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EMERGENCE TIME AND MATING-RELATED BEHAVIOR OF THE COTTON BOLLWORM, *HELIOTHIS ARMIGERA*, (LEPIDOPTERA: NOCTUIDAE) IN REVERSED PHOTOPERIOD[1]

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Rong Kou and Yien-Shing Chow (1987) Emergence time and mating-related behavior of the cotton bollworm, *Heliothis armigera*, (Lepidoptera: Noctuidae) in reversed photoperiod. *Bull. Inst. Zool., Academia Sinica* **26**(2): 179-186. A study of sex pheromonal behavior of the cotton bollworm, *Heliothis armigera* (Hübner), was conducted at 20-22°C, under a reversed 14L: 10D photoperiod. Some females started calling at 2 days postemergence. As moths aged, calling commenced earlier in the scotophase and the 6-day-old females called at the earliest. Mating percentage also coincided with female calling during the scotophase; 4-day-old adults mated at the earliest and exhibited the shortest mating duration. Different sex ratio have no effect on female calling, but high mating percentage might be obtained at sex ratio of female : male=1:2.

The cotton bollworm, *Heliothis armigera* (Hübner), is a polyphagous pest of field crops and seriously interferes with tomato and corn production in Taiwan and Australia (Rothschild *et al.* 1982, Talekar *et al.* 1983). No effective control method is available at the present time. Its sex pheromone components have been first identified as (Z)-11-hexadecenal (Piccardi *et al.* 1977) and later (Z)-11-hexadecen-1-ol, (Z)-9-hexadecenal, hexadecenal and 1-hexadecanol are added (Nesbitt *et al.* 1979). In field trapping experiment, only (Z)-11-hexadecenal and (Z)-9-hexadecenal are needed, addition of (Z)-11-hexadecen-1-ol would reduce the number of insects caught (Nesbitt *et al.* 1980). Thus the true mating response of this pest is very complicated. As little information is available on the sex pheromonal behavior of this pest, such as diel rhythm of female calling and the complementary rhythm of male response, or on the criteria of the physiological age of moths, this study was undertaken to define physiological age of the cotton bollworm and examined the effects of age and sex ratio on sex pheromone-related behaviors.

MATERIALS AND METHODS

Insects

Cotton bollworm pupae used in this study were supplied by the Asian Vegetable Research and Development Center, Tainan, Taiwan. Pupae were held separately in growth chambers at 25°C under a reversed 14L: 10D photoperiod with scotophase beginning at 9 a. m.. All the experiments were conducted at 20 to 22°C under a dim red light in a dark room.

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Circadian rhythm of emergence

Moths emergence time was determined by placing 300 pupae of each sex under the same condition as described above, and emerged moths were removed and counted at 2-h intervals from 9 a. m. to 7 p. m. each day.

Female calling and adult mating

Tested moths were aged from 1-day-old to 6-day-old. Ten males and eight females of each age group were placed in a 30×30 ×30 cm transparent plastic box 1 h before scotophase, a 15 cm high potato plant, *Lycopersicon esculentum*, was set inside the box. Calling females were easily recognized as they extruded their ovipositors and raised their wings slightly above the abdomen. The number of calling females and mating pairs were recorded at hourly interval through scotophase. The age effect on mating duration was also recorded. At the end of observation, tested females were dissected in order to determine the percentage of successful spermatophore transfer.

Comparison of calling behavior and mating ability between 3-day-old and 6-day-old adults

In the above study, 6-day-old females showed high calling and low mating percentages. Does the sex pheromone released have low effect? or the sex pheromone concentration is too high to guide the male to mate? or the 6-day-old males have low mating ability? For answering these questions, eight 6-day-old males were paired with eight 3-day-old females, and eight 6-day-old females were paired with eight 3-day-old males. Females calling and adults mating were observed in hourly interval. The percentage of successful spermatophore transfer was recorded.

Effect of sex ratio on female calling and mating frequency

Sex ratio of female : male was fixed at 1:1, 1:2 and 2:1, with eight 3-day-old individuals represent the ratio of 1. The percentage of females calling and adults mating were recorded in hourly interval.

Data analysis

Student-Newman-Keul's test (Steel and Torrie 1960) was applied in the data analysis for the present study.

RESULTS

Circadian rhythm of emergence

Under the reversed 14L:10D photoperiod, the largest number of moths emerged during the first 2 h after the last light period. More moths emerged between 4 h and 10 h after scotophase, a small number emerged between 9 p. m. and 7 a. m., the photophase (Fig. 1). All the moths which emerged within 2 hours after scotophase was determined to be of the same physiological age, and only these were used in the following tests.

Female calling and adult mating

Females calling percentage varied with age (Fig. 2A). None of the 1-day-old females exhibited calling behavior. A small proportion of the 2-day-old females exhibited weak calling, with the ovipositor partially extended, only within 6-8 h after scotophase. Strong calling, ovipositor fully extended, exhibited from the 3-day-old females with calling behavior started at 4 h, and reached peak at 6 h after scotophase. Calling behavior of 4-day-old females started at 2 h, and reached peak at 6-7 h after scotophase. For 5-day-old females, calling started and reached peak (37.5%) at 4 h after scotophase. A high proportion of 6-day-old females started calling at 2 h after scotophase, continued at a high level through the scotophase and terminated after the initiation of photophase. The fact that older females called sooner than the younger ones which can also be shown with the $\bar{x}$ calling time, the point at which half of the calling-hours exhibited for the day was reached (Fig. 2A). The $\bar{x}$ calling time was not significantly different among different age groups.

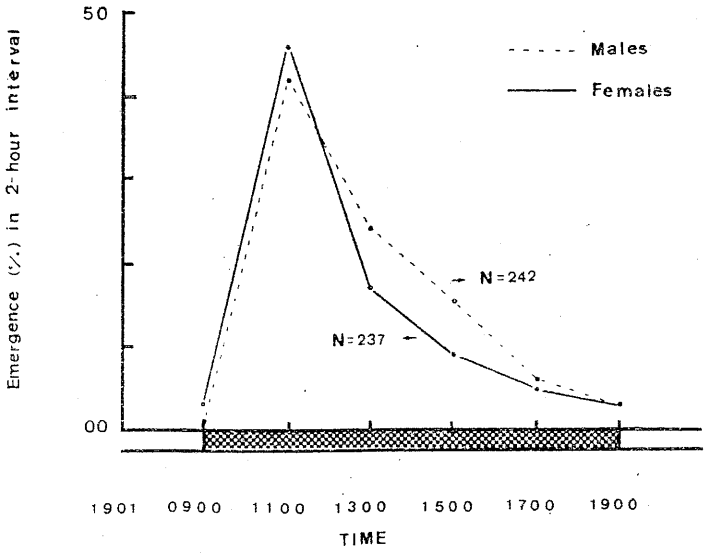

Fig. 1. Demonstration of circadian rhythm of emergence in *Heliothis armigera*. All pupae were held under 25°C, scotophase from 0900 to 1900 (shading denotes scotophase). "N" refers to the total number of moths emerged. Data are plotted at the end of each time interval.

A small proportion of 2-day-old adults started mating behavior late in the scotophase whereas high percentage of 3-day-old and 4-day-old adults started earlier in the scotophase. In the 5-day-old and 6-day-old adult groups, mating behavior started late and with a declined mating percentage (Fig. 2B). The $\bar{x}$ mating time, the point at which half of the mating-hours exhibited for the day was reached, was at 8 h after scotophase for the 2-day-old adults, then shifted to earlier hours for the older adults (Fig. 2B). The average mating duration of each group was 94.0± 4.0 min, 82.4±14.3 min, 74.8±20.0 min, 84.6± 14.4 min, and 90.0±15.0 min for the 2-day-old, 3-day-old, 4-day-old, 5-day-old, and 6-day-old adults, respectively. Both $\bar{x}$ mating time and mating duration were not significantly different among different age groups.

Comparison of calling behavior and mating ability between 3-day-old and 6-day-old adults

The percentage of successful spermatophore transfer was 50.0% for 3-day-old females+6-day-old males group and 62.5% for the group with reversed age of sexes. Calling behavior started earlier in older 6-day-old females than in the younger 3-day-old females (Fig. 3A). The $\bar{x}$ calling time was 5.9 h and 5.3 h after scotophase for the 3-day-old and 6-day-old females, respectively (Fig. 3A). Mating started at 1 h and 3 h, reached peak (25%) at 7 h and 6-7 h after scotophase for 3♀-6♂ and 6♀-3♂, respectively (Fig. 3B). Both $\bar{x}$ calling time and $\bar{x}$ mating time were not significantly different between these two treatments.

Effect of sex ratio on female calling and mating frequency

Both the onset of calling and the $\bar{x}$ calling time were the same, with sex ratio of ♀:♂ =1:2 or 2:1 when under the same density (24 individuals/box), at 1 h and 5.9 h after scotophase. But which were late at 3 h and 6.6 h after scotophase, respectively, when sex ratio was 1:1 at lower density (16 individuals /box) (Fig. 4A). Percentage of successful spermatophore transfer was 81.3%, 50.0% and 12.5% for the sex ratio of ♀:♂=1:2, 1:1 and 2:1, respectively.

Initiation of mating, which was delayed with the increased female ratio, started at 2 h, 5 h and 7 h after scotophase at the sex

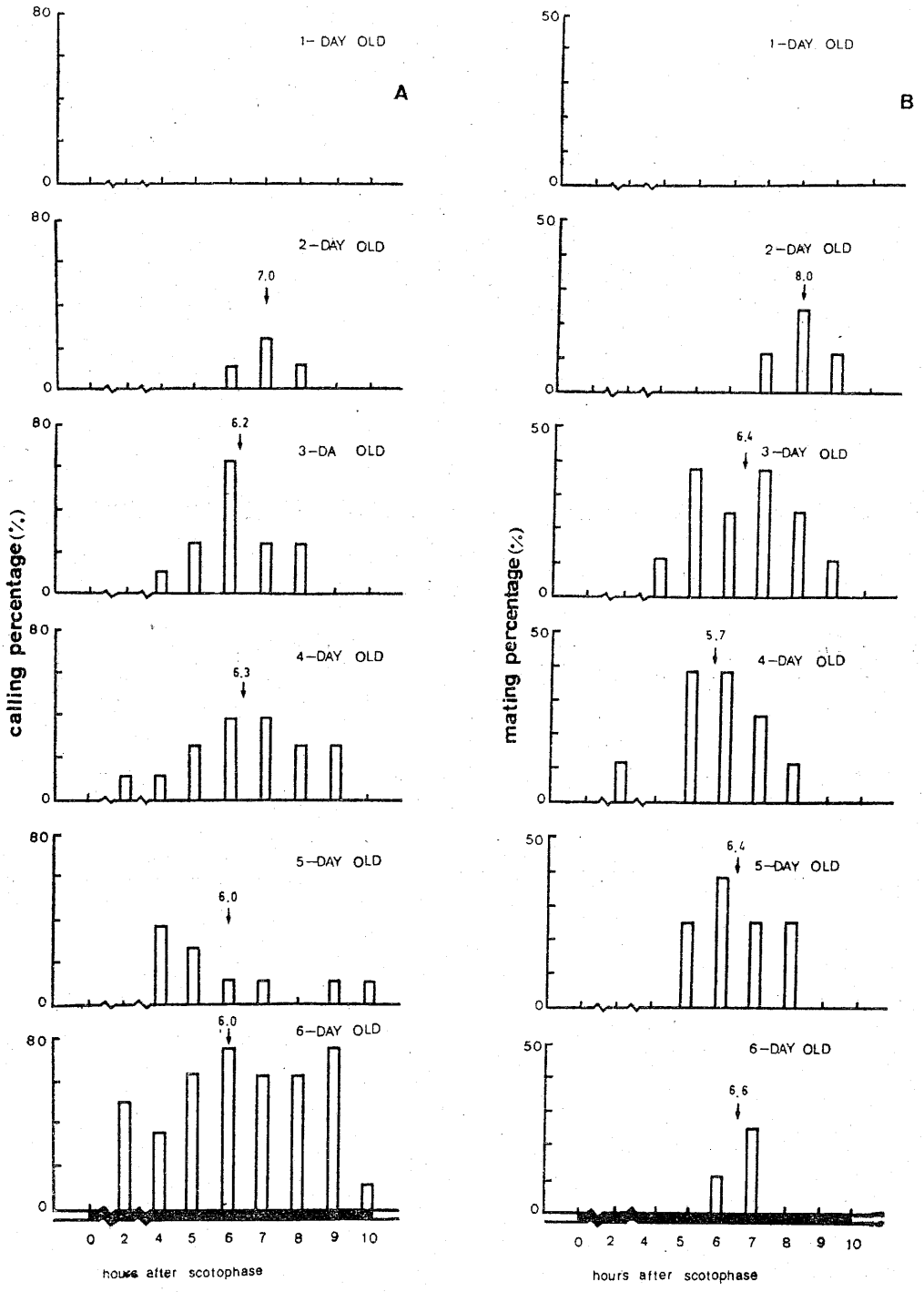

Fig. 2. A. Age effect on female calling of *Heliothis armigera.*
B. Age effect on mating of *Heliothis armigera.*
* Arrows indicated $\bar{x}$ calling time or $\bar{x}$ mating time which was not significantly different among the six age groups (ANOVA, p=0.05; Student-Newman-Keul's test).
* Shading on the abscissa denotes scotophase. Temperature was 20-22°C.

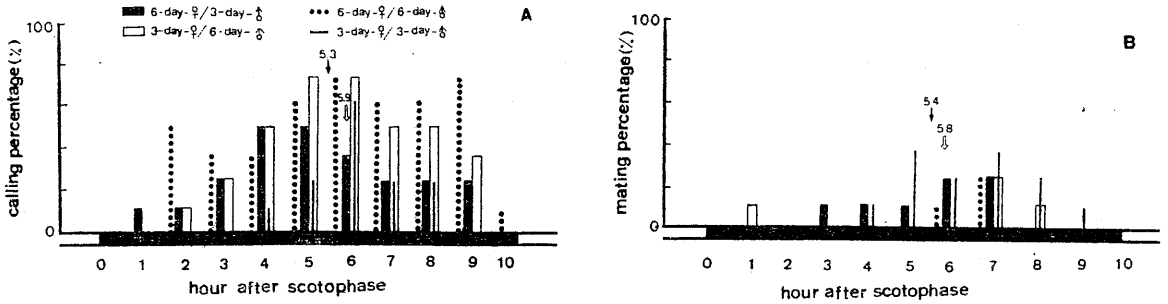

Fig. 3. A. Effect of two different adult ages on female calling of *Heliothis armigera.*
B. Effect of two different adult ages on mating of *Heliothis armigera.*
* White arrows represent $\bar{x}$ calling time or $\bar{x}$ mating time of 3-D-♀/6-D-♂.
Black arrows represent $\bar{x}$ calling time or $\bar{x}$ mating time of 6-D-♀/3-D-♂.
* The $\bar{x}$ calling time or $\bar{x}$ mating time was not significantly different among the two age groups (ANOVA, p=0.05; Student-Newman-Keul's test).
* Shading on abscissa denotes scotophase. Temperature was 20-22°C.

ratio of ♀ : ♂ =1:2, 1:1 and 2:1, respectively (Fig. 4B). The $\bar{x}$ mating time was shifted from 6.1 h to 6.5 h and 8.3 h after scotophase at above sex ratios.

Both $\bar{x}$ calling time and $\bar{x}$ mating time were not significantly different among different sex ratios.

DISCUSSION

Sex pheromonal response have been studied for a long time based on moths age, but few informations are available on the criteria of moths physiological age. It is commonly observed under laboratory conditions that *Heliothis* spp. moths tend to eclose within a few hours of the initiation of darkness (Callahan 1958, Hennebery and Clayton 1984, Roush and Schneider 1985). In the present study, the largest number of *H. armigera* moths also eclosed within 4 h of the initiation of scotophase (Fig. 1) under a reversed photoperiod. Therefore, moths which emerged within this narrow time period was decided to be at the same physiological age.

Based on the physiological age, our results indicated that almost all calling activity occurred within 2–10 h after scotophase. This is well coincided with field study of *H. armigera* by Kravchenko (1981) who noted that intensive secretion of pheromone by the females occurred at 3-9 h after sunset. Our results also presented that patterns of calling and mating varied with the age. There were no calling and mating activities observed on day one, as postulated by Shorey (1974) that females of certain noctuid moth species neither matured their eggs nor contained detectable sex pheromone in their glands until some time following emergence. As moths aged, calling commenced earlier in the scotophase, 6-day-old females called at the earliest. Similar changes in calling behavior with age was recorded in the sweet potato leaf folder, *Brachmia macroscopa* (Hirano and Muramoto 1976), the black cutworm, *Agrotis ipsilon* (Swier *et al.* 1977), *Yponomeuta viginti-punctatus* (Retzius) (Hendrikse 1978), the rice stem borer, *Chilo suppressalis* (Walker) (Kanno 1979) and the armyworm, *Pseudaletia unipuncta* (Turgeon and McNeil 1982). Swier *et al.* (1977) suggested that by calling earlier in the scotophase older females increase their probability of mating by being the first to attract males, while Hendrikse (1978) suggested that there may be a change in the amount of pheromone produced associated with age. Mating behavior also changed with moths age (Fig. 2B), as moths aged, mating commenced earlier in the scotophase, 4-day-old adults mated earliest (2 h after scotophase). Similar changes was also recorded in

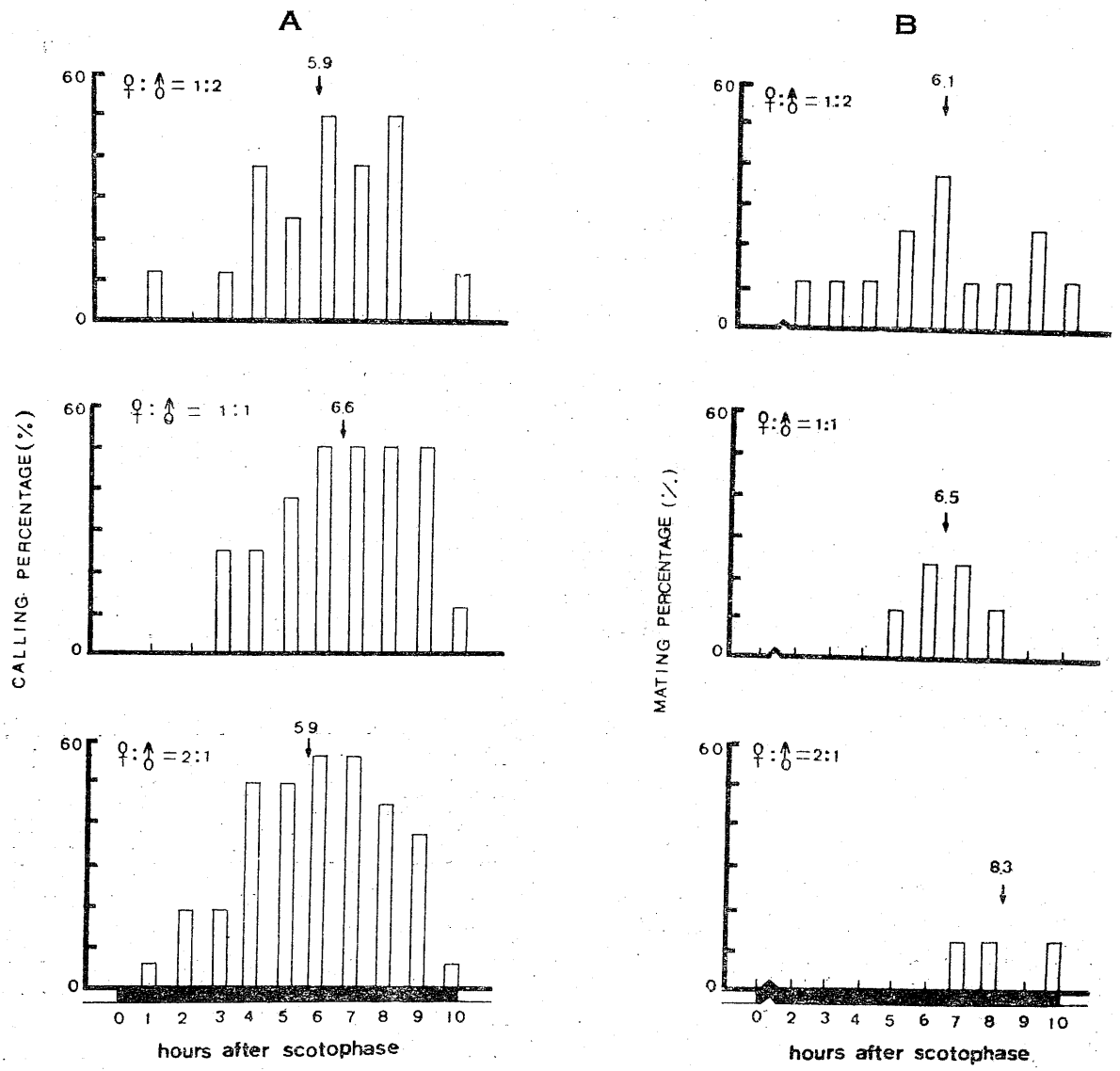

Fig. 4. A. Effect of different sex ratios on female calling of *Heliothis armigera.*
B. Effect of different sex ratios on mating of *Heliothis armigera.*
* Arrows indicated $\bar{x}$ calling time or $\bar{x}$ mating time which was not significantly different among the three sex ratios. (ANOVA, p=0.05; Student-Newman-Keul's test).
* Shading on abscissa denotes scotophase. Temperature was 20-22°C.

the smaller tea tortrix, *Adoxophyes orana* (Tamaki *et al.* 1969), the tabacco cutworm, *Spodoptera litura* (Yushima *et al.* 1973), the sweet potato leaf folder, *Brachmia macroscopa* (Hirano and Muramoto 1976), the tea tortrix moth, *Homona magnanima* (Noguchi 1979). Our results also showed that 6-day-old males may have low mating ability, which is reasonable to be expected since the longevity of males was around 7-10 days in the laboratory.

Callahan (1958) indicated that a higher mating percentage might be obtained in the corn earworm *Heliothis zea* by putting more males than females in the same cage. But in *Spodoptera litura*, the mating rate didn't

show any tendency to vary regularly in accordance with the change in sex ratio or density (Otake and Oyama 1973). The present study showed that more males (♀:♂=1:2) resulted in a higher percentage of mating (81.3%) than fewer males (♀:♂=2:1) (12.5%) when under the same density, and an intermediate mating rate (50.0%) was shown with the normal 1:1 sex ratio.

Sex pheromone production in the female corn earworm, *Heliothis zea*, was controlled by a hormonal substance produced in the brain, this hormonal substance is released into the haemolymph to stimulate pheromone production only in the scotophase, and the stimulatory activity was also detected in the brain of male corn earworm moths and other moths (Raina and Klun 1984). Further study is needed to understand whether a similar hormone existed in the cotton bolloworm moth. The present study which concerning the criteria of the cotton bollworm's physiological age and the relationship between age and the mating-related behaviors should be helpful in further study of the bioassay technique in sex pheromone research.

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蕃茄夜蛾 (*Heliothis armigera* Hübner) 之羽化及其交配有關行爲

寇　融　周延鑫

本研究係報導蕃茄夜蛾，*Heliothis armigera* (Hübner)，於 20-22°C，逆光週期之性費洛蒙行爲學，雌蛾求偶行爲始於羽化後第 2 日，並隨日齡增加而於暗期中提前，以 6 日齡蟲爲最早。交配行爲亦隨日齡增加而提前，以 4 日齡蟲具最高交配率。不同性比對雌蛾求偶行爲無影響，雌雄性比爲 1:2 時，可得最高交配率。