU unit. The paired (block) design used in the tests should minimise the impact of these environmental variations.

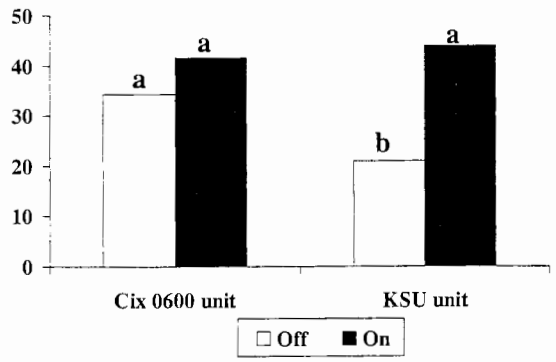

Fig. 7. Percentage of *P. interpunctella* adults found on the enclosure floor. Means with different letters above bars were significantly different ($P=0.05$; paired t-test)

Conclusions

Ultrasound produced from a commercial device and a novel unit had a significant impact on *P. interpunctella* reproduction. Our results suggest that ultrasound technology has potential in the development of strategies for behavioural management of this economically important insect pest.

Acknowledgements

We thank Dr Rennie Roesli for reviewing the manuscript and Dr George Milliken for statistical advice on adult distribution data. Research reported here was supported by funds from USDA/CSREES under Agreement No. 00-51101-9674, and Weitech, Inc., Sisters, Oregon, USA.

References

Acharya, L., McNeil, J.N., 1998. Predation risk and mating behavior: The responses of moths to bat-like ultrasound. Behavioral Ecology 9, 552–558.

Agee, H.R., 1969. Response of flying bollworm moths and other tympanate moths to pulsed ultrasound. Annals of the Entomological Society of America 62, 801–807.

Baker, T.C., Cardé, R.T., 1978. Disruption of gypsy moth male sex pheromone behavior by high frequency sound. Environmental Entomology 7, 45–52.

Belton, P., Kempster, R., 1962. A field test on the use of sound to repel the European corn borer. Entomologia Experimentalis et Applicata 5, 281–288.

Butt, B.A., Cantu, E., 1962. Sex determination of lepidopterous pupae. United States Department of Agriculture, Agricultural Research Service Report 33–75, pp. 1–7.

Conner, W.E., 1999. 'Un chant d'appel amoureux': acoustic communication in moths. Journal of Experimental Biology 202, 1711–1723.

Huang, F., Subramanyam, Bh., Taylor, R., in press. Ultrasound affects spermatophore transfer, larval numbers, and larval weight of *Plodia interpunctella* (Hûbner) (Lepidoptera: Pyralidae). Journal of Stored Products Research.

Keckler, D., 1995. Surfer for Windows, version 6. Golden Software, Golden, Colorado, USA.

Lollis, D.W., 1971. The auditory (ultrasonic) reception and behavior of two stored products pests: the almond moth, *Cadra cautella*, and the Indian meal moth, *Plodia interpunctella* (Lepidoptera, Pyralidae). Ph.D. dissertation, University of Oklahoma, pp.62.

Lum, P.T.M., 1979. Degeneration of ova in the bulla seminalis of Lepidoptera. Journal of Insect Physiology 25, 595–599.

Mullen, M.A., Tsao, C.H., 1971. Tympanic organ of Indian meal moth, *Plodia interpunctella* (Hübner), almond moth, *Cadra cautella* (Walker) and tobacco moth, *Ephestia elutella* (Hübner) (Lepidoptera: Pyralidae). International Journal of Insect Morphology and Embryology 1, 3–10.

Payne, T.L., Shorey, H.H., 1968. Pulsed ultrasonic sound for control of oviposition by cabbage looper moths. Journal of Economic Entomology 61, 3–7.

Rydell, J., Skals, N., Surlykke, A., Svensson, M., 1997. Hearing and bat defense in geometrid winter moths. Proceedings of the Royal Entomological Society of London B. 264, 83–88.

SAS Institute., 1990. SAS/STAT User Guide, version 6. 4th edition, SAS Institute, Cary, North Carolina, USA.

Sinha, R.N., Watters, F.L., 1985. Insect Pests of Flour Mills, Grain Elevators, and Feed Mills and Their Control. Research Branch, Agriculture Canada, Publication No. 1776E.

Spangler, H.G., 1988. Moth hearing, defense, and communication. Annual Review of Entomology 33, 59–81.

Subramanyam, Bh., Cutkomp, L.K., 1987. Total lipid and free fatty acid composition in male and female larvae of Indian-meal moth and almond moth (Lepidoptera: Pyralidae). Great Lakes Entomologist 20, 99–102.

Trematerra, P., Pavan, G., 1995. Ultrasound production in the courtship behavior of *Ephestia cautella* (Walk.) *E. kuehniella* Z. and *Plodia interpunctella* (Hb) (Lepidoptera: Pyralidae). Journal of Stored Products Research 31, 43–48.

ADVANCES IN STORED PRODUCT PROTECTION

Proceedings of the 8th International Working Conference on Stored Product Protection

22 – 26 July 2002
York, UK

Edited by P.F. Credland, D.M. Armitage, C.H. Bell,
P.M. Cogan and E. Highley

CAB INTERNATIONAL

CABI Publishing is a division of CAB International

CABI Publishing
CAB International
Wallingford
Oxon OX10 8DE
UK

Tel: +44 (0)1491 832111
Fax: +44 (0)1491 833508
E-mail: cabi@cabi.org
Web site: www.cabi-publishing.org

CABI Publishing
44 Brattle Street
4th Floor
Cambridge, MA 02138
USA

Tel: +1 617 395 4056
Fax: +1 617 354 6875
E-mail: cabi-nao@cabi.org

A catalogue record for this book is available from the British Library, London, UK.
A catalogue record for this book is available from the Library of Congress, Washington, DC, USA.

ISBN 0 85199 691 4

Technical editing and production by Clarus Design Pty Ltd, Canberra, Australia; sales@clarusdesign.com
Printed and bound in the UK by Biddles Ltd, Guildford and King's Lynn, from copy supplied by the editors.