

## Behavior and Circadian Rhythm of Emergence, Copulation and Oviposition in the Oriental Tobacco Budworm, *Heliothis assulta* Guenee

담배나방의 우화, 교미 및 산란의 행동과 일일리듬

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**ABSTRACT** The behavior and circadian rhythm of emergence, copulation and oviposition in the Oriental tobacco budworm (*Heliothis assulta* Guenee) were studied to obtain the following results. More than three quarters of adults emerged within 3 hours after the light-off. It took about 33 min for an adult to expand and fan its wings after emergence. Adult male showed a sequential pattern of mating behavior, such as antennal movement, wing elevation and vibration, extension of hairpencils, and tapping of female ovipositor leading to a copulation. However, adult female revealed a rather simple behavior, such as protraction and retraction of her terminal abdomen and vibration of wings. All of the mating took place during the early part of the scotophase, with the peak from 1 hr to 2 hrs after the light-off. But the mating peak in virgin females tended to advance with age. Adult females showed the highest rate of mating among 24 hr-olds and the rate declined with age. The rate of first matings in males gave a very similar pattern to that of females. The duration of copulation was  $77 \pm 14$  min. Oviposition occurred throughout the entire scotophase, with the peak during the first 2 hrs. The most number of eggs deposited daily was seen on the 3rd day after the mating. Mating rate, number of eggs laid and longevity were compared under different sex ratio. Unmated adults lived longer than mated adults.

**KEY WORDS** Oriental tobacco budworm, *Heliothis assulta*, emergence, copulation, oviposition

**抄 錄** 사육실조건(25±1°C, 광주기 16L : 8D, 상대습도 60%)에서 조사된 담배나방(*Heliothis assulta*)의 우화, 교미 및 산란의 행동과 일일리듬은 다음과 같다. 불꺼진 후 3시간 이내에 75% 이상이 우화하였으며, 우화후 날개확장과 경화까지는 약 33분이 소요되었다. 성충 수컷은 교미때까지 촉각 운동, 날개 진동, hairpencils 확장, 암컷 산란관 두드림 등 일련의 행동을 보였으나, 암컷은 복부끝의 수축과 이완, 날개진동 등의 다소 단순한 행동을 보였다. 모든 교미는 암기간 전반기에 주로 일어났으며, 불꺼진 2시간 전후에서 최대피크를 보였으나, 처녀 암컷의 경우 연령증가에 따라 빨라지는 경향을 보였다. 교미율은 24시간된 미교미암컷에서 가장 높았으며, 연령증가에 따라 감소되는데 같은 경향이 미교미수컷에서도 나타났다. 교미지속기간은 평균  $77 \pm 14$  분이였다. 산란 역시 암기간 전체를 통하여 일어났지만, 불꺼진 후 2시간 이내에 최적피크를 보였으며, 산란수는 교미후 3일에서 가장 많았다. 교미율, 산란수, 수명은 성비에 따라 달랐으며, 교미한 성충의 수명이 교미하지 않은 것보다 더 짧았다.

**檢 索 語** 담배나방, 우화, 교미, 산란, 행동, 리듬

The Oriental tobacco budworm (*Heliothis assulta* Guenee) is widely distributed in Korea, Japan, China, Australia and Africa and causes serious damage to the hot pepper in Korea. It is difficult to control *H. assulta*

larvae in the field, because of their feeding habit within fruits of the hot pepper and their resistance against several insecticides. When the field is heavily infested, it is required to apply insecticides frequently, which causes serious adverse effects such as the mammalian toxicity and chemical residue in

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the environment.

To cope with this problem, some researchers began to show an interest in the fundamental biology such as endocrinology, emergence rhythm, mating behavior, etc. On the basis of basic knowledge, some applied research has been focused on the development of insect growth regulators and synthetic sex pheromones to selectively control the target pest without toxic effects to nontarget organisms and/or the environment.

The possibility of using sex pheromones in insect pest control has been much discussed (Mitchell 1975, Tumlinson et al. 1976, Silverstein 1981). However, in order to use the synthetic sex pheromones in the field the first requirement is that the mating behavior of the target pest must be understood. Therefore, this study was initiated to investigate the behavior and circadian rhythm of emergence, copulation and oviposition in the Oriental tobacco budworm.

## MATERIALS AND METHODS

The Oriental tobacco budworm larvae were collected in Suwon area and reared on tobacco leaves. Adults from these larvae were used as the test insects. The observations during the entire scotophase were made with a flashlight equipped with a red filter. All experiments were conducted at  $25 \pm 1^\circ\text{C}$ , 60% RH and 16L : 8D photoperiodic regime.

### Behavior and Rhythm of Emergence

The time of moth emergence was determined by placing more than 50 male or female pupae in the plastic boxes. The time of moth emergence was regarded as the time when the old pupal cuticle is cast off completely. Observation was made at 1 hr intervals from the light-off to the end of the dark period, since it was found in a preliminary experiment that most of adult emer-

gence occurred during this period. Emerged moths were removed at each observation time. This experiment was replicated 2 times.

Behavior of the imago at emergence was studied from ten male or female pupae placed in a polystyrene cage (30×30×35cm) equipped with a pleated cardboard to which the adults could cling. An 8% sugar solution was also supplied in the polystyrene cage.

### Behavior and Rhythm of Adult Mating

Three pairs of adults were placed in the polystyrene cage (30×30×35cm) containing a bottle of 8% sugar solution. The precopulatory behavior of adult moths was recorded on an audio tape and later transcribed and depicted.

The rate of first matings in virgin females was examined at different ages. In this case the same number of males as females was introduced. The males were also of the same age as the females. But, when the first mating rate of virgin males was examined they were paired with 24 hr-old females. Observation in this experiment was made at half-hour intervals throughout the entire scotophase.

Depending on the sex ratio (female : male = 3 : 1, 2 : 1, 1 : 1, 1 : 2, 1 : 3), mating rate, number of eggs laid and longevity of the mated adult were determined and also compared with those of the unmated adult. This experiment was replicated 4 times.

### Ovipositional Behavior and Rhythm of Adults

Upon emergence, the mated adult moths were placed in the polystyrene cage (30×30×35cm) together with 8% sugar solution for drinking and tobacco leaves for oviposition. The ovipositional behavior was described. The number of eggs was counted on the fresh tobacco leaves that were changed

every 2 hours.

**RESULTS AND DISCUSSION**

**Adult Emergence and Wing Expansion**

*Heliothis assulta* adult emerged from 1 hr before the light-off to the end of scotophase (Fig. 1). The highest hourly percentage of adult emergence was recorded from 1 hr to 2 hrs after the light-off. More than three quarters of all adult emergence took place within 3 hrs after the light-off. These results were similar to those reported by Lingren(1978) and Kim and Boo(1986). Thus this rhythmicity of adult emergence at night will be associated with nocturnal behavior of these insects.

After emergence, the adult showed a typical behavior for wing expansion(Fig. 2). Their wing pads upon emergence averaged only 3—4mm in length (Fig. 2.A). After emergence adult moths became very active and walked around as if searching for a suitable structure to cling to for a normal wing expansion. It took about 20 min for

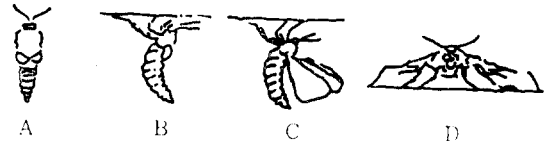


Fig. 2. Wing expansion behavior of an adult after emergence.

adult moths to find a suitable place to cling (Fig. 2.B). About 33 min after emergence the wings were completely expanded and tanned (Fig. 2.C). After wing expansion, the full length of wings was 14—15mm. This postemergence behavior was similar to that described for *Heliothis zea* (Callahan 1958) and *Dioryctria abietella* (Fatzinger & Asher 1971). However, emerged *H. assulta* moths did not always require, but preferred to have a villous surface to cling to for a normal wing expansion. Some moths were observed to have crumpled, deformed wings. Callahan(1958) stated that moths of *H. zea* required a villous surface to cling to for a normal wing expansion. These deformities did not, however, appear to be correlated with the type of surface to cling to for a normal wing expansion. Thus these deformities remain to be elucidated. In addition, total time required from emergence to completion of wing expansion and tanning in *H. assulta* was intermediate between *H. zea*(43min) and *D. abietella*(19min).

**Mating Behavior**

Their mating behavior was similar to that of other noctuids. Figure 3 shows typical positions of the tobacco budworm moths before, during and after copulation. The activities of males and females before copulation can be sequentially described as follows:

A. Female behavior 13—20 min before copulation: The female repeatedly extended and retracted her terminal abdomen including the ovipositor. It is likely that she

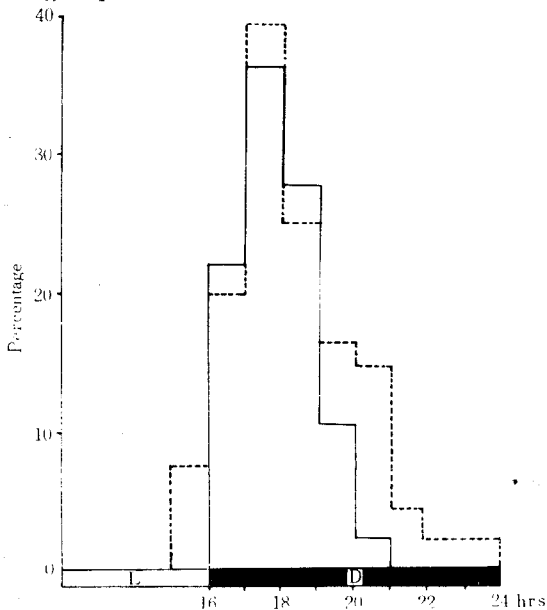


Fig. 1. Temporal patterns of emergence (···) and mating (—) in *Heliothis assulta* adults (L: light period, D: dark period).

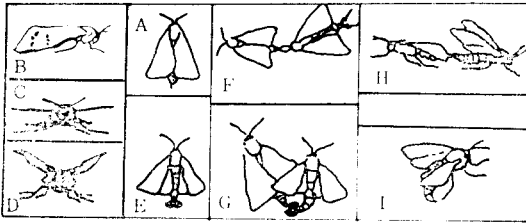


Fig. 3. Typical positions of the tobacco budworm moths before, during and after copulation (see the text for details).

was releasing sex pheromone from the gland located between the 8th and 9th abdominal segment (Moon et al. 1986). Before this behavior, the female displayed similar behavior to the males as described in the following 3 steps (B, C & D).

- B. Male behavior 12–18 min before copulation: The antennae were brought forward and raised 5–15° above and lateral to the body axis. This behavior must have been initiated by stimulation from female's precopulatory behavior.
- C. Male behavior 11–16 min before copulation: The male raised his wings to a horizontal position and vibrated them intermittently.
- D. Male behavior 9–13 min before copulation: The male further raised his wings to 45° above the horizontal axis and vibrated them. He also repeated protraction and retraction of his hairpencils.
- E. Male behavior 5–8 min before copulation: The male extended and spread his hairpencils.
- F. Male behavior 3–5 min before copulation: The male approached the female from behind and tapped her extended ovipositor (about 2–3mm) with his antennae once or twice.
- G. Male behavior 1–2min before copulation: The male arched his abdomen toward the female genitalia and extended his hairpencils so as to grasp her genitalia.

H. Typical copulation posture: After connection, the head of the male was positioned in the direction opposite to that of the female.

I. After mating a female showed an ovipositing posture.

Males did not attempt to copulate with females that failed to either vibrate her wings or to extend her ovipositor. This adult mating behavior was similar to that of other lepidopteran species (Agee 1969, Fatzinger & Asher 1971, Teal & Byers 1980). In addition, the paired moths exhibited little locomotor activity throughout periods of copulation, although they readily walked around when disturbed.

All of the mating took place during the early part of the scotophase (Fig. 1). The highest percentage of adult mating occurred during one hour from 1 hr to 2 hrs after the light-off. As a whole, about 86 per cent of all adult matings took place during the first three hrs after the light-off. The calling rhythm of female *D. abietella* has been reported to follow an endogenous circadian rhythm (Fatzinger 1973). The mating time was determined by female rhythm of sex pheromone release (Shorey & Gaston 1965). Thus the mating time in *H. assulta* will be determined by female rhythm of sex pheromone release.

The duration of copulation in *H. assulta* widely varied from 50min to 120min, but was concentrated between 60min and 90min (Fig. 4). The present result was similar to that (from 45 to 90min.) of *H. zea* (Agee 1969), but was slightly different from that (99±4min.) of *D. abietella* (Fatzinger & Asher 1971).

Adult females showed the highest rate of mating 24 hrs after emergence, and the rate declined with age (Fig. 5). Shorey et al. (1968), in females of 7 species representing 3

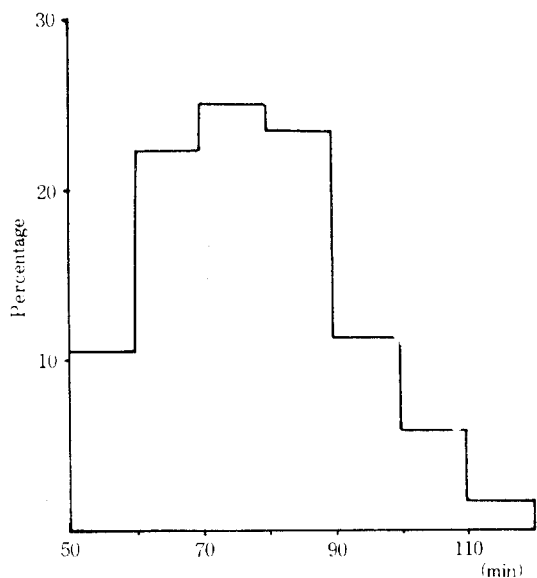


Fig. 4. Duration of copulation in *H. assulta*.

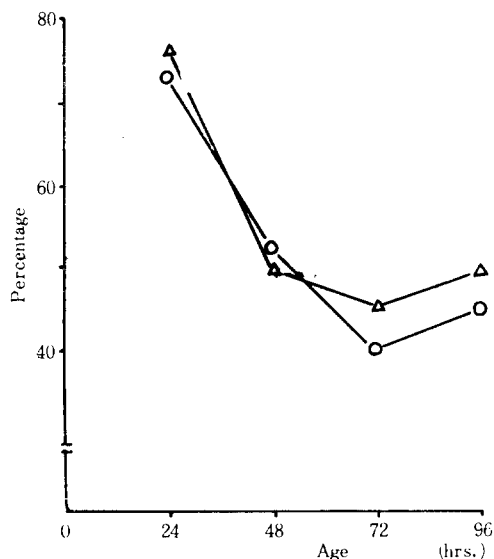


Fig. 5. The rate of first matings in virgin females (△—△) and males (○—○) at different ages. Numbers and ages of females were equal to those of males in the virgin female test. Numbers of males, 24 hr-olds, was equal to that of females in the virgin male test.

subfamilies of Noctuidae, reported that for each species rapid production of sex pheromone occurred within one day preceding or following emergence from pupa. Mating could be determined depending on the amount of sex pheromone released. Therefore, we

think that the highest mating occurred in 24 hr-old virgin females due to the highest quantity of sex pheromone at this age. But the temporal patterns of the first matings in virgin females tended to advance with age. For example, 24hr-old females showed their peak of mating 2 hrs after the light-off. But 96 hr-olds had the peak during the first hour right after the light-off (Fig. 6). This result was similar to those examined for *Heliothis armigera* (Kou & Chow 1987), *Agrotis ipsilon* (Swier et al. 1977) and *Chilo suppressalis* (Kanno 1979). In their studies, they reported that the initiation time of mating was advanced with female age. This phenomenon of the temporal patterns was correlated with increased probability of mating, by being the first female to attract males (Swier et al. 1977, Kanno 1979).

The rate of first matings in males also gave a very similar pattern to that of the virgin females (Fig. 5) showing the highest rate among 24 hr-olds and then decreasing with their age. This mating rate is always about equal to or lower than of females of the same age. Therefore it may be concluded that the mating rate is governed by the release rhythm of female sex pheromone and also by the males' own mating tendency.

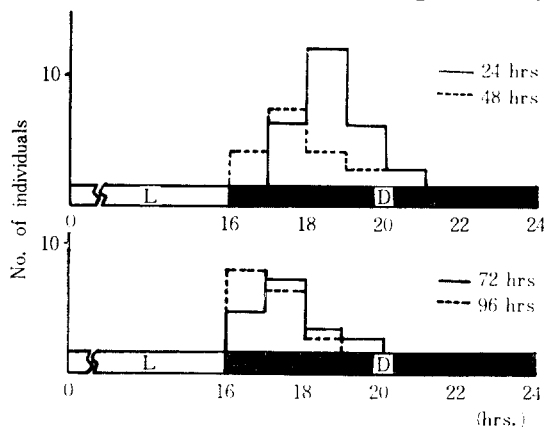


Fig. 6. Temporal patterns of the first matings in virgin females of the different ages(1—4 days) after emergence(16L/8D).

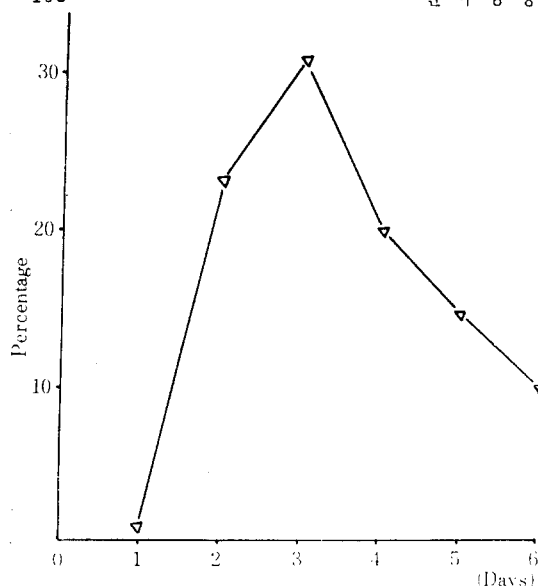


Fig. 7. Circadian rhythm in oviposition of *H. assulta*(16L/8D).

But it is not clear from this experiment what governs the males' mating tendency and what makes their tendency decrease with their age, instead of increase observed in many other insect species.

### Oviposition

Mated *H. assulta* females oviposited throughout the entire scotophase, with the peak (52%) during the first 2 hrs (Fig. 7). They tended to lay the most number of eggs on the 3rd day after the mating (Fig. 8). No oviposition was ever observed on the night of the the mating. Females rarely began to oviposit without feeding beforehand. In some cases (about 0.8% only), they oviposited during the light period. This result was similar

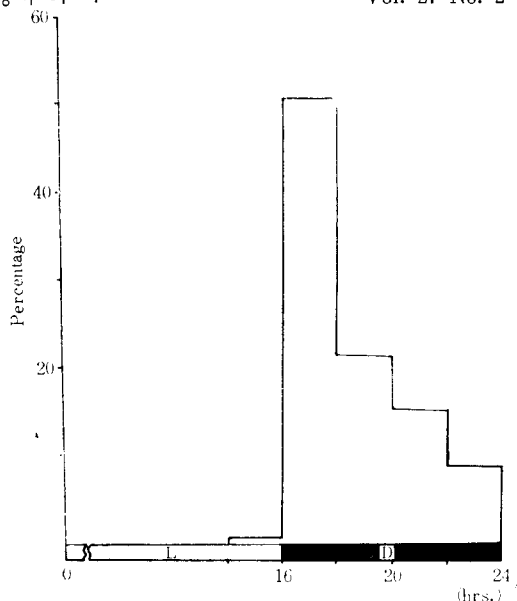


Fig. 8. Daily oviposition pattern after copulation in *H. assulta*.

to that obtained for *H. virescens* (Callahan 1958). The females, while looking for a suitable spot, arched the abdomen into a sickle shape and walked rapidly over tobacco leaves. It took approximately 4—9 seconds to deposit one egg and eggs were deposited sporadically around the outer edge of the leaves. Very few eggs were laid on the glass or the corner of the cage.

### Sex Ratio and Longevity

When the ratio of female to male was changed (Table 1) the mating rate of the female increased with increasing numbers of males. However, the mean number of eggs laid by a female did not show much difference, except for a case of the highest male

Table 1. Mating rate, number of eggs laid, longevity of the mated adult depending on the sex ratio

Sex ratio (♀ / ♂)	Mating rate /female(%)	Mean no. eggs /female	Average longevity(days)	
			Female mated	Male mated
3 : 1	33.3	467.5 ± 53.0	12.0 ± 0.7	14.4 ± 1.5
2 : 1	50.5	450.3 ± 11.0	9.7 ± 0.5	10.0 ± 0.8
1 : 1	76.7	412.0 ± 70.8	8.3 ± 0.4	9.8 ± 1.5
1 : 2	100.0	451.3 ± 80.7	10.7 ± 2.4	11.7 ± 2.4
1 : 3	100.0	611.8 ± 123.9	11.3 ± 4.5	12.7 ± 2.1

\* All experiments were replicated 4 times.

Table 2. Comparison in longevity of the mated and unmated adults

		Replication					Average (days)
		1	2	3	4	5	
Female	Unmated	12	13	14	15	14	13.6 ± 1.0
	Mated	8	10	12	11	11	10.4 ± 1.4
Male	Unmated	13	14	15	16	15	14.6 ± 1.0
	Mated	10	11	13	12	13	11.8 ± 1.2

to female ratio. On the other hand, average longevity was the shortest at 1 : 1 ratio and became longer as the numbers of either sex increased (Table 1). In a similar experiment with *H. virescens* Guerra et al. (1972) reported that fecundity decreased but number of matings per female increased, as the ratio of the male to female in a laboratory cage increased. Adult longevity was the shortest (6.6 days) at the highest ratio of male to female (9 : 1), while the longest longevity (9.8 days) was obtained at 1 : 1 ratio (Guerra et al. 1972). This difference, with the exception of the mating per female, remains to be elucidated.

When the mated and unmated adults were compared (Table 2), the longevity of the mated was shorter than that of unmated adults. This result also contrasts to that obtained for *H. virescens* (Callahan 1958). This could be due to the fact that the mated adults are more active and also need more nutrition for egg development and oviposition.

#### ACKNOWLEDGMENT

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