

Calling and Mating Behavior of *Diaphania angustalis* (Lepidoptera: Crambidae)

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Abstract

Diaphania angustalis Snellen (Lepidoptera: Crambidae) has emerged as a very important pest of blackboard tree, *Alstonia scholaris* (L.) R. Br. (Apocynaceae), in China during the last two decades. Understanding its biology and behavior is crucial for designing effective and environmentally friendly pest management strategies. Under laboratory conditions [25–28°C, 12:12 (L:D) h, 75–80% RH], adults emerged during both light and dark hours with peak emergence occurring between the first and fifth hours of scotophase, and unmated males and females lived for a mean (\pm SE) 5.4 ± 0.4 and 5.3 ± 0.7 d, respectively. Female calling behavior was observed only during scotophase (peaking in the fifth hour) by 1- to 5-d-old females, and mating activities occurred 2–5 d after emergence, peaking on day 3. These behavioral characteristics could inform control programs targeting adults.

Key words: *Diaphania angustalis*, emergence, calling behavior, mating behavior

The blackboard tree, *Alstonia scholaris*, is a tropical evergreen tree native to Australasia, the Indian subcontinent, Indomalaya, and Malesia (Macabeo et al. 2005, Jajetia and Baliga 2006, Jonganurakkun et al. 2007) and is a valued medicinal plant and street tree in China (Wang et al. 2014). This tree frequently suffers damage from *Diaphania angustalis* Snellen (Lepidoptera: Crambidae) in Yunnan, Guangdong, Guangxi, and Sichuan provinces in China (Li et al. 2006; Huang et al. 2007; Chen 2009, 2016; Shu and Li 2014). Outbreaks of this pest in China have been common since the early 2000s after the host tree *A. scholaris* became widely planted for commercial use and for landscaping (Li et al. 2006, Chen 2009). The *D. angustalis* larvae feed on mesophyll leaf tissues, and damage can cause early senescence and complete defoliation during heavy infestations. Chemical control with organophosphates, pyrethroids, and biopesticides is recommended for managing *D. angustalis* (Huang et al. 2007, Chen 2009, Lei and Xie 2009), but concerns over insecticide resistance and environmental contamination have been raised. Alternative and safer control methods are needed for *D. angustalis* management.

The morphology and life history of *D. angustalis* have been partially described (Lei and Xie 2009, Cai et al. 2012). The pest has 6–7 generations per year in China (Chen 2009). Females deposit their eggs on the leaves, and the larvae complete six instars. The larvae begin

activity when the new leaves grow out, and the sixth instar larvae roll a leaf to form a chamber in which they overwinter as either a larva or pupa. The adult females disperse before calling or mating. Recently, our group studied female sex pheromone production and identified the major components (Ma et al. 2017). However, there is little information on the *D. angustalis* reproductive behavior. In this study, we investigated *D. angustalis* adult emergence, longevity, and calling and mating behavior. Our long-term goal is to develop environmentally friendly control strategies based on this insect's biology and behavior.

Materials and Methods

Insects

Diaphania angustalis larvae (third and later instars) and host leaves were hand-removed from infested *A. scholaris* trees in July and August 2016 in Conghua (23.57°N, 113.55°E), Guangzhou, China. Trees were 5–6 yr old and 16–20 cm diameter at 1.3 m height. Larvae were fed fresh *A. scholaris* leaves replaced daily and reared in transparent plastic rearing cages (60 × 30 × 30 cm) until pupation. The rearing cages were held in the laboratory at 25–28°C, with a 12:12 (L:D) h photoperiod (scotophase: 20:00–8:00 h), and 75–80% RH. Upon adult emergence, moths were supplied with 15% honey solution on cotton, replaced daily.

Diurnal Rhythm of Adult Emergence

Emergence of adults (193 males and 219 females) from a pool of 420 pupae, held in a 1.5-liter plastic jar under conditions described above, was recorded hourly over a 24-h period. During the observation period, the emerged adults were removed from the jars, counted, and placed in new cages hourly. A flashlight covered with red translucent plastic was used to aid observation during scotophase and reduce light disturbance of the insects.

Calling Behavior

The calling behavior of virgin female moths was recorded 1–5 d post-eclosion during scotophase. Calling females were characterized by an upward-curved abdomen, a protruded ovipositor, and frequent up-and-down movements of the antennae. No calling behavior was observed during photophase in preliminary observations. The observation was repeated for three batches of insects ($n = 20$, all in a single container) during July–August 2016. Newly emerged virgin female moths were kept in 1.5-liter plastic containers containing fresh *A. scholaris* leaves to provide a host stimulus to the insects. Observations were made under red light. The starting time, posture, length of calling, and number of calling females were recorded.

Mating Behavior

Twenty newly emerged (<24 h old), virgin moths of each sex were placed in a clear Perspex cage (200 × 40 × 40 cm). An observation was made every 30 min during scotophase until a mating was observed. Afterward, the container was monitored continuously for moths initiating mating, and the duration of these copulations was recorded. Additionally, the number of mating pairs was recorded daily for 5 d. This experiment was repeated for 3 batches ($n = 20$ for each batch) during July–August 2016.

Oviposition

Ten mated females (emerged on the same day) were placed together in a clear Perspex cage (60 × 60 × 60 cm) with an *A. scholaris* seedling (25–30 cm in height). The date of oviposition, the number of

eggs, and the dates of egg hatch were recorded daily. This experiment was repeated three times (30 females in total).

Longevity

Ten males, 10 females (both sexes <24 h old), and 10 pairs of mated males and females were each placed in each Perspex cage. The mated moths had emerged on the same day. They were provided with 15% honey solution as supplementary nutrition. The number of dead moths was recorded each day until all the moths died. This experiment was repeated three times, and a total of 120 moths were observed.

Statistical Analysis

The proportion of calling female moths, and measurements of mating behavior, mating duration, and longevity of adults were log-transformed before being subjected to analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) test to separate the means ($\alpha = 0.05$). All analyses were performed using SPSS version 17.0 software (SPSS, Inc., Chicago, IL).

Results

Diurnal Rhythm of Adult Emergence

First instar larvae pupated after 15–18 d, and adults emerged approximately 8–10 d after pupation. Although males and females emerged both during photophase and scotophase, the majority (81%) emerged during scotophase (Fig. 1). Female emergence peaked 3–4 h and male emergence 4–5 h into scotophase.

Calling Behavior

Female moths began calling 2–3 h into scotophase. Each calling period lasted for 20–30 min based upon observation of 60 females. Each female exhibited calling behavior one or two times per day. The percentage of females exhibiting calling behavior during a 24-h period varied with their age ($F = 59.86$; $df = 4,10$; $P < 0.001$), with 3-d-old moths being the most active (Fig. 2). Less than 15% of the 1-d-old or 5-d-old females exhibited calling behavior. Female

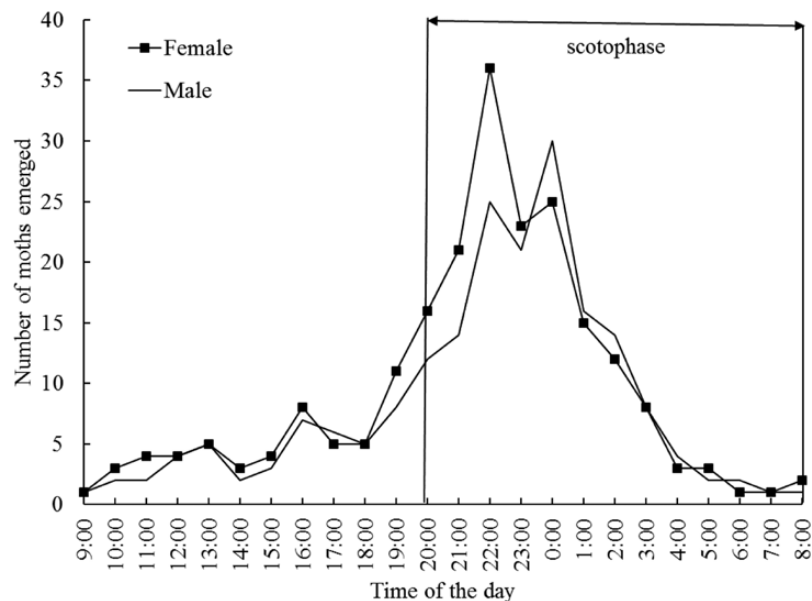


Fig. 1. The diurnal rhythm of *Diaphania angustalis* adult emergence based upon 193 males and 219 females.

calling behavior also varied with the time into scotophase (Fig. 3A; $F = 91.69$; $df = 11,24$; $P < 0.001$). Peak calling behavior occurred 5 h into scotophase. No calling behavior was observed before 3 h and after 8 h into scotophase.

Mating Behavior

Moths were first observed mating on the second day after emergence. Before mating, quiescent males became active and excited

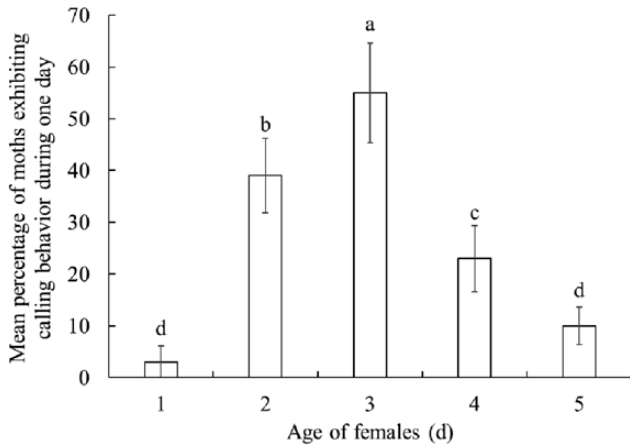


Fig. 2. Effect of age on female calling under laboratory conditions. Means are based upon three batches of 20 moths per batch. Bars with different letters are significantly different (Tukey's HSD test).

presumably when they sensed the sex pheromone released by females. They then responded to the calling females by fanning their wings rapidly in an elevated position and approaching the females. The male and female then made contact with their antennae. If they accepted each other (the acceptance rate was 88%), they would initiate copulation. Otherwise, the male typically flew away from the female and made contact with another female. Mating varied with the hour of scotophase ($F = 106.9$; $df = 11,24$; $P < 0.001$) and was observed only between hour 3 and 8 (Fig. 3B), with peak mating activity 5 h into scotophase. Mating activity varied with moth age ($F = 72.56$; $df = 4,10$; $P < 0.001$), and peak mating activity occurred on the third day after emergence (Fig. 4). After mating, both males and females would rest or feed. The observed longest mating duration was 135 min by a 5-d-old pair. The shortest mating duration was 66 min by a pair of 3-d-old moths. The mean mating duration varied significantly with the age of moths in the mating pair (Fig. 5; $F = 35.6$; $df = 4,10$; $P < 0.001$).

Oviposition

Mated females began to oviposit on the second day after mating. Eggs were deposited on host leaves individually or in clutches. Each female laid an average of 65.3 ± 2.8 eggs. The hatching rate was 92%. The duration of egg stage was 3.9 ± 0.7 d based on observation of 588 eggs.

Longevity

The longevity of unmated and mated moths is shown in Table 1. There were no significant differences in their longevity ($F = 2.87$; $df = 3,8$; $P = 0.07$).

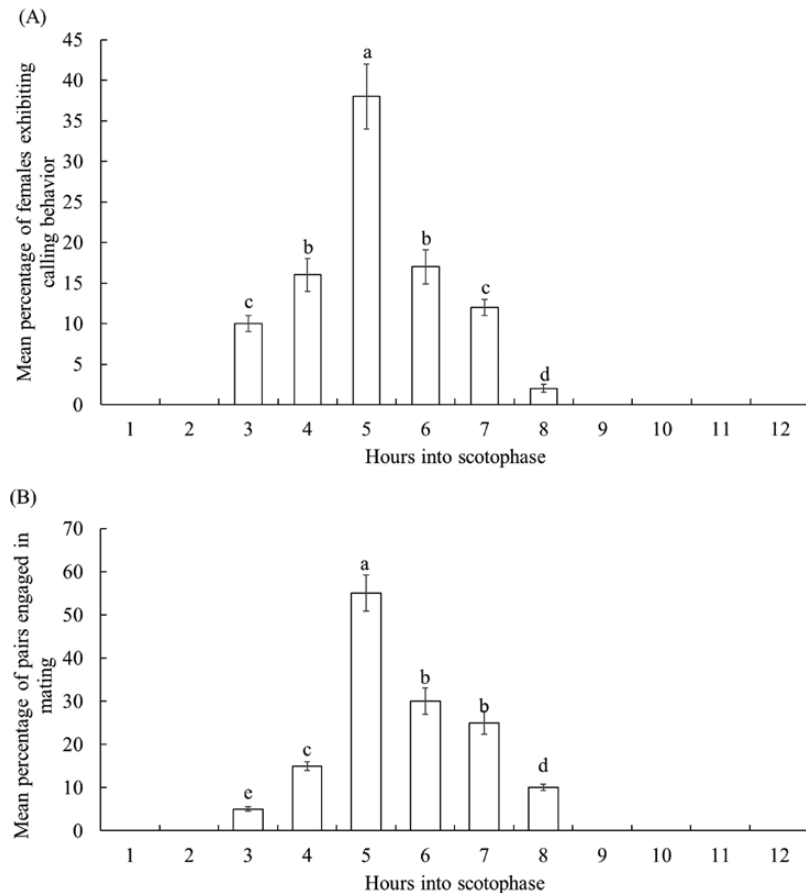


Fig. 3. Incidence of calling and mating by *Diaphania angustalis* at different times during scotophase. Bars with different letters are significantly different (Tukey's HSD test). (A) Mean percentage of females exhibiting calling behavior. (B) Mean percentage of pairs engaged in mating.

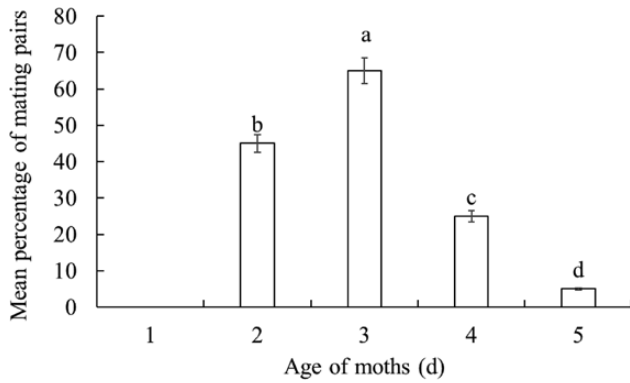


Fig. 4. Effect of age of *Diaphania angustalis* on mating frequency under laboratory conditions. Bars with different letters are significantly different (Tukey's HSD test).

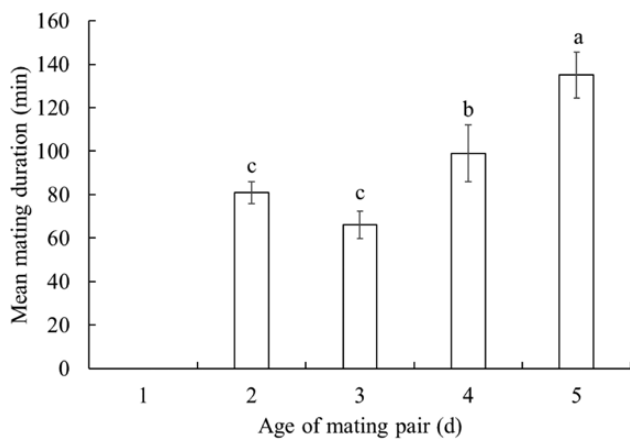


Fig. 5. Effect of age of *Diaphania angustalis* on mating duration under laboratory conditions. Bars with different letters are significantly different (Tukey's HSD test).

Discussion

In lepidopterans, emergence, calling and mating behavior of moths follow a specific periodicity and diurnal rhythm (Kumara et al. 2015, Zhou et al. 2016). In the present study, results showed that *D. angustalis* emerged during light and dark hours but preferred dark hours, which is similar to other species in the same genus such as *D. perspectalis* (Walker) (Lepidoptera: Phycitidae) (She and Feng 2006) and *D. glaucalis* (Guenée) (Lepidoptera: Pyraustinae) (Liu et al. 2014). Female calling and adult mating behavior were limited to hours in scotophase, and this is also the same as *Euzophera batanagensis* (Caradja) (Lepidoptera: Pyralidae) (Liu et al. 2008) and *D. nitidalis* (Stoll) (Lepidoptera: Pyralidae) (Valles et al. 1992), but is different from other lepidopterous insects such as *Parocneria orientalis* (Chao) (Lepidoptera: Lymantriidae) and *Paranthrene regalis* (Butler) (Lepidoptera: Sesiidae) which only mated during photophase (Zhou 1991, Zang et al. 2012). Our observations of emergence timing of *D. angustalis* adults revealed that males and females emerged synchronously, and the peak of moth emergence occurred during the middle of scotophase.

Female *D. angustalis* displayed only one calling posture characterized by a raised (20–30°) abdomen and protrusion of the ovipositor. This posture exposes the pheromone gland, and calling intervals are the best time to extract sex pheromone (Ma et al. 2017). The characteristic posture in *D. angustalis* females during calling is also observed in other moths such as *Lasiognatha cellifera* (Meyrick) (Lepidoptera: Tortricidae) (Chang et al. 2015), *Diatraea indigenella* (Dyar and

Table 1. Longevity (d) of adult *Diaphania angustalis* from laboratory observations

Rearing group	<i>n</i>	Range	Mean
Unmated females	30	2–7	5.4 ± 0.4
Unmated males	30	1–6	5.3 ± 0.7
Mated females	30	3–6	4.7 ± 0.4
Mated males	30	2–5	3.8 ± 0.6

Heinrich) (Lepidoptera: Crambidae) (Palacio-Cortés et al. 2014), and *D. indica* (Saunders) (Lepidoptera: Pyralidae) (Kinjo and Arakaki 1997), *Condylorrhiza vestigialis* (Guenée) (Lepidoptera: Crambidae) (Ambrogi et al. 2009), *Atheloca subrufella* (Hulst) (Lepidoptera: Phycitidae) (Nascimento et al. 2016), and *Opisina arenosella* (Walker) (Lepidoptera: Oecophoridae) (Kumara et al. 2015).

Most *D. angustalis* females required 3 d to become sexually mature, and this is common for many insects as they need to obtain nourishment for ovary development after emergence (Qin and Guo 1994). Previous observations indicated that peak calling behavior in *D. angustalis* coincides with peak female sex pheromone production (Ma et al. 2017). A similar pattern has been reported in other species, such as *Cnephasia jactatana* (Walker) (Lepidoptera: Tortricidae) (Jimenezperez and Wang 2004), *Helicoverpa assulta* (Guenée) (Lepidoptera: Noctuidae) (Kamimura and Tatsuki 1993), and *Micromelalopha troglodyta* (Graeser) (Lepidoptera: Notodontidae) (Chen et al. 2014). The observed pattern of calling activity suggests that the best method to extract sex pheromone glands is to utilize 3-d-old females during fifth hour of scotophase.

Behavioral patterns involved with reproduction are important components in an insect's life history. The current study could help identify or enhance novel *D. angustalis* management strategies that utilize the sex pheromone to attract and kill adult males or disrupt mating behavior. The current study provides information on when sex pheromone-baited traps would be most effective for trapping *D. angustalis*. Further investigations on its sex pheromone communication, such as effective distance of synthetic sex pheromones and effect of synthetic sex pheromone on mating success, could provide practical knowledge for designing environmentally friendly *D. angustalis* control strategies.

Acknowledgments

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