

Dim Light During Scotophase Enhances Sexual Behavior of the Oriental Tobacco Budworm *Helicoverpa assulta* (Lepidoptera: Noctuidae)

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Source: Florida Entomologist, 98(2) : 690-696

Published By: Florida Entomological Society

URL: <https://doi.org/10.1653/024.098.0244>

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Dim light during scotophase enhances sexual behavior of the oriental tobacco budworm *Helicoverpa assulta* (Lepidoptera: Noctuidae)

Huiting Li, Shuo Yan, Zhen Li, Qingwen Zhang, and Xiaoxia Liu*

Abstract

The role of light in sexual behavior of lepidopteran species has been studied for many years because of the wide variation in the habits of moths and butterflies. Light level is important for mating of butterflies, but information on the potential role of light on sexual behavior in nocturnal moths is scanty. This study was conducted to determine the relationship between light intensity and sexual behavior in a nocturnal moth species. The mating frequency and the hourly variations of both calling behavior and sex pheromone titer in the oriental tobacco budworm *Helicoverpa assulta* (Guenée) (Lepidoptera: Noctuidae) were determined under several light intensities (0.0, 0.5, 5.0, and 50.0 lux). We found that 1) high-intensity light (50.0 lux) suppressed calling behavior, pheromone production, and mating; 2) low-intensity light (0.5 lux) significantly promoted female calling and mating in less time (1 h) than in complete darkness (0.0 lux); and 3) no relationship existed between sex pheromone production and calling behavior. These results suggested that low-intensity light (0.5 lux) promoted mating. This study provides reliable background information for using sex pheromones in conjunction with light traps for integrated management of nocturnal moths.

Key Words: calling behavior; mating; sex pheromone; spermatophore; (Z)-9-hexadecenal

Resumen

Se ha estudiado el papel de la luz en el comportamiento sexual de las especies de lepidópteros por muchos años debido a la amplia variación en los hábitos de polillas y mariposas. El nivel de luz es importante para el apareamiento de las mariposas, sin embargo, la información sobre el posible papel de la luz sobre el comportamiento sexual en las polillas nocturnas es escasa. Se realizó este estudio para determinar la relación entre la intensidad de la luz y el comportamiento sexual en una especie nocturna de polilla. Se determinaron la frecuencia de apareamiento y las variaciones horarias tanto de comportamiento de llamar y el título de la feromona sexual del gusano oriental de la yema del tabaco, *Helicoverpa assulta* (Guenée) (Lepidoptera: Noctuidae) bajo varias intensidades de luz (0.0, 0.5, 5.0 y 50.0 lux). Se encontró que 1) la luz de alta intensidad (50.0 lux) suprimió el comportamiento de llamar, la producción de feromonas y el apareamiento, 2) la luz de baja intensidad (0.5 lux) promovió significativamente la llamada de la hembras y el apareamiento en menos tiempo (1 h) que en la más completa oscuridad (0.0 lux).

3) También se encontró ninguna relación entre la producción de feromonas sexuales y el comportamiento de llamar. Estos resultados sugieren que la luz de baja intensidad (0.5 lux) promueve el apareamiento. Este estudio provee una información de base confiable para el uso de feromonas sexuales en conjunto con trampas de luz para el manejo integrada de las mariposas nocturnas.

Palabras Clave: comportamiento de llamar; apareamiento; feromona sexual; espermatóforo; (Z)-9-hexadecenal

Light is one of the major signals in the environment involved in a variety of life processes of almost all living organisms. The role of light in mating success has been investigated in *Drosophila* species and butterflies (Sakai et al. 1997a, 1997b, 2002; McDonald & Nijhout 2000; Sweeney et al. 2003). Grossfield (1971) reported that many *Drosophila* species showed a characteristic degree of reduced mating success in the dark as compared with that in light, a phenomenon he called "light-dependency of mating." In contrast to butterflies, the temporal synchronization of mating behaviors between female and male moths is mediated by sex pheromones, and the variation in calling behavior and sex pheromone production may affect chemical communication, which in turn impacts the mating success. Previous studies illustrated that the daily rhythm of sexual activity, such as calling activity and phero-

mone production and release, was usually dependent upon endogenous (neural, hormonal) and exogenous factors (photoperiod, temperature) (Hollander & Yin 1982; Raina & Klun 1984; Delisle & McNeil 1987; Raina 1993). Sex pheromone level and calling percentage usually decrease during the photophase, falling to nearly zero by the onset of the scotophase, before increasing again during the scotophase (Schal & Cardé 1986; Delisle & McNeil 1987; Kou 1992; Kamimura & Tatsuki 1994; Dong & Du 2001; Kawazu & Tatsuki 2002; Xiang et al. 2010; Kawazu et al. 2011), and the circadian rhythm of female calling and sex pheromone production and release may be entrained by the photoregime (Kamimura & Tatsuki 1994; Kawazu et al. 2011).

The oriental tobacco budworm *Helicoverpa assulta* Guenée (Lepidoptera: Noctuidae) is a widespread pest, causing great economic

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losses both from direct yield reduction and from the cost of chemicals, application, and scouting required to control them (Fitt 1989). Widely grown transgenic crops have controlled the pest's damage, but the potential ecological consequences and human health effects cannot be ignored (Shelton et al. 2002). Because of the conspicuous phototactic behavior of moths, light trapping is another option, which is used to capture moths in fields (Nowinszky & Puskás 2011; Yan et al. 2012). However, information on the potential role of light in the sexual behavior of these nocturnal moths is scanty. Most nocturnal moths mate at dawn and dusk, suggesting that dim light facilitates mating activity. Rutowski (1991) outlined the hypothesis that environmental temperature might restrict mating activity, but light level also had an independent effect on mating behavior (McDonald & Nijhout 2000).

Our aim was to determine the relationship between light intensity and sexual behavior of *H. assulta*. Thus we undertook a series of experiments to determine the mating frequency and the hourly variations of both calling behavior and sex pheromone titer in the sex pheromone glands under different light intensities. Finally, based on a better understanding of the nocturnal moth's sexual behavior, we suggest approaches for the joint use of the sex pheromone and light traps in the integrated management of nocturnal moth species.

Material and Methods

INSECT REARING AND EXPERIMENTAL LIGHT CONDITION

Larvae of the *H. assulta* used in this work were originally collected in a tobacco field in Xuchang, Henan Province, China, and were maintained for successive generations in the laboratory at 26 ± 1 °C, $70 \pm 10\%$ RH, and a 16:8 h L:D photoperiod. Larvae were reared on an artificial diet (Wu & Gong 1997). Pupae were segregated by sex, and female and male pupae were separately placed in holding cages ($20 \times 25 \times 30$ cm), the walls of which were made of screen mesh. Moths were supplied with 10% honey solution. Moths that emerged during the scotophase were designated as 0-d-old, 1-d-old, 2-d-old, and so forth on subsequent days. Most female moths started calling on the 2nd or 3rd day following emergence (Kou & Chow 1987; Hou & Sheng 2000; Murata et al. 2006; Ming et al. 2007), and therefore we used 2-d-old virgin moths in the study for all experiments.

Trials were conducted in light chambers ($60 \times 40 \times 60$ cm) with white light (milky color, broad spectrum) obtained from 15 W incandescent bulbs (Philips 15W, Royal Dutch Philips Electronics Ltd., Amsterdam, Holland) controlled by an "intelligent" regulator (for gradual increase/decrease in light intensity). The walls, ceilings, and floors of the light chambers were covered with white paper (Omkar & Pathak 2006), and frosted glass was used to obtain uniform illumination. Target light intensities of 0.0, 0.5 ± 0.1 , 5.0 ± 1.0 , and 50.0 ± 2.0 lux (beginning from onset of scotophase to onset of photophase), measured with a TES model 1330A illumination meter (TES Electrical Electronic Corp, Taipei, Taiwan), were used instead of scotophase to evaluate the impacts of light intensity on sexual behavior in *H. assulta*. The light intensity of photophase was 500.0 ± 10.0 lux.

OBSERVATION OF CALLING BEHAVIOR

One 2-d-old virgin female at a time was held in a light chamber at each intensity of illumination (0, 0.5, 5.0, or 50.0 lux) at the beginning of the scotophase and allowed to move freely. Previous observations had confirmed that no moths called during the photophase. We observed calling behavior every 5 min during the scotophase with a

red lamp (approximately 0.5 lux). Calling was recognized by the extrusion of the ovipositor beyond the tip of the abdomen, but females that showed obvious oviposition behavior (laying eggs) were excluded (Kamimura & Tatsuki 1993, 1994). For each virgin female, the following behavioral parameters were recorded: the percentage calling, the mean onset time of calling, the mean duration of calling, and the mean number of calling bouts (Ming et al. 2007). A total of 37 to 40 females were analyzed in each treatment.

SEX PHEROMONE EXTRACTION AND ANALYSIS

In this experiment, the ovipositors and associated pheromone glands of *H. assulta* were extracted at 1 h intervals during 8 h scotophase in light chambers (8 time points). One 2-d-old virgin female was released in one light chamber (0, 0.5, 5.0, or 50.0 lux) at a time at the beginning of the scotophase and allowed to move freely. All 80 females were individually placed in the chambers during a few days. Ten samples were measured for each time point. Therefore, 10 females were kept in their chambers for 1 hour, another 10 for 2 hours, until 8 hours had elapsed. Pheromone extraction was performed as described by Kawazu & Tatsuki (2002), Ming et al. (2007), and Lima et al. (2008). For each analysis, the ovipositors and associated pheromone glands of 10 virgin females were excised and individually placed in 5 μ L of hexane containing 5 ng of the internal standard tridecyl acetate (13:Ac) (Kaimura & Tatsuki 1994; Ming et al. 2007), and then sealed and left at room temperature for 15 min. The amount of (Z)-9-hexadecenal (Z9-16:Ald), the main pheromone component of *H. assulta* (Ming et al. 2007), was determined by a Hewlett-Packard HP 5890 Series II Plus gas chromatograph (GC) (Hewlett-Packard Development Company L.P., California, USA) equipped with a capillary column (50QC2/BPX70-0.25, SGE), a flame ionization detector (FID), and a splitless injector system. The column was kept at 100 °C for 3 min, increased at 10°C/min to 250 °C, and then to the final temperature for 5 min. Nitrogen was used as the carrier gas at a column head pressure of 200 kPa. Z9-16:Ald was identified relative to the retention time of the known compound, and quantified by comparing the area under the curve to that of 13:Ac.

MATING SUCCESS UNDER VARIOUS LIGHT INTENSITIES

Preliminary observation indicated that few moths mated during the photophase; thus the mating trial was conducted during scotophase. We examined mating success at 2 time points (1 h and 8 h into the scotophase) under 4 light intensities. For each time point, 10 pairs of virgin moths were randomly paired at the onset of scotophase, and each pair was held separately in a light chamber (0, 0.5, 5.0, or 50.0 lux). At the end of the trial, all female moths were dissected to detect spermatophores to determine whether they had mated (Dong et al. 2005; Su et al. 2006). Mating frequency was calculated as the percentage of inseminated females relative to the total number of dissected females. A total of 10 females were analyzed for each time point under 1 light intensity, and each treatment was repeated 9 times.

DATA ANALYSES

All statistical analyses were conducted using SPSS 16.0 Windows (IBM, Armonk, New York, USA) (SPSS 1998). Descriptive statistics were given as mean values and standard errors of the mean. Data were analyzed by 1-way ANOVA, and means were compared by the Tukey HSD test. In all tests, *P* values < 0.05 were considered significant.

Results

EFFECTS OF LIGHT INTENSITY ON FEMALE CALLING BEHAVIOR

As shown in Fig. 1, calling by *H. assulta* females occurred throughout the scotophase. The percentage of females calling usually increased with the passage of time in the scotophase and peaked at 5 to 7 h. The percentage of calling females at any given time in the scotophase remained the highest in the low-intensity light (0.5 lux) treatment.

Light intensity greatly affected the parameters of female calling behavior, including the mean onset time of calling, the mean duration of calling, and the mean number of calling bouts (Table 1). In low-intensity light (0.5 lux), the onset time of calling was the earliest ($F_{3,98} = 13.506$, $P < 0.001$), the mean duration of calling was the longest ($F_{3,98} = 22.682$, $P < 0.001$), and the mean number of calling bouts was the largest ($F_{3,152} = 25.324$, $P < 0.001$).

EFFECTS OF LIGHT INTENSITY ON SEX PHEROMONE TITER

Light intensity and the length of time into the scotophase both showed significant effects on the sex pheromone titer (Z9-16:Ald)

(Fig. 2). The pheromone titer in low-intensity light (0.5 lux) increased dramatically after the beginning of the scotophase, peaked 4 h later, remained at this level for 3 h, and then decreased stably for the remainder of the scotophase ($F_{7,72} = 18.969$, $P < 0.001$), which showed a similar temporal pattern to that in the dark ($F_{7,72} = 23.200$, $P < 0.001$). In the light of 5.0 and 50.0 lux, the sex pheromone titers remained lower than that in low-intensity light and did not change appreciably during the entire scotophase (5.0 lux: $F_{7,72} = 0.930$, $P = 0.489$; 50.0 lux: $F_{7,72} = 1.108$, $P = 0.368$).

EFFECTS OF LIGHT INTENSITY ON THE INCIDENCE OF MATING

As shown in Fig. 3, light intensity significantly affected the incidence of mating. At 1 h into the scotophase, the incidence of mating was significantly higher in low-intensity light (0.5 lux) than under darkness (0.0 lux), indicating low-intensity light could promote mating in a shorter amount of time (1 h) than under darkness (1 h: $F_{3,32} = 14.258$, $P < 0.001$). At 8 h into the scotophase, there was no significant difference in the incidence of mating between the low-intensity light (0.5 lux) and dark treatments (8 h: $F_{3,32} = 16.608$, $P < 0.001$).

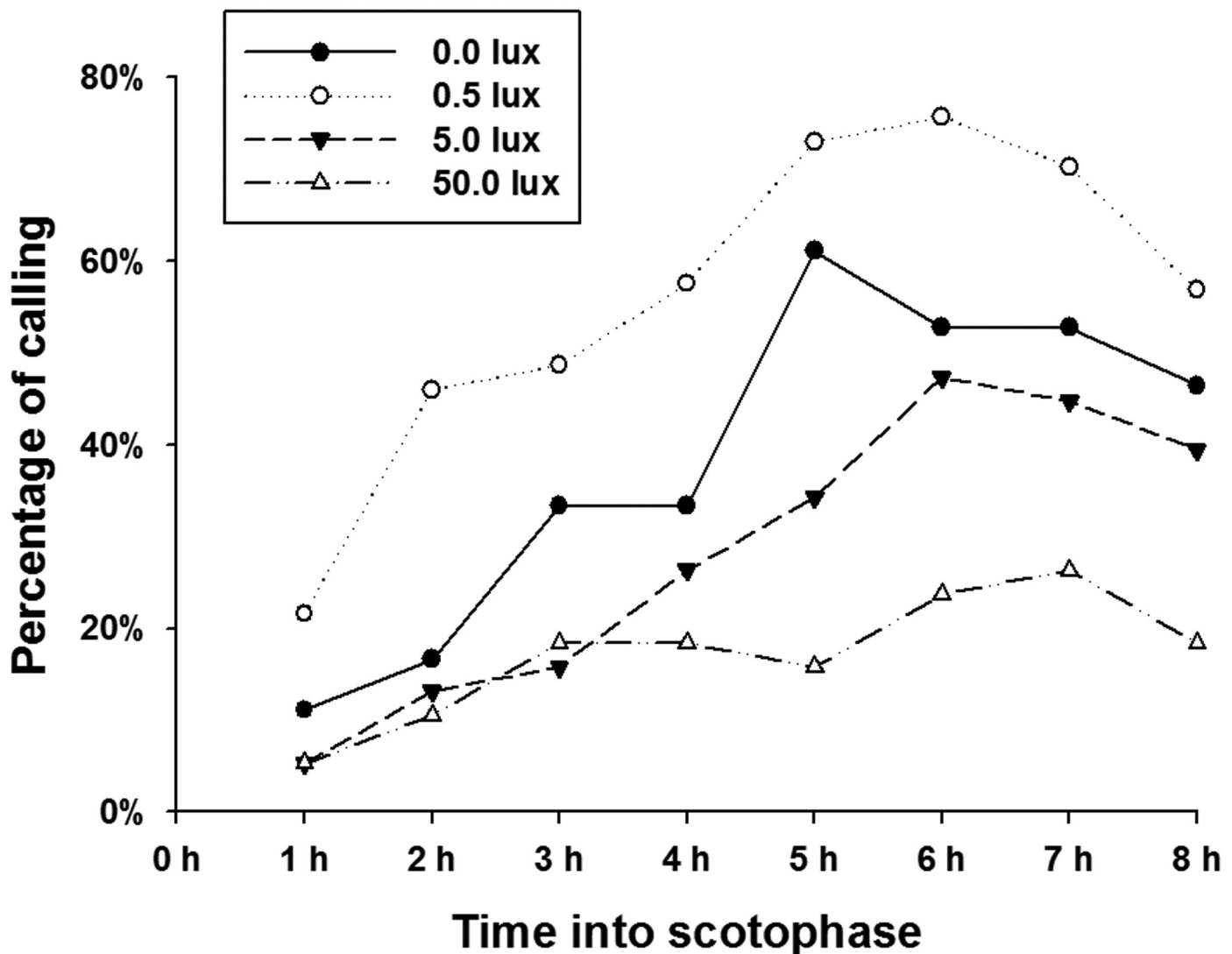


Fig. 1. Effects of light intensity and time into scotophase on percentage of virgin females calling. Thirty-seven to 40 female moths were used in each treatment. Percentage of calling = $100 \times$ number of calling females at the time point / total number of females.

Table 1. Effects of light intensity on the calling behavior of *Helicoverpa assulta* virgin females.

Light intensity (lux)	No. of females	Called at least once (%)	Mean onset time of calling (min)	Mean duration of calling (min)	Mean no. of calling bouts
0.0	40	70	167.50 ± 20.48 bc	120.89 ± 15.58 b	3.58 ± 0.49 b
0.5	39	84.6	101.06 ± 15.63 c	237.12 ± 20.73 a	6.44 ± 0.56 a
5.0	40	67.5	249.26 ± 19.14 a	85.74 ± 13.31 bc	2.40 ± 0.42 bc
50.0	37	37.8	239.29 ± 24.17 ab	41.43 ± 7.89 c	1.08 ± 0.27 c

Calling behavior was monitored every 5 min during the scotophase with a red lamp (approximately 0.5 lux). Means ± SE in columns followed by different letters are significantly different (Tukey multiple comparison test, $P < 0.05$).

Discussion

In the current study, we found that female *H. assulta* calling was inhibited in high-intensity light (50.0 lux), whereas calling behavior was the most frequent in low-intensity light (0.5 lux) based on the record of the calling parameters. Secondly, we found that the female calling during the scotophase was the most active from 5 to 7 h into either the dark condition (0.0 lux) or low-intensity light (0.5 lux). Likewise, Web-

ster & Conner (1986) found that calling of *Spilosoma congrua* Walker (Lepidoptera: Arctiidae) persisted after the onset of a low-intensity light photophase or when the scotophase was prolonged, and they found that high-intensity light inhibited calling. *Helicoverpa assulta* calling behavior becomes irregular under continuous light, and the percentage calling decreases (Kamimura & Tatsuki 1994). Our study is the first to confirm that low-intensity light (0.5 lux) promotes female calling, and that calling is suspended when the light intensity increases

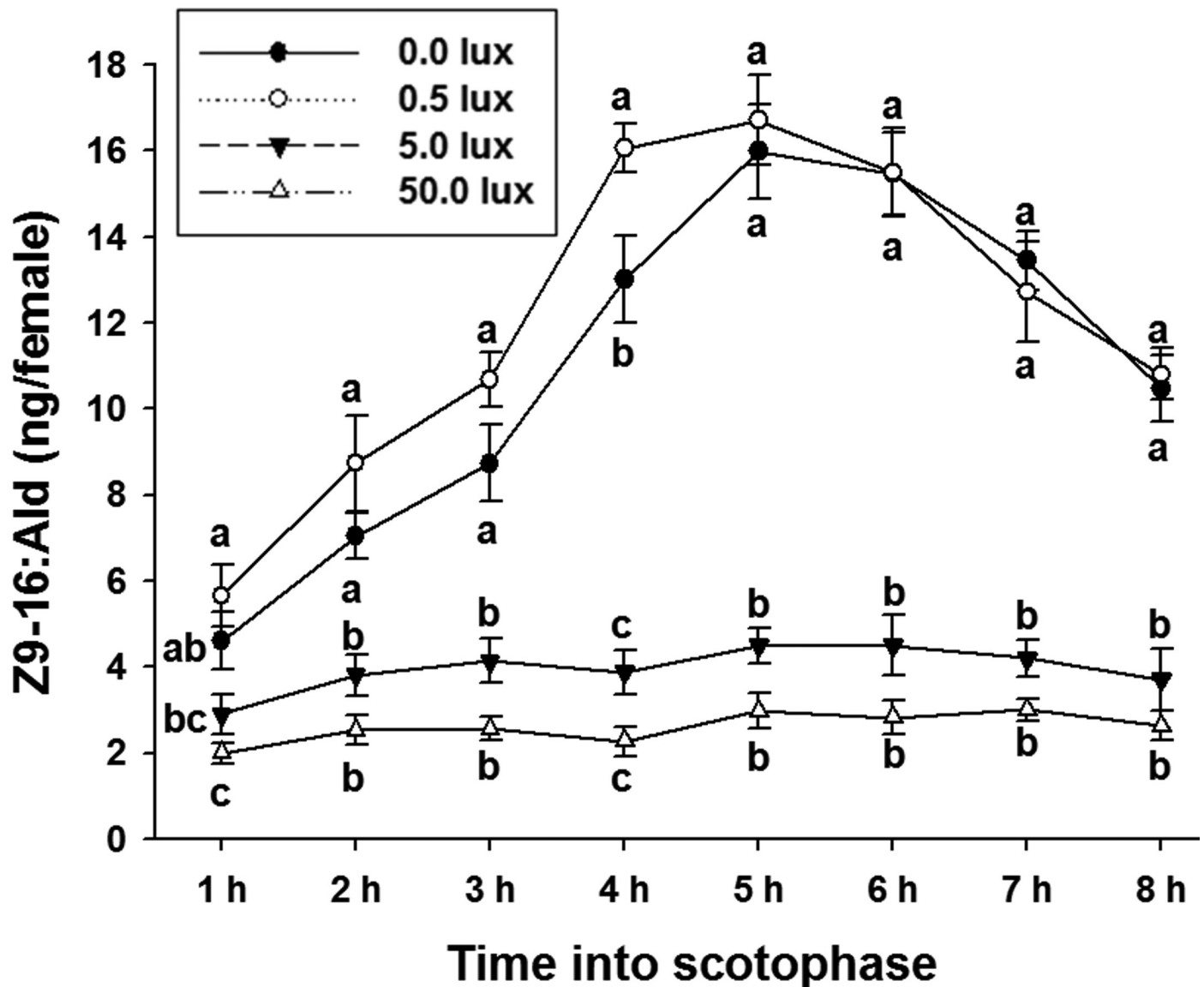


Fig. 2. Effects of light intensity and time into scotophase on sex pheromone (Z9-16:Ald) titer in pheromone glands of *Helicoverpa assulta*. Data in the same column followed by different letters are significantly different (Tukey multiple comparison test, $P < 0.05$). Each treatment was replicated 10 times ($N = 10$).

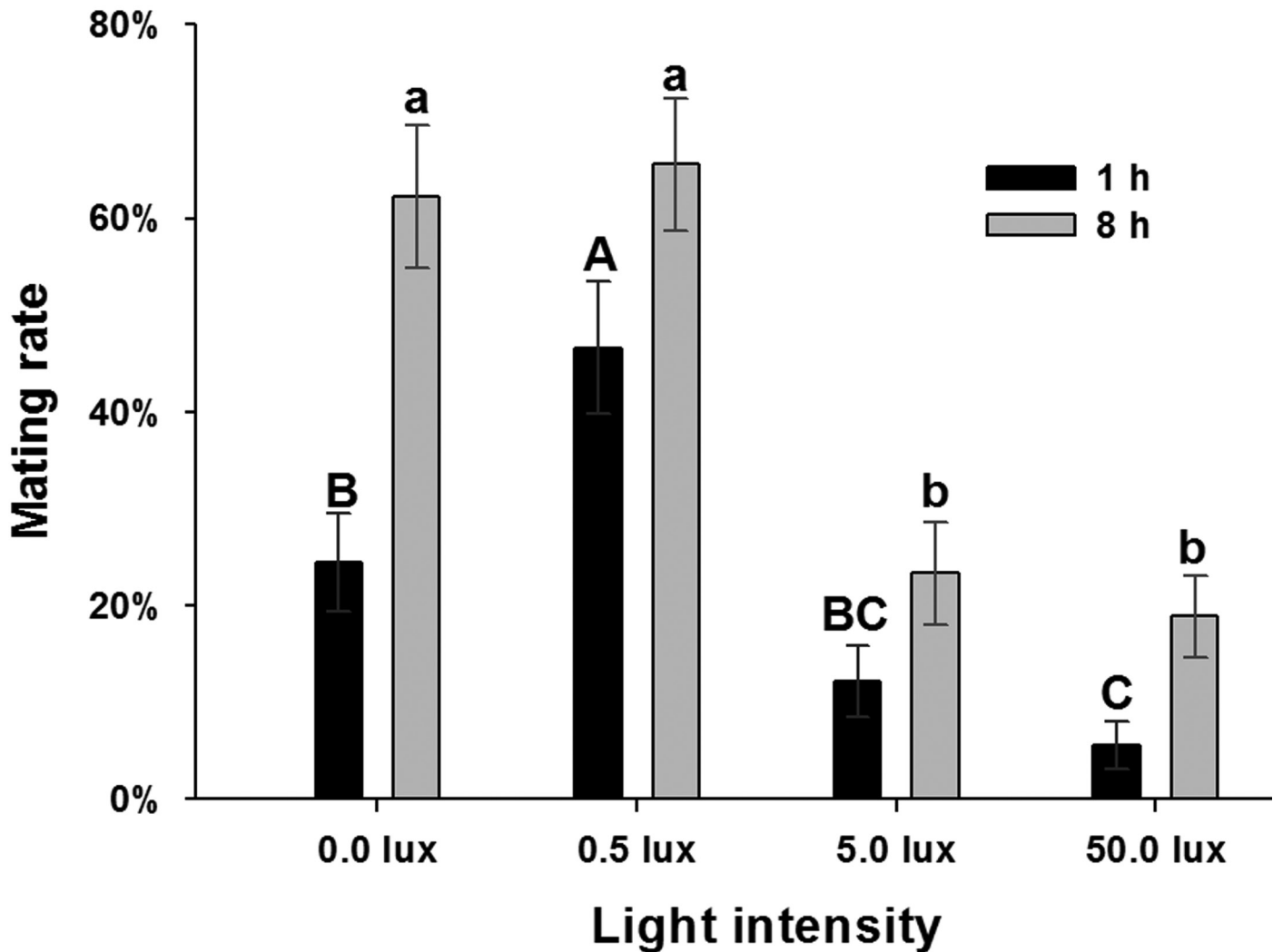


Fig. 3. Effects of light intensity on the mating rate (%) of *Helicoverpa assulta*. Each value is the mean \pm SE of 9 collections. Capital and lowercase letters above each bar indicate significant differences at 1 h and 8 h into scotophase, respectively (Tukey multiple comparison test, $P < 0.05$).

to a specific level. Whether low-intensity light (0.5 lux) is significant for other sexual behaviors remains to be determined.

We did not find that low-intensity light (0.5 lux) could promote sex pheromone production more than darkness. The production of Z9-16:Ald was the highest under darkness and low-intensity light (0.5 lux), and production was inhibited by light of 5.0 and 50.0 lux. Fluctuations of Z9-16:Ald under darkness and low-intensity light (0.5 lux) were similar to findings of Ming et al. (2007). They found that the amount of Z9-16:Ald in *H. assulta* peaked 4 h after the lights were turned off, and remained at this level for 4 h. In contrast, Kamimura & Tatsuki (1993) found that the sex pheromone titer of *H. assulta* reached a maximum at 2 h after lights had been turned off and remained at this level for 4 h, and they found that only a small amount of sex pheromone was detected during the photophase. Sex pheromone level usually increases during the scotophase and decreases during the photophase (Schal & Cardé 1986; Raina et al. 1991; Kou 1992; Dong & Du 2001; Kawazu & Tatsuki 2002; Kawazu et al. 2011).

Under darkness and low-intensity light (0.5 lux), the highest amounts of Z9-16:Ald in the pheromone gland of *H. assulta* occurred a little earlier than the peak of female calling (Figs. 1 and 2). However, there was no relationship between sex pheromone production and calling behavior. More than 40% females called while the amount of

Z9-16:Ald was low in the light of 5.0 lux (Figs. 1 and 2). Similar to our study, Ming et al. (2007) found that the peak of pheromone production in *H. assulta* occurred about 2 h before the peak of calling under 16:8 h L:D, whereas Kamimura & Tatsuki (1993, 1994) found that maximal pheromone titer and calling activity occurred simultaneously in *H. assulta* under 15:9 h L:D. *Lymantria dispar* L. (Lepidoptera: Erebidiae) females that called during the early part of their first photophase had virtually no extractable pheromone (Raina 1993). Thus, pheromone biosynthesis and calling in moths appear to be 2 independently controlled processes, which have a circadian periodicity (Delisle & McNeil 1987; Raina 1993; Fónagy 2009).

Canale et al. (2013) found that integration of visual and chemical stimuli were fundamental for mate location and courtship in *Psytalia concolor* (Szepliget, 1910) (Hymenoptera: Braconidae). Interestingly, Burks et al. (2011) found that weak illumination in the final hour of the scotophase promoted mating in navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae), whereas weak illumination all night did not increase the mating frequency. In the current study, we found that low-intensity light (0.5 lux) could promote mating in an amount of time 1 h shorter than under darkness. Possibly darkness influences the recognition of a sexual partner, and therefore, low-intensity light promotes mating in a relatively short time. Visual factors might

be more important than the sex pheromone in the recognition of sex partners at close range, and the long-wavelength-sensitive *opsin* gene might be important for moth copulation (Yan et al. 2014). Certainly, morning twilight may be required for mating behavior of certain species (Burks et al. 2011). Meanwhile, greater sexual activity in the low-intensity light might promote mating. In addition, mating of *H. assulta* was reduced significantly in high-intensity light (50.0 lux) compared with that observed under darkness and low-intensity light (0.5 lux), which is consistent with the phenomenon that moths usually mate at dawn and dusk (Topper 1987).

Based on our experiments, we suggest that light traps in current use not only attract moths from long distances, but also inhibit moth copulation at relatively short distances. However, further research will be needed to evaluate the actual situation in fields. Our present study provides some background information for using sex pheromones in conjunction with light traps for integrated management of nocturnal moths.

In certain lepidopteran species, male moths may also produce sex pheromones (Lecomte et al. 1998), and the volatile chemicals released by male abdominal hairpencils—acting as aphrodisiacs—can improve mating success with conspecific females and play a role in mating choice and species isolation (Hillier & Vickers 2004, 2011). Upwind flight of males toward a pheromone source can be inhibited by light (Liu et al. 2004). Thus, light intensity might affect the sexual behavior of male *H. assulta*, and in mating trials the sexual behaviors of both males and females may be affected by light intensity. This study revealed that *H. assulta* was highly sensitive to light intensity, which played an important role in sexual behavior. Because of the potential ecological risks of transgenic plants and chemical control, light and sex pheromone trapping are commonly used to capture moths in fields (Voerman & Rothschild 1978; Ryall et al. 2012; Nowinszky & Puskás 2011; Yan et al. 2012). Our study provides a greater understanding of the sexual behavior of nocturnal moths, and is beneficial for integrated management of nocturnal moths. However, several aspects are not yet clear, including variations in the ability of males to inseminate females under different light intensities, and whether light intensity influences the sex pheromone acceptance by males. These questions represent future research directions.

Acknowledgments

We are grateful to Hui Ni and Jialin Zhu for their valuable comments on the manuscript. This research was supported by Natural Science Foundation of China (31371943).

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