Effects of Ultrasound on the Fecundity and Development of the Cotton Bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)[1]

Yu-Ping Zha,[2,3] Jing-Yuan Chen,[3] Zhan-Bao Jin,[4] Cheng-Bin Wang,[4] and Chao-Liang Lei[4]

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ABSTRACT Noctuid moths possess tympanal hearing organs capable of detecting ultrasonic echolocation calls of hunting bats, which leads to the supposition that ultrasound may be an environmental stress factor for these insects. Effects of non-bat ultrasound from a commercial ultrasound-producing device, LCH20 (Lihui, Inc., Wuhan, China), on the fecundity of adult *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) were investigated in the laboratory. In each of the three trials, 10 newly emerged pairs of male and female moths were introduced into cages with or without added ultrasound. The ultrasound device produced frequencies between 33 and 69 kHz and a 97 dB sound pressure level at a distance of 50 cm. In the cage with ultrasound, female moths had 11.7% more spermatophores and produced 54.5% more eggs than those not exposed to ultrasound. There were no differences between the daily development patterns of larval *H. armigera* in the absence or presence of ultrasound, however the pupation rate was reduced by 14.5% in the presence of ultrasound. This is the first report that ultrasound affects the mating, oviposition, and development in *H. armigera*.

KEY WORDS *Helicoverpa armigera*, Lepidoptera, ultrasound, fecundity, development

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Noctuid moths have tympanal ears on the lateroposterior edge of their metathoracic segment that can detect the echolocation calls of insectivorous bats (Waters & Jones 1996, Roeder 1998, Miller & Surlykke 2001). These ears are able to detect ultrasound in the range of 10 to 100 kHz, with an optimal frequency range between 20 and 50 kHz (Fullard 1988, Norman & Jones 2000). Noctuid moths with ears show a series of evasive behaviors when exposed to bat-like ultrasounds (Spangler 1988, Roeder 1998). Noctuid moths typically have a two-phased behavior in response to the calls of an approaching bat. They typically turn and fly away when bats are distant, but when they are close, the moths show zigzag and looping flights, power dives, or passive falls to avoid the bats (Zha et al. 2009).

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[1] Accepted for publication 6 November 2013.
[2] Corresponding author; zha_yuping@hotmail.com
[3] Hubei Province Academy of Forestry, Wuhan, 430075, Peoples Republic of China.
[4] Key laboratory of Insect Resource Utilization & Sustainable Pest Management of Hubei Province, Huazhong Agricultural University, Wuhan 430070, Peoples Republic of China.

Certain noctuid moths have evolved the ability to use ultrasound for intraspecific communication (Conner 1999, Waters 2003). Males of *Thecophora fovea* (Tr.) (Lepidoptera: Noctuidae) are able to produce ultrasonic pulse trains consisting of 10–12 ms-long pulses with the main energy around 32 kHz for several minutes (Surlykke & Gogala 1986). It is thought that ultrasound production in *T. fovea* functions in long-distance calling. Males of *Hecatesia exultans* Walker (Lepidoptera: Noctuidae) produce pure in-flight ultrasonic tones centered at 30 kHz through castanet-like structures on the forewings. These males respond to heterospecifics, dead conspecifics of either sex, and paper models of conspecifics by modifying their calls (Conner 1999). Males of *H. thyridion* Feisthamel call while defending their mating territories (Surlykke & Fullard 1989).

The cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), is a significant agricultural pest in China as well as many other countries (Fitt 1989, Harsulkar et al. 1999). The bollworm is a widespread polyphagous pest that uses more than 60 crops as host plants, and it has high mobility and fecundity (Fitt 1989). *Helicoverpa armigera* has an optimal auditory frequency at 15–30 kHz with high sensitivity (Fullard et al. 2007). Males and females of *Helicoverpa armigera* are both able to produce ultrasonic signals in the range of 20–40 kHz and have similar signal characteristics (Xue et al. 1996). We found that stress caused by exposure to ultrasound could alter the cholinergic (Zha et al. 2008) and the antioxidant enzyme (Zha & Lei 2012) systems in *H. armigera*. The objective of the present study was to investigate how non-bat ultrasound stress influences the fecundity of *H. armigera*. This information could be helpful for exploiting ultrasound technology to manipulate *H. armigera* behavior for pest management.

Materials and Methods

Insects. *Helicoverpa armigera* were reared in a climate chamber with 27 $\pm$ 1°C, 65 $\pm$ 5% RH, and a L14:D10 light cycle. Following the protocol of Wu & Gong (1997), larvae were reared in culture plates with an artificial diet. Pupae were segregated by sex into separate test tubes. Adult moths were held in 100 ml clear plastic containers and fed 10% honey solution in absorbent cotton.

Ultrasonic devices. The commercial ultrasonic device, LHC20 (Lihui, Inc., Wuhan, China), has been used for arthropod pest management. This device generated peak frequencies at 33 and 69 kHz during our experiments. It produced a 97 dB sound pressure level (SPL) at a distance of 50 cm from the source. The waveform plot showed the ultrasound pulse width was 0.02 s. Distributions of SPL within a cage ranged between 90 and 97 dB, and those in the non-exposed cage approached 0 dB.

Test procedures. Three replications of a paired test were conducted to evaluate *H. armigera* responses to ultrasound. Two identical cages measuring 30 cm $\times$ 30 cm $\times$ 40 cm were built using Plexiglas covering five sides, with one side covered with gauze. The LHC20 ultrasonic device was mounted 50 cm in front of the gauze side of one cage. The other cage was positioned 50 cm behind the commercial ultrasonic device, with one of the Plexiglas sides facing the device. Ten newly emerged pairs of male and female *H. armigera* adults were released into each cage. The ultrasonic unit was turned on immediately after moth introduction and was kept on until termination of the test; no ultrasound units were placed in front of the control cage. Tests were terminated when all moths were dead. The gauze coverings on one side of the cages were replaced by new ones every day. Eggs were collected from the gauze coverings and egg production was recorded daily. Larval hatch was recorded twice a day, and larvae were collected. All dead female moths were preserved in vials containing 99% ethanol for later dissection under a stereomicroscope to count spermatophores (Huang et al. 2003).

Two groups of newly hatched *H. armigera* larvae of similar size were placed in separate test containers. One group of fifty larvae was exposed to ultrasound from the LHC20 device at 8:00 and 17:00 for 0.5 h each time. The other group of larvae was placed in similar conditions without ultrasound, and both groups of fifty larvae were reared normally. Beginning 4 d after experiment initiation, all larvae were weighed and pupae were counted and removed daily. This experiment was replicated three times.

Sixty male pupae and sixty female pupae that had pupated on the same day were selected. One group of 30 male pupae and 30 female pupae was exposed to the ultrasound from the LHC20 device until the termination of the test, whereas the other group of 30 male pupae and 30 female pupae were not exposed to ultrasound. Adult eclosion was recorded daily. This experiment was replicated three times.

Data analysis. Differences in the number of spermatophores per female, number of larvae, larval weight, egg viability, rate of pupation, and adult emergence in the presence and absence of ultrasound were determined using paired *t*-tests (SPSS 11.0; IBM Corp., Armonk, NY). Data for egg deposition were analyzed by one-way analysis of variance (ANOVA) using GraphPad Prism (version 4.0 for Mac, GraphPad Software, San Diego, CA).

Results and Discussion

Exposure to ultrasound significantly increased spermatophore transfer (t = 23.5, df = 2, P = 0.002), oviposition (t = 2.92, df = 2, P = 0.036), and percent pupation (t = −40.9, df = 2, P = 0.001) (Table 1, Figure 1). There was no measurable effect of ultrasound exposure on larval weight (t = 0.060, df = 2, P = 0.958) or percent adult eclosion (t = −3.27, df = 2, P = 0.082).

Our results clearly showed that the number of spermatophores per female and the number of eggs laid increased significantly in response to ultrasound stress from a commercial device. In *H. armigera*, the presence of a spermatophore in the female indicates a single successful mating (Wang et al. 1999). The increased number of spermatophores in females exposed to ultrasound could be due to an increase in the number of successful matings. These results are different from previous studies that concluded that ultrasound could disrupt courtship and mating behaviors of *H. armigera* (Agee & Webb 1969) and other moths, such as *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) (Huang et al. 2003, Huang & Subramanyam 2004), *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), and *Pseudaletia unipuncta* (Haworth) (Lepidoptera: Noctuidae) (Acharya & McNeil 1998). The results also showed that there is no significant difference between the daily development patterns of larval *H. armigera* in the absence and presence of ultrasound. However, there is a significant difference between the pupation rate in the absence or presence of ultrasound (Table 1).

Table 1. Reproductive and development performance of *Helicoverpa armigera* in the presence and absence of ultrasound produced by a commercial device.

	Mean ± SEM[a]				
Treatment	No. spermatophores/female[b]	No. eggs[b]	Larval weight (mg)[c]	Percent pupation[b]	Percent eclosion
Ultrasound	1.43 ± 0.08	5788 ± 889	419.0 ± 1.8	77.7 ± 0.9	73.8 ± 4.5
Untreated control	1.28 ± 0.07	3746 ± 705	418.5 ± 11.7	92.2 ± 0.7	80.4 ± 6.5

[a]Each mean is based on n = 3 replication.
[b]Significantly different according to a paired-*t* test ($P = 0.05$) (SPSS 11.0; IBM Corp., Armonk, NY).
[c]Larval weight after nine days of treatment.

A possible reason for a difference between the result of our present study and those of Agee & Webb (1969) could be that the latter used trains of ultrasound resembling bat echolocation calls, whereas the ultrasound used in the present study was a constant non-bat sound. Bat-like ultrasound makes moths display evasive behaviors, but the non-bat ultrasound may be regarded only as background noise by *H. armigera*.

There are no published reports of increases in insect fecundity under noise stress, but other stressors (e.g., temperature, starvation, insecticide) have been reported to induce similar changes in fecundity (Nemoto 1993, Zhang 2007). For example, the reproductive capacity of newly emerged adults of *Lissorhoptrus oryzophilus* Kuschel (Coleoptera: Curculionidae) was studied under different temperatures with starvation stress (Zhang 2007). Zhang (2007) concluded that the stressors, high temperature and starvation, promoted the reproductive

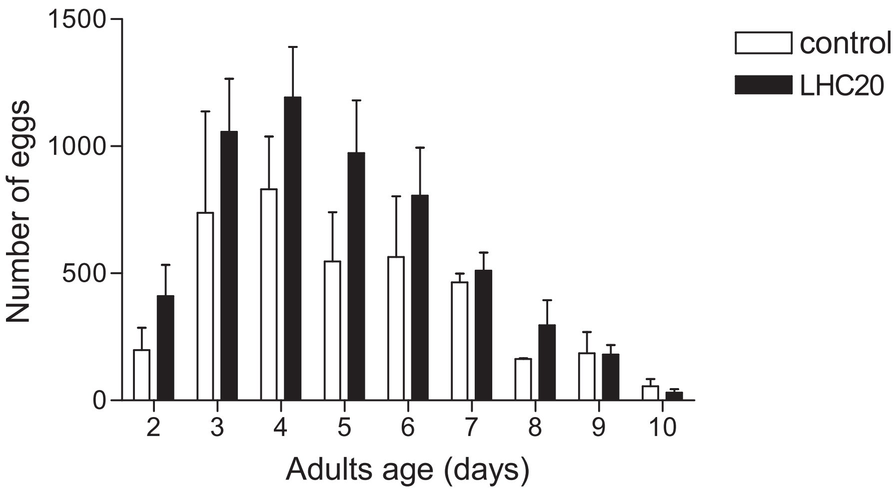

Fig. 1. Daily oviposition pattern of *Helicoverpa armigera* in the absence and presence of ultrasound produced by a commercial device.

capacity of *L. oryzophilus*. In addition, our laboratory experiments have shown that the moths of *H. armigera* have increased mating under light stress (unpublished data). Therefore, we propose that the increase in oviposition in *H. armigera* is attributed to compensatory mechanisms. Our research showed that moths quickly habituate to continual sounds and will produce compensatory mechanisms rendering these "non-bat sounds" ineffectual as a control agent. Rather than being a possible control mechanism, non-bat ultrasounds produced in these frequency ranges could actually increase fecundity of this pest.

It is reported that larval Lepidoptera can produce sounds that are used during interactions between conspecifics (Yack et al. 2001, Fletcher et al. 2006, Brown et al. 2007), but there is no evidence of ultrasound retarding larval development (Huang et al. 2003). We speculated that the impact of ultrasound on cotton bollworm larvae and pupae is not the same as the impact on adults, and in this study, ultrasound had little effect on *H. armigera* in the larval and pupal stages. However, it is possible that ultrasound could be used as a mechanical wave, with the physical stimulus affecting the cotton bollworm larvae and pupae.

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